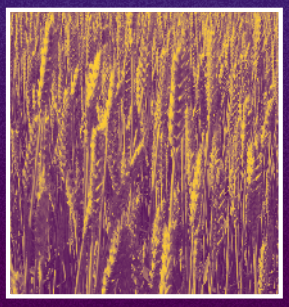




# RESEARCH FOR RURAL DEVELOPMENT 2011

*Volume No 1*

*Annual 17th International Scientific Conference Proceedings*

Latvia University of Agriculture

# RESEARCH FOR RURAL DEVELOPMENT 2011

**Annual 17th International Scientific Conference Proceedings**

Volume No 1

**Jelgava 2011**

**Research for Rural Development 2011**
Annual 17th International Scientific Conference Proceedings (Volume No1).
Jelgava, LLU, 2011, 208 pages
ISSN 1691-4031

**ORGANIZING COMMITTEE**

**Ausma Markevica**, Mg.paed., Mg.sc.soc., Head of the Post-graduate Studies Department,
Latvia University of Agriculture

**Aida Radzevičiūtė**, Manager of the Department of the Investments and Development,
Lithuanian University of Agriculture

**Nadežda Karpova-Sadigova**, Mg.sc.soc., main specialist of Post-graduate Studies Department,
Latvia University of Agriculture

**EDITORIAL BOARD**

**Chairman**
Professor **Zinta Gaile**, Dr.agr., Latvia University of Agriculture

**Members**
Associate professor **Andra Zvirbule-Bērziņa**, Dr.oec., Latvia University of Agriculture
Professor **Tālis Juhna**, Dr.sc.ing., Riga Technical University
Professor **Irina Arhipova**, Dr.sc.ing., Latvia University of Agriculture
Associate professor **Gerald Assouline**, Dr.sc.soc., Director of QAP Decision, Grenoble, France
Professor **Inga Ciproviča**, Dr.sc.ing., Latvia University of Agriculture
Associate professor **Aivars Kaķītis**, Dr.sc.ing., Latvia University of Agriculture
Associate professor, **Antanas Dumbrauskas**, Dr.sc.ing., Lithuanian University of Agriculture
Senior researcher, **Āris Jansons**, Dr.silv., Latvian State Forest Research Institute "Silava"

**TECHNICAL EDITORS**
Santa Treija
Inga Skuja

**© Latvia University of Agriculture, 2011**

The Proceedings of previous Annual International Scientific Conferences "Research for Rural Development" published by Latvia University of Agriculture since 1994 and are included in to databases **CABI** (Commonwealth Agricultural Bureaux International), **AGRIS** (International Information System for the Agricultural Sciences and Technology) and others non-profit basis.
The Latvian Council of Science accepted Proceedings of International Scientific Conferences "Research for Rural Development" as generally recognized and reviewed scientific publication in 2003.

Editorial office: Latvia University of Agriculture, Lielā ielā 2, Jelgava, LV-3001, Latvia
Phone and fax: + 371 630 05607; e-mail: Ausma.Markevica@llu.lv

Printed and bound in Jelgavas tipogrāfija

**Supported by**:

## EDITORIAL

With this issue of 2011, we bring 72 proceedings of the 122, which started life as presentations at the Annual 17th International Scientific Conference "Research for Rural Development 2011" held at the Latvia University of Agriculture, in Jelgava, on 18 to 20 May 2011.

In the retrospect of four months later, we can count the Conference as a great success. The theme – Research for Rural Development - attracted participation of 252 researchers with very different backgrounds. There were 18 presentations from different universities of Lithuania, 2 from Estonia, 3 from Poland, 1 from Spain, 1 from Ukraine, 2 from Russia and 95 from Latvia.

Four independent reviewers estimated each article.

The proceedings of the Annual 17th International Scientific Conference "Research for Rural Development 2011" is intended for academics, students and professionals researching in the area of crop production, animal breeding, agricultural engineering, agrarian and regional economics, food sciences, veterinary medicine, forestry, wood processing, water management, environmental engineering, landscape architecture, information and communication technologies.

The proceedings will also be useful for researchers in educational sciences.

# WINTER OILSEED RAPE (*BRASSICA NAPUS* L.) AUTUMN GROWTH

***Oskars Balodis, Zinta Gaile***
*Latvia University of Agriculture*
*balodis.oskars@inbox.lv*

## Abstract

Lack of data about effect of meteorological conditions, sowing date, sowing rate, fungicide as growth regulator application and other agroecological factors on rape autumn growth is observed in Latvia. The aim of our research, started in autumn 2007 and continued up to autumn 2010 in Research and Study farm 'Vecauce', was to investigate the influence of agroecological factors (sowing date, sowing rate and fungicide (as growth regulator in autumn)) application as well as meteorological conditions on autumn plant growth of two types of winter rape varieties. Winter rape biometrical parameters were estimated, and meteorological parameters (hydrothermal coefficient (HTC) and growing degree days (GDD)) were calculated. Four year results showed that winter oilseed rape seed germination was affected by precipitation and air temperature around the sowing time. Calculated GDD correlated with plant biometrical parameters during trial years. HTC showed moisture effect on rape emergence time. Sowing date was important factor which had strong and significant impact on biometrical parameters of rape plants in autumn. Earlier sowing date increased height of growth point, root neck diameter, plant and root mass, and main root length significantly for both cultivars in four trial years. Such agro-ecological factor as sowing rate (plant density) affected plant biometrical parameters only in some trial years. Plant weight was significantly influenced by sowing rate for both cultivars if sowing rates were equal. Other important parameter – height of growth point – was not significantly influenced by sowing rate for both cultivars when similar sowing rates were used in all years; exception was year 2009 for 'Californium'.
**Key words**: winter rape, sowing date and rate, growing degree days, hydro-thermal coefficient.

## Introduction

Winter oilseed rape (*Brassica napus* L.) has become one of the basic crops in modern crop farming in Europe and Latvia. No decrease in oilseed rape cropping area has been observed during the last twelve years, and area under rape reached 109 400 ha in Latvia. Latvia, especially its central region, has suitable soils and meteorological conditions for winter rape cultivation. Proportion sown with winter rape is increasing (from 43% in 2005 to 66% in 2007 from the total rape sowing area) in central Latvia region – Zemgale; at the same time, if winter is hard, spring oilseed rape proportion from the total area can again increase (winter rape – 61% in 2010 from the total area sown with rape according to data of Central Statistical Bureau of Latvia). For good rape wintering, meteorological conditions in autumn as well as technology used are important factors.

Meteorologist M. Rummukainen (2010) mentions that drought is becoming a factor to be considered also in the Nordic and Baltic Sea region. Various climate extremes also seem to change. Some examples are frequency of intensive precipitation, cold spells, and heat waves. Therefore analyzing winter oilseed rape and also other winter crop growth during autumn effect of meteorological condition on plant development has to be more carefully analyzed. Hydrothermal coefficient (HTC) is one of the tools for interpretation of meteorological conditions' effect on plant development used in wheat and other crops and even in forestry. HTC till now is less used for oilseed rape growing issue interpretation (Ozolincius et al., 2005; Povilaitis and Lazauskas, 2010).

Rape seed germination and early development mainly is affected by soil moisture and temperature. Important rape plant development functions such as evapotranspiration, photosynthesis, water and nutrient absorption and other biological and chemical activities are regulated by temperature (Rapacz, 1998; Diepenbrock, 2000). Temperature effect on rape plant development can be interpreted as Growing Degree Days (GDD) and Corn Heat Units (CHU); both measures of heat accumulated by a crop over a period of time. GDD are much simpler to calculate and prove a good estimation of accumulated heat for most cool season crops like oilseed rape and wheat (Bonhomme, 2000). GDD calculation is more popular in Canada and United States, but less popular in Europe. To evaluate the risks of sowing time selection, the 'typical' GDD for specific area should be known. Such information about 'typical' GDD in rape growing areas of Latvia is not found while enough climate data websites are available for Canada with GDD maps. Some researches about winter oilseed rape autumn development with specific accent on GDD in a region close to Latvia are found (Sidlauskas and Rife, 1999; Laaniste et al., 2007).

Growth, development and seed yield of winter oilseed rape are closely related to sowing date and plant (sowing) density. A number of environmental factors interact with optimal sowing like soil temperature and moisture level affecting germination (BBCH 00–09) and seedbed preparation. Some objectives must be taken into account when choosing a sowing date: (1) ensuring that high-quality germination and emergence are possible; (2) optimizing the weight and number of the harvested plant parts; and (3) timing the cycle so as to limit the incidence of periods unfavourable to the crop (various stresses, parasites, frost, drought, etc.) (Fleury, 1986 cited by Rathe et al., 2006).

Plant density has been observed to have a huge influence on growth, development and seed yield of winter oilseed rape (Leach et al., 1999; Diepenbrock, 2000). Stand establishment generally varies depending on different

important circumstances, such as soil condition and water availability during germination as well as weather conditions (Mendham et al., 1981). Optimal plant density varies depending on oilseed rape growing region. Optimal plant density is about 80–150 plants $m^{-2}$ before winter and 60–80 plants $m^{-2}$ at the beginning of spring, respectively. Lower seeding densities (60–70 seeds $m^{-2}$) are required for hybrids, as mentioned by W. Sauermann (1995) (cited by Diepenbrock, 2000) in Germany, but optimum crop density for successful wintering in Nordic states is reported to be 30 to 70 plants $m^{-2}$ (Velicka, 2003). Required optimal densities are changing in length of time together with availability of more knowledge of rape yield formation.

In agro-ecological conditions of Latvia, data on winter rape plant autumn development is little documented. The aim of currently described section of our research was to investigate the influence of agricultural practice (sowing date and sowing rate) and meteorological conditions on autumn plant development two types of winter rape varieties. Part of research was described already in 2009 (Balodis and Gaile, 2009) using data about plant biometrical parameters in 2007 and 2008. Now research is complete, and new data, analyses and conclusions are added in the current paper.

## Materials and Methods

Four-year (starting from 2007/2008 to 2010/2011) experiments were carried out in the Research and Study farm 'Vecauce' (latitude: N 56º 28´, longitude: E 22º 53´) of Latvia University of Agriculture. Three-factor field trial using two types of winter rape (*Brassica napus* ssp. oleifera) cultivars (line 'Californium' and hybrid 'Excalibur') was carried out. The paper is focused on plant autumn growth results for all four seasons. The following factors were investigated:

Factor A – sowing date:
1st – called 1st August,
2nd – called 10th August,
3rd – called 20th August,
4th – called 1st September,
5th – called 10th September.

Factor B – sowing rate (120, 100, 80, 60 germinable seeds per $m^2$ – 'Californium'; 80, 60, 40, 20 germinate able seeds per $m^2$ – 'Excalibur' in 2007/2008 and in 2008/2009). In seasons 2009/2010 and 2010/2011, the sowing rates were supplemented to equals – 120, 100, 80, 60, 40, 20 germinable seeds per $m^2$ for both cultivars.

Soil at the trial site was strongly altered by cultivation in 2007 and 2010, and by soil-gleyic in 2008 and 2009; grading composition was loam; pH KCl = 6.7 to 7.4; content of available for plants K was 103 to 194 mg $kg^{-1}$, and P – 100 to 136 mg $kg^{-1}$; humus content – 25 to 38 g $kg^{-1}$. Pre-crop was cereal mixture for silage in all years. Traditional soil tillage with mould-board ploughing was used, rototilling was used before sowing. The crop was fertilized with a complex mineral fertilizer at the rate of N–12 to 28 kg $ha^{-1}$, P–18 to 30 kg $ha^{-1}$, and K–79 to 103 kg $ha^{-1}$ before sowing depending on a year. Sowing was done according to the previously described design. Weeds were controlled using herbicide Butisan Star s.c. (metasachlor, 333 g $L^{-1}$ + kvinmerac, 83 g $L^{-1}$),– 2.5 L $ha^{-1}$, except autumn and in 2010 when 3.0 L $ha^{-1}$ were applied. The herbicide was applied when the rape was fully germinated in plots of first three sowing dates in 2007 and 2008. For plots of 4th and 5th sowing date, the herbicide was not used in autumn 2007 (Lontrel 300 s.c. (clopiralid, 300 g $L^{-1}$), 0.5 L $ha^{-1}$, was used in spring 2008), but in autumn 2008 and in all plots in 2009 and 2010 Butisan Star s.c. was used directly after sowing.

Rape plant density was established by counting plants in one constant 0.5 $m^2$ area of each plot in autumn. At the end of autumn vegetation, 10-plant samples were taken randomly from each plot for biometrical analysis. Number of leaves per plant (No), leaves', plant and roots' weight (g), root length (cm), diameter of root neck (mm), and height of growth-point (mm) were measured in laboratory. Ten-plant samples from plots of first, second and third sowing date and all plants sown on 4th and 5th date were taken for from 1.0 $m^2$ dry matter content analyses. Dry matter of leaves was determined by drying at temperature of 105 °C for 2 hours (ISO 6496: 1999). The dry matter yield of rape leaves per $m^2$ was calculated according to plant number per 1 $m^2$.

To describe conditions of autumn growth, meteorological parameters were analyzed. Meteorological data were collected from automatically working meteorological station approximately 1 km from trial site. Hydrothermal coefficient (HTC) was calculated using (1) formula of G. Selianinov:

$$HTC = \frac{R \times 10}{\sum t} \quad (1)$$

where R – is the sum of precipitation (mm) during (ten-day) period;
$\sum t$ – the sum of the average daily temperatures during the same period.

The growing degree days (GDD) were calculated using (2) formula:

$$GDD = \frac{(T_{\max} + T_{\min})}{2} - T_{base} \quad (2)$$

where $T_{max}$ – daily maximum temperature;
$T_{min}$ – daily minimum temperature;
$T_{base}$ – base temperature (5 ºC).

ANOVA procedures were used for processing the experimental data.

Meteorological conditions in the autumn of all trial years were considerably different. October 2009 and 2010 was characterized by very low mean air temperatures. August 2009 and September 2008 were relatively dry, but wet August 2010 was extremely.

Vegetative period (mean temperature below 5 ºC for at least 3 days) ended on 4th of November in 2007; and 2008 and renewed for eight to five days period up to 4th

December; at 1st of November in 2009; (two-week period in the middle of the October registered with very low temperatures); at 7th of November in 2010.

## Results and Discussion

*Oilseed rape autumn growth depending on meteorological conditions*

Winter oilseed rape seed germination (BBCH 09) length in days was different depending on the sowing year and the sowing time because of different soil moisture. A more even seed germination was observed in autumn 2007 (seeds germinated in 8 to 10 days). The longest germination time was observed in plots sown on 1st August in 2008 and 2009 (15 and 16 days), and in plots sown on 10th September in 2008, 2009 and 2010 (14 to 17 days).

Our observations showed that appropriate soil moisture and productive precipitations were very significant for successful field-germination of seed. Water stress expressed as hydrothermal coefficient gives clear explanation about seed germination speed. Dependence on soil moisture, especially at the beginning of August when mean air temperature is high and soil surface dries quickly, is critical for seed germination. The first decade in August was critical (HTC below 1.0) because of amount of precipitation in all four trial years; even in years 2008 and 2009 lack of precipitation was observed in the ten-day period before 1st sowing date, which that explains the slow seed germination (Fig. 1).

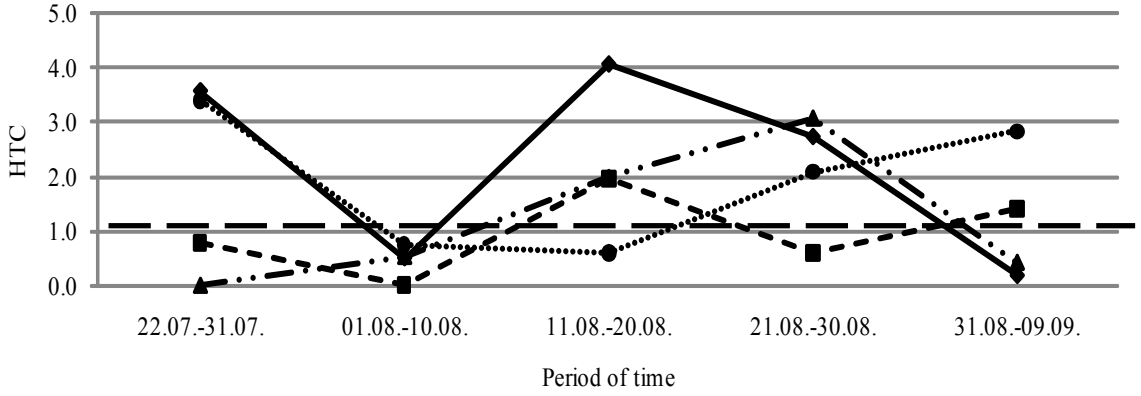



Figure 1. Water stress expressed as hydrothermal coefficient in RSF 'Vecauce' in autumn 2007, 2008, 2009 and 2010. ( —— - year 2010; —·—·— - year 2009; – – – - year 2008; ····· - year 2007; —— —— - threshold).

The role of temperature in seed germination increases with later sowing date. Longer time for seed germination was dependent on the air and soil temperature, which agrees with data of R.E. Blackshaw (1991) who emphasizes the role of moisture and temperature role for seed germination – in dry and hot conditions seed germination was delayed for three days, but in cool conditions – for up to six days. On the last sowing date, in the first decade of September, the importance of precipitation decreased because of a lower air temperature that reduced water evaporation; however seed germination still had connection to HTC values (see Fig. 1) when longest seed germination was observed in 2010 (17 days, HTC 0.2) and fastest germination – in 2007 (9 days, HTC 2.8). Difference in germination length between the cultivars was not observed.

Oilseed rape plants require a specific number of GDD to develop from growth stage to growth stage between emergence, flowering and maturity. Also the specific amount of GDD has to be accumulated before oilseed rate wintering. In our experiment GDD had various values because of different meteorological conditions in all trial years. Four years' data indicated that sowing time on 1st August (478 GDD till the end of vegetation period as four-year average) was risky because the rapeseed plants tended to overgrow, especially in autumn 2007 (567 GDD) when the height of root column, which is the most vulnerable part of the rapeseed plant, reached up to 5 cm above the soil surface. This agrees with Laaniste et al. (2007) who reported overgrown plants in Estonian conditions when 515 GDD were accumulated. On the other hand, if sowing was performed later than 1st September, especially in years 2009 (60 GDD) and 2010 (38 GDD), only 3-4 small leaves formed which were exposed to the danger for winter-kill. The autumn development of oilseed rape plants may affect not only their wintering and subsequent vegetative renewal in spring, but also the yield. A number of leaves was affected by accumulated GDD in three trial autumns (2008, 2009, and 2010 – Fig. 2) only for hybrid cultivar 'Excalibur'. The plant produced a sufficient number of leaves for successful wintering (five leaves according to Velicka et al., 2006).

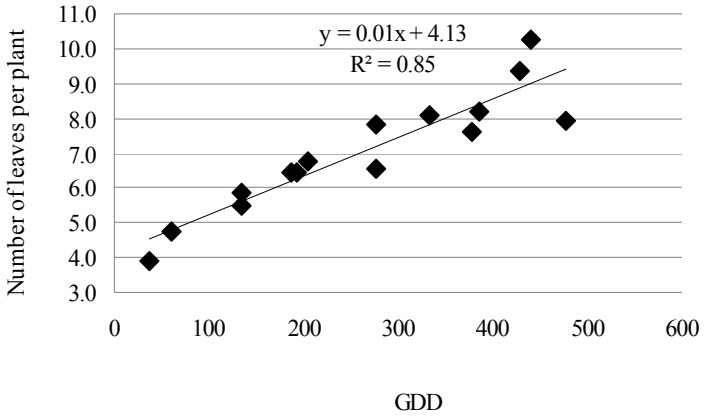



Figure 2. The relationship between GDD (x) and number of leaves per plant (y) for 'Excalibur' in years 2008, 2009, and 2010 ($p<0.05$).

More important is leaf area expansion before wintering, as declared by G. Sidlauskas and C. Rife (1999). They admitted that only 5 leaves reached the maximum leaf area during autumn vegetative growth (the plant received 552 GDD until the end of vegetative period). In our trial it was observed that especially in the last sowing dates the plant leaves did not reach full expansion and leaf area was dramatically lower, which was proved by calculating the last dry mass (DM) from 1 $m^2$ (Fig. 3). A significant relationship between GDD and root neck diameter was observed for both cultivars in all trial years ($y = 0.0153 + 0.4783x$ and $R^2=0.89$ for 'Californium'; $y = 0.0204x + 0.6800$ and $R^2=0.93$ for 'Excalibur'). A significant ($p<0.05$) linear relationship was also observed between GDD and height of growth point, root mass and length.

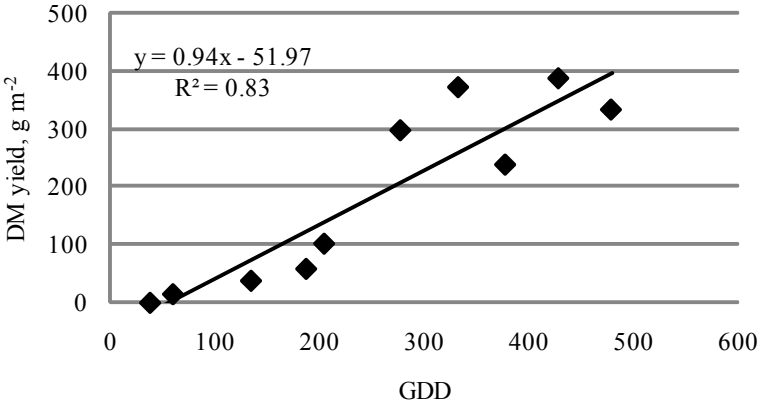



Figure 3. The relationship between GDD (x) and DM mass of oilseed rape leaves (y) at the end of autumn growth for 'Excalibur' in years 2009 and 2010.

Plant DM mass of leaves per 1 $m^2$ at the end of vegetation in five sowing dates had strong relationship with GDD, which underlines to the importance of GDD in plant preparation for successful wintering (Fig. 3). Correlation between GDD for 'Californium' was not statistically significant ($p>0.05$). The trend (Fig. 2) shows relationship that DM of aboveground vegetation depends mostly on the accumulation of GDD which agrees with G. Sidlauskas and C. Rife (1999). Also GDD relationships with plant fresh mass, plant leaf number, and root mass were observed in our experiment. More investigations should be performed on GDD impact on winter rape dry mass indices of different plant parts.

*Effect of sowing date*

Important plant parameters (plant fresh mass, height of growth point, root neck diameter, number of leaves per plant, shoot mass and length) that characterize plant autumn development were measured each experimental year. From the four-year results (2007-2010) it is evident that winter rape biometrical parameters were influenced by the sowing date, rate and fungicide application in autumn period, as well as by used cultivar. Sowing date had the most important and significant ($p<0.05$) impact on plant parameters if compared with sowing rate (density). A significant ($p<0.05$) impact of sowing date on all measured winter rape parameters in autumn 2007, 2008, 2009 and

2010 was noted. The proportion of sowing date influence (η%) on plant parameters showed that in all experimental years greater effect of the sowing date was on the height of growth point and root neck diameter (Table 1).

Table 1

**Proportion (η%) of sowing date influence (p<0.05) on winter oilseed rape plant parameters of cultivars 'Californium' and 'Excalibur' in autumns 2007 – 2010**

| Evaluated parameters | 2007 | | 2008 | | 2009 | | 2010 | |
|---|---|---|---|---|---|---|---|---|
| | C† | E‡ | C† | E‡ | C† | E‡ | C† | E‡ |
| Height of growth point | 92 | 95 | 84 | 90 | 93 | 90 | 95 | 95 |
| Root neck diameter | 82 | 81 | 89 | 89 | 89 | 78 | 94 | 86 |
| Plant mass | 70 | 55 | 71 | 79 | 72 | 53 | 83 | 63 |
| Number of leaves | 70 | 66 | 43 | 58 | 73 | 34 | 79 | 59 |
| Root mass | 77 | 66 | 84 | 87 | 80 | 63 | 87 | 69 |
| Root length | 82 | 68 | 82 | 94 | 73 | 90 | 88 | 92 |

C† – line cultivar 'Californium; E‡ – hybrid cultivar 'Excalibur'

Four-year experiments showed that extremely high growth point was determined for early sownrape (e.g. 1st August; 43.0 mm for 'Californium', 66.4 mm for 'Excalibur' in 2007), and lowest height of growth point was observed on the last sowing date (e.g. 10th September; 2.0 mm for 'Californium', 2.2 mm for 'Excalibur' in 2010). Optimal height of growth point in our region would not be higher than 30 mm according to results of researchers from Eastern Europe countries (Velicka, 2003; Velicka et al., 2006; Becka et al. 2004); however but experience shows that plants with higher plant parameter values which could be theoretically risky for overwintering have survived well in specific conditions. In our experiment plants tended to overgrow in year 2007 because of the height of growth point reached up above the soil surface, which is risky for overwintering according to Laaniste (2007). In experimental years with equal sowing rates for both cultivars (2009 and 2010), higher average height of growth point was observed for 'Excalibur' (e.g., 1st August – 44.8 mm in 2010 and 33.6 mm in 2009). Four-year experiment gives evidence that height of growth point decreased in later sowing dates and was especially affected by meteorological conditions in a specific autumn. Also lower height of growth point was observed for line-type cultivar if compared to hybrid cultivar.

Plant leaf number (leaf area is even more important) is also a parameter that influences plant survival during autumn and affects the seed yield (because of more plant branching). Results of Estonian researchers (Laaniste et al., 2007) indicate that plants before wintering reach 9 - 11 leaves if sown at the beginning of August and 3 - 4 leaves if sown at the end of August. Accordingly, it was found that the 7-8 leaf stage proved to be the most optimal for successful overwintering. Sowing date significantly (p<0.05) affected the number of leaves per plant for both cultivars in all trial years (Table 1). In 2009-2010 (equal sowing rates were used for both cultivars), also a tendency was observed that hybrid cultivar 'Excalibur' developed more leaves than line cultivar 'Californium'.

Table 2

**Average number of leaves depending on the sowing date in years 2009 and 2010**

| Sowing date | Californium | | Excalibur | |
|---|---|---|---|---|
| | 2009 | 2010 | 2009 | 2010 |
| 1st August | 7.6 | 7.2 | 8.9 | 7.2 |
| 10th August | 6.5 | 7.5 | 6.9 | 7.6 |
| 20th August | 6.5 | 5.7 | 7.0 | 6.5 |
| 1st September | 5.1 | 4.9 | 6.2 | 5.5 |
| 10th September | 3.7 | 3.4 | 4.8 | 3.8 |
| $RS_{0.05}$ | 0.42 | 0.37 | 0.76 | 0.46 |

Significant differences in the number of leaves were not noted between the plants sown on 10th and 20th August 2009, but in the year 2010 – between 1st and 10th August (Table 2). It is with certainty evident than in late sowing dates of 2009 and 2010 the number of leaves was lower than in years 2007 and 2008 (e.g., sown on 10th of September– on average 4.5 leaves for 'Californium', and leaves 5.5 for 'Excalibur' in 2007; 5.4 leaves for 'Californium', and 5.9 leaves for 'Excalibur' in 2008). Importance of number of leaves difference between plants sown in different dates

could affect plant wintering and has to be analyzed with wintering issue in spring.

Sowing date affected also the root neck diameter significantly ($p<0.05$) for both varieties in all trial years, and sowing date impact on this parameter was within $\eta$ – 78.1% for 'Excalibur' in 2009 and $\eta$ – 94.3% for 'Californium' in 2010 (Table 1). Root neck diameter decreased with later sowing date (on average from all trial years, from 7.2 mm in plants sown on 1st August to 1.8 mm in plants sown on – 10th September for 'Californium'; 9.8 mm for plants sown on 1st August to 2.3 mm – 10th September for 'Excalibur'). Root neck diameter of plants sown on last two sowing dates was less than 5 mm in all trial years, which might be risky for good rape wintering according to Velicka et al. (2006). The same tendency was observed also for root neck diameter. This parameter in of hybrid cultivar 'Excalibur' was greater than in line cultivar 'Californium', which was especially marked in the year 2010 then October was very cool and 'Excalibur' developed larger root neck diameter in the last two sowing dates (3.0 mm in plants sown on 1st September, and 1.4 mm in plants sown on 1st September for 'Excalibur', and 1.8 mm and 1.2 mm for 'Californium' respectively).

Average fresh plant weight from all trial years was from 90.4 g in plants sown on 1st August 2007 to 0.8 g in plants sown on 10th September 2010 for 'Excalibur and', 42.3 g in plants sown on 19th August on 2008 to 0.5 g in plants sown 0n 10th September 2010 for 'Californium'. Plant weight parameter showed a very similar tendency, and also the values of plant weight were similar in years 2009 and 2010 (equal sowing rates for both cultivars). Root weight and root length also were significantly ($p<0.05$) influenced by the sowing date for both cultivars in all trial years. The same tendency was observed that root weight and root length decreased in later sowing dates: average root weight from all years: was within 5.3 g in plants sown on 1st August and 0.1 g in plants sown on 10th September for 'Californium, and with in 12.7 g in plants sown on 1st August and 0.2 g in plants sown on 10th September for 'Excalibur'; average length was within 20.9 cm in plants sown on 1st August and 9.2 cm in plants sown on 10th September for 'Californium, and within 23.8 cm in plants sown on 1st August and 10.6 cm in plants sown on 10th September for 'Excalibur'.

*Effect of sowing rate*

Plant development parameter in autumn – plant weight – was significantly ($p<0.05$) influenced by sowing rate in some cases (for 'Excalibur' in years 2007, 2009, and 2010; for 'Californium' only in years 2009 and 2010). Use of equal sowing rates for both cultivars in 2009 and 2010 allows discussing sowing rate (plant density) impact on plant weight with more certainty. Plant weight using sowing rate of 120 germinable seeds per 1 $m^2$ was nearly three times smaller than using 20 germinable seeds per 1 $m^2$ for both cultivars (Fig. 4). Also plant weight was significantly ($p<0.05$) influenced by sowing rate in years 2009 and 2010 (Fig. 4) for both cultivars.

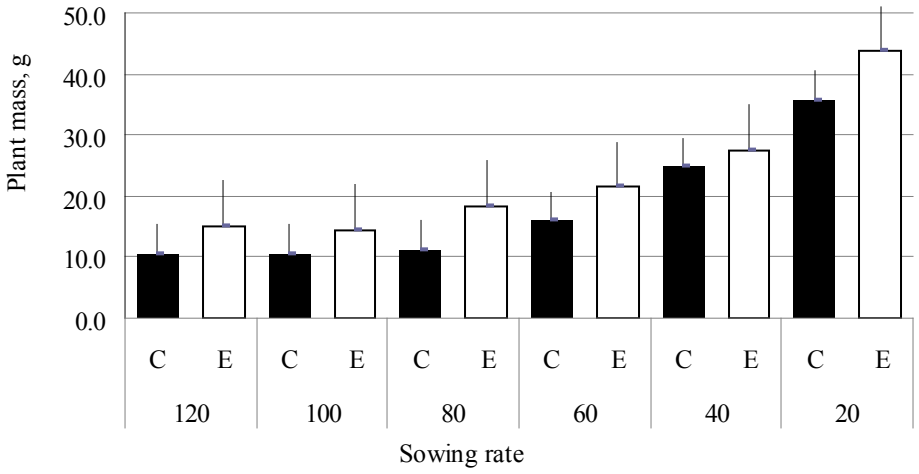



Figure 4. Sowing rate influence on winter rape plant mass of cultivars 'Californium' and 'Excalibur' in autumn 2010 (■ – 'Californium' (C); □ –'Excalibur'(E)).

Four-year results showed that height of growth point was not significantly influenced the by sowing rate for both cultivars when equal sowing rates were used, the exception was year 2009 for 'Californium' when sowing rate showed significant ($p<0.01$) impact on height of growth point using rates from 20 up to 120 germinable seeds per 1 $m^2$.

Sowing rate significantly ($p<0.05$) influenced winter oilseed rape root weight and root neck diameter for cultivar 'Californium' in the years 2009 and 2010 (when low sowing rates were used) and for 'Excalibur' in 2007, and

in 2009 as well as 2010 when also high sowing rates were used. Root length was not significantly ($p>0.05$) influenced by the sowing rate in all trial years for both cultivars.

## Conclusion

1. Four-year results showed that winter oilseed rape seed germination was affected by the amount of precipitation and air temperature around the sowing time. Seed germination was delayed for some days during drought (HTC<1) on some sowing dates in several trial years. The accumulated GDD during autumn vegetation correlated with plant biometrical parameters during trial years.
2. Sowing date was an important factor which had a strong and significant impact on rape plant development in autumn. Sowing on earlier dates significantly increased the height of growth point, root neck diameter, plant and root mass, and main root length for both cultivars (line and hybrid) in four trial years.
3. Sowing rate (plant density) affected plant biometrical parameters only in some trial years. Plant weight was significantly influenced by the sowing rate for both cultivars when sowing rates were equal. The height of growth point was not significantly influenced by sowing rate for both cultivars even if equal sowing rates were used in all years; exception was year 2009 for 'Californium'.

## Acknowledgements

The study was supported by ESF Project 2009/0225/1DP/1.1.2.1.2/09/IPIA/VIAA/129.

## References

1. Balodis O., Gaile Z. (2009) Influence of agroecological factors on winter oilseed rape *(Brassica napus* L.) Autumn growth. In: Gaile Z., Ciproviča I., Kaķītis A., et al. (eds) Research for Rural Development – 2009. *International Scientific Conference Proceedings*, Jelgava, LLU, pp. 36-43.
2. Becka D., Vasak J., Kroutil P., Stranc P. (2004) Autumn growth development of different winter oilseed rape variety types at three input levels. *Plant and Soil Environment*, 50, pp. 168-174.
3. Blackshaw R.E. (1991) Soil temperature and moisture effects on downy brome vs. winter canola, wheat and rye emergence. *Crop Science*, 31, pp. 1034-1040.
4. Bonhomme R. (2000) Bases and limits to using 'degree day' units. *European Journal of Agronomy*, 13, pp. 1-10.
5. Diepenbrock W. (2000) Yield analysis of winter oilseed rape (*Brassica napus* L.): a review. *Field Crop Reaserch*, 67, pp. 35-49.
6. Laaniste P., Joudu J., Eremeev V., Maeorg E. (2007) Sowing date influence on winter oilseed rape overwintering in Estonia. *Acta Agriculturae Scandinavica. Section B-Soil and Plant Science*, 57, pp. 342-348.
7. Leach J.E., Stevenson H.J., Rainbow A.J., Mullen L.A. (1999) Effects of high plant population on growth of winter oilseed rape (*Brassica napus*). *Journal of Agriculture Science,* 132, pp. 173-180.
8. Mendham N.J., Shipway P.A., Scott R.K. (1981) The effect of delayed sowing and weather on growth, development and yield of winter oil-seed rape (*Brassica napus*). *Journal of Agriculture Science*, Cambridge, 96, pp. 389-416.
9. Povilaitis V., Lazauskas S. (2010) Winter wheat productivity in relation to water availability and growing intensity. *Žemdirbystė–Agriculture*, 97 (3), pp. 59-68.
10. Ozolincius R., Stakenas V., Serafinaviciute B. (2005) Meteorological factors and air pollution in Lithuanian forests: possible effects on tree condition. *Enviromental pollution*, 137 (3), pp. 587-595.
11. Rapacz M. (1998) The effects of day and night temperature during early growth of winter oilseed rape (*Brassica napus* L. var. *oleifera* cv. *Gorczanski*) seedlings on their morphology and cold acclimation responses. *Acta Physiologiae Planitarium*, 20 (1), pp. 67-72.
12. Rathke G.-W., Behrens T., Diepenbrock W. (2006) Integrated nitrogen management strategies to improve seed yield, oil content and nitrogen efficiency of winter oilseed rape (*Brassica napus* L.): a review. Agriculture, Ecosystems and Environment, No.117, pp. 80-108.
13. Rummukainen M. (2010) Climate outlook for the Baltic sea region. *NJF report* 6. (1.) pp. 12-13.
14. Sidlauskas G., Rife C. (1999) Environmental and agronomic factors affect on the growth of rape leaves in autumn. *Proceedings of 10th International Rape Congress, Canbera, Australia, 1999*. Available at: www.regional.org.au/au/gcirc/index/references.htm, 2 March 2011.
15. Velicka R. (2003) *Rape:* Summary of the monograph, presented for habilitation confer. Lithuanian University of Agriculture, Kaunas, 78 p.
16. Velicka R., Marcinkeviciene A., Raudonis S., Rimkeviciene M. (2006) Integrated evaluation of rape readiness for overwintering. *Acta Agriculturae Scandinavica Section B-Soil and Plant Science*, 56, pp. 110-116.

# IDENTIFICATION AND CONTROL OF RAPE STEM WEEVIL *CEUTORHYNCHUS* SPP. IN WINTER OILSEED RAPE IN LATVIA

***Ingrida Grantiņa[1], Ilze Apenīte[2], Ināra Turka[1]***
*[1]Latvia University of Agriculture*
*[2]Latvian Plant Protection Researche Centre*
*ingrida.grantina@llkc.lv; ilze.apenite@laapc.lv*

**Abstract**

Research on identification of *Ceutorhynchus* spp. on winter oilseed rape in Latvia was done during the vegetation season of 2009 and 2010. Field trials were located in the Research and Study Farm "Peterlauki", Jelgava municipality, Latvia (56°32'17.38N, 23°43'17.65E). Four species of brassica stem weevils were identified: cabbage stem weevil *Ceutorhynchus pallidactylus* Marsh., syn. *C. quadridens* Pan., rape winter stem weevil *C. picitarsis* Gyll., blue stem weevil *C. sulcicollis* Pay., and *C. typhae* Herb. One of the most common species in winter oilseed rape was cabbage stem weevil *C. pallidactylus.*

Use of a sweep net for identification of particular species cannot be applied, as in practice no specimens have been collected by this method. One of the most appropriate methods for identification of *Ceutorhynchus* spp. on winter oilseed rape has been yellow sticky traps and water traps that have been used to collect the greatest number of specimens (24) during the vegetation season.

First pests appear during the 2nd decade of April, when the plant reaches 33 BBCH. Pest control using systemic insecticide Proteus 110 OD (tiakloprid 100 g $L^{-1}$, deltametrin 10 g $L^{-1}$) with a dose of 0.75 L $ha^{-1}$ was applied when the plant reached 39-44 BBHC. Despite the high population density (2 to 10 insects on 40 plants), significant ($p<0.05$) decrease in pest damage by 43-51%, when compared to untreated area, was recorded during vegetation seasons of 2009 and 2010. Pest control with a systemic insecticide when the plant is in the stem elongation phase provided a significant ($p<0.05$) yield increase of 0.5 to 0.7 t $ha^{-1}$.

**Key words**: weevil, *Ceutorhynchus* spp., winter oilseed rape.

## Introduction

Cultivated areas of winter and spring rapeseed in Latvia have reached 100 thousand ha. It is forecasted that the areas will continue to increase. Oilseed rape takes a solid place in the crop rotations of arable farms throughout Europe, and almost in all cases its cultivation is connected with intensive application of plant protection products. This is a result of increase in disease and weed pressure, and also distribution of pests.

Cabbage stems weevil *Ceutorhynchus pallidactylus* Marsh., syn. *Ceutorhynchus quadridens* Pan., and rape stem weevil *Ceutorhynchus napi* Gyll. (Toshova et al., 2009; Williams, 2004) have been mentioned as one of the most harmful pests in Europe and Northern America. Less damage is caused by rape winter stem weevil *Ceutorhynchus picitarsis* Gyll., blue stem weevil *Ceutorhynchus sulcicollis* Pay., and *Ceutorhynchus typhae* Herb. (Alford, 2003; Veromann et al., 2006). *Ceutorhynchus* spp. are the most important pests for oilseed rape in Germany, where they can cause severe yield losses (Büch and Katzur, 2005; Aljmli, 2007). Weevils are comparably small bugs with the length of 1.3 to 7 mm (2-3 mm in most cases) and are commonly found throughout the world (Toshova et al., 2009). More than one third of *Ceuthorhynchus* are monophagous or oligophagous on *Brassicaceae* species (Toshova et al., 2009; Evans, 2007). Some of *Ceuthorhynchus* species can be mentioned as especially harmful for crucifers, as their larvae and imago target specific plant parts. Larvae of *C. picitarsis* (Gyllenhal, 1837) damage leaf stems; larvae of *C. pallidactylus* (Marsham, 1802), *C. typhae* (Herbst, 1795) and *C. sulcicollis* (Paykull, 1800) damage plant stalks, and *C. obstrictus* (Marsham, 1802) damage rapeseed in pods (Toshova et al., 2009). Weevil imago feed on oilseed rape leafs, stalks, buds and flowers (Toshova et al., 2009). Crucifer stem weevil is univoltine. Adults exit from hibernation in March/April and migrate to oilseed rape fields. Most harmful invasion time of brassica stem weevil starts with the beginning of stem elongation and lasts until appearance of flower buds (BBCH 30 – 50) (Aljmli, 2007), when imago can be observed. In the later plant development stages damage is caused by emerged larvae, who tunnel inside the stalks and leaf stems to feed. Feeding goes on until the 2nd decade of June, when openings are created in stalks and the larvae drop out on the soil. Due to the pest damage plants have smaller size, wither away and die (Kelm and Klukowski, 2000). The necessity of controlling this particular pest depends on a specific situation (Hiiesaar et al., 2003).

Imago of brassica stem weevil on oilseed rape can be identified by using a sweep net, shake cone, water traps, sticky traps and performing insect counts on the plants (Free and Williams, 1978; Free and Williams, 1979; Tarang et al., 2004; Walszak et al., 1998). In Czech Republic, Austria, Germany and Poland pest emergence and control of pest population is determined by using yellow water traps, and treatment is recommended when the threshold number has been exceeded for over 3 days (Biocontrol-based..., 2010). Water traps have been used to sample all coleopterous pests of oilseed rape (Alford, 2003; Walczak et al., 1998). Sticky traps are more often used for flying insects and have been extensively used to monitor several different species of pests on oilseed rape (Alford, 2003). Sweep netting has been used for sampling of many kinds of oilseed rape pest.

This method is most efficient when the crop canopy is dry and when there is little or no wind (Alford, 2003).

The aim of the research was to identify the best timing for collection of brassica stem weevil and to determine the most efficient control measures in winter oilseed rape in Latvia. Collected data will help to set the most efficient timing for control measures of brassica stem weevil on winter oilseed rape.

## Materials and Methods

Field trials were located in the Research and Study Farm "Peterlauki", Jelgava municipality, Latvia (56°32'17.38N, 23°43'17.65E), using winter oilseed rape *Brassica napus* variety 'Adder'. Sowing was done on 15 August in 2008 and 12 August in 2009. Agrotechnics: previous crop – black fallow, ploughing and cultivation were done twice in autumn. Field trial was set up in two variants (control and treatment), six randomized replications. Plot size was $5 \times 10 = 50$ m$^2$, isolation bands – 0.01 ha$^{-1}$. When count of *Ceutorhynchus palidactylus* average reached 1 imago on 40 plants, treatment with Proteus 110 OD (tiakloprid 100 g L$^{-1}$, deltametrin 10 g L$^{-1}$) was done on 6 May.

Counting of brassica stem weevil was done by using four methods:

recording of stem weevil – average on 40 plants in plot;

yellow glue trap – yellow glue trap (10 × 25 cm) in every plot;

yellow water trap – yellow water trap (Ø 30 cm, 10 cm high) in every plot;

sweep net – where every sample was obtained with the help of 150 insect net sweeps (25 sweeps per every plot) of the winter oilseed rape stand.

Samples were taken starting with the 2nd decade of April (33 BBHC) until the 1st decade of June (71 BBHC) in 5-day intervals – 20.04., 25.04., 01.05., 05.05., 10.05., 15.05., 20.05., 25.05., 01.06., 05.06., 10.06., 15.06. in 2009, and 20.04., 28.04., 06.05., 11.05., 17.05., 21.05., 27.05., 02.06., 09.06. in 2010.

Plant volume invaded by brassica stem weevil (P, %) was calculated by using the following Formula (1):

$$P = (n \times 100)/ N, \quad (1)$$

where: n = number of insects;

N = total number of sampled stems

(Интегрированные системы защиты..., 2005).

Correlation analyses were used to determine the dependence between the factorial (X), which is the number of insects, and the resultative attribute (Y), which is yield. To evaluate the collected data, the estimated value was compared to the critical value. Obtained results were analysed with the necessary probability level ($p<0.05$) and actual number of samples.

Weather data received from the Jelgava HMS of Latvian Environment, Geology and Meteorological Agency were compared to the long-term average data.

The weather in vegetation season of April 2009 was mild and dry. Second and third decade of April was without rain. In April, the average daily air temperature was higher than the common norm. This was the 2nd driest April in Latvia during 86 years. Also in May the weather was mild and dry. In May, the average daily air temperature was higher than the common norm, and the average precipitation was not sufficient for development of winter oilseed rape. In June and July, the air temperature and precipitation were sufficient for development of winter oilseed rape.

In 2010, the average air temperature in May was + 1.3 °C higher than perennial mean. In the third decade of May, the air temperature was on - 0.2 °C lower than perennial mean. Precipitation level was 51.31% of the norm in the second decade of May. In June, the average daily air temperature was higher than the norm. Precipitation was below the long-term average. In July, the average daily temperature was higher than perennial norm (+ 4.4 °C), and precipitation was 319% of perennial norm. This was the hottest July in Latvia during 95 years. In the second and third ten day period of July there were rainstorms.

High average air temperature and low participation level in May of 2009 and 2010 was favourable for the spread of pests and caused problems for pest control.

## Results and Discussion

Four brassica stem weevil species have been identified in Latvia: cabbage stem weevil *C. pallidactylus* Marsh., syn. *C. quadridens* Pan., rape winter stem weevil *C. picitarsis* Gyll., blue stem weevil *C. sulcicollis* Pay., and *C. typhae* Herb. First imago of the different species mentioned above has been observed in different plant development stages. Imago is observed when the air temperature exceeds + 6 °C. They feed both on wild and cultivated cruciferous plants. Females make an ovipositor bite either in leaf stem or stalk and lay 1-2 eggs. In approximately 10 days the larvae emerge. Larvae feed and damage leaf stems and stalks for the next three to five weeks. Afterwards they leave the plant and pupate in the soil. Only one generation of brassica stem weevils is possible in Latvian climatic conditions. By the end of the summer imago develops from the pupa and feeds and damages crucifer leafs. In late August insects head to overwintering sites in field margins and forest edges (Alford, 2003; Toshova et al., 2009; Hiiesaar et al., 2003).

In vegetation season of 2009, first imago of brassica stem weevils were identified on April 20 (BBHC 35) on yellow sticky traps. On the plants and in the yellow water traps the first insects were counted only 5 days later – April 25, BBHC 39 (Table 1).

Table 1

**Efficiency of different identification methods of brassica stem weevils on winter oilseed rape in vegetation season of 2009**

| Sampling dates | Phenological growth stages (BBCH) | Number of insects | | | |
|---|---|---|---|---|---|
| | | On single plant | Average per one | | 150 sweeps of insect net |
| | | | yellow sticky trap | yellow water trap | |
| 15 April | 33 | 0 | 0 | 0 | 0 |
| 20 April | 35 | 0 | 3 | 0 | 0 |
| 25 April | 39 | 2 | 22 | 2 | 0 |
| 01 May | 44 | 3 | 4 | 0 | 0 |
| 05 May | 51 | 2 | 3 | 0 | 0 |
| 10 May | 57 | 1 | 2 | 0 | 0 |
| 15 May | 60 | 0 | 3 | 1 | 0 |
| 20 May | 67 | 1 | 3 | 0 | 0 |
| 25 May | 69 | 2 | 0 | 1 | 0 |
| 01June | 70 | 3 | 0 | 1 | 0 |
| 05 June | 71 | 2 | 0 | 0 | 0 |
| 10 June | 72 | 1 | 0 | 1 | 0 |
| 15 June | 74 | 1 | 0 | 0 | 0 |

In vegetation season of 2010, the first imago of brassica stem weevil in the trial field was observed one week later by help of the yellow water trap in the end of April (28 April), and after a week in early May (06 May) it was recorded on the plants. Late appearance of the pest can be explained by the low average air temperature and frequent precipitation in the last decade of April (Table 2).

Table 2

**Efficiency of different identification methods of brassica stem weevils on winter oilseed rape in vegetation season of 2010**

| Sampling dates | Phenological growth stages (BBCH) | Number of insects | | | |
|---|---|---|---|---|---|
| | | On single plant | Average per one | | 200 sweeps of insect net |
| | | | yellow sticky trap | yellow water trap | |
| 20 April | 33 | 0 | 0 | 0 | 0 |
| 28 April | 35 | 0 | 0 | 3 | 0 |
| 06 May | 39 | 1 | 0 | 25 | 0 |
| 11 May | 44 | 3 | 1 | 16 | 0 |
| 17 May | 51 | 2 | 1 | 3 | 0 |
| 21 May | 67 | 1 | 4 | 0 | 0 |
| 27 May | 69 | 0 | 2 | 0 | 0 |
| 02 June | 70 | 0 | 4 | 0 | 0 |
| 09 June | 71 | 0 | 0 | 0 | 0 |

By using the sweep netting, no brassica stem weevils were collected during vegetation seasons in both years – 2009 and 2010. In order to control the population of brassica stem weevil, systemic insecticide Proteus 110 OD was used by applying a dose of 0.75 L $ha^{-1}$.

In 2009, the first control measures of brassica stem weevils were made 10 days after observation of the first insects on 30 April- BBHC 44. The 10 imago were counted on 40 plants, which may be considered as a very heavy infestation – according to the critical thresholds developed by A. Priedītis the control measures should be taken when there is one imago counted on 40 plants (Priedītis, 1999). The control was done according to the insect counts on the yellow sticky traps and yellow water traps. In 2010, control measures using insecticides were done one week later than in 06 August in 2009. - BBCH 39, when one to two insects were counted on 40 plants. Control measures could not be implemented any earlier as there was minor, but frequent precipitation. With the decrease of precipitation and increase of the average air temperature at the end of 1st decade of May, massive emergence of brassica stem weevils was observed in the 2nd decade of May (11 may) when

8 insects per 25 plants were counted. This measurement is close to the critical threshold set in Poland – 6 insects on 25 plants (Garbe et al., 1996; Alford, 2003). The high population density in the trial field may be explained by the close distance to oilseed rape field in the previous season (~50 m), where some of the insects overwintered.

According to the amount of insect-invaded plants it can be noted that there has been an increase of pests by 48% in 2010 if compared to 2009 (untreated variant). This can be explained by the absence of proper crop rotation in the trial field (Fig. 1). Most of the Latvian farmers cultivating oilseed rape face the same problem.

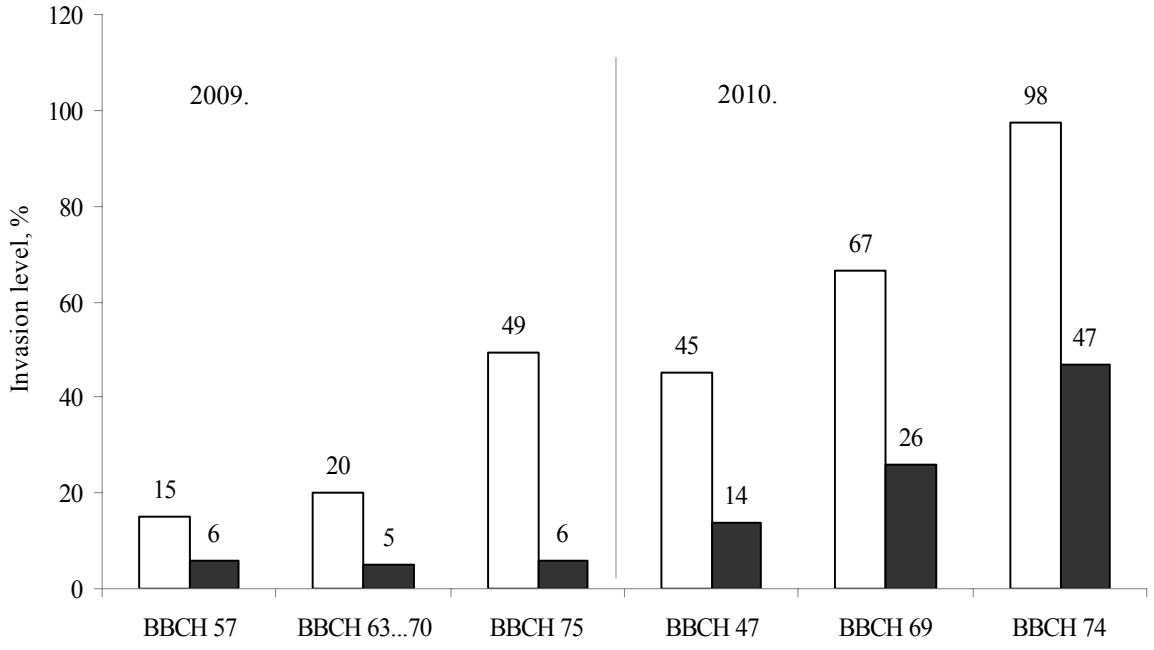



Figure 1. *Brassica* stem weevil invaded plants (%) on winter oilseed rape in in vegetation seasons of 2009 and 2010.

2009 vegetation season LSD 95% - 45.47
2010 vegetation season LSD 95% - 24.6

Despite the high density of brassica stem weevil on winter oilseed rape it has been possible to significantly ($p<0.05$) decrease the pest damage in 2009 and 2010 – by 43 – 51% (BBHC 74 development of the pods), when compared to the untreated area. Systemic insecticide Proteus 110 OD impacted the hatched larvae by slowing down their feeding and further development.

Pest control of brassica stem weevil with a systemic insecticide Proteus 110 OD provided a significant ($p<0.05$) yield increase of 0.5 to 0.7 t $ha^{-1}$ or ~ 19% if compared to control (Table 3).

Table 3

**Influence of brassica stem weevil on the yield of winter oilseed rape in vegetation season of 2009 and 2010**

| Variants | 2009 | | 2010 | |
|---|---|---|---|---|
| | Yield, | Compared to control, | Yield, | Compared to control, |
| | t $ha^{-1}$ | % | t $ha^{-1}$ | % |
| Control | 2.6 | 100 | 3.2 | 100 |
| Proteus 110 OD | 3.1 | 119 | 3.8 | 119 |
| $LSD_{0.05}$ | 0.31 | | 0.26 | |
| $r_{yx}$ | -0.99 | | -0.99 | |

## Conclusions

1. Four species of brassica stem weevils have been identified on oilseed rape (*C. pallidactylus, C. picitarsis*, *C. sulcicollis*, *C.typhae*). *C. pallidactylus* is the most common species found on oilseed rape.
2. In Latvia sweep netting cannot be used for counting of brassica stem weevils, as no insects have been captured by this method.
3. Pest control of brassica stem weevil with a systemic insecticide Proteus 110 OD on winter oilseed rape (BBHC 39-44) provided a significant ($p<0.05$) yield increase of 0.5 to 0.7 t $ha^{-1}$ (~19%) when compared to untreated areas.
4. Suitable methods for counting of brassica stem weevils in winter oilseed rape were use of yellow sticky traps and water traps.

## References

1. Alford V. (2003) *Biocontrol of oilseed rape pests.* Blackwell Publishing, Oxford, 355 p.
2. Aljmli F. (2007) *Classification of oil seed rape visiting insects in relation to the sulphur supply.* Dissertation thesis. Technische Universität Braunschweig, 189 p.
3. *Biocontrol-based Integrated Managment of Oilseed Rape Pests* (2010) Williams I.H. (ed.) Springer Science+Busness Media, [s. l.], 455 p.
4. Büchs W., Katzur K. (2005) Regulierung von Schadinsekten durch pflanzenbauliche Maßnahmen im ökologischen Rapsanbau. (Plant protection measures for control of pest populations in oilseed rape). In: *Entomologie im Pflanzen- und Vorratsschutz*. Sektion 8, S. 221. (in German).
5. Evans A. (2007) *Stem boring pests of winter oilseed rape*: Available at: http://www.sac.ac.uk/consulting.pdf, 24 August 2009.
6. Free J.B., Williams I.H. (1978) The insect pest of oil seed rape. In: *Proceedings of the International Rapeseed Conference.* Malmo, Sweden, June 12 – 16. Vol. 1, pp. 310-314.
7. Free J.B., Williams I.H. (1979) The distribution of insect pests on crops of oilseed rape (*Brassica napus* L.) and the damage they cause. *Journal of Agricultural Science,* Vol. 92, pp. 139-149.
8. Garbe V., Broschewitz B., Erichsen E., Hobfeld R., Lauenstein G., Steinbach P., Ulber B., Zellner M. (1996) Scadensschwellen bei Rapsschadlingen. Instrumente einer wirtschaftlichen Winterrapsproduktion. (Weevils in oilseed rape pests. Tools for economic winter oilseed rape production). *Raps*, No. 14, S. 58-63. (in German).
9. Grantiņa I., Turka I. (2010) Krustziežu stublāju un sēklu smecernieki *(Ceuthorhynchus* spp.*)* ziemas rapša sējumos. (Cruciferous Stem and Seed Weevils *(Ceuthorhynchus* spp.*)* in Winter Oilseed Rape) No: *Ražas svētki 'Vecauce-2010'. Zinātniskā semināra rakstu krājums*. LLU, Jelgava, 38-41. lpp. (in Latvian).
10. Hiiesaar K., Metspalu L., Lääniste P., Jõgar K., Jõudu J. (2003) Insect pests on winter oilseed rape studied by different catching methods. *Agronomy Research*, Vol.1, No.1, pp. 17-29.
11. Kelm M., Klukowski Z. (2000) The effect of stem weevil (*Ceutorhynchus pallidactylus* Marsh.) infestation on oilseed rape yield. *IOBC/ WPRS Bulletin,* Vol. 21 (5), pp. 147-151.
12. Priedītis A. (1999) *Kultūraugu kaitēkļu kritiskie sliekšņi ķīmisko un bioloģisko aizsardzības pasākumu pamatošanai.* (Critical thresholds for justifying of chemical and biological plant protection measures). LLU, Jelgava, 16 lpp. (in Latvian).
13. Rubenis J. (1987) *Agronomisko izmēģinājumu metodikas praktikums. (*Practicum of methodology agronomical trials). Rīga: Zvaigzne, 158 lpp. (in Latvian).
14. Tarang T., Verroman E., Luika A., Williams S.I. (2004) On the target entomofauna of an organic winter oilseed rape field in Estonia. *Latvia Entomology*, No. 41, pp. 100-110.
15. Toshova T., Subchev M., Toth M. (2009) The diversity of species of *Ceuthorhynchinae* captured in traps in the region of Sofia, Bulgaria. *Bulletin of Insectology,* Vol. 62 (1), pp. 27-33.
16. Veromann E., Luik A., Metspalu L., Williams I. (2006) Key pests and their parasitoids on spring and winter oilseed rape in Estonia. *Entomologica Fennica,* Vol. 17, pp. 400-404.
17. Walczak B., Kelm M., Klukowski Z., Smart L.E., Ferguson A.W., Williams I.H. (1998) Effect of trap design and 2-phenylethyl isothiocyanate on catches of stem weevils (*Ceutorhynchus pallidactylus* Marsh. and *C. napi* Gyll.) in winter oilseed rape. *IOBC/WPRS Bulletin,* Vol. 21, pp. 141-146.
18. Williams I.H. (2004) Advances in Insect Pest Management in Europe. In: *Insect Pest Management*, Springer-Verlag, Berlin, pp. 181-208.
19. *Интегрированные системы защиты сельскохозяйственных культур от вредителей, болезней и сорняков.* (Integrated crop protection systems from pests, diseases and weeds). (2005) Беларусская наука, Минск, 462 с. (in Russian).

# INFLUENCE OF WARMTH CONDITIONS CHARACTERIZING PARAMETERS ON YIELD AND CHEMICAL COMPOSITION OF MAIZE IN LATVIA

***Jānis Bartuševics, Zinta Gaile***
*Latvia University of Agriculture*
*janis.bartusevics@gmail.com*

**Abstract**

Maize yield and quality are affected by many factors, including production practices, diseases, pests, and differences in the climate. Usually it is not possible to do much to change the effect of temperature, but it is important to grow better adapted or characterized with right maturity rating hybrids. The paper is aimed to determine the impact of accumulated growing degree days (GDD) on maize development, organic dry matter yield, ODM and chemical composition of maize hybrids. A field trial was carried out in the Research and Study farm 'Vecauce' of the Latvia University of Agriculture (LLU) from 2008 till 2010. Ten (in 2008), eleven (in 2009) and fifteen (2010) maize hybrids with different maturity ratings according to FAO numbers (FAO 180–340) were harvested at three different times beginning on 5 September at fourteen-day intervals. GDD were calculated when maize reached a certain growth stage (full emergence, first tassels, full tassels, first ears, full ears, specific harvest date) to characterize conditions for maize growing in a specific year. Composition of fresh maize was analyzed for all hybrids using standard methods. Results were statistically processed using methods of correlation and regression analysis. Our results presented evidence that ODM yield in 2008 and 2009 on last harvest dates was lower than that on the first harvest date in 2010. Organic dry matter yield was higher in years when more GDD were accumulated. Results also showed that GDD negatively correlated with the total N, protein, fiber, cellulose, hemi-cellulose, NDF, and ADF concentration.
**Key words**: maize hybrid, growing degree days, organic dry matter, chemical composition.

## Introduction

Many of the world's most important crops such as maize, sorghum, rice are now cultivated with good success outside their zones of origin. Temperature is one of the most important environmental factors that determines the rate of plant growth and development. Also maize growth depends on the temperature. Maize requires a specific amount of heat to develop from one growth stage to another. The accumulated heat is one of the most important environmental factors for the development of the maize plant. Maize hybrids with different maturity rating require a different total number of growing degree days (GDD) for growth, development and maturity. Maize is characterized by high optimum temperature needed for germination and growth and belongs to the thermophilic plant species.

Climate is one of the most important limiting factors for agricultural production: frost risk during the growing period and low and irregular precipitation with high risks of drought during the growing period are common problems in agriculture (Moonen et al., 2002).

Environmental factors of critical importance for maize growth between planting time and emergence are temperature, moisture, and physical condition of the seedbed. Cold soil temperatures may lead to slow maize emergence, reduced stands and seedling vigor, and delayed maturity (Griffith et al., 1973). The maize crop can tolerate a wide range of temperatures (from 5 to 45 °C), but very low or very high temperatures can have a negative effect on the yield (Quansah, 2010).

The temperature regime is a key factor in maize adaptation, and the use of the accumulated heat units (GDD) is of great importance. Research has shown that measuring the heat accumulated over time provides a more accurate physiological estimate than counting calendar days (Edmond, 2004).

Maize is a crop with a rapid growth rate that yields best under moderate to warm temperatures. Cool temperatures slow down the progress to maturity and high temperatures hasten maturity (Brown, 1997).

Temperature also affects final leaf number (Hunter and Tollenaar, 1983) and leaf canopy development, which influences crop leaf area index. It has also been documented that low air temperatures reduce radiation use efficiency (RUE) in maize (Andrade et al., 1993).

Temperatures below and around the minimum temperature for germination and growth may cause various types of physiological damage in maize. These low-temperature effects are often referred to as chilling injury. Chilling injury is physiological damage caused by temperatures between 0 and about 12 °C (Miedema, 1982).

Crop growing season and plant developmental stages are other important factors that influence the forage quality (Darby and Lauer, 2002). Incidence of stress at any growth stage may influence the feed and forage digestibility and reduced nutrient intake. Thus, morphological development can be used to accurately predict the forage quality of any crop throughout the season (Mahmood et al., 2010).

The paper is aimed to determine the impact of accumulated growing degree days on maize development, organic dry matter yield, and chemical composition of maize hybrids.

## Materials and Methods

A three-factor field trial was carried out during 2008-2010 in the Research and Study farm 'Vecauce' (latitude:

N 56° 28´, longtitude: E 22° 53´) of the Latvia University of Agriculture. Trials were arranged in four-replication randomized blocks with plot size of 16.8 $m^2$. Row width was 0.7 m. Planted population density was 83300 plants per ha. Original seed of ten maize hybrids (Factor A) in 2008, of eleven maize hybrids in 2009, and of fifteen maize hybrids in 2010 with different maturity ratings defined by FAO number were used: Tango* (standard, FAO 210), Target[a] (FAO 180), Estelle[a] (FAO 200), Salgado* (FAO 200), Silas* (FAO 210), Turini[a] (FAO 220), Marco[b] (FAO 220), Progress[b] (FAO 220), Ceklad* (FAO 235), Ronaldinio** (FAO 240), Bombastic[b] (FAO 240), Celio* (FAO 250), KX A8151** (FAO 250), Cemet* (FAO 260), Fernandez** (FAO 260), Paroli[b] (FAO 260), Celido* (FAO 270), and Cefran** (FAO 340). The seven hybrids marked with asterisk were used in all trial years, four hybrids marked with two asterisks – in 2009-2010, but others – only in one year – 2008 ([a]) or 2010 ([b]). Soil at the site was strongly altered by cultivation: sand loam with pH KCl – 6.7, available for plants content of P – 112 mg $kg^{-1}$, K – 99 mg $kg^{-1}$, and humus content – 19 g $kg^{-1}$ in 2008, sand loam with pH KCl – 6.4, available for plants content of P – 129 mg $kg^{-1}$, K – 143 mg $kg^{-1}$, and humus content – 21 g $kg^{-1}$ in 2009; and sod-gleyic sand loam with pH KCl – 7.2, available for plants content of P – 232 mg $kg^{-1}$; K – 190 mg $kg^{-1}$, and humus content – 26 g $kg^{-1}$ in 2010. Traditional soil tillage was used: mould-board ploughing in previous fall, cultivation and rototilling before sowing in spring. The following fertilizers were given: 148 kg $ha^{-1}$ N (18+70+60), 34 kg $ha^{-1}$ P, 75 kg $ha^{-1}$ K. Maize was sown on May 6. Planting was carried out by a hand-handled planter at a 3-4 cm depth. Weeds were controlled by spraying herbicides: Arrat d.g. (tritosulfuron 250 g $kg^{-1}$; dicamba 500 g $kg^{-1}$) 200 g $ha^{-1}$ and Titus 25 d.g. (rimsulfuron 250 g $kg^{-1}$) 30-50 g $ha^{-1}$ together with surfactant in 2008 and 2009, and Maisters OD 61 s.c. (foramsulfuron, 30 g $L^{-1}$, + jodosulfuron-methyl-sodium, 1 g $L^{-1}$) 1.5 L $ha^{-1}$ + Estet 600 e.c. (2.4 – D, 600 g $L^{-1}$) 0.5 L $ha^{-1}$ in 2010 were applied when maize reached 3-6 leaves stage. In addition, Lontrel s.c. (clopiralid, 300 g $L^{-1}$) 0.4 L $ha^{-1}$ together with Estet e.c. (2.4 – D, 600 g $L^{-1}$) 0.5 L $ha^{-1}$ were applied in 2009 for control of *Tussilago farfara* and *Artemisia vulgaris*. Mechanical weeding was used for the remained weeds in later maize development stages (in July of all years). Harvesting was done at three different times beginning on 5 September in 2008, on 4 September in 2009, and on 3 September in 2010 at fourteen-day intervals. The yield was accounted from 0.7 $m^2$ at first and second harvest times, and from 8.4 $m^2$ at last harvest time. Samples were taken for every hybrid from every replication.

Composition of fresh maize was analyzed for all hybrids using standard methods: dry matter (DM) (samples were dried up to constant weight at 105 °C) and organic dry matter (ODM) (calculated from DM and ash content) content; all other components were calculated in g $kg^{-1}$ on DM basis: total N (by Kjeldahl method), crude protein (by multiplying total N with the coefficient 6.25; ISO 5983), crude fiber (ISO 5498:1981), cellulose (calculated from NDF and ADF), hemi-cellulose (calculated from NDF and ADF), crude fat (ISO 6492:1999), neutral detergent fiber (NDF) (LVS EN ISO 16472:2006), acid detergent fiber (ADF) (Forage analyses, USA, method 4.1:1993), crude ash (XA) (ISO 5984), and total carbon (C) (CS – 500 method). Results were statistically processed using methods of correlation and regression analysis.

Daily maximum and minimum temperatures were recorded by an automatic weather station located near to the field experiment. Sum of precipitation during the same period (May-September) was 230 mm in 2008, 327 mm in 2009, and 454 mm in 2010. June and September of 2008 were cool, and the season in general was dry if compared with long-term average data; sum of active temperature was 1943 °C. Start of the season (May, June) in 2009 was too cold (Table 1) and unsuitable for the development of maize, but average day and night temperature in September was warmer if compared with long-term average data, active temperature sum per season was 2037 °C. The vegetation period in 2010 was very suitable for development of maize.

Table 1

**The average day and night temperature and precipitation during maize growing season in 2008-2010 and in comparison with long term average**

| Month | Long-term average temperature | Temperature, ºC | | | Long-term average precipitation | Precipitation, mm | | |
|---|---|---|---|---|---|---|---|---|
| | | 2008 | 2009 | 2010 | | 2008 | 2009 | 2010 |
| May | 11.2 | 11.3 | 11.0 | 11.9 | 43 | 24.2 | 18.0 | 72.6 |
| June | 15.1 | 14.6 | 13.7 | 14.6 | 51 | 44.2 | 95.0 | 37.8 |
| July | 16.6 | 17.1 | 17.1 | 20.8 | 75 | 56.8 | 136.0 | 131.8 |
| August | 16.0 | 16.4 | 15.8 | 18.2 | 75 | 90.2 | 38.8 | 133.4 |
| September | 11.5 | 10.6 | 12.9 | 10.8 | 59 | 14.8 | 39.8 | 78 |

For characterizing conditions for maize growing in specific year, growing degree days (GDD) were calculated when maize reached a certain growth stage (full emergence, first tassels, full tassels, first ears, full ears, specific harvest date). To calculate daily GDD accumulation, the average temperature for that day was taken (lowest plus highest, divided by 2) and subtracted the base temperature, which for maize is 10 °C. If the lowest temperature for day is

above 10 °C and the highest is 32 °C or lower, then this calculation can be done using actual temperature. If the lowest temperature is less than 10 °C, then 10 °C was used as the low temperature in the equation. If the highest temperature is above 32 °C, then 32 °C was used as the high temperature in the equation (Hoeft et al., 2000).

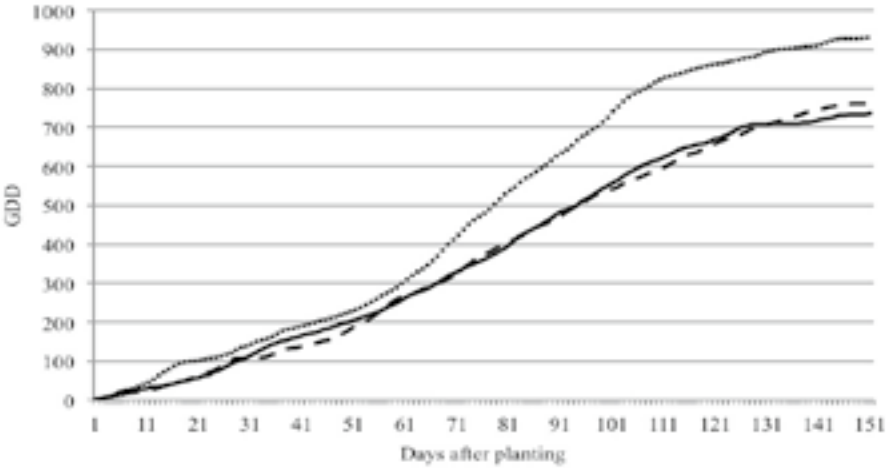



Figure 1. GDD accumulation in period between planting and last harvest date in all years: — 2008; -- 2009; ····· 2010.

In 2010, accumulated GDD between planting and last harvest date was greater (931) if compared to 2009 (761) and 2008 (735) respectively (Figure 1). Figure 1 shows that all the season of 2010 was characterized by better warmth conditions if compared with 2009 and 2008.

**Results and Discussion**

Average organic dry matter (ODM) content per all harvest times and all years of early ripening (FAO 180-239) maize hybrids was 257.2 g $kg^{-1}$, but of medium ripening maize hybrids (FAO 240 – 340) at the same harvest dates per all years - 237.1 g $kg^{-1}$. There was a significant correlation between accumulated growing degree days (GDD) and ODM content of early ripening (FAO 180-239) maize hybrids ($r=0.77 > r_{0.05}=0.28$, $p<0.05$) and also of medium ripening (FAO 240-340) hybrids ($r=0.84 > r_{0.05}=0.26$, $p<0.05$). Our results showed that GDD highly correlated ($r=0.81 > r_{0.05}=0.26$, $p<0.05$) with ODM yield of maize hybrids with FAO 240-340 (Figure 2). Range of ODM yield of maize hybrids per all three harvest dates and all years was wide (from 7.8 t $ha^{-1}$ (Celio at 663 GDD) to 21.8 t $ha^{-1}$ (Fernandez at 931 GDD)).

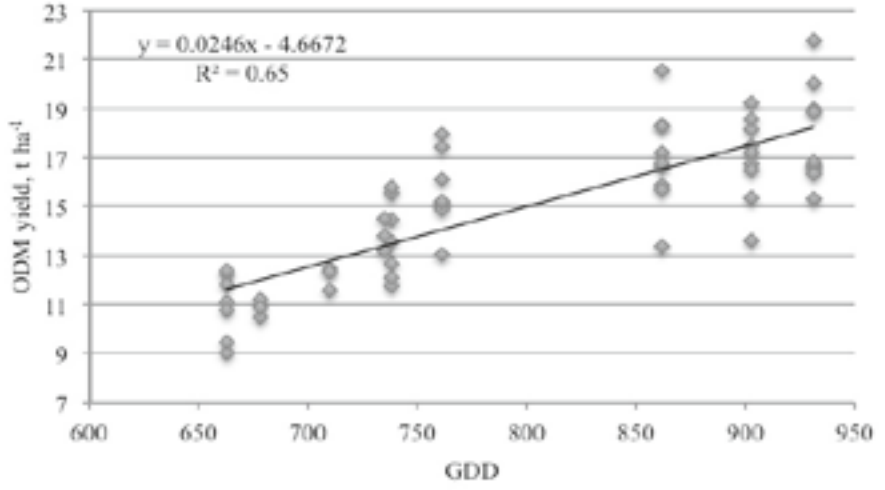



Figure 2. The relationship between GDD (x) and ODM yield (y) of maize hybrids with FAO 240-340 in all years.

According to findings of T. Amon et al. (2004), crude protein, crude fiber and cellulose content declined in the course of the vegetation period. Hemi–cellulose and starch content increased. Our results showed that GDD negatively

correlated with the total N, protein, fiber, cellulose, hemicellulose, NDF and ADF concentration, which means that the content of mentioned parameters decreased during plant development. Crude fat, ODM, total carbon, and crude ash positively correlated with the number of GDD. If we correlate the accumulated GDD with protein content (maize hybrids with FAO 180-239), the value is low ($r=/-0.26/ < r_{0.05}=0.28$). In other words, it is not correct to use GDD for prediction of the protein content in maize silage.

Our results showed that hybrids with FAO 180-239 on average per all years reached crude fat content of 22.62 g $kg^{-1}$, but later maturity hybrids (FAO 240-340) - only 20.05 g $kg^{-1}$. The results (Figure 3) represented a high correlation ($r=0.90 > r_{0.05}=0.26$) between GDD and fat content of fresh medium ripening maize samples. Whereas content of fat ranged from 7.2 g $kg^{-1}$ to 34.9 g $kg^{-1}$ if all hybrids per all years were analyzed. The average fat content of fresh maize was highest on the last harvest date.

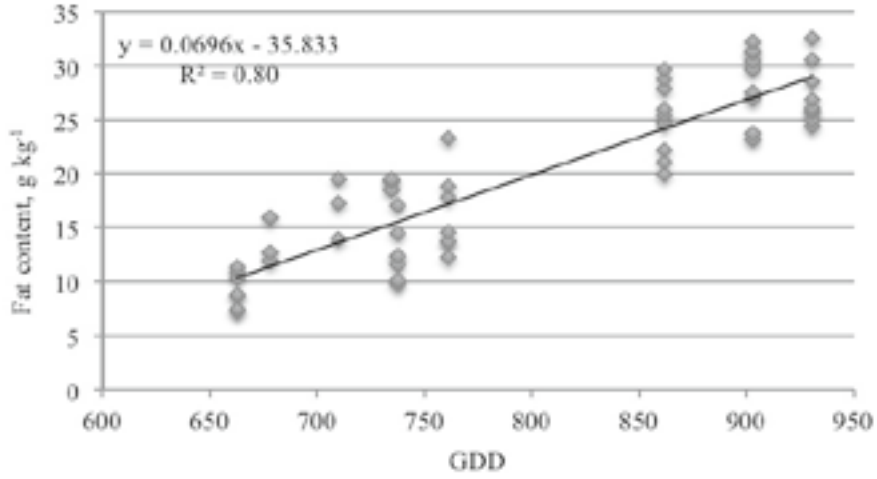



Figure 3. The relationship between GDD (x) and fat content (y) of maize hybrids with FAO 240-340 in all years.

Also a high correlation ($r=/-0.83/ > r_{0.05}=0.26$) between GDD and cellulose content of fresh medium ripening maize samples was found (Figure 4). The average cellulose content of fresh maize was the highest on the first harvest date.

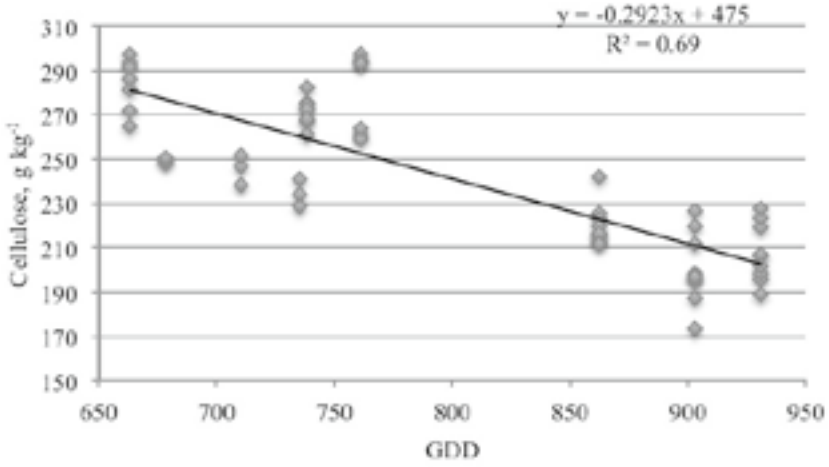



Figure 4. The relationship between GDD (x) and cellulose content (y) of maize hybrids with FAO 240-340 in all trial years.

The number of GDD which was needed for maize development from sowing to emergence, first tassels, full tassels, first ears and full ears were calculated using temperature data of all years.

The critical base temperature for germination of maize is 9.8 °C, and for emergence the crop needs to accumulate about 60 °C. Ideally this period should not exceed 10-12 days in order to minimize seed damage and optimize crop establishment (Garcia, 2010). Our results demonstrated that between sowing date and emergence were accumulated from 44 (in 2008) up to 69 (in 2010) GDD were accumulated. Maize emergence was noted after

16 days in the years 2008 and 2009, but some days earlier (after 13 days) in 2010 when more GDD were accumulated even per this shorter period.

Very important is the flowering time of maize because by it harvesting maturity for silage making can be approximately predicted. Our results showed that between sowing date and full ears, 20-41 GDD more were needed for maize hybrids characterized by FAO 240-340 (525-571 GDD) if compared with those characterized by FAO 180-239 (484-551) (Table 2). The average tassel emergence date for the early ripening maize (FAO 180-239) hybrids was 31 July in 2009, which moves the full ears stage later (August 12). When season was warmer (2010), the full ears flowering of medium ripening maize (FAO 240-340) occurred already on 28th of July when 571 GDD were accumulated after planting. In practice, maize needs to accumulate between 1000 °C to 1500 °C GDD to mature, depending on the hybrid (Garcia, 2010). Our results showed that in years which were characterized by temperature close to long term average (2008-2009) it was possible to accumulate up to 750-800 GDD if maize harvesting could be delayed until first decade of October. The total number of accumulated GDD from sowing until early October slightly exceeded 900 only in 2010 (Fig. 1), which was warmer (Table 1) if compared with long-term average data.

Table 2

**Growing degree days to reach different growth stages**

| Stage | FAO | 2008 | | 2009 | | 2010 | |
|---|---|---|---|---|---|---|---|
| | | GDD | Date | GDD | Date | GDD | Date |
| Emergence | 180-340 | 44 | 22.05. | 46 | 22.05. | 69 | 19.05. |
| First tassels | 180-239 | 415 | 26.07. | 448 | 31.07. | 474 | 19.07. |
| | 240-340 | 402 | 25.07. | 483 | 05.08. | 501 | 22.07. |
| Full tassels | 180-239 | 444 | 30.07. | 468 | 04.08. | 510 | 23.07. |
| | 240-340 | 454 | 01.08. | 514 | 09.08. | 530 | 25.07. |
| First ears | 180-239 | 434 | 29.07. | 478 | 05.08. | 478 | 20.07. |
| | 240-340 | 445 | 31.07. | 511 | 09.08. | 507 | 23.07. |
| Full ears | 180-239 | 484 | 04.08. | 532 | 12.08. | 551 | 26.07. |
| | 240-340 | 525 | 10.08. | 568 | 19.08. | 571 | 28.07. |

Our results showed that ODM yield in 2008 and 2009 on the last harvest dates (3 October – 735 GDD, and 5 October–761 GDD, respectively) was lower than that on the first harvest date (3 September – 862 GDD) in 2010 (Table 3). ODM yield of early ripening (FAO 180-239) maize hybrids at the first harvest time in 2010 (3 September) on average was 14.71 t $ha^{-1}$, but that of medium ripening maize hybrids (FAO 240 – 340) on the same harvest date was 16.97 t $ha^{-1}$. Average ODM content on 3 September 2010 despite the more accumulated GDD was lower if compared with early October in 2008 and 2009 when 127-101 GDD less were accumulated (Table 3). It can be explained by drying down of maize during September in 2008 and 2009; in addition, frosts were observed before harvesting in October 2009. Maize hybrids still looked green with high moisture level on 3 September 2010.

Table 3

**Organic dry matter content and yield of maize at different growing degree days in all years**

| Year | GDD | FAO 180-239 | | FAO 240-340 | |
|---|---|---|---|---|---|
| | | ODM, % | ODM, t $ha^{-1}$ | ODM, % | ODM, t $ha^{-1}$ |
| 3 October 2008 | 735 | 27.84 | 12.95 | 23.87 | 13.84 |
| 5 October 2009 | 761 | 27.50 | 14.61 | 24.25 | 15.68 |
| 3 September 2010 | 862 | 25.67 | 14.71 | 23.60 | 16.97 |

If above average temperatures occur and the sum of GDD reaches 850 or more already until early September, better ODM yields with good quality can be obtained using medium ripening (FAO 240-340) maize hybrids. Also some researchers have mentioned that for biogas production slightly later (by 30-50 FAO units) hybrids as traditionally grown can be used (Degenhardt, 2005; cited by Schittenhelm, 2008). As Global Climate Change is occurring we will possibly have challenges to grow such hybrids with good success in Latvia during next decade as it was widely discussed during NJF Seminar No 430 'Climate Change and Agricultural Production in the Baltic Sea Region' held in Uppsala, 2010 (e.g., Svensson, 2010).

## Conclusions

From three trial years the growing season 2010 was the warmest. It was noted that hybrid maturity rating (earliness) did not affect the length of maize seedlings' emergence; it was affected by accumulated growing degree days and for emergence maize needed 44-69 GDD. Entering in flowering phase was affected as well by hybrid earliness: early maturity hybrids (characterized by FAO 180-239) entered into full flowering when 484-551 GDD depending on a year were accumulated, but medium ripening hybrids (characterized by FAO 240-340) – when 525-571 GDD were accumulated. GDD can be used as a predictor of maize developmental progress. We recommend maize for silage harvest as late as possible in the autumn, which will increase ODM yield per hectare. Still yield of maize was obtained higher when season was warmer; and it was even possible to obtain a higher yield in early September (in 2010) when the season was warmer than in early October (2008, 2009) when the season was characterized by temperature close to the long-term average data.

Warmth conditions of season (characterized by GDD) affected significantly also chemical composition of maize. Our results showed that accumulated GDD negatively correlated with the total N, protein, fiber, cellulose, hemicellulose, NDF and ADF concentration, but positively – with the fat, total carbon and ash concentration of fresh maize.

## Acknowledgments

This publication has been prepared within the framework of the ESF Project „Attraction of human resources to the research of the renewable energy sources", Contract No. 2009/0225/1DP/1.1.1.2.0/09/APIA/VIAA/129.

## References

1. Amon T., Amon B., Kryvoruchko V., Zollitsch W., Potsch E. (2004) Biogas Production from Maize and Clover Grass Estimated with the Methane Energy Value System. Available at: www.nas.boku.ac.at/fileadmin/_/H93/H931/AmonPublikationen/biogas_production_maize_and_clover_methane_energy_system.pdf, 14 March 2011.
2. Andrade F.H., Uhart S.A., Cirilo A.G. (1993) Temperature affects radiation use efficiency in maize. *Field Crops Research,* 32, pp. 17-25.
3. Brown D.M. (1997) Crop Heat Units for Corn and Other Warm Season Crops in Ontario. Available at: http://www.uoguelph.ca/plant/research/homepages/ttollena/research/assets, 9 March 2011.
4. Darby M., Lauer J.G. (2002) Harvest Date and Hybrid Influence on Corn Forage Yield, Quality, and Preservation. Available at: http://agron.scijournals.org/cgi/content/full/94/3/559, 9 March 2011.
5. Edmond M.M. (2004) Agroclimatic characterization of Lesotho for dryland maize production. Available at: http://etd.uovs.ac.za/ETD-db//theses/available/etd-08232005-104915/unrestricted /MOELETSIME.pdf, 9 March 2011.
6. Garcia Y. (2010) *Growing maize for silage. A guide for dairy farmers*. Available at: www.futuredairy.com.au/documents/growingmaizeforsilage.pdf, 9 March 2011.
7. Griffith D.R., Mannering J.V., Galloway H.M., Parson S.D., Richey C.B. (1973) Effect of eight planting systems on soil temperatures, percent stand, plant growth and yield of corn on five Indiana soils. *Agronomy Journal*, 65, pp. 321-326.
8. Hoeft R.G., Nafziger E.D., Johnson R.R., Aldrich S.R. (2000) *Modern corn and soybean production*. First edition. MCSP Publications. Champaign, IL, 353 p.
9. Hunter R.B. and Tollenaar M. (1983) Effect of temperature during grain filling on whole plant and grain yield in maize (*Zea mays* L.). *Canadian Journal of Plant Science*, 63, pp. 357-363.
10. Mahmood S., Javed F., Basra S.M.A. (2010) Heat stress effects on forage quality characteristics of maize (*Zea mays*) cultivars. Available at: www.fspublishers.org/ijab/past-sues/IJABVOL_12_NO_5/11.pdf, 9 March 2011.
11. Miedema P. (1982) The effects of low temperature on *Zea mays*. *Advances in Agronomy,* 35, pp. 93-128.
12. Moonen A.C., Ercoli L., Mariotti M., Masoni A. (2002) Climate change in Italy indicated by agrometeorological indices over 122 years. *Agricultural and Forest Meteorology,* 111(1), pp. 13-27.
13. Quansah G.W. (2010) Effect of organic and inorganic fertilizers and their combinations on the growth and yield of maize in the semi-deciduous forest zone of Ghana. Available at: http://dspace.knust.edu.gh/dspace/bitstream/123456789/2070/1/fulltxt.pdf, 9 March 2011.
14. Schittenhelm S. (2008) Chemical composition and methane yield of maize hybrids with contrasting maturity. *European Journal of Agronomy*, 29 (2008), pp. 72-79.
15. Svensson H. (2010) The effect of climate change in agriculture – an overview. In: Climate Change and Agricultural Production in the Baltic Sea Region. *NJF Report*, Vol. 6, No 1 (2010), pp. 23-24.

# EFFECT OF DIFFERENT PLANT PRODUCTION METHODS ON YIELD AND QUALITY OF PEA CULTIVAR 'MADONNA'

***Berit Tein, Vyacheslav Eremeev, Indrek Keres***
*Estonian University of Life Sciences*
*btein@emu.ee*

## Abstract

The yield and quality (1000 kernel weight, protein, nitrogen, phosphorus and potassium content) of peas was studied in cultivar 'Madonna'. The peas (*Pisum Sativum* L.) were part of the five-year crop rotation experiment where red clover (*Trifolium pratense* L.), winter wheat (*Triticum aestivum* L.), peas, potato (*Solanum tuberosum* L.) and barley (*Hordeum vulgare* L.), were following each other. There were two production variants which followed the crop rotation. In one variant mineral fertilizers and pesticides were used, and the other variant was conversion to organic without any synthetic agrochemicals. In mineral fertilizing variants the background in all variants was N20, P25, and K95 kg $ha^{-1}$. The previous crop was winter wheat which received in fertilized variants mineral fertilizers with the background of P25 and K95 kg $ha^{-1}$, the N amount varied from 0 to 150 kg $ha^{-1}$. The aim of this paper was to study a mineral fertilizing after-effect on the peas which followed the winter wheat. Herbicide MCPA 750 was used. In conversion to organic, the pea grains which followed the crop rotation and mineral fertilizing N150 after-effect variants had higher yield, protein and nitrogen content compared to the other variants where mineral fertilizing after-effect was investigated. Phosphorus contents were higher in N0, and conversion to organic variants. Potassium content remained lower in N100, and N150 mineral fertilizing after-effect variants. The 1000 kernel weight was significantly higher in the variant of conversion to organic compared to the other variants.

**Key words**: 1000 kernel weight, conversion to organic, mineral fertilizing after-effect, protein, *Pisum sativum* L.

## Introduction

Over the last few decades, consumer demand for healthier food and government policies focused on environmentally sustainable agricultural systems have both promoted a rapid expansion of organic farming (Järvan and Edesi, 2009). It is a well known consumers' belief that crops grown under organic conditions are healthier and with better quality than conventionally grown products, but an improved nutritional profile of organic vs. conventional crops has not been ascertained (Gennaro and Quaglia, 2003).

Field peas (*Pisum sativum* L.) are cultivated mainly to use them as animal feed and human food. As a leguminous plant peas are also good soil nutrients because their remains are rich in protein, so they can improve soil quality even during a longer period of time (Sanchez, 2004) making them a valuable previous and following crop. Peas are important link in crop rotation because of their ability to fixate atmospheric nitrogen which makes peas vital to organic farming (Luik et al., 2008). Leguminous plants are less demanding to soil nitrogen reserve because they get 70-80% of the necessary nitrogen from the air and the rest will come from soil (Jaama and Lauk, 1999) therefore leguminous plants require less mineral fertilization.

Winter wheat (*Triticum aestivum* L.) is a high nutrient-demanding and nutrient-using crop. Its nutrient demand is depending on yield – 60-140 kg of N $ha^{-1}$, 17-33 kg of P $ha^{-1}$, and 40-95 kg of K $ha^{-1}$ (Kärblane and Kevvai, 1999). The previous crop of winter wheat was red clover (*Trifolium pratense* L.) which enriches the soil with organics and nitrogen. In a sowing year, red clover leaves to the soil up to 160 kg of N $ha^{-1}$ in to the soil (Viil and Võsa, 2005) and has a positive effect on the formation of productivity elements of crops not only in the first year but also in the second year, which determines the productivity of the crop rotation links (Skuodienė and Nekrošienė, 2007).

L.A. McLean et al. (1974) have reported that the protein contents of field peas are very dependent on the N status of the soil and are efficient converters of soil N to seed protein. Pea protein content can be increased 20-30% (200-300 g $kg^{-1}$) by application of nitrogen fertilizers. The average protein content of peas is 21-26% (210-260 g $kg^{-1}$) (Kalev and Narits, 2004). Pea plants are assimilating 11-20% (110-200 g $kg^{-1}$) of phosphorus and 50-60% (500-600 g $kg^{-1}$) of potassium from the soil which has been given there with mineral fertilizers (Kärblane et al., 1996).

1000 kernel weight, measure of the size of a grain, depends on the variety of genetic characteristics, on growth conditions, and on fertilization (Koppel and Ess, 2007).

The aim of this paper was to study whether there is a mineral fertilizing after-effect on the pea yield and quality when the previous crop has received high amounts of mineral nitrogen.

## Materials and Methods

Field trials with the pea cultivar 'Madonna' (bred in Germany) were carried out on the experimental fields of the Department of Field Crops and Grassland Husbandry, Estonian University of Life Sciences in 2009. There were five treatments – second-year conversion to organic (Conv. Organic CR), $N_0P_0K_0$, $N_{20}P_{25}K_{95}$ (N50), $N_{20}P_{25}K_{95}$ (N100), and $N_{20}P_{25}K_{95}$ (N150). Values in brackets show the previous crop (winter wheat) mineral fertilizers nitrogen amounts which were applied in the same test block. The peas were part of the five-year crop rotation experiment where red clover, winter wheat, peas, potato (*Solanum tuberosum* L.), and barley (*Hordeum vulgare* L.) were following each other. Peas were sown according to the norm: 227 kg $ha^{-1}$,

80 germinate able seeds per 1 $m^2$. Peas were fertilized with Kemira Grow How Power N:P:K – 5:14:28. The previous crop winter wheat was fertilized with different fertilizers: Kemira Grow How Power N:P:K – 5:14:28, and ammonium nitrate 34.4 N:P:K – 34.4:0:0. The different winter wheat fertilized variants were: $N_0P_0K_0$, $N_{50}P_{25}K_{95}$, $N_{100}P_{25}K_{95}$, and $N_{150}P_{25}K_{95}$. The pea variants that had received $N_0P_0K_0$ and mineral fertilizers including with mineral fertilizers after-effect, where sprayed with herbicide MCPA 750 (preparation norm – 0.7 L $ha^{-1}$, active ingredient 750 g $L^{-1}$ dimethylamine salt). The experiments were laid out in four replications. The size of each test plot was 60 $m^2$. The soil of the experimental field was *Stagnic Luvisol* by World Reference Base (2002) classification (Deckers et al., 2002), the texture of which is sandy loam with a humus layer of 20-30 cm.

The soil analyses were carried out at the laboratories of the Department of Soil Science and Agrochemistry, Estonian University of Life Sciences. The trial soil was slightly acidic – pH KCl 6.0, carbon – 13.8 g $kg^{-1}$, mobile phosphorus – 0.103 g $kg^{-1}$ (ammonium lactate), mobile potassium – 0.179 g $kg^{-1}$ (ammonium lactate), calcium – 0.980 g $kg^{-1}$, magnesium – 0.164 g $kg^{-1}$, and nitrogen – 1.29 g $kg^{-1}$ of soil.

The average temperatures of many years and the average temperatures in 2009 were similar. May and July had less precipitation compared to the average of 1966-1998, but there was abundant rainfall in June and August (Table 1).

Table 1

**Average monthly temperatures (°C) and precipitation (mm) in Estonia during the vegetation period**

| Month | Temperatures, °C | | Precipitation, mm | |
|---|---|---|---|---|
| | Average of 2009* | Average of 1966-1998** | Sum of 2009* | Average sum of 1966-1998** |
| May | 11.5 | 11.6 | 13.4 | 55.0 |
| June | 13.8 | 15.1 | 137.4 | 66.0 |
| July | 16.9 | 16.7 | 54.6 | 72.0 |
| August | 15.4 | 15.6 | 89.2 | 79.0 |
| May-August | 14.4 | 14.8 | 294.6 | 272.0 |

* – according to the Eerika weather station

** – Jaagus, 1999

The yield was determined by weighing after harvesting and drying the grains. Yield data and grain quality parameters were adjusted to 14% moisture content. Yield, 1000 kernel weight, protein, N, P, and K contents were calculated as average of 4 replications. 1000 kernels were counted manually and then weighed. In order to get the protein content percentage, total nitrogen content from grains was determined by using the Kjeldahl method, and then for getting the protein content percentage, the number of nitrogen content was multiplied with the coefficient 6.25. Acid digestion by sulfuric acid solution (Methods of Soil and Plant Analysis, 1986) was used to determine phosphorus and potassium content in plant material. After the digestion the content of total phosphorus was determined colorimetrically. Total potassium content was determined by flame photometer.

Experimental data were processed by Statistica 7.0 software (Anova, Fisher LSD test) (Statsoft, 2005).

## Results and Discussion

Study results in 2009 showed that there were no statistical differences between the variants, but there was observed an upward trend in the yields (Fig. 1). The average yields of pea cultivar 'Madonna' in 2009 were 2.75-3.72 t $ha^{-1}$. In variant Conv. Organic CR there were more root nodules that were initiated by Rhizobia bacteria which fixate the soil air nitrogen in a form that the plant can absorb and use nutrients more effectively and therefore the yields are higher. It has been studied that the use of mineral nitrogen significantly reduces the root nodulation of grain legumes (Abdel Wahab et al., 1996). If there is not enough root nodules to help the plant to supply the soil with air nitrogen then the plant gets its nutrients directly from the soil and from mineral fertilizers without the help of root nodules. Therefore the yield was also higher in the variant which followed the N150 after-effect.

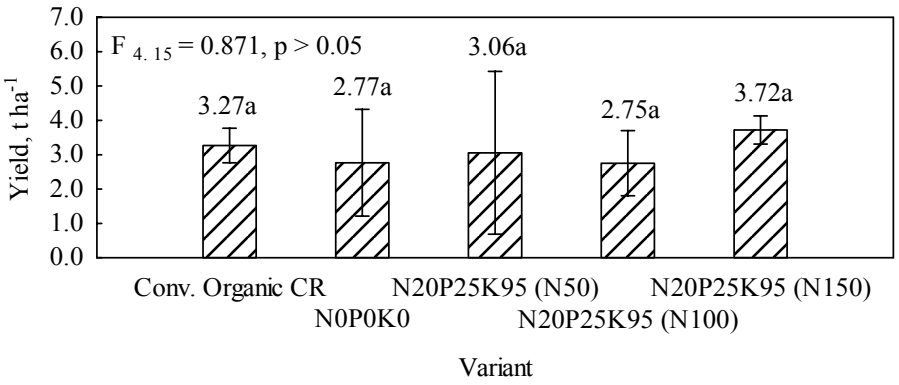



Figure 1. Average yield of pea cultivar 'Madonna' in 2009, t $ha^{-1}$. Means followed by a different letter in the same row are significantly different ($p<0.05$). Values in brackets show the previous (crop winter) wheat mineral fertilizers nitrogen amounts which were applied in the same test block.

The fertilization decreased substantially the 1000 kernel weight of pea grains (Fig. 2). The 1000 kernel weight was highest in variant Conv. Organic CR – 283.8 g. The fertilized variants had almost a 50-g-lower 1000 kernel weight. We observed visually that there were fewer pods in the Conv. Organic variant than in other variants, and therefore the grains were able to grow larger and weigh more. This also explains why the yield was one of the highest in Conv. Organic CR variant.

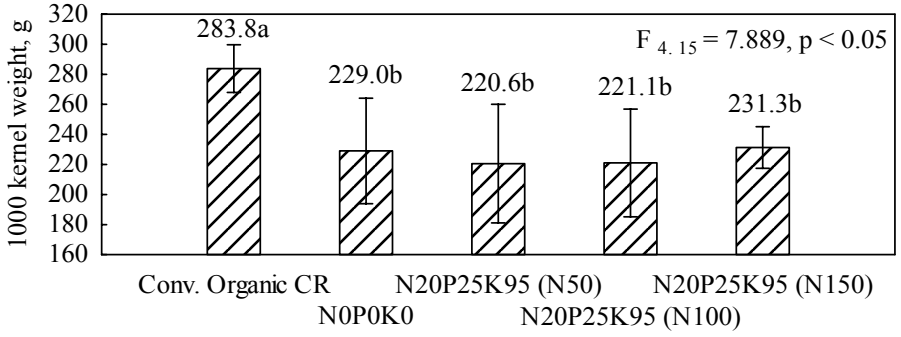



Figure 2. Average 1000 kernel weight of pea cultivar 'Madonna' in 2009, g. Means followed by a different letter in the same row are significantly different ($p<0.05$). Values in brackets show the previous crop (wheat mineral) fertilizers nitrogen amounts which were applied in the same test block.

Grain nitrogen (Table 2) and protein (Fig. 3) content can be viewed together. The higher the nitrogen content in grains the higher the protein content in grains. This can be explained by the same reasons as in the yield part, and, moreover the protein content as well as nitrogen content in grains are particularly sensitive to environmental stress – rainfall, light intensity, length of growing season, length of day, and temperature, as well as agronomic factors such as plant density, weeds, and soil fertility (McLean et al., 1974; Holl and Vose, 1980). The protein and nitrogen contents were lowest in N50 after-effect variant (nitrogen content – 24.75 g $kg^{-1}$, and protein content – 154.7 g $kg^{-1}$). In other variants, average nitrogen contents in grains were between 27.0-29.0 g $kg^{-1}$, and protein contents between 170.0-180.0 g $kg^{-1}$.

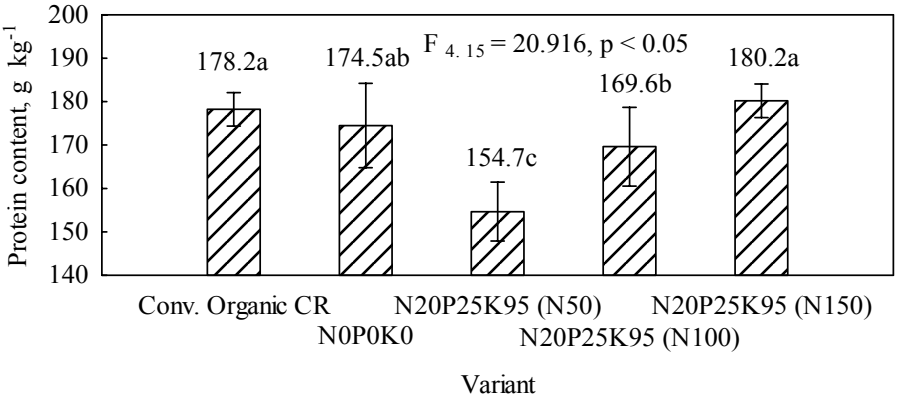



Figure 3. Average protein content in pea cultivar 'Madonna' in 2009, g $kg^{-1}$. Means followed by a different letter in the same row are significantly different ($p<0.05$). Values in brackets show the previous (crop winter) wheat mineral fertilizers nitrogen amounts which were applied in the same test block.

The average phosphorus and potassium contents were lower in variants that received mineral fertilizers (Table 2). The previous crop was winter wheat which needs a lot of nutrients. The higher the yield the more nutrients are required. Previous crop winter wheat uses a lot of potassium and phosphorus in order to grow high yields (Kärblane and Kevvai, 1999).

Table 2

**Average nitrogen, phosphorus and potassium content in grains of peas cultivar 'Madonna', g $kg^{-1}$, in 2009**

| Variant | Nitrogen content, g $kg^{-1}$ | Phosphorus content, g $kg^{-1}$ | Potassium content, g $kg^{-1}$ |
|---|---|---|---|
| Conv. Organic CR | 28.52a | 4.87a | 10.24a |
| $N_0P_0K_0$ | 27.92ab | 4.72b | 10.41a |
| $N_{20}P_{25}K_{95}$ (N50) | 24.75c | 3.98c | 10.33a |
| $N_{20}P_{25}K_{95}$ (N100) | 27.14b | 3.55d | 10.07ab |
| $N_{20}P_{25}K_{95}$ (N150) | 28.83a | 3.41e | 9.75b |

Explanation: Values in brackets show the previous (crop winter) wheat mineral fertilizers nitrogen amounts which were applied in the same test block.
Means followed by a different letter in the same column are significantly different ($p<0.05$)

Other researches have shown that the average phosphorus content grain can reach 6.3 g $kg^{-1}$, and potassium content 8.3 g $kg^{-1}$ (Wang and Daun, 2004). In our experiment, the phosphorus content in grain was slightly lower ranging between 3.41 g $kg^{-1}$ (N150 after-effect) and 4.87 g $kg^{-1}$ (Conv. Organic CR), but potassium content was slightly higher ranging between 9.75 g $kg^{-1}$ (N150 after-effect) and 10.41 g $kg^{-1}$ ($N_0P_0K_0$).

## Conclusions

1. The average yields of pea cultivar 'Madonna' in 2009 were 2.75-3.72 t $ha^{-1}$. In conversion to organic, the pea grains which followed the crop rotation and mineral fertilizing N150 after-effect variants had higher yield compared with the other variants. The yield was the lowest in mineral fertilizing variant N100 after-effect (2.75 t $ha^{-1}$). There were no statistical differences between the variants.
2. The 1000 kernel weight was statistically higher in variant Conv. Organic CR – 283.8 g. The fertilized variants had almost a 50-g-lower 1000 kernel weight.
3. The protein and nitrogen contents were the lowest in the N50 after-effect variant (nitrogen content – 24.75 g $kg^{-1}$, and protein content – 154.7 g $kg^{-1}$). In other variants, average nitrogen content in pea grains was between 27-29 g $kg^{-1}$, and protein content – between 170-180 g $kg^{-1}$. There were statistical differences in nitrogen as well as in protein content in grains between the variants.
4. The phosphorus content in pea grains was ranging between 3.41 g $kg^{-1}$ (N150 after-effect) and 4.87 g $kg^{-1}$ (Conv. Organic CR), and potassium content between 9.75 g $kg^{-1}$ (N150 after-effect) and 10.41 g $kg^{-1}$ ($N_0P_0K_0$). Phosphorus and potassium content in grains had statistical differences between the variants.

**References**

1. Abdel Wahab A.M., Zahran H.H. and Abd-Alla M.H. (1996) Root-Hair infection and nodulation of four grain legumes as affected by the form and the application time of nitrogen fertilizers. *Folia Microbilogica*, 41(4), pp. 303-308.
2. Deckers J.A., Driessen P., Nachtergaele F.O.F. and Spaargaren O. (2002) World reference base for soil resources–inanutshell.In:MicheliE.,NachtergaeleF.O., Jones R.J.A. and Montanarella L. (eds) *Soil Classification 2001. European Soil Bureau Research Report* No. 7, EUR 20398 EN, pp. 173-181.
3. Gennaro L. and Quaglia G. (2003) Food safety and nutritional quality of organic vegetables. *Acta Horticulture*, 61, pp. 675-680.
4. Holl F.B. and Vose J.R. (1980) Carbohydrate and protein accumulation in the developing field pea seed. *Canadian Journal of Plant Science*, 60, pp. 1109-1114.
5. Jaagus J. (1999) Uusi andmeid Eesti kliimast (New Data about the Climate of Estonia). In: Jaagus J. (ed.) *Studies on Climate of Estonia, Tartu: Publications Instituti Geographici Universitatis Tartuensis* 85, pp. 28-38. (in Estonian).
6. Jaama E. and Lauk E. (1999) Teraviljade kasv ja arenemine (The development and growth of cereals). In: Older H. (ed.) *Teraviljakasvatuse käsiraamat (Cereal growing manual)*, Eesti Maaviljeluse Instituut, Saku, Eesti, pp. 26-42. (in Estonian).
7. Järvan M. and Edesi L. (2009) The effect of cultivation methods on the yield and biological quality of potato. *Agronomy Research,* 7(1), pp. 289-299.
8. Kalev S. and Narits L. (2004) Weather effects on yield and quality of different field pea types. *Journal of Agricultural Science*, XV (1), pp. 3-11.
9. Koppel R. and Ess M. (2007) Talinisu sortide omadused ning agrotehnilised võtted kvaliteetse talinisu kasvatamiseks (Winter wheat varieties characteristics and agro techniques in order to grow high-quality winter wheat crops). *Millest sõltub teravilja saagikus (The dependence of cereal yields),* Jõgeva Sordiaretuse Instituut, Jõgeva, Eesti, pp. 67-84. (in Estonian).
10. Kärblane H., Kevvai L. and Kalmet R. (1996) Mineraalväetised (Mineral fertilizers). In: Kärblane H. (ed.) *Taimede toitumise ja väetamise käsiraamat (Plant nutrition and fertilization handbook)*, EV Põllumajandusministeerium, Tallinn, Eesti, pp. 149-196. (in Estonian).
11. Kärblane H. and Kevvai L. (1999) Teraviljade taimetoitainete vajadus (Nutrient demandings of cereals). In: Older H. (ed.) *Teraviljakasvatuse käsiraamat (Cereal growing manual)*, Eesti Maaviljeluse Instituut, Saku, Eesti, pp. 193-213. (in Estonian).
12. Luik M., Mikk M. and Vetemaa A. (2008) *Mahepõllumajanduse alused (Basics of organic farming*). 174 p. (in Estonian).
13. *Methods of Soil and Plant Analysis* (1986) Agricultural Research Centre, Department of Soil Science. Jokioinen, Finland, 44 p.
14. McLean L.A., Sosulski F.W. and Youngs C.G. (1974) Effects of nitrogen and moisture on yield and protein in field peas. *Canadian Journal of Plant Science*, 54, pp. 301-305.
15. Sanchez E. (2004) The organic way – selecting green manure crops for soil fertility. *Virginia vegetable, small fruit and speciality crops*, 3 (4), pp. 11-13.
16. Skuodienė R. and Nekrošienė R. 2007) Impact of perennial legumes and timothy as green manure on productivity of *Secale cereale* L. and x *Triticosecale* Wittm and on occurrence of cereal diseases. *Agronomy Research*, 5 (1), pp. 59-71.
17. *STATSOFT* (2005) Statistica 7.0. Copyright 1984-2005. Tulka, OK, USA. 716 pp.
18. Viil P. and Võsa T. (2005) Liblikõielised haljasväetised (Leguminous green manures). Available at: http://www.eria.ee/public/files/Infoleht_148.pdf , 15 March 2011. (in Estonian).
19. Wang N. and Daun J.K. (2004) Effect of variety and crude protein content on nutrients and certain antinutrients in field peas (*Pisum sativum*). *Journal of the Science of Food and Agriculture*, 84 (9), pp. 1021-1029.

# PRODUCTION OF BIO-ETHANOL FROM WINTER CEREALS

***Inga Jansone, Zinta Gaile***
*Latvia University of Agriculture*
*inga.jansone@e-apollo.lv*

**Abstract**

Renewable energy resources play an important role in energy production both in Latvia and in the world. Bio-ethanol is used as a substitute for oil products in various countries of the world. It is produced from the plants containing starch: cereals, potatoes, beet, maize. The task of the research was to evaluate the suitability of different varieties of winter wheat, triticale and rye for extraction of bio-ethanol in Latvia. The research was carried out at the State Stende Cereals Breeding Institute in 2009, and the following varieties and lines of cereals were examined: winter wheat varieties 'Mulan', 'Skalmeje', and the line '99-115', developed at the State Stende Cereals Breeding Institute; winter triticale varieties 'SW Valentino', 'Dinaro', and the line '0002-26', developed at the State Priekuli Plant Breeding Institute; winter rye varieties 'Matador', 'Placido' $F_1$, and 'Dankowskie Nowe'. The content of crude protein and starch of grains, the grain yield, and the bio-ethanol yield were determined. The highest bio-ethanol yield was acquired from the winter wheat and triticale varieties that had the highest starch content and the lowest crude protein content of grains. The best results were obtained from winter wheat line '99-115' and variety 'Mulan' (409.4 and 406.8 L $t^{-1}$), triticale variety 'Dinaro' (423.3 L $t^{-1}$), and winter rye variety 'Matador' (370.1 L $t^{-1}$.)
**Key words:** winter wheat, winter triticale, winter rye, crude protein, starch.

## Introduction

The renewable energy resources have the priority in the future energy production. The tendency in the world is to promote the use of biofuels that significantly reduce the greenhouse gas emission. By ratifying the Kyoto Protocol and introducing the Action plan for energy efficiency, the European Union (EU) made the commitment to achieve the long-term goals, including the goal to reduce the general level of EU greenhouse gas emission and increase the share of renewable energy in the entire energy consumption of the EU till 2020 (Directive..., 2009). Latvia, similarly to the majority of countries in the world, has also committed itself to implement international obligations related to the prevention of the global climate change.

The use of bio-ethanol for renewable energy resources is one of the solutions for saving the environment and reducing the global warming. It also reduces the necessity to import oil products and provides diversification of fuels as well as ensures the security of supply.

Bio-ethanol is increasingly used as a substitute for oil products in various countries of the world. According to the data for the year 2009, the total amount of bio-ethanol produced in the world was 74 billion litres. The biggest producers of bio-ethanol were the United States of America and Brazil. The EU is mentioned as the third biggest producer of bio-ethanol. In 2009 it produced 3.7 billion litres of bio-ethanol. Among the EU countries, France and Germany are the ones that produce the greatest amount of bio-ethanol. Latvia produced 15 millions litres of bio-ethanol in 2009 (Production of..., 2009).

Bio-ethanol is extracted from plants that contain starch, for example, cereals, potatoes (*Solanum tuberosum* L.), beet (*Beta vulgaris saccharifera* L.), maize (*Zea mays* L.). In Latvia, bio-ethanol is produced from cereals. The research data from other countries shows that cereals are suitable for production of bio-ethanol (Clarke et al., 2008; Wang et al., 1997, 1998; Müllerovs and Mikulins, 2008).

The aim of the research was a suitability of several varieties of winter wheat (*Triticum aestivum* L.), triticale (*Triticosecale* Wittm), and rye (*Secale cereale* L.) for extraction of bio-ethanol in Latvia.

## Materials and Methods

The research was carried out at the State Stende Cereals Breeding Institute in the vegetation period of 2009/2010. Soil at the trial site was sod-podzolic loam with the following characteristics: pH KCL 5.6 – 6.0, content of organic substance 22 – 26 g $kg^{-1}$, content of plant-available P – 100 mg $kg^{-1}$, K – 150 mg $kg^{-1}$.

Field experiments were carried out in the breeding crop rotation fields, placed randomly in 4 replications with the plot area of 12 $m^2$.

The following varieties and lines were examined during the research: wheat varieties 'Mulan' and 'Skalmeje', and the line '99-115', developed at the State Stende Cereals Breeding Institute; winter triticale varieties 'SW Valentino', 'Dinaro', and line '0002-26', developed at the State Priekuli Plant Breeding Institute; winter rye varieties 'Matador', 'Placido' $F_1$, and 'Dankowskie Nowe'.

The **previous crop** in the experiment field was white mustard used for green manure.

**Soil tillage**. The pre-crop – mustard – was worked into the soil, using the KUHN plough VARIMASTER.

**The sowing rate** for population rye ('Matador', 'Dankowskie Nowe') and triticale was 400 germinating seeds per 1 $m^2$, for hybrid rye ('Placido' $F_1$) – 200 germinating seeds per 1 $m^2$, and for winter wheat – 450 germinating seeds per 1 $m^2$. The sowing was carried out on 18 September of 2009. Complex fertiliser N4-P20-K20 in the amount of 300 kg $ha^{-1}$ was used as a basic fertiliser. In the spring of 2010 ammonium nitrate (N 34%) was used as top-fertiliser in the following amounts:

at the renewal of vegetation:
- for winter wheat – 90 kg N ha$^{-1}$;
- for triticale and rye – 60 kg N ha$^{-1}$;

at the growing stages 31 – 32 for all examined winter cereals – 60 kg N ha$^{-1}$.

The winter cereals were harvested on 8 August using the harvester WINTERSTEIGER DELTA, which also determined the weight of grains (kg), and moisture (%). After drying the samples, they were cleaned with MINI PETKUS MP100. The yield of winter cereals (t ha$^{-1}$) was recalculated at 14% of moisture and 100% purity.

The analyses of grain quality were carried out in the Laboratory of Grain Technology and Agro-chemistry of the State Stende Cereals Breeding Institute. The content of crude protein and starch was determined by using INFRATEC ANALYZER 1241.

The ethanol was extracted at the Institute of Microbiology and Biotechnology of the University of Latvia. A modified method was used for the extraction of ethanol. The method is based on the fermentation of the grain sample with alcohol yeasts (*Saccharomyces cerevisiae*) (Vigants et al., 2008).

The formulas (1)-(3) recommended by the Institute of Microbiology and Biotechnology of the University of Latvia were used in order to calculate the theoretical ethanol outcome (L t$^{-1}$) and yield (L ha$^{-1}$).

Theoretical outcome of ethanol from starch:

$$Et=\frac{C\times 1.11\times 2\times 46}{180.16} \quad (1),$$

where: $Et$ – theoretica l outcome of ethanol, g g$^{-1}$;
1.11 – factor for starch recalculation into glucose;
2 – stechiometrical factor for the summary reaction equation of glucose conversion into ethanol;
46 – molar mass of ethanol;
180.16 – molar mass of glucose;
$C$ – starch, g g$^{-1}$.

Outcome of ethanol, L t$^{-1}$:

$$E=\frac{Et}{0.789}\times 1000 \quad (2),$$

where: $E$ – outcome of ethanol, L t$^{-1}$;
$E_t$ – acquired outcome of ethanol, g g$^{-1}$;
0.789 – ethanol density, g ml$^{-1}$.

Ethanol yield, L ha$^{-1}$:

$$E_r=E\times R \quad (3),$$

where: $E_r$ – ethanol yield, L ha$^{-1}$;
$E$ – outcome of ethanol, L t$^{-1}$;
$R$ – grain harvest, t ha$^{-1}$.

The meteorological data obtained at the Stende station of hydrometeorology were compiled for the period of the vegetation and hibernation period. The weather conditions during the season of 2009/2010 were suitable for germination and tillering of winter cereals. The thick cover of snow at the beginning of the winter was beneficial for wintering. Rather dry and hot weather was registered during summer. It reduced the potential yield of winter cereals because grain was dried before full maturation. During the harvest the amount of precipitation increased, hampering the harvesting of grain and leaving an impact on their quality.

The statistical evaluation of data was carried out by using methods of dispersion, correlation and regression analysis, as well as descriptive statistics.

## Results and Discussion

*Grain yield*

The grain yield in trials in 2009/2010 was comparatively good. It ranged from 8.46 to 8.90 t ha$^{-1}$ (Table 1). The average grain harvest of the examined species of winter cereals did not have significant differences ($p>0.05$).

Table 1

**Average grain yield and quality, and bio-ethanol outcome of winter cereals**

| Species | Crude protein, g kg$^{-1}$ | Starch, g kg$^{-1}$ | Yield, t ha$^{-1}$ | Bio-ethanol outcome, L t$^{-1}$ | Bio-ethanol yield, L ha$^{-1}$ |
|---|---|---|---|---|---|
| Winter wheat | 126.6 | 676.8 | 8.46 | 400.1 | 3386 |
| Winter triticale | 117.1 | 647.7 | 8.74 | 411.9 | 3607 |
| Winter rye | 104.8 | 614.9 | 8.90 | 367.3 | 3270 |
| $LDS_{0.05}$ | 3.53 | 11.94 | 0.71 | 10.07 | 369.18 |

Evaluating the results of different varieties of the same species, the average yield of the examined wheat varieties and lines was 8.46 t ha$^{-1}$ (Table 1). Better grain yield was obtained from the line '99-115' – 9.00 t ha$^{-1}$. Yield of varieties 'Skalmeje' and 'Mulan' was 0.81 and 0.80 t ha$^{-1}$ lower (8.18 and 8.19 t ha$^{-1}$, respectively) in comparison to the yield of the line '99-115' (Table 2). The average yield of the examined triticale varieties and lines was 8.74 t ha$^{-1}$. A

significantly ($p<0.05$) better grain yield was obtained from triticale variety 'Dinaro' – 9.65 t ha$^{-1}$. The variety 'Dinaro' also had a good yield during the research in previous years (Jansone et al., 2010). The yield of triticale variety 'SW Valentino' and the line '0002-26' was 0.93 and 1.79 t ha$^{-1}$ lower (8.72 and 7.86 t ha$^{-1}$ respectively).

Table 2

**Grain yield and quality of varieties and lines of winter cereals**

| Varieties and lines | Yield, t ha$^{-1}$ | Crude protein, g kg$^{-1}$ | Starch, g kg$^{-1}$ | Bio-ethanol outcome, L t$^{-1}$ | Bio-ethanol yield, L ha$^{-1}$ |
|---|---|---|---|---|---|
| Wheat | | | | | |
| Skalmeje | 8.18 | 123.7 | 675.8 | 384.0 | 3141 |
| Mulan | 8.19 | 128.7 | 676.8 | 406.8 | 3332 |
| L. 99-115 | **9.00** | 127.5 | 678.0 | 409.4 | 3684 |
| $LDS_{0.05}$ | 1.16 | 9.56 | 38.89 | - | - |
| Triticale | | | | | |
| Dinaro | **9.65** | 111.1 | 666.5 | 423.3 | 4085 |
| SW Valentino | 8.72 | 115.8 | 642.8 | 408.7 | 3564 |
| L. 0002-26 | 7.86 | 124.5 | 634.0 | 403.7 | 3173 |
| $LDS_{0.05}$ | 0.78 | 4.48 | 10.26 | - | - |
| Rye | | | | | |
| Placido | **9.69** | 102.8 | 610.5 | 365.0 | 3537 |
| Matador | 8.48 | 101.8 | 617.0 | 370.1 | 3138 |
| Dankowskie Nowe | 8.54 | 110.0 | 617.3 | 366.9 | 3133 |
| $LDS_{0.05}$ | 0.49 | 1.98 | 3.07 | - | - |

The hybrid rye variety 'Placido' F1 had significantly ($p<0.05$) higher level of yield in comparison to population rye varieties 'Matador' (-1.21 t ha$^{-1}$) and 'Dankowskie Nowe' (-1.15 t ha$^{-1}$).

*Yield quality*

According to the data of other researchers (Reaker et al., 1998; Clarke et al., 2008), a higher starch and lower crude protein content of grain has impact on the bio-ethanol outcome. In our research, the crude protein and starch content in the grain of the varieties and lines of winter cereals also was evaluated. Among the species the rye had the lowest average crude protein content – 104.8 g kg$^{-1}$ (Table 1). The winter wheat had the highest average crude protein content (126.6 g kg$^{-1}$). Although the wheat grain contained the highest amount of crude protein, they also had the highest starch content of all the species – 676.8 g kg$^{-1}$. During the evaluation of the rye it was possible to find the same correlation in reverse: the grain that had the lowest crude protein content also had the lowest starch content – 614.9 g kg$^{-1}$. The quality parameters for triticale were in the middle between the wheat and rye quality parametrs. The starch content is dependent on the weather and cultivation conditions (Kučerov, 2007).

Evaluating the species separately, no substantial differences in the crude protein and starch content of grain were discovered among the examined varieties and lines of winter wheat. The crude protein content ranged from 123.7 to 128.7 g kg$^{-1}$, and the starch content ranged from 675.8 to 678.0 g kg$^{-1}$. Evaluation of the wheat varieties by taking into account the crude protein content of the grain showed they are suitable for baking high-quality bread.

Evaluating triticale varieties and lines it was found that the crude protein and starch content of the grain depended greatly on the chosen variety. The variety 'Dinaro' that also had the highest starch content in the research of previous years (Jansone et al., 2010), had the highest starch content (666.5 g kg$^{-1}$) and the lowest crude protein content (111.1 g kg$^{-1}$). The variety 'SW Valentino' and the line '0002-26' were found to have significantly ($p<0.05$) lower starch content and substantially ($p<0.05$) higher crude protein content than 'Dinaro'. L. Krejčirova and I. Capouchova (2008) who have carried out research in the Czech Republic on the suitability of triticale for bio-ethanol production have acquired data on starch content of different triticale varieties. The starch content differed depending on the variety, the place, and the year of cultivation. The Czech research showed that the starch content of one variety ranged from 673.3 to 693.9 g kg$^{-1}$.

As regards the rye, the varieties 'Matador' and 'Dankowskie Nowa' were found to have significantly ($p<0.05$) higher starch content – 617.0 and 617.3 g kg$^{-1}$ respectively. The starch content of the hybrid variety

'Placido' $F_1$ was slightly lower – 610.5 g $kg^{-1}$. The varieties 'Matador' and 'Placido' $F_1$ had the lowest crude protein content – 101.8 and 102.8 g $kg^{-1}$ respectively. It was slightly higher for the variety 'Dankowskie Nowa' – 110.0 g $kg^{-1}$.

*Ethanol outcome (L $t^{-1}$) and ethanol yield (L $ha^{-1}$)*

Grain ensures high ethanol outcome. Comparing the species, the trials showed that the highest ethanol outcome (L $t^{-1}$) was obtained from triticale – 411.9 L $t^{-1}$. Triticale can provide the ethanol yield in the amount of 3607 L $ha^{-1}$ with the yield level of 8.74 t $ha^{-1}$, which is the highest result among all the examined species of winter cereals (Table 1). S. Wang et al. (1997) have stated that the bio-ethanol outcome from triticale grain is 362-367 L $t^{-1}$. F. Sosulski and K. Sosulski (1994) mention that the outcome of commercial bio-ethanol from wheat ranges from 324 to 364 L $t^{-1}$. It was found that the ethanol outcome of winter wheat (400.1 L $t^{-1}$) is 11.8 L $t^{-1}$ lower than that of triticale, and with the yield level of 8.46 t $ha^{-1}$ it is possible to obtain ethanol yield at the amount of 3386 L $ha^{-1}$ .

The use of rye in production of bio-ethanol is economically rentable and a good alternative for production of biofuels. According to the data of different researches, the bio-ethanol outcome from rye can be at the amount of 362-409 L $t^{-1}$ (Wang et al., 1997, 1998). During our research the ethanol outcome acquired from rye was 367.3 L $t^{-1}$. If the grain yield is 8.91 t $ha^{-1}$, rye can provide the ethanol yield at the amount of 3270 L $ha^{-1}$. According to the data of D'Appolonia (Д'Апполониа, 1978), the rye grain contains more starch granules of the size 30-40 and 20-30 μm. The size of starch granules influences the technology of grain processing for extraction of ethanol. The research has shown that the starch granules of the size >10 μm (type A) contain slightly higher amounts of amylose. In order to split amylose in ethanol production technology it is necessary to use more energy (Smith et al., 2006). This explains why rye varieties have lower ethanol outcome.

In order to produce bio-ethanol, it is customary to use wheat varieties suitable for fodder and technical purposes and have high starch content. The results of researches from other countries indicate that the highest ethanol outcome can be acquired from the soft wheat (*Triticum aestivum* L.) varieties (Smith et al., 2006; Sosulski and Sosulski, 1994). Out of all the examined varieties and lines the highest ethanol outcome was obtained from the new wheat line '99-115' and the variety 'Mulan' (409.4 and 406.8 L $t^{-1}$ respectively). The line '99-115' provided the highest ethanol yield – 3684 L $ha^{-1}$, due to highest grain yield.

According to the data of the research carried out by J. Kučerov (2007), the ethanol yield of triticale can be affected by several factors: firstly, the choice of variety, because different varieties have different ethanol outcome, and secondly, the choice of geographical location and agro-ecological differences depending on the year. According to the results of our research it can be noted that the highest ethanol outcome and yield was obtained from triticale variety 'Dinaro' (423.3 L $t^{-1}$ and 4085 L $ha^{-1}$ respectively), which was logical, since this variety had the highest starch content and the best yield.

The highest ethanol outcome among the rye varieties was obtained from the variety 'Matador' – 370.1 L $t^{-1}$. The highest ethanol yield was obtained from the rye variety 'Placido' $F_1$ – 3537 L $ha^{-1}$, because it had the highest grain yield (9.69 t $ha^{-1}$).

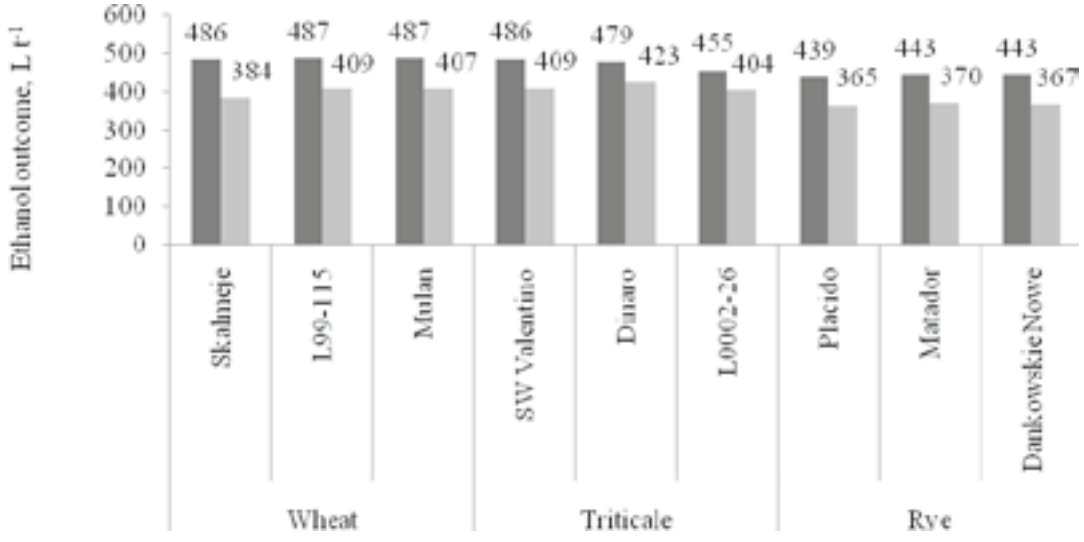
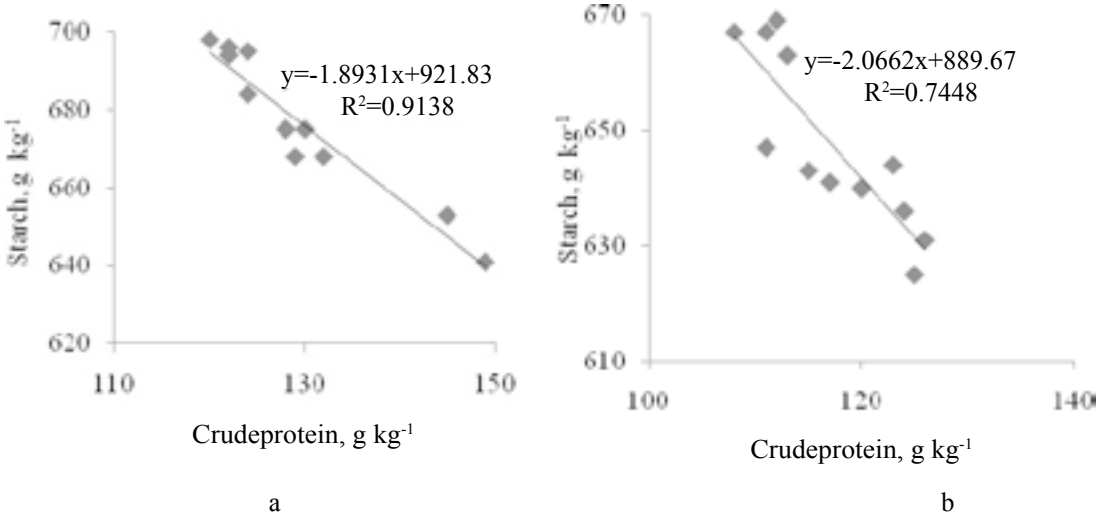



Figure 1. Theoretically calculated and practically obtained ethanol outcome, L $t^{-1}$, from winter cereals.
theoretically calculated ethanol outcome, L $t^{-1}$, practically obtained ethanol outcome, L $t^{-1}$

Evaluating the theoretically calculated and practically obtained ethanol outcome, the practically obtained outcome from all the examined varieties of winter cereals was 11-20% lower than the calculated outcome (Fig. 1). It can be explained by losses in the process of ethanol production (Dale and Tyner, 2006).

The most important qualitative characteristics that have to be taken into account while evaluating the suitability of species for bio-ethanol production, is the correlation between starch and crude protein. In order to determine the strength of correlation between starch and crude protein for each species, the analysis of data linear regression was carried out. Correlation between starch and crude protein content in triticale grains is described by a regression equation y = - 2.066x + 889.6. The determination coefficient $R^2$=0.744 shows that in 74% of cases the changes in starch content can be explained by the changes in crude protein content (Fig. 2).




a b

Figure 2. Correlations between crude protein and starch content in wheat (a) and triticale (b) grains.

Correlation between crude protein and starch content in wheat grain is described by a regression equation y = -1.893x + 921.8, $R^2$=0.913 (Fig. 2), but a significant correlation between these indices was not observed for rye ($p>0.05$) in this research period.

Correlation analysis was carried out comparing quality indicators of the winter cereals' yield and ethanol outcome. A positive correlation was determined between grain yield and ethanol yield ($r = 0.795 > r_{0.05} = 0.468$), between ethanol outcome and starch content ($r = 0.7122 > r_{0.05} = 0.468$), and between ethanol outcome and ethanol yield ($r = 0.633 > r_{0.05} = 0.468$), as well as a negative correlation between grain yield and crude protein content of grain ($r = -0.550 > r_{0.05} = 0.468$) was found for all the species of winter cereals. Evaluating the correlations it was confirmed that in order to obtain high ethanol outcome it is necessary to use the varieties of winter cereals with high starch content. A good grain yield is also necessary for obtaining a high ethanol yield. The analysis of correlations within the species shows that rye displayed deviations from the general tendency. The analysis of correlations for rye showed that there is a close correlation only between grain and ethanol yield ($r = -0.832 > r_{0.05} = 0.811$). No correlations between ethanol outcome and other indicators were found for rye. Such finding suggests paying attention to further investigations on the size of starch granules.

## Conclusions

1. The average amount of grain yield of the examined winter cereal species did not differ significantly: it ranged from 8.46 to 8.91 t $ha^{-1}$. Evaluating the examined varieties and lines of each species, the best yield for wheat was obtained from the line '99-115' (9.00 t $ha^{-1}$), for triticale – the variety 'Dinaro' showed a significantly ($p<0.05$) better grain yield (9.65 t $ha^{-1}$), and for rye the best grain yield was obtained from the hybrid variety 'Placido' $F_1$ (9.69 t $ha^{-1}$).
2. In order to extract bio-ethanol it is important to use species and varieties of winter cereals with low crude protein content and high starch content. The results of the research showed that winter triticale practically provided the highest ethanol outcome (411.9 L $t^{-1}$) and ethanol yield (3607 L $ha^{-1}$). The average starch content of winter triticale was lower than that of winter wheat, while the grain yield was lower than the average yield of rye.
3. Of all the examined varieties and lines of winter wheat the highest bio-ethanol outcome was obtained from the new line '99-115' (409.4 L $t^{-1}$) and the variety 'Mulan' (406.8 L $t^{-1}$). The line '99-115' provided the highest ethanol yield (3684 L $ha^{-1}$), because this line had the best grain yield. As regards winter triticale, the highest ethanol outcome and yield was acquired from the variety 'Dinaro' (423.3 L $t^{-1}$ and 4085 L $ha^{-1}$ respectively), because this variety was observed to have

the highest starch content and the best grain yield. The highest ethanol outcome from winter rye was obtained from the variety 'Matador' (370.1 L $t^{-1}$), and the highest ethanol yield – from the hybrid rye variety 'Placido' $F_1$ (3537 L $ha^{-1}$), which provided the best grain yield.

4. Having evaluated the correlations between the grain quality indices, yield and ethanol outcome, it was found that the highest ethanol yield for winter wheat and winter triticale can be acquired from the varieties that provide the highest grain yield, high starch content and low crude protein content of the grain. As regards rye, the research has to be continued.

**Acknowledgements**

We express our gratitude to the employees of the State Stende Cereals Breeding Institute for their support during the field and laboratory experiments.

**References**

1. Clarke S., Kindred D., Weightman R., Dyer C., Sylvester – Bradley R. (2008) Growing Wheat for Alcohol and Bioethanol Production in the North East. *The Final Report of ADAS Project XAA1500 conducted for NEPIC between September 2008 to October 2008*. Available at: www.adas.co.uk, 12 January 2010.
2. Directive of the European Parliament and Council No 2009/28/EC (2009). Available at: http://eurlex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:140:0016:0062:LV:PDF, 9 September 2010.
3. Jansone I., Malecka S., Miglane V. (2010) Suitability of winter triticale varieties for bioethanol production in Latvia. *Agronomy Research*, 8, pp. 573-582.
4. Krejčířová L., Capouchová I. (2008) Quality of Winter Wheat and Triticale for Bioethanol Production. *In: Field Crop Production: Proceedings. 43rd Croatian and 3rd International Simposium on Agriculture*. Opatia, pp. 601-603. Available at http://sa.agr.hr/2008pdf/sa2008_0516.pdf, 25 January 2011.
5. Kučerov J. (2007) The Effect of Year, Site and Variety on the Quality Characteristics and Bioethanol Yield of Winter Triticale. *Journal of the Institute of Brewing,* 133, pp. 142-146.
6. Müllerov J., Mikulin M. (2008) Production and utilization of bio-fuels in conditions of the Slovak Republic. *Ročnik* 3. Čislo 2, pp. 69-74.
7. Production of bioethanol in the UE (2009) Available at: www.plateforme-biocarburants.ch/en/infos/eu-bioethanol.php, 3 January 2011.
8. Reaker M.Ö., Gaines C.S., Finney P.L., Donelson T. (1998) Granule Size Distribution and Chemical Composition of Starches from 12 Soft Wheat Cultivars. *Cereal Chemestry*, 75 (5), pp. 721-728.
9. Dale R.T., Tyner W.E. (2006) Economic and Technical Analysis of Ethanol Dry Milling: Model user's manual. Agricultural Economics Department Purdue University. Available at: http://ageconsearch.umn.edu/bitstream/28658/1/sp060005.pdf, 15 February 2011.
10. SmithT.C.,KindredD.R.,BrosnanI.M.,WeightmanR.M., Shepherd M., Sylvester – Bradley R. (2006) Wheat as a feedstock for alcohol production. *The Home-Grown Cereals Authority*. *Research Review,* 61. London, UK, pp. 89.
11. Sosolski K., Sosulski F. (1994) Wheat as a feedstock for fuel ethanol. *Applied Biochemistry and Biotechnology*, 45 (6), pp. 169-180.
12. Vigants A., Lukjanenko J., Upite D., Kaminska E., Bekers M. ( 2008 ) Jerusalem artichoke based substrates as raw material for ethanol production by *Z.mobilis* and *S.cerevisiae*. Proceedings of *16th European Biomass Conference &Exibition*, Valencia, Spain, pp. 1610-1612.
13. Wang S., Thomas K.C., Ingledew W.M., Sosolski K., Sosulski F. (1997) Rye and Triticale as Feedstock for Fuel Ethanol Production. *Cereal Chemistry*, 74 (5), pp. 621-625.
14. Wang S., Thomas K.C., Ingledew W.M., Sosolski K., Sosulski F. (1998) Production of fuel ethanol from rye and triticale by very-high-gravity (VHG) fermentation. *Applied biochemestry*. 69 (3), pp. 157-175.
15. Д'Апполониа Б.Л. (1978) Обзор данных о крахмале тритикале. В кн.: *Тритикале – первая зерновая культура, созданная человеком*. перевод с английского Евгенева М.Б., под редакцией Гужова Ю.Л.Б. (Triticale: First Man-Made Cereals). Москва: Колос, 88-194 с. (in Russian).

# THE EVALUATION OF OIL-FLAX (*LINUM USITATISSIMUM* L.) QUALITY PARAMETERS FOR BIOFUEL PRODUCTION

***Liena Poiša, Aleksandrs Adamovičs***
*Latvia University of Agriculture*
*lienapoisa@inbox.lv*

## Abstract

Oil-flax has a wide range of utilization possibilities, inclusive of bioenergy production. In our research evaluated out two oil-flax varieties 'Scorpion' and 'Flanders' after the calorific value, oil, lignin, and ash content; and also the chemical composition in 2008 -2010. The results show that oil-flax shoves have a high calorific value and high lignin content but a low temperature for ash fusion. It was established that the growth year and some unexplored factors have influence on the ash content. The oil content in the seeds was 336-458 g $kg^{-1}$, depending on the chosen variety. The flax shoves can be used as a supplement for biofuel combustion, as they have a low fusion temperature, a high calorific value, but a comparatively low yield makes oil-flax unprofitable to grow for biofuel production.
**Key words**: *Linum usitatissimum* L., shove, lignin, ash, oil, calorific value.

## Introduction

Classification of energy crops – dedicated energy crops - can be divided into three subgroups based on the utilization of the plant materials in the conversion process to bioenergy/biofuel: 1) sources of sugar and starches (non-structural carbohydrates); 2) ligno-cellulosic feedstock; and 3) sources of vegetable oils (Biomass...., n.y.). Oil-flax is an annual plant that requires more warmth but less moisture in comparison to fibre flax. From the oil-flax it is possible to obtain: technical fibre, shoves, oil cake, and straw. All parts of oil-flax can be used (Ivanovs and Stramkale, 2001; Stramkale et al., 2008; Груздевене et al., 2009).

The flax shoves are considered as waste products, which are left behind after the cellulose fibre is separated from the flax stems. The shove yield is 2.5 tonnes to one tonne of fibre produced (Cox et al., 1999).

In the world, oil-flax has been studied as one of the energetic plants, and it has been found that other crops are the most promising (Biomass...., n.y.). Studies in Latvia have not been conducted on the possibilities of using linseed biofuel production, although there are studies on oil content and oil composition.

The aim of the research is to evaluate the utilization of oil-flax for bioenergy production.

## Materials and Methods

Annual crop – oil-flax (*Linum usitatissimum* L.) from *Linacea* family – was tested in the Latvia and under the conditions described in Table 1. The trial was carried out in Agricultural Science Centre of Latgale in 2008-2010. The nitrogen supplementary fertilizer and agrochemicals were given to the oil-flax in the fir-tree phase (Growth Stages (GS) 4), and insecticide – at GS 1 and GS 4.

Table 1

**Oil-flax trial methods**

| Parameters | | Trial year | | |
|---|---|---|---|---|
| | | 2008 | 2009 | 2010 |
| Soil type | | Humi-podzolic gley soil | | |
| Soil composition | pH | 7.3 | | 7.0 |
| | OM, % | 3.8 (Tyrin's method) | | 6.5 (Tyrin's method) |
| | P, mg $kg^{-1}$ | 36 (DL method) | | 63 (DL method) |
| | K, mg $kg^{-1}$ | 54 (DL method) | | 98 (DL method) |
| Pre-crops | | Spring rape | | Winter wheat |
| Complex fertilizers | N:P:K, kg $ha^{-1}$ | 6:11.3:24.9, total 300 (N=29.03; P=54.9; K=120.5) | | |
| Sowing time | | 9th May | 4th May | 6th May |
| Sowing rate | kg $ha^{-1}$ | 70 | | |
| Varieties | | 'Scorpion' | 'Scorpion', 'Flanders' | |
| N fertilizer rate | kg $ha^{-1}$ | N0, N60, N80, N100 | | |
| Harvesting time | | 23rd September | 21st September | 10th August |
| Trial plots | $m^2$ | 7.5 | | |
| Replication | | 4 | | |
| Agro-chemicals | Insecticide | Fastaks 50 e.c. (alfa- cipermetrin, 50 g $L^{-1}$) 0.3 L $ha^{-1}$ | | |
| | Herbicide | Glins 75 d.g. (hlorsulfuron, 750 g $kg^{-1}$) 10 g $ha^{-1}$ | | |
| | | Lontrels 300 s.c. (clopiralid, 300 g $L^{-1}$) 0.3 L $ha^{-1}$ | | MCPA Super 500 s.c. (MCPA, 500 g $L^{-1}$) 1.0 L $ha^{-1}$ |

The flax samples were harvested by hand in the growth stage of the early yellow ripeness. The plants were tied up in bundles and left in the field for 5-8 days. When flax was dry, it was crushed with the machine Eddi, and after that the pods were cleaned through a sieve. The seeds were cleaned with a sample cleaner MLN, weighed (accuracy ± 0.001 g) and the seed yield was established taking 100% purity and 9% moisture content.

The sample of 10 g of stems was weighed (accuracy ± 0.0001 g) then scutched with the tool ЛМ-3, broken and shaken until the shoves were withdrawn and weighed again. The result was calculated by the formulae (Freimanis et al., 1980). Five percent may be dust etc., these substances were eliminated from the shove content (formulae 2).

$$C = 100\frac{S}{L} \quad (1)$$

where C – fibre content, %; S – strow mass, g;
L – fibre mass, g.

$$K = 95\text{-}C \quad (2)$$

where K – shove content, %; C – fibre content, %.

The meteorological conditions for the growth period of oil-flax are shown in Figure 1. The weather conditions during the trial years were different. In 2008, in Latvia the air temperature in the 3rd ten-day period of May was close to the long-term average, but there was no rain. Also in June and July there was no precipitation. In July the average daily temperature corresponded to the long-term average, and the amount of precipitation was abundant. The amount of precipitation in the 1st and 3rd ten-day period of July was only 40% of the long-term average, but in the second ten-day period it was 173% of the long-term average. In the first ten-day period of August, the daily temperature was 15.8 °C, close to the long-term average, but the rainfall was 86% of the long-term average. The weather conditions during trial years were different. In May 2009, the temperature was the same as the long-term average, but the amount of precipitation was only 32% of the long-term average. There was rain in June and July. The amount of precipitation in August was only 22%, and in September – 52% of the long-term average. In July and August 2010, the amount of precipitation was only 30% of the long-term average, but temperature was about 3.8-4.8 °C higher than the long-term average.

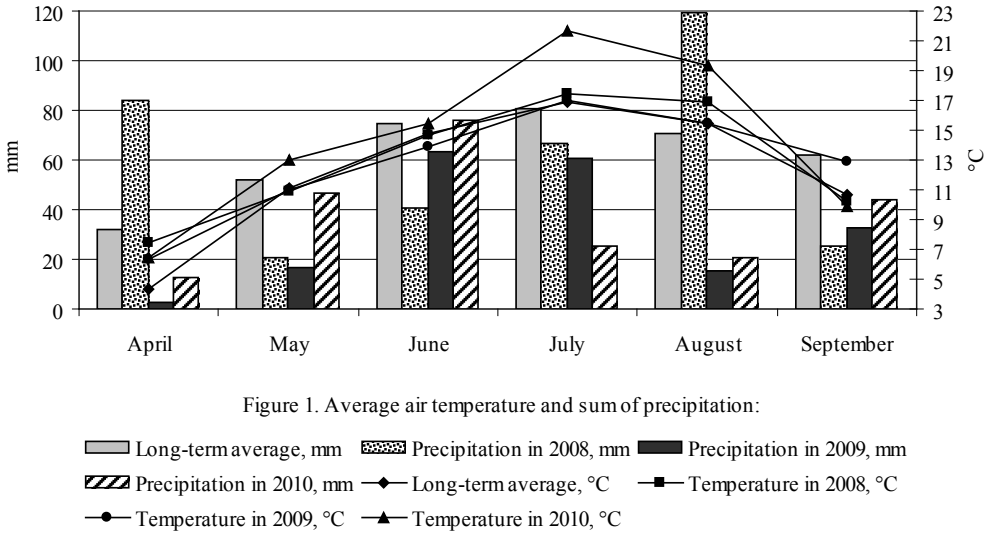



Figure 1. Average air temperature and sum of precipitation:

The following parameters were tested: 1) moisture content in shoves, according to standard ISO 589-81; 2) ash content for dry material, according to standard ISO 1171-81; 3) gross calorific value ($Q_{gr.d}$) with V (volume)=constant for dried fuel at 105 ºC, according to standard LVS CEN/TS 14918; 4) net calorific ($Q_{net}$) value with V=constant, according to standard LVS CEN/TS 14918; 5) ash behaviour when melting in an oxidising atmosphere, according to standard ISO 540; 6) potassium (K), calcium (Ca), sodium (Na) and silicon (Si) concentrations in mineralized samples in shoves were determined with the inductively coupled plasma optical emission spectrometer Perkin Elmer Optima 2100 DV with concentrated nitric acid; 7) oil content in the seed samples was determined by the grain analyser Infratec 1241tm, which has a specially adapted system, built-in for the analysis of oil content for flax and hemp; 8) determination of lignin of shoves was performed using Clason's method published by Ģ. Zaķis. Clason-lignin is cleaned with 72% sulfuric acid (Zaķis, 2008).

The MS Excel programme was used for data statistical processing. The ANOVA method and correlation and regression analysis were used. The test of statistically significant differences (LSD 0.05) with the Fisher criterion (F-test) and factor density influence was used for the analysis of mean differences.

**Results and Discussion**

Previous research in Latvia shows that agrometeorological conditions for the 2008 and 2009 growth period did not influence the oil content for the variety 'Scorpion' (Poiša et al., 2010). The air temperature in the 2010 growth period was higher than the long-term average (Fig. 1) the result of which was that the oil content was only 84% of the previous year result (Table 2). The variety's or genotype's, as a factor, influence was essential ($p<0.05$) for the oil content in the flax seeds. The average oil content in the seeds was 415 g $kg^{-1}$, which corresponds to the data in the literature on the subject (Ivanovs and Stramkale, 2001; Ульянчик and Лукашик, 2008). For the variety 'Scorpion' the oil content was within the range of 33.6-43.8%, and for the variety 'Flanders' the range was 449-458 g $kg^{-1}$. The standard error for the oil content of variety 'Scorpion' was greater than for 'Flanders', which means that the oil content in the seeds of variety 'Scorpion' has a greater variation than that in variety 'Flanders'.

Table 2

**Oil content in oil-flax seed DM, g $kg^{-1}$**

| Variety | Year | | N fertilizer rate, kg $ha^{-1}$ | | | |
|---|---|---|---|---|---|---|
| | | | N0 | N60 | N80 | N100 |
| Scorpion | 2008 | Average ± standard error | 438±7.1 | 416±7.2 | 397±10.5 | 383±8.3 |
| | | min | 427 | 413 | 361 | 351 |
| | | max | 454 | 434 | 432 | 416 |
| | 2009 | Average ± standard error | 425±8.8 | 416±8.4 | 408±7.4 | 398±10.1 |
| | | min | 395 | 391 | 384 | 352 |
| | | max | 448 | 447 | 425 | 439 |
| | 2010 | Average ± standard error | 358±9.8 | 351±9.5 | 342±4.9 | 336±7.8 |
| | | min | 339 | 332 | 334 | 325 |
| | | max | 379 | 366 | 350 | 347 |
| Flanders | 2009 | Average ± standard error | 449±6.5 | 453±7.2 | 456±6.6 | 458±7.9 |
| | | min | 435 | 447 | 451 | 449 |
| | | max | 457 | 465 | 463 | 474 |
| | 2010 | Average ± standard error | 453±1.2 | 453±1.7 | 452±1.4 | 451±1.9 |
| | | min | 451 | 450 | 450 | 448 |
| | | max | 455 | 455 | 455 | 455 |

For the variety 'Scorpion', the seed yield increased from 0.09 to 0.59 t $ha^{-1}$ when N fertilizer was used, and the flax straw yield increased from 0.24 to 0.71 t $ha^{-1}$ in 2008 (Fig. 2). As the air temperature was higher in 2010 and there was a precipitation deficit in the second half of the summer (Fig. 1), the flax ripened earlier, which in its turn reduced the yield. Extracted oil varied from 0.36 to 0.88 t $ha^{-1}$, which is comparatively low, as other oil plants have a higher oil yield. Therefore it is better not to use oil-flax as a biofuel, as it can be better utilized elsewhere.

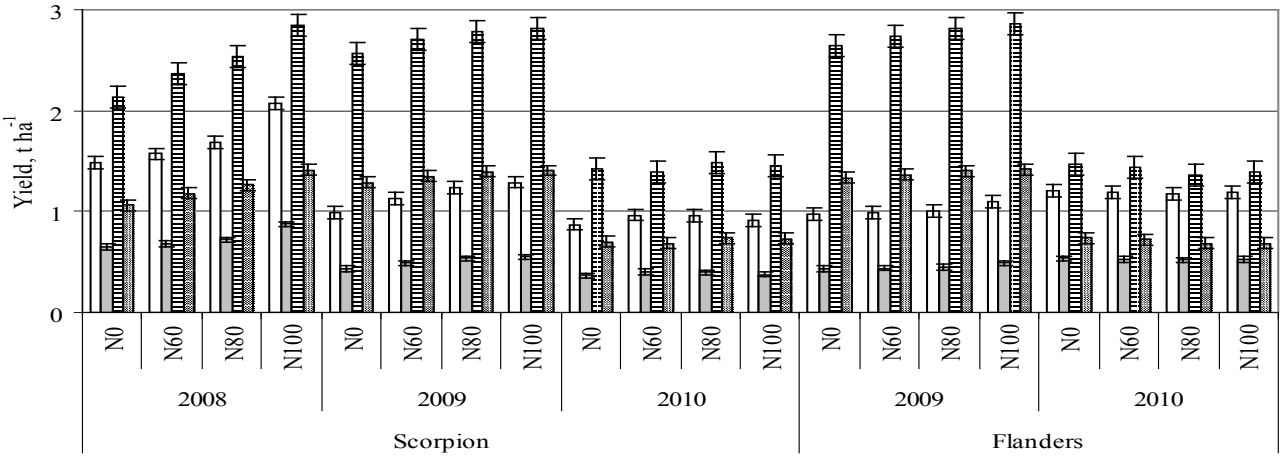



Figure 2. Oil-flax yield depending on the oil-flax variety, growing year, and N fertilizer rate, where □ Seed yield, ▩ Oil yield, ▤ DM yield, ▨ Shove yield.

Ash represents the mineral content of biomass that depends on the soil and environmental conditions. In general, the ash content of crop materials biomass is significantly higher than that of wood (Volynets and Dahman, 2011).

The ash content in the flax shoves is from 15 g $kg^{-1}$ (Ross and Mazza, 2010; Tamaki and Mazza, 2010). Our research shows that ash content in the shoves varied from 12.7 g $kg^{-1}$ to 36.0 g $kg^{-1}$ (Fig. 3). Which was significantly influenced ($p<0.05$) by the growth year ($\eta=82\%$), and by the interaction between the growth year, the variety, and the small N fertilizer rate ($\eta=1.5\%$).

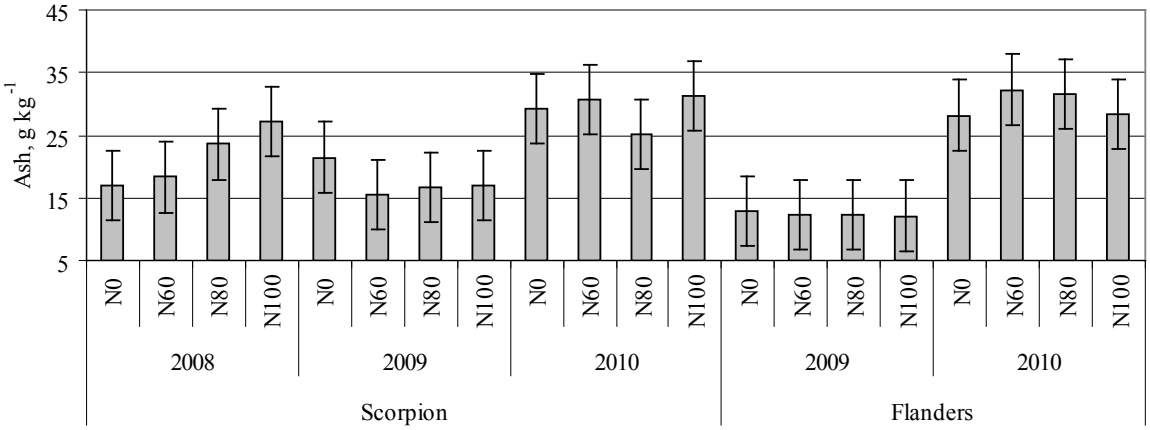



Figure 3. Ash content depending on the oil-flax variety, growing year, and N fertilizer rates.

Biomass ashes have a relatively low fusion temperature the deformation temperature (Dt) is normally in the range from 750 to 1000 °C, in comparison with coal which Dt exceeds 1000 °C; because the ash chemical and mineralogical composition for the coal and biomass is very different (Baxter and Koppejan, 2005). In our research for the oil-flax shoves the deformation temperature was 670 °C, which was not influenced by the growth year, the N supplementary fertilizer, or the chosen variety. The ash sphere temperature formation start was at 780-990 °C. The flax shove ash fusion temperature was much lower than for coal (1400 °C) (Magasiner et al., 2002).

In 2010, the gross calorific value - for the oil-flax shoves was from 18.2 to 19.4 MJ $kg^{-1}$, and the net calorific value from 15.45 to 16.08 MJ $kg^{-1}$. Nevertheless, the large amount of alkaline metals in the biomass, which causes corrosion deposit in the boilers, makes this type of biomass unsuitable for combustion (Wright et al., 2000; Volynets and Dahman, 2011).

A significant ($p<0.05$) close linear negative correlation was observed between Ca (x) and K content in the oil-flax shoves (y) ($r=0.771$; $n=10$), and the relationship is reflected in the regression equation $y = -0.60x + 10.92$; $R^2=0.59$.

The variety, the conditions of growth year, and the N supplementary fertilizer rate significantly ($p<0.05$) influenced the amount of chemical elements Ca, K, Na, Si - in the oil-flax shoves (Fig. 4 - 5). The higher level of K, Ca, Mg and Na in the biomass can be explained by the use of pesticides and fertilizers (Wright et al., 2000).

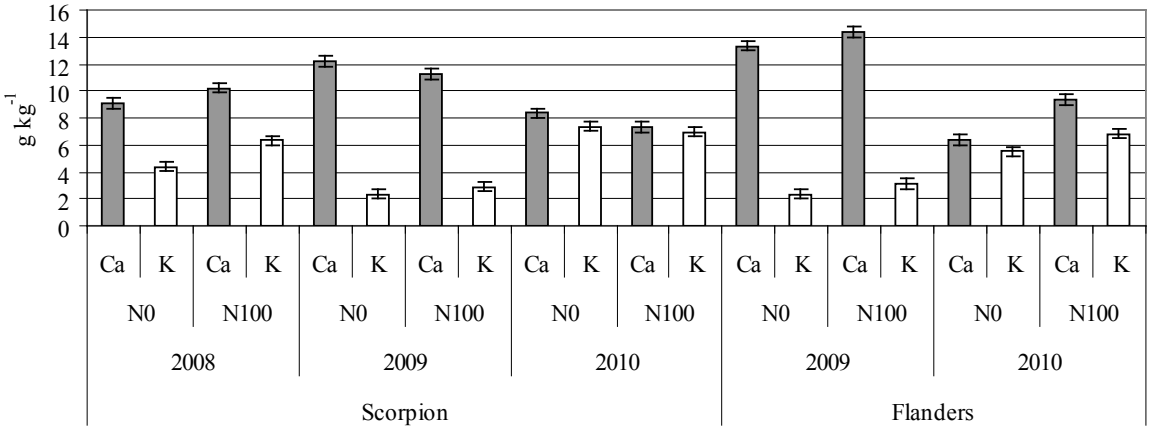



Figure 4. Potassium (K), and calcium (Ca) amount in oil-flax shoves depending on the oil-flax variety, growing year, and N fertilizer rates.

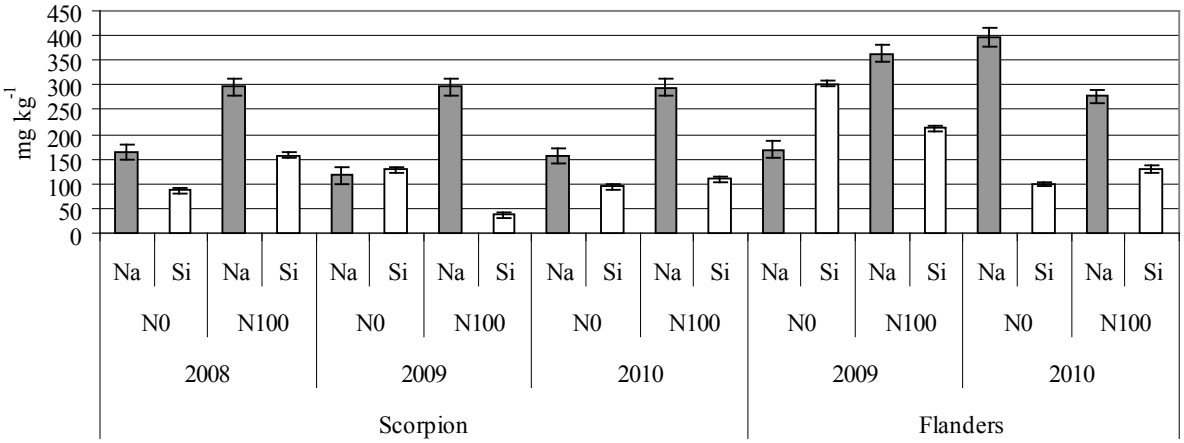



Figure 5. Sodium (Na) and silicon (Si) amount in oil-flax shoves depending on the oil-flax variety, growing year, and N fertilizer rates.

The high lignin content is detrimental for the quality requirements of textiles, cellulose and paper products, as lignin has a dark colouring, and during bleaching it tends to darken as autoxidation and /or photo oxidation tends to age it (Ross and Mazza 2010). The flax shoves have a typically high lignin content (230-310 g $kg^{-1}$), and high also cellulose (530 g $kg^{-1}$) and hemicellulose (240 g $kg^{-1}$) content (Tamaki and Mazza, 2010; Ross and Mazza, 2010.)

In our research, the lignin content in the oil-flax shoves was as follows: for 'Flanders' from 222 g $kg^{-1}$ to 256 g $kg^{-1}$, and for 'Scorpion' from 245 g $kg^{-1}$ to 271 g $kg^{-1}$. It was not influenced by the rate of N supplementary fertilizer.

The flax shoves can be used as a supplement for biofuel combustion, as they have a low fusion temperature, and a high calorific value. But a comparatively low yield makes oil-flax unprofitable to grow for biofuel production.

## Conclusions

1. The variety's or genotype's, as a factor, influence is significant ($p<0.05$) on the oil content of the oil-flax seeds. The variety 'Scorpion' had an oil content within the range of 336-438 g $kg^{-1}$, and the variety 'Flanders' - 449 to 458 g $kg^{-1}$. The oil yield was from 0.36 to 0.88 t $ha^{-1}$.
2. The ash content in the shoves varied from 12.7% to 360 g $kg^{-1}$, and was influenced by the conditions of the growth year.
3. For the oil-flax shoves the ash deformation temperature was 670 °C, the start of the sphere formation temperature was at 780-990 °C, which is a lot less than for coal (1400 °C).
4. In 2010, the gross calorific value ranged from 18.2 to19.4 MJ $kg^{-1}$ for the shoves, and the net calorific value - from 15.45 to 16.08 MJ $kg^{-1}$.
5. The lignin content for the variety 'Flanders' was from 222 g $kg^{-1}$ to 256 g $kg^{-1}$, and for the variety 'Scorpion' from 245 g $kg^{-1}$ to 271 g $kg^{-1}$. As the shoves are a waste by-product, they can be a good supplement for the manufacture of biofuel.

## Acknowledgements

The study was supported by ESF Project 2009/0225/1DP/1.1.1.2.0/09/APIA/VIAA/129. The authors would like to thank the Agricultural Science Centre of Latgale for oil-flax installation assistance, the of the Chemistry Laboratory of the Rēzekne Higher Education Institution and assistants S. Augule for her help with the metal analysis. L. Poiša would also like to thank the Vītols Fund and the LAB-AN (Latvian Agronomic Society – Foreign Department) for granting her a bursary.

## References

1. Baxter L., Koppejan J. (2005) Biomass-coal co-combustion: opportunity for affordable renewable energy. *Fuel*, 84, pp. 1295-1302.
2. Biomass Energy (n.y.) Available at: http://www.seco.cpa.state.tx.us/publications/renewenergy/biomassenergy.ph, 25 January 2011.
3. Freimanis P., Holms I., Jurševskis L., Lauva J., Ruža A. (1980) *Augkopības praktikums* (Practicum of crop production). Rīga, Zvaigzne, 326 lpp. (in Latvian).
4. Ivanovs S., Stramkale V. (2001) *Linu audzēšanas un novākšanas tehnoloģijas* (Technology of flax growing and harvesting). LLU Ulbrokas Zinātnes centrs, 191 lpp. (in Latvian).
5. Magasiner N., van Alphen M., Inkson M., Misplon B. (2002) Characterising Fuels For Biomass – Coal Fired Cogeneration. *International Sugar Journal*, 104, No. 1242, pp. 251-267.
6. Poiša L., Adamovičs A., Stramkale V., Komlajeva L. (2010) Eļļas lini atjaunojamās enerģijas iegūšanai (Oil-flax for renewable energy). *Latgale National Economy research*, *Journal of Social Sciences*. No. 1 (2), 290-300. lpp. (in Latvian).

7. Ross K., Mazza G. (2010) Characteristics of Lignin from Flax Shoves as Affected by Extraction Conditions. *International Journal of Molecular Sciences*, 11, pp. 4035-4050.
8. Stramkale V., Poiša L., Vikmane M., Stramkalis A., Komlajeva Ļ. (2008) Eļļas linu audzēšana un izmantošanas iespējas Latvijā (Oil-flax breeding and application opportunity in Latvia). *Opportunities and challenges of national economic development*: Proceedings of the International Conference, April 17, 2008, Rēzekne, 412-420. lpp. (in Latvian).
9. Tamaki Y., Mazza G. (2010) Measurement of structural carbohydrates, and micro-components of straw and shoves: Effects of extractives, particle size and crop species. *Industrial Crops and Products*, 31, pp. 534-541.
10. Volynets B., Dahman Y. (2011) Assessment of pretreatments and enzymatic hydrolysis of wheat straw as a sugar source for bioprocess industry. *International Journal of Energy and Environment*, 2, pp. 727-446.
11. Zaķis Ģ. (2008) *Koksnes ķīmijas pamati* (Wood Chemistry Basics). LV Koksnes ķīmijas institūts, Rīga, 199 lpp. (in Latvian).
12. Wright I.G., Leyens C., Pint B.A. (2000) *An Analysis of the potential for deposition, erosion, or corrosion in gas turbines fueled by the products of biomass gasification or combustion*, ASME Paper No. 2000-GT-0019.
13. Груздевене Э., Янкаускиене З., Манкевичене А. (2009) Влияние условий окружающей среды и генотипа на урожай и качество семян льна масличного (Influence of environmental conditions and genotype on the oil-flax yield and seed quality). *Enviroment. Technology. Resources*: Proceedings of the 7th International Scientific and Practical Conference, June 25-27, 2009, Vol. 1, Rezekne, c. 154-159. (in Russian).
14. Ульянчик В.И., Лукашик Г.Н. (2008) Влияние ростовых веществ и микроудобрений на продуктивность и качество льна масличного. *Льноводство: реалии и перспективы*: сборник научных материалов международной научно-практической конференции на РУП „Институт льна" 25-27 июня 2008 года. Могилев: Могилевская областная укрепнная типография имени Спиридона Соболя, с. 184-190. (in Russian).

# EVALUATION OF SOME QUALITATIVE CHARACTERISTICS OF NEW PLUM CULTIVARS

***Ilze Grāvīte[1,2], Edīte Kaufmane[2], Mintauts Āboliņš[1]***
*[1]Latvia University of Agriculture*
*[2]Latvia State Institute of Fruit Growing*
*ilze.gravite@lvai.lv*

**Abstract**

A study was done at the Latvia State Institute of Fruit-Growing, evaluating new domestic plum (*Prunus domestica* L.) selections, for which in 2008 -2010 some fruit quality characteristics were studied.

In result of evaluation four new cultivars were selected from the breeding material and in 2010 handed in for cultivar registration in Latvia. Cultivar 'Ance' is early ripening one month before 'Victoria'. Cultivar 'Adele' is medium ripening one week before 'Victoria'. Fruits keep well in cool storage. Cultivar 'Sonora' is medium ripening one week after 'Victoria' and self-fertile. Fruits of this cultivar keep well in cool storage, too. Cultivar 'Lotte' is medium-late ripening two weeks later than 'Victoria', and is partially self-fertile.

Average of three years, the highest soluble solids content was found for cultivar 'Adele' (13.28 Brix%), but cultivar 'Sonora' had the total content of acids (1.28 g 100 $g^{-1}$).

Significant changes in flesh firmness were observed at different storage times for cultivars 'Ance' and 'Lotte'. Significant variations in the total content of acids were determined for 'Ance'. Whereas cultivar 'Sonora' demonstrated substantial fluctuations in soluble solids content.

Correlations between firmness and soluble solids content were observed for cultivars 'Ance' (r=-0.731), 'Adele' (r=-0.436) and 'Sonora' (r=0.526). Cultivar 'Lotte' produced correlations between firmness and total content of acids (r=-0.536).

Significance of interaction was determined within cultivars, years, and years × cultivars.

**Key words**: *Prunus domestica* L., flesh firmness, total content of acids, soluble solids.

## Introduction

Currently in the world the plum crop is about 10 million tons. However, over the last ten years *Prunus domestica* L. plantations in many parts of Europe fell sharply on account of virus disease PPV (*Plum Pox Virus*), and only in recent years after introduction of virus resistant cultivars the area again renewed (Ramming and Cociu, 1990; Hartmann et al., 2010). The harvest period of plums is from June to October. Plum fruits have rather rich biochemical content. By virtue of the natural fibre, especially valuable is their ability to cleanse the intestinal tract, improving digestion and peristalsis, which is the defense against arteriosclerosis, rheumatism and gout, as well as helps reduce blood cholesterol levels. Contributing to the excess of sodium salts and water clearance from the body, plums are a good tool for hypertension and the cardiovascular diseases, as well as to renal insufficiency. In the morning on an empty stomach to drink prune juice perfectly cleanses the body. Most of fresh fruit is water on average 84%, while dry matter is 16%, most of which are carbohydrates (Herrmann, 2001).

Plum biochemical composition is variable and differs between cultivars grown in Latvia and, for example, Germany. It depends on the solar intensity and day length. Of carbohydrates, plums mainly contain disaccharides (sucrose, which splits to monosaccharides as fructose and glucose) and polysaccharides (starch, cellulose, pectins). Pectins are partially soluble in water, but in concentrated sugar solution form a gel. Latvian-growing cultivars contain glucose, fructose and sucrose in relatively uniform quantities (2.7:2.1:2.8 g 100 $g^{-1}$ of edible part) (Kaufmane et al., 2007), whereas in Germany K. Herrmann observed much higher levels of sucrose (on average, 7.3 g 100 $g^{-1}$ of edible part), and less glucose (mean 2.3 g 100 $g^{-1}$ of edible part) and fructose (0.94 g 100 $g^{-1}$ of edible part). Most of the minerals found in plums are potassium (after German data - up to 300 mg 100 $g^{-1}$ of edible part) and phosphorus (up to 26 mg 100 $g^{-1}$ of edible part) (Herrmann, 2001). Plums also contain significant quantities of minerals Na, Mg, Ca, Fe, Cu, Cl, J, S, etc. (Кретович, 1986). In Russia (Moscow region), solids and total content of acids in local cultivars have been studied. On average, solids were 6.3 – 10.7 Brix%, emphasizing the importance of climatic conditions and soil properties. Sugar content in Russian cultivars was similar to that in Latvian cultivars, i.e. 2.5 – 9.5 g 100 $g^{-1}$ of glucose; 1.0 – 4.5 g 100 $g^{-1}$ of fructose and 0.6 – 7.0 g 100 $g^{-1}$ sucrose. The total content of acids depended on the cultivar, and on average was from 0.16 to 1.32 g 100 $g^{-1}$ (Анзин и др., 1956)

Organic acid content in fruits depends on the characteristics of the cultivar. Fluctuations in acid composition from 0.4 to 3.5 g 100 $g^{-1}$ are mainly determined by climatic conditions and plant health. Russian researchers have looked for a relationship how the cultivar of pollination affects fruit quality (Кондратьев и др., 1971)

Vitamin C in Latvian plums maker up to 9.5 mg 100 $g^{-1}$, in German plums – up to 14 mg 100 $g^{-1}$ of edible part. Plums contain quite a lot of vitamins - $B_1$, $B_2$, $B_6$, A, E and PP. Aroma compounds of plums are few and last for

a very short time. More aroma is found in acidic fruits of plums and prunes, especially in those which grow further north – if plums receive intense heat and solar light, they are sweeter, but less aromatic. Vitamin content in fruits strongly depends on the cultivar, the growing conditions, and the fruit maturity (if a plum is completely mellow, vitamin C is very low) (Kaufmane et al., 2007; Herrmann, 2001).

In the process of fruit ripening, the enzymes react with their substrates: starch is split down to glucose, which partially isomerizes into fructose, cellulose and pectin partially pull down, and the fruit becomes soft; chlorophyllases pull down chlorophyll, and immediately afterwards carotene and xanthophylls form the yellow ground colour. Later develop anthocyanins, which generate red colours in fruits (Baltess, 1998).

The aim of this study was to introduce with modifications of fruits' quality characteristics during storage of new Latvian cultivars.

## Materials and Methods

The study was carried out at the Latvia State Institute of Fruit-Growing in Dobele, geographical position: East longitude 23°17.888`; North latitude 56°36.6333`. The plum trial was established in 1998 – 1999. The soil in the trial was sod-podzolic and carbonate with sandy clay loam, with organic matter content - 2.7%, soil reaction was slightly acidic (pH KCl – 6.1), with an average to low P content (96.4 mg $kg^{-1}$), average K content (278.1 mg $kg^{-1}$) and low Mg content (274 mg $kg^{-1}$). Soil analysis was conducted in 2010 by, Agrochemical Research Centre, Ltd. The trial was carried out with four perspective cultivars 'Ance', 'Adele', 'Sonora' and 'Lotte'. Three trees per each cultivar grafted on seedlings of *Prunus cerasifera* were planted at distance of 3 × 5 m.

The study was evaluating new domestic plum (*Prunus domestica* L.) selections, for which some fruit quality characteristics were studied in 2008 – 2010.

The study included measurements of some qualitative characteristics of the fruits immediately after harvesting, after one week, and after two weeks of storage in refrigerator (storage temperature + 3 ± 1 ºC, humidity 89 ± 2% RH). A total of 60 fruits were harvested at random at one time. Each measurement of week was carried out in 20 replications. Analysis included:

- the total content of acids (g 100 $g^{-1}$) (further in text TA) was determined by titrating with 0.1 N NaOH (Khan et al., 2008);
- firmness (kg $cm^{-2}$) of fruit – with a digital penetrometer (instrument error ± 0.01 g $cm^{-2}$) following the standard BS EN 12143 (July, 2001);
- soluble solids content (Brix%) of fresh fruits (further in text SSC) (ISO 2173: 2003) – at 20 °C with a digital refractometer ATAGO N20 (instrument error ± 0.01 Brix%) according to standard BS EN 12147 (July, 2001).

Estimation of the connection between the observed characteristics was done using analysis of variance (significance level $\alpha$=0.05). Features of bilateral impact significance were determined by Pearson correlation. Differences between cultivars and replicate measurements were compared using descriptive statistics, and additional analysis of variance was done using Tukey test by which it is possible to group the results into significantly different groups, as designated by small letters of the alphabet $^{abcd}$, in which $^{a}$ always means lowest value.

## Results and Discussion

The requirements of fruit market are targeted mainly at a few individual characteristics: fruit colour (in Latvian market plums with yellow fruits have an especially good demand, unlike rest of Europe where the preferred are blue plums (prunes)), taste of fruit, and stone adherence from fruit flesh. For new plum cultivars, an essential trait is potentially higher fruit firmness that increases transportability and ensures longer consumption time. An assessment of disease resistance, tree vegetative characteristics, and fruit organoleptic rating (1 – 5 scale) were done before a qualitative evaluation of plum trial.

'Ance' – a cultivar of very early ripening time (first week of August). Fruits ripen about a month before 'Victoria' (a very popular cultivar grown in many orchards of the world). Self-sterile. The flavour varies between years, from good to very good. Fruit skin is thin, yellow with reddish blush. The suture line is inconspicuous. The stone is easily separated from flesh. The average yield from the year 2008 to 2010 was 43 kg per tree.

'Adele' – a cultivar of middle ripening time. Fruits ripen about a week before 'Victoria'. Self-sterile. Fruit skin is thin, yellow with reddish over-colour on part of fruit. Stone separation from flesh is good. Over colour is well pronounced, if the tree is trained to provide sunlight access into the canopy. The average yield from the year 2008 to 2010 was 20 kg per tree.

'Sonora' – a cultivar with an average of late ripening time. Fruits ripen about a week after 'Victoria'. Self-fertile. Fruitlets are self-thinning. The fruit skin is rather thin, with reddish ground colour and purple bloom. The suture line is semi-pronounced. Flavour is good. Stone separation from flesh at full maturity is good. The fruits are very attractive. The average yield from the year 2008 to 2010 was 25 kg per tree.

'Lotte' – a cultivar with late ripening time. Fruits ripen about two weeks after 'Victoria'. Flavour is very good and notably sweet. Fruit skin is rather thin, purplish blue with grayish blue bloom. Stone separation from flesh is semi good. The average yield from the 2008 to 2010 was 18 kg per tree.

For the first time, some of qualitative characteristics of the new cultivars were analyzed in 2008. Analyses were done for freshly harvested fruits, and then again after one and two weeks (in figures of measurement periods called – first, second, third time) (Fig. 1). In assessing the sustainability of fruit firmness (average of the three-year period) during storage, it was significantly stable for

cultivars `Adele` and` Sonora` – within a week firmness had not changed. Small firmness changes could be observed after two weeks. Significant and rapid changes in firmness occurred in cultivars 'Ance' and 'Lotte', which obviously will need earlier harvesting and marketing time. Although this reduces fruit eating quality, it is excusable for the total harvest time to be prolonged.

If the firmness fluctuations are small, the fruit shelf-life can be long enough. The more rapid are changes in firmness, the shorter the shelf-life period. A pronounced correlation of firmness and SSC was observed in cultivar 'Lotte' for which it is essential to harvest fruit at full maturity, or suffer a loss of taste. As other plum researchers have admitted, this increasingly proves the hypothesis that if determination of maturity of plums is inaccurate, fruit quality decreases and consumers desire to buy products diminishes (Vangdal et al., 2007; Crisosto and Kader, 2000). Long-time studies in Latvia on cultivars from Estonia, Lithuania, Russia and Sweden during 1999-2004 showed that fruit firmness ranged between 1.20 – 2.69 kg $cm^{-2}$ (Kaufmane et al., 2010).

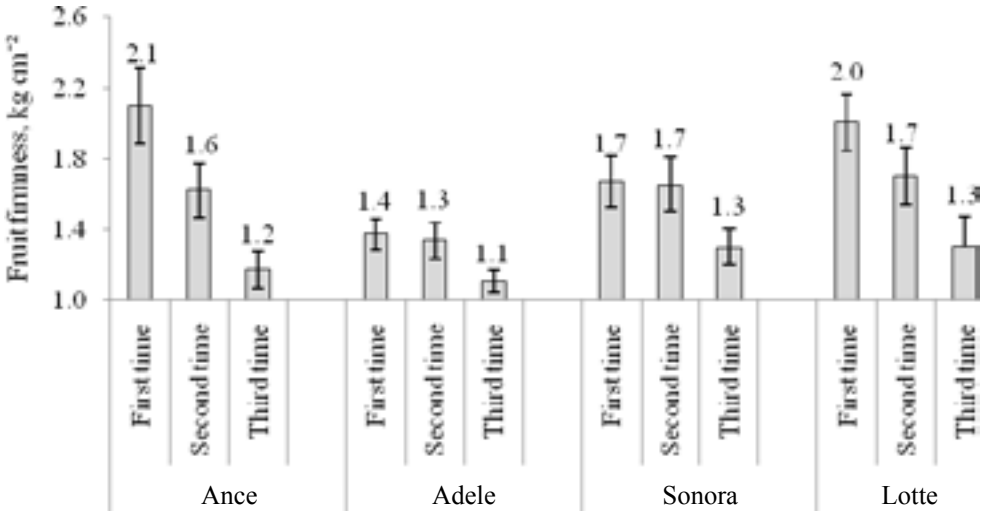



Figure 1. Changes in fruit firmness at different storage times.

During storage there were no significant differences in SSC between periods of measurement (Fig. 2) for cultivars 'Ance' and 'Adele', but during this time they had significant decrease in the TA (Fig. 3). Cultivar 'Sonora' had significant fluctuations in SSC between measurement periods. Currently there is no exact explanation as to why there have been such changes during all three years. To search for an explanation of this, we will continue to extend further studies about reduction of sugar in the period of fruit storage. SSC of the cultivar 'Lotte' changed, but these changes over the years didn't show significant differences.

The average SSC of the studied cultivars was 12.2 Brix%. Average SSC of Latvian growing *Prunus domestica* L. cultivars was found to be 15 – 17 Brix% (Kaufmane et al., 2010). From evaluated cultivars, the highest value of SSC was found in 'Ance' and 'Adele'. SSC of plum fruits determines their sweetness the higher the SSC, the sweeter the fruits.

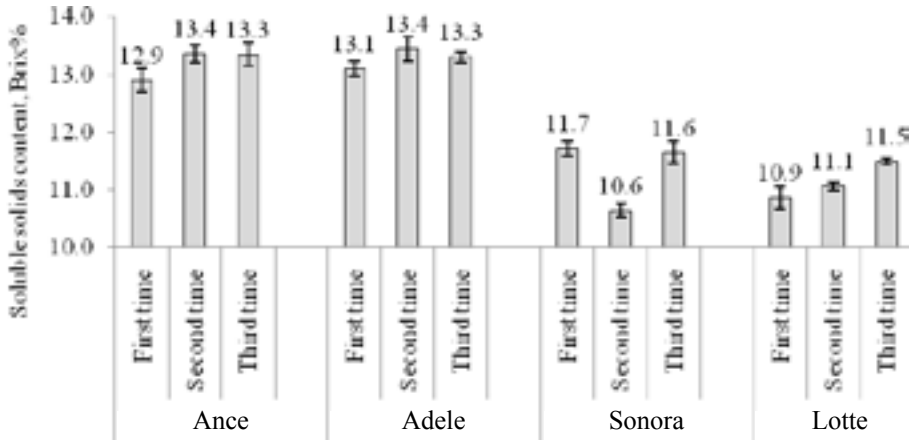



Figure 2. Changes in soluble solids content at different storage times.

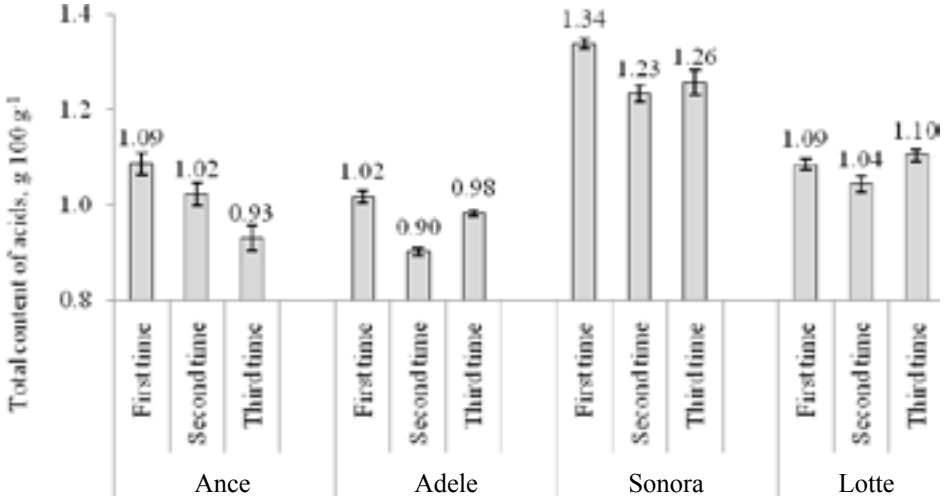



Figure 3. Changes in total content of acids at different storage times.

Evaluating the average difference between cultivars (Table 1), fruit firmness was significantly different for the cultivar 'Adele' it had softer fruit, while the other cultivars over the years didn`t show significant differences. The SSC was similar in cultivars 'Adele' and 'Ance' (on average 13.2 and 13.3 Brix% respectively), which was significantly higher than the other two analyzed cultivars. 'Sonora' had significantly different and higher TA content than other cultivars. The data can be compared with studies conducted in Dobele in 1990s, when SSC of cultivar 'Minjona' was 13.2 Brix%, but of 'Victoria' – 14.9 Brix%, whereas TA content was 1.34 and 1.48 g 100 $g^{-1}$ respectively (Skrīvele et al., 1998). Average TA of other Latvian growing *Prunus domestica* L. research cultivars was found to be 1.15 – 2.11 g 100 $g^{-1}$ (Kaufmane et al., 2010).

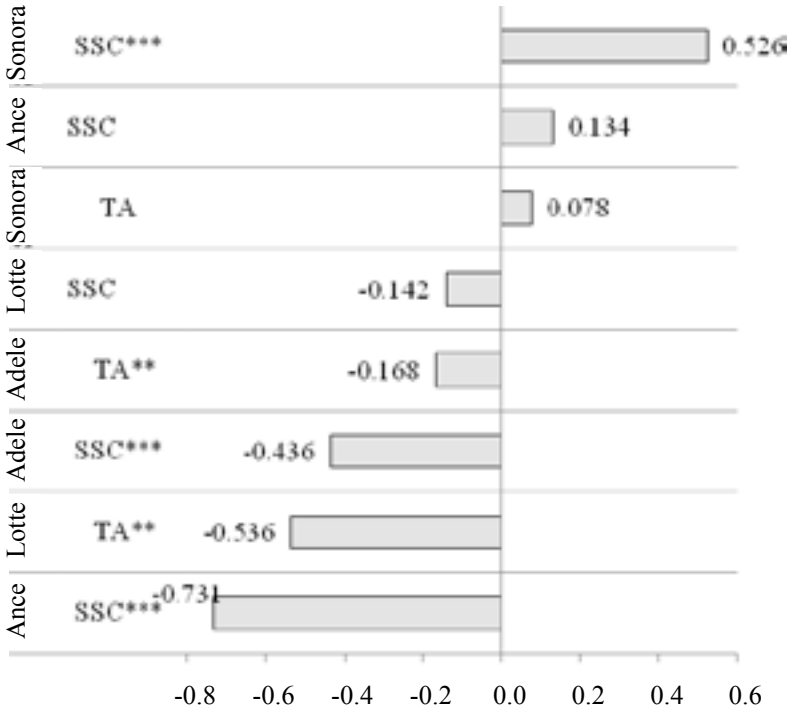
Table 1

**Analysis of mean values of fruit quality characteristics using the Tukey criterion**

| Cultivar | Firmness, kg $cm^{-2}$ | SSC, Brix% | TA, g 100 $g^{-1}$ |
|---|---|---|---|
| 'Adele' | $1.27^{a}$ | $13.28^{c}$ | $0.97^{ab}$ |
| 'Sonora' | $1.54^{b}$ | $11.34^{b}$ | $1.28^{d}$ |
| 'Ance' | $1.63^{b}$ | $13.20^{c}$ | $1.01^{b}$ |
| 'Lotte' | $1.67^{b}$ | $11.14^{a}$ | $1.08^{bc}$ |
| p-value<0.001 | | | |

Cultivar 'Lotte' showed a medium negative correlation (r=-0.536). For these cultivars, as firmness decreased, also the content of TA increased.

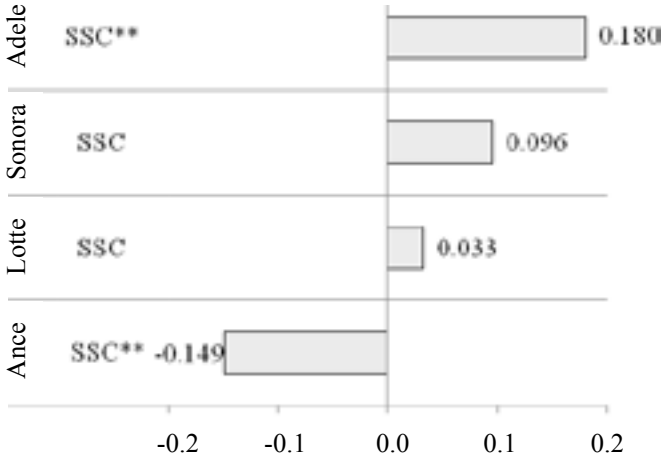
Fruit firmness showed a significant correlation ($p<0.001$) with SSC: for cultivar 'Sonora' – a medium positive correlation (r=0.526), for cultivar 'Adele' - a medium negative correlation (r=-0.436), for cultivar 'Ance' - a negative correlation (r=-0.731).




** p-value < 0.005; *** p-value < 0.001; $r_{yx}$; y – firmness

Figure 4. Correlation between soluble solids content (x), total content of acids (x), and fruit flesh firmness (y).

Cultivar 'Ance' had significant, but weak negative correlations between TA and SSC (Fig. 5) (r=-0.149); ($p<0.05$). Cultivars 'Sonora' and 'Lotte' did not show any significant correlations between TA and SSC.




**- p-value < 0.005; *** p-value < 0.001; $r_{yx}$; y – total content of acids

Figure 5. Correlation between soluble solids content and total content of acids.

After evaluating interactions between factors of research with $p<0.001$, significant effect on biochemical parameters was found for both factors (cultivars and year), which shows heterogeneity of the cultivars. Also for the interaction among these factors the effect was significant.

## Conclusions

1. Fruit quality parameters varied between the years differently for different cultivars. For cultivars whose measurements were fluctuating, the reason could be non-uniform maturity of fruit samples (not for all

cultivars it is easy to visually estimate the same degree of maturity, especially for blue fruits). Results were better for cultivars whose degree of firmness was decreasing slower. The smallest changes in firmness showed cultivars 'Adele' and 'Sonora'. During the study period, these cultivars demonstrated a very good keeping quality, and density changes within two weeks were not significant. Fruits of cultivars 'Ance' and 'Lotte' consumable in shorter period - fruit qualitative characteristics diminished faster.

2. Both cultivars and years and the interaction between these factors showed a significant impact on the qualitative parameters of the new plum cultivars.
3. It is difficult to explain why there were fluctuations during the shelf-life in SSC for cultivar 'Sonora', and in TA for 'Adele'. After the single-factor variance analysis, they were significant: $p<0.05$.
4. Following the test results and their mathematical analysis it can be concluded that the time of fruit harvesting and storage life has very significant impact on plum fruit quality.

**Acknowledgements**

The authors gratefully acknowledge the funding from Latvian Council of Science and the European Union and European Structural Fund (grant No. 2009/0180/1DP/1.1.2.1.2/09/IPIA/VIAA/017 'Support for Doctoral Study Programs' contract No. 04.4-08/EF2.D1).

**References**

1. Baltess V. (1998) *Pārtikas ķīmija* (*Food Chemistry).* Rīga, Latvijas Universitāte, 477 lpp. (in Latvian).
2. Crisosto C.H., Kader A.A. (2000) Plum and Fresh Prune Postharvest Quality Maintenance Guidelines. In: Hui Y.H. (eds) *Handbook of fruits and fruit processing*, Blackwell publishing, pp. 553-564.
3. Hartmann W., Neumüller M., Treutter D. (2010) Breeding for sharka resistance and high Fruit Quality in European plum (*Prunus domestica*) at Weihenstephan: breeding strategy and selection tools. In: Sottile F. (eds) *IX ISHS International Symposium on Plum and Prune genetics, breeding and pomology*, Dipartimento di Colture Aboree, University of Palermo, 20 p.
4. Herrmann K. (2001) *Inhaltsstoffe von Obst und Gemüse.(Ingredients of fruits and vegetables).* Stuttgart (Hohenheim), Ulmer, S. 200. (in German).
5. Kaufmane E., Skrivele M., Rubauskis E., Ikase L. (2007) The yield and fruit quality of two plum cultivars on different rootstocks. *Journal article*. *Sodininkyste ir Darzininkyste*, Lietuvos Sodininkystes ir Darzninkystes Institutas *Scientific works of the Lithuanian Institute of Horticulture*, 26 (3), pp.10-15.
6. Kaufmane E., Ikase L., Seglina D. (2010) Pomological Characteristics of Dessert Plum Cultivars in Latvia. In: Sottile F. (eds) *IX ISHS International Symposium on Plum and Prune genetics, breeding and pomology*, Dipartimento di Colture Aboree, University of Palermo, pp. 337-344.
7. Khan A.S., Singh Z., Abbasi1 N.A., Swinny E.E. (2008) Pre- or post-harvest applications of putrescine and low temperature storage affect fruit ripening and quality of 'Angelino' plum. *Journal of the Science of Food and Agriculture,* 88, pp. 1686-1695.
8. Ramming D.W., Cociu V. (1990) Plums. In: Moore J.N., Ballington J.R. (eds) *Genetic Resources of Temperate Fruit and Nut Crops*. First International Society for Horticulturae Science of Wageningen, the Netherlands, pp. 235-282.
9. Skrīvele M., Kaufmane E., Ikase L. (1998) `Lāse` and `Minjona` - two new, promising Latvian plum varieties. In: Ikase L. (eds) Fruit Growing Today and Tomorrow. *Proceeding of the International Scientific Conference dedicated to the 90th birthday of the breeder A.Spolītis and 100th birthday of the breeder V.Vārpa,* Dobele Horticultural Plant Breeding Experimental Station, Latvia, pp. 42-50.
10. Vangdal E., Flatland S., Nordbø R. (2007) Fruit quality changes during marketing of new plum cultivars (*Prunus domestica* L.). *Horticulturae science* (Prague), 34 (3), pp. 91-95.
11. Анзин Б.Н., Еникеев Х.К., Рожков М.И. (1956) *Слива (Plum)*. Москва, Колос, Russia, 460 с. (in Russian).
12. Кондратьев В.Д., Милованова Л.В., Семенченко С.Н. (1971) *Влияние опылителей на урожай сливы и качество плодов (Effect of pollination on yield and fruit quality of plums*). Кишинев, Бельцкая типография, Russia, 58 с. (in Russian).
13. Кретович В.Л. (1986) *Биохимия растений (Plant biochemistry)*. Москва, Высшая школа, Russia, 503 с. (in Russian).

# INFLUENCE OF ROOTSTOCK ON WINTERING AND HEALTH STATUS OF PLUM CULTIVAR 'VICTORIA'

***Dzintra Dēķena[1,2], Ina Alsiņa[2]***
*[1]Pūre Horticultural Research Centre*
*[2]Latvia University of Agriculture*
*dzintra.dekena@puresdis.lv; ina.alsina@llu.lv*

## Abstract

The choice of rootstock is the main precondition for establishing of high yielding and sustainable plum orchards. Therefore influence of rootstock on the plum cultivars wintering in Latvia climatic conditions becomes more and more actual. Investigation was carried out with the aim to clarify influence of rootstock on the wintering and health status of plum cultivar 'Victoria'. Investigation was carried out at Pūre Horticultural Research Centre during two different wintering seasons of 2008/2009 and 2009/2010, in the orchard planted in 2001. The winter of 2008/2009 was characteristic with sharp temperature fluctuations, but winter of 2009/2010 was snowy with stable low temperatures. Sixteen widely used rootstocks known in Europe were included. Plum general tree health status (scored by 1-5 points) and percentage of dead trees were evaluated. No statistically significant differences among rootstocks were established according to the evaluation of tree health status. Differences in wintering ability among rootstocks were stated between two years of investigation. Trees grafted on Brompton seedlings and St. Julien d`Orleans demonstrated the highest winter hardiness (4.3 points). No one dead tree was registered on these two rootstocks. Rootstock GF8/1 was the least suitable for Pūre conditions – with general health status scored at 2.3 points after severe winter of 2009/2010, and only 25% of alive trees.
**Key words**: *Prunus domestica, Prunus cerasifera,* wintering, tree health status.

## Introduction

The choice of a rootstock is the main precondition for establishing of high yielding and sustainable orchards. The lack of appropriate rootstocks is one of the main reasons limiting development of intensive plum orchards in Latvia. Compatibility of rootstock and cultivar is the main condition influencing tree productivity and yield comprising parameters. A grafted tree is a complex organism consisting of two different genotypes – rootstock and cultivar. Therefore tree life, resistance to unfavourable weather conditions, crown size, precocity, and yielding intensity depend not only on rootstock or cultivar, but also on their interaction (Wertheim, 1998).

Caucasus plum (*Prunus cerasifera* Ehrk) has been the most used rootstock in Latvia during last several decades. However, it does not meet the demands of intensive orchard because of its vigorous habit (Grzyb et al., 1998). It is not well appropriate also for hobby gardens, if they are located in moist areas. As an additional drawback should be mentioned incompatibility of this rootstock with some cultivars. In Pūre Horticultural Research Centre there was observed insufficient health evaluation of trees grafted on Caucasus plum as rootstock (Lepsis et al., 2008).

Myrobolana and dwarfing Wangenheims Zwetche as rootstocks are used in other European countries (Rozpara and Grzyb, 2007). Pixy is investigated as a dwarfing rootstock in intensive orchards in Europe (Sosna, 2002). Also several other plum rootstocks have been included in the investigations in Europe, but there have not been performed investigations on these rootstocks in Latvia till now.

In Latvia cultivars of European plum (*Prunus domestica*) are popular and very broadly grown, therefore the widespread cultivar 'Victoria' belonging to this group was included in the investigation of different rootstocks. The aim of the investigation was to clarify the influence of different rootstocks on the winter hardiness of cultivar `Victoria` in Latvia conditions. Data obtained during two vegetation seasons (2009 and 2010) and consequently after two wintering seasons of 2008/2009 and 2009/ 2010 are discussed.

## Materials and Methods

A plum orchard was established in Pūre Horticultural Research Centre, Latvia, in 2001. In the investigation, cultivar 'Victoria' was grafted on 16 different rootstocks well known in Europe.

Eight vegetatively propagated rootstocks were included in the investigation**:** St. Julien A, Brompton, Ackermann, Pixy, GF8/1, G5/22, GF 655/2, and Hamyra; as well as eight generative propagated rootstocks: St. Julien INRA2, St. Julien d Orleans, St. Julien Noir, Brompton, Wangenheims Zwetche, St. Julien Wädenswill, Myrobolana, and *Pr. Cerasifera* var *divaricata.*

Plants were planted at 3 × 5 m density, in four replications, three trees per plot. Soil was sandy loam on dolomite consisting loamy mother rock, pH KCl 7.2. Content of plant available phosphorus was 183 mg $kg^{-1}$ and potassium – 215.6 mg $kg^{-1}$. Irrigation in orchard was not available. Weeds in spaces between rows were moved, and herbicides were applied in strips to control the weeds.

General health status of trees was scored in the vegetation period of 2008, 2009, and 2010 by the following scale: 0 – tree completely dead, 1 – tree has lost ability to grow, 2 – overground part is completely damaged, but new

shoots are developed, 3 – two and three years old braches and trunks are damaged, 4 – only annual shoots were damaged, 5 – tree in excellent condition. Average yield per tree (kg) was analysed only for data of the year 2008 due to strong regeneration pruning in the spring of 2009, therefore there was no significant yield for the cultivar in 2009. The number of dead trees was registered starting from 2001 till the end of investigation – the year 2010.

Statistical analysis of results was performed by using ANOVA.

Meteorological data were obtained from automatic meteorological station 'Lufft', registering meteorological conditions each 10 minutes. Actual meteorological data were compared to long-term data.

Average air temperature in 2008 – 2010 and long-term temperature are displayed in Figure 1. Years of investigations were characterised by different meteorological conditions. In 2008, relatively sharp temperature fluctuations were registered during the first three months: in January from -14.1 °C to + 7.9 °C, but at the 2nd part of March from - 11.6 °C to + 15.1 °C. Also the January of 2010 was cold, when the 3rd decade was the coldest and the temperature dropped down to - 28.6 °C.

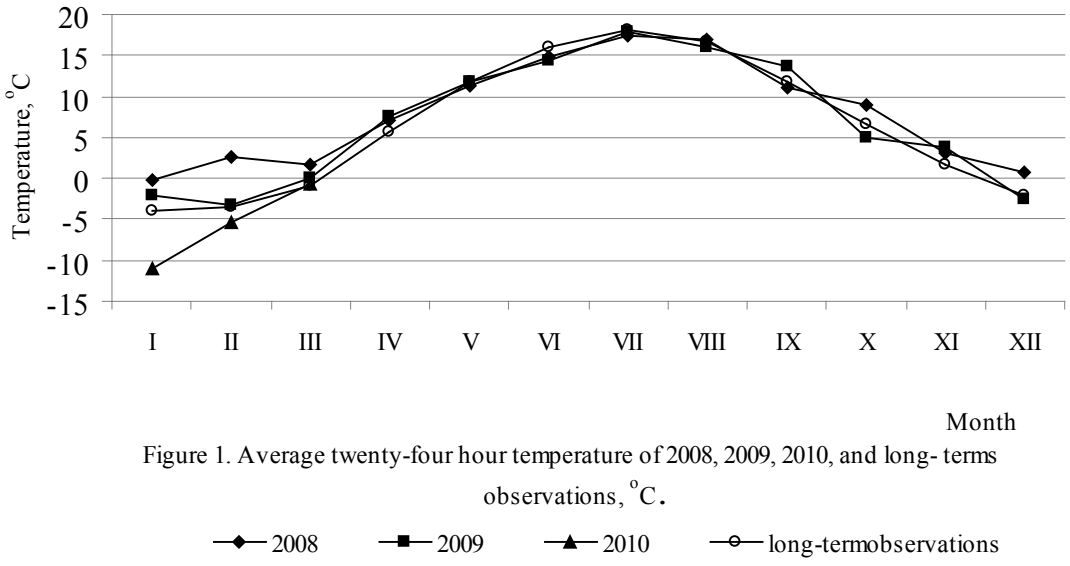



Figure 1. Average twenty-four hour temperature of 2008, 2009, 2010, and long- terms observations, °C.

The average amount of precipitation in 2008 – 2010 and long-term observations are illustrated in Figure 2. The wintering period of 2008 was rich in precipitation. The total amount of precipitation was 426 mm according to observations of the local meteorological station in Pūre. A lot of precipitation was observed also in June and July of 2009 – 81.4 and 107 mm respectively. That is much higher than in long-term observations.

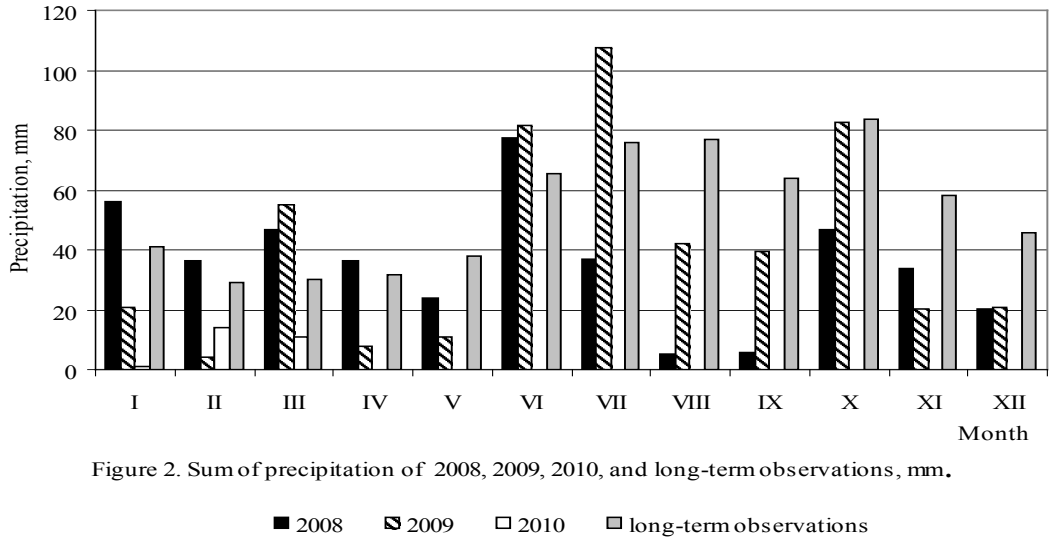



Figure 2. Sum of precipitation of 2008, 2009, 2010, and long-term observations, mm.

**Results and Discussion**

In 2008, relatively sharp temperature fluctuations were registered during the first three months, when in January temperature ranged from - 14.1 °C to + 7.9 °C, but at the 2nd part of March – from - 11.6 °C to + 15.1 °C. Nevertheless temperature fluctuations did not influence wintering of flower buds, plum blossoming was good, and the following yield developed very well. The highest yield in 2008 was registered for trees on the rootstock Hamyra – 60.7 kg per tree (Fig. 3). The yield exceeding 50 kg per tree was obtained from trees grafted on St. Julien d` Orleans, GF 655/2, St. Julien Noir, *Prunus cerasifera*, and Ackermann. The lowest yields were obtained from the trees grafted on G5/22 and Pixy (36.7 and 40.3 kg per tree respectively). Tree health status was evaluated as good because the 1st part of the summer was warm and rich in precipitation.

August was warm, but with insignificant amount of precipitation – 5mm. Also in September was registered low precipitation – only 5.8 mm. It could influence wintering ability of some rootstocks, especially those which are sensitive to insufficient moisture with shallow root system. Also intensity of yielding can influence negatively the wintering ability of trees in the succeeding winter, especially it is characteristic for cultivar `Victoria` (Jänes and Kahu, 2008). In 2009, during wintering period a rapid decrease in air temperature was observed – down to - 21.8 °C, which could negatively influence tree wintering.

This statement partly was approved in the season of 2009. Trees suffered less in 2009 after the relatively mild winter of 2008/2009. The regeneration pruning was performed in spring, which facilitated development of leaf surface during the following summer, and also the yield was not high. Good health status was observed for trees grafted on Wangenheims Zwetche (4.0 points), although in 2008 average yield for trees on this rootstock was 45.1 kg per tree.

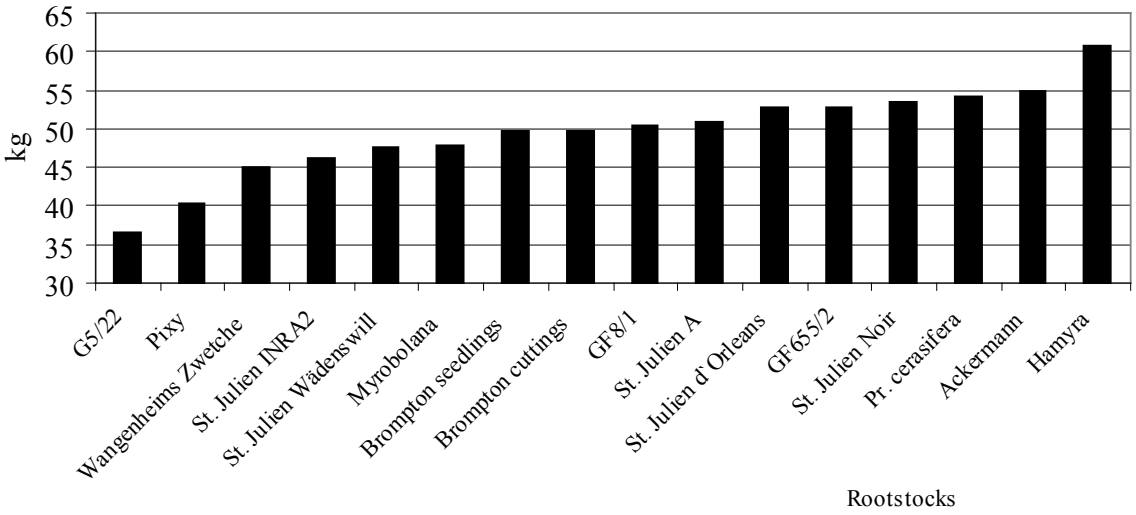



Figure 3. Average yield kg per tree in 2008.

In November of 2009, precipitation was relatively low (20.4 mm) if compared with long-term observations. A rapid decrease in temperature was observed in December (-22.7 °C in the 2nd decade), which could influence plum wintering processes negatively.

Also January of 2010 was cold, and the 3rd decade of the month was the coldest, when temperature dropped to - 28.6 °C. February was relatively cold, with minimal temperature - 21.6 °C, but in the 3rd decade temperature increased to + 3.5 °C causing very sharp temperature fluctuations, which can cause tree damages. April, too, had changeable weather, which could cause mechanical injuries to trees. Nevertheless, tree health status in 2010 was evaluated as relatively good, despite unfavourable wintering conditions. Statistically significant differences among rootstocks were not found according to the evaluation of tree health status (p=0.62).

Rootstocks were evaluated more or less differently for a three-year time period of the investigation according to health status and number of dead trees. In 2010, trees grafted on St. Julien d` Orleans and Brompton seedlings were in the better condition (evaluated at 4.3 points). Also other researchers have reported about good wintering ability of trees grafted on these rootstocks (Lepsis et al., 2008). Comparing the two wintering seasons in Pūre, it is clearly visible that health evaluation of trees grafted on GF 8/1 has decreased with each year – in spring of 2008 – 3.1 points, but in 2010 – only 2.1 points. From the year of planting the orchard, 25% of trees were dead for the cultivar 'Victoria' on this rootstock. However, in Estonia small injuries have been registered for trees on rootstock GF 8/1 (Jänes et al., 2007).

Table 1

**Tree health status (in points) and percentage of dead trees for cultivar 'Victoria'**

| Rootstock | Year | | | Dead trees, % |
|---|---|---|---|---|
| | 2008 | 2009 | 2010 | |
| Ackermann | 3.8 | 4.0 | 3.8 | 0.0 |
| St. Julien INRA2 | 3.8 | 3.7 | 3.5 | 8.3 |
| Brompton seedlings | 4.1 | 4.4 | 4.3 | 0.0 |
| Myrobolan | 3.8 | 3.3 | 3.2 | 16.7 |
| GF8/1 | 3.1 | 2.3 | 2.1 | 25.0 |
| G5/22 | 3.9 | 3.8 | 3.6 | 0.0 |
| St. Julien d`Orleans | 4.2 | 4.4 | 4.3 | 0.0 |
| Brompton cuttings | 3.9 | 3.8 | 3.5 | 16.7 |
| St. Julien Noir | 4.2 | 4.0 | 3.9 | 8.3 |
| St.Julien Wädenswill | 4.2 | 3.6 | 3.4 | 0.0 |
| Wangenheims Zwetche | 4.1 | 4.0 | 4.0 | 0.0 |
| St. Julien A | 3.7 | 3.3 | 3.2 | 16.7 |
| Pixy | 3.7 | 3.5 | 3.2 | 8.3 |
| Hamyra | 4.2 | 3.7 | 3.7 | 8.3 |
| *P. cerasifera var. divaricata* | 3.7 | 3.4 | 3.2 | 8.3 |
| GF655/2 | 3.9 | 3.9 | 3.8 | 0.0 |
| $LSD_{0.05}$ | 0.56 | 0.62 | 0.92 | - |

Different results are also reported for trees grafted on *Prunus cerasifera* which has unsatisfactory wintering results in Pūre. In Estonia its wintering ability is evaluated higher (Jänes et al., 2007), which indicates the possible influence of soil and microclimatic conditions on the wintering ability of trees grafted on *Prunus cerasifera*. The possible cause of such differences can be the relatively long vegetation period of the rootstock, which influences tree wintering ability in the fluctuating meteorological conditions so frequently observed in Latvia.

In the investigations in Poland, widely grown dwarfing rootstock Pixy had average wintering results. It should be taken into account that in the case of sufficient wintering good yields from trees grafted on this rootstock could be obtained by planting trees in increased density (Grzyb and Sitarek, 1998). In addition there is advisable watering system because of shallow root system of the rootstock (Grzyb et al., 1998). Sensitivity to moisture conditions could be the reason for insufficient wintering ability of the rootstock in Pūre in 2008/2009 when low precipitation in August and September of 2008 was observed (Figure 2).

In 2010, 16.7% of dead trees were registered on the vegetatively propagated Brompton, Myrobolan and St. Julien A. Trunk injuries were observed for trees grafted on Brompton vegetatively propagated rootstock after the severe winter of 2009/2010 with sharp temperature fluctuations in March. Good overwintering was observed for trees grafted on GF 655/2. Similar results are reported on this rootstock also in other researches (Lepsis et al., 2008).

Since planting in 2001, no one dead tree was observed in Pūre for cv. 'Victoria' grafted on Ackermann, Brompton seedlings, G5/22, St. Julien d` Orleans, St. Julien Wädenswill, Wangenheims Zwetche, and GF655/2. This indicates the possible compatibility of the cultivar and the rootstock and/or good adaptation to Pūre climatic conditions. Overall, tree winter hardiness evaluations were less satisfactory on all rootstocks included in the investigation after the winter of 2009/2010 with severe temperature conditions.

## Conclusions

1. The highest wintering and better health status were observed for cultivar 'Victoria' grafted on rootstocks St. Julien d`Orleans and Brompton seedlings. This proves good compatibility of the cultivar and the rootstocks and ability to overcome different overwintering conditions.
2. The worse health status and more dead trees were observed for trees grafted on GF8/1, which indicates the unsuitability of the rootstock to the climatic conditions in Pūre.
3. The highest yields in 2008 were obtained from trees grafted on Hamyra rootstock.

## References

1. Grzyb Z.S., Sitarek M. (1998) Growth and cropping of plums grafted on Pixy rootstock and planted in differentiated density. *Acta Horticulturae,* Vol.1, No. 478, pp. 103-106.
2. Grzyb Z.S., Sitarek M., Kozinski B. (1998) Effect of different rootstocks on growth, yield and fruit quality of four plum cultivars (in Central Poland). *Acta Horticulturae,* Vol.1, No. 478, pp. 239-242.
3. Jänes H., Kahu K. (2008) Winter injuries of plum cultivars in winters 2005 – 2007 in Estonia. In**:** Dimza I., Eihe M., Ikase L. et al. (eds) Proceedings of

international scientific conference. *Sustainable fruit growing: from plant to product*. Latvia State Institute of Fruit Growing, Jurmala-Dobele, pp. 149-154.

4. Jänes H., Klaas L., Pae A. (2007) Winter Hardiness of Plum on Different Rootstocks in Winter 2002/2003 in Estonia. *Acta Horticulturae* Vol.1, No. 734, pp. 295-298.
5. Lepsis J., Dēķena Dz., Dēķens V. (2008) Evaluation of European plum rootstocks in Latvia. In**:** Dimza I., Eihe M., Ikase L. et al. (eds) Proceedings of international scientific conference. *Sustainable fruit growing: from plant to product*. Latvia State Institute of Fruit Growing, Jurmala – Dobele, pp. 77-82.
6. Lepsis J., Drudze I., Dekens U. (2004) The evaluation of different plum and pear rootstocks in the nursery. *Acta Horticulturae*, Vol. 1, No. 658, pp. 167-171.
7. Rozpara E., Grzyb Z. (2007) Growth, yield and fruit quality of eighteen plum cultivars grafted on two rootstocks. *Acta Horticulturae*, Vol.1, No. 734, pp. 157-161.
8. Sosna I. (2002) Growth and croping of four plum cultivars on different rootstocks in south western Poland. *Journal of fruit and ornamental plant research*. Vol. 10, pp. 95-103.
9. Wertheim S.J. (1998) European plum. In: *Rootstock Guide*. Fruit research station Wilhelminadorp, the Netherlands, pp. 115-137.

# INFLUENCE OF WOODCHIP MULCH AND DRIP IRRIGATION ON FRUIT QUALITY OF SOUR CHERRIES

***Daina Feldmane***
*Latvia University of Agriculture*
*daina.feldmane@inbox.lv*

**Abstract**

The contents of soluble solids, acids and their ratio as well as phenol content are important indices for quality evaluating of sour cherry fruits. These parameters are influenced by the cultivar and growing conditions. The trial was carried out at the Latvia State Institute of Fruit- Growing to evaluate the effect of woodchip mulch and drip irrigation on fruit quality of sour cherry cultivars 'Bulatnikovskaya', 'Desertnaya Morozovoi', 'Latvijas Zemais', 'Orlica', 'Shokoladnica', 'Tamaris', and 'Zentenes'. The biochemical composition of the fruits was analyzed in 2009 and 2010. The content of soluble solids was determined with the method of refractometry; the content of total acids by titrating with 0.1N NaOH; the total content of phenols with the method of spectrometry. The content of total soluble solids in sour cherry fruits was decreased by use of woodchip mulch. The content of acids, the ratio of soluble solid content to the acid content and the content of the phenols was not significantly influenced by woodchip mulch and drip irrigation. The cultivar 'Desertnaya Morozovoi' showed the highest soluble solid content in the fruits, the cultivar 'Latvijas Zemais' – the highest acid content, and cultivar 'Tamaris' – the highest phenol content in the fruits.

**Key words**: soluble solids, acids, phenols, cherry woodchip mulch, drip irrigation.

## Introduction

Sour cherries are valuable fruits which are used for the processing as well as for consuming in fresh way. The content of soluble solids, the content of the acids and their ratio are essential parameters for the evaluation of sour cherry fruits. High soluble solid to acid ratio is desirable, because it is precondition of balanced taste of fruits (Voća et al., 2008). Nevertheless, acids are also necessary for taste providing. Sour cherries contain dehydroascorbic acid which is available as vitamin C (Jacob et al., 2003), and phenolic acids (Prior and Cao, 2000) which have positive influence on the health.

Another important parameter of fruit quality is the content of phenols. In human organism, free radicals have oxidative properties and mostly their influence is injurious. The scavenging of them done by antioxidants is important in health protection, and phenols are compounds with high antioxidative activity. The phenols advance the prevention of cardiovascular diseases, cancer, arthritis, disease of Alcheimer (Shi et al., 2005). Sour cherries are rich source of phenols (Dietrich et al., 2003; Rimpapa et al., 2007). The positive effect of sour cherries to the health is proven in several researches. The anthocyans (class of phenolic compounds) obtained from sour cherries reduce pain (Tall et al., 2004). Sour cherry juice decreases the strength loss and muscle pain after physical training (Conolly et al., 2006).

The fruit quality parameters have varied significantly among the cultivars of sour cherries. The content of soluble solids and acids and their ratio are influenced significantly by both yielding and pollinating cultivar (Ansari and Davarybejad, 2008). The significant differences in the phenol content among cherry cultivars were detected in Croatia (Šimunić et al., 2005), Latvia (Ruisa et al., 2008), and Hungary (Papp et al., 2010).

The changes in growing conditions can cause significant changes in biochemical properties of fruits. The decrease in the content of soluble solids and acids in sour cherry fruits has been caused by increase in the amount of precipitation (Szabo 2007 cited from Banati et al., 2010; Poll et al., 1999). Similarly, the content of soluble solids has been lower if trees received full irrigation rate or excessive irrigation compared with deficit irrigation in peach fruits (Crisosto et al., 1994) as well as in sour cherry fruits (Papenfuss, 2010). But, in Turkey, different irrigation levels for sweet cherries did not affect significantly the content of soluble solids and acids in the fruits (Demirtas et al., 2008). The content of soluble solids and acids can be influenced also by other factors. M. Ansari and G. H. Davarnejad have proved the effect of pollinating cultivar on sour cherry fruit quality, including biochemical properties (Ansari and Davarnejad, 2008).

The total content of phenols as well as the content of anthocyans can be influenced by the Sun energy. The content of phenols and anthocyans in cherry fruits has been higher in the year with more hours of sunshine in the vegetation period (Ruisa et al., 2008; Pedisić et al., 2010; Poll et al., 2003). Ultra-violet illumination has caused accumulation of the flavonoid compounds (belonging phenols) in the cells of eggplants (Takeda et al., 1994). Tree covering, in contrary, did not influence phenol content in fruits of sweet cherries (Usenik et al., 2009). The synthesis of phenols is one of plant responses to drought stress. Accordingly, an increased content of phenols has been detected in leaves of strawberries after drought period (Borkowska et al., 2004) as well as in leaves of cherries having the disturbance of the water transport (Schmid and Feucht, 1986). But there is no information about effect of soil moisture regulation on the biochemical composition of sour cherry fruits. However,

other factors can influence the content of phenols too. Therefore the accumulation of phenols can be advanced by nutrient deficiency and graft incompatibility (Dirr et al., 1994) as well as by infection by fungus *Blumeriella jaapii* (Rehm) (Niederleitner et al., 1994).

The aim of the research is to estimate the influence of woodchip mulch and drip irrigation on the fruit quality parameters (total content of soluble solids, total content of the acids, total soluble solids to total acids ratio, total content of the phenols) of sour cherry cultivars grown in Latvia.

## Materials and Methods

The trial was established at the Latvia State Institute of Fruit-Growing. The treatment of soil moisture influencing regimes had three variants: woodchip mulch in the tree strips, drip irrigation, and the control – bare soil without irrigation. There were three replications for the mulch and the control variants, and four replications for the irrigation variant. Seven sour cherry plants (one of each cultivar) were planted in every replication.

Sour cherries were planted in spring of 2007 on clayic Podzoluvisol soil with the content of P – 53 mg $kg^{-1}$, content of K – 124 mg $kg^{-1}$, pH 6.4. The planting distance was 4 × 4 m. The macronutrients were given yearly as 12 g $m^{-2}$ of N, 5 g $m^{-2}$ of P, and 10 g $m^{-2}$ of K in the tree strips. Nitrogen fertilizer was given in spring (in April), phosphorus and potassium ferilizers were given in October.

Weeds were controlled both by removing them and spraying with herbicide Basta® (soluble concentrate, active ingredient glufosinate-ammonium 200 g $L^{-1}$) in 1 m wide strips along the trees. Perennial grasses were sown in the space between strips.

The volumetric soil moisture was measured with device Theta Probe type ML2x once every 7 – 11 days. In variant with drip irrigation soil moisture was provided, about 200 ml $L^{-1}$.

The total amount of precipitation during the period of active vegetation in Dobele was 275 mm in 2009, and 470 mm in 2010 (these indices are based on data of Latvian Agency of Environment, Geology and Meteorology). In 2009, drip irrigation was done 12 times. The total amount of used water was 876 L per 4 m long tree strip. In 2010, drip irrigation was not done, because soil was moist during all vegetation season. The sum of temperatures in active vegetation period was 2454 °C in 2009, and 2604 °C in 2010.

The fruits of following sour cherry cultivars were tested for their composition in 2009 and in 2010: 'Desertnaja Morozovoi' and 'Tamaris', 'Bulatnikovskaya', 'Latvijas Zemais', 'Orlica', 'Shokoladnica', 'Zentenes'. The biochemical content of fresh cherry fruits was determined at the Laboratory of Biochemistry of the Latvia State Institute of Fruit-Growing. Each analysis was performed in 3 replications.

The following characteristics were determined for sour cherry cultivars:

- the content of soluble solids ($^{o}$Brix) was determined at the temperature of 20 °C with a digital refractometer ATAGO N20 (deviation of measuring instrument face value ±0.1%) (ISO 2173:2003);
- the content of total acids (g 100 $g^{-1}$) was determined by titrating with 0.1N NaOH (ISO 750 – 1998);
- the total content of phenols (mg 100 $g^{-1}$) was determined with the method of spectrometry, by using spectrometer UV-1650-PC at wave length of 765 nm.

Data were statistically processed using analysis of variance and Duncan test for *post hoc* analysis.

## Results and Discussion

*The content of total soluble solids (TSS)*

The content of the total soluble solids varied significantly among cultivars. The highest content of TSS was observed for the cultivars 'Desertnaya Morozovoi' and 'Shokoladnica' (on average, 16.9 and 16.3 °Brix, respectively) in both years (Fig. 1). It was significantly higher than the content of TSS for other cultivars. A comparatively high content of total soluble solids was typical also for cultivar 'Bulatnikovskaya' (on average, 15.5 °Brix), which was at same as level with sour cherry cultivar 'Érdi Bőtermő', grown and investigated in Iran (Ansari and Davarynejad, 2008).

The average content of TSS ranged from 13.1 °Brix to 14.5 °Brix for the other cultivars, where the cultivar 'Tamaris' had the less soluble solids in the fruits. The range of total soluble solids of sour cherry cultivars in our investigation was similar with that of the cultivar 'Oblacinska' (12.0 – 16.0 °Brix), investigated in Serbia (Miletic et al., 2009), and the average TTS content in our investigation (14.7 °Brix ) was similar with that of the cultivar 'Montmorency' (14.43 °Brix), investigated in USA (Chaovanalikit and Wrolstad, 2004).

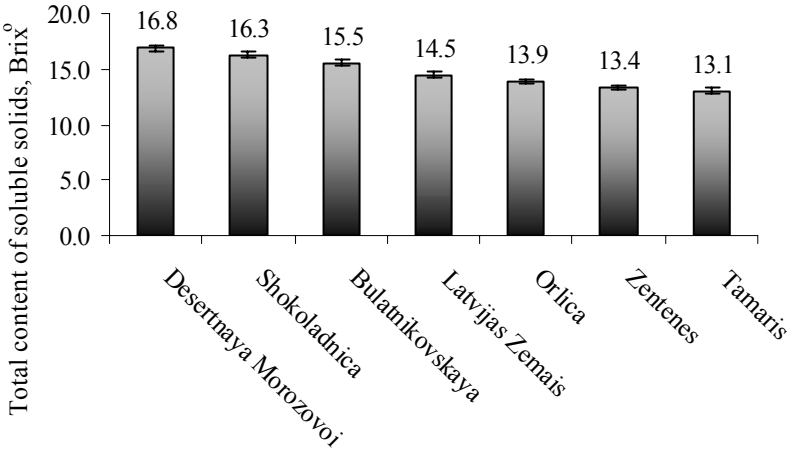



Figure 1. The average total soluble solid content of the sour cherry cultivars in 2009 – 2010.

In general, sour cherries (except the cultivar 'Shokoladnica') grown with woodchip mulch had a significantly lower content of total soluble solids in the fruits compared to the control. Such influence was observed both in 2009 and in 2010 (Tab. 1).

But difference in TSS content between drip irrigation and control variants was not significant – like for sweet cherries in investigation of C. Demirtas (Demirtas et al., 2008). Obviously, differences in soil moisture in drip irrigation and control did not reach such level so that they could influence the accumulation of the TSS. In our investigation, the average soil moisture in cherry fruit growing and ripening time was 184 ml $L^{-1}$ in the control variant in 2009; it was significantly less than in drip irrigation variant – 218 ml $L^{-1}$. In 2010, the average soil moisture in the previous mentioned period did not differ significantly between the control and drip irrigation variants, and it was 232 – 236 ml $L^{-1}$.

Table 1

**The influence of soil moisture treatment and growing year on the content of total soluble solids in sour cherry fruits**

| Soil moisture treatments | The content of total soluble solids, °Brix | | |
|---|---|---|---|
| | in 2009 | in 2010 | on average |
| Woodchip mulch | 14.7[a] | 14.0[a] | 14.4[a] |
| Drip irrigation | 15.3[b] | 14.7[b] | 14.9[b] |
| Control | 15.5[b] | 14.3[b] | 14.8[b] |
| On average | 15.1* | 14.3** | 14.7 |

Means in the column marked with the same letter and means in the row marked with the same symbol did not differ significantly at $p<0.05$.

Sour cherry fruits had higher content of the total soluble solids in the year 2009 than in 2010.

The soil moisture in woodchip mulch variant was higher than in drip irrigation and control variants in both years, and the amount of precipitation in 2010 was larger than in 2009. Therefore precipitation and soil moisture could significantly increase the water content in cherries coincidently decreasing the content of TSS. It complies with other investigations where additional moisture – irrigation or precipitation - caused decreased content of total soluble solids in the fruits (Szabo, 2007 cited by Banati et al., 2010; Poll et al., 2003; Crisosto et al., 1994; Papenfuss, 2010).

However, in our investigation, the cultivar 'Shokoladnica' responded with significantly increased content of TSS in drip irrigation variant compared with the control and woodchip mulch in both years.

*The content of total acids (TA)*

The cultivar 'Latvijas Zemais' had more sour fruits than other cultivars – the content of total acids was significantly higher compared with other cultivars in both years – on average, 1.86 g 100 $g^{-1}$ (Tab. 2). It was similar to the content of TA of cultivar 'Oblacinska' (2.09 – 1.84 g 100 $g^{-1}$) grown in Serbia (Miletic et al., 2009). The lowest content of TA was observed for the cultivars 'Desertnaya Morozovoi' and 'Tamaris' – on average, 1.19 and 1.29 g 100$g^{-1}$, respectively, which was less than for the other cultivars.

Similarly, content of total acids varied from 1.21 to 1.91 g 100 $g^{-1}$ (1.21% to 1.91%) in the fruits of sour cherry cultivars grown in Iran (Ansari and Davarynejad, 2008).

In general, the influence of soil moisture treatment on the TA content in sour cherry fruits was not significant in

both years. Nevertheless, in 2009, the cultivar 'Zentenes' responded with increased content of TA to control variant, the cultivar 'Desertnaya Morozovoi' had increased content of TA in the woodchip mulch variant, but cultivar 'Latvijas Zemais' – in the drip irrigation variant. No significant differences in TA content were detected for the sour cherry cultivars depending on soil moisture treatment in 2010.

Table 2

**The content of total acids in the fruits of sour cherry cultivars in 2009 and 2010**

| Cultivars | The content of total acids, g 100 $g^{-1}$ | | |
|---|---|---|---|
| | in 2009 | in 2010 | on average |
| 'Latvijas Zemais' | 1.91[a] | 1.82[a] | 1.86[a] |
| 'Shokoladnica' | 1.74[b] | 1.69[ab] | 1.71[b] |
| 'Orlica' | 1.64[bc] | 1.64[b] | 1.64[bc] |
| 'Zentenes' | 1.50[cd] | 1.63[b] | 1.56[cd] |
| 'Bulatnikovskaya' | 1.43[d] | 1.56[b] | 1.50[d] |
| 'Tamaris' | 1.34[d] | 1.23[d] | 1.29[e] |
| 'Desertnaya Morozovoi' | 1.01[e] | 1.39[c] | 1.19[e] |
| On average | 1.50* | 1.57** | 1.54 |

Means in the column marked with the same letter and means in the row marked with the same symbol did not differ significantly at $p<0.05$.

The total acid content ranged from 1.47 g 100 $g^{-1}$ in woodchip mulch variant in 2009 to 1.59 g 100 $g^{-1}$ in drip irrigation variant in 2010. The average content of total acids in 2009 was significantly less than in 2010. It was contrary to other investigations where decrease in TA content was observed in years with more precipitation (Szabo, 2007 cited by Banati et al., 2010; Poll et al., 2003). Increased content of acids in 2010 could be explained with high soil moisture and air temperatures which caused active transpiration. It could enhance the uptake of potassium which stimulates the accumulation of organic acids in the fruits (Habib, 2000).

*The ratio of total soluble solids to total acids (TSS/TA)*

The cultivar 'Desertnaya Morozovoi' had the significantly highest TSS/TA ratio (on average, 14.6) in the fruits in both years (Fig. 2). It slightly exceeded the TSS/TA ratio of cultivar 'Erdi Botermo' grown in Iran (10.58 - 13.80) which was the highest in the investigation of M. Ansari and G.H. Davarynejad (Ansari and Davarynejad, 2008).

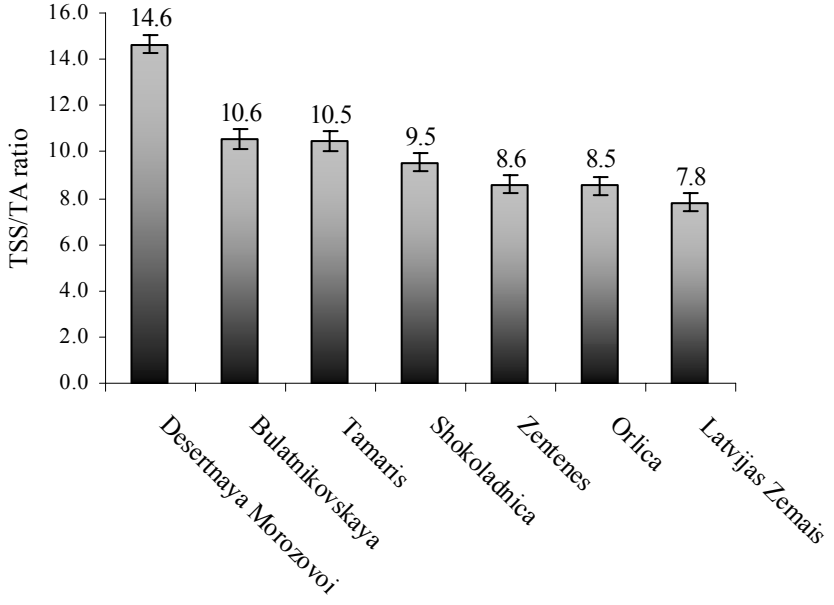



Figure 2. The average total soluble solid content to total acid content ratio of sour cherry cultivars in 2009 – 2010.

The cultivars 'Bulatnikovskaya', 'Tamaris', and 'Shokoladnica' had a significantly higher ratio of TSS/TA in the fruits than the cultivars 'Latvijas Zemais', 'Orlica', and 'Zentenes'. The cultivar 'Cigany Meggy' grown in Iran had ratio of TSS/TA from 7.65 to 9.80 in the fruits and it was considered as not appropriate (Ansari and

Davarynejad, 2008). Such level of TSS/TA ratio had the cultivars 'Shokoladnica', 'Latvijas Zemais', 'Orlica', and 'Zentenes'.

The average TSS/TA ratio was significantly higher in 2009 (10.7) than in 2010 (9.4). In 2009, no significant differences in the TSS/TA ratio were detected among soil moisture treatment variants for most of sour cherry cultivars except for the cultivar 'Desertnaya Morozovoi'. This sour cherry cultivar had significantly higher TSS/TA ratio in control variant compared with mulch variant. TSS/TA ratio in drip irrigation did not differ significantly from the other variants. The soluble solid-acid ratio was not significantly influenced by soil moisture treatment in 2010 for all cultivars.

*The content of total phenols*

High content of total phenols (TP) in the fruits was typical for the cultivar 'Tamaris' (Fig. 3), for which the average content of total phenols was 339.8 mg 100 $g^{-1}$ and it was significantly more than TP for other cultivars in both years.

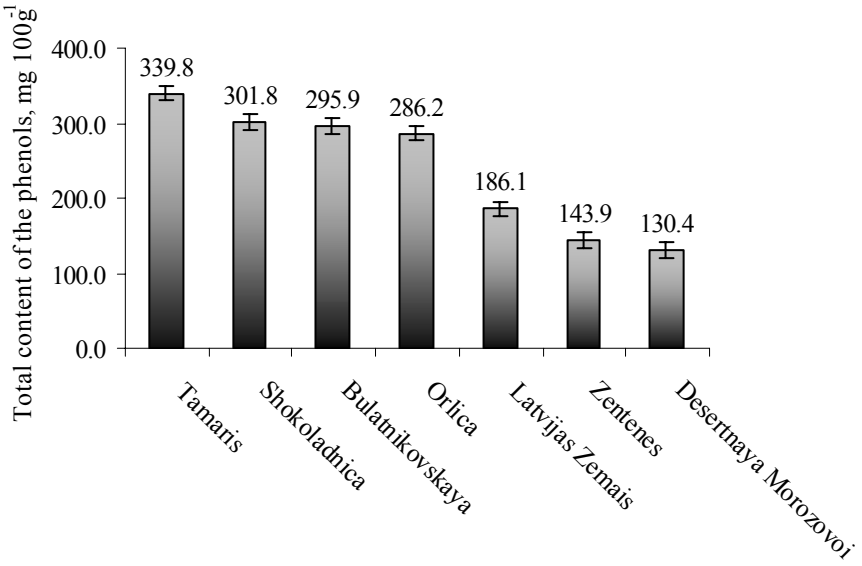



Figure 3. The average total phenol content of the sour cherry cultivars in 2009 – 2010.

The TP content in the fruits of cultivar 'Tamaris' was close to that of the cultivar 'Montmorency' (400 mg $g^{-1}$) tested in USA (Chaovalanikit and Wrolstad, 2004).

The fruits of the cultivars 'Shokoladnica', 'Bulatnikovskaya' and 'Orlica' also had a comparatively high content of TP: from 286.2 to 301.8 mg 100 $g^{-1}$on average, which is significantly more than for of the cultivars 'Latvijas Zemais', 'Zentenes', and 'Desertnaya Morozovoi'. Sour cherry fruits in Croatia contained 141.6 mg 100 $g^{-1}$ of TP, on average (Jakobek et al., 2009), which is similar with the content of TP in the cultivars 'Zentenes' and 'Desertnaya Morozovoi'.

In general, the content of total phenols was influenced neither by soil moisture treatment, nor by the growing year. The average content of phenols ranged from 221.8 mg 100 $g^{-1}$ (drip irrigation, 2010) to 248.8 mg 100 $g^{-1}$ (control, 2010). In 2009, a significantly decreased content of TP in woodchip mulch variant was observed for the cultivars 'Shokoladnica' and 'Zentenes' but a significantly increased phenol content in drip irrigation variant was detected for the cultivar 'Tamaris'. However, such influence did not continue in 2010. Similar situation was observed in the research carried out in Hungary. There were no significant differences in the polyphenol content between sour cherries grown conventionally or organically, but significant differences were caused by growing year (Nagy-Gasztonyi et al., 2010).

## Conclusions

1. The content of total soluble solids in sour cherry fruits was decreased by use of woodchip mulch but was not influenced by use of drip irrigation for six of seven cultivars ($p \leq 0.05$).
2. The content of total acids, ratio of total soluble solid content to total acid content, and the content of phenols were not significantly influenced by woodchip mulch and drip irrigation ($p \leq 0.05$).
3. The biochemical content of sour cherry fruits varied among the cultivars and the growing years. The biochemical peculiarities of the cultivars did not change depending on the growing years:
   - the cultivar 'Desertnaya Morozovoi' had fruits with the highest total soluble solids content, lowest acid content, and highest soluble solid to acid ratio ($p \leq 0.05$);
   - the cultivar 'Latvijas Zemais' had the significantly highest total acid content ($p \leq 0.05$);
   - the cultivar 'Tamaris' had fruits with the highest total phenol content ($p \leq 0.05$).

**Acknowledgements**

This study was supported by Europe Social Fund project "Support for the implementation of doctoral studies at the Latvia University of Agriculture", the agreement No. 04.4-08/EF2.D1.12.

**References**

1. Ansari M., Davarynejad G.H. (2008) Marked improvement of Hungarian sour cherries by cross-pollination II: fruit quality. *Asian Journal of Plant Sciences,* 7(8), pp. 771-774.
2. Bánáti D., Tóth-Markus M., Adányi N., Boross F., Vámos-Falusi Z., Daood H.G., Szabó T., Nyéki J. (2010) Composition and sensory properties of sour cherry cultivars. *International Journal of Horticultural Science,* 16(3), pp. 19-23.
3. Borkowska B., Sowik I., Michalczuk B. (2004) Chlorophyll fluorescence as a tool for evaluation of plant susceptibility to abiotic and biotic stresses. *Scientific Works of the Lithuanian Institute of Horticulture and Lithuanian University of Agriculture. Horticulture and Vegetable Growing,* 23(2), pp. 143-148.
4. Chaovanalikit A., Wrolstad R.E. (2004) Total anthocyanins and total phenolics of fresh and processed cherries and their antioxidant properties. *Journal of Food Science,* 69, pp. 67-72.
5. Connolly D.A.J., McHugh M.P., Padilla-Zakour O.I. (2006) Efficacy of a tart cherry juice blend in preventing the symptoms of muscle damage. *British Journal of Sports Medicine,* 40, pp. 679-683.
6. Crisosto C.H., Johnson R.S., Luza J.G., Crisosto G.M. (1994) Irrigation regimes affect fruit soluble solids contrentation and rate of water loss of 'O'Henry' peaches. *HortScience*, 29(10), pp. 1169-1171.
7. Demirtas C., Candogan B.N., Soylu A., Erturk U., Yazgan S. (2008) Effects of different irrigation levels on the vegetative growth, flower bud formation and fruit quality of sweet cherry in western part of Turkey. *Journal of Food, Agriculture and Environment*, 6(2), pp. 173-177.
8. Dietrich H., Rechner A., Patz S.D. (2003) Influence of processing method on the phenolic antioxidants of fruit juices (Elderberry, blackberry, morello cherry, goosberry). *Flüssiges Obst,* 70(12), pp. 714-712.
9. Dirr U., Feucht W., Treutter D. (1994) Effect of nutrient defficiency on accumulation and leakage of the stress metabolite prunin. *Acta Horticulturae,* 381, pp. 398-404.
10. Habib R. (2000) Modeling fruit acidity in peach trees effects of nitrogen and potassium nutrition. *Acta Horticulturae*, 512, pp. 141-148.
11. Jacob R.A., Spinozzi G.M., Simon V.A., Kelley D.S., Prior R.L., Hess-Pierce B., Kader A.A. (2003) Consumption of cherries lowers plasma urate in healthy women. *Journal of Nutrition,* 133, pp. 1826-1829.
12. Jakobek L., Šeruga M., Šeruga B., Novak I., Medvidović-Kosanović M. (2009) Phenolic compound composition and antioxidant activity of fruits of *Rubus* and *Prunus* species from Croatia. *International Journal of Food Science and Technology,* 44, pp. 860-868.
13. Miletic R., Rakicevic M., Pesakovic M. (2009) Pomological and technological properties of fruits of 'Oblacinska' sour cherry during harvesting period. *Acta Horticulturae,* 825, pp. 521-525.
14. Nagy-Gasztonyi M., Sass-Kiss A., Tmskzi-Farkas R., Bnti D., Daood H.G. (2010) Liquid chromatographic analysis of phenolic compounds in organically and conventionally grown varieties of sour cherries. *Chromatographia,* 71, pp. 99-102.
15. Niederleitner S., Zinkernagel V., Treutter D., Feucht W. (1994) Accumulation of flavanols in cherry leaves after infection by the fungus *Blumeriella jaapii*. *Acta Horticulturae,* 381, pp. 767-770.
16. Papp N., Nyéki J., Szabó Z., Stefanovits-Bányai E., Szabó T., Hegedűs A. (2010) Large variations in antioxidant capacity and contents of Hungarian sour and sweet cherry cultivars. *International Journal of Horticultural Science,* 16(3), pp. 25-28.
17. Papenfuss K.A. (2010) Regulated deficit irrigation of 'Montmorency' tart cherries. Available at: http://digitalcommons.usu.edu/cgi/viewcontent.cgi?article=1531&context=etd , 9 March 2011.
18. Pedisić S., Dragovic-Uzelac V., Levaj B., Škevin D. (2010) Effect of maturity and geographical region on anthocyanin content of sour cherries (*Prunus cerasus* var. *marasca*). *Food Technology and Biotechnology,* 48(1), pp. 86-93.
19. Poll L., Petersen M. and Nielsen G.S. (2003) Influence of harvest year and harvest time on soluble solids, titrateable acid, anthocyanin content and aroma components in sour cherry (*Prunus cerasus* L. cv. 'Stevnsbær'. *European Food Research and Technology*, 216(3), pp. 212-216.
20. Prior R.L. and Cao G. (2000) Flavonoids: diet and health relationships. *Nutrition in Clinical Care*, 3(5), pp. 279-288.
21. Rimpapa Z., Toromanovic J., Tahirovic I., Sapcanin A., Sofic E. (2007) Total content of phenols and anthocyanins in edible fruits from Bosnia. *Bosnian Journal of Basic Medical Sciences,* 7(2), pp. 117-120.
22. Ruisa S., Krasnova I., Feldmane D. (2008) Research on biochemical composition of cherries in Latvia. In: Dimza I., Eihe M., Ikase L., Lanauskas J., Libiakova G., Lopez M., Stanys V., Tirzītis G., Venskutonis R., Zurawicz E. (eds). *Proceedings of international scientific conference 'Suistainable Fruit Growing: From Plant To Product'* pp. 258-264.
23. Scmid P.P.S., Feucht W. (1986) Kohlenhydrate, Chlorophyll, Prolin und Polyphenole in Blättern von Kirschkombinationen (*Prunus avium* L. auf *P. cerasus* L.) mit unterschiedlichen Streßsymptomen

(Carbohydrates, chlorophyll, proline and polyphenols in the leaves of combinations of cherries (*Prunus avium* L. on *P. cerasus* L.) with different symptoms of the stress) *Angewandte Botanik,* 60, S. 365-372. (in German).

24. Shi J., Nawaz H., Pohorly J., Mittal G., Kakuda Y., Jiang Y. (2005) Extraction of polyphenolics from plant material for functional foods-engineering and technology. *Food Reviews International,* 21, pp. 139-166.
25. Šimunić V., Kovač S., Gašo-Sokač D., Pfannhauser W., Murković M. (2005) Determination of anthocyanins in four Croatian cultivars of sour cherries (*Prunus cerasus*). *European Food Research and Technology,* 220, pp. 575-578.
26. Takeda K., Tochika Y., Fukazawa R., Mori T. (1994) Flavonoids as UV-protectant. *Acta Horticulturae,* 381, pp. 348-354.
27. Tall J.M., Seeram N.P., Zhao C., Nair M.G., Mayer R.A., Raja S.N. (2004) Tart cherry anthocyanins supress inflammation-induced pain behavior in rat. *Behavioural Brain Research,* 153, pp. 181-188.
28. Voća S., Dobričević N., Dragović-Uzelac V., Duralija B., Družić J., Čmelik Z., Skendrović Bajobelić M. (2008) Fruit quality of new early ripening strawberry cultivars in Croatia. *Food Technology and Biotechnology,* 46, pp. 292-298.
29. Usenik V., Zadravec P., Štampar F. (2009) Influence of rain protective tree covering on sweet cherry fruit quality. *European Journal of Horticultural Science*, 74(2), pp. 49-53.

# POTASSIUM REMOVAL WITH GRASS IN AN APPLE ORCHARD UNDER INFLUENCE OF MULCH AND IRRIGATION

***Valentīna Surikova, Aldis Kārkliņš***
*Latvia University of Agriculture*
*valentina.surikova@lvai.lv*

**Abstract**

Potassium (K) is one of the most important nutrients necessary for many life functions of plants, like shoot growth, fruit and flower bud set, and fruit size. The aim of this study was to determine the content of potassium in orchard lawn for reduction of potassium fertilizer application and to include the potassium from mown grass into K balance and turnover calculation. The investigation was done at the Latvia State Institute of Fruit-Growing in Dobele in 2009, on the basis of an established field experiment planted in 1997 with apple (*Malus domestica* Borh.) cultivar 'Melba' (rootstock B9), trees spaced at 1.5 × 4 m distances. Three different treatments of soil moisture management were compared: control, sawdust mulch, and fertigation. Soil of the experimental plot was Pisocalcic Cutanic Luvisol (Hypereutric, Hyposkeletic); loam. Organic matter – 25 g $kg^{-1}$, soil reaction pH – 6.5. Plant-available P was 130.9, K – 157.7, and Mg – 102.2 mg $kg^{-1}$. Inter-row strips were covered with grass vegetation (*Lolium perenne* L. and *Poa pratensis* L.). Grass samples were collected during cutting, 3 times per season of 2009: May 19, June 20, and August 11. The uptake and removal of potassium was calculated as kilograms per hectare area. The concentration of potassium in the lawn and the height of grass growth were significantly influenced by the mowing time and the soil moisture treatment. These results can be a base for further studies of potassium turnover in an orchard, as well as for fertilizer planning and management.
**Key words**: *Malus domestica* Mill., mineral nutrition, nutrient uptake.

## Introduction

Potassium (K) is one of the most important nutrients necessary for many life functions of plants, like shoot growth, fruit and flower bud set, and fruit size. Potassium facilitates the water supply in cells, and accumulation of carbohydrates. The amount of potassium influences also fruit colour, tree winter hardiness, and disease resistance. If there is not enough potassium, brown necrotic spots appear on leaf margins, older leaves can even die, and plants become susceptible to fungal diseases (Nosal et al., 1990). Lack or surplus of potassium in the soil greatly depends on the type of farming and technologies used. It has been found that at farms where post-harvest residues remains are left on the field and ploughed down, the total loss of potassium is significantly lower, because potassium returns to the circulation (Līpenīte and Kārkliņš, 2006).

Development of integrated fruit growing in Latvia makes some restrictions for use of mineral fertilizers. These restrictions are fixed in regulations of the Latvia Council of Ministers, which have been worked out on the basis of EU guidelines as well as on the fruit and berry integrated production guidelines which provide the measures for recording of used fertilizers and mechanisms of control. The main idea is to minimaze the use of chemical substances in fruit growing and conform its use with soil conditions. Therefore regulations require the farmers to compose annual fertilizing plans based on actual (or planned) nutrient removal, therefore relevant data sets should be developed taking into consideration the modern technologies of orchard crop growing.

The rapidly increasing price of mineral fertilizers stimulates the producer, without loss of yield and income, to choose more rational growing technologies with a suitable fertilization system. It could be stated as minimal inputs for planned yield goal but taking into consideration the maintenance of soil fertility status. If the mown grass is left in the orchard, not only the nutrients come back to the turnover, but also the content of humus in the soil will increase. Thus the buffer capacity of soil increases which in turn preserves nutrients from leaching, as well as improves soil aeration so influencing positively not only the growth of apple-tree roots, but also microbiological processes in soil, increasing and preserving sustainable soil fertility (Hoagland et al., 2008). This has become especially important during the latest years, with serious concern for environment and development of organic and integrated fruit growing where mineral fertilizers are used as little as possible.

To provide the practical information for fertilizer planning it is necessary to clarify the quantity of potassium found in the mown grass depending on technologies used for water supply – mulching of soil around trees or establishment of irrigation systems, which may significantly influence the grass biomass as well as concentration of potassium in grass. The aim of this study was to determine the content of potassium in orchard lawn for reduction of potassium fertilizer application and to include the potassium from mown grass into K balance and turnover calculation.

## Materials and Methods

The investigation was carried at the Latvia State Institute of Fruit–Growing, Dobele, in 2009. A field trial in three replications was set up on the basis of an orchard established in 1997, for cultivar 'Melba' on rootstock B9 (planting pattern 1.5 × 4 m). Three kinds of soil water

treatment in tree strips were compared: (1) control – no water regulation, (2) sawdust mulch, and (3) fertigation, e.g. drip irrigation with fertilizer additives. In the mulching treatment, soil surface was covered with a 10–20–cm layer of sawdust which was renewed every three years. In the irrigation treatment, 'Den' type pipelines with built-in drippers spaced 0.38 cm apart were used. The irrigation provided effective moistening of a 1–m–wide zone in sandy loam soil, which makes about 25% of orchard area.

For the lawn sown in the inter-row strips, *Lolium perenne* L. and *Poa pratensis* L. in proportion 1:3 were used. The tree strip in the control and drip irrigation treatments was 1 m wide, and during the growth season it was maintained free from grasses. The inter-row strips were 3 m wide. The grass during the experiment was mown regularly (3 – 5 times per season). The apple-trees were trimmed as a slender spindle. The average yield was 20 t $ha^{-1}$ annually.

Soil of the experimental plot was Pisocalcic Cutanic Luvisol (Hypereutric, Hyposkeletic), fine sandy loam/loam. Organic matter content in soil was 25 g $kg^{-1}$ (according to Tyurin method, wet combustion), soil reaction was pH 6.5 (in 1*M* KCl suspension, potentiometrically). Organic matter – 25 g $kg^{-1}$, soil reaction pH–6.5. Plant-available P was 130.9, K – 157.7, and Mg – 102.2 mg $kg^{-1}$ (according to Egner–Rheem or DL method). This is a typical automorphic soil with relatively good water storage and water supply capacity.

Grass samples were collected during cutting, 3 times per season of 2009: May 19, June 20, and August 11. From the start of the growing season till May 19 the average air temperature was 13.6 °C, precipitation – 9.3 mm; till June 21 – 14.8 °C and 93 mm; and till August 11 – 18 °C and 96 mm correspondingly. Grass samples were collected at distances of 0 – 15 cm, 15 – 30 cm, and 30 – 45 cm from the grass-free tree strip. During each sampling the height of grass growth was measured. Potassium content in grass was determined using flame photometrics method. Removal of K was calculated as kilograms per hectare area (kg $ha^{-1}$) (Kārkliņš, 1998).

The results of the investigation were analyzed using dispersion analysis *ANOVA*, as well as descriptive statistics *(Descriptive statistic)*. To compare the data from two sample groups, the Fisher criterion was used.

## Results and Discussion

Soil moisture management positively influenced the growth of grass in inter-row strips. The applied moisture treatments and mowing time significantly influenced the height of orchard grass growth ($p<0.05$) (Figure.1).

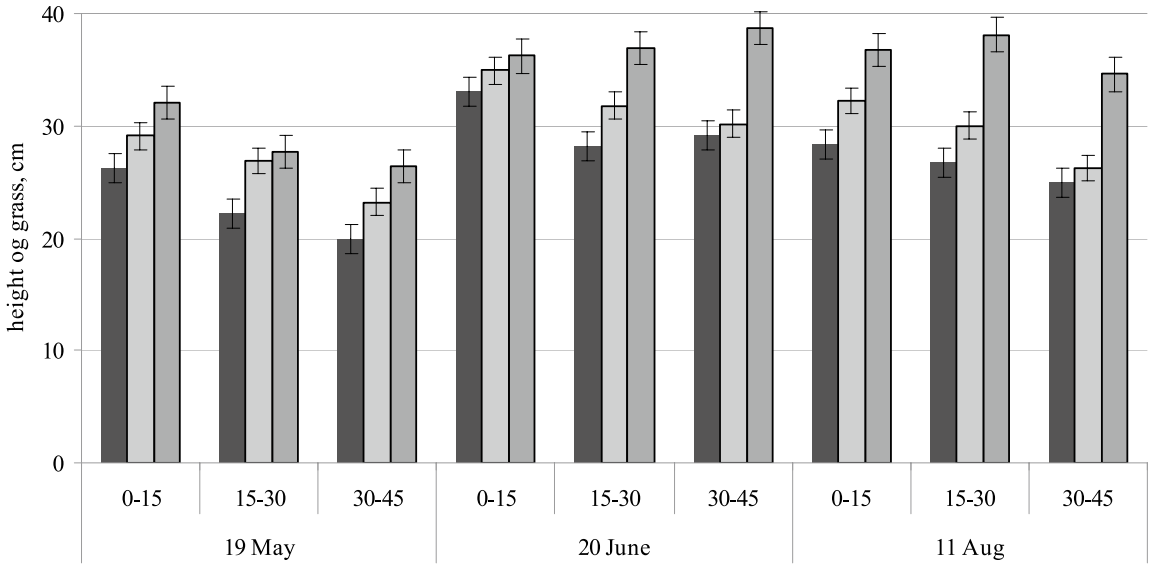



Figure 1. Height of orchard lawn at different distances from the tree strip depending on moisture treatment and mowing time, cm.
– control – mulch – fertigation

During the first mowing of grass, the growth at a 0 – 15 cm distance from the tree strip was the highest in the fertigation treatment and the shortest – in the control treatment, and this difference was statistically significant. A similar situation was observed also at a 30 – 45 cm distance from the tree strip. Whereas at 15 – 30 cm from the tree strip the shortest growth also was in the control treatment, but the growth in mulch and fertigation treatments did not differ significantly. Yet significant influence of fertigation on grass growth in these treatments was found during the second and third cutting time. This means that the influence of fertigation may appear later, besides, it must be taken

into account that from the beginning of vegetation till the first mowing the precipitation was very low, which can explain the significantly lower grass growth in the control treatment. It is possible that in the control treatment the uptake of nutrients was limited as a result of the drought, as also shown by other investigations (Shengzuo et al., 2008).

During the second cut, no significant differences between treatments were found at 0 – 15 cm from the tree strip. This may be explained by the fact that fertilizer was applied in the tree strips at the beginning of the growing season. By increase of precipitation, the fertilizer uptake by grass near to the tree strip was facilitated in comparison with the first mowing time. At 15 – 45 cm from the tree strip, significantly higher grass growth was found in the fertigation treatment, while in control and mulch treatments the results showed no significant difference.

During the third cut significant differences were found between treatments at 0 – 15 cm from the grass-free tree strip. This can be explained by the positive effect of mulch and fertigation on soil moisture, as well as by the relatively high air temperature during this period. Besides, in all treatments a certain tendency was observed – along with increase of distance from the tree strip, the grass growth decreased. These differences may be the result either of the applied soil moisture treatment or the specifics of fertilization in an orchard. Fertilizer was not applied in the whole area, but only in the tree strips, which means that closer to the tree strips the inter-row grass growth could receive more nutrients along with increased uptake due to higher moisture and temperature.

The results of the investigation showed that the content of potassium in the orchard lawn grown in was influenced by the applied soil moisture regulation treatments – sawdust mulch and fertigation ($n=54$, $p<0.05$) (Figure 2).

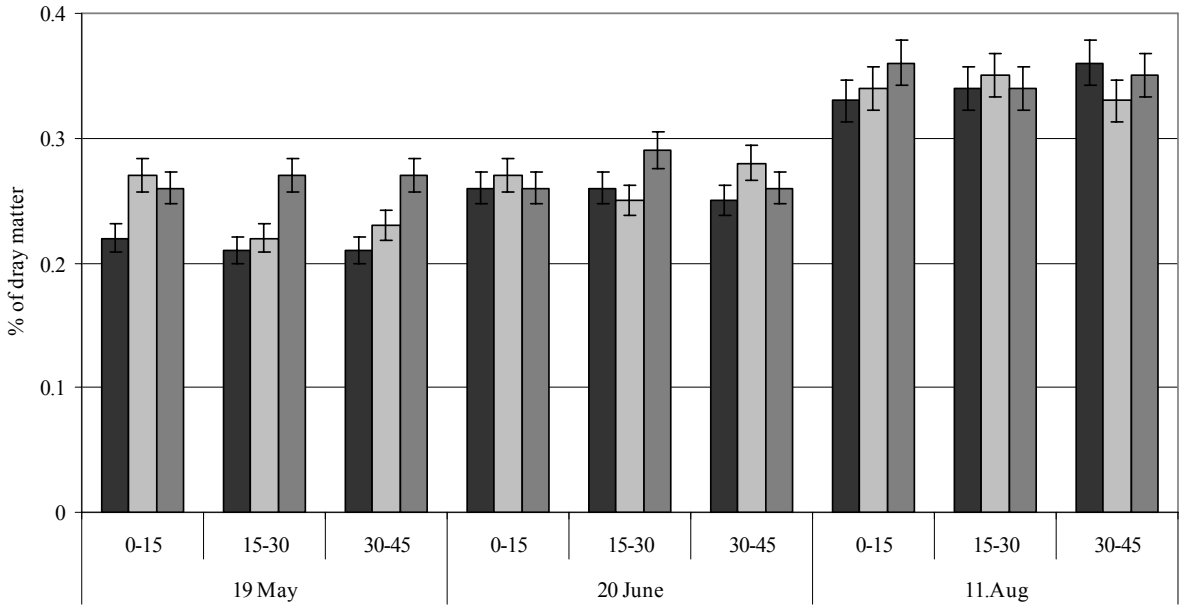



Figure 2. Content of K in orchard lawn dry matter at different distances from the tree strip depending on moisture treatment and cutting time, g $kg^{-1}$.
– control – mulch – fertigation

The concentration of potassium in grass was the lowest during the first cut. At 0 – 15 cm from the tree strip the lowest potassium content was found in the control treatment. In the mulch and fertigation treatments, the content of potassium was 16% higher. In control and fertigation treatments, the potassium content at 15 – 45 cm from the tree strip was similar to that at 0 – 15 cm from the tree strip, while in the mulch treatment it was 28% lower, and the difference was significant ($p<0.05$).

During the second cut, the concentration of potassium in grass was higher by 5 – 15%, yet no significant differences were found between the first and third mowing times ($p>0.05$), except in the mulch treatment where increase in potassium content was significant. The relative increase in potassium concentration may be explained by changes in precipitation and temperature during this period. The average air temperature increased for 1.2 °C. Changes in potassium concentration did not correlate with changes in precipitation, the latest increased even tenfold during this period. On June 14, very high amount of rainfall was observed – 59.5 mm, which should increase the concentration of potassium in plants, as moisture has positive influence on it (Malaguti et al., 2006). Probable that the 4 days which passed between the strong rain and the mowing of grass were not enough to influence the potassium concentration, although it has been found that potassium moves in plants rather quickly (Adamec, 2002).

During the third cut, the content of potassium in grass

was by 55% higher than during the first cut and 30% higher than during the second cut. The differences were significant ($p<0.05$). During the third cut, the potassium content in grass had a tendency to increase along with the distance from the tree strip. The increase inpotassium concentration was especially expressed in the control treatment, although no statistically significant differences were found. It can be concluded with 95% probability that for the formation of 1 ton of grass dry mass, from 22 to 40 kg of potassium are required. Results of this study comply with the conclusions of other researchers (Līpenīte and Kārkliņš, 2001) that grasses need on average 27.4 kg of potassium for the production of 1 t of dry mass.

Still it is not possible to ascertain that the concentration of potassium in the lawn grass was influenced only by the mowing time. Theoretically the concentration of potassium in plants should decrease during the growing season (Nurzinski et al., 1990), but in this study it was an opposite – the concentration increased. These contradictions may be explained by the fact that the grass was cut down several times during the season, which did not allow grass to go through all developmental stages; besides it was not in the same stage of development during all mowing times. The grass mown on May 20 had already reached beginning of flowering, but on August 11 the mown grass was at a much earlier stage. However, the development stages of grass were not different among moisture regulation treatments.

The concentration of potassium could be influenced not only by air temperature and precipitation during the growth of grass, but also by other factors. Yet at all mowing times there was observed a tendency of mulch reducing the potassium concentration in the lawn.

Although the potassium concentration during the first cut was lower in the control and mulch treatments (Figure 2), the removal of potassium was the lowest in the control treatment ($p<0.05$); besides it was notably different from the mulch treatment where the potassium removal was 2 times higher, and from the fertigation treatment where the removal was 3 times higher. During the second cutting of grass, significant differences were found between the control and fertigation treatments. Fertigation increased the removal of potassium 2 times ($p<0.05$) as compared to the control (Table 1). During the third mowing significant differences were established among all three treatments ($p<0.05$): lower removal of potassium was found in the control treatment, in the mulch treatment it was 56% higher, while in the fertigation treatment – 2 times higher.

Table 1

**Grass biomass and potassium uptake, kg ha$^{-1}$**

| Cut | Treatment | | | | | |
|---|---|---|---|---|---|---|
| | control | | mulch | | fertigation | |
| | biomass | K uptake | biomass | K uptake | biomass | K uptake |
| 1 | 282.40$^{a}$ | 5.90$^{a}$ | 365.50$^{b*}$ | 8.37$^{ab*}$ | 359.33$^{b*}$ | 9.04$^{a*}$ |
| 2 | 443.54$^{b}$ | 11.17$^{b}$ | 503.39$^{c*}$ | 13.13$^{bc}$ | 542.12$^{c*}$ | 14.19$^{b}$ |
| 3 | 494.27$^{c}$ | 16.54$^{c}$ | 503.48$^{c}$ | 16.49$^{b}$ | 567.69$^{c}$ | 19.29$^{c*}$ |
| Per season, kg ha$^{-1}$ | 1220.21 | 33.61 | 1372.38 | 37.99 | 1469.14 | 42.52 |

a, b, c, – significantly different within columns ($p<0.05$)
$^{*}$ – significantly different within rows ($p<0.05$).

Such differences were observed because the biomass of the mown grass significantly varied between moisture regulation treatments and mowing times. Till May 20 when the grass was cut for the first time, the precipitation since the start of growth season was only 9.3 mm, therefore in the fertigation and mulch treatments where the soil moisture conditions were presumably better the grass biomass was higher. These results comply with the results of other researchers showing that plant biomass significantly increases when fertigation is used (Hornig and Bünemann, 1993).

No similar studies have been done in Latvia, so there are no data about the rate of the decomposition of cut grass and return of potassium into the natural turnover, but researchers in other countries (Shengzuo et al., 2008; Tagliavini et al., 2008) have found that potassium returns into circulation already 1 – 2 years after grass mowing. Besides it has been investigated (Cazzato et al., 2004; Eason et al., 1991) that throwing of cut grass onto the tree strips significantly increases the amount of organic matter in soil, which is favourable for the potassium turnover and availability to plants.

It should be added that the results of the study could be influenced by weather conditions and other uncontrollable factors, therefore here are only some tendencies discussed. Yet a similar investigation in Latvian conditions was performed for the first time, and the results may become a base for further studies of potassium turnover in an orchard as well as for fertilization planning.

## Conclusions

Mulching of tree strips in an apple orchard significantly reduced the concentration of potassium in the orchard lawn and the height of the cut grass growth.

The potassium concentration in orchard lawn was significantly influenced by the time of cut during the

growing season and the height of grass which in turn was determined by air temperature and precipitation, stage of grass development, and other factors.

Annual removal of potassium with the biomass of orchard lawn in the control treatment was 33.61 kg $ha^{-1}$, in the mulch treatment it was 14% higher, and in the fertigation treatment 26% higher ($p<0.05$).

**Acknowledgements**

The investigation was supported by the European Social Fund.

I would like to thank the Vītols Fund and the LAB-AN (Latvian Agronomic Society – Foreign Department) for granting me a bursary.

**References**

1. Adamec L. (2002) Leaf Absorbtion of Mineral Nutrients in Carnivorous Plants Stimulates Root Nutrient Uptake. *New Phytologist,* 155 (1), pp. 89-100.
2. Cazzato E., Anesse V., Colreto A. (2004) Effects of Clipping Management on Some Aspacts of Tall Fescue (*Fescue arundinacea*) turf. *Acta Horticulturae*, 661, pp. 301-307.
3. Eason W.-R., Newman E.-I., Chuba G.-H. (1991) Specificity of Interplant Cycling of Nutrients: the Role of Mycorrhizas. *Plant and Soil*., 137, pp. 267-274.
4. Hoagland L., Carpenter-Boggs L., Granastein D., Mazzola M., Smith J., Peryea F., Reganold J.P. (2008) Orchard Floor Management Effect on Nitrogen Fertility and Soil Biological Activity in a Newly Established Organic Apple Orchard. *Biology and Fertility of Soils*., 45, pp. 11-18.
5. Hornig R., Bünemann G. (1993) Fertigation and Controlled Strip Cover by Weeds in Apple Orchrards. *Acta Horticulturae*, 335, pp. 65-72.
6. Kārkliņš A. (1998) *Aprēķinu metodes agroķīmijā* (Calculation Methods in Agrochemistry). Jelgava, LLA, 55 lpp. (in Latvian).
7. Līpenīte I., Kārkliņš A. (2001) Calculation of Plant Nutrient Removal for the Specification of Normatives. *Proccedings. of the Latvia University of Agriculture*, 4, pp. 9-16.
8. Līpenīte I., Kārkliņš A. (2006) Plant Nutrient Balance Studies in Farms of Latvia VI. Farm `Tereni`. *Proccedings. of the Latvia University of Agriculture*, 18, pp. 9-16.
9. Malaguti A., Rombola M., Quartieri A., Lucchi C., Inderst B., Marangoni M., Tagliavini D. (2006) Effect of the Rate of Nutrients by Fertigation and Broadcast Application in `Gala` and `Fuji` Apple. *Acta Horticulturae*, 721, pp. 165-172.
10. Nosal K., Poniedzialek W., Kropp K., Porebski S. (1990) Effectiveness of Nitrogen and Potassium Fertilization of Apple Trees. *Acta Horticulturae*, (274), pp. 211-214.
11. Nurzinski J., Kepta M., Komosa A., Kozera G. (1990) Seasonal Changes of N, P, K, Ca and Mg Content in Apple Tree Leaves During Vegetation Period. *Acta Horticulturae*, (274), pp. 365-373.
12. Shengzuo F., Huayong L., Baodong X. (2008) Decomposition and Nutrient Release of Four Potential Mulching Materials for Poplar Plantations. *Agroforesty Systems,* 74, pp. 27-35.
13. Tagliavini M., Tonon G., Scandellari F., Quinones A., Palmieri S., Menarbin G., Gioacchini P., Masia A. (2008) Nutrient Recycling During the Decomposition of Apple Leaves (*Malus domestica*) and Mowed Grasses in an Orchard. *Agriculture, Ecosystems and Environment*, (118), pp. 191-200.

# CHANGES IN SERUM IMMUNOGLOBULINS CONCENTRATION OF NEWBORN CALVES

***Indra Eihvalde, Daina Kairiša***
*Latvia University of Agriculture*
*indra.eihvalde@gmail.com; daina.kairisa@llu.lv*

**Abstract**

The changes in the serum immunoglobulins concentration were investigated in newborn heifers from birth to 7 days of age. The mothers of calves were determined serum and colostrum immunoglobulins (Ig) concentration. The research was carried out at the Latvia University of Agriculture (LLU), in dairy farm 'Līgotnes'of the Research and Study Farm 'Vecauce', in a loose housing system with 530 cows, of which 430 were milking cows. The cows during the dry period were kept tethered in the farm. Eighteen heifers and their dams (Latvian Brown and Holstein breeds) which calved from 30 November 2010 to 23 January 2011 were used in this study. The calves' serum total immunoglobulin concentration at birth was 9.0 ± 1.31 mg $dL^{-1}$ (or 0.09 mg $mL^{-1,}$), and after 24 hours (h) it increased significantly to 4.3 mg $dL^{-1}$ (or 0.043 mg $mL^{-1}$), $p<0.05$. The immunoglobulin G (IgG) concentration in calf serum after birth was 6.8 ±1.50 mg $dL^{-1}$ (or 0.068 mg $mL^{-1}$), and after 1 day and after 7 days it did not change significantly. The average Ig serum concentration in cows was 12.6 ± 1.36 mg $dL^{-1}$ (or 0.126 mg $mL^{-1}$), and Ig concentration in colostrum was 18.5 ± 1.68 mg $mL^{-1}$. On average, close correlation (r=0.56) was found between cow blood serum IgM and colostrum IgM. Correlation between cow and calf IgG serum concentration proved to be weak (r=0.49).

**Key words**: calves, cows, serum, colostrum immunoglobulins.

## Introduction

The calves are born without their own immunoglobulins, hence they get them by drinking colostrum (Jezek and Klinkon, 2004). Colostrum is the first natural food for the newborn calf. It is secreted during the first few days after calving. The importance of colostrum for the health of calves has been known for a long time (Pakkanen and Aalto, 1997). The high level of immunoglobulins is only in the first milking colostrum (Quigley and Martin, 1994; Jezek and Klinkon, 2004). Bovine colostrum is a very rich source of immunoglobulins and their absorption is essential to provide passive immunity after birth. These antibodies protect newborn calves against infectious enteric and respiratory diseases, which are principal reasons for mortality of calves (Pakkanen and Aalto, 1997). The most important aspect of colostral feeding period is timely provision of an adequate amount of hight quality colostrum. The first feeding has the greatest influence on Ig absorption. The timing of colostral feeding is critically important because the intestine of the newborn is capable of absorbting large molecules for not more than 24 hours after birth. At least 100 g of pure Ig must be ingested at each of the first two feedings of colostrum to achieve a blood concentrations of at least 1.0 mg $dL^{-1}$ (or 0.01 mg $mL^{-1}$) of IgG to reduce the incidence of disease and death. During the first 3 days calves should be fed high quality colostrum. The recommended volume is 1.5 $L^{-1}$ of colostrum for first feeding. The temperature of the colostrum should be 39 °C to ensure good digestion and acceptance (Brant et al., 2001). Immunoglobulins are proteins which are critical to the identifying and detroying pathogens in the animal. There are three major types of Ig in colostrum of cattle: IgG, IgM, and IgA. There are two isotypes of IgG: $IgG_{1,}$ and $IgG_2$ (Quigley, 2001) These Ig work together to provide the calf with passive immunity until the calf´s own active immunity develops. Colostrum contains 75 mg $mL^{-1}$ IgG, 5 mg $mL^{-1}$ IgA, and 4.9 mg $mL^{-1}$ IgM (Zītare, 2001). Most of the bovine immunoglobulins are IgG, they are transfered from the blood of the cow into colostrum by a higly specific transport mechanism. This mechanism moves large amounts of IgG from blood into the udder. Serum IgG concentrations of the dam decline precipitously, beginning about 2 and 3 weeks prior to calving. IgM and IgA are synthesized by the plasmacytes in the mammary gland (Quigley, 2001). Maternal immunoglobulins are not transfered across the placenta to the fetus in cattle and calves are born with very low concentrations of serum immunoglobulins (Pakkanen and Aalto, 1997). Optimal supply with colostrum of good quality is important for calf health, but good management is also essential. The aim of the research was to estimate the cows' and newborn calves' blood serum and colostrum Ig concentration.

## Materials and Methods

The research was carried out at the Latvia University of Agriculture (LLU), in dairy farm 'Līgotnes' of the Research and Study Farm 'Vecauce', in a loose housing system with 530 cows, of which 430 were milking cows. The cows during the dry period were kept tethered in the farm. Eighteen heifers and their dams (Latvian Brown and Holstein breeds) which calved from 30 November 2010 to 23 January 2011 were used in this study. The calves were housed in individual boxes 2 months after birth. All time they had a straw bed, which was regulary changed. First five days of life they got their dams' colostrum: maximum 4 – 6 $L^{-1}$ twice a day. Calves suckled milk from a nipple pail. From first week of life, calves had free access to the starter and hay. After calving, blood samples from calves and their dams were collected. Blood samples of calves

were collected from the jugular vein into vacutainer tube (5 $mL^{-1}$) at birth, 24 h, and 7 days after calving. Blood samples of cows were collected from the tail vein shortly after partum. Blood was separated for 15 min by centrifugation, chilled (approximaly at 4 °C), and stored prior to the analysis for immunoglobulins (IgA, IgM, and IgG) by Immunturbidimetric method in the Laboratory 'Egila Gulbja Laboratorija' in Riga. Colostrum samples (500 $mL^{-1}$) were obtained immediately after calving, and after 12 h. Quality of colostrum was estimated using a colostrometer by company 'DeLaval' (Biogenics – Colostrometer, 1980). Before evaluation, colostrum was heated till 22 °C. The colostrometer evaluates colostrum's quality (or Ig concentration) as superior – 50 to 140 mg $mL^{-1}$, moderate – 30 – 50 mg $mL^{-1}$, or inferior < 30 mg $mL^{-1}$. These quality levels are also coded by colour on the colostrometer as green, yellow, and red (Fleenor, Stott, 1980). Colostrum was devided into two 50 $mL^{-1}$ samples, stored frozen at 20 °C, and shipped to the Laboratory of Food processing of the Faculty of Food Technology of the LLU. Colostrum samples were analysed for immunoglobulins (IgA, IgM, and IgG) by Turbidimetric method. Statistical analysis of the data was perfomed by using Microsoft Excel. Statistical analysis of results was perfomed by using a T-test. Statistical significance ($p<0.05$) was indicated with different alphabet letters in superscript.

## Results and Discussion

In this research, Ig concentration in cow blood serum was determined shortly after calving. The results showed that total cow serum Ig concentration was 12.6 ± 1.36 mg $dL^{-1}$ (or 0.126 mg $mL^{-1}$) at various intervals - 5 – 24 Ig mg $dL^{-1}$ (or 0.05 – 0.24 mg $mL^{-1}$) (Table 1).

Table 1

**Levels of immunoglobulins in cow (n=18) blood serum**

| Parameters | $\bar{x} \pm s_{\bar{x}}$ | Min | Max | Cv% |
|---|---|---|---|---|
| Lactation | 2.6 ± 0.23 | 1 | 4 | 8.8 |
| Total Ig, mg $d^{L-1}$ | 12.6 ± 1.36 | 5 | 24 | 45.7 |
| IgM, mg $dL^{-1}$ | 6.6 ± 0.97 | 1 | 15 | 62.5 |
| IgG, mg $dL^{-1}$ | 6.0 ± 0.82 | 1 | 17 | 57.7 |

The average age of the cows was 2.6 lactations, and the coeficient of variation was 8.8%, which indicates that the model group is leveled. The chosen model group shows the cows' average age in the farm - 2.4 lactations. IgA was not observed in blood serum. It is mentioned in the literature that immunoglobulin gets into cow udder with blood and synthesides. Compared with blood serum, IgA concentration in colostrum is higher (Neilands, Zītare, 1988). In our research, IgG concentration contained 6.0 ± 0.82 mg $dL^{-1}$ (or 0.06 mg $mL^{-1}$). The first milking immunoglobulin concentration was 88.1 mg $mL^{-1}$ (Table 2), which was superior quality of colostrum (Fleenor and Stott., 1980), but after 12 h it was decreased significantly (52.1 ± 4.95 mg $mL^{-1}$). In publications scientists has found that high level of immunoglobulins is only in the first milking colostrum (Quigley and Martin, 1994; Jezek and Klinkon, 2004).

Table 2

**Levels of immunoglobulins in cow (n=18) colostrum**

| Parameters | 1st milking | | 2nd milking (after 12 hours) | |
|---|---|---|---|---|
| | $\bar{x} \pm s_{\bar{x}}$ | Cv% | $\bar{x} \pm s_{\bar{x}}$ | Cv% |
| Ig used by colostrometer, mg $mL^{-1}$ | 88.1 ± 5.27 [a] | 25.4 | 52.1 ± 4.95 [b] | 40.3 |
| Total Ig, mg $mL^{-1}$ | 18.5 ± 1.68 | 38.4 | 17.0 ± 1.63 | 40.7 |
| IgA, mg $mL^{-1}$ | 2.6 ± 0.11 | 18.0 | 2.6 ± 1.17 | 28.7 |
| IgM, mg $mL^{-1}$ | 2.9 ± 0.12 | 17.4 | 2.9 ± 0.11 | 15.9 |
| IgG, mg $mL^{-1}$ | 13.0 ± 1.63 | 53.6 | 11.5 ± 1.64 | 60.5 |

[a, b] – averages of immunoglobulin level with different superscripts differ significantly ($p<0.05$)

In the present study, cow age did not significantly affect Ig concentration in colostrum ($t_{Stat} = 34.0 > t_{Critical} = 4.2$ where $p=1.75>0.05$), correlation was r=-0.34. This is contrary to the data in the literature, where the colostral immunoglobulin level rises with the number of lactations (Avendano-Reyes and Saucedo-Quintero, 2004; Eihvalde and Kairiša 2010). In our research, concentration of IgA, IgM un IgG remained at the same level for the next 12 h,

which confirms of other researchers findings (Elfstrand et al., 2002). The total Ig concentration in colostrum was 18.5 mg $mL^{-1}$; wheares various literature sources indikate, that the total amount of colostrum Ig can be 93 – 101 mg $mL^{-1}$, IgA - 1.6 – 6.2 mg $mL^{-1}$, IgM - 3.4 – 6.1 mg $mL^{-1}$ and IgG - 54 – 93 mg $mL^{-1}$ (Pakkanen and Aalto, 1997; Elfstrand et al., 2002; Quigley et al., 1995). In our study, total Ig and IgG was several times less than found in the literature.

The research shows how Ig concentration in cow blood serum influences Ig concentration in colostrum (Table 3).

Table 3

**Correlation coefficient (r) between cows serum Ig and colostrum Ig concentration in cows (n=18)**

| Serum | Colostrum | | | |
|---|---|---|---|---|
| | Total Ig, mg $mL^{-1}$ | IgA, mg $mL^{-1}$ | IgM, mg $mL^{-1}$ | IgG, mg $mL^{-1}$ |
| Total Ig, mg $dL^{-1}$ | - 0.08 | 0.19 | 0.58* | - 0.13 |
| IgM, mg $dL^{-1}$ | 0.00 | 0.16 | 0.56* | 0.01 |
| IgG, mg $dL^{-1}$ | - 0.22 | 0.13 | 0.30 | - 0.22 |

* $p<0.05$

A negative correlation was observed between the cow blood serum Ig and colostrum Ig concentration, which shows that the concentration of cow blood serum Ig did not affect the concentration of Ig in colostrum. Negative correlation was between IgG concentration in the cows blood and colostrum (r=-0.22). It is contradiction to how scientists say. They say that the primary immunoglobulin in bovine colostrum is IgG, which is derived from maternal serum IgG (Weaver et al., 2000). An average close correlation (r=0.56) was found between cow blood serum IgM and colostrum IgM, which demonstrates, that concentration of IgM in colostrum is affected by the concentration of IgM in the cow blood serum.

Health of some days old calves can be evaluated by immunoglobulins concentration in blood serum (Figure 1).

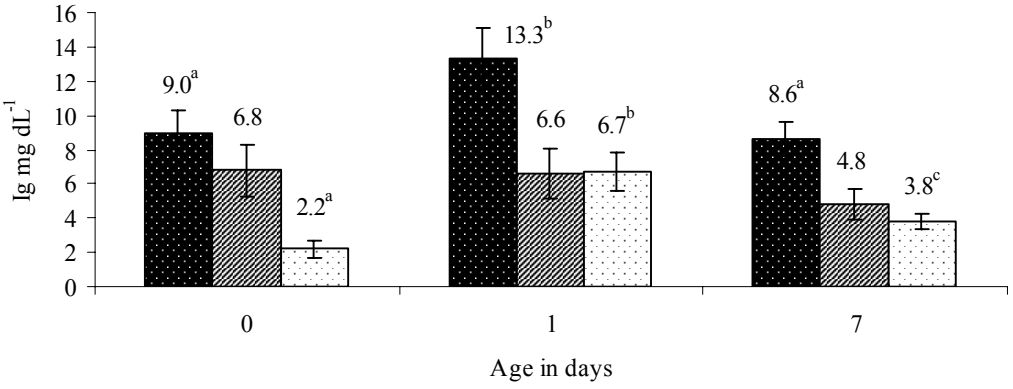



Figure 1. Changes in calf serum Ig after birth (n=18):
[a, b, c] – averages of immunoglobulin level with different superscripts differ significantly ($p<0.05$).
lg (total) lg G lg M

The success of passive immunity transfer is typically assessed by measuring calf blood serum IgG concentrations at 24 to 48 hours after partus. In our research, total Ig concentration in blood of the newborn calf was 9.0 ± 1.31 mg $dL^{-1}$ (or 0.09 mg $mL^{-1}$), and after 24 hours it increased significantly to 4.3 mg $dL^{-1}$ (or 0.043 mg $mL^{-1}$), $p<0.05$. It is mentioned in the literature that in the blood serum of newborn calves Ig cannot be found at all or can be found in small amounts - about 5 mg $dL^{-1}$ (or 0.05 mg $mL^{-1}$) (Trubka, 1999). In our research (24 hours after birth), correlation between Ig concentration in colostrum and in blood serum the correlation was negative, which confirms inferior Ig absobtion in calves blood. IgG concentration in the calf blood serum after birth was 6.8 ± 1.50 mg $dL^{-1}$ (or 0.068 mg $mL^{-1}$), and after 1 day and 7 days it did not change significantly. In the literature reported, that the blood serum IgG concentration was higher than 10 mg $mL^{-1}$ by 24 hours after birth, it provides successful passive immunity. (Shea et al., 2009). Calves with high serum immunoglobulins have lower mortality rates than calves with serum IgG < 10 mg $mL^{-1}$ (Godden, 2009). Whereas IgM concentration in the blood serum at the

calf birth was 2.2 ± 0.50 mg $dL^{-1}$ (or 0.022 mg $mL^{-1}$), after 24 hours it increased significantly to 4.5 mg $dL^{-1}$ (or 0.045 mg $mL^{-1}$) ($p<0.05$), but after 7 days it decreased significantly to 2.9 ± 0.43 mg $dL^{-1}$ (or 0.29 mg $mL^{-1}$). Ig concentration in calves blood clarifies the risk factor of calves diseases. Blood serum concentration in calves may be influenced by many factors, including calf age at first feeding, sex of the calf, body weight, amount of Ig consumed, colostrum quality, and method of colostrum feeding (Gvozdic et al., 2008). The IgA concentrations in calves serum were not found.

The research shows how Ig concentration in cows' blood serum influences Ig concentration in new-born calf blood serum (Table 4).

Table 4

**Correlation coefficient (r) between cows and newborn calves Ig serum concentration shortly after calving**

| Calf serum | Cow serum | | |
|---|---|---|---|
| | Total Ig, mg $dL^{-1}$ | IgM, mg $dL^{-1}$ | IgG, mg $dL^{-1}$ |
| Total Ig, mg $dL^{-1}$ | 0.45 | 0.20 | 0.51* |
| IgM, mg $dL^{-1}$ | - 0.02 | 0.06 | - 0.12 |
| IgG, mg $dL^{-1}$ | 0.41 | 0.15 | 0.49* |

* $p<0.05$

Our research showed that between the cow and the calf IgG concentration in blood serum there was weak correlation (r=0.49), which confirms researches of other scientists, that transplacental transport of proteins is a selective process, singly only IgG, and not IgM or IgA, cross the placental barrier (Klaus et al., 1969).

## Conclusions

The average Ig concentration in cows serum was 12.6 ± 1.36 mg $dL^{-1}$ (or 0.126 mg $mL^{-1}$). In first milking, colostrum Ig concentration was 18.5 ± 1.68 mg $mL^{-1}$, but in the 2nd milking colostrum was by 1.5 mg $mL^{-1}$ lower. An average close connection (r=0.51) was found between cows blood and colostrum IgM concentration. The newborn calves blood serum at birth contained 9.0 ± 1.31 mg $dL^{-1}$ (or 0.09 mg $mL^{-1}$) Ig, 24 hours after birth Ig concentration was higher than 4.3 mg $dL^{-1}$ (or 0.043 mg $mL^{-1}$), there were significant differences ($p<0.05$). A statistically significant correlation was (r=0.49) detected between IgG concentration in cow and in calf serum. Whereas IgA was not found in the cow and calf blood serum. Neonates with not adequate Ig concentration in blood serum can easily suffer from diseases if placed in a filthy environment or exposed to hidly virulent organisms.

## References

1. Avendano - Reyes L., Saucedo-Quintero J.S. (2004) Colostrum immunoglobulins transference in Holstein cattle according the age of the dam. *American Society of Animal Science*, 55, pp. 322-324.
2. Biogenics – Colostrometer (1980) Available at: http: // www.colostrometer.com/support.asp?ID=2,11February 2011.
3. Brand A., Noordhuizen J.P.T.M., Shukken Y.H. (2001) *Heard Health and Production Management in Dairy Practice,* Wageningen, Netherlands, 90 p.
4. Eihvalde I., Kairiša D. (2010) Immunoglobulin level in colostrum of dairy cows at different age. *Proceeding of the 15th Baltic animal breeding conference,* Riga, pp. 56-60.
5. Elfstrand L., Lindmark-Mansson H., Paulsson M., Nyberg L., Akesson B. (2002) Immunoglobulins, grow factors and grow hormone in bovine colostrum and the effects of processing. *International Dairy Journal,* 12, pp. 879-887.
6. Fleenor W.A., Stott G.H. (1980) Hydrometer test for estimation of immunoglobulin concentration in bovine colostrum. *Journal of Dairy Science*, 63, pp. 973-977.
7. Godden S.M., Haines D.M., Hagman D. (2009) Improving passive transfer of immunoglobulins in calves I: Dose effect of feeding a comercial colostrum replacer. *Journal of Dairy Science,* 92, pp. 1750-1757.
8. Gvozdič D., Stojič V., Šamans H., Fratrič N., Dakovič N. (2008) Apparent efficiency of immunoglobulin absorption in newborn calves orally treated with zeolite. *Acta Veterinaria (Beograd),* 58, pp. 345-355.
9. Jezek J., Klinkon M. (2004) Influence of colostral immunity on gain and health status in calves. *Acta agriculturae slovenica*, 8, pp. 179-184.
10. Klaus G.G.B., Bennett A., Jones E.W. (1969) A Quantitave Study of the Transfer of Colostral Immunoglobulins to the Newborn Calf. *Journal of Immunology,* 16, pp. 293.
11. Martin K.R., Quigley J.D. (1994) Immunoglobulin concentration, specific gravity, and nitrogen fractions of colostrum from Jersey cattle. *Journal of Dairy Science*, 77, pp. 264-269.
12. Neilands J., Zītare I. (1988) Imūndeficīta etiopatoģenēze jaundzimušiem teļiem (New born calve's immunodeficiency etiopathogenesis). Proceedings of *International conference*, 1998.gada 14. aprilī, LLU Zinātnes centrs 'Sigra', Sigulda, 61-63. lpp. (in Latvian).

13. Pakkanen R., Aalto J. (1997) Grow Factors and Antimicrobial Factors of Bovine Colostrum. *International Dairy Journal,* 7, pp. 285-297.
14. Quigley J. (2001) A primer on colostral immunoglobulins. Calf Notes. Available at: http:// www.calf notes.com, 20 January 2010.
15. Quigley J.D., Martin K.R., Dowlen H.H. (1995) Concentration of Trypsin Inhibitor and Immunoglobulins in Colostrum of Jersey Cows. *Journal of Dairy Science,* 78, pp. 1573-1577.
16. Shea E.C., Whitehouse N.L., Erickson P.S. (2009) Effects of colostrum replacer supplemented with lactoferrin on the blood plasma immunoglobulin G concentration and intestinal absorption of xylose in the neonatal calf. *Journal of Animal Science,* 87, pp. 2047-2053.
17. Trubka R. (1999) Hipogammaglobulinēmijas etiopatoģenēze un diagnostika teļiem (Hypogammaglobulinemia in calf: etiopathogenesis and diagnostic). *Veterinārmedicīnas raksti* 1999, Jelgava, 328-333. lpp. (in Latvian).
18. Weaver D.M., Turler J.W., VanMertre D.C., Hostetler D.E., Barrington G.M. (2000) Passive Transfer of Colostrum Immunoglobulins in Calves. *Journal Veterinary Internal Medicine,* 14, pp. 569-577.
19. Zītare I. (2001) Svarīgākie nosacījumi, lai iegūtu veselu teļu un izaudzētu labu govi (Most important concditions to get a healthy calf and breed a good cow). *Latvijas Piensaimnieks,* 3, 23-25. lpp. (in Latvian).
20. Степановиц Н. (1998) *Состояние естественной резистентности и иммунологической реактивности у новорожденных телят при колибактериозе* (Natural resistence conditions and immunological influence of newborn calves in colibacteriotical situations). Диссертация резуме, Государсвеннй Аграрный Университет Молдови. с. 228. (in Rusian).

# EFFECT OF ORAL GLYCEROL ON THE LACTATION PERFORMANCE OF DAIRY COWS IN POSTPARTUM PERIOD

***Marko Kass[1,2], Tiia Ariko[2], Meelis Ots[1,2], David Arney[2], Olav Kärt[2]***
*[1]Bio-Competence Centre of Healthy Dairy Products, Estonia*
*[2]Estonian University of Life Sciences*
*marko.kass@emu.ee*

**Abstract**

The treatment of ketosis with glycerol was first introduced in the 1950s. Currently, the availability of glycerol as a feed supplement for dairy cattle has increased due to the rapid expansion of the biodiesel industry. It has been suggested that glycerol can be used as a dietary glucose precursor for dairy cows in a similar way to propylene glycol. This study examined the effect of orally administrated crude glycerol on milk production and composition, and energy-status related blood metabolites of primiparous (n=4) and multiparous (n=4) Holstein dairy cows in the first three weeks of lactation. The treatment group cows were given an oral drench of 500 mL of crude glycerol once a day before the morning feeding. Long-term oral drenching of crude glycerol had no effect on dry matter intake (DMI), but there was a positive effect on milk yield. Most milk composition values were not affected by the treatment, but treatment groups had lower milk protein levels. Blood glucose and insulin concentrations were declined with glycerol administration.
**Key words**: glycerol, oral drenching, dairy cows.

## Introduction

The high yielding cow is unable, insofar as is necessary, to co-ordinate her metabolism to support milk secretion, causing a decrease in production and an increasing frequency of metabolic disorders, such as ketosis. There are a number of additives used as a preventive tool – propylene glycol, glycerol, and calcium propionate. The treatment of ketosis with glycerol was first introduced in the 1950s (Johnson, 1951; Johnson, 1954). Glycerol, as a by-product of the biodiesel industry, has been produced by a reaction that utilizes a base-catalyzed transesterification of oil (rapeseed oil) in the formation of methyl and ethyl fatty acid esters in the production process (Thompson and He, 2006). It has been suggested that glycerol can be used as a dietary glucose precursor for dairy cows in a similar way to propylene glycol. Recent studies of this are different in their methods of administration, quantity, delivery period and quality of crude glycerol (Stokes et al., 2002; DeFrain et al., 2004; Ogborn, 2006). However, there is a lack of information in the literature regarding oral glycerol administration over the longer period. Also, there is no data on how glycerol administration in the postpartum period affects the lactation performance of dairy cows at different parities; primiparous Holstein cows mobilize fewer body reserves than multiparous cows (Gallo et al., 1996; Friggens et al., 2007). The objective of this study was to study the effect of oral administration of glycerol on the performance of cows with different parity.

## Materials and Methods

The study was carried out at the Märja experimental farm of the Estonian University of Life Sciences. This study examined the effect of orally administrated crude glycerol on milk production and composition and energy-status related blood metabolites of primiparous (n=4) and multiparous (n=4) Holstein dairy cows in the first three weeks of lactation. Cows were grouped (2 + 2) based on body weight (BW) and body condition score (BCS) according to the system proposed by Edmonson et al. (1989). The mean BW was 598 ± 11 kg for primiparous and 619 ± 25 kg for the multiparous cows. The mean BCS was 3.44+0.06 for primiparous and 3.31 + 0.12 for the multiparous cows.

The cows were fed according to Estonian feeding standards. Both groups were given a basal diet containing concentrate feed (rapeseed cake – 270, wheat – 240, wheat bran – 101, corn – 90, corn feed – 85, soyabean meal – 80, sliced fodder beet – 60, barley – 50, salt – 9, palm oil – 8, limestone 5, and Premix Ko 2 g $kg^{-1}$), minerals, and forage silage (*ad libitum*) according to nutritional requirements. Concentrate feed and silage were fed separately. Silage was removed and measured before the subsequent milking. Twice weekly a sample of silage was taken for chemical composition analysis (AOAC, 2005). The treatment group was given an oral drench of 500 mL of crude glycerol (82.6% glycerol, 9.3% salts, 7.1% water, 0.6 crude fat, and 0.4% methanol) once a day just before the morning feeding. DMI and milk yield were recorded daily.

Milk and blood samples were collected on two consecutive days, once a week. Milk samples were combined proportionally according to milk yield, and kept at +4 °C until analysis on the following morning. The milk fat, protein, and lactose contents were measured at the Milk Analysis Laboratory of the Animal Recording Centre, using an automated infrared milk analyser (System 4000; Foss Electric, Hillerød, Denmark). Blood samples were taken, before administration of glycerol, from the coccygeal vein and were kept frozen at -22 °C till analysis.

Analyses of variance, t-tests and correlation analyses were used to evaluate the relationships between the different parameters (SAS Systems 9.1 and Excel statistical tools). To interpret the results, the following criteria of significance were used: significant ($p<0.05$), tendency ($p<0.1$), and not significant ($p>0.1$).

## Results and Discussion

Oral drenching of crude glycerol had no affect on BW change (p=0.331), nor for BCS change (p=0.536). However, multiparous cows lost less BCS (0.50) than primiparous cows (0.75) during the first three weeks of lactation. There were no effects of treatment on DMI (p=0.928), but there was an effect of lactation (p=0.029). There was a trend for a decrease of DMI in the third week in primiparous cows. Ogborn (2006) has found that cows in second or greater lactations showed a decreased postpartum DMI with glycerol as an oral drench for five days. Oral drenching of crude glycerol had a positive effect on milk yield (p=0.030); mean milk yields were 30.75 kg for control and 32.04 kg for treatment cows. Additionally, mean DMI and milk yields were higher in multiparous cows. As found in the previous study, Ogborn (2006), there was no change in milk and ECM yields after the short-term administration of glycerol. Energy corrected milk-yield (p=0.623), milk fat (p=0.89), and milk lactose contents (p=0.090) were not affected by treatment. Treatment cows from both parities had lower milk protein contents (p=0.01) than the control cows. The mean protein percentage in milk for primiparous cows in the control group was 3.57, and in treatment group – 3.20; in multiparous cows the values were 3.50 and 3.36 respectively. Treatment cows had a lower level of blood glucose (p=0.01) and insulin (p=0.01) concentrations. The mean concentration of blood glucose (mg $dL^{-1}$) for primiparous cows in the control group was 85.7, and in the treatment group – 75.6; in multiparous cows the values were 93.1 and 80.0 respectively. In contrast, both Linke et al. (2004) and Goff and Horst (2001) reported an increase in blood glucose levels after oral administration of glycerol. Ogborn (2006) found that short-term (5 days) oral drenching of glycerol had no effect on blood glucose, as the samples were collected at the same time as in the previous study. However, DeFrain et al. (2004) also detected a decrease in the blood plasma glucose concentration, and assumed that glycerol fed in the postpartum period was mostly used as an energy source by the rumen microorganisms, instead of entering the gluconeogenic pathways. Concentrations of blood nonesterified fatty acids were neither affected by treatment (p=0.622), nor parity (p=0.305).

## Conclusion

Long-term oral drenching of crude glycerol had no effect on DMI, but there was a positive effect on milk yield. Most milk composition values were not affected by treatment, but treatment groups had lower milk protein levels. Blood glucose and insulin concentrations declined with glycerol administration. The changes in measured parameters over time acted in the same way for both parities, as expected multiparous cows produced more milk as they consumed more dry matter.

## Acknowledgements

Founding was supported by the Bio-Competence Centre of Healthy Dairy Products (Kreutzwaldi 1, Tartu, Estonia). Appreciation is expressed to all personnel at the Department of Nutrition and Animal Products Quality, Estonian University of Life Sciences. This research was supported by the European Social Fund's Doctoral Studies and Internationalisation Programme DoRa.

## References

1. AOAC (2005) Official Methods of Analysis of AOAC INTERNATIONAL, 18th ed. Association of Official Analytical Chemists International, Gaithersburg, MD, USA, Available at: http://www.aoac.org/omarev1/front_18th_ed.pdf, 22 February 2011.
2. DeFrain J.M., Hippen A.R., Kalscheur K.F. and Jardon P.W. (2004) Feeding glycerol to transition dairy cows: effects on blood metabolites and lactation performance. *Journal of Dairy Science,* 87, pp. 4195-4206.
3. Edmonson A.J., Lean I.J., Weaver L., Farver T. and Webster G. (1989) Development of a Body Condition Scoring Chart. *Journal of Dairy Science,* 72, pp. 68-76.
4. Friggens N.C., Berg P., Theilgaard P., Korsgaard I.R., Ingvartsen K.L., Løvendahl P. and Jensen J. (2007) Breed and parity effects on energy balance profiles through lactation: Evidence of genetically driven body energy change. *Journal of Dairy Science*, 90. pp. 5291-5305.
5. Gallo L., Carnier P., Cassandro M., Mantovani R., Bailoni L., Contiero B. and Bittante G. (1996) Change in body condition score of Holstein cows as affected by parity and mature equivalent milk yield. *Journal of Dairy Science*, 79, pp. 1009-1015.
6. Goff J.P. and Horst R.L. (2001) Oral glycerol as an aid treatment of ketosis/fatty liver complex. *Journal of Dairy Science*, 84 (Suppl. 1), pp. 153.
7. Johnson R.B. (1951) New Methods of Treating Ketosis. A Preliminary Report. *The North American Veterinarian*, 31, pp. 327-332.
8. Johnson R.B. (1954) The treatment of ketosis with glycerol and propylene glycol. *Cornell Veterinarian,* 44, pp. 6-21.
9. Linke P.L., DeFrain J.M., Hippen A.R. and Jardon P.W. (2004) Ruminal and plasma responses in dairy cows to drenching or feeding glycerol. *Journal of Dairy Science*, 87 (Suppl. 1), pp. 343.
10. Ogborn K.L. (2006) Effects of method of delivery of glycerol on performance and metabolism of dairy cows during the transition period. MS Thesis. Cornell University, Ithaca, New York, U.S.A, 75 p.
11. Stokes S.R., Kaiser G.E., Goff J.P. and Brawley C.L. (2002) Effects of oral drenching of glycerol on blood parameters and milk production in dairy cattle at parturition. *Journal of Dairy Science*, vol. 85, (Suppl. 1), pp. 190.
12. Thompson J.C. and He B.B. (2006) Characterization of crude glycerol from biodiesel production from multiple feedstocks. *Applied Engineering in Agriculture*, vol. 22, pp. 261-265.

# RELATION BETWEEN MILK PROTEIN AND UREA CONTENT IN DIFFERENT FARMS

***Diana Ruska, Daina Jonkus***
*Latvia University of Agriculture*
*delta@e-apollo.lv*

## Abstract

Milk production and milk composition are of prime economic importance for farmers. It is well known in dairy management that the balanced feeding and holding technology is an important lever by which milk production and milk composition can be modified. The objectives of this work are to establish relation among milk protein and urea content in different farms. Four farms represent three cow breeds (Holstein Black and White, Latvian Brown, and cross breed XP). Individual cow milk samples (n=8400) were collected monthly from September 2009 to November 2010. Milk samples were analyzed for total protein, casein, fat, lactose, and urea content with instrumental infrared spectroscopy method. The average milk yield in farms was significantly different (from 26.8 to 16.0 kg per control day), and average protein content varied from 3.32 g $kg^{-1}$ to 3.62 g $kg^{-1}$. The urea content in cow milk was between 21.3 to 42.6 mg 100 $mL^{-1}$. The average protein content was higher and significantly ($p<0.05$) different in first yield level (up to 15 kg) for all farms. Overall, in different farm and milk yield levels correlation between protein and urea was low or very low. In the farm C, average urea content ranged between 30.0 and 60.0 mg 100 $mL^{-1}$, which indicated problems in feeding or management in the farm. It was established that milk productivity traits significantly ($p<0.05$) varied in farms with different dairy cow holding and feeding technologies and milk protein and urea content significantly ($p<0.05$) varied for cows with different milk yield per day.
**Key words**: dairy cow, milk yield, protein and urea content.

## Introduction

Milk is a complex biological fluid consisting of fats, proteins, minerals, vitamins, enzymes, and lactose. The composition of milk varies according to the breed, the genetic background of the animal, the stage of lactation, the nutritional quality of the animal's feed, the milking technology, and the incidence of disease such as mastitis and general environmental conditions (Coballero et al., 2003; Savickis et al., 2010).

The most important milk components for cheese and curd production are milk proteins. Now in Latvia the milk payment system is based on the content of total protein in milk, and on milk amount. Therefore task of the Latvian breeding programmes will be high milk yields with high protein content.

Normal bovine milk contains 30 to 35 g of protein $kg^{-1}$. Milk total proteins are composed of casein, whey proteins, and non-protein nitrogen (Depeters and Cant, 1992). The two principal types of milk proteins are caseins and whey proteins. Caseins constitute 76 to 86% of the total milk protein. Whey proteins represent 14 to 24% of milk proteins and are in solution in the serum phase of milk (Hui, 1993).

Researchers (Joudu et al., 2008) have found that the content of proteins and caseins in different breed's cow milk is different. Observe that Estonian Red breeds cow milk content highest protein and casein that Estonian Holstein breeds cow milk. On the contrary S.M. Carroll et al. (2006) did not find affect from Holstein, Jersy, and Brown Swiss cows breed or diet in milk total nitrogen and urea nitrogen content.

Urea is therefore a normal constituent of milk and comprises part of the nonprotein nitrogen fraction. Although opinions do vary to some extent, milk urea levels between 20 and 30 mg 100 $mL^{-1}$ are generally considered as normal for cow's milk. Urea accounts for roughly 50% of the non-protein nitrogen fraction in herd bulk milk of dairy cows, although this may vary from 35 to 65%. For milk from individual cows, this variation may be even larger (Bijgaart, 2003). The urea content may be used to monitor nutritional status of lactating dairy cows and improve dairy herd nutrition.

The objective of this study was to evaluate the relation between milk protein and urea content in different farms with different holding technologies.

## Materials and Methods

In the study, individual cow milk samples (n=8400) were collected monthly from four dairy farms (Farm A-D) from September 2009 to November 2010. Dairy herds represent three breeds: Holstein Black and White (HB), Latvian Brown (LB), and cross breed XP (cross breed from HB and LB). Average lactation number for cows in farm A was 2.37, farm B – 1.97, farm C – 3.47, and farm D – 2.16.

Dairy farms were with different number of animals in herds and with different milking and holding technologies. Farms A and C had a small (26 and 19 cows accordingly) number of animals and the traditional holding technology in the pasture-based seasonal dairying system. In these farms cows were managed in one feeding group. Whereas farms B and D were big farms (320 and 150 cows accordingly) with a balanced feeding and total mixed ration in all years without pasture period. Management in these farms was organized in feeding groups according to lactation stage. Milking frequency was two times per day. Farm B had one cow group with robotic milking. The herds were under official performance and pedigree recording.

The monthly control milk samples were analyzed for

total protein, casein, fat, lactose, and urea content. All parameters were analyzed in accredited milk quality laboratory SIA 'Piensaimnieku Laboratorija' with instrumental infrared spectroscopy method with FOSS instrument CombiFoss FC.

Data regarding breed of cows and date of milk analysis were available from monthly records of the herds from state agency "Agricultural Data Centre" program.

Control day milk yield was grouped into six levels: 1st – ≤ 15.0 kg, 2nd – from 15.0 to 19.9 kg, 3rd – from 20.0 to 24.9 kg, 4th – from 25.0 to 29.9 kg, 5th – from 30.0 to 34.9 kg, and 6th – 35 kg and more.

The statistical analyses were performed using SPSS program package and Microsoft Excel for Windows.

The obtained data were analyzed using descriptive statistics and Pearson correlation analysis. The significance of the differences between the samples was assessed using ANOVA.

## Results and Discussion

The study results were analyzed separately for each farm to evaluate cow milk productivity traits in the different farms (Table 1).

Table 1

**Average milk productivity and quality traits during the research**

| Traits | Farms | | | |
|---|---|---|---|---|
| | A (n=387) | B (n=5539) | C (n=280) | D (n=2194) |
| Milk yield, kg | 24.4±0.35$^{a}$ | 23.1±0.10$^{b}$ | 16.0±0.34$^{c}$ | 26.8±0.19$^{d}$ |
| Protein content, g kg$^{-1}$ | 3.32±0.021$^{a}$ | 3.62±0.006$^{b}$ | 3.61±0.031$^{b}$ | 3.49±0.009$^{c}$ |
| Casein content, g kg$^{-1}$ | 2.57±0.015$^{a}$ | 2.78±0.004$^{b}$ | 2.74±0.023$^{b}$ | 2.68±0.006$^{c}$ |
| Fat content, g kg$^{-1}$ | 4.14±0.042$^{a}$ | 4.55±0.014$^{b}$ | 4.49±0.053$^{b}$ | 4.09±0.016$^{a}$ |
| Lactose content, g kg$^{-1}$ | 4.70±0.009$^{a}$ | 4.81±0.002$^{b}$ | 4.69±0.016$^{a}$ | 4.78±0.005$^{c}$ |
| Urea content, mg 100 mL$^{-1}$ | 21.3±0.35$^{a}$ | 26.4±0.13$^{b}$ | 42.6±0.86$^{c}$ | 26.2±0.15$^{b}$ |

$^{a, b, c, d}$ – milk productivity and quality traits with unequal letter, difference significantly between farms ($p<0.05$)

Average cow milk yield in farms significantly differed (from 26.8 to 16.0 kg per control day). The lowest milk yield was in farm C with LB breed cows, where cows were managed in one feeding group. The highest milk yield was in farm D with several breeds' cows, from which HM breed cows predominated and management in this farm was organized in feeding groups according to lactation stage.

Between farms was statistical significantly difference in milk constitute and quality. The farm B cow milk had highest protein, fat, casein and lactose content (3.62 g kg$^{-1}$, 4.55 g kg$^{-1}$, 2.78 g kg$^{-1}$, and 4.81 g kg$^{-1}$). Farm B had LB and HB breed cows, and management in this farm was organized in feeding groups according to lactation stage.

The average cow milk protein (3.57 g kg$^{-1}$) and fat (4.41 g kg$^{-1}$) content for all farms was higher than average milk recording results in Latvia in the year 2010 (3.31 and 4.29 g kg$^{-1}$ accordingly).

The urea content in farms ranged between 21.3 and 42.6 mg 100 mL$^{-1}$. The average urea content in farm C was significantly higher (42 mg 100 mL$^{-1}$) than in other farms, which indicates problems in cow feeding balance and management. Also Lithuanian researchers (Savickis et al., 2010) have established influence to urea content in cow milk from farm.

The next in study were established that total protein and urea content in milk had influence from milk yield level and farms (Table 2).

Table 2

**Average protein and urea content in milk of cows with different milk yield per day**

| Farms | Traits | Milk yield, levels | | | | | |
|---|---|---|---|---|---|---|---|
| | | 1 (≤15.0) | 2 (15.0-19.9) | 3 (20.0-24.9) | 4 (25.0-29.9) | 5 (30.0-34.9) | 6 (35.0 ≥) |
| A | Protein, g kg$^{-1}$ | 3.93±0.077 $^{a}$ | 3.58±0.039 $^{b,A}$ | 3.32±0.029 $^{c,A}$ | 3.18±0.029 $^{d,A}$ | 3.03±0.040 $^{e,A}$ | 3.00±0.561$^{e,A}$ |
| | Urea, mg100 mL$^{-1}$ | 19.4±1.37 $^{A}$ | 20.5±0.72 $^{A}$ | 21.9±0.64 $^{A}$ | 21.2±0.72 $^{A}$ | 22.4±1.05 $^{A}$ | 22.2±1.43 $^{A}$ |
| B | Protein, g kg$^{-1}$ | 4.07±0.019$^{a, A}$ | 3.80±0.010 $^{b,B}$ | 3.58±0.088 $^{c,B}$ | 3.46±0.009 $^{d,B}$ | 3.34±0.011 $^{e,B}$ | 3.20±0.016 $^{f,B}$ |
| | Urea, mg100 mL$^{-1}$ | 23.3±0.31 $^{a,B}$ | 25.3±0.27 $^{b,B}$ | 26.3±0.25 $^{c,B}$ | 28.1±0.28 $^{d,B}$ | 27.5±0.37 $^{d,B}$ | 29.4±0.52 $^{e,B}$ |
| C | Protein, g kg$^{-1}$ | 3.91±0.043 $^{a,B}$ | 3.39±0.043 $^{b,C}$ | 3.29±0.032 $^{b,A}$ | 3.22±0.032 $^{b,A}$ | 3.40±0.204 $^{b,B}$ | - |
| | Urea, mg100 mL$^{-1}$ | 37.8±0.93 $^{a,C}$ | 44.8±1.65 $^{b,C}$ | 45.4±2.31 $^{b,C}$ | 57.2±4.17 $^{c,C}$ | 63.9±3.54 $^{c,C}$ | - |
| D | Protein, g kg$^{-1}$ | 3.95±0.039$^{a,B}$ | 3.72±0.019 $^{b,D}$ | 3.56±0.017 $^{c,B}$ | 3.44±0.015 $^{d,B}$ | 3.33±0.016 $^{e,B}$ | 3.21±0.013 $^{f,B}$ |
| | Urea, mg100 mL$^{-1}$ | 23.3±0.49 $^{a,B}$ | 24.4±0.39 $^{a,B}$ | 26.2±0.32 $^{b,c,B}$ | 27.2±0.31 $^{c,B}$ | 27.4±0.36 $^{c,B}$ | 26.7±0.31 $^{c,C}$ |

$^{a, b, c, d, e, f}$ – milk productivity and quality traits with unequal letter, difference significantly between milk yield levels group ($p<0.05$);

$^{A, B, C, D}$ – milk productivity and quality traits with unequal letter, difference significantly between farms ($p<0.05$).

The average protein content was higher and significantly different in first milk yield level in all farms. Observed decrease in second milk yield level (15.0 – 19.9 kg) of the average protein content, but it was highest than third till sixth milk yield level and significantly different in farms A, B and D. A significant difference between six yield levels was establish in farms B and D where higher average cow milk protein content (4.07 and 3.95 g kg$^{-1}$ accordingly) was in first milk yield level, gradually decreasing to sixth milk yield level.

The milk urea content was significantly different in farms A and C in all milk yield levels, but no difference was observed of cow milk in farm B and D. Difference in milk urea content between milk yield levels was not observed in farm A. It should be pointed out that the average milk urea content had tendency to increase in all study farms from first milk yield level (≤15.0 kg) to the sixth (35.0 kg and more). The results of this study confirm previous researcher (Oltner et al., 1985) that milk yield still higher than milk urea content increase.

To evaluate relation between cow milk protein and urea content was estimated correlation in all farms and milk yield levels (Table 3).

Overall, in different farm and milk yield levels correlation between cow milk protein and urea content was low or very low. Average closely negative significant correlation (-0.491) was in farm A for cows with milk yield level up to 15 kg. These are stronger correlations than those reported by J.D. Ferguson et al. (1997) -0.138 for milk protein content. In farms B and D, for the same milk yield level, a very low significantly positive correlation (0.129 and 0.229 accordingly) was observed. Correlation was closely negative (-0.449) in farm C with milk yield level from 30 to 35 kg.

Table 3

**Correlation between cow milk protein and urea content in farm and milk yield level**

| Farms | Milk yield, levels | | | | | |
|---|---|---|---|---|---|---|
| | 1 (≤15.0) | 2 (15.0-19.9) | 3 (20.0-24.9) | 4 (25.0-29.9) | 5 (30.0-34.9) | 6 (35.0 ≥) |
| A | -0.491** | -0.097 | 0.080 | 0.053 | 0.138 | 0.013 |
| B | 0.129** | -0.030 | 0.041 | 0.028 | 0.011 | 0.012 |
| C | 0.075 | 0.146 | 0.353* | -0.341 | -0.449 | - |
| D | 0.229** | 0.154** | 0.021 | 0.014 | -0.034 | -0.017 |

*$p<0.05$, **$p<0.01$.

Researchers (Eicher et al., 1999) have observed the relationships between milk urea and protein content in respect of the factors parity, daily milk yield and days postpartum also vary considerably among herds. E.Z.M. Oudah (2008) confirms a very low negative correlation among milk protein and urea content in the lower test-day

milk yield. Canadian researchers have found negative relationships between milk protein and milk urea nitrogen content in dairy cow milk (Arunvipas et al., 2003). Whereas W. Richardt (2002) has established positive correlation among milk protein and urea content in the cow milk.

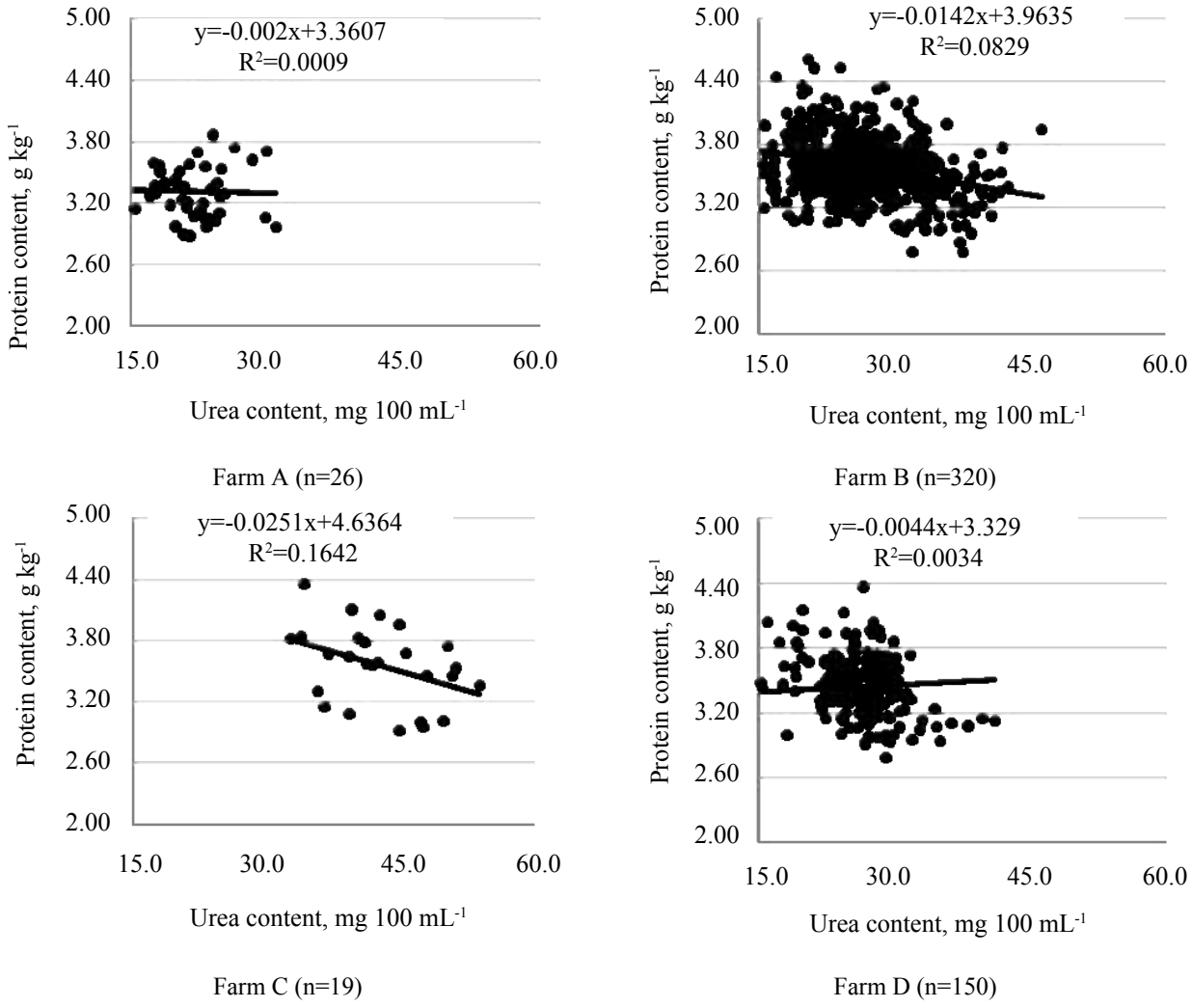



Figure 1. Relationship between average individual cow milk protein and urea content during research.

Many researchers (Depeters and Cant, 1992; Bijgaart, 2003) confirm that normal milk urea content in milk is from 15.0 to 30.0 mg 100 $mL^{-1}$. The measurements of milk urea content could be used to assess the adequacy of protein feeding in dairy cows and the efficiency of N utilization for milk production (Jonker et al., 1998; 2002; Nousiainen et al., 2004). Basing on measurements from each farm and average cow milk protein and urea content for each cow in a farm, the relationship between them was estimated. In farm C, average milk urea content varied between 30.0 and 60.0 mg 100 $mL^{-1}$, which indicates problems in feeding or management in the farm (Figure 1). In farm B, significantly influence milk protein content from milk urea content varied that suggest regression analyze ($R^2$=0.0829).

## Conclusions

1. It was established that milk productivity traits significantly ($p<0.05$) varied in farms with different dairy cow holding and feeding technologies.
2. In farms A, B, and D, milk urea content was not higher than the allowable level (from 15.0 to 30.0 mg 100 $ml^{-1}$) which suggests about balanced feeding or good management in the farm.
3. The milk protein and urea content significantly ($p<0.05$) varied for cows with different milk yield per day the cow with the highest milk yield had the lowest protein and highest milk urea content.
4. The evaluation between milk protein and urea content were significant from low negative (r=-0.491) to low positive (r=0.353) was established.
5. In farm B, variations in milk urea content significantly influenced changes in milk protein content, which was confirmed by regression analysis ($R^2$=0.0829).

**Acknowledgements**

This study was supported by ESF project agreement No. 2009/0180/1DP/1.1.2.1.2/09/IPIA/VIAA/017, contract No. 04.4-08/EF2.D2.26.

**References**

1. Arunvipas P., Dohoo I.R., Van Leeuwen J.A., Keefe G.P. (2003) The effect of non-nutritional factors on milk urea nitrogen levels in dairy cows in Prince Edward Island, Canada. *Preventive veterinary medicine*, 59, pp. 83-93.
2. Bijgaart H. van den (2003) Urea. New applications of mid-infra-red spectrometry. *Bulletin of the IDF* 383, pp. 5-15.
3. Carroll S.M., Depeters E.J., Taylor S.J., Rosenberg M., Perez-Monti H., Capps V.A. (2006) Milk composition of Holstein, Jersy, and Brown Swiss cows in response to increasing levels of dietary fat. *Animal Feed Science and Technology*, 131, pp. 451-473.
4. Coballero B., Truco L., Finglas P. (2003) Encyclopedia of food sciences and nutrition. *Elsever Science*, 6, pp. 3947-3949.
5. Depeters E.J., Cant J.P. (1992) Nutritional factors influencing the nitrogen composition of bovine milk - a review. *Journal Dairy Science,* 75, pp. 2043-2070.
6. Eicher R., Bouchard E., Bigras-Poulin M. (1999) Factors affecting milk urea nitrogen and protein concentrations in Quebec dairy cows. *Preventive Veterinary Medicine*, 39, pp. 53-63.
7. Ferguson J.D., Thomsen N., Slesser D., Burris D. (1997) Pennsylvania DHIA milk urea testing. *Journal Dairy Science,* 80, (Suppl. 1), pp. 161.
8. Hui Y.H. (1993) *Dairy Science and Technology Handbook: Principles and Properties.* Volume 1, 404 p.
9. Jonker J.S., Kohn R.A., Erdman R.A. (1998) Using milk urea nitrogen excretion and utilization effiency in lactating dairy cows. *Journal Dairy Science*, 81, pp. 2681-2692.
10. Jonker J.S., Kohn R.A., High J. (2002) Use of milk urea nitrogen to improve dairy cow diets. *Journal Dairy Science*, 85, pp. 939-946.
11. Joudu I., Henno M., Kaart T., Pussa T., Kart O. (2008) The effect of milk protein contents on the rennet coagulation properties of milk from individual dairy cows. *International Dairy Journal*, 18, pp. 964-967.
12. Nousiainen J., Shingfield K.J., Huhtanen P. (2004) Evaluation of milk urea nitrogen as a diagnostic of protein feeding. *Journal Dairy Science,* 87, pp. 386-398.
13. Oltner R., Emanuelson M., Wiktorsson H. (1985) Urea concentrations in milk in relation to milk yield, live weight, lactation number and amount and composition of feed given to dairy cows. *Livestock Production Science*, 12, pp. 47-57.
14. Oudah E.Z.M. (2008) Phenotypic relationships among somatic cell count, milk urea content, test-day milk yield and milk protein percent in dairy cattle. Available at: http://www.lrrd.org/lrrd20/8/ouda20132.htm, 26 October 2009.
15. Savickis S., Juozaitiene V., Juozaitis A., Zilaitis V., Sederevicius A., Sauliunas G. (2010) Influence of genetic and non-genetic factors on milk urea of cows. *Veterinarija ir zootechnika*, 50, pp. 81-87.
16. Richardt W. (2002) Neue Grenzwerte für den Milchharnstoffgehalt – Entwicklung eines dynamischen Modells (New limits on the milk urea content - the development of a dynamic model). Available at: http://www.portal-rind.de/index.php?name=News&file=article&sid=28, 26 October 2009. (in German).

# ENVIRONMENTAL FACTORS AFFECTING PERFORMANCE TRAITS IN LATVIAN SHEEP POPULATION

***Anita Puste[1], Daina Kairiša[1], Rita Sarma[2]***
*[1]Latvia University of Agriculture*
*[2]State agency 'Agricultural Data Centre'*
*Anita.Puste@ldc.gov.lv*

## Abstract

This study investigates different environmental effects on birth weight, weaning weight, and pre-weaning daily gain using different statistical models in the Latvian sheep population. The coefficients of determination ($R^2$) were used to estimate the extent of different non-genetic factors influencing birth weight, weaning weight, and pre-weaning daily gain. Data were collected of 4460 lambs born in 36 herds during 2008 to 2010. The birth weight (on average 4.1 ± 0.01 kg), weaning weight (on average 21.0 ± 0.07 kg) and pre-weaning daily gain (on average 242.1 ± 0.93 g) was significantly affected by type of birth and sex, lambing age of dam (covariate) and herd, year, season of birth ($p<0.01$). Coefficients of determination were estimated from 0.14 to 0.42 for birth weight, from 0.13 to 0.49 for weaning weight, and from 0.10 to 0.46 for pre-weaning daily gain. The results show that when the fixed effects of HYS (interaction between the herd, year, and season of birth) were included it improved the quality of the statistical model most of all, because higher determination coefficients were obtained for all traits: 0.42 for birth weight, 0.49 for weaning weight, and 0.46 for pre-weaning daily gain.

**Key words**: weight, daily gain, performance traits, environmental factors.

## Introduction

Sheep farming is one of the oldest agricultural branches in Latvia. Historically, the main goal was to provide country with a good quality of sheep (particularly lamb) meat, also to produce wool and fur. Today, the sheep breeding becomes more and more popular, because it is a complementary component of a mixed farming system. The high fertility and short generation intervals make it popular among breeders.

Economically important traits in the sheep industry are birth weight, weaning weight, and pre-weaning daily gain. These traits are influenced by several genetic and environmental factors (sex of lamb, type of birth, seasonal variation during different years). Environmental factors influence the estimation of breeding value. Investigation and determination of environmental factors that have effect on traits and correction of records for these factors cause estimated genetic parameters and breeding value to show animal's genetic potential (Rashidi et al., 2008). The significant influences of environmental factors on body weight at the various ages can be explained in part by differences in year, male and female endocrine system, limited uterine space and inadequate availability of nutrients during pregnancy, competition of milk between twins, maternal effects and maternal ability of dam in different ages (Mohammadi et al., 2010).

A. Hussain (2006) found that the influence of year of birth, sex, and type of birth on birth weight was significant. The age of dam (covariate) affected birth weight of lambs and was significant ($p<0.01$) too. Also S.Alipour and M.A.Edriss (1997) reported that there were highly significant year and sex effects on body lamb weight at birth.

S.Alipour and M.A.Edriss (1997) reported that year of birth, sex, age of dam, and type of birth significantly affected weaning weight. P.Akhtar et al. (2001) reported that the effect of type of birth of lamb and age of the dam on weaning weight was not significant.

According to P.Akhtar et al. (2001) and A.Hussain (2006), pre-weaning daily gain was affected significantly by year and season of birth, and sex of lamb ($p<0.01$). However, G.D.Snowder and L.D.Van Vleck (2003) reported that type of birth, sex of lamb, and age of dam had no significant effect on pre-weaning daily gain.

The influence of environmental factors on the productivity of sheep has not been widely studied in Latvia, therefore included it in our study.

The aim of the study was to determine the influence of environmental factors on three performance traits using different statistical models in the Latvian sheep population.

## Material and Methods

The data for this study were collected from the Latvian State agency 'Agricultural Data Centre' which is responsible for the data processing of the sheep recording results. Performance traits examined in the present study were birth weight, weaning weight (weight at 70 days of age), and pre-weaning daily gain (the average daily gain from birth to weaning). Recording data collected from 4460 lambs located in 36 herds (on average 6 to 582 lambs in one herd). Animals were born in the period from 2008 to 2010 (79% of lambs born in 2010, of witch 86% of lambs were born in first four months of the year). The twinning rate in this data set was 70%, but 20% of all lambs born as singles. The sex ratio males and females was 49.6:50.4, respectively. Latvian dark-head sheep breed is the main sheep breed in Latvia, and 94% of all lambs of this study

belong to this breed. Other breeds of the present study are Oxforddown, Suffolk, and German Merino Local, since these breeds have been more recently used like sire breeds to improve quality of lamb in Latvia.

Each observed value of the trait was assumed to the different statistical model. As shown in Table 1, interaction between the birth type of lamb and sex as the fixed effect and lambing age of dam as covariate is included in all statistical models: the fixed herd of birth effect in the 1st model, the fixed herd×year (interaction between the herd and the year of birth) effect in the 2nd model, the fixed HYS (interaction between the herd, the year and the season of birth) effect in the 3rd model, and the fixed TOB (birth type of lamb)×sex effect in the 4th model.

Table 1

**Gradation classes of fixed factors and covariates**

| Symbol | Factors (fixed or covariates) included in the models | Number of gradation classes |
|---|---|---|
| (Herd) | the herd of birth (fixed) | 36 (2nd model) |
| (Herd×Year) | interaction between the herd and year of birth (fixed) | 59 (3rd model) |
| (HYS) | interaction between the herd, year and season of birth (fixed) | 92 (4th model) |
| (TOB×Sex) | interaction between the birth type of lamb and sex (fixed) | 6 (all models) |
| (Age) | calving age of dam (covariate) | 4 (all models) |

The year of birth was divided into 2 classes (year1st 2008/2009 birth; 2nd birth year 2010). The birth year was divided into 2 seasons (1st season from October to March, 2nd season from April to September). The age of dam (13 – 123 months) was divided into 4 classes.

The quality of the model was determined by calculating the determination coefficient (CD), using the GLM procedure of SAS statistical package (SAS, 1998).

## Results and Discussion

The results of this study showed that economically important traits in sheep breeding are birth weight, weaning weight, and pre-weaning daily gain. These traits are affected not only by genetic factors but also by environmental factors as herd, sex, age, season, age of dam, type of birth, etc**.** Therefore, it is essential to estimate the extent of all such factors so that the genetic variation among animals can be used to design breeding plans for the improvement.

Mean performance values for three traits are summarized in Table 2. The results showed that the average birth weight was 4.1 ± 0.01 kg, the weaning weight was 21.0 ± 0.07 kg, and the pre-weaning daily gain was 242.1 ± 0.93 g.

The lambs born in 2010 were slightly heavier (4.1 ± 0.01 kg) than the lambs born in years 2008 and 2009 (4.10 ± 0.013 kg) ($p<0.01$). The lambs born during autumn season were heavier (4.2 ± 0.03 kg) than the lambs born during the spring season (4.1 ± 0.01 kg) ($p<0.01$). Lambs born as single were heavier (4.6 ± 0.03 kg) than twins (4.0 ± 0.01 kg) and triplets (3.7 ± 0.03 kg) ($p<0.01$). The male lambs were also heavier than females. The average birth weight for male lambs was 4.2 ± 0.02 kg, and for females – 4.0 ± 0.02 kg ($p<0.01$).

The birth weight of lambs varied significantly depending on the type of birth, and the sex of the lamb born. This is due to the reason that lambs born as single have to better opportunities in the dam's uterus than the multiple births. Male lambs are heavier than female lambs, witch is due to the reason that the gestation period for male lambs is slightly longer (1-2 days) as compared to female lambs (Hussain, 2006).

Weaning weight of the lamb indicates the mothering ability of dam as well the growth potential inherited by the lamb. The highest value for weaning weight (21.8 ± 0.17 kg) was in 2008/2009, while lower (20.8 ± 0.08 kg) was in 2010. The data revealed that the autumn-born lambs weighed heavier (23.2 ± 0.18 kg) than spring born lambs (20.6 ± 0.07 kg) ($p<0.01$). Weaning single-born lambs were heavier at (24.0 ± 0.17 kg) than twins (20.4 ± 0.08 kg) and triplets (19.5 ± 0.19) ($p<0.01$). Similarly, the male lambs were also heavier than the females (21.6 ± 0.10 vs. 20.5 ± 0.09 kg) ($p<0.01$).

The finding of L.Carrillo and J.U.Segura (1993) also reported that the male lambs weighed more than female lambs at weaning, but single-born lambs were heavier than twins and triplets. A.Hussain (2006) analyzed variation of weaning weight in different studies and reported that wide variation observed in the weaning weight of sheep suggested that there is a scope for improvement in this trait by adopting different breeding methods.

The pre-weaning daily gain ranged from 239.1 ± 1.00 g (in 2010) to 253.2 ± 2.23 g (in 2008/2009). Autumn-born lambs have a higher (271.3 ± 2.44 g) pre-weaning daily gain than spring-born lambs (236.4 ± 0.97 g) ($p<0.01$). Single-born lambs also have a higher pre-weaning daily gain (277.9 ± 2.16 g) than twins (234.5 ± 1.05 g) and triplets (226.5 ± 2.60 g). Male lambs have a higher pre-weaning daily gain (248.8 ± 1.40 g) than female lambs (235.5 ± 1.21 g) ($p<0.01$).

M.Nawaz and M.K.Ahmad (1998) reported that single lambs grew 23% faster than twins, and twin lambs grew 11% faster than triplets. The superiority of singles over triplets was 37% in the pre-weaning daily weight gain. Spring-born lambs grew 33% faster ($p<0.01$) than autumn-born lambs, but males grew 14% faster than females ($p<0.01$). A.Hussain (2006) pointed out that the contradictions in different studies may be due to different breeds of sheep, as well as, climatically and ecological differences where sheep farming had been practiced.

Table 2

**Mean performance values for three traits**

| Factors | Gradation classes | n | Performance traits ($\bar{x} \pm s_{\bar{x}}$) | | |
|---|---|---|---|---|---|
| | | | birth weight (kg) | weaning weight (kg) | pre-weaning daily gain (g) |
| Year of birth | 2008/2009 | 936 | $4.0 \pm 0.03^{a}$ | $21.8 \pm 0.17$ | $253.2 \pm 2.23$ |
| | 2010 | 3524 | $4.1 \pm 0.01^{a}$ | $20.8 \pm 0.08$ | $239.1 \pm 1.01$ |
| Season of birth | 1: spring | 3727 | $4.1 \pm 0.01^{b}$ | $20.6 \pm 0.07^{b}$ | $236.4 \pm 0.97^{b}$ |
| | 2: autumn | 733 | $4.2 \pm 0.03^{b}$ | $23.2 \pm 0.18^{b}$ | $271.3 \pm 2.44^{b}$ |
| Birth type | Singe | 870 | $4.6 \pm 0.03^{c}$ | $24.0 \pm 0.16^{c}$ | $277.9 \pm 2.16$ |
| | Twin | 3122 | $4.0 \pm 0.01^{c}$ | $20.4 \pm 0.08^{c}$ | $234.5 \pm 1.05$ |
| | Triplet | 468 | $3.7 \pm 0.03^{c}$ | $19.5 \pm 0.19^{c}$ | $226.5 \pm 2.60$ |
| Sex | Male | 2212 | $4.2 \pm 0.02^{d}$ | $21.6 \pm 0.10^{d}$ | $248.8 \pm 1.40^{d}$ |
| | Female | 2248 | $4.0 \pm 0.02^{d}$ | $20.5 \pm 0.09^{d}$ | $235.5 \pm 1.21^{d}$ |
| On average: | | 4460 | $4.1 \pm 0.01$ | $21.0 \pm 0.07$ | $242.1 \pm 0.93$ |

[a, b, c, d,] – Performance traits marked with identical letter differ significantly ($p<0.01$) between the birth years ([a]), birth season ([b]), birth type ([c]), or sex ([d])

The interaction effect between the birth type of lamb and sex, the effect of lambing age of dam and the effect of the herd of birth or the interaction effect between the herd and year of birth or the interaction effect between the herd, year and season of birth on birth weight, weaning weight and pre-weaning daily gain were significant in all cases ($p<0.01$).

The significant effect of year, sex, birth type and age of dam on the birth weight of lambs as obtained in the present study agrees with the findings of other researchers (Hussain, 2006; Babar et al., 2004; Alipour and Edriss, 1997).

The present study only partially confirmed investigations of S.Alipour and M.A.Edriss (1997) that year of birth, sex, age of dam and type of birth significantly affected the weaning weight, and research of P.Akhtar et al. (2001) that the effect of type of birth of the lamb and age of the dam on the weaning weight was not significant.

The greatest effect on the pre-weaning daily gain was significantly observed by year and season of birth and sex of lamb ($p<0.01$) thus confirming findings of P.Akhtar et al. (2001) and A.Hussain (2006).

Coefficients of determination were estimated from 0.14 to 0.42 for birth weight, from 0.13 to 0.49 for weaning weight, and from 0.10 to 0.46 for pre-weaning daily gain using different statistical models (Table 3).

Table 3

**Coefficient of determination ($R^2$) for performance traits**

| Information about different models | $R^2$ | | |
|---|---|---|---|
| fixed effects and covariates | birth weight | weaning weight | pre-weaning daily gain |
| (TOB×Sex) + (Age) | 0.14 | 0.13 | 0.10 |
| (TOB×Sex) + (Age) + (Herd) | 0.38 | 0.44 | 0.40 |
| (TOB×Sex) + (Age) + (Herd*Year) | 0.41 | 0.46 | 0.43 |
| (TOB×Sex) + (Age) + (HYS) | 0.42 | 0.49 | 0.46 |

The higher determination coefficients for all performance traits were obtained when the interaction effect between the herd, year and season of birth was included in the statistical model. Although the largest part of the lambs were born during spring the season, it is possible to improve the quality of the statistical model.

The results of this study confirmed that environmental factors are significant sources of variation for different performance traits and therefore play an important role in expression of genetic potential. Therefore, effects of environmental factors need to be considered for estimation of the breeding value.

## Conclusions

1. The data revealed that the male lambs weighed more than female lambs at birth ($4.2 \pm 0.02$ kg) and weaning ($21.6 \pm 0.10$ kg), and they had a higher pre-weaning daily gain ($248.8 \pm 1.40$ g) ($p<0.01$). Single-born lambs also were heavier than twins and triplets and had a higher pre-weaning daily gain: the average birth weight was $4.6 \pm 0.03$ kg ($p<0.01$), weaning weight – $24.0 \pm 0.16$ kg ($p<0.01$), and pre-weaning daily gain – $277.9 \pm 2.16$ g.
2. The results showed that the birth weight, weaning weight, and pre-weaning daily gain was significantly influenced by various environmental factors: type of

birth and sex, lambing age of dam and herd, season of birth ($p<0.01$).

3. The highest determination coefficients were obtained using the statistical model with fixed HYS (interaction between the herd, the year, and the season of birth) effect (0.42, 0.49, and 0.46, respectively for birth weight, weaning weight, and pre-weaning daily gain).

## References

1. Akhtar P., Ahmad Z., Mohiuddin G., Ali S. and Javed K. (2001) Environmental factors affecting preweaning growth traits of Hissardale sheep in istan. *Pakistan Journal of Veterinary*, 21 (1), pp. 17-21.
2. Alipour S. and Edriss M.A. (1997) Estimation of genetic and environmental factors affecting some of the economical traits in Fars Karakul (Gray) breed of sheep. *Jornal of Agriculture Sciences – Islamic Azad University***,** 3 (11/12), pp. 69-77.
3. Babar M.E., Ahmad Z., Nadeem A. and Yaqoob M. (2004) Environmental factors affecting birth weight in Lohi sheep. *Pakistan Journal of Veterinary*, 24 (1), pp. 5-8.
4. Carrillo L. and Segura J.C. (1993) Environmental and genetic effects on pre-weaning growth performance of Hair sheep in Mexico. *Tropical Animal Health and Production*, 25 (3), pp. 173-178.
5. Hussain A. (2006) *Genetic evaluation of Thalli sheep in Pakistan*. Ph.D. Thesis. University of Agriculture, Pakistan, 130 p.
6. Mohammadi K., Beygi Nassiri M.T., Fayazi J. and Roshanfekr H. (2010) Effects of Environmental Factors on Pre-Weaning Growth Traits in Zandi Lambs. *Journal of Animal and Veterinary Advances*, 9 (5), pp. 903-906.
7. Nawaz M. and Ahmad M.K. (1998) Comparison of Lohi and crossbred ewes: Productive and reproductive traits. *Proceedings of the 6th World Congress on Genetics Applied to Livestock Production*, Armidale, NSW, Australia, 24, pp. 185-188.
8. Rashigi A., Mokhtari M.S., Jahanshahi A.S. and Abadi M.R.M. (2008) Genetic parameter estimates of pre-weaning growth traits in Kermani sheep. *Small Ruminant Research*, 74, pp. 165-171.
9. SAS 1998 (1998) SAS User's guide SAS Institute Inc., Gary, North Carolina. http://support.sas.com/documentation/cdl/en/statugsurvival/61761/PDF/default/statugsurvival.pdf, 9 March 2011.
10. Snowder G.D. and Van Vleck L.D. (2003) Estimates of Genetic parameters and selection strategies to improve the economic efficiency of postweaning growth in lambs. *Journal of Animal Science***,** 81, pp. 2704-2713.

# THE INFLUENCE OF COW FEED ENRICHED WITH CARROTS ON MILK QUALITY AND NUTRITIONAL VALUE

***Unigunde Antone[1,2], Vita Šterna[2], Jeļena Zagorska[1]***
*[1]Latvia University of Agriculture*
*[2]Agency of the Latvia University of Agriculture 'Research Institute of Biotechnology and Veterinary Medicine 'Sigra"*
*u.antone@gmail.com; vita.sterna@inbox.lv; jelena.zagorska@llu.lv*

## Abstract

β-Carotene (BC) and α-tocopherol in milk fat have positive implications in human nutrition, besides the specifically protecting polyunsaturated fatty acids from oxidation. To determine the possible effect on some milk components, nutritional value and lipid stability of including carrots in the diet of lactating cows, ten cows were selected in a dairy farm and divided into 2 groups (control and experimental) by 5 cows in each. In experimental group's diet additionally 7 kg carrots per cow per day were included. Individual cow milk samples were obtained 1 day before feed enrichment, in days 7, 24, 35, 42 (during the feed enrichment), and 1 week after feed enrichment. Analyses of milk fat, protein content, somatic cell count (SCC), retinol, BC, vitamin C, tocopherols, immunoglobulins (Ig), lysozyme, fatty acids (FA) were made, and milk yield was measured. Supplying cow diet with carrots showed a tendency to improve milk quality by promoting the faster decrease of SCC, and significantly ($p<0.05$) increasing Ig and lysozyme content, thus potentially improving milk nutritional value. The vitamin content rose significantly ($p<0.05$) by retinol and tocopherol contents, but β-carotene content increase in milk was not observed. Also milk fatty acid (FA) stability changes during 5 day storage in temperature of 4 - 6 °C were not observed.

**Key words**: dairy products, nutritional value, antioxidants, carotenoids, forages.

## Introduction

The oxidative and lipolytic stability of milk and dairy products are of concern to the dairy industry. The deterioration processes can result in strong off-flavours and in decreased nutritional quality of dairy products, making them unacceptable to consumers. The oxidative stability of milk and dairy products is the result of delicate balance between anti- and pro-oxidative processes in milk influenced by factors such as degree of FA unsaturation, content of antioxidants as tocopherols and carotenoids, dissolved oxygen, storage temperature, metal prooxidants (especially copper) (Bergamo et al., 2003; Havemose et al., 2004; Smet et al., 2008; Żegarska, 2003). Carotenoids besides their well-known function as vitamin A precursors have other valuable properties. These properties include their anti-oxidant functions (radical and singlet oxygen quenching), role in cell differentiation, precursor of nuclear receptor ligand, and induction of cell-communication (Hulshof et al., 2006).

Concerning milk nutritional value, milk fat is one of the main sources of retinol, BC, vitamin E in the human diet; better absorption of BC from milk than from many other foods is also known (Swensson et al., 2007; Żegarska, 2003). Higher levels of α-tocopherol and BC in milk fat have positive implications in human nutrition: besides the specifically protecting polyunsaturated FA, it reduces cholesterol oxidation, and, therefore, its cytotoxicity and atherogenecity (Bergamo et al., 2003).

Carotenoids are abundant in plant material and appear in milk as the result of their ingestion by cow. There are often seasonal and regional variations of retinol and carotenoid concentrations, which reflect the nature of the forage used. However, BC is easily oxidized and concentrations decrease quickly during storage. Thus, to achieve a high-concentration of BC in milk, the importance of a cow diet with high-quality roughage, especially silage or pasture, cannot be underestimated (Swensson et al., 2007). Since animals cannot synthesize carotenoids and animal feed is generally poor in carotenoids, about 30 - 120 ppm of total carotenoids, are added to animal feed to improve animal health, enhance meat colour and quality, and increase vitamin A levels in milk and meat (Ananda and Vadlani, 2010). Animals in transition period or challenged with stress may benefit of a feeding strategy including antioxidants to inhibit free radical attacks and enhance the antioxidant status. High producing dairy cows are prone to oxidative stress, and the situation can be exacerbated under certain environmental, physiological, and dietary conditions. However, specific feeding strategies may contribute to enhance immunity and the antioxidant status (Petit, 2009). It has been demonstrated that carotenoids and retinol are able to reduce mastitis in dairy cows although the effect of BC was not systematic. Carotenoids also have a positive role in fertility independent of the role of retinol (Chew, 1995; Weiss, 2010).

The spontaneous oxidized flavour (SOF) of milk has been known for many years, predominantly as a seasonal problem arising in spring. It tends to occur in well-managed, high-yielding dairy herds. It has been suggested that the susceptibility of milk to SOF depends on the balance between α-tocopherol, BC and the polyunsaturated FA in the neutral lipids and phospholipids (Żegarska, 2003). Several reports have highlighted the beneficial action of α-tocopherol supplementation in cows feed on spontaneous oxidized flavour-related problems in milk,

but the influence of carotenoids is not as clearly defined (Nozière et al., 2006).

Carrot roots are rich source of carotenoids. Carotenes are the main representatives of carotenoids in carrot roots and constitute about 5.33 mg 100 $g^{-1}$ (Kotecha et al., 1998) or BC - 5.650 – 16.300 mg 100 $g^{-1}$ (Danish Food Composition Databank, 2009). The proportions of individual carotenoids reported are BC (45 - 80 g 100 $g^{-1}$), α-carotene (15 - 40 mg 100 $g^{-1}$), γ-carotene (2 - 10 mg 100 $g^{-1}$), and others (3 - 6 mg 100 $g^{-1}$) from the sum of all carotenoids. Thiamine, riboflavin, niacin, folic acid, and vitamin C are also present in appreciable amounts in carrot roots. Carrots are also good sources of carbohydrates and minerals like calcium, phosphorus, iron, and magnesium (Kotecha et al., 1998). In absorption of carotenoids as fat soluble constituents the presence of lipids is important. Fats and oils have been included in concentrate mixtures for dairy cows principally to increase the energy density of the diet, enabling high yielding cows in early lactation to attain their full milk yield potential. More recently lipids were included in an attempt to alter the fatty acid (FA) composition of milk fat, thereby improving its nutritional and physical properties. However, lipids, especially those containing polyunsaturated FA, have an adverse effect on rumen microflora and fibre digestibility, and at high levels in the diet usually result in reduced yields of milk and milk constituents. In practical diets the levels of fat added to concentrates generally do not exceed 5 g 100 $g^{-1}$ (Murphy et al., 1990).

The aim of this study was to assess the influence of cow feed enrichment with carotenoids on milk quality, nutritional value and lipid stability.

## Materials and Methods

***Experimental design***. Ten cows were selected in a conventional dairy farm "Strautiņi" and divided into control group (CG) and experimental group (EG) by 5 cows in each. Cow breed (Holstein, Latvian Brown), lactation number (3 - 5), stage of lactation and productivity were as similar as possible in both groups. The basic feed was equal in both groups, i.e. silage was fed to ad libitum and rapeseed animal feed – 2 kg per cow per day. In the EG diet additionally 7 kg carrots per cow per day were included (Table 1). Both groups also received rapeseed oil 100 g per cow per day, mixed in dry forage for better carotenoid absorption. The carotenoids content in cow feed is given in Table 1.

Table 1

**The characterization of cow feed**

| Cow groups | Basic feed | Additional feed | Content of carotenoids, mg per cow per day | | |
|---|---|---|---|---|---|
| | | | β-carotene | α-carotene | Total carotenes |
| Control | Silage – ad libitum; rapeseed animal meal – 2 kg per cow per day | Rapeseed oil – 100 g per cow per day | 207 | - | 225 |
| Experimental | | Carrots – 7 kg, rapeseed oil – 100 g per cow per day | 1090 | 221 | 1325 |

***Milk sample collection and storage***. Individual cow milk samples were obtained in days 0 (1 day before additional feed enriched with carotenoids was administered to the herd), 7, 24, 35, 42 (during the feed enrichment), and 1 week after special feeding (day 56) from afternoon milking. Equal amounts of each cow's milk were pooled together getting 1 sample of each group that were immediately cooled to 4 - 8 °C temperature and transported to the laboratory next morning. Milk was stored at 4 - 6 °C temperature until analyses of milk fat, protein, SCC, and FA, or frozen at – 18 °C temperature until analyses of retinol, α- and γ-tocopherols, BC, Ig, and lysozyme.

***Feed analyses***. Total carotene content in feed was determined by the extraction of carotene from the sample (3 g) with petroleum ether (50 ml) and measuring the concentration on the photometer FEK-56 M by the wave length 450 nm, in accordance with ГОСТ 13496.17-95 method.

The extraction of total lipids from feed for analyses of α- and β- carotene concentration was performed by the method of Hara and Radin, 1978. Concentration of α- and β- carotene in feed was determined by HPLC using the technique consisting of a Waters Alliance 2695 HPLC with photodiode array detector, monitoring between 280 and 600 nm, using a 150 x 4.6 mm, RP C18 column and Empower Pro software. The flow rate was 2 ml $min^{-1}$ and the mobile phase consisted of acetonitrile, methanol-acetate ammonium 50 m*M*, dichloromethane and water (70:15:10:5). Concentration of carotenoids was calculated by using external standards.

***Milk vitamin A, tocopherol, and BC analyses***. Milk (10 ml) was mixed with 10 ml of isopropanol and 5 ml of hexane: toluene (10: 8 $vv^{-1}$). The tubes were centrifuged at temperature of 4 °C at 2800 rpm for 5 min. The top layer was transferred to a clean container, residue mixed with 3 ml of hexane: toluene mix, centrifuged, extraction repeating 3 times. Supernatants were collected, evaporated under a gentle stream of nitrogen on a warm plate at 40 °C until dryness and dissolved in 5 ml of ethanolic butylhidroxitoluene (2 g $l^{-1}$) and 5 ml 2 *M* saturated potassium hydroxide solution. Samples were placed in a water bath at 40 °C for 30 min, then cooled in an ice water

bath. The samples were added to 10 ml deionized water, centrifuged. The extraction was repeated 3 times, and supernatants were collected. The extracts were evaporated to dryness under $N_2$. The residues were dissolved in 2 ml of methanol, filtered, and 80 µl was injected for HPLC analysis using the technique consisting of a Waters Alliance 2695 HPLC with diode array detector, vitamin A monitoring at 325 nm, tocopherols at 292 nm, and BC at 475 nm.

***Vitamin C or L-ascorbic acid in milk*** was determined in accordance with method of Tillmans (Matiseks et al., 1998). All reagents and standards for vitamin analyses were at least analytical grade from Sigma Aldrich.

***Analyses of milk FA***. Milk fat was obtained by centrifugation of milk 15 min at 4 °C and 3500 rpm. The upper cream layer was centrifuged 30 min at 40 °C and 13000 rpm. 7-15 mg of the upper butter oil layer was mixed with 1 ml of hexane and 10 µl of Na methilate (12.5 g 100 $ml^{-1}$ $wv^{-1}$), shaken 1 min, left for 10 min in room temperature, and centrifuged 5 min at 4 °C and 13000 rpm. The upper layer was used for further analysis by gas-liquid chromatography using an ACME model 6100, GLC (Young Lin Instrument Co.) gas chromatograph fitted with flame ionization detector, automatic sample injector, and a 30 m long, 0.25 mm i.d. Alltech AT-FAME analytical column. The carrier gas (He) flow rate was 2 ml $min^{-1}$. The injector and detector temperatures were 225 °C and 250 °C, resp. The oven temperature was programmed from 50 °C (initial delay 4 min) till 170 °C at a rate of 8 °C $min^{-1}$ (held 15 min), till 240 °C at a rate of 6 °C $min^{-1}$. Peaks were identified according to similar peak retention times using standard mixture Supelco FAME Mix C4-C24, Sigma Aldrich. Results were evaluated with a conventional integrator program (Autochro-2000, Young Lin Instrument Co.)

FA content was analyzed in days 1 and 5 to compare the lipid stability in milk stored in 4-6 °C temperature (poured into 25 ml glass bottles, in dark). The sum of polyunsaturated FA (linoleic C18:2 *cis* n-6, C18:2 *trans* and linolenic acids C18:3 *cis* n-6, and n-3) in storage days 1 and 5 were compared.

The above mentioned feed and milk analyses were carried out in the Scientific Laboratory of Biochemistry and Microbiology of the Research Institute of Biotechnology and Veterinary Medicine 'Sigra' of the LLU.

***Immunoglobulin (IgA, IgG, IgM) and lysozyme*** concentrations were determined by turbodimetric method (Грант Х., 1973), using pH-meter Jenway 3520 and spectrophotometer Jenway 6705 UV/VIS (UK) in the Petera Delles Food Processing laboratory of the Faculty of Food Technology of the LLU.

***Milk fat and protein content*** was determined by automated infrared analysis using Milcoscan equipment (Standard method ISO 9622-1999), and SCC was determined by "Somacount 300" in the laboratory of Milk Quality Control of the Sigulda CMAS. ***Milk yield*** was registered once a month from cow supervision data.

Samples were analyzed at least in triplicate. The results were calculated, analyzed, and graphs were made using MS Office program Excel or Microsoft Windows for SPSS software packages.

## Results and Discussion

The average milk yield, fat, protein and SCC content before (D0) and during (D7, 24, 35, and 42) the cow feed enrichment (D7, 24, 35, 42) are given in Table 2.

Table 2

**Characterization of milk before and during feed enrichment with carotenoids**

| Parameters | Control group | | Experimental group | |
|---|---|---|---|---|
| | Before enrichment | During enrichment | Before enrichment | During enrichment |
| Milk yield, kg $day^{-1}$ | $27.65 \pm 1.131^{a}$ * | $26.12 \pm 1.895^{ac}$ ** | $22.08 \pm 1.626^{b}$ * | $23.44 \pm 0.962^{c\ b}$** |
| Fat content, g 100 $g^{-1}$ | $4.78 \pm 0.239^{a}$ | $3.66 \pm 0.218^{b}$ | $4.49 \pm 0.225^{ac}$ | $4.28 \pm 0.180^{c}$ |
| Protein content, g 100 $g^{-1}$ | $3.05 \pm 0.153^{a}$ | $2.92 \pm 0.046^{a}$ | $3.53 \pm 0.177^{b}$ | $2.98 \pm 0.155^{a}$ |
| Somatic cell count, $10^3$ $ml^{-1}$ | $246 \pm 12.300^{a}$ | $152.20 \pm 35.172^{c}$ | $397 \pm 19.850^{b}$ | $128.00 \pm 72.365^{c}$ |

Means within rows marked with the same letter did not differ significantly at $p<0.05$.
* – The average milk yield from January and February months.
** – The average milk yield from March and April months.

Before feed enrichment (D0) the average milk yield was significantly ($p<0.05$) lower in EG, but protein content and SCC were significantly ($p<0.05$) higher in EG in milk as in CG. Comparing results before and during experiment, milk yield remained similar in both groups; fat content significantly ($p<0.05$) decreased in CG, but in EG remained similar; protein content significantly ($p<0.05$) decreased in EG, but in CG remained similar; SCC significantly ($p<0.05$) decreased in both groups. During feed enrichment there were no significant ($p<0.05$) differences on average milk yield, protein content and SCC between both groups. However, the fat content was significantly ($p<0.05$) higher in EG milk during the feed enrichment. The SCC in all milk samples did not exceed 400 000 $ml^{-1}$, and, during the experiment, decreased in both groups (Figure 1) that can be related with the lactation period and seasonal changes (Piena Lopkopība, 2001). Fluctuations of results can be also related to cow health, mobility, other factors, as well as analytical accuracy. As seen from trend line slope coefficients, the tendency of milk SCC decrease was greater

in EG: -51.46 versus -21.26 of CG. This tendency of SCC decrease is in accordance with previous findings (Chew, 1995), showing that feed enrichment with carotenoids have positive role on cow health, especially mastitis prevention.

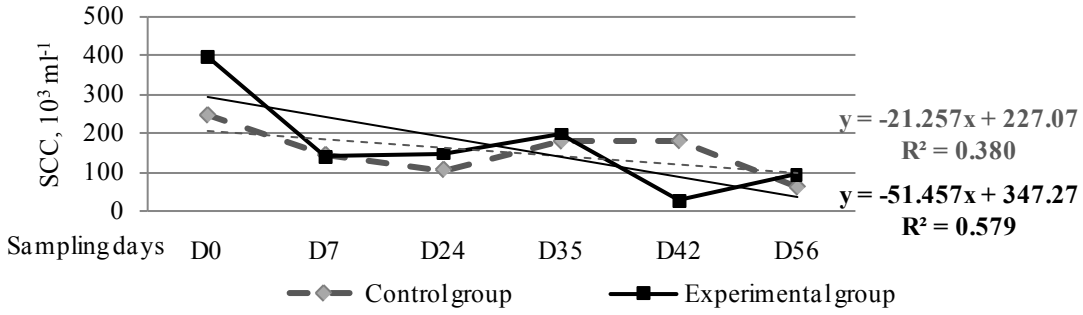



Figure 1. Somatic cell count of milk during the experiment.

In next figures (2 and 3) we can see that Ig concentration was significantly higher ($p<0.05$) in EG milk, as in CG milk in D24, 42 and 56, but the lysozyme concentration – in D24, 35, 56 and, particularly, in D42 when the duration of feed enrichment was the most lengthy. The decrease of Ig in D35 can be related with sample storage or analytical error (similar tendency is seen in both groups). The higher amounts of antimicrobial proteins in EG milk can be derived from feed enrichment with carotenoids thus strengthening cow health. In D7 a higher lysozyme and Ig concentration in EG milk was not observed yet, showing that the favourable effect can not be obtained immediately, but after a longer adaptation period to feed enrichment. As seen from Fig. 4, the growth of PB in this sampling day was significantly higher in EG milk, as in CG milk.

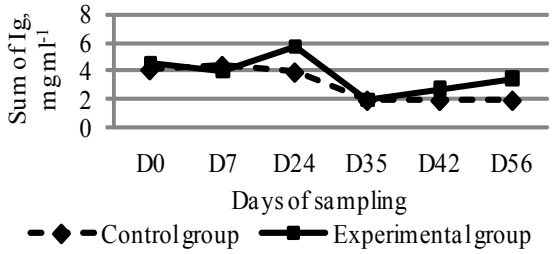



Figure 2. The sum of immunoglobulins in milk.

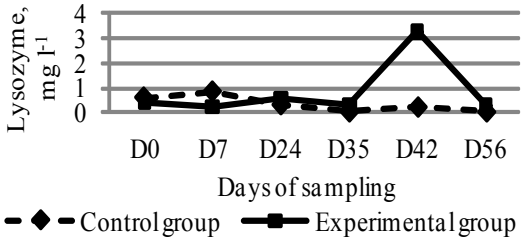



Figure 3. Lysozyme content in milk.

In spite of a wide diversity of xanthophylls and carotenes in forages, BC (especially the all-*trans* isoform) is the main circulating carotenoid in bovines (Nozière et al., 2006). The 9-*cis* and 13-*cis* isoforms have been also observed, as well as α-carotene and lutein (Nozière et al., 2006). In our experiment the BC was measured as the main representative of milk carotenoids. As seen from Figure 4, the content of BC in milk of both groups was significantly ($p<0.05$) lower during and after feed enrichment, as before.

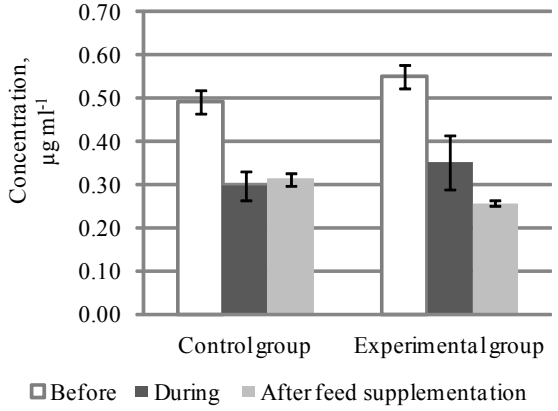



Figure 4. β-Carotene content in milk.

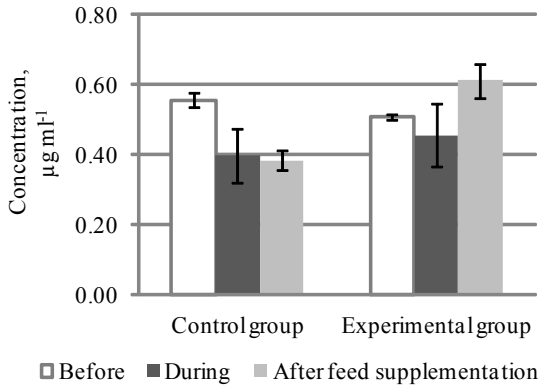



Figure 5. Retinol content in milk.

The BC decrease by 36% in EG, and by 39% in CG looks surprisingly, because the concentration of BC in milk is highly dependent on the concentration of BC in the diet. However, different studies confirm that carotenoid transfer from diet to milk is relatively low (Nozière et al., 2006). The low content of BC in EG milk could be connected with BC conversion and accumulation processes in cow body, and may depend on both - the initial retinol and the BC status of the animals. Carotenoid availability for secretion in milk is governed by their transport into lymph and plasma, their metabolism within tissues (especially conversion into vitamin A and by utilization as pigments or antioxidants), as well as their storage in adipose tissues or secretion into bile by the liver. The extent of carotenoid degradation by microorganisms in the rumen also remains uncertain because of the wide range of results. Also a specific effect of stage of lactation has not been clearly established, and this could be an important factor affecting concentrations of BC, and other micronutrients, in milk (Nozière et al., 2006). Other possible factors, as analytical error also have to be mentioned.

BC concentrations measured in milk of both groups during and after feed enrichment were similar or higher to earlier findings – BC in Netherlands raw milk in March was 0.186 ± 0.021 μg $g^{-1}$ (Hulshof et al., 2006), but in France BC in milk of cows whose diet was based on hay and grass-silage in March and April was (0.056-0.142) ± 0.008 μg $g^{-1}$ (Nozière et al., 2006). BC concentration in milk of cows fed grass-clover silage was 0.440 ± 0.023 μg $ml^{-1}$, but in milk of cows fed hay was 0.264-0.445μg $ml^{-1}$ (Havemose et al., 2006). In our experiment the concentration of BC measured in day 0 was remarkably higher (0.490 ± 0.027 for CG and 0.550 ± 0.028 μg $ml^{-1}$ for EG), as literature data. BC is one of the most fluctuating milk constituents and its content depends on cow breed, feeding regimen, lactation period, health status, milk fat content, as mentioned before (Nozière et al., 2006).

Comparing the retinol content in milk during the experiment, we can see that it significantly ($p<0.05$) decreased in CG milk, while in EG milk remained similar (Fig. 5), and after the end of feed enrichment slightly but significantly increased, and was significantly ($p<0.05$) higher than that in CG milk. That can be explained by the fact that for retinol synthesis for EG cows BC is more available. The results of other authors show, that retinol content in Swedish raw milk was 0.307 μg $ml^{-1}$ in March (Swensson et al., 2007), retinol in Netherlands' raw milk in March was 0.396 ± 2.8 μg $100g^{-1}$ (Hulshof et al., 2006). According to Haug et al. (2007), the milk retinol content is about 0.280 μg $ml^{-1}$. During our experiment the retinol content in both groups' milk was similar or above previously mentioned quantities.

The sum of α- and γ-tocopherols was determined (see Figure 6). Vitamin E is not a single compound; it includes tocopherols and tocotrienols. In whole milk, α-tocopherol is the major form of vitamin E (> 85 g 100 $g^{-1}$); γ-tocopherol and α-tocotrienol are present to a lesser extent, about 4 g 100 $g^{-1}$ each of the sum of tocopherols and tocotrienols (Haug et al., 2007). According to Haug et al. (2007), the vitamin E concentration in milk is about 0.6 μg $ml^{-1}$, but may increase 3-4 folds by proper feeding regimes. Before the experiment the sum of α- and γ-tocopherols was significantly higher ($p<0.05$) in CG milk than in EG milk. During the experiment it changed significantly ($p<0.05$) in milk of both groups, decreasing in CG milk, but increasing in EG milk. An adverse shift occurred after the end of feed enrichment. Results are not easily explicable because carrots are not regarded as important source of tocopherols, still they contain a small amount of α-tocopherol, i.e. 0.320 - 0.950 mg 100 $g^{-1}$ (Danish Food Composition Databank, 2009). Oil supplementation also could enhance the absorption of this vitamin from feed. Compared to previous literature data, the sum of tocopherols in both groups before, during and after feed enrichment was rather below 0.6 μg $ml^{-1}$.

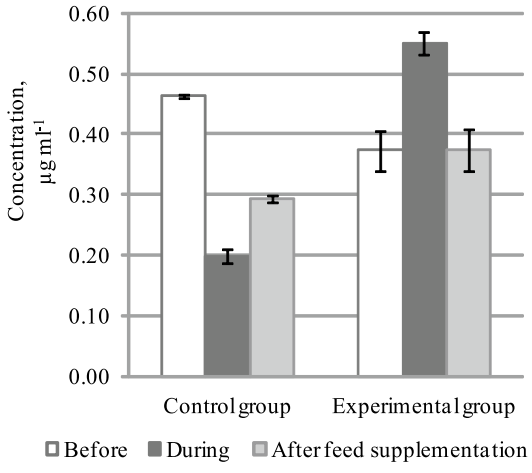



Figure 6. The sum of α- and γ-tocopherols in milk.

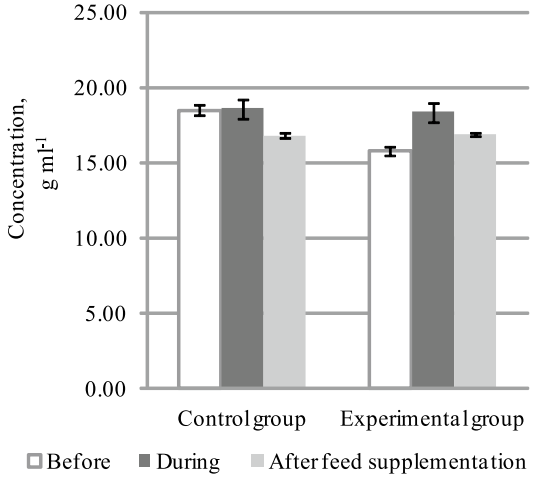



Figure 7. Vitamin C content in milk.

Concerning vitamin C, which also has antioxidant activity (Coultate, 2002; Swensson et al., 2007), its content in milk before additional feeding was slightly higher ($p<0.05$) in CG milk (Fig. 7). During and after the feed enrichment the average vitamin C content resembled in both groups' milk without significant differences. The average vitamin C concentration in milk was 17.49 ± 1.153 μg $ml^{-1}$, that conforms to literature data, i.e. 10-25 μg $g^{-1}$ (Walstra, 2006).

Milk lipid stability was investigated by the storage stability of polyunsaturated FA. Comparing the polyunsaturated FA content in milk stored in 4 - 6 °C temperature in days 1 and 5, there no significant changes of it in milk of both groups (Figure 8) were observed. This can be explained with the induction period when the oxidation process is still slow due to slow oxygen uptake and milk lipids are still protected against oxidative damage due to sufficient antioxidant concentrations (Coultate, 2002). Compared with many edible fats, milk fat is relatively resistant to oxidation because of its low polyunsaturated FA content, and high proportion of saturated FA, and the presence of natural antioxidants, principally α-tocopherol and BC (Żegarska, 2003). The average content of polyunsaturated FA during the feed enrichment was similar in milk of both groups (2.35 ± 0.198 g 100 $g^{-1}$ of total fatty acids in EG and 2.19 ± 0.183 g 100 $g^{-1}$ in CG).

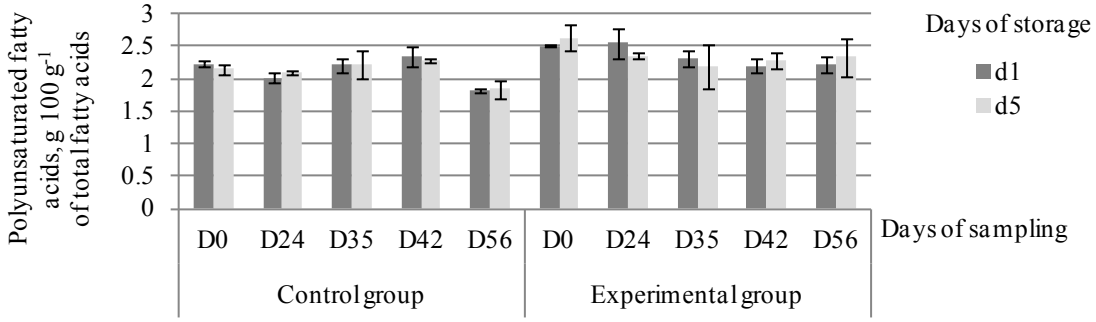



Figure 8. Polyunsaturated fatty acid content in milk stored in 4-6 °C temperature in days 1 and 5.

However, under more unfavorable storage conditions, such as light or high temperature influence, or in a longer period, as in such dairy products, as butter or ice cream storage, milk lipid stability potentially can be ameliorated by increased antioxidant vitamin concentrations.

## Conclusions

Enriching cow diet with carrots showed a tendency to promote the decrease of somatic cell count in milk, significantly ($p<0.05$) increase immunoglobulin and lysozyme content, thus potentially improving milk nutritional value. Milk retinol and tocopherol content in EG milk rose significantly ($p<0.05$), but β-carotene content decreased by 36 and 39% in EG and CG milk, accordingly. Polyunsaturated fatty acid stability changes during 5 day storage in temperature of 4 - 6 °C were not observed.

## Acknowledgements

The authors gratefully acknowledge the partial financial support provided by the European Social Fund through the project 1.1.2.1.2. "Support for doctoral studies in LLU" (agreement 2009/0180/1DP/1.1.2.1.2/09/IPIA/ VIAA/017).

## References

1. Ananda N., Vadlani P.V. (2010) Production and optimization of carotenoid-enriched dried distiller's grains with solubles by Phaffia rhodozyma and Sporobolomyces roseus fermentation of whole stillage. *Journal of Industrial Microbiology and Biotechnology*, 37(11), pp. 1183-1192.
2. Bergamo P., Fedele E., Iannibelli L., Marzillo G. (2003) Fat-soluble vitamin contents and fatty acid composition in organic and conventional Italian dairy products. *Food Chemistry,* 82, pp. 625-631.
3. Chew B.P. (1995) Antioxidant vitamins affect food animal immunity and health. *Journal of Nutrition,* 125, pp. 1804S-1808S.
4. Coultate T.P. (2002) *Food. The Chemistry of Its Components,* 4th ed., RSC, 432 p.
5. Danish Food Composition Databank, Technical University of Denmark (DTU), National Food institute, Version 7.01 March 2009, Available at: http://www.foodcomp.dk/v7/fcdb_details.asp?FoodId=1129, 16 February 2011.
6. Hara A., Radin N.S. (1978) Lipid extraction of tissues with a low-toxicity solvent, *Analytical Biochemistry,* 90, pp. 420-426.
7. Haug A., Hostmark A.T., Harstad O.M. (2007) Bovine milk in human nutrition – a review. *Lipids in Health and Disease*, 6:25. Available at: http://www.lipidworld.com/content/6/1/25, 19 January 2011.
8. Havemose M.S., Weisbjerg M.R., Bredie W.L.P., Poulsen H.D., Nielsen J.H. (2006) Oxidative Stability of Milk Influenced by Fatty Acids, Antioxidants, and Copper Derived from Feed. *Journal of Dairy Science,* 89, pp. 1970-1980.
9. Havemose M.S., Weisbjerg M.R., Bredie W.L.P., Nielsen J.H. (2004) Influence of feeding different types of roughage on the oxidative stability of milk. *International Dairy Journal*, 14, pp. 563-570.

10. Hulshof P.J.M., van Roekel-Jansen T., van de Bovenkamp P., West C.E. (2006) Variation in retinol and carotenoid content of milk and milk products in the Netherlands. *Journal of Food Composition and Analysis,* 19, pp. 67-75.
11. ISO 9622-1999 (2000) Whole milk - Determination of milkfat, protein and lactose content - Guidance on the operation of mid-infrared instruments, 34 p.
12. Kotecha P.M., Desai B.B., Madhavi D.L. (1998) Carrot. In: Salunke D.K. and Kadam S.S. (eds) *Handbook of Vegetable Science and Technology.* Marcel Dekker, NY, pp. 119-139.
13. Matiseks R., Šnēpels F.M., Šteinere G. (1998) *Pārtikas analītiskā ķīmija. Pamati, metodes, lietošana. (Analytical chemistry of food. Basics, methods, application)*. 2. izd., LU, Rīga, 456 lpp. (in Latvian).
14. Mullan W.M.A. (2003) Major antimicrobial proteins in milk. Available from: http://www.dairyscience.info/exploitation-of-anti-microbial-proteins/52-antimicrobial proteins.html?showall=1, 19 February 2011.
15. Murphy J.J., McNeill G.P., Connolly J.F., Gleeson P.A. (1990) Effect on cow performance and milk fat composition of including full fat soyabeans and rapeseeds in the concentrate mixture for lactating dairy cows. *Journal of Dairy Research*, 57, pp. 295-306.
16. Nozière P., Graulet B., Lucas A., Martin B., Grolier P., Doreau M. (2006) Carotenoids for ruminants: From forages to dairy products. *Anim. Feed Sci. Technol.*, 131, pp. 418-450.
17. Nozière P., Grolier P., Durand D., Ferlay A., Pradel P., Martin B. (2006) Variations in Carotenoids, Fat-Soluble Micronutrients, and Color in Cow's Plasma and Milk Following Changes in Forage and Feeding Level. *Journal of Dairy Science,* 89, pp. 2634-2648.
18. Petit H.V. (2009) Antioxidants and dairy production: the example of flax. *Revista Brasileira de Zootecnia,* 38, pp. 352-361.
19. *Piena lopkopība. Rokasgrāmata. (Dairy Cattle-breeding. Manual)*. (2001) Jemeļjanovs A. (red.), ZI Sigra, Sigulda, 192 lpp. (in Latvian).
20. Smet K., Raes K., De Block J., Herman L., Dewettinck K., Coudijzer K. (2008) A change in antioxidative capacity as a measure of onset to oxidation in pasteurized milk. *International Dairy Journal,* 18, pp. 520-530.
21. Swensson C., Lindmark-Mansson H. (2007) The prospect of obtaining beneficial mineral and vitamin contents in cow's milk through feed. Journal of Animal and Feed Sciences, 16, Suppl. 1, pp. 21-41.
22. Walstra P., Wouters J.T.M., Geurts T.J. (2006) *Dairy Science and Technology,* 2d ed., CRC, pp. 95.
23. Weiss V.P. (2010) Antioxidant Nutrients and Milk Quality. Available at: http://www.extension.org/pages/Antioxidant_Nutrients_and_Milk_Quality, 21 January 2011.
24. Żegarska Z.A. (2003) Milk Lipids. In: Sikorski Z.E., Kolakowska A. (eds) *Chemical and Functional Properties of Food Lipids.* CRC Press, 400 p.
25. ГОСТ 13496.17-95. (1997) *Корма. Методы определения каротина.* (Feedstuffs. Carotene Determination Methods). 8 с. (in Russian).
26. Грант Х. (1973) *Турбодиметрический способ количественного опредиление лизоцима с применением спектрофотометра СФУА, Лабораторное дело* (Turbodimetric Method for lysozyme Determination with SFUA Spectrophotometer, A Laboratory Manual). Х. Грант, Л.И. Яворковский, И.А. Блумберга (ред.), 300-304 с. (in Russian).

# ANTIRADICAL ACTIVITY OF DIFFERENT BARLEY VARIETIES AND MALT TYPES

***Ilona Dabina – Bicka, Daina Karklina, Zanda Kruma***
*Latvia University of Agriculture*
*ilona.dabina@inbox.lv*

**Abstract**

Cereal grains have long been thought to be less important sources of antioxidants than fruits and vegetables although they contain many antioxidants and are major dietary components worldwide. The aim of the current research was to study and compare an antioxidant activity (AOA) and total phenolic content (TPC) of different barley varieties and malt types as well as to evaluate possible interconnection between TPC and AOA of barley and malt samples.

The research was carried out on four lines of hull-less barley '3528'; 'L-400'; '3475'; '3537' and one variety of flaky barley 'Klass' grains, which were cultivated in Latvia in 2010, and their corresponding malt. Commercial sorts of malt - Pilsener, Munich, Caramel and Dark were used in the research to compare with the malt produced in the laboratory scale.

The antioxidant potential of barley and their products is analyzed by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays. Total phenolic content of barley and malt samples was determined according to the Folin-Ciocalteu spectrophotometric methods.

The values of DPPH radical scavenging activity for 5 barley samples ranged from 4.57 to 5.89 μmol TE $g^{-1}$ DW. The total amount of phenols ranged from 1.96 to 2.43 mg GAE $g^{-1}$ DW for unprocessed barley samples and from 2.5 to 3.4 mg GAE $g^{-1}$ DW for their corresponding malt. TPC of malt commercial sort ranged from 3.5 to 6.7 GAE $g^{-1}$ DW. The increase of TPC for commercial malts is strongly related with Maillard reaction products.

**Key words**: barley; hull-less barley; malt; phenols; antioxidants

## Introduction

A broad definition of an antioxidant is: a substance that, present at low concentration compared with those of an oxidizable, substrate significantly delays or prevents oxidation of that substrate. However, the way antioxidants can exert their antioxidant activity differs among compounds. Phenolic compounds are known to quench superoxide radicals and to inhibit lipid peroxidation. Melanoidins (brown, nitrogenous polymers and co-polymers) are the final products of the Maillard reaction and are known to scavenge free and active oxygen, act as reducing and chelate metals. However, pro-oxidant activity has been reported as well (Preedy, 2009).

Interest in barley (*Hordeum vulgare* L.) as a food grain is emerging. Over the past decade an increasing interest in barley for human consumption has been observed, mainly due to its content of health-related bioactive components. The health benefits normally associated with barley are attributed to high amounts of dietary fibre. However, antioxidants or phenolic structured antioxidant compounds are also detected in barley (Holtekjolen et al., 2006), and recent studies have shown that cereals contain more phytochemicals than previously considered.

Barley is grown in many parts of the world both for food and for feed uses. In many countries it is mostly used as a feed crop as well as for brewing and production of ethanol but in small quantities barley is used in the production of soups, dressings, baby foods and speciality items. Whole-grain cereals are a major source of polyphenols, especially phenolic acids such as ferulic, vanillic, caffeic, syringic, sinapic and p-coumarin acids. All of them have potentially antioxidant properties due to the presence of an aromatic phenolic ring that can stabilize and delocalize the unpaired electron within its aromatic ring. However, their mechanisms of action are not fully elucidated (Fardet et al., 2008), but antioxidant properties of phenols works as a food preservation (to inhibit lipid oxidation), or for disease prevention (Liu and Yao, 2007).

Phenolic acids are the major phenilpropanoid components in barley and different kind of these phenolics are found in different fraction of cereals (Antoine et al., 2004). In different barley varieties, the starchy endosperm contains low level of poliphenols, whereas the outer layers of the grain (pericarp, aleurone layer, and germ) contain the highest level of poliphenols (Holtekjolen et al., 2006). Lately more and more new barley varieties are selected and one of those is hull-less barley.

In the food industry, hull-less barley (*Hordeum vulgaris* L.) is acknowledged as more valuable and more economical, compared with covered barley. Selected hull-less barley varieties are able to pass flaky barley criteria; moreover, the amount of extract substances in hull-less barley is higher by 4-5% compared to malting barley (Dabina – Bicka et al., 2010). However hull-less barley is hull free which contains most of the existing phenol components.

Malting barley and the malting process can have impact on beer instability owing to the presence of pro-oxidant and anti-oxidant activities. Polyphenols and phenolic acids present in malt are natural antioxidants, capable of delaying; retarding or preventing oxidation processes, and therefore thought to have a significant effect in malting and brewing as inhibitors of oxidative damage. About 80% of beer polyphenols originate from malt and the remaining 20% come from hop (Dvorakova et al., 2008).

The aim of the current research was to study and

compare an antioxidant activity and total phenolic content of different barley varieties and malt types as well as to evaluate possible interconnection between TPC and AOA of barley and malt samples.

## Materials and Methods

*Barley and malt samples*

The research was carried out on hull-less barley (four lines '3528'; 'L-400'; '3475'; '3537', further in text abbreviated: A; C; D; B, respectively) and flaky barley (one line 'Klass') grains, which were harvested in Latvia in 2010. The following technology was used for malt production: washing and steeping of grains ($H_2O$ t = 17 ± 2 °C) until moisture content is reached 38 – 40%. Afterwards the grains were placed for germination from four to six days at a temperature of 19 ± 1° C. The kilning of the germinated grains was completed in eight hours in a laboratory kiln. Grains in a thin layer were spread on sieves in a chamber-type drier with hot air circulation at a temperature of 50 °C to 80 °C till in the grains a constant moisture content was achieved (5 ± 1%).

In this study experimentally produced malt from hull-less barley was compared to commercial sorts of malt. Four kinds of malt, which are produced in "Viking Malt" (Lithuania) – Pilsner, and "Slodownia Strzegom" (Poland) - Munich, Light caramel and Black. Kilning and roasting temperatures were the following: Pilsner – 75 °C; Munich –100 °C; Caramel – 150 °C and Black – 230 °C.

*Chemicals*

Gallic acid, Folin-Ciocalteus phenol reagent, 2,2-diphenyl-1-picrylhydraziyl (DPPH) and (±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) were purchased from Sigma-Aldrich (Switzerland). All other chemicals and solvents ($Na_2CO_3$, ethanol, acetone) are used in the research were with the highest commercial grade and are obtained from BARTA a CIHLAR spol.s.r.o. (Czech Republic).

*Preparation of extracts from barley and malt*

Barley and malt was finely ground in the laboratory mill CIATRONIC KSW 2669. Four grams of grounded samples were extracted for 10 minutes in the ultrasound bath (ULTRASONS, SELECTA P) with 40 ml of solvent. To reach a compromise between alcoholic and acetone extractions, a 7/7/6 ethanol/acetone/water (v/v/v) mixture was tested (Bonoli et al., 2004). After centrifugation at 3000 $min^{-1}$ for 10 min using a centrifuge MEDITRONIC BL-C, the supernatant once more was removed and the extraction was repeated. The supernatant was collected in a 50 ml volumetric flask and refilled by solvent till the marked line (Jakobsone, 2008).

*Determination of total phenolic content (TPC)*

The TPC of the barley and malt extract was determined according to the Folin-Ciocalteu spectrophotometric method (Singleton et al., 1999) with some modifications. First, 0.25 ml of sample was transferred to a 25.0-ml volumetric flask containing 6 ml of $H_2O$, to which was subsequently added 1.25 ml of undiluted Folin-Ciocalteu reagent. After 1 min, 3.75 ml of 20% aqueous $Na_2CO_3$ was added, and the volume was made up to 25.0 ml with $H_2O$. The control sample contained all the reaction reagents except the extract. After 2 h of incubation at 25 °C, the absorbance was measured at 760 nm using a spectrophotometer JENWAY 6300. Total phenols were expressed as gallic acid equivalents (Damien Dorman et al., 2004).

*Determination of DPPH radical scavenging activity*

Antioxidant activity of the barley and malt extract were measured on the basis of scavenging activities of the stable 2,2-diphenyl-1-picrylhydraziyl (DPPH) radical as outlined by Yu et al., (2003). Briefly, barley or malt extracts diluted in ethanol/acetone/water (v/v/v) (1:1). The antioxidant reaction was initiated by transferring 0.5 ml of grain extract into a sample cavity containing 3.5 ml of freshly prepared $DPPH^{\cdot}$ methanol solution (0.004 g $DPPH^{\cdot}$ to 100 ml methanol). After 30 min of incubation in the dark at room temperature, the absorbance was measured at 517 nm using a spectrophotometer JENWAY 6300. For determination of dilution ratio the inhibition of DPPH radical was calculated as a percentage (%) using the formula:

$$\text{Percentage inhibition (\%)} = [(A_{control} - A_{sample})/ A_{control}] \times 100$$

where: $A_{control}$ is the absorbance of the control reaction (containing all reagents except test compounds),

$A_{sample}$ is the absorbance of the test compound.

Percentage inhibition of DPPH radical is acceptable within range of 45 – 90%.

Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The Trolox calibration curve was plotted as a function of the absorbance to concentration of Trolox. The final results were expressed as micromoles of Trolox equivalents (TE) per gram of dry weight (μmol TE $g^{-1}$ DW) (Zhao et al., 2008).

*Statistical analysis*

The differences in the antioxidant activity and total phenol amount of samples are presented as a mean ± standard deviation (SD). The differences between independent groups were specified by two way analysis of variance (ANOVA), and value of $P<0.05$ was regarded as statistically significant. In case of establishing statistically significant differences, homogeneous groups were determined by Tukey's multiple comparison test at the level of confidence $\alpha=0.05$.

## Results and Discussion

Relatively stable organic radical DPPH has been used widely for the determination of antioxidant activity of pure antioxidant compounds of barley and malt extracts. For evaluation of antiradical activity of barley and malt, different malting barley varieties and malt types were measured and compared with their DPPH radical scavenging activities. Results are expressed as micromoles of Trolox equivalent per gram of dry weight of samples (μmol TE $g^{-1}$ DW) and are shown in Fig. 1. All malting barley varieties exhibited strong DPPH radical scavenging activity at the test concentration. The values of DPPH

radical scavenging activity for 5 barley samples ranged from 4.57 to 5.89 µmol TE $g^{-1}$ DW. Obtained results are lower compared to those reported by Zhao et al., 2008. The significant differences in DPPH radical scavenging activity for different barley varieties suggested that variety might have significant influences on the antioxidant activity of malting barley. According to Ragaee et al., (2006) the antioxidant activity in scavenging DPPH radical of barley was higher than other cereals, like wheat, corn and rye, and it is important for nutrition because it comprises significant part of our daily intake.

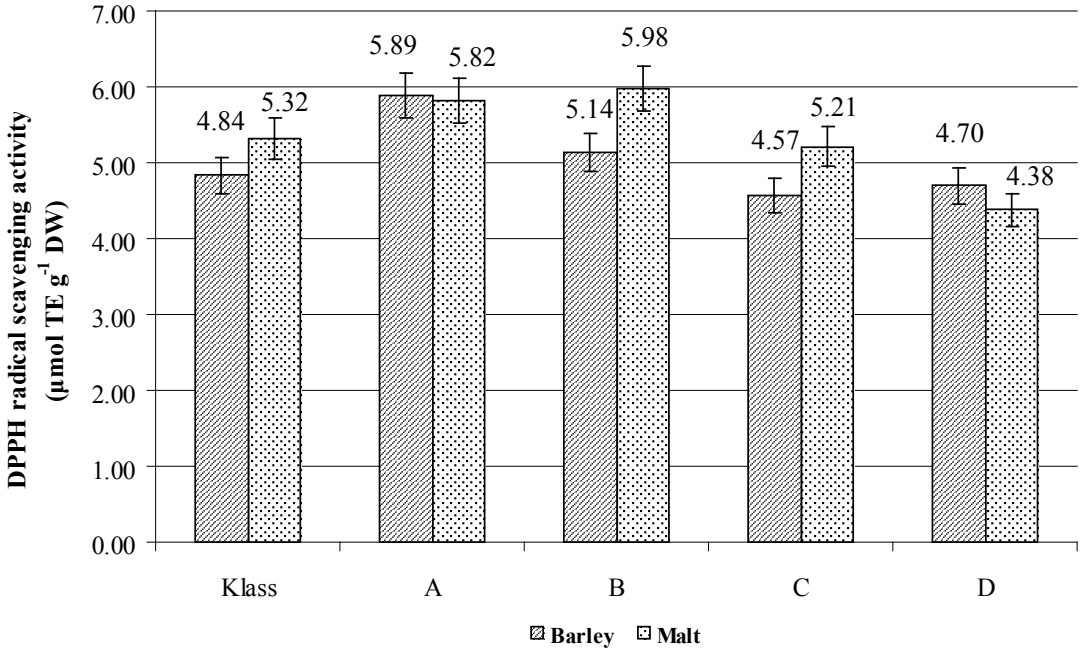



Figure 1. DPPH radical scavenging activities in barley grains and malt.

The TPC of flaky and hull-less barley varieties and their corresponding malt are presented in Table 1. A close linear correlation in the phenolic content (r=0.90) was found between the barley and malt (Palmer, 2006). Similar trend was observed on barley and their corresponding malt antiradical activity, respectively Klass – 0.91 and a line of hull-less barley B – 0.86, and C – 0.89.

Table 1

**TPC of flaky barley 'Klass' and hull-less barley lines and their corresponding malt**

| Varieties or line | TPC, mg GAE $g^{-1}$ DW | |
|---|---|---|
| | Barley | Malt |
| Klass | 2.06 | 3.40 |
| 3528 (A) | 2.27 | 3.21 |
| 3537 (B) | 2.27 | 2.98 |
| L-400 (C) | 1.96 | 2.53 |
| 3475 (D) | 2.43 | 3.29 |

During malting change of DPPH radical scavenging activity for different barley varieties were not disparity, with the exception of line B and C. The majority of malts have higher antioxidant activities than their corresponding barley, but no qualitative differences were found as Goupy et al., (1999) reported. The cereals with higher TPC values were not necessarily better in DPPH inhibition. According to Dordevic et al., (2010), ferulic acid, the main phenolic acid in cereal grain, showed a weak antiradical effect in experiments with the DPPH radical, which may explain the discrepancies. In addition, although the Folin-Ciocalteu method is widely used to determine total phenolic contents in botanical and biological samples, it has its own limitations.

As shown in the research of Dvorakova et al. (2008), the highest antioxidant activities were detected for the hull-less barley lin, than those for hulled varieties. But obtained results did not match with the given statement. DPPH scavenging activity of hull-less barley lines A and B are higher than flaky barley 'Klass', but hull-les barley lines of C and D are lower than varieties of flaky barley.

Four kinds of commercial malt, which are produced in different kilning and roasting temperatures, were analyzed of DPPH radical scavenging activity and total phenolic content.

The influence of kilning temperature on antioxidant activity is shown in Fig. 2 and on total phenolic content – Fig. 3. The increase of antioxidant activity could come from development of such non-enzymatic browning products as Maillard products; which can also act as antioxidants, particularly melanoidins (Goupy et al., 1999). Moreover, kilning leads to more friable tissues and probably allows better extraction of phenolic acids, mostly present in the outer layers of the grain (Dvorakova et al., 2008). This result highlights the hypothesis that after kilning there is possible better extraction of flavonoids and phenolic acids. .

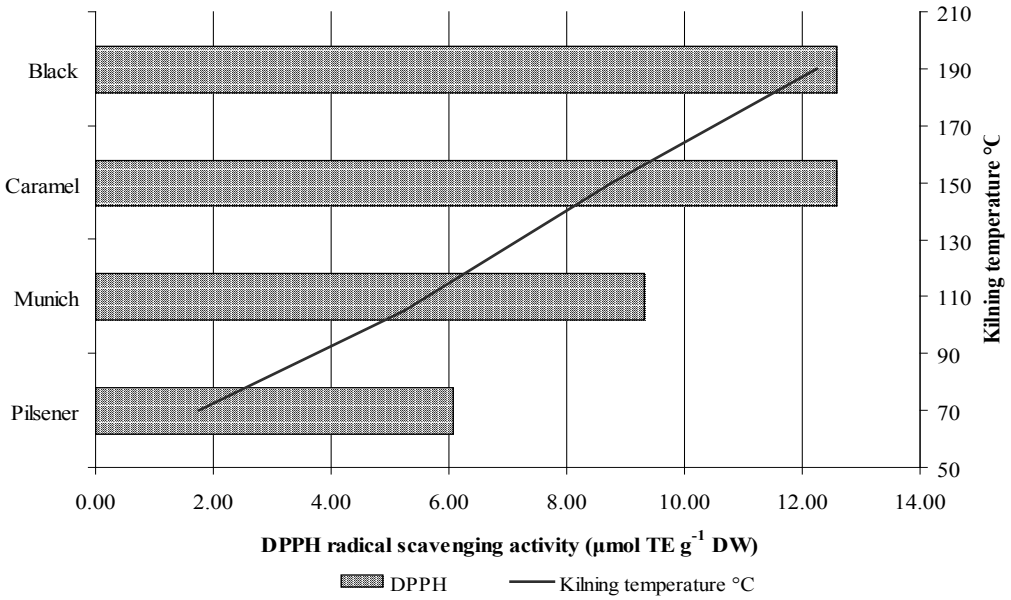



Figure 2. The influence of kilning on DPPH radical scavenging activity of commercial malts.

Stronger scavenging effects on the DPPH radical were found for Caramel and Black malt 12.58 µmol TE $g^{-1}$ DW. The DPPH radical scavenging effect observed in this work is in agreement with literature data reported by Randhir et al., (2007), where a substantial increase in antioxidant activity due to the thermal processing was observed in all samples. Results showed that higher malt kilning temperature resulted in higher antiradical activity, respectively, (Fig.2.) with increase of kilning temperature per 80 – 150 °C, antiradical activity increased for 50% (from 6.06 till 12.58 58 µmol TE $g^{-1}$ DW). Studies indicate that the reactions mechanism of the antioxidant and DPPH depends on structural conformation of the antioxidants, hence perhaps thermal processing alters the phenolic structure resulting in improved antioxidant function. Other factors for improved antioxidant activity could be due to the additive and synergistic effects between other phytochemicals and thermally altered phenolics (Randhir et al., (2007).

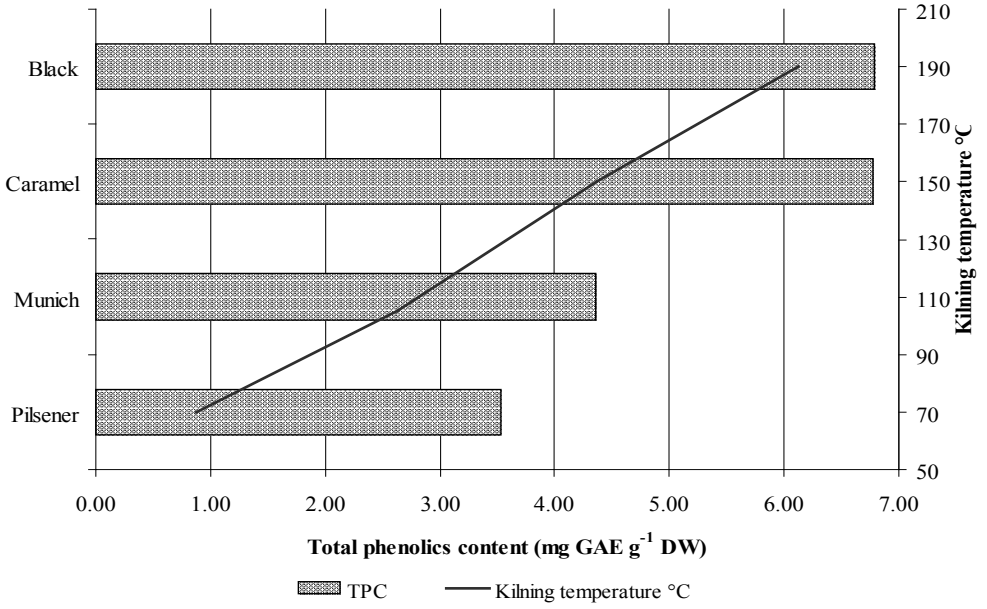



Figure 3. The influence of kilning on Total phenolic content of commercial sort of malts.

Polyphenols are known to have antioxidant activity and thus, possible health benefits, and barley is therefore claimed to be good sources of natural antioxidants (Holtekjolen et al., 2006) The current research showed that the malt kilned at the lower kilning temperature contains lower content of TPC comparing to those that have been kilned at the higher kilning temperature. There we can assume that TPC has an increase by increasing kilning temperature (Fig. 3). Positive correlation between kilning temperature and TPC of malt was observed. The values of TPC for four evaluated, commercial malt samples ranged from 3.53 to 6.78 mg GAE $g^{-1}$ DW.

Dvarokova et al., (2008) reported TPC of barley ranged from 0.7 to 1.5 mg GAE $g^{-1}$ DW, but their corresponding malt from 1.6 to 2.7 mg GAE $g^{-1}$ DW. Differences could be explained by used variety, kilning temperature, phenolic compounds extraction method and solvent.

The nature of the phenolic material in wort is influenced strongly by how strongly the malt has been kilned (Briggs et al., 2004). Barley malt contributes phenolic and polyphenolic compounds to the early stages of the brewing process. The malt phenolics, upon processing, polymerize to give rise to beer polyphenolics that furnish colour, impart astringent taste, as well as a browning substrate, and participate in precipitation of poorly coagulable beer proteins (Shahidi and Naczk, 1995). Higher levels of antioxidant substances in the beer retard deterioration processes. On the other hand, proanthocyanidins from barley malt influence the development of haze in beer, because 80% of these phenolics present in regular beer are derived from barley malt (Shahidi and Naczk, 1995).

## Conclusions

Even if the amount of phytochemicals is higher in the hulled barley compared to the hull-less varieties of barley, the industry should consider the hull-less varieties. The necessarily of pearling of the hulled varieties will decrease the amounts of antioxidants in these genotypes considerably compared to the hull-less samples. Furthermore, the variations observed in the amount of phytochemicals within the different types of barley should provide an advantage for breeders to produce barley varieties of high antioxidant levels for food uses.

The analysis of five different varieties of barley did not allow a clear correlation between the level of phenolic compounds and the antioxidant activity to be demonstrated because the differences between the samples were not significant. However, in the case of commercial type of malt, a great increase of total phenolics also led to an importance increase of antiradical activity. Thus, even if phenolic compounds are not the only components responsible for the antioxidant activity of barley and malt, they could play a major role.

Studies addressing the impact of thermal processing on total phenolics and antioxidant activity in foods are becoming more important due to its role in human health and disease management. It is essential to understand how to optimize levels of beneficial phenolics linked to health-relevant functionality in commercially processed grains. In general, thermal processing improved the total phenolic content and antioxidant activity in barley. Therefore, the antioxidant activity of cereal products is far from negligible.

**Acknowledgements**

This research has been carried out within the framework of the ESF Project "Formation of the Research Group in Food Science", Contract No. 2009/0232/1DP/1.1.1.2.0/09/APIA/VIAA/122.

**References**

1. Antoine C., Peyron S., Lullien-Pellerin V., Abecassis J., Rouau X. (2004) Wheat bran tissue fractionation using biochemical markers. *Journal of Cereal Science*, 39, pp. 387-393.
2. Bonoli M., Verardo V., Marconi E., Caboni M.F. (2004) Antioxidant Phenols in barley (*Hordeum vulgare* L.) Flour: comparative Spectrophotometric study among extraction methods of free and bound phenolic compounds. *Journal of Agricultural and Food Chemistry*, 52, pp. 5195-5200.
3. Briggs D.E., Boulton C.A., Brookes P.A., Stevens R. (2004) The science of mashing. In: *Brewing science and practice*, Woodhead publishing limited and CRC press LLC, pp. 86-170.
4. Dabina–Bicka I., Karklina D., Rakcejeva T., Sniedzane R., Kviesis J. (2010) Dynamics of Vitamins C and E in barley products during malting. *Research for Rural development 2010,* International Scientific Conference Proceedings, LLU, Jelgava, Latvia, pp. 111-115.
5. Damien Dorman H.J., Bachmayer O., Kosar M., Hiltunen R. (2004) Antioxidant properties of aqueous extracts from selected *Lamiaceae* species grown in Turkey. *Journal of Agricultural and Food Chemistry*, 52, pp. 762-770.
6. Dordevic T.M., Siler-Marinkovic S.S., Dimitrijevic-Brankovic S.I. (2010) *Food Chemistry*, 119, pp. 957-963.
7. Dvorakova M., Douanier M., Jurkova M., Kellner V., Dostalek P. (2008) Comparison of antioxidant activity of barley (*Hordeum vulgare* L) and malt extracts with the content of free phenolic compounds measured by high performance liquid chromatography coupled with coularray detector. *Journal of the Institute of Brewing*, 114 (2) pp. 150-159.
8. Fardet A., Rock E., Remesy C. (2008) Is the in vitro antioxidant potential of whole-grain cereals and cereal products well reflected in vivo? *Journal of Cereal Science*, 48, pp. 258-276.
9. Goupy P., Hugues M., Boivin P., Amiot M.J. (1999) Antioxidant composition and activity of barley (*Hordeum vulgare*) and malt extracts and of isolated phenolic compounds. *Journal of Science of Food and Agriculture*, 79, pp. 1625-1634.
10. Holtekjolen A.K., Kinitz C., Knutsen S.H. (2006) Flavanol and bound phenolic acid contents in different barley varieties. *Journal of Agriculture and Food Chemistry*, 54, pp. 2253-2260.
11. Jakobsone I. (2008) Pārtikas produktu uzturvērtības noteikšana. (Determination of Food Nutritive Value). Latvian University Akademiskais apgads, Riga, Latvia, pp. 148-149. (in Latvian).
12. Liu Q., Yao H.Y. (2007) Antioxidant activities of barley seeds extracts. *Food Chemistry*, 102. pp. 732-737.
13. Liu R.H. (2007) Whole grain phytochemicals and health. *Journal of Cereal Science*, 46, pp. 207-219.
14. Palmer H.G. (2006) Barley and malt. In: *Handbook of Brewing.* Edited by Davidson P.M., Dreher M. et al., Taylor&Francis Group, 6000 Broken Sound Parkway NW, pp. 139-159.
15. Preedy V.P., Rajendram R. (2009) Ethanol in Beer: Production, Absorption and Metabolism. In: *Beer in Health and disease prevention*. Preedy V.P. (ed.) Elsevier Inc pp. 429-440.
16. Ragaee S., Abdel-Aal E.M., Noaman M. (2006) Antioxidant activity and nutrient composition of selected cereals for food use. *Food Chemistry*, 98, pp. 32-38.
17. Randhir R., Know Y., Shetty K. (2007) Effect of thermal processing on phenolics, antioxidant activity and health-relevant functionality of select grain sprouts and seedlings. *Innovative Food Science and Emerging Technologies*, 9, pp. 355-364.
18. Shahidi F., Naczk M. (1995) Phenolic compounds of beverages. In: *Food phenolics, source, chemistry, effects, applications.* Technomic Publishing Company, Inc.: Lancaster, PA, pp. 128-136.
19. Singleton V.L., Orthofer R., Lamuela-Raventos R.M. (1999) Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods in Enzymology*, 29, pp. 152-178.
20. Yu L., Haley S., Perret J., Harris M., Wilson J., Haley S. (2003) Antioxidant properties of bran extracts from Akron wheat grown in different locations. *Journal of Agriculture and Food Chemistry*, 51, pp. 1566-1570.
21. Zhao H., Fan W., Dong J., Lu J., Chen J., Shan L., Lin Y., Kong W. (2008) Evaluation of antioxidant activities and total phenolic contents of typical malting barley varieties. *Food Chemistry*, 107, pp. 296-304.

# DETECTION OF VOLATILE COMPOUNDS DURING WHEAT DOUGH FERMENTATION

***Martins Sabovics, Evita Straumite, Ruta Galoburda***
*Latvia University of Agriculture*
*martins.sabovics@inbox.lv*

## Abstract

Taste, smell and the flavour are the most important attributes determining the quality of bread or baked cereal products in general. Bread flavour is composed of hundreds of volatile and non-volatile compounds, i.e. many alcohols, ketones, aldehydes, acids, esters and other compounds. Many researchers have been studying volatile compounds in different breads worldwide, but in Latvia only few studies are conducted on volatile compounds in bread and its production stages. The aim of this research was to analyse the composition of volatile compounds during wheat dough fermentation. Experiments were done in 2011 and carried out in the Laboratory of Bread Technology and Laboratory of Packing Material Investigations at the Department of Food Technology in the Latvia University of Agriculture. An investigation of volatile compounds was done using solid-phase microextraction (SPME) and gas-chromatography mass-spectrometry (GC-MS). Volatile compounds were analyzed on the $10^{th}$, $20^{th}$ and $30^{th}$ minutes of wheat dough fermentation. In a fermentation process of wheat dough totally 15 volatile compounds were detected. Eight of them were alcohols, two aldehydes, two ketones, one ester, one acid and one terpene. Three volatile compounds – 1-octanol, caryophyllene and acetophenone, were detected in the dough samples only after 30 minutes of fermentation – those were not detected at the earlier stages of fermentation. The peak areas of 11 volatile compounds increased, but peak area of one volatile compound decreased along the fermentation time. The study proved that solid-phase microextraction can be used for detection of volatile compounds in wheat dough fermentation process.
**Key words**: wheat dough, volatile compounds, solid-phase microextraction, gas-chromatography.

## Introduction

Wheat (*Triticum aestivum*) is a raw material for various staple foods in many parts of the world. It is grown throughout the world and is adaptable to the wide range of environmental conditions. Wheat based foods are major source of nutrients such as carbohydrates, protein, vitamins and minerals in many regions of the world. It is extensively used in many parts of the world for the preparation of different types of bread and many other products (Kent and Evers, 1994). In many European regions for wheat and rye bread making is used sourdough. In Latvia sourdough is typically used for rye bread production, but yeast is used for wheat bread production. Sourdough is a key element in traditional rye bread baking, where it contributes significantly to the process ability, flavour and texture (Poutanen et al., 2009).

Taste, smell and the flavour are undoubtedly the most important attributes determining the quality of bread or baked cereals in general and one of the most important attributes influencing the acceptance of the consumer (Schieberle, 1996; Hansen and Schieberle, 2005). Flavour is usually the result of the presence, within complex matrices, of many volatile and non-volatile components possessing diverse chemical and physicochemical properties whereas the non-volatile compounds contribute mainly to the taste, the volatile ones influence both taste and aroma. A vast array of compounds may be responsible for the aroma of the food products, such as alcohols, aldehydes, esters, dicarbonyls, short to medium-chain free fatty acids, methyl ketones, lactones, phenolic compounds and sulphur compounds (Gatfield, 1988; Urbach, 1997).

The compounds which contribute to the aroma of food are usually present in trace to ultra-trace amounts comprising a diverse range of classes of chemical compounds. This chemical diversity may be further enhanced by subsequent processing. The physical properties of these compounds are equally diverse extending from that of the permanent gases to substances with boiling points exceeding several hundred degrees. This facilitates separations but complicates simultaneous recovery of the full range of aroma compounds. Methods used to assess the aroma compounds contributing to flavour in cereals are reviewed (Zhou et al., 1999). The ingredients and various technological processes (dough fermentation and the baking process) play a vital role in the development of characteristic flavour of the bread. The formation of volatile compounds in dough is started during lactic acid and alcoholic fermentation (Hansen and Hansen, 1994; Hansen and Schieberle, 2005). Aroma compounds in typical wheat bread are 3-methylbutanal, 2,3-butandione, 3-methylbutanol, acetic acid,

1-octen-3-one, 1-hexanol, 1-propanol, 2-methyl-1-butanol, 2-phenylethanol, ethyl acetate etc. (Schieberle, 1996; Poinot et al., 2008), but in bread made by sourdough composition of aroma compounds are propanol, pentanol, ethyl acetate, heptanal, acetic acid, ethanol, 2-pentylfurane, acetaldehyde, 3-methyl-1-butanal etc. (Damiani et al., 1996; Hansen and Schieberle, 2005; Guerzoni et al., 2007)

Many researchers have been studying volatile compounds in different breads worldwide, but only few studies are conducted on formation of volatile compounds in wheat bread and rye bread made by scald and sourdough technologies used in Latvia. Solid-phase microextraction (SPME) can be used for investigation of volatile compounds in wheat dough fermentation process.

Solid-phase microextraction is a modern, fast, sensitive, solvent-free and economical sample preparation technique before analysis through gas chromatography (Arthur and Pawliszyn, 1990; Hook et al., 2002). This technique has been developed to combine sampling and sample preparation in one step to analyse volatile compounds in bread crumb. Three different types of fibres can be used to determine the volatile compounds. Carboxen/Polydimethylsiloxane showed the best extraction efficiency for volatile analysis (Ruiz et al., 2003).

The aim of this research was to analyze the composition of volatile compounds during wheat dough fermentation.

## Materials and Methods

Experiments were carried out in the Laboratory of Bread Technology and Laboratory of Packing Material Investigations at the Department of Food Technology in the Latvia University of Agriculture in 2011.

For this research wheat flour (550$^{th}$ type of flour), purchased from joint stock company 'Rigas Dzirnavnieks' (Latvia) was used. Wheat flour (550$^{th}$ type) is especially strong flour which is suitable for baking. Properties of 550$^{th}$ type of flour: ash content 0.62 g 100 g$^{-1}$, Falling Number 280 s, quantity of protein – 29 g 100g$^{-1}$, stability of protein – 5 min, water absorption – 58%. Other raw materials – sugar (Danisco Sugar 'Dansukker', Finland), salt ('Artimsol', Ukraine) and dried yeast (S.I.Lesaffre 'San-instant', France) – were purchased from a local retail store. The recipe for experimental bread making is presented in Table 1.

Table 1

**Wheat dough recipe per 250 g of flour amount**

| Raw material | Amount |
|---|---|
| Wheat flour, 550$^{th}$ type, g | 250.0±1 |
| Salt, g | 3.0±1 |
| Sugar, g | 3.5±1 |
| Dried yeast, g | 3.5±1 |
| Water, mL | 160.0±1 |

The research structure is presented in Fig. 1. Water temperature for dough mixing was adjusted to 37.5 ± 0.3 °C. Dough mixing time 2 minutes (the first step – slow) and 8 minutes (the second step - fast). The dough samples were analyzed after 10, 20 and 30 minutes of yeast fermentation in triplicates.

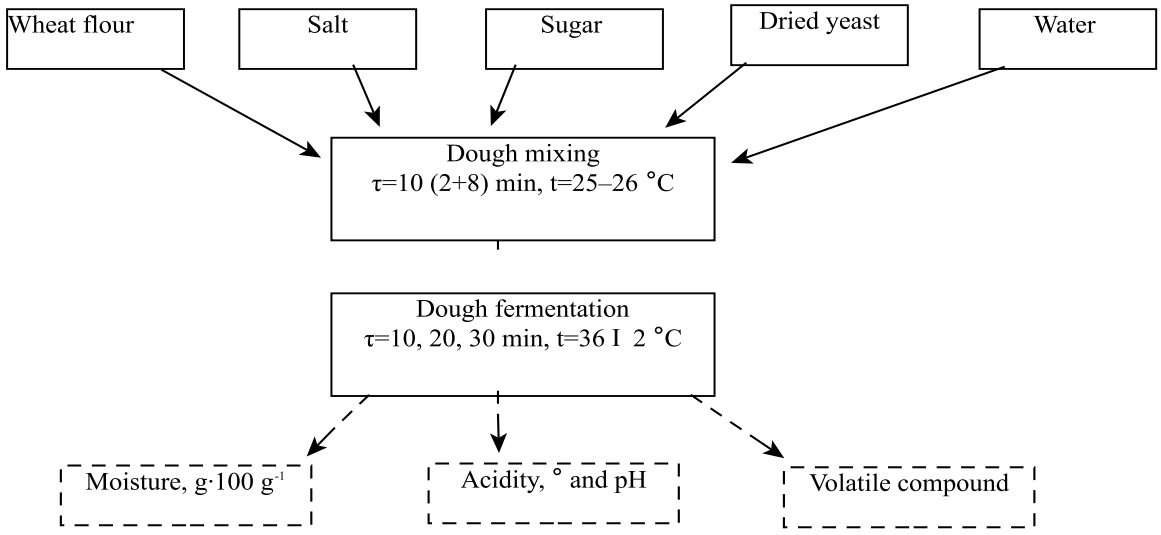



Figure 1. Preparation and analysis of dough samples.

Digital pH meter 'Jenway 3510' (Barloworld Scientific Ltd., UK) was used for pH measurement of dough. For pH measurement 5.00 ± 0.07 g of a sample was mixed with 50 mL of distilled water and stirred for two minutes in a 100 mL beaker (Standard method: AACC 02-52). Acidity was measured by titration against standardized 0.1 N NaOH and 1% phenolphthalein alcohol solution presence until pink colour (AACC 02-31). Moisture content was analysed drying sample in a moisture scale 'Precisa XM 105' (Precisa Instruments AG, Switzerland)

at the temperature of +110 ± 1 °C until constant weight (Express method).

For detection of volatile compounds 50.0 ± 0.1 g of mixed wheat dough was used, the sample was weight into a 250 mL glass container equipped with aluminium lid with 1 mm diameter hole in the middle for solid-phase microextraction (SPME) fibre. The container was placed in a water bath 'Clifton Food Range' (Nickel-electro Ltd, UK) at water temperature 36 ± 2 °C for fermentation process (Figure 2).

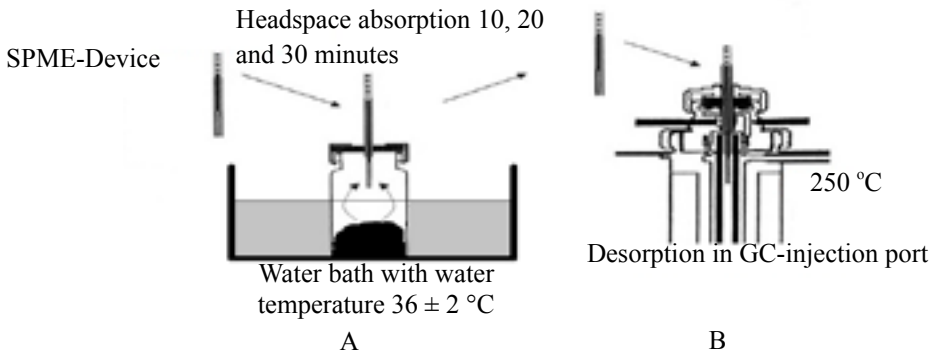



Figure 2. Volatile compound absorption (A) and desorption (B) process (Drawn by Sporkert and Pragst, 2000).

Volatile compounds were extracted from wheat dough samples using SPME fibre in a combination with gas chromatography/mass spectrometry. The SPME fibre itself is a fused-silica optical fibre, coated with a thin polymer film – Carboxen/Polydimethylsiloxane (CAR/PDMS). The SPME device is a modified syringe (Figure 3) consisting of manual fibre holder and a fibre assembly, which includes a 1 – 2 cm long retractable SPME fibre (Vas and Vékey, 2004).

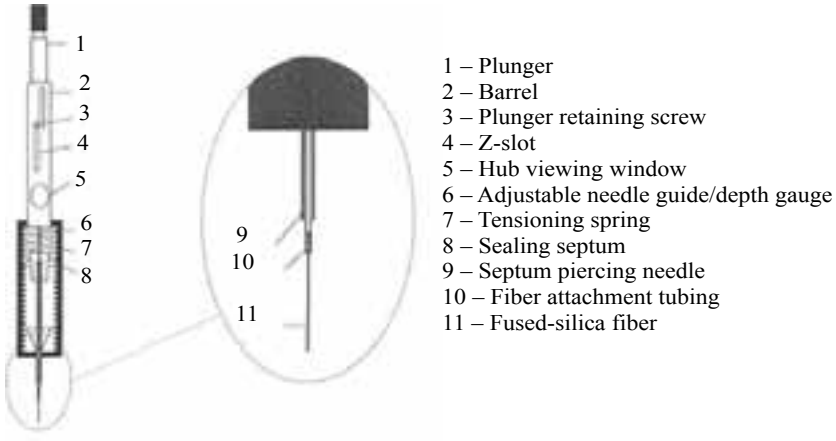



Figure 3. Schematic diagram of a commercial SPME device (Reprinted by Vas and Vékey, 2004).

The film thickness is 85 μm with biopolar polarity (Supelco, Inc., USA). Volatile compounds from fibre were thermally desorbed in the injector of a gas chromatograph-mass spectrometer 'Clarus 500 GC/MS'(PerkinElmer, Inc., USA). Before usage, the fibers were conditioned according to the manufacturer's instructions (30 min at 250 °C). The SPME extraction time was done for different duration of dough fermentation: the first - extraction time is 10 min at 36±2 °C without pre-incubation, the second – 20 min and the third – 30 min.

Volatile compounds from fiber were thermally desorbed in the injector of gas chromatograph/mass spectrometer. Separation of volatiles was carried out on the Elite-Wax (PerkinElmer, Inc., USA) capillary column (60 m × 0.25 mm i.d., DF 0.25 μm). The details of the program used in GC–MS analysis are as follows: the initial temperature was 40 °C, held for 7 min, then ramped from 40 °C to 160 °C at a rate of 6 °C $min^{-1}$ and from 160 °C to 210 °C at a rate of 10 °C $min^{-1}$ with a hold time for 15 min. The total run time was 47 min for a sample. Mass spectrometer in Electron impact Ionization mode was set on 70 eV as the electron energies, while the ion source temperature was set to 250 °C and inlet line temperature 250 °C. Injections were performed in splitless mode and helium (He) was used as carrier gas at a constant flow of 1 mL $min^{-1}$. Acquisition parameters in full scan mode: scanned m/z 40-300. Compounds were identified by comparison of their mass spectra with mass spectral library Nist98. Gas chromatograph-mass

spectrometer 'Clarus 500 GC/MS' was used for the qualitative analysis of volatile compounds in fermented wheat dough and in the results there are shown peak areas of volatile compounds.

Statistical analysis - means, standard deviation of the means were derived with Microsoft Office Excel 2007 (Microsoft Corporation, Redmond, WA).

## Results and Discussion

Water plays a very significant role in bread making. In quantity, water ranks second as an ingredient in bread dough. The amount of added water determines dough consistency and it is also essential for the gelatinization of starch and for the heat-setting of proteins. The wheat flour moisture was 11.6 ± 0.15 g 100 $g^{-1}$. In dough making process water in amount of 64% from wheat flour mass was added. Flour moisture and added water amount formed dough moisture to 29.60 ± 0.13 g 100 $g^{-1}$. Dough moisture is summarized in Figure 4.

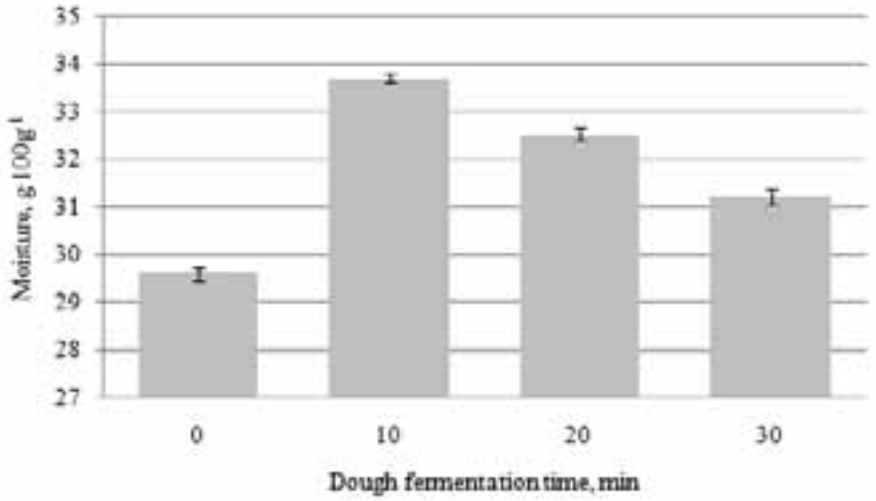



Figure 4. Moisture content in fermented wheat dough.

The results show that the highest moisture content (33.71 ± 0.10 g 100 $g^{-1}$) was in dough after 10 minutes of fermentation, but in the further process (after 20 and 30 min) moisture of dough decreased to 32.52 ± 0.14 g 100 $g^{-1}$ and 31.22 ± 0.15 g 100 $g^{-1}$, respectively. This could be explained by the wheat flour ability to bind water at the beginning of fermentation, but in the next minutes moisture was released from dough when temperature was rising to 36 ± 2 °C.

Fresh dough had an initial pH 5.833 ± 0.049. Dough pH, acidity and volatile compounds total peak area are summarized in Table 2.

Table 2

**pH, acidity and total peak area of volatile compounds in fermented dough**

| Fermentation time, min | pH | Acidity, ° | Total peak area (×$10^6$) of volatile compounds |
|---|---|---|---|
| 10 | 5.645±0.042 | 1.6±0.1 | 122.19 |
| 20 | 5.575±0.021 | 1.8±0.1 | 200.89 |
| 30 | 5.399±0.038 | 2.0±0.1 | 316.73 |

Flour and yeast contained small amounts of bacteria, which can produce acetic and lactic acids as their main metabolic products (Sluimer, 2005). Because of acid formation in a dough, fermentation pH value decreased. Freshly prepared dough acidity was 1.0 ± 0.1°, but after 30 minutes of fermentation the dough acidity increased to 2.0 ± 0.1°. Comparing the pH value in dough after 10 minutes or 30 minutes of fermentation, the pH decrease from 5.645 ± 0.042 to 5.399 ± 0.038 was observed. Wheat dough acidity possibly increased due to the formation of various acids in a fermentation process.

The volatile profile of different bread types has been widely investigated during the past years. These studies demonstrated that bread flavour is composed by different volatile compounds, belonging to several chemical classes, mainly heterocyclic compounds, alcohols, aldehydes, ketones, etc. Depending on the characteristic of each kind of bread, volatile compounds are present in well defined ratios (Grosch and Schieberle, 1997; Rehman et al., 2006; Bianchi et al., 2008).

Analysing the volatile compounds after 10, 20 and 30 minutes of wheat dough fermentation overall was identified

8 alcohols, 2 aldehydes, 2 ketones, 1 terpene, 1 ester and 1 acid. In wheat dough after 10 minutes of fermentation 11 volatile compounds were detected, after 20 minutes – 12, but after 30 minutes – 15 volatile compounds. The volatile compounds that were found in wheat dough fermentation time are presented in Table 3 as ($\times 10^6$) of peak area.

The peak areas of detected 11 volatile compounds increased in dough fermentation process, but peak area of acetic acid decreased. The highest peak area of acetic acid was detected after 10 minutes (3.84) of wheat dough fermentation, but the lowest after 30 minutes of fermentation (3.03). This can be explained that in the wheat dough fermentation process, alcohols and acids which further can be transformed to ethyl acetate detected after 20 and 30 minutes of fermentation can be formed from yeast. Ethanol was not detected in wheat dough after 30 minutes of fermentation as a volatile compound, but it can be formed from sugars in the fermentation process. Ethyl acetate peak area increased by 2.91 during fermentation between 20 and 30 minutes. After 30 minutes of fermentation 3 volatile compounds (1-octanol, caryophyllene and acetophenone) which were not detected after 10 and 20 minutes of fermentation were identified.

Table 3

**Volatile compounds in wheat dough at different stages of fermentation**

| Compounds | Retention time (min) | Peak area ($\times 10^6$) depending on dough fermentation time | | |
|---|---|---|---|---|
| | | 10 min | 20 min | 30 min |
| 4-amino-1-pentanol | 4.77 | 11.64 | 15.53 | 23.77 |
| 2-ethyl-butanal | 6.09 | 0.79 | 0.86 | 1.63 |
| Ethyl acetate | 7.80 | n.d. | 1.92 | 4.83 |
| 4-penten-2-ol | 9.25 | 76.00 | 129.91 | 211.80 |
| 2-methyl-1-propanol | 14.60 | 1.46 | 2.90 | 3.80 |
| 1-ethoxy-2-propanol | 16.89 | 10.48 | 11.16 | 19.23 |
| 3-methyl-1-butanol | 18.09 | 13.73 | 27.69 | 36.68 |
| 1-pentanol | 19.28 | 0.41 | 0.72 | 1.03 |
| 3-hydroxy-2-butanone | 20.54 | 0.57 | 0.90 | 1.59 |
| 1-hexanol | 21.94 | 2.03 | 4.22 | 6.91 |
| Nonanal | 23.14 | 0.51 | 0.61 | 1.17 |
| Acetic acid | 24.52 | 3.84 | 3.16 | 3.03 |
| 1-octanol | 26.58 | n.d. | n.d. | 0.79 |
| Caryophyllene | 27.81 | n.d. | n.d. | 1.04 |
| Acetophenone | 28.96 | n.d. | n.d. | 1.00 |

n.d. – not detected

Researchers describe that the volatile compounds in bakery products are formed in mixing, fermentation (yeast) and baking process. Added raw materials and their amount has great effect on product`s aroma formation (Martínez-Anaya, 1996; Thiele et al., 2002; Sabovics et al., 2010). The highest peak area value (211.80) among all detected volatile compounds had 4-penten-2-ol (alcohol) indentified after 30 minutes of fermentation, but the lowest peak area value (0.41) was detected for 1-pentanol (alcohol) after 10 minutes of fermentation. From 3 volatile compounds detected only after 30 minutes of fermentation, the highest peak area value (1.04) showed caryophyllene (terpene), but the lowest (0.79) – 1-octanol (alcohol).

In all dough samples 11 common volatile compounds (Table 3) were found: alcohols (4-amino-1-pentanol, 4-penten-2-ol, 2-methyl-1-propanol, 1-etoxy-2-propanol, 3-methyl-1-butanol, 1-pentanol, 1-hexanol), aldehydes (2-ethylbutanal, nonanal), ketone (3-hydroxy-2-butanone) and acid (acetic acid). Alcohols can be formed in alcoholic fermentation when yeast can produce long-chain and complex alcohols, otherwise aldehydes and ketones can be formed from alcohols. Acetic acid is formed in dough fermentation process from yeast. Yeast secondary metabolism can form 3-methyl-1-butanol, which gives malty flavour to dough. The amount of flavour compounds formed in dough can be affected by yeast amount and activity, fermentation time and fermentation temperature. All of the detected volatile compounds can produce dough aroma: 2-methyl-1-propanol produce whiskey odour, 4-penten-2-ol – fruity, 1-pentanol – sweet, 3-hydroxy-2-butanone – like butter or yogurt, 1-hexanol – freshly mown grass and nonanal - strong fruity or floral odour (Schieberle, 1996; Zhou et al., 1999; Kulp et al., 2003).

Volatile compounds detected after 30 minutes of

dough fermentation adds more specific aroma to dough, because caryophyllene give woody spicy and hay odour, acetophenone - spicy (almond, nuts), fruity (cherry, strawberry), but 1-octanol can give to yeast fermented wheat dough fresh like orange-rose, waxy and sweet odour (Schieberle, 1996; Zhou et al., 1999; Kulp et al., 2003).

## Conclusion

1. The highest moisture content 33.71 ± 0.10 g 100 $g^{-1}$ was in dough after 10 minutes of fermentation, but in the further process (after 20 and 30 min) moisture of dough decreased to 32.52 ± 0.14 g 100 $g^{-1}$ and 31.22 ± 0.15 g 100 $g^{-1}$.
2. Solid-phase microextrection in combination with GC/MS can be used for detection of volatile compounds in fermented wheat dough.
3. After 30 minutes of fermentation three compounds – 1-octanol, caryophyllene and acetophenone, which were not present in samples tested after 10 or 20 minutes of fermentation were detected.
4. In all dough samples (after 10, 20 or 30 minutes of fermentation) totally 15 volatile compounds out of which 11 volatile compounds were common: alcohols (4-amino-1-pentanol, 4-penten-2-ol, 2-methyl-1-propanol, 1-etoxy-2-propanol, 3-methyl-1-butanol, 1-pentanol, 1-hexanol), aldehydes (2-ethylbutanal, nonanal), ketone (3-hydroxy-2-butanone) and acid (acetic acid) were detected.

## Acknowledgment

This research has been prepared within the framework of the ESF Project „Formation of the Research Group in Food Science", Contract No. 2009/0232/1DP/1.1.1.2.0/09/APIA/ VIAA/122.

## References

1. Arthur C.L., Pawliszyn J. (1990) Solid phase microextraction with thermal desorption using fused silica fibers. *Analytical Chemistry*, 62, pp. 2145-2148.
2. Bianchi F., Careri M., Chiavaro E., Musci M., Vittadini E. (2008) Gas chromatographic-mass spectrometric characterization of the Italian Protected Designation of Origin 'Altamura' bread volatile profile. *Food Chemistry*, 110, pp. 787-793.
3. Damiani P., Gobbetti M., Cossignani L., Corsetti A., Simonetti M.S., Rossi J. (1996) The sourdough microflora. Characterization of hetero- and homofermentative lactic acid bacteria, yeast and their interactions on the basis of the volatile compounds produced. *Food Science and Technology*, 29, pp. 63-70.
4. Gatfield I.L. (1988) Production of flavour and aroma compounds by biotechnology. *Food Technology,* 10, pp. 110-122.
5. Grosch W., Schieberle P. (1997) Flavour of cereals products – A review. *Cereal Chemistry*, 74, pp. 91-97.
6. Guerzoni M.E., Vernocchi P., Ndagijimana M., Gianotti A., Lanciotti R. (2007) Generation of aroma compounds in sourdough: effects of stress exposure and lactobacilli-yeast interactions. *Food Microbiology*, 24, pp. 139-148.
7. Hansen A., Hansen B. (1994) Influence of wheat flour type on the production of flavour compounds in wheat sourdough. *Journal of Cereal Science*, 19, pp. 185-190.
8. Hansen A., Schieberle P. (2005) Generation of aroma compounds during sourdough fermentation: applied and fundamental aspects. *Trends in Food Science and Technology*, 16 (1-3), pp. 85-94.
9. Hook G.L., Kimm G.L., Hall T., Smith P.A. (2002) Solid-phase microextraction (SPME) for rapid field sampling and analysis by gas chromatography-mass spectrometry (GC-MS). *Trends in Analytical Chemistry*, 21, pp. 534-543.
10. Kent N.L., Evers A.D. (1994) *Technology of cereals: an Introduction for Students of Food Science and Agriculture*, 4th Edition, Pergamon Press, Oxford, UK, 334 p.
11. Kulp K., Lorenz K.J. (2003) *Handbook of Dough Fermentation*, Marcel Dekker, Inc., New York, USA, 328 p.
12. Martínez-Anaya M.A. (1996) Carbohydrate and nitrogen related components in wheat sourdough processes: a review. *Advances in Food Science*, 18, pp. 185-200.
13. Poinot P., Arvisenet G., Grua-Priol J., Colas D., Fillonneau C., Le Bail A., Prost C. (2008) Influence of formulation and process on the aromatic profile and physical characteristics of bread. *Journal of Cereal Science*, 48, pp. 686-697.
14. Poutanen K., Flander L., Katina K. (2009) Sourdough and cereal fermentation in a nutritional perspective. *Food Microbiology*, 26, pp. 693-699.
15. Rehman S., Peterson A., Piggott J.R. (2006) Flavour in sourdough breads: A review. *Trends in Food Science and Technology*, 17, pp. 557-566.
16. Ruiz J.A., Quilz J., Mestres M., Guash J. (2003) Solid phase micro-extraction method for head space analysis of volatile compounds in bread crumb. *Cereal Chemistry*, 80, pp. 255-259.
17. Sabovics M., Straumite E., Galoburda R. (2010) Analysis of Volatile Compounds in Flour Blend from Non-traditional Cereals in Latvia. Available at: http://www.foodinnova.com/foodInnova/docu2/36.pdf, 5 March 2011.
18. Schieberle P. (1996) Intense aroma compounds – useful tools to monitor the influence of processing and storage on bread aroma. *Advances in Food Science*, 18, pp. 237-244.
19. Sluimer P. (2005) *Principles of Breadmaking. Functionality of Raw Materials and Process Steps,* American Association of Cereal Chemists, Inc., Minnesota, USA, 212 p.

20. Sporkert F., Pragst F. (2000) Use of headspace solid-phase microextraction (HS-SPME) in hair analysis for organic compounds. *Forensic Science International,* 107(1-3), pp. 129-148.
21. Thiele C., Ganzle M.G., Vogel R.F. (2002) Contribution of sourdough lactobacilli, yeast and cereal enzymes to the generation of amino acids in dough relevant for bread flavor. *Cereal Chemistry,* 79, pp. 45-51.
22. Urbach G. (1997) The flavour of milk and dairy products: II. Cheese: Contribution of volatile compounds. *International Journal of Dairy Technology*, 50, pp. 79-89.
23. Vas G., Vékey K. (2004) Solid-phase microextraction: a powerful sample preparation tool prior to mass spectrometric analysis. *Journal of Mass Spectrometry*, 39, pp. 233-254.
24. Zhou M.X., Robards K., Glennie-Holmes M., Helliwell S. (1999) Analysis of volatile compounds and their contribution to flavor in cereals. *Journal of Agricultural and Food Chemistry*, 47(10), pp. 3941-3953.

# ANTHOCYANIN CONTENT IN LATVIAN CRANBERRIES DRIED IN CONVECTIVE AND MICROWAVE VACUUM DRIERS

***Karina Ruse, Tatjana Rakcejeva, Ruta Galoburda, Lija Dukalska***
*Latvia University of Agriculture*
*karinaruse@inbox.lv*

**Abstract**

The current research focuses on the study of anthocyanin content changes in Latvia wild growing as well as cultivated cranberries during convective and microwave vacuum drying. The research was accomplished on fresh Latvian wild and cultivated cranberries. The berries before drying in a convective drier were pre-treated using perforating, steam-blanching and halving; berries dried in microwave vacuum drier – using steam-blanching and halving; part of berries was dried in microwave vacuum drier without pre-treatment (whole berries). For air drying experiments, a convective dryer "Memmert" (Model 100-800) was used. For drying experiments in microwave vacuum a dryer „Musson-1" was used. Anthocyanin was determined by means of spectrophotometric method. Data are expressed as mean ± standard deviation; variance analysis, homogeneity were used for the evaluation of changes of anthocyanin in cranberries during drying depending on pre-treatment methods. The initial content of anthocyanin among wild and cultivated fresh cranberries was differing: very similar anthocyanin content was detected in cranberry cultivars 'Pilgrim' and 'Early Black', it was on average three times higher comparing to wild fresh cranberries. However, the lowest anthocyanin content was detected in wild fresh cranberries – 306.81 ± 4.19 mg $\oplus 100g^{-1}$ (in dry matter). With the probability of 95%, detected by means of the analysis of variance, it may be presumed, that pre-treatment method of cranberries influenced anthocyanin changes during convective and microwave vacuum drying ($p=0.001, <=0.05$). Halving is advisable as a pre-treatment method for berries processing in a convective or microwave drier, because decrease in anthocyanin content is smaller.

**Key words**: anthocyanin, cranberries, pre-treatment, convective drying, microwave vacuum drying.

## Introduction

The cranberry, *Vaccinium macrocarpon* Ait., accumulates some of the highest concentrations of phenolic compounds, with demonstrable human health related benefits including antioxidant status, antiviral, and anticancer properties. Studies on cranberries have focused mainly on the anthocyanin fraction due to the high concentration found in berries and importance as a berries' quality (Vedenskaya and Vorsa, 2004). Recent studies have shown anthocyanin, proanthocyanidins from cranberries are active components in molecular mechanism behind various health benefits of cranberries (Lacombe et al., 2010).

Anthocyanins (from Greek anthos, a flower; and kyanos, dark blue) are the largest and most important group of water-soluble and vacuolar pigments in nature. They comprise a major flavonoid group that is responsible for cyanic colours ranging from salmon pink through red and violet to dark blue of most flowers, fruits and leaves of angiosperms commonly found in nature (Andersen and Jordheim, 2006; Delgado-Vargas and Paredes-López, 2003). The most significant function of anthocyanin is their ability to impart colour to the plants or plant products in which they occur (Kong et al., 2003).

The red colour of cranberries is due to the presence of four major anthocyanin pigments: cyanidin-3-galactoside (Cy-3Ga), peonidin-3-galactoside (Pn-3-Ga), cyanidin-3-arabinoside (Cy-3-Ar), peonidin-3-arabinoside (Pn-3-Ar) and two minor anthocyanin: cyanidin-3-glucoside and peonidin-3-glucoside (Sapers and Hargrave, 1987).

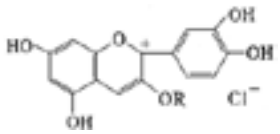



Figure 1. The structure of anthocyanin (Антоцианы, 2011).

The biosynthesis of anthocyanin has been characterized in great detail. The basic anthocyanin molecule is comprised of two aromatic rings and oxygen containing heterocyclic ring (Figure 1). One of the aromatic rings is derived from phenylalanine and the second ring from the action of chalcone synthase (CHS), condensing one molecule of p-counaroyl-coA with three molecules of malonyl-coA to produce tetrahydroxy chalcone (Winkel-Shirley, 2001; Grotewold, 2006; Yu et al., 2006). CHS is the first committed enzyme in the anthocyanin biosynthetic pathway. The regulation of anthocyanin biosynthesis has also been studied thoroughly and comprises basic-helix-loop-helix (bHLH) transcription factors, interacting with R2R3 MYB (LhMYB6 and LhMYB12) transcription factors to activate either all or part of the anthocyanin genes (Allan et al., 2008).

According to Lee (2009), anthocyanin constitutes pigments with a wide range of biological activities including antioxidant (Tsuda et al., 2003), anti-inflammatory (Wang and Mazza, 2002; Youdim et al., 2002), anticancer (Hou, 2003), antimutagenic (Peterson and Dwyer, 1998) and

α-glucosidase inhibition (Matsui et al., 2001).

Drying is one of the oldest methods of food preservation, and it is a food processing operation mainly because undesirable changes in quality (Wang and Xi, 2005).

Many authors have studied the influence of temperature in the anthocyanin stability from different sources proving that heating has a detrimental effect on the anthocyanin content (Ikeda et al., 2009; Jimenez et al., 2010; Lin et al., 2009; Ochoa et al., 1999; Rodrigues et al., 2009; Sadilova et al., 2009). Anthocyanin is highly unstable and easily susceptible to degradation whose colour stability is strongly affected by pH, temperature, anthocyanin concentration, oxygen, light, enzymes, and other accompanying substances such as ascorbic acid, sugars, sulphites, co-pigments, metallic ions, among others (Wilska-Jeszka, 2007).

Different drying methods are used for drying fruits, berries and vegetables. Air-drying is the most common method in the drying of foodstuffs. However, this method leads to serious injuries such as worsening of the taste, colour and nutritional content of the product, decline in the density and water absorbance capacity and shifting of the solutes from the internal part of the drying material to the surface due to the long drying period and high temperature. Microwave drying has the specific advantage of rapid and uniform heating due to the penetration of microwaves into the body of the product. Microwave energy is capable of polarizing substances. The electrons in the polarized substance are in motion due to the conversion of electromagnetic energy embedded in the substance into kinetic energy. Electrons bump into each other during this electron movement and their energy is converted to heat energy as a result of friction. Thus, the moisture was removed from the product in the microwave drying (Alibas, 2007).

Compared with convective atmospheric drying, vacuum drying has some distinctive characteristics such as higher drying rate, lower drying temperature and oxygen deficient processing environment etc., these characteristics may help to improve the quality and nutritive value of the dried products. Presently, vacuum drying has been applied to dry various food materials, the vacuum drying kinetics of many fruits and vegetables has been investigated and the effect of vacuum drying conditions on the drying process and the qualities of dried products have been evaluated (Wu et al., 2007).

For the better water evaporation during drying process there are known many vegetable and fruit pre-treatment methods such as halving or slicing (Hui et al., 2006), blanching in hot water (Mayer-Miebach and Spieß, 2003), steam-blanching in order to inactivate enzymes activity (Llano, 2003) and perforation with needle (of 1 mm diameter) (Shi et al., 1997).

The current research focuses on the study of anthocyanin.

## Materials and Methods

The research was accomplished on fresh in Latvia wild growing (*Vaccinium oxycoccus* L.) and cultivated (*Vaccinium macrocarpon* Ait.) cranberries harvested in Kurzeme region in the first part of October, 2010 and immediately used in current drying experiment. Cranberry cultivars were: 'Early Black', 'Ben Lear', 'Stevens', 'Bergman' and 'Pilgrim'.

Three methods were used for pre-treatment of berries: perforation, halving and steam-blanching. The berries before drying in a convective drier were pre-treated using all three methods and berries dried in microwave vacuum drier – using two pre-treatment methods – steam-blanching and halving. Part of berries was dried in microwave vacuum drier without pre-treatment (whole berries).

Perforation of berries (3.000 ± 0.001 kg) was realised manually by a needle (1 mm diameter) about 20 pricks equally on all berry surface; halving (3.000 ± 0.001 kg) was realised manually by knife; steam-blanching (3.000 ± 0.001 kg) was realised using "TEFAL VC4003 VITAMIN+" (Tefal, China) vessel at temperature + 94 ± 1 °C.

Drying conditions were selected in accordance to results of previous experiments for maximal biological compounds preservation in berries during processing in elevated temperatures (Dorofejeva et al., 2010).

For air drying experiments, a convective dryer "Memmert" Model 100-800 (Memmert GmbH Co. KG, Germany) was used; drying parameters were as follows: temperature 50 ± 1 °C and air flow velocity 1.2 ± 0.1 $m \oplus s^{-1}$. Berries were placed on a perforated sieve (diameter − 0.185 m), with the diameter of the holes – 0.002 m.

For drying experiments in vacuum, a microwave dryer „Musson-1" (OOO Ingredient, Russia) was used (at 2450 MHz frequency and length of waves − 12.5 cm) (Vacuum microwave drier MUSSON-1, 2007). The power of installed magnetrons each of four is 640 W. The necessary amount of microwave energy (magnetron minutes) was calculated. The following drying conditions for processing of cranberries in microwave vacuum drier were selected: the first drying stage at 4 magnetrons – energy of 2100 kJ, the second stage at 3 magnetrons – energy of 2520 kJ, the third stage at 2 magnetrons – 1260 kJ and the fourth stage at 1 magnetron – 756 kJ. Temperature in microwave vacuum drier was 36 ± 2 °C.

Anthocyanin were determined by means of "Spectrophotometer Anthocyanin Determination Method" (Bordignon-Luiz et al., 2007) using a device 6705 UV/VIS Spectrophotometer YENWAY.

Data are expressed as mean ± standard deviation; variance analysis, homogeneity were used for the evaluation of changes of anthocyanin in cranberries during drying depending on pre-treatment methods. Each experiment was carried out in triplicate.

## Results and Discussion

During the current research it was established that the initial content of anthocyanins in wild and cultivated fresh cranberries was different, which mainly depended on varieties' individuality and growing conditions.

Very similar anthocyanin content was detected in cranberry cultivars 'Pilgrim' and 'Early Black', 898.66 ± 12.69 mg $100g^{-1}$ and 839.59 ± 8.53 mg ⊕$100g^{-1}$ in dry matter respectively (Table 1); the anthocyanin content in analysed berries was on average three times higher compared to wild fresh cranberries. However, the lowest anthocyanin content was detected in wild fresh cranberries – 306.81 ± 4.19 mg ⊕$100g^{-1}$ (in dry matter) (Table 1).

Low anthocyanin content in wild cranberries could be explained mainly by growing conditions of berries, ie., in berries grown in greenwood bog in a sunny place without pronounced wind (air temperature was elevated); as a result, smaller amount of anthocyanin was formed. It is possible that fertilizer presence positively influenced anthocyanin formation in cultivated berries during growing process too.

Table 1

**Anthocyanin content in dry matter of fresh cranberries**

| No. | Cranberry cultivar | Content of anthocyanin, mg $100g^{-1}$ |
|---|---|---|
| 1 | Wild | 306.81±4.19 |
| 2 | 'Stevens' | 612.98±6.69 |
| 3 | 'Bergman' | 674.34±6.79 |
| 4 | 'Ben Lear' | 492.56±4.74 |
| 5 | 'Pilgrim' | 898.66±12.69 |
| 6 | 'Early Black' | 839.59±8.53 |

For the shelf life extension, cranberries were dried using convective or microwave vacuum drying methods.

The temperature in convective dryer for maximum preservation of biologically active compounds was established of 50 ± 1 °C (Doymaz, 2008; Dorofejeva et al., 2010).

Anthocyanin are highly unstable molecules and easily susceptible to degradation through factors such as light, pH, temperature, sulphite, ascorbic acid, enzymes, among others (Wesche-Ebeling and Argaiz-Jamet, 2002). In scientific literature it was found that the stability of anthocyanin and all pigment content in foods decreaseduring processing and storage as temperature increases (Bobbio and Mercadante, 2008).

The mechanical and thermal pre-treatment of berries was used because the berries' waxy skin presents a high resistance to water vapour transfer. In the present research it was found out that there was no pronounced correlation between changes in content of anthocyanin, cranberry cultivar and pre-treatment method of cranberries, when microwave vacuum drying was applied (Figure 2).

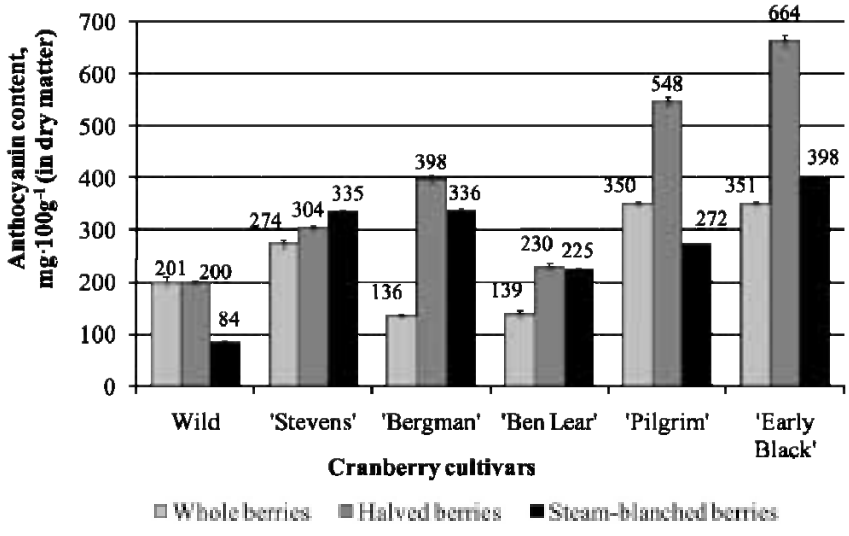



Figure 2. Anthocyanin content in microwave vacuum dried cranberries of different cultivars depending on the pre-treatment method.

The results of present experiments demonstrate that for maximum preservation of anthocyanin in berries during microwave vacuum drying pre-treatment of berries is necessary. Smaller loss of anthocyanin was found only if wild cranberries dried as whole berries: the anthocyanin content decreased 1.53 times (by 65%) comparing to anthocyanin content in fresh wild cranberries. The halving of berries prior to drying reduces the loss of anthocyanin in cranberry cultivars 'Bergman', 'Pilgrim' and 'Early Black' during microwave vacuum drying (Figure 2), i.e. anthocyanin content decreased by 1.69, 1.64 and 1.26 times (by 59%, 61% and 79%) respectively comparing to fresh berries (Table 1). The decrease of anthocyanin content in cranberry cultivars 'Stevens' and 'Ben Lear' pre-treated by halving or by steam-blanching was very similar (Figure 2), therefore for drying process optimisation the halving is advisable as pre-treatment method for berries. It is necessary to observe minimal juice losses during berries halving, mainly because berries tightness. During short time steaming only micro crevices are formed on berries surface for better moisture migration in future drying process. With the probability of 95%, detected by means of the analysis of variance, it may be presumed that pre-treatment method of cranberries influenced anthocyanin decrease (the anthocyanin content decrease − mg $\oplus 100g^{-1}$ was used as the dependent variable) in berries during microwave vacuum drying ($p=0.00 < =0.05$).

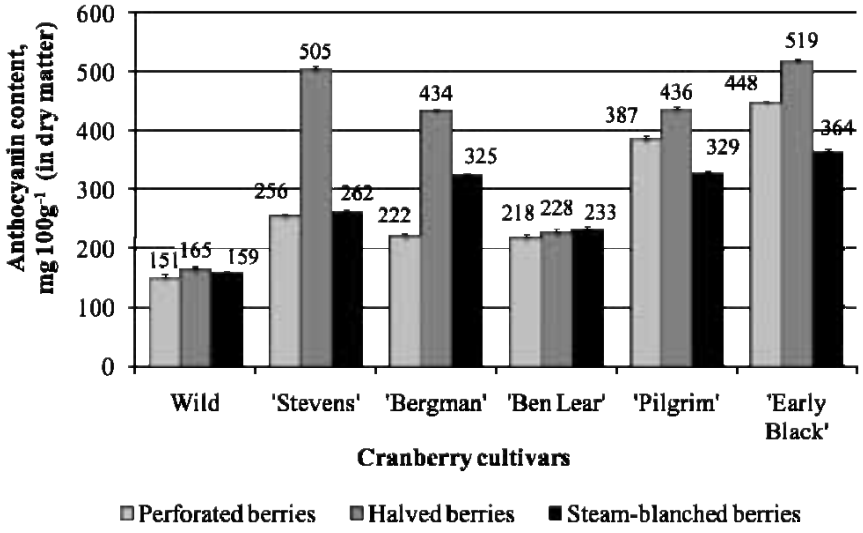



Figure 3. Influence of pre-treatment method on the anthocyanin content in several cultivars of convective dried cranberries.

In the present research it was found that there is no pronounced correlation between changes in content of anthocyanin, cranberry cultivars and pre-treatment method of cranberries, when drying in a convective drier was applied (Figure 3).

In present experiments it was established that pre-treatment does not significantly influence loss of anthocyanins in wild cranberries and cranberry cultivar 'Ben Lear' – the loss of anthocyanins was very similar, ie., approximately 50% decrease compared to initial anthocyanin content in fresh cranberries was observed. Anthocyanin losses in berries pre-treated by perforating and steam-blanching were significant (Figure 3) for cranberry cultivars 'Stevens', 'Bergman', 'Pilgrim' and 'Early Black' compared to anthocyanin content in berries pre-treated by other methods. Therefore, the pre-treatment methods such as perforating and steam-blanching of berries are not recommendable for preservation of anthocyanin during convective drying of cranberries. Such pre-treatment processes are difficult and labour-consuming and the possible economical effect is not beneficial. Halving, as a pre-treatment method for anthocyanin preservation in berries during convective drying is more recommendable for cranberry cultivars 'Stevens', 'Bergman', 'Pilgrim' and 'Early Black'; i.e. anthocyanin content in berries decreased during drying by 1.21, 1.55, 2.06 and 1.62 times (by 82%, 64%, 49% and 62%) respectively compared to the initial content of anthocyanin in fresh berries.

As in experiments of berries drying in microwave vacuum drier, with the probability of 95%, detected by means of the analysis of variance, it may be presumed, that pre-treatment method of cranberries influenced anthocyanin decrease (the anthocyanin content decrease − $mg \cdot 100g^{-1}$ was used as the dependent variable) during berries convective drying ($p=0.001 < =0.05$).

As a result, halving is advisable as a pre-treatment method for berries processing before both convective and microwave vacuum drying, because decreases in anthocyanin content (Figure 4) are smaller. It could be explained by anthocyanin location in the skin of berries, ie., if berries are dried whole or perforated, the skin of berries reduce the water evaporation, therefore the temperature on

the skin increases. As a result, anthocyanin destroys. If berries are halved, the moisture evaporation from the inside of berries is easier; thus, the temperature on the surface of a berry does not increase and loss of anthocyanin is smaller.

After mathematical data processing, it was established that halving of cranberry cultivar 'Ben Lear' (Figure 4) influences the changes in anthocyanin content similar in microwave vacuum or convective driers and there is not found any substantial difference ($p=0.807$, $< =0.05$). However, there is found substantial difference ($p=0.001$, $< =0.05$) in anthocyanin content loss in wild, 'Stevens', 'Bergman', 'Pilgrim' and 'Early Black' using different drying methods.

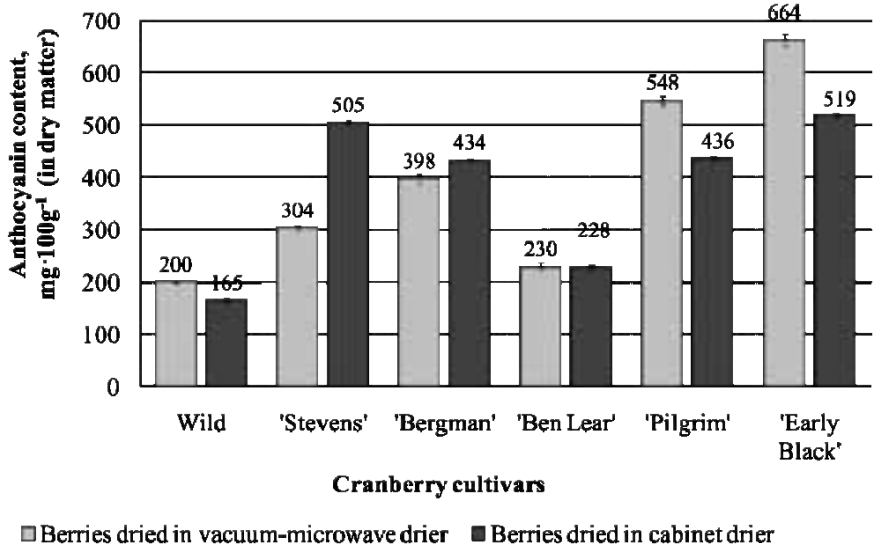



Figure 4. Anthocyanin content in halved convective and microwave vacuum dried cranberries.

During current experiments it was proved, that halving of wild, 'Ben Lear', Pilgrim' and 'Early Black' cranberry cultivars do not significantly influence the anthocyanin content loss during drying process in microwave vacuum dryer whereas halving of cranberry cultivars 'Stevens' and 'Bergman' does not significantly influence the anthocyanin content loss during drying in convective dryer that mainly depends on variety individuality and chemical composition of berries.

## Conclusions

The highest anthocyanin content was detected in fresh cranberry cultivars 'Pilgrim' and 'Early Black' (898.66 ± 12.69 and 839.59 ± 8.53 mg 100 $g^{-1}$ in dry matter respectively); the lowest − in wild fresh cranberries (306.81 ± 4.19 mg 100 $g^{-1}$ in dry matter) Halving of berries reduces the loss of anthocyanin in cranberry cultivars 'Bergman', 'Pilgrim' and 'Early Black' during microwave vacuum drying − anthocyanin content decreased by 59%, 61% and 79% respectively compared to initial content in fresh cranberries. The loss of anthocyanin in cranberry cultivars 'Stevens' and 'Ben Lear' pre-treated by halving or by steam-blanching was very similar.

The perforating and steam-blanching pre-treatment methods do not preserve the anthocyanin in convective dried cranberries. Halving, as a pre-treatment method influence the anthocyanin preservation in convective dried cranberry cultivars 'Stevens', 'Bergman', 'Pilgrim' and 'Early Black' − anthocyanin content decreased by 82%, 64%, 49% and 62% respectively compared to the initial content of anthocyanin in fresh berries.

During current experiments it was proved that halving of wild, 'Ben Lear', Pilgrim' and 'Early Black' cranberry cultivars do not significantly influence the anthocyanin content loss during drying in microwave vacuum dryer whereas halving of cranberry cultivars 'Stevens' and 'Bergman' variety does not significantly influence the anthocyanin content loss during drying in convective dryer.

## Acknowledgements

The research and publication has been prepared within the framework of the ESF Project "Formation of the Research Group in Food Science", Contract No. 2009/0232/1DP/1.1.1.2.0/09/APIA/VIAA/122.

## References

1. Alibas I. (2007) Microwave, air and combined microwave – air-drying parameters of pumpkin slices. *Food Science and Technology*, Volume 40, Issue 8, pp. 1445-1451.
2. Allan A.C., Hellens R.P. and Laing W.A. (2008) MYB transcription factors that colour our fruit, *Journal of Trends Plant Science*, 13, pp. 99-102.
3. Andersen O.M. and Jordheim M. (2006) The anthocyanins. In O.M. Andersen, & K.R. Markham (eds), Flavonoids: *Chemistry, biochemistry and applications*. Boca Raton, FL: CRC Press, Taylor & Francis Group, pp. 471-551.

4. Bobbio F.O. and Mercadante A.Z. (2008) Anthocyanins in foods: occurrence and physicochemical properties. In: F.O. Bobbio & A.Z. Mercandante (eds). *Food colorants: chemical and functional properties*, 1 Boca Raton: CRC Press. pp. 241-276.
5. Bordignon-Luiz M.T., Gauche C., Gri E.F. and Falcao L.D. (2007) Colour Stability of Anthocyanins from Isabel Grapes *(Vitis labrusca L.)* in *Model Systems, LWT-Food Science and Technology,* 40(4), pp. 594-599.
6. Delgado-Vargas F. and Paredes-López O. (2003) Anthocyanins and betanals. In F. Delgado-Vargas and Paredes-López O. (Eds.), *Natural colorants for food natraceutical uses* Boca Raton: CRC Press. pp. 167-211.
7. Doymaz İ. (2008) Convective drying kinetics of strawberry. *Chemical Engineering and Processing: Process Intensification*, 47(5), pp. 914-919.
8. Dorofejeva K., Rakcejeva T., Skudra L., Dimins F. and Kviesis J. (2010) Changes in physically – chemical and microbiological parameters of Latvian wild cranberries during convective drying. *Research of rural development 2010.* Volume 1. pp. 132-137.
9. Grotewold E. (2006) The genetics and biochemistry of floral pigments. *Annual Review of Plant Biology*, 57, pp. 761-780.
10. Hou D.X. (2003) Potential mechanisms of cancer chemoprevention by anthocyanins. *Current Molecular Medicine*, 3, pp. 149-159.
11. Hui H.Y., Barta J., Pilar Cano M., Gusek T., Sidhu J.S. and Sinha N. (2006) *Handbook of fruits and fruit processing*, Blackwell Publishing, USA, 697 p.
12. Ikeda T., Yamazaki K., Kumakura H. and Hamamoto H. (2009) Effect of high temperature on fruit quality of pot-grown strawberry plants. *Acta Horticulturae,* 842, pp. 679-682.
13. Jimenez N., Bohuon P., Lima J., Dornier M., Vaillant F. and Perez A.M. (2010) Kinetics of anthocyanin degradation and browning in reconstituded blackberry juice trade at high temperatures (100 – 180 °C). *Journal of Agricultural and Food Chemistry*, 58, pp. 2314-2322.
14. Kong J.-M., Chia L.-S., Goh N.-K., Chia T.-F. and Brouillard R. (2003) Analysis and biological activities of Anthocyanins. *Rhytochemistry*, 64, pp. 923-933.
15. Lacombe A., Wu V.C.H., Tyler S. and Edwards K. (2010) Antimicrobial action of the American cranberry constituents; phenolics, anthocyanins, and organic acids, against Escherichia coli O157:H7. *Food Microbiology*, 139, pp. 102-107.
16. Lee J.H., Kang N.S., Shin S.O., Shin S.H., Lim S.G. and Suh D.Y. (2009) Characterization of Anthocyanins in the black soybean (Glycine max L.) by HPLC DAD-ESI/MS analysis. *Food Chemistry*, 112, pp. 226-231.
17. Llano K.M., Haedo A.S., Gerschenson L.N. and Rojas A.M. (2003) Mechanical and biochemical response of kiwifruit tissue to steam blanching. *Food Research International*, 36(8), pp. 767-775.
18. Matsui T., Ueda T., Oki T., Sugita K., Terahara N. and Matsumoto K. (2001) α-Glucosidase inhibitory action of natural acylated Anthocyanins. 2. α-Glucosidase inhibition by isolated acylated Anthocyanins. *Agricultural and Food Chemistry*, 49, pp. 1952-1956.
19. Mayer-Miebach E. and Spieß W.E.L. (2003) Influence of cold storage and blanching on the carotenoid content of *Kintoki* carrots. *Food Engineering*, 56(2-3), pp. 211-213.
20. Ochoa M.R., Kesseler A.G., Vullioud M.B. and Lozano J.E. (1999) Physical and chemical characteristics of raspberry pulp: Storage effect on composition and color. *LWT – Food Science and Technology,* 32, pp. 149-153.
21. Peterson J. and Dwyer J. (1998) Flavonoids: Dietary occurrence and biochemical activity. *Nutrition Research*, 18, pp. 1995-2018.
22. Rodriguez A.S., Perez–Gregorio M.R., Gracia-Falcon M.S. and Simal-Gandara J. (2009) Effect of curing and cooking on flavonols and Anthocyanins in traditional varieties of onion bulbs. *Food Research International,* 42, pp. 1331-1336.
23. Sadilova E., Stintzing F.C., Kammerer D.R. and Carle R. (2009) Mitrix dependent impact of sugar and ascorbic acid addition on color and anthocyanin stability of black carrot, eldenberry and strawberry single strength and from concentrate juice upon thermal treatment. *Food Research International.* 42, pp. 1023-1033.
24. Sapers G.M. and Hargrave D.L. (1987) Proportions of individual anthocyanins in fruits of cranberry cultivars. Available at: http://wyndmoor.arserrc.gov/Page/1987%5C5137.pdf, 22 February 2011.
25. Shi J.X., Maguer L.M., Wang S.L. and Liptay A. (1997) Application of osmotic treatment in tomato processing: effect of skin treatments on mass transfer in osmotic dehydration of tomatoes. *Food Research International*, 30, pp. 669.
26. Tsuda T., Horio F. and Osawa T. (2003) Dietary cyaniding 3-O-β-D-glucoside-rich purple colour prevents obesity and ameliorates hyperglycemia in mice. *Nutrition*, 133, pp. 2125-2130.
27. Vacuum microwave drier MUSSON-1. (2007) Technical specification. St.Petersburg, 49 c. (in Russian).
28. Vedenskaya I.-O. and Vorsa N. (2004) Flavonoid composition over fruit development and maturation in American cranberry, *Vaccinium macrocarpon* Ait. *Plant Science*, 167, pp. 1042-1054.
29. Wang J. and Mazza G. (2002) Effects of anthocyanins and other phenolic compounds on the production of tumor necrosis factor alpha in LPS/IFN-gamma-activated RAW 264.7 macrophages. *Agricultural and Food Chemistry*, 50, pp. 4183-4189.
30. Wang J. and Xi Y.S. (2005) Drying characteristics and drying quality of carrot using a two-stage microwave process. *Food Engineering*, 68, pp. 505-511.

31. Wesche-Ebelings P. and Argaiz-Jamet A. (2002) Stabilization mechanisms for anthocyanin the case for copolymerization reactions. In J. Welti-Chanes, G.V. Barbosa-Cánovas and J.M. Aguilera (eds), *Engineering and food for the 21st century* Boca Raton: CRC Press. pp. 141-150.
32. Wilska-Jeszka J. (2007) Food colorants. In Z.E. Sikorski (ed.), Chemical and functional properties of food components *Boca Ration: CRC Press.* pp. 245-274.
33. Winkel-Shirley B. (2001) Flavonoid biosynthesis. A colourful model for genetics, biochemistry, cell biology, and biotechnology. *Plant Physiology*, 126, pp. 485-493.
34. Wu L., Orikasa T., Ogawa Y. and Tagawa A. (2007) Vacuum drying characteristics of eggplants. *Food Engineering*, 83(3), pp. 422-429.
35. Youdim K.A., McDonald J., Kalt W. and Joseph J.A. (2002) Potential role of dietary flavonoids in reducing microvascular endothelium vulnerability to oxidative and inflammatory insults. *Nutritional Biochemistry*, 13, pp. 282-288.
36. Yu O., Matsuno M. and Subramanian S. (2006) Flavonoid compounds in flowers: genetics and biochemistry, in: J.A. Teixeira de Silva (Ed.). *Floriculture, Ornamental and Plant Biotechnology*, 1, Global Science Books, Ltd., UK, 646 p.
37. Антоцианы (Anthocyanin). Available at: http://bse.sci-lib.com/article063467.html, 21 February 2011. (in Russian).

# BEHAVIOUR OF INOCULATED WILD *L. MONOCYTOGENES* IN SLICED VACUUM-PACKED COLD SMOKED PORK

***Indulis Siliņš[1,2], Edgars Liepiņš[1]***
*[1]Latvia University of Agriculture*
*[2]Food and Veterinary Service, Dienvidzemgale Branch, Latvia*
*forest.con@apollo.lv; Edgars.Liepins@llu.lv*

## Abstract

The non-spore forming gram-positive bacterium *Listeria monocytogenes* is a food pathogen bacterium and a causative agent of listeriosis. The aim of the study was to determine the survival limits of *L. monocytogenes* inoculated in manufactured vacuum-packed cold smoked pork depending on shelf time, supported by water activity ($a_w$) and pH values. Enumeration of *L. monocytogenes* colony forming units per gram (cfu $g^{-1}$) was done according to ISO standard. Water activity ($a_w$) and pH values in pork samples were more or less constant and supported *L. monocytogenes* growth. The behaviour of *L. monocytogenes* in cold-smoked sliced pork by shelf time, when environmental factors changed minimally and supported growth, largely depended on the initial contamination level. A lag-phase of bacterial growing process before exponential growth rate of inoculated *L. monocytogenes* depended on initial cell concentration and had 10 days step level if storage temperature was approximately 5 °C. A significant Pearson's correlation ($p<0.01$) was established between the microbiological test values of *L. monocytogenes* count changes in sliced and packed cold-smoked pork during storage time of 60 days. The main parameter which maintained negative polynomial growth rate of *L. monocytogenes* in cold-smoked pork was the decrease of live cell concentration in samples below lg 2.0. The experiments were done at the Faculty of Veterinary Medicine of the Agricultural University of Latvia and at a sausage manufacturer's laboratory.

**Key words**: *Listeria monocytogenes*, cold smoked pork, water activity, pH.

## Introduction

*Listeria monocytogenes* can be found in a large number of food products. Meat and processed meat products such as cold smoked sausages, beef, and pork are part of major products associated with listeriosis (Thevenot et al., 2005). Unlike many other food pathogens, *L. monocytogenes* infection has a high mortality rate – 20 - 30% (Farber and Peterkin, 1991). In the United States, a zero tolerance of *L. monocytogenes* in ready-to-eat foods has been prescribed for several years (Shank et al., 1996), but the European Union regulation accepts concentration of *L. monocytogenes* in ready-to-eat food to 100 colony forming units (cfu) per gram (Anonymous, 2005). An important characteristic of *L. monocytogenes* is the capability to survive and grow under refrigeration, condition which is usually an obstacle for the growth of most foodborne pathogens (Lunde´n et al., 2003). K. Glass and M. Doyle (1989) found out that *L. monocytogenes* decrease level in fermented sausages and ham would be 1-2 lg (sausages) in 14 days, and 2-3 lg (ham) in 28 days. This enhances the growth conditions of psychotropic pathogens such as *Listeria*, allowing them to grow to dangerous levels (Francis and O'Beirne, 1998). *L. monocytogenes* strains may persist in food-processing plants for months or even years despite regular sanitation procedures (Miettinen et al., 1999; Lunde´n et al., 2003, 2005) and are to be categorized into persistent strains according to the frequency of the strain and the duration of the contamination. The persistent strains showed higher tolerance to acidic conditions (Lunde'n et al., 2008). Nowadays, 13 different serotypes of *L. monocytogenes* have been identified of which serotypes 1/2a, 1/2b, 1/2c, and 4b hold over 95% and are isolated from food and patients (Doumith et al., 2004).

Slicing is the last processing step for many types of food prior to packaging, and becomes an important stage to monitor *L. monocytogenes* contamination during the industrial production of sliced meat products. In the study on cross-contamination of *L. monocytogenes* between processing equipment and daily meats, C. Lin et al. (2006) found that slicing did play a significant role in microbial transfer from equipment to sliced meats. They found that the degree of transfer correlated with the numbers of *Listeria* inoculated onto the slicer blade, where the inoculum levels were from 1 to 3 lg cfu $g^{-1}$. In recent publications (Vorst et al., 2006; Sheen and Hwang, 2008) it was reported that the cutting force, fat, and moisture content were significant factors affecting *L. monocytogenes* transfer. K. Aarnisalo et al. (2007) showed that the transfer of *L. monocytogenes* during slicing was affected by initial inoculum level, temperature, and attachment time, and concluded that the total *Listeria* transfer count was lower when the inoculum level was lower, the temperature was cooler, and the attachment time was longer.

The newest papers described that the growth of *L. monocytogenes* ceased at a cell concentration of about $10^2$ cfu $ml^{-1}$ when natural microflora of foods, as lactic acid bacteria, entered stationary phase (Al-Zeyara et al., 2011).

*Listeria monocytogenes* is able to multiply in different kinds of food because it can survive and grow at relatively low water activity ($a_w$). R. Petran and E. Zottolla (1989) observed the growth of *L. monocytogenes* at the minimum $a_w$ of 0.92. Below this minimum $a_w$ level, cell death is proportionate to water activity (Miller, 1992).

Important consideration for growth and survival of *L.*

*monocytogenes* is pH. The organism has a possible range of growth from pH 4.1 to 9.6, with an optimum range of 6.0 - 8.0 (Jay, 2000). This pH range is dependent upon various factors, including incubation temperature, available nutrients, moisture % content, and product composition. M. Parish and D. Higgins (1989) found that lower pH had a deleterious effect on viability of *L. monocytogenes*, although a lag period occurred at 4 °C before cell reduction occurred. They concluded that low pH products were of concern in *L. monocytogenes* outbreaks under contamination followed by consumption of the product soon after the purchase, as the lag effect would prevent immediate cell death.

According to the existing literature resource data, many recent studies in food safety have investigated non-thermal processing of ready-to-eat food products, but there is little information about survival of *L. monocytogenes* found in different cold smoked meat products when water activity and pH values support *L. monocytogenes* growth. To be able to reduce pathogen counts, in this case *L. monocytogenes* would be beneficial to both food processors and consumers. Therefore, the aim of the study was to determine the survival limits of *L. monocytogenes* inoculated in manufactured vacuum-packed cold smoked pork depending on storage time, supported by water activity ($a_w$) and pH values.

## Materials and Methods

The experiments were done at the Faculty of Veterinary Medicine at the Agricultural University of Latvia and at the laboratory of a meat product manufacturer in 2009 - 2010.

Sliced, vacuum-packed cold-smoked pork samples (totally 252 pcs) were obtained from a meat product factory in packs of 100 g on the day of packaging. Samples (slices) for independent experimental series (S1, S2, and S3) were prepared from a single piece of cold-smoked pork in weight 2.5 – 3.0 kg by a fully automatic slicer with an integrated scale, series A 510 (Bizerba, Germany). The slicer was equipped with a 420 – mm –diameter ground knife (round blade) and operated at maximum 250 slices per minute. The detailed configuration of the slicer can be found at www.bizerba-openworld.com, where a model A 510 is displayed. The pork was sliced without chilling at room temperature of 5 ± 1 °C.

Individual packs were inoculated internally between pork slices with a cocktail of local (domestic) strains of *L. monocytogenes* and further stored after collateral ear resumption. For each of three experimental series, four decimal dilutions from initial culture, obtained approximately 8.0 lg (cfu $ml^{-1}$), were prepared. Consequently, each of experimental series consisted of four lines (L) of six samples (triplicated) with initial inoculate culture concentration according to theoretically calculated lg 4.0 (L1), 3.0 (L2), 2.0 (L3), and 1.0 (L4) (cfu $g^{-1}$). Inoculated samples were stored in a laboratory ice-box at 5±1 °C temperature conditions for 10, 20, 30, 40, 50, and 60 storing days and then detected for *L. monocytogenes* count, cfu $g^{-1}$, according to adapted in Latvia Standard LVS EN ISO 11290-2:1198 A:2005 "Microbiology of food and animal feeding stuffs. Horizontal method for the detection and enumeration of *Listeria monocytogenes*. Part 2: Enumeration method". Three batches of cold-smoked pork were investigated (a total of 72 samples, and 9 control samples) and the mean values of lg (cfu $g^{-1}$) were estimated in each of them, in addition to pH and water activity ($a_w$) changes at the storing time. Each experimental batch was free of *L. monocytogenes* before culture inoculation, detected by plate count method. Inoculation was prepared from persistent *L. monocytogenes* strains (serotypes 1/2a, and 4b) originally isolated from surfaces and meat products of the mother factory (Bērziņš et al., 2010). *L. monocytogenes* strains were incubated in half-Fraser base medium for 18 h at 37 °C. The fresh concentrated culture of the selected strains was prepared by sterile half-Fraser broth (CM0895, SR0166E, Oxoid), and then samples of vacuum-packed cold-smoked pork wire inoculated portionally (1 ml of inoculate in 100 g of sample = 0.1 ml of inoculate between slices) according to the scheme of the experiment. The samples were analyzed by numbering *L. monocytogenes* by a nine-tube most-probable-number (MPN) method. For analysis, 10 g of a carefully mixed cold-smoked pork slice samples were blended with 90 ml of sterile buffered peptone water in a laboratory blender (Stomacher 400, Interscience, France) for 1 min. Decimal dilutions were made to obtain samples of 1, 0.1, 0.01, 0.001, and 0.0001 g. To determine the MPN, three consecutive dilutions were used. Afterwards, 0.1 ml of each target dilution was outspread on two LM-selective plates (PALCAM, Oxoid) and incubated for 48 h at 37 °C. For confirmation of *L. monocytogenes*, five typical colonies from two selective plates at each sampling time were streaked on sheep blood agar plates and incubated for 24 h at 37 °C. Catalase-positive, gram-positive rods produced hemolysis on sheep blood agar (CAMP-test), which was considered *L. monocytogenes* (McKellar, 1994). Total count (cfu $g^{-1}$) of *L. monocytogenes* in cold-smoked pork samples was calculated by a classical formula given in Enumeration method standard.

Ingredients and preparation of cold-smoked pork were: pig meat – raw defrosted loin (musculus *Longissimus dorsi*) ~3.0 kg each piece, and 3.25 kg salt, and species summary for 20 pieces dry salting in a single box for 2-3 weeks.

*pH measuring*. Individual packages of cold-smoked pork were measured every time when a microbiological analysis was done, and then mean pH value was calculated. The pH-meter Testo 205 (Testo AG Germany) with automatic temperature compensation was applied. Meter calibration was done according to 2 point method with pH standard solutions 4.01 and 7.00.

*Water activity measuring*. $a_w$ was measured by means of PawKit (Decagon) water activity meter. Calibration of the device was done with saturated NaCl (sodium chloride) 6.0 molar standard solution (0.760 $a_w$ at 20 °C). Samples for water activity measuring were collected in original polyethylene vessels with caps and measured when it they had reached room temperature.

*Statistical analysis*. All experiments were performed three times, and tests were triplicated. The results represent the mean ± standard deviations. Means were compared by Student's t test. Differences were considered statistically significant when $p<0.05$. Statistical analysis was conducted with SPSS 17.0 (SPSS, Chicago, Ill., USA). Tables and chart figures were done by means of MS Excel 2007 software.

## Results and Discussion

The results of three experimental series (S1, S2, and S3) were consolidated. Mean values of a total plate count lg (cfu $g^{-1}$) of inoculated *L. monocytogenes* in control samples were: lg 4.18, 4.32, and 4.58 (L1), lg 3.20, 3.29, and 3.50 (L2), lg 2.28, 2.62, and 2.78 (L3), and lg 1.82, 1.87, and 1.87 (L4). The samples of cold-smoked pork had a mean initial pH value of 5.70 ± 0.04, which agrees with the results found by V. Paleari et al. (2003). The mean value of an initial water activity was 0.93 which decreased in the product minimally during shelf life. In all series, $a_w$ and pH values were inconsequentially changed on ranges 0.19 pH and 0.01 $a_w$ and were speculated as constant environment parameters in sample storage time. $a_w$ value in all series was within the interval 0.92 – 0.93 with statistical mean of 0.925 ± 0.0023. pH values interval in each series was $pH_{s1}$ = 5.50 – 5.69, $pH_{s2}$ = 5.55 – 5.68, and $pH_{s3}$ = 5.58 – 5.72, with statistical mean pH = 5.59 ± 0.055. It is proved that the concrete value ranges of $a_w$ and pH supported *L. monocytogenes* growth in cold-smoked meat products (Augustin et al., 2005). The initial and ending results of *L. monocytogenes* count and changes in all three experimental series are shown in Table 1.

Table 1

**The total plate count (quantity ± SD) – initial and ending values lg (cfu $g^{-1}$) of inoculated *L. monocytogenes* during 60 days storage time of cold-smoked pork**

| Series and lines | Total plate count lg(cfu $g^{-1}$) ± standard deviation (SD) | | Growth estimation |
|---|---|---|---|
| | Beginning of experiment, $0^{th}$ day | End of experiment, $60^{th}$ day | |
| S1L1 | 4.18 ± 0.020 | 5.84 ± 0.000 | increased |
| S1L2 | 3.20 ± 0.057 | 4.78 ± 0.017 | increased |
| S1L3 | 2.28 ± 0.020 | 3.28 ± 0.025 | increased |
| S1L4 | 1.87 ± 0.020 | 1.34 ± 0.025 | decreased |
| S2L1 | 4.32 ± 0.036 | 5.88 ± 0.030 | increased |
| S2L2 | 3.29 ± 0.026 | 4.75 ± 0.056 | increased |
| S2L3 | 2.62 ± 0.040 | 3.61 ± 0.118 | increased |
| S2L4 | 1.87 ± 0.072 | 1.31 ± 0.023 | decreased |
| S3L1 | 4.58 ± 0.036 | 6.02 ± 0.049 | increased |
| S3L2 | 3.55 ± 0.017 | 4.77 ± 0.047 | increased |
| S3L3 | 2.78 ± 0.025 | 4.24 ± 0.198 | increased |
| S3L4 | 1.82 ± 0.040 | 1.32 ± 0.023 | decreased |

The results of microbiological tests were rather similar in all experimental series, the linear correlation coefficient 'r' between *L. monocytogenes* count between series was within the interval 0.993 – 0.998, therefore it is illustrated by the fourth separate chart only of S1 series in Figure 1.

In three experimental variants (L) of four (S1L1, S1L2, and S1L3), shown in Figure 1, in all series the initial concentration of inoculated *L. monocytogenes* was more than 2.0 lg (cfu $g^{-1}$), and the growth of bacteria – increased of total plate count, was seen. The microbiological tests L4 line samples showed phase-down of inoculated *L. monocytogenes* in comparison with initial count values < 2.0 lg (cfu $g^{-1}$) and stopped decreasing at lg 1.34. It can been explained as an initial inoculated count factor influence necessary for bacteria adaptation in concrete environment conditions.

As the environmental conditions in all experimental series were comparatively the same, the correlation factor

(r=0.999) between growth determination ($R^2$) and initial *L. monocytogenes* concentration lg (cfu $g^{-1}$) confirm an initial bacterial count as an important factor of count change possibilities if the environment conditions are rather constant.

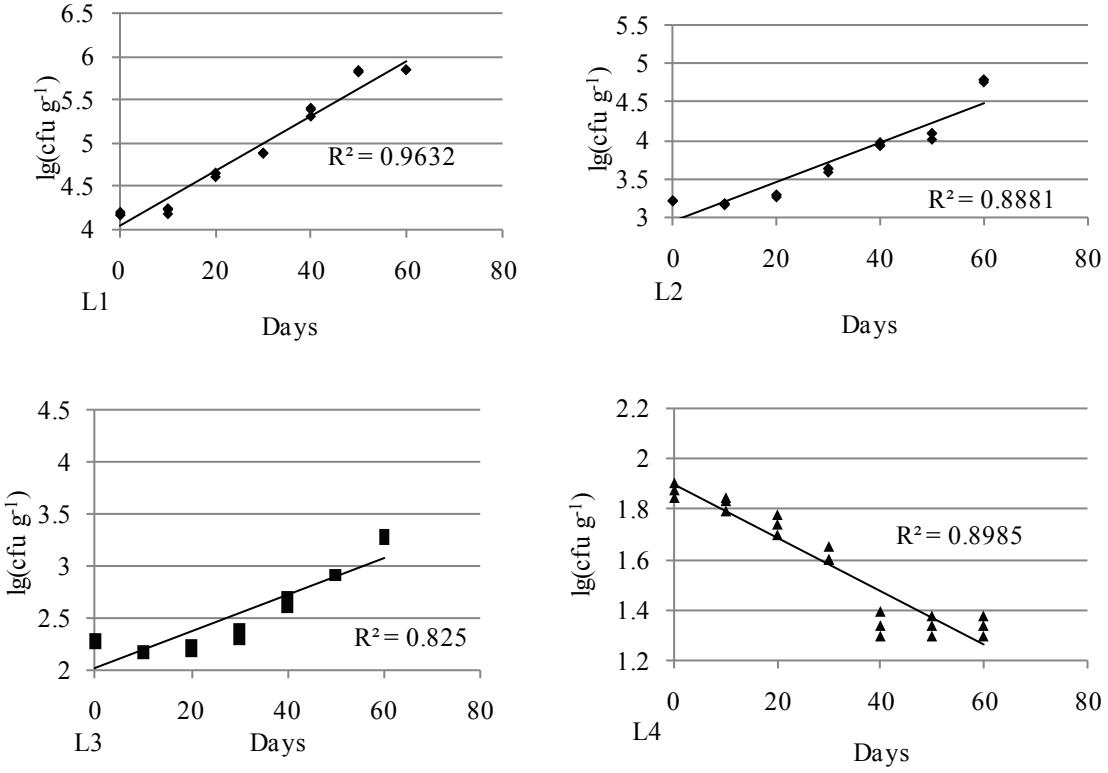



Figure 1. The growth lines of *L. monocytogenes* in cold-smoked sliced pork determined by storage time factor and depending on initial innoculated *L. monocytogenes* count lg(cfu $g^{-1}$) L1 – L4, experimental series S1.

In Figure 1 it can be seen that on L1, L2, and L3 lines *L. monocytogenes* count increase begins not directly after inoculation, but some time later and the lesser the initial bacteria concentration the longer time is needed. At L1 line, the lag-phase was approximately 10 days, at L2 line ~20 days, and at L3 line ~30 days. This suggests that *L. monocytogenes* lag-phase in cold-smoked pork, stored at 5 ± 1.0 °C, can be approximately 30 days if the initial concentration of contamination is higher than 2.0 lg (cfu $g^{-1}$). The step of lag-phase decrease can be approximately 10 days, if contamination level increases by lg 1.0.

The *L. monocytogenes* growth between L1, L2, and L3 lines was significantly ($p<0.01$) correlative that was proved by the following correlation coefficients: $r_{L1:L2}=0.77$, $r_{L1:L3}=0.88$, and $r_{L2:L3}=0.97$.

The phase-down of *L. monocytogenes* lg (cfu $g^{-1}$) in L4 line preferably conforms with polynomial characteristics ($R^2=0.94$), but in Fig. 1 is shown (L4) as a linear connection allowing comparison with other lines (L1, L2, and L3). As in all experimental series, at the beginning of storage time, the initial concentration of *L. monocytogenes* in L4 line was < lg 2.0, witch allows suggesting that a minimal count of bacteria in a food product is necessary to begin the growing process, which was in case of cold-smoked pork when general factors, such as $a_w$ and pH, supported bacterial cell count increase. This minimal count of *L. monocytogenes* living cells, measured in cfu $g^{-1}$, which possible necessary for growth starting in could-smoked pork, would be approximately lg 2.0. The idea of necessary minimal initial cell concentration is partly in compliance with B. Carpentier and O. Cerf's (2011) conclusion about the necessity of some minimal *L. monocytogenes* cell count for beginning and supporting the persistence.

The results of study give the theoretical supporting and real guidance for manufacturers appointing the products safe storage time prognosis, if the storage conditions and bacterial contamination level is known.

## Conclusions

The behaviour of *L. monocytogenes* in cold-smoked sliced pork through storage time when environmental factors change minimally and support the growth, largely depends on an initial contamination level.

The main parameters maintained negative polynomial ($R^2=0.94$) and negative linear ($R^2=0.90$) growth rate of *L. monocytogenes* in cold-smoked pork, which is the decrease of live cell concentration in a sample below lg 2.0.

A lag-time of bacterial growth process before

exponential growth rate of inoculated *L. monocytogenes* depended on the initial cell concentration and had a 10-day step, if storage temperature was approximately 5 °C.

A significant Pearson's correlation ($p<0.01$) was established between microbiological tests of *L. monocytogenes* count changes (increasing) in sliced and packed cold-smoked pork during a 60-day storage time, when an initial bacterial count was higher than lg 2.0.

**References**

1. Aarnisalo K., Sheen S., Raaska L. and Tamplin M. (2007) Modeling transfer of *Listeria monocytogenes* during slicing of „gravad" salmon. *International Journal of Food Microbiology*, 118, pp. 69-78.
2. Al-Zeyara S.A., Jarvis B. and Mackey B.M. (2011) The inhibitory effect of natural microflora of food on growth of *Listeria monocytogenes* in enrichment broths. *International Journal of Food Microbiology*, 145, pp. 98-105.
3. Anonymous (2005) Commission regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. *Official Journal of the European Union*, L338, pp. 1-25.
4. Augustin J.C., Zuliani V., Cornu M. and Guillier L. (2005) Growth rate and growth probability of *Listeria monocytogenes* in dairy, meat and seafood products in suboptimal conditions *Journal of Applied Microbiology*, 99, pp. 1019-1042.
5. Bērziņš A., Hellström S., Siliņš I. and Korkeala H. (2010) Contamination Patterns of *Listeria monocytogenes* in Cold-Smoked Pork processing. *Journal of Food Protection*, 73, pp. 2103-2109.
6. Carpentier B. and Cerf O. (2011) Persistence of *Listeria monocytogenes* in food industry equipment and premises. *International Journal of Food Microbiology*, 145, pp. 1-8.
7. Doumith M., Buchrieser C., Glaser P., Jacquet C. and Martin P. (2004) Differentiation of the major *Listeria monocytogenes* serovars by multiplex PCR. *Journal of Clinical Microbiology*, 42, pp. 3819-3822.
8. Farber J.M. and Peterkin P.I. (1991) *Listeria monocytogenes*, a food-borne pathogen. *Microbiology and Molecular Biology Reviews*, 55, pp. 476-511.
9. Francis G.A., O'Beirne D. (1998) Effects of storage atmosphere on *Listeria monocytogenes* and competing microflora using a surface model system. *International Journal of Food Microbiology,* 33, pp. 465-476.
10. Glass K.A. and Doyle M.P. (1989) Fate of *Listeria monocytogenes* in processed meat products during refrigerated storage. *Applied and Environmental Microbiology,* 55, pp. 1565-1569.
11. Jay J.M. (2000) Foodborne Listeriosis. In: *Modern Food Microbiology*, 6th ed., J. Jay eds, Aspen Publishers, Maryland, pp. 488-510.
12. Lin C., Takeuchi K., Zhang L., Dohm C.B., Meyer J.D., Hall P.A. and Doyle M.P. (2006) Cross-contamination between processing equipment and deli meats by *Listeria monocytogenes*. *Journal of Food Protection*, 69, pp. 71-79.
13. Lunde´n J., Autio T. and Korkeala H. (2003) Persistent and nonpersistent *Listeria monocytogenes* contamination in meat and poultry processing plants. *Journal of Food Protection*, 66, pp. 2062-2069.
14. Lunde´n J., Bjorkroth J. and Korkeala H. (2005) Contamination routes and analysis in food processing environments. In: Lelieveld H.L.M., Mostert M.A. and Holah J. (eds) *Handbook of Hygiene Control in the Food Industry*, Woodhead Publishing Limited, Cambridge, pp. 539-555.
15. Lunde'n J., Tolvanen R. and Korkeala H. (2008) Acid and heat tolerance of persistent and nonpersistent *Listeria monocytogenes* food plant strains. *Letters in Applied Microbiology*, 46, pp. 276-280.
16. McKellar R.C. (1994) Use of the CAMP Test for Identification of *Listeria monocytogenes*. *Applied and Environmental Microbiology*, 60, pp. 4219-4225.
17. Miettinen M.K., Björkroth K.J. and Korkeala H.J. (1999) Characterization of *Listeria monocytogenes* from an ice-cream plant by serotyping and pulsed-field gel electrophoresis. *International Journal of Food Microbiology*, 46, pp. 187-192.
18. Miller A.J. (1992) Combined water activity and solute effects on growth and survival of *Listeria monocytogenes* Scott A. *Journal of Food Protection,* 55, pp. 414-418.
19. Palearia V.M.M., Berettaa T. and Bersania M. (2003) Cured products from different animal species. *Meat Science,* 63, pp. 485-489.
20. Parish M.E and Higgins D.P. (1989) Survival of *Listeria monocytogenes* in low pH model broth systems. *Journal of Food Protection,* 52, pp. 144-147.
21. Petran R.L. and Zottola E.A. (1989) A study of factors affecting growth and recovery of *Listeria monocytogenes* Scott A. *Journal of Food Science,* 54, pp. 458-460.
22. Shank F.R., Eliot E.L., Wachsmuth I.K. and Losikoff M.E. (1996) US position on *Listeria monocytogenes* in foods. *Food Control*, 7, pp. 229-234.
23. Sheen S. and Hwang C.A. (2008) Modeling transfer of *Listeria monocytogenes* from slicer to deli meat during mechanical slicing. *Foodborne Pathogens and Disease,* 5, pp. 135-146.
24. Thevenot D., Delignette-Muller M.L., Christieans S. and Ver-nozy-Rozand C. (2005) Fate of *Listeria monocytogenes* in experimentally contaminated French sausages. *International Journal of Food Microbioogy,* 101, pp. 189-200.
25. Vorst K., Todd E.C.D. and Ryset E.T. (2006) Transfer of *Listeria monocytogenes* during mechanical slicing of turkey breast, bologna, and salami. *Journal of Food Protection*, 69, pp. 619-626.

# SENSORY EVALUATION OF ROASTED MARINATED VENISON

***Laima Šiliņa, Ilze Grāmatiņa***
*Latvia University of Agriculture*
*ilzegramatina@inbox.lv*

**Abstract**

Marinating is a simple technological treatment used to improve the tenderness and flavour of meat by soaking it in an aqueous solution which is composed of different ingredients. That also increases water brinding capacity of meat, thus reducing cooking losses and improving meat juiciness. The aim of current research was to investigate a degree of preference of marinated roasted venison meat. The red deer (*Cervus elaphus*) meat samples were obtained from a local farm "Saulstari 1". The experiments were carried out in the Latvia University of Agriculture, Faculty of Food Technology. Prepared samples were marinated at 4 ± 2 °C temperature in the refrigerator for 48 ± 1 h. After marinating, venison meat samples were wrapped in aluminium foil, and cooked on a pre-heated double hotplate grill at 200 ± 2 °C temperature until ready to eat (45 ± 2 min). The cooked hot meat samples were sensory evaluated. The sensory evaluation was carried out by using the nine point hedonic scale – ISO 4121:2003. The sensory evaluation of meat sample with thyme and juniper berries showed that the degree of preference was not so high (approximately – 6.0 points). For the venison meat preliminary treatment mayonnaise and tomato sauce marinades possibly could be recommended for acquiring better sensory properties of ready to eat product.

**Key words:** venison, marinating.

## Introduction

Venison originally is described as meat of any game animal killed by hunting, and was applied to any animal from the families *Cervidae* (deer), *Leporidae* (hares), and *Suidae* (wild pigs), and certain species of the genus *Capra* (goats and ibex), such as elk, red deer, fallow deer, roe deer, moose, reindeer / caribou, pronghorn, brown hare, arctic hare, wild boar and ibex, but its usage is now almost entirely restricted to the flesh of various species of deer. The world demand for animal products in human diets is steadily and substantially increasing (Corbett, 2001).

The main and most important factors influencing changes in consumer demand for meat and meat products are (Resurrecion, 2003) – increased health concerns, change in demographic characteristics, the need for convenience and increased eating out, change in distribution and change in relative prices. As a result of these changes in the demand for meat, interest in new red meat products such as venison which claimed to be low-fat red meat and particularly convenience-oriented product, has dramatically increased in recent years (Dahlan and Norfarizan-Hanoon, 2007). The meat is seen to be a major source of fat in the diet and especially of saturated fatty acids, which have been implicated in diseases associated with modern life, especially in developed countries. Venison has gained an increase in popularity in resent years, due to the meat's lower fat content (Dahlan and Norfarizan-Hanoon, 2008). Venison is lower in calories, cholesterol and fat than common cuts of beef, pork or lamb (Drew et al., 1991; Wood et al., 2003). Venison can be consumed as steaks, roasts, sausages and ground meat (Dryden, 1997). Attributes of rusa venison were reviewed by G.Mc.L. Dryden (Dahlan and Norfarizan-Hanoon, 2008). In brief, rusa deer carcasses yield more lean meat than cattle, and typically have little fat (520 – 960 mg $kg^{-1}$) in carcasses of entire stags (Sookhareea et al., 2001). Possibly because of its low fat content, venison does not always score highly for organoleptical properties (Sookhareea et al., 1993), perhaps because much of the carcass fat is structural.

Marinating is the process of soaking foods in a seasoned, often acidic liquid before cooking. The origins of the word respond to the use of brine (*aqua marina*) in the pickling process, which led to the technique of adding flavor by immersion in liquid (Ergezer and Gokce, 2011).

The liquid in question, the "marinade", must be either acidic with ingredients such as vinegar, lemon juice, or wine or enzymatic (made with ingredients such as pineapple or papaya). Along with these liquids, a marinade often contains oils, herbs and spices to further flavor the food items. It is commonly used to flavor foods and to tenderize tougher cuts of meat (Lemos et al., 1999). Acid-based marinades both tenderize and flavour many different types of foods, not just meats and seafood. Acids such as citrus juices, pineapple, yogurt, buttermilk, and wine tenderize by denaturing or unwinding protein strings. They also add flavour to the end product. In meats, the acid causes the tissue breakdown, allowing more moisture to be absorbed and producing a juicier end product. However, too much acid can be detrimental to the end product. A good marinade will have a delicate balance of spices, acids, and oil. It is generally not recommended that raw marinated meats be frozen, as the marinade can break down the surface and make the outer layer turn mushy (Alvarado and McKee, 2007; Ergezer and Gokce, 2011). Also, sodium chloride and sugar are considered important ingredients of marinades, and they mainly improve meat tenderness. Marinating also increases water binding capacity of meat, thus reducing cooking losses and improving meat juiciness. Marinades are incorporated into meat by soaking texture

and moisture retention; to enrich the meat flavour; to tenderize the fibers of muscle foods; and to preserve the products over a longer time (Alvarado and McKee, 2007). Several methods were used to marinate meat, including immersion of meat in the marinade, injection and tumbling with a marinade or combination of injecting and tumbling. Immersion, the oldest method, consists of submerging the meat in the marinade and allowing the ingredient to penetrate the meat through diffusion with the passage of time (Ergezer and Gokce, 2011). In the present research based on the scientific literature studies, immersion method for meat marinating was chosen.

Therefore, the aim of current research was to investigate degree of preference of marinated roasted venison meat.

## Materials and Methods

The red deer (*Cervus elaphus*) meat samples were obtained from a local farm "Saulstari 1", located in Sigulda region. "Saulstari 1" was the first private deer garden in Latvia which has been working since 1995. There are more than 230 fallow-deer and European stag (white and red) are grazing in 140 ha lair area. Deer were slaughtered at 35 months of age. The experiments were carried out in the Latvia University of Agriculture, Faculty of Food Technology. Four marinade types were used: red wine marinade, mayonnaise marinade, tomato sauce marinade, vinegar marinade. Composition of marinades are summarized in Table 1.

Table 1

**Recipes of marinades**

| Type of marinades (label) | Label of samples | Composition of marinades |
|---|---|---|
| Red wine (A) | 1A | Red wine, garlic, salt, black pepper |
| | 2A | Red wine, onion, horseradish, parsley, sweet pepper, basil, black pepper, rosemary, salt |
| | 3A | Red wine, onion, mayonnaise, mustard, garlic, salt, black pepper |
| | 4A | Red wine, honey, salt, black pepper, juniper berry, thyme |
| | 5A | Red wine, onion, vinegar, garlic, parsley, sweet pepper, basil, black pepper, rosemary, salt |
| Mayonnaise (B) | 1B | Mayonnaise, salt, black pepper |
| | 2B | Mayonnaise, onion, parsley, paprika, basil, black pepper, rosemary, salt |
| | 3B | Mayonnaise, mustard, salt |
| | 4B | Mayonnaise, salt, thyme, black pepper, juniper berry |
| | 5B | Mayonnaise, garlic, salt, powder of red sweet pepper, black pepper |
| Tomato sauce (C) | 1C | Tomato sauce, lemon, salt, vinegar, black pepper |
| | 2C | Tomato sauce, garlic, salt, black pepper |
| | 3C | Tomato sauce, mayonnaise, onion, garlic, salt, black pepper |
| | 4C | Tomato sauce, salt, thyme, black pepper, juniper berry |
| | 5C | Tomato sauce, lemon, onion, parsley, sweet pepper, basil, black pepper, rosemary, salt |
| Vinegar (D) | 1D | Onion, vinegar, lemon, salt |
| | 2D | Onion, vinegar, garlic, salt, black pepper |
| | 3D | Onion, vinegar, salt, thyme, black pepper, juniper berry |
| | 4D | Onion, vinegar, salt, powder of red sweet pepper, black pepper |
| | 5D | Tomato sauce, mayonnaise, vinegar, lemon, onion, parsley, paprika, basil, black pepper, rosemary, salt |

Venison meat marinating included the following steps:

1) *Longissimus dorsi* muscle from venison saddle cuts were manually divided in 0.250 ± 0.002 kg pieces with a knife;
2) pieces of *Longissimus dorsi* muscle were packaged in plastic bags and labelled;
3) the packaged meat samples were stored in freezer at -20 ± 2 °C temperature for two weeks;
4) meat sample before marinating was defrosted at 4 ± 2 °C temperature in the refrigerator for 24 ± 1 h;
5) after defrosting, venison meat was divided into pieces of size 2 × 3 cm and additives according prescription were added (Table 1);
6) prepared samples were marinated at 4 ± 2 °C temperature in the refrigerator for 48 ± 1 h;
7) after marinating, venison meat samples were wrapped

in aluminium foil, and cooked on a pre-heated double hotplate grill at 200 ± 2 °C temperature until ready to eat (45 ± 2 min).

After cooking the marinated venison meat samples were sensory evaluated hot only. The sensory evaluation was carried out using the nine point hedonic scale (1 – dislike extremely and 9 – like extremely). The nine point hedonic scale was used in order to determine the degree of preference of products (Meilgard et al., 1991; Poste and Mackei, 1991; ISO 4121:2003). The sensory evaluation was organized in four sessions, ie., one type of marinated meet was evaluated in each session, same 27 panellists participated in it. The panellists received equally prepared cooked samples and questionnaires, instructions for the evaluation procedure.

The analysis of variance (ANOVA) and Tukey's test were used for the analysis of acquired sensory data.

## Results and Discussion

Twenty samples of marinated roasted venison meat were prepared for the sensory evaluation.

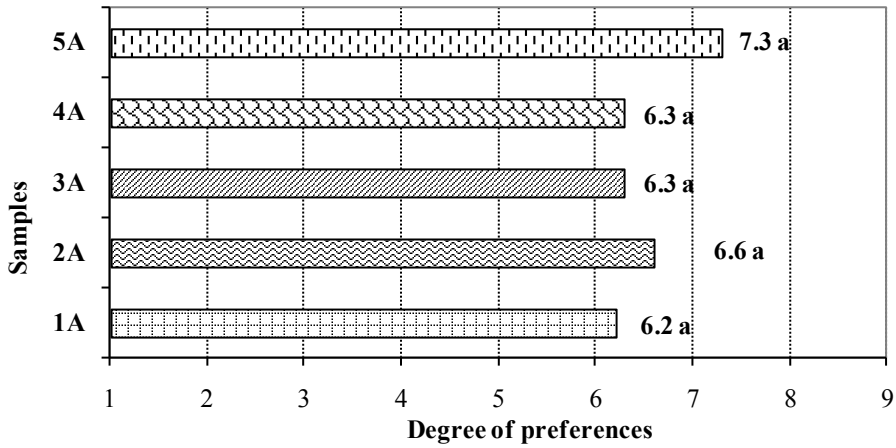
Five samples in red wine marinade (A), five samples in mayonnaise marinade (B), five samples in tomato sauce marinade (C) and five samples in vinegar marinade (D). The results of analysis of variance of meat samples in red wine marinade of hedonic evaluation are given in Table 2.

Table 2

**Results of analysis of variance of meat samples in red wine marinade using hedonic scales (α≤0.05)**

| Source of variation | Degree of freedom, df | Sum of squares, SS | Mean square, MS | Variance ratio, F |
|---|---|---|---|---|
| Samples of meat in red wine marinade | 4 | 19.45 | 4.86 | 2.03 |
| Panellists | 26 | 126.80 | 4.88 | 2.04 |
| Error | 69 | 165.35 | 2.40 | – |
| Total | 99 | 311.60 | – | – |

The results of analysis of variance indicate that $F_{cal} = 2.03 < F_{crit} = 2.50$; therefore, it can be concluded that there are no significant differences among the five samples of red wine marinade in the degree of preference. The results of hedonic evaluation of marinated roasted venison in red wine marinade are given in Figure 1.




* – values, marked with the same subscript letters, are not significantly different (p>α0.05).

Figure 1. Results of hedonic scores for marinated roasted venison in red wine marinade.

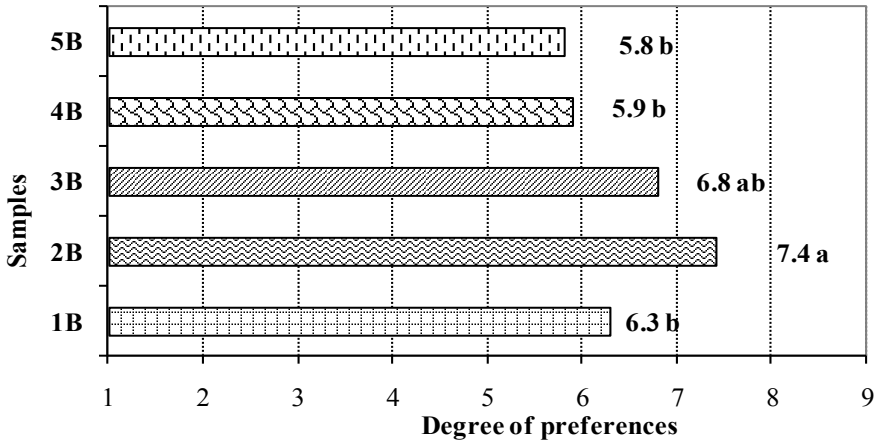
The hedonic scores of marinated roasted venison meat samples in red wine marinade are within the scale interval from "like slightly" to "like very much" (6.2 – 7.3). The results of analysis of variance of meat samples in mayonnaise marinade hedonic evaluation are shown in Table 3.

Table 3

**Results of analysis of variance of meat samples in mayonnaise marinade using hedonic scales (α≤0.05)**

| Source of variation | Degree of freedom, df | Sum of squares, SS | Mean square, MS | Variance ratio, F |
|---|---|---|---|---|
| Samples of meat in mayonnaise marinade | 4 | 50.18 | 12.54 | 4.50 |
| Panellists | 26 | 86.81 | 3.34 | 1.20 |
| Error | 69 | 192.22 | 2.79 | – |
| Total | 99 | 329.21 | – | – |

The current results (see Table 3) of analysis of variance demonstrate that $F_{cal} = 4.50 > F_{crit} = 2.50$, that proves the significant differences among the marinated roasted venison meat samples. The Tukey's test shows which samples panellists like best and how these five marinated roasted venison meat samples are arranged according to the degree of liking. The results of hedonic evaluation of marinated roasted venison in mayonnaise marinade are shown in Figure 2.




* – values, marked with the same subscript letters, are not significantly different (p>α0.05).

Figure 2. Results of hedonic scores for marinated roasted venison in mayonnaise marinade.

Tukey's test results demonstrated that the highest degree of preference was of sample 2B (7.4). Hedonic rating indicates that the degree of preference for five samples of meat processed in mayonnaise marinade are 5.8 – 7.4, that means the interval from "neither like nor dislike" to "like very much" on the scale. In the scientific literature (Baše and Kisele, 2007) data was found on suitability of thyme and juniper berries for venison marinating and its positive influence on flavour of ready product. However, in our experiments the sensory analyses of meat samples with these additives (4B sample) show that panellists did not give high scores, they did not like meat with thyme and juniper berries. The results of analysis of variance of meat samples in tomato sauce marinade of hedonic evaluation are given in Table 4.

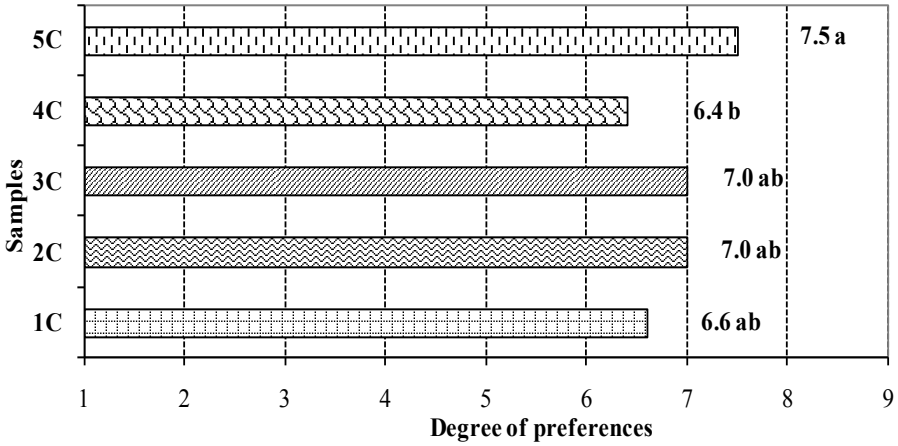
Table 4

**Results of analysis of variance of meat samples in tomato sauce marinade using hedonic scales (α≤0.05)**

| Source of variation | Degree of freedom, df | Sum of squares, SS | Mean square, MS | Variance ratio, F |
|---|---|---|---|---|
| Samples of meat in tomato sauce marinade | 4 | 19.38 | 4.84 | 2.64 |
| Panellists | 26 | 96.86 | 3.73 | 2.03 |
| Error | 69 | 126.62 | 1.84 | – |
| Total | 99 | 242.86 | – | – |

The obtained results of analysis of variance demonstrate (Table 4) that $F_{cal}$= 2.64 > $F_{crit}$= 2.50, which allows to conclude that there are significant differences among five analysed samples. Tukey's test establishes which samples panellists like best and how the samples are arranged according to the degree of preference.

The results of hedonic evaluation of marinated roasted venison in tomato sauce marinade are shown in Figure 3.




* – values, marked with the same subscript letters, are not significantly different (p>α0.05).
Figure 3. Results of hedonic scores for marinated roasted venison in tomato sauce marinade.

Tukey's test results indicate that panellist more like the meat sample 5C (7.5). Hedonic rating demonstrates that degree of preference for five samples in tomato sauce marinade processed meat is 6.4 – 7.5, that means the interval from "like slightly" to "like very much" on the scale. The results can mainly be explained with possible negative effect on product sensory properties of spices such as thyme and juniper berries used in marinade preparation.

The results of analysis of variance of hedonic evaluation of meat samples in vinegar marinade are given in Table 5.

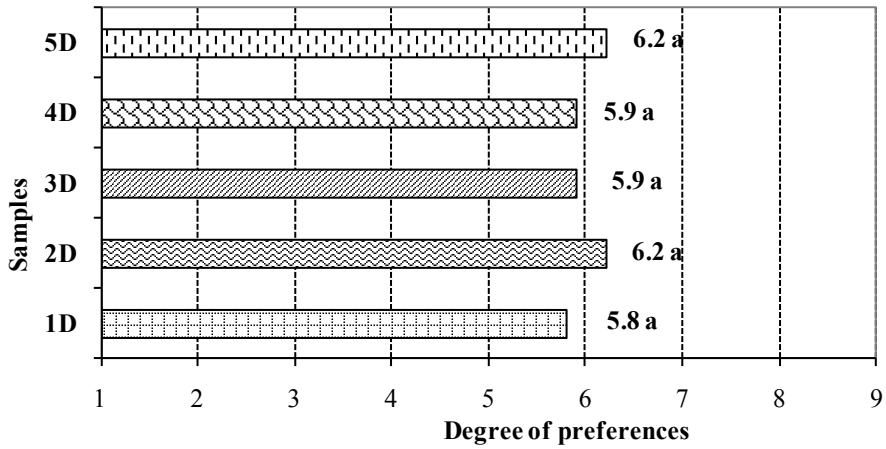
Table 5

**Results of analysis of variance of meat samples in vinegar marinade using hedonic scales (α≤0.05)**

| Source of variation | Degree of freedom, df | Sum of squares, SS | Mean square, MS | Variance ratio, F |
|---|---|---|---|---|
| Samples of meat in vinegar marinade | 4 | 3.51 | 0.88 | 0.43 |
| Panellists | 26 | 152.59 | 5.87 | 2.87 |
| Error | 69 | 140.89 | 2.04 | – |
| Total | 99 | 296.99 | – | – |

The results of analysis of variance indicate that $F_{cal}$ = 0.43 < $F_{crit}$ = 2.50, which indicates that there are no significant differences in the degree of preference among these five samples of meat in vinegar marinade.

The results of hedonic evaluation of marinated roasted venison in vinegar marinade are shown in Figure 4.




* – values, marked with the same subscript letters, are not significantly different (p>α0.05).
Figure 4. Results of hedonic scores for marinated roasted venison in vinegar marinade.

The hedonic scores of current five samples is on the scale interval from "neither like nor dislike" to "like slightly" (5.8 – 6.2). Therefore, there are no substantial differences in the degree of preference between samples of marinated roasted venison in vinegar marinade.

## Conclusion

1. The hedonic evaluation of roasted venison meat in mayonnaise marinade is in the scale interval from "neither like nor dislike" to "like very much" (5.8 – 7.4). However, five samples of meat in tomato sauce marinade are evaluated on the scale interval from "like slightly" to "like very much" (6.4 – 7.5).
2. The sensory analyses of meat samples with thyme and juniper berries allows to conclude that those were not acceptable in the preference for panellists.
3. For the venison meat preliminary treatment mayonnaise and tomato sauce marinades possibly could be recommended for acquiring better sensory properties of ready to eat product.

## Acknowledgement

This research and publication has been prepared within the frame of the State Research Programme "Sustainable use of local resources (earth, food, and transport) – new products and technologies (NatRes)" (2010 - 2013) Project No. 3 "Sustainable use of local agricultural resources for development of high nutritive value food products (Food)".

Grateful acknowledgments from the authors are extended to the researcher Evita Straumīte for valuable scientific advice.

## References

1. Alvarado C. and McKee S. (2007) Marination to Improve Functional Properties and Safety of Poultry Meat. *The Journal of Applied Poultry Research*, 16, pp. 113-120.
2. Baše A. un Kisele R. (2007) Medījumi. Apstrāde un receptes. (Game. Processing and Recipes). Design & Printing Services, Cēsis, Latvia, 174. lpp. (in Latvian).
3. Corbett J.L. (2001) The challenging demand for increases in meat and milk production: enhancing the contributions of grazing livestock. *Asian-Australasian Journal of Animal Science*, 14, pp. 1-12.
4. Dahlan I. and Norfarizan-Hanoon N.A. (2007) Fatty acid profiles and cholesterol composition of venison from farmed deer. *Journal of Animal and Veterinary Advances*, 6 (5), pp. 650-657.
5. Dahlan I. and Norfarizan-Hanoon N.A. (2008) Chemical composition, palatability and physical characteristics of venison from farmed deer. *Animal Science Journal*, 79, pp. 498-503.
6. Drew K.R., Stevenson J.M., Fennessy P.F. (1991) Venison-a marketable products. *Proceeding of a Deer course for Veterinarians*, 8, pp. 31-35.
7. Dryden G.Mc.L. (1997) Venison in the human diet-is venison a low-fat meat? *Proceeding of Nutrition Society of Australia*, 21, pp. 44-51.
8. Ergezer H. and Gokce R. (2011) Comparison of marinating with two different types of marinade on some quality and sensory characteristics of turkey breast meat. *Journal of Animal and Veterinary Advances*, 10, pp. 60-67.
9. ISO 4121:2003 Sensory analysis – Guidelines for the use of quantitative response scales. Available at: http://www.iso.org/iso/iso_catalogue/catalogue_tc/catalogue_detail.htm?csnumber=33817, 9 March 2011.
10. Lemos A.L.S.C., Nunes D.R.M and Viana A.G. (1999) Optimization of the still-marinating of chicken parts. *Meat Science*, 52, pp. 227-234.
11. Meilgard M., Civvile G.V. and Carr B.T. (1991) *Sensory evaluation techniques*, 2th ed., CRC Press, inc., Boca Roton, USA, 354 p.

12. Poste L.M. and Mackie D.A. (1991) *Laboratory methods for sensory analysis of food*, Agriculture Canada, Ottowa, USA, 90 p.
13. Resurreccion A.V.A. (2003) Sensory aspects of consumers choices for meat and meat products. *Meat Science*, 66, pp. 11-20.
14. Sookhareea R., Taylor D.G., Woodford K.B., Shorthose W.R., Dryden G.Mc.L. and Chen T. (1993) A study of carcase characteristics and meat quality of castrate and entire *Java rusa* stags. MEAT '93. *Australian Meat Industry Research Conference Gold Coast Session*, 8B, pp. 1-5.
15. Sookhareea R., Woodford K.B. and Dryden G.Mc.L. (2001) The effect of castration on growth and composition of *Java rusa* stags. *Asian-Australasian Journal of Animal Science*, 14, pp. 608-614.
16. Wood J.D., Richardson R.I., Nute G.R., Fisher A.V., Compo M.M., Kasapidou E., Sheard P.R. and Enser M. (2003) Effects of fatty acids on meat quality: a review. *Meat Science*, 66, pp. 21-32.

# EVALUATION OF QUALITY INDICES OF STRAWBERRY MASS

***Sigita Boca, Imants Skrupskis, Evita Straumite***
*Latvia University of Agriculture*
*sigita.boca@apollo.lv*

**Abstract**

Color, texture and aroma are the main quality attributes of food influencing consumer acceptability of food products. Color of the product is one of the factors affecting hedonic evaluation. Strawberry mass for a consumer is characteristic by its distinct red color. The stability of anthocyanins becomes most significant in the case of color quality. To investigate color quality, it is necessary to measure color, as well as pigment concentration. Fresh and thermally treated strawberry mass was analyzed. Formation and stability of anthocyanins, which is the main color formation substance of berries, are determined by different factors. Anthocyanin colors can be enhanced and stabilized by the addition of different natural or artificial acidifiers. Data of the research indicate that anthocyanin amount during storage period decreases. After 2 days storage their amount increased, but afterwards decreased, both, in fresh and thermally treated mass. The taste evaluation of strawberry mass was essentially affected by the enhancers added.

The aim of research was to analyze the quality parameter – color of strawberry mass with added acidifier which further could be used in production of different products for the needs of public catering, e.g. in production of desserts. The anthocyanin content in strawberry mass with acidifiers was determined to see the organic acid impact to color stability. Research shows, that natural enhancers – quince and sea buckthorn juices used in the research, did not substantially affect each other's pH in the product.

**Key words**: strawberry mass, color, enhancers, fresh, thermally treated.

## Introduction

Strawberry is one of the world's largest fruit crops (Doymaz, 2008). The strawberry (*Fragaria*) is a genus of plants in the family *Rosaceae* and the fruit of these plants. There are more than 20 named species and many hybrids and cultivars. The most common strawberries grown commercially are cultivars of the Garden strawberry, *Fragaria ananassa* (Oszmianski et al., 2009). Strawberries assigned to consumption in fresh state have better taste and aroma when they are collected in consumption ripeness state (whole surface is colored uniformly). Application of suitable processing technology is necessary because of excessive production of fresh strawberries (Alvarez, 1995). In food technology strawberries have many applications. Preserved fruit occurs as frozen and dried food, which is obtained with the use of different methods: osmotic dehydration, as fruit charge in dairy industry, in fruit-vegetable industry to juice production, jams and in distilling of alcohol (El-Beltragy, 2006). Each of these methods of preserving and processing causes quality changes in comparison to raw material (Moreno, 2000).

The quality of food products, inconformity with consumers' requirements and acceptance, is determined by their sensory attributes, chemical composition, physical properties, and level of microbiological and toxicological contaminants, shelf life, packaging and labeling (Costell, 2002). Sensory evaluation concerns the interpretation of what the senses – sight, olfaction, taste, touch, audition – inform about the product (Giboreau et al., 2007). The sensory properties of food are extremely important, because these properties determine consumer acceptance. This is why sensory tests are essential in terms of quality (Kuti et al., 2004).

Color, texture and aroma are the main quality attributes of food influencing consumer acceptability of food products. During processing these attributes may be lost or altered depending on the water content in foods. Many approaches have been taken to improve the quality of fruit products. One common approach is to apply less invasive process and the other is to use specific additives (Kopjar et al., 2008).

As color is one of the most important quality properties characterizing quality parameters of fruits and berries, in this research greater attention was paid to color stability and durability.

The color of food has always been a value of quality. Today, the attractive red color of food products, like strawberry products, is an important quality parameter influencing consumer behavior. Obtaining a strong and stable color of fruit and berry products, however, is problematic during processing and storage. The need to avoid the use of synthetic colorants and move towards the use of natural food colors has also increased research in this field during the past decades. Rein (2005) in his dissertation clarified that anthocyanins are natural pigments widely distributed in nature. They are responsible for such colors as red, purple and blue in fruits and berries, anthocyanins are mainly located in the peel. However, the stability of anthocyanins becomes most significant also in this case, as well as in the case of color quality. Anthocyanins are highly unstable and easily susceptible to degradation. As mentioned Rein (2005) in his dissertation, stability of anthocyanins is affected by pH, storage temperature, presence of enzymes, light, oxygen, structure and concentration of the anthocyanins, and the presence of other compounds such as other flavonoids, proteins, and minerals.

In order to improve and stabilize color of berries, natural berry juices were used in the research:

sea buckthorn ('*Hippophae rhamnoides*') juice contains soluble sugars - glucose, fructose, vitamins (A, K, E and C, $B_1$, $B_2$), essential fatty acids Omega 3, 6, 7 and 9; fatty acids, organic acid- quinic acid, malic acids; oxalic and citric acid, tartaric acid, succinic acid, lipids, free amino acids- apartic acid, threonine; valine, methionine, leucine, lysine, trytophan, isoleucine, phenylalanine, carbohydrates, folic acids, tocopherols and flavanoids, phenols, terpenes and tannins (Beveridge et al., 2002); Japanese quince ('*Chaenomeles japonica*') juice contains soluble sugars-glucose and fructose, organic acids-malic, tartaric and citric acids, tanning agents, volatile oils, adding piquant aroma and sourish smack, essential amino acids, micro and macro elements, vitamins: C, $B_1$, $B_2$, PP, H, folacin (Hellin et al., 2003).

The aim of the research was to analyze the quality parameter – color of strawberry mass with added acidifier which further could be used in production of different products for the needs of public catering, e.g. in production of desserts. In the research, thermal treatment for strawberry mass was carried out in order to analyze further sensory properties, anthocyanin, phenol and color stability, pH and soluble matter, both, for fresh strawberry mass with enhancers and thermally treated one.

**Sf –** fresh strawberry mass **without enhancers**

**Hf –** fresh strawberry mass **with sea buckthorn juice**

**Qf –** fresh strawberry mass **with Japanese quince juice**

**Af –** fresh strawberry mass **with L (+) - Ascorbic acid, Sodium salt**

**Asf –** fresh strawberry mass with **Ascorbic acid**

*Methods*

Total phenol content was determined by the photometric method with Folin-Ciocalteau reagent and the absorbance of the blue color was measured at 760 nm. The phenolic contents of the fruits were expressed as gallic acid equivalents GAE/FW 100 g (Singleton et al., 1999).

The content of total anthocyanin (mg $100g^{-1}$) was determined by the conventional method by using a spectrometer UV-1650-PC at wave length 535 nm (Moor et al., 2005).

The pH is measured using a pH meter 3510 (Jenway) according to LVS EN 1132:2001.

Color was determined during the storage in CIE L*a*b* color system using Color Tec PCM/PSM device. The measured parameters were L* for lightness, a* for redness, and b* for yellowness. Hue angle (h°) is derived from the two coordinates a* and b*. The calculations of Hue angle (h°) were made with the following equation:

$$h = \arctan (b^*/a^*) \quad (1)$$

Results were processed with Microsoft Office Excel 2007 programmer.

The hedonic evaluation and line scale methods were used based on ISO 4121:2003 "Sensory analysis – Guidelines for the use of quantitative response scales". A 9-point hedonic scale (9 – extremely like, 5 – neither like nor dislike, and 1 – extremely dislike) was used to determine the degree of acceptance of the products. Panellists evaluated intensity of sensory properties (aroma, colour, taste, consistence and after taste), the line scale (ISO 4121:2003) was used. A panel of 28 panellists, consisting of 21 females and 7 males at age from 24 to 68, took part in this study.

The results were processed by mathematical and statistical methods. Data were subjected to one way analysis of variance (ANOVA) and Two-Way analysis of variance (ANOVA), by Microsoft Office Excel 2007, significance was defined at $p<0.05$.

## Materials and Methods

The research was carried out at the Latvia State Institute of Fruit Growing and laboratories of the Faculty of Food Technology in Latvia University of Agriculture.

*Materials*

Frozen strawberries, the variety '*Polka*', were used as a raw material for the research. The object of the research is fresh (f) and thermally treated (h) – heated at the temperature +80 °C – strawberry mass. The berry juices: sea buckthorn and Japanese quince were used as strawberry mass natural enhancers and Ascorbic acid, L (+) Ascorbic acid, Sodium salt were used as artificial enhancers. All enhancers were used with concentration of 5% of strawberry mass.

Fresh and thermally treated product (heated at the temperature + 80 °C) was analyzed. Berry mass was stored for 8 days at the temperature + 4 °C. Measurements were taken at 2 - day interval. Chemical, physical and sensory indices of the product – pH, soluble dry matter, anthocyanins, phenols and color, smell, taste, consistency intensity were determined as quality evaluation.

Notations used in the research:

**Sh –** heated strawberry mass **without enhancers**

**Hh –** heated strawberry mass **with sea buckthorn juice**

**Qh –** heated strawberry mass **with enhancers Japanese quince juice**

**Ah –** heated strawberry mass **with L (+) - Ascorbic acid, Sodium salt**

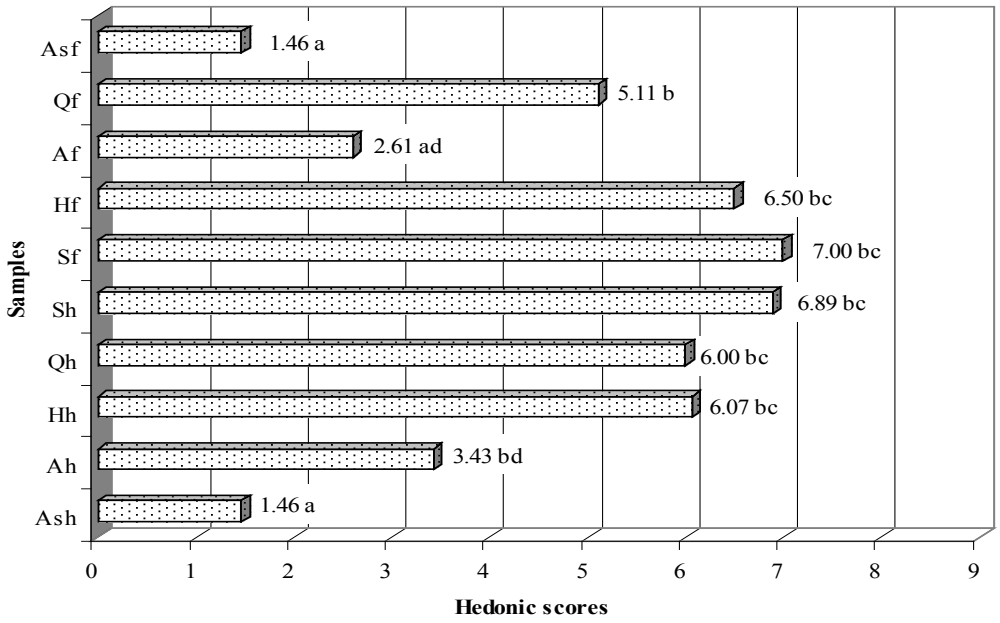
**Ash –** heated strawberry mass **with Ascorbic acid**

## Results and Discussion

Formation and stability of anthocyanins is determined by different factors. Formation of anthocyanins is enhanced by the presence of monosaccharides and disaccharides. It is established that varieties of darker red color have higher antioxidative activity than the lighter ones (Courtney and Rui, 2002). Anthocyanin colors can be enhanced and stabilized by the addition of different natural or artificial

acidifiers. Natural berry juice and artificial acidifier - L (+) - Ascorbic acid, Sodium salt impact to color stability of strawberry mass was analyzed and compared in the research.

Many different types of sensory methods have been proposed and used to evaluate and control the sensory quality of foods. The results of the analysis of variance show that $F_{cal} = 51.15 > F_{crit} = 1.92$; it means that there are significant differences in the degree of liking among the strawberry mass. The degree of liking of strawberry mass, evaluated by hedonic scores is presented in Figure 1.




Values, marked with the same letters, are not significantly different (p>α0.05)

Figure1. Evaluation results of strawberry mass using 9-point hedonic scale.

According to the hedonic scale panellists evaluated strawberry mass with enhancers in the range from 1 (dislike extremely) to 7 (like moderately). Results of the hedonic scores showed that the panellists liked (p>0.05) samples Sf (fresh strawberry mass without enhancers) and Sh (heated strawberry mass without enhancers) the most because they had pleasant, slightly sour taste with the most distinct strawberry taste. Analysis shows that there is no significant difference in hedonic scores among the samples Hh, Hf, Qh, Qf, Sh and Sf (p>0.05), which is strawberry mass with natural enhancers and strawberry mass without enhancers. The panellists liked the least Ash (heated strawberry mass with Ascorbic acid) and Asf (fresh strawberry mass with Ascorbic acid) (p>0.05), because they were too sour and the taste (aftertaste) of citric acid was felt. The sample Sf (fresh strawberry mass without enhancers) did not differ in degree of liking from the samples Sh (heated strawberry mass without enhancers) and Hh, Hf, Qh, Qf (fresh and heated strawberry mass with natural enhancers), but significant difference exists between samples Ash, Asf, Af and Ah (fresh and heated strawberry mass with artificial enhancers) in the degree of liking. The panelists evaluated degree of liking separately for every characteristic feature.

Strawberry mass for a consumer is characteristic by its distinct red color and strawberry taste. The assessment results of the intensity of sensory properties – aroma, colour, taste, consistency and aftertaste of strawberry mass are presented in Figure 2.

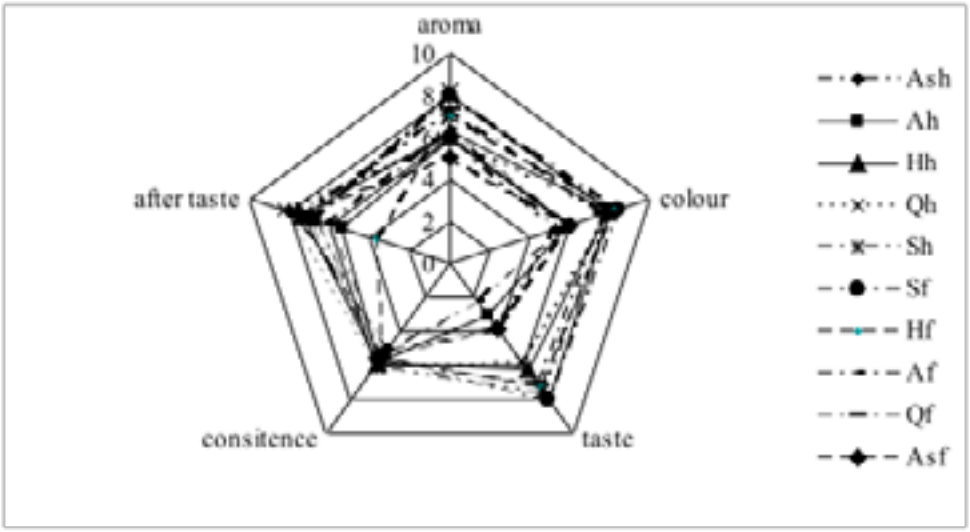



Figure 2. Intensity of sensory properties of strawberry mass.

Evaluation of intensity of sensory properties of strawberry mass shows that there is no significant difference ($F_{cal} = 1.34 < F_{crit} = 1.92$) in consistency, but there is significant difference in intensity of aroma, colour, taste and aftertaste. The main differences among the samples for colour, aroma, taste and aftertaste intensity were expressed to the strawberry mass with natural enhancers (Hh, Hf, Qh, Qf), but the lowest intensity of sensory properties were expressed to the strawberry mass with artificial enhancers ( Ash, Asf, Af, Ah). The least distinct aftertaste was identified to the sample Hf (fresh strawberry mass with sea buckthorn), but by adding Ascorbic acid (As) and L (+) – Ascorbic acid Sodium salt (A) to the strawberry mass, aftertaste became more intense. It was likely caused by characteristic properties of artificial enhancers added.

As panellists considered fresh and heated strawberry mass with Ascorbic acid (Ash and Asf) being too acid and unsuitable for nutrition, then for further research only fresh and heated strawberry mass with L (+) – Ascorbic acid Sodium salt (Af and Ah) was used as artificial enhancers for the strawberry mass. Therefore, color durability and stability in the strawberry mass depending on the added acidifier will be analyzed further in the research.

Attractive color is one of the most important sensory characteristics of fruit and berry products. The color of red berry products is unstable and susceptible to degradation. The main color formation substances of berries are anthocyanins of phenol group, which are the red color formers (Ancos et al., 2000). Polyphenols and anthocyanins present in different berries work as collectors of peroxide radicals. Anthocyanins are a group of many diverse chemical compounds with variable properties, therefore not only the general content is significant, but also their composition. More valuable is delphinidin as it is more stable in processing. Strawberries improve blood composition and help the body to release from residues of chemical substances and their compounds. Strawberries contain a substance with anti-cancer activity, which can protect cells from the influence of synthetic and natural cancerogenic substances (Melo et al., 2000).

Rein (2005) in the dissertation wrote that fortification of fruit and berry juices with ascorbic acid is a common method to protect against oxidation and to increase the nutritional value of a food product. The ascorbic acid is thought to have several different roles in anthocyanin color stability. Ascorbic acid enhances polymer pigment formation and bleaches anthocyanin pigments. Also the formation of hydrogen peroxide from ascorbic acid oxidation can influence anthocyanin stability (Talcott et al., 2003). Anthocyanins are also considered to be protected by ascorbic acid against enzymatic degradation. Fruit treatment by organic acid reduces oxidative changes of the color. Whereas ascorbic acid may have a protective effect with regard to anthocyanins because it reduces the o-quinones formed before their polymerization. However, ascorbic acid as well as products of its degradation increases the anthocyanin degradation rate where the fortification with ascorbic acid accelerated anthocyanin degradation in strawberry mass (Wilska-Jeszka, 2007).

The anthocyanin content in strawberry mass with acidifiers was determined to see the organic acid impact to color stability. In literature strawberries (*Fragaria ananassa*), having quite a light red hue, contain anthocyanins between 10-80 mg 100 $g^{-1}$ wrote Cordenunsi et al., 2003, but in this research the anthocyanin content is 20.2 mg 100 $g^{-1}$. In our research, the anthocyanin amount in strawberry mass with added acidifiers is indicated in Figure 3.

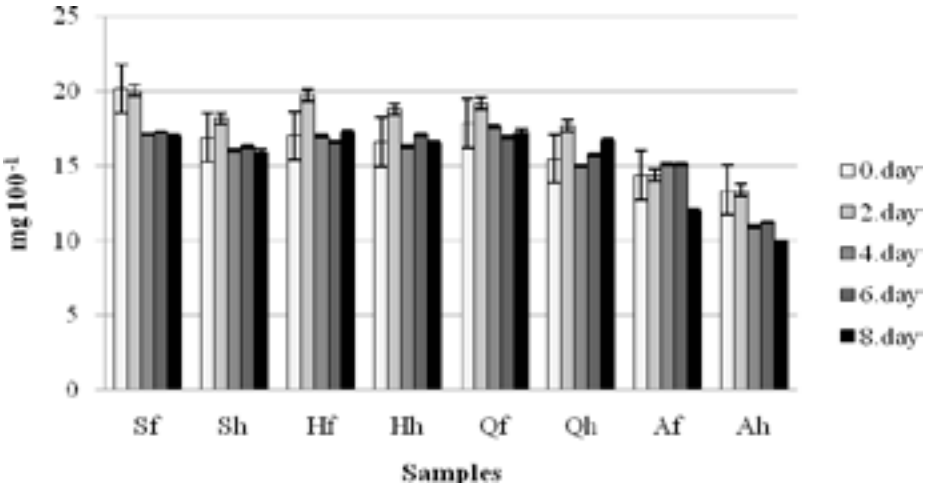



Figure 3. Anthocyanins in strawberry mass with enhancers.

Data of the Figure indicate that anthocyanin amount during storage decreases. After 2-day- storage their amount increases, but afterwards decreases, both, in fresh and thermally treated mass. Anthocyanin amount in fresh strawberry mass is larger than in a thermally treated mass. It might prove that during thermal treatment with the increase of temperature anthocyanin amount decreases. Anthocyanin stability is affected by temperature. Cavalcanti et al., 2010 wrote that degradation rate of anthocyanins increases during processing and storage as the temperature rises. Decomposition of anthocyanins depends also on the temperature and heating duration (Melo et al., 2000).

The stability of anthocyanins is affected by many factors – pH, storage temperature, presence of enzymes, light, oxygen, structure and concentration of the anthocyanins, and the presence of other compounds such as other flavonoids, proteins, and minerals.

Anthocyanins show great susceptibility toward pH being more stable in acidic media at low pH values than in alkaline solutions with high pH values. The taste evaluation of strawberry mass was essentially affected by the enhancer added. As the Fig. 2 shows the control samples Sf and Sh (7.1 – 7.2) and Hf- fresh strawberry mass with sea buckthorn juice (7.3) had the highest degree of liking. As the next best degree of liking by pannelists was Qf – fresh strawberry mass with Japanese quince juice (6.5). Natural enhancers – quince and sea buckthorn juices used in the research, did not substantially affect each other's pH in the product. The initial higher pH value of the control samples (Sf and Sh) can be explained by the fact that there is no enhancer added. Fig. 4 shows pH value analyzed in the research.

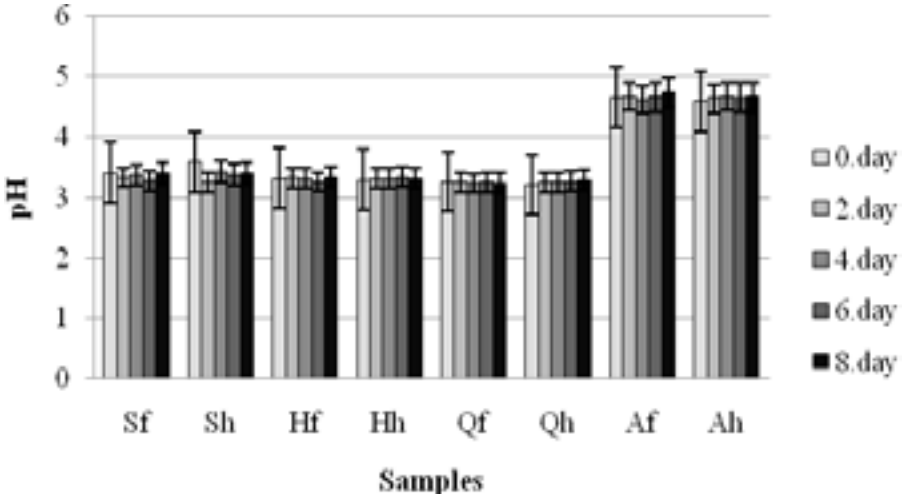



Figure 4. pH value of strawberry mass with enhancers.

The pH data found out in the research indicate that pH in strawberry mass with natural enhancers ranges from pH 3 till 3.6, but in strawberry mass with added Ascorbic acid, Sodium salt pH is 4.6 – 4.7. It means that by using natural enhancers anthocyanins are more stable and color is more intense, but by using artificial enhancer the medium becomes more alkaline at the presence of soda and salt.

To investigate color quality it is necessary to measure color, as well as pigment concentration. The CIEL a*b* system is very effective for measuring color differences and tracking color changes during processing and storage. Color is three dimensional, where L*= lightness with

100= absolute white and 0=absolute black. Hue angle (h°) is derived from the two coordinates a* and b* and determined as arctan b*/a*(formula 1). The values of Hue angle is shown in Table 1.

Table 1

**Color stability determination with Hue angle**

| Day | Samples | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Sf | Sh | Hf | Hh | Qf | Qh | Af | Ah |
| 0 | 41.4 | 40.9 | 41.0 | 40.0 | 44.3 | 46.0 | 31.7 | 48.9 |
| 2 | 46.4 | 44.2 | 45.0 | 44.9 | 42.5 | 43.2 | 37.0 | 61.5 |
| 4 | 38.9 | 41.3 | 42.7 | 40.9 | 43.2 | 39.2 | 31.1 | 60.4 |
| 6 | 46.2 | 43.5 | 43.8 | 42.9 | 42.2 | 43.3 | 36.2 | 61.9 |
| 8 | 43.5 | 43.0 | 42.2 | 36.5 | 33.3 | 40.8 | 38.8 | 64.1 |

Changes of the h° angle in Table 1. indicate that strawberry mass with added sea buckthorn juice (Hf and Hh) initially gives a positive result for anthocyanin stability, although after 8 - day storage the color of heat treated mass changes for 10% (h°=36.5), but that of fresh mass – only for 2% (h°=42.2). Fresh strawberry mass with added quince juice (Qf and Qh) during the first 6 days change color the least and is the most stabile. Whereas H angle for the strawberry mass with added artificial enhancer accelerates quickly – heat treated mass for 27% and fresh one for 22% that indicates essential change in color stability.

When comparing s taste and nutritional value among the juices used in the research, it has to be pointed out that sea buckthorn juice is one of the most valuable, rich in vitamins and other biologically active substances. Quince juice and sea buckthorn juice contain a little bit higher soluble dry matter content as the control samples, which affect taste properties of the product and liking rate. In general, it can be concluded that researched natural juices (sea buckthorn and quince) are recommended for usage in public catering as healthy acidifiers, color and taste enhancers in making desserts from different berries.

## Conclusions

1. The main differences among the samples for color, aroma, taste and consistency intensity were expressed to the strawberry mass with natural enhancers (Hh, Hf, Qh, Qf), but the lowest intensity of sensory properties was expressed to the strawberry mass with artificial enhancers (Ash, Asf, Af, Ah).
2. Analysis shows that there is no significant difference in hedonic scores among the samples Hh, Qh, Sh, Sf, Hf and Qf ($p>0.05$), which are samples with natural enhancers and strawberry mass without enhancers.
3. Anthocyanin amount in fresh strawberry mass is larger than in a thermally treated mass. Color is stable in acid medium, what is provided by natural juices. It indicates that strawberry mass with added sea buckthorn and Japanese quince juice initially gives a positive result for anthocyanin stability during storage.
4. For production of desserts thermal treatment was used causing color changes. For public catering it is recommended to use strawberry mass with natural enhancers.

## References


1. Alvarez C.A. (1995) Air dehydration of strawberries: Effects of blanching and osmotic pretreatments on the kinetics of moisture transport. *Journal of Food Engineering*, 25, pp. 167-178.
2. Ancos B., Ibanez E., Reglero G., Cano M.P. (2000) Frozen storage effects on anthocyantins and volatile compounds of raspberry fruit. *Journal of Agricultural and Food Chemistry*, 48, pp. 873-879.
3. Beveridge T., Harrison J.E., Drover J. (2002) Processing Effects on the Composition of Sea Buckthorn Juice from Hippophae rhamnoides L. Cv. Indian Summer *Journal of Agricultural and Food Chemistry*, *50,* pp. 113-116.
4. Cavalcanti R.N., Santos D.T., Meireles M.A.A. (2010) Non-thermal stabilization mechanisms of anthocyanins in model and food systems. An overview. *Food Research International*, doi: 10.1016/j/foodres. Elsevier Ltd., 11 p.
5. Cordenunsi B.R., Nascimento J.R.O., Lajolo F.M. (2003) Physico-chemical changes related to quality of five strawberry fruit cultivars during cool-storage. *Food Chemestry,* 83, pp. 167-173.
6. Costell E. (2002) A comparison of sensory methods in quality control. *Food Quality and Preference*, Volume 13, Issue 6, pp. 341-353
7. Courtney W. and Rui H.L. (2002) Antioxidant Capacity and anticancer properties of red raspberries. *Acta Horticulture*, 585, ISHS, pp. 451-457.
8. Doymaz İ. (2008) Convective Drying Kinetics of S trawberry. *Chemical Engineering and Processing,* Process Intensification, Volume 47, Issue 5, pp. 914-919.
9. El-Beltragy A. (2006) Solar drying characteristics of strawberry. *Journal of Food Engineering,* 78, pp. 456-464.
10. Giboreau A., Dacremont C., Egoroff C., Guerrand S., Urdapilleta I., Candel D. and Dubois D. (2007) *Food Quality and Preference*, Volume 18, Issue 2, pp. 265-274.
11. Hellin P., Vila R., Jordan M.J., Laencina J., Rumpunen K., Ros J.M. (2003) Characteristics and composition of chaenomeles fruit juice. I/In: *Japanese quince*

- *Potential fruit crop for Northern Europe. Sid*., pp. 127-139.
12. Kopjar M., Piližota V., Hribar J., Simčič M., Zlatič E. and Nedić Tiban N. (2008) Influence of trehalose addition and storage conditions on the quality of strawberry cream filling, *Journal of Food Engineering*, Volume 87, Issue 3, pp. 341-350.
13. Kuti T., Hegyi A. and Kemény S. (2004) Analysis of sensory data of different food products by ANOVA. *Chemometrics and Intelligent Laboratory Systems*, Volume 72, Issue 2, pp. 253-257.
14. Melo M.J., Moncada M.C., Pina F. (2000) On the red colour of raspberry (*Rubus idneus*). *Tetrahedron Letters*, 41, Issue 12, pp. 1987-1991.
15. Moor U., Karp K., Põldma P. and Pae A. (2005) Cultural Systems Affect Content of Anthocyanins and Vitamin C in Strawberry Fruits. *European Journal of Horticultural Science,* 70 (4). S., ISSN 1611-4426, pp. 195-201.
16. Moreno J. (2000) Effect of blanching/osmotic dehydration combined methods on quality and stability of minimally processed strawberries. *Food Research International*, 33, pp. 609-616.
17. Oszmiański J., Wojdyłoa A., Kolniak J. (2009) Effect of l-ascorbic acid, sugar, pectin and freeze–thaw treatment on polyphenol content of frozen strawberries. *Food Science and Technology*.Volume 42, Issue 2, pp. 581-586.
18. Rein M. (2005) Copigmentation reactions and color stability of berry anthocyanins. *Dissertation*. University of Helsinki, Department of Applied Chemistry and Microbiology, Food Chemistry Division, 87 p.
19. Singleton V.L., Orthofer R., Lamuela-Raventos R.M. (1999) Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteau reagent. *Methods in Enzymology*, 299, pp. 152-178.
20. Talcott S.T., Brenes C.H., Pires D.M., Del Pozo-Insfran D. (2003) Phytochemical stability and color retention of copigmented and processed muscadine grape juice. *Journal of Agricultural and Food Chemistry,* 51, pp. 957-963.
21. Wilska-Jeszka J. (2007) Food colorants. In Z.E. Sikorski (ed.), *Chemical and functional properties of food components*, Boca Raton: CRC Press, pp. 245-274.

# ASSESSMENT OF APPLE CULTIVAR QUALITY AND SELECTION OF THE MOST SUITABLE APPLE CULTIVARS FOR FRESH CUT SALAD PRODUCTION

***Inta Krasnova[1,2], Daina Karklina[1], Dalija Seglina[2], Karina Juhnevica[2], Jorens Kviesis[2]***
*[1]Latvia University of Agriculture*
*[2]Latvia State Institute of Fruit Growing*
*inta.krasnova@lvai.lv*

## Abstract

Apples (*Malus domestica* L.) are the most consumed fruits in Latvia. Apples are used as one of ingredients for the fresh cut fruit salad preparation. The evaluation of physical, chemical and sensory properties is important; they could influence the nutritional value of fruit salads. The objective of this research is to select for fresh cut salad production the most appropriate apple cultivars grown in Latvia and evaluate their physical, chemical and sensory properties.

Ten commercial apple cultivars grown in Latvia were selected for experiments: 'Zarya Alatau', 'Saltanat', 'Belorusskoe Malinovoe', 'Auksis', 'Antei', 'Sinap Orlovskii', 'Orlik', 'Korichnoe Novoe', 'Alesya', 'Kovalenkovskoe'.

The research was carried out in the Latvia State Institute of Fruit Growing (LSIFG), the years 2010 – 2011. The apple physical indices (average mass, diameter of fruit, flesh consistency and colour) and chemical parameters (total sugar, soluble solids content, titratable acidity ratio between soluble solids and titratable acidity) were analyzed as well. The sensory properties were determined using quantitative descriptive analysis and affective method by hedonic scale.

The physical and chemical properties of fruits were analyzed and for fresh cut fruit salad production as the best the following apple cultivars: 'Zarya Alatau' and 'Sinap Orlovskii' were selected; however, the cultivars 'Antei', 'Auksis' and 'Alesya' could be successfully used for mentioned aim as well.

**Key words**: apple, physical, chemical, sensory properties.

## Introduction

Apples are the most consumed fruits in Latvia (Feliciano et al., 2010). Diet rich vegetables and fruits can provide human body of high level natural antioxidants, and therefore reduce the risk of chronic and aging related diseases. Boyer and Liu also reported that by including apples in daily nutrition, it is possible to reduce obesity level (Boyer and Liu, 2004).

The apple quality is affected by growth and storage conditions. Fruit chemical composition depends on the variety, climate conditions, place of growth, soil compound and harvesting time. An important indicator of the fresh apple quality is the firmness of pulp and the crispness. Firmness of apple pulp affects the cells tissue structure, which form cells at the different molecular levels properly. This is one of the most important organoleptic parameters, which the consumers accept by sensory evaluation. Firmness of pulp is the uppermost indicator for the reason why apples are consumed (Varela et al., 2007). The sensory acceptance of apple liking is affected by soluble solids and titratable acid ratio. The pleasant taste of apples is created by a well-balanced sugar and acid composition (Peirs et al., 2003; Harker et al., 200).

Apples are widely used in fresh cut salad production. Fresh cut apples have became a well-known snack in the EU school program, where they are successfully used for childrens' food (EU school lunch programs for children's overweight reduction). Fresh cut fruits and fruit salads are popular and occupy an important place on the marketing, in coffee-bars and restaurants. Within next few years it is expected, that the demand of healthy and ready for use fresh cut fruits will increase (Quevedo et al., 2009).

The browning of fresh cut fruits is one of the main problems, which has to be prevented and controlled in the salad processing and during shelf-life (Lamikanra, 2002). The colour values L * and b * reflect the apple flesh quality, which indicate colouring during the storage after slicing. Studies show, that the visual appearance of fresh cut fruits and vegetables significantly affects their distribution. When purchasing, consumers' first attention is drawn to the fresh cut fruit colour and only after that they think about the shelf life deadline, nutritional value and price (Suttirak and Supranee, 2010).

Numerous studies have noted that the fresh cut salad quality depends on the fruit cultivar. The objective of this research is to select the most appropriate apple cultivars for fresh cut salad production grown in Latvia and evaluate their physical, chemical and sensory properties=

## Materials and Methods

The experiments were carried out at the Latvia State Institute of Fruit-Growing (LSIFG), the years 2010 – 2011. Ten commercial apple cultivars grown in Latvia were selected for experiments: 'Zarya Alatau', 'Saltanat', 'Belorusskoe Malinovoe', 'Auksis', 'Antei', 'Sinap Orlovskii', 'Orlik', 'Korichnoe Novoe', 'Alesya', 'Kovalenkovskoe'. The apples after harvesting were stored for four months at the temperature + 4 °C and air relative humidity (RH) 90%. Mean sample of 10 apples from each cultivar was selected for analyzing. The selected samples before analyzing were stored for 3 hours at room

temperature 20 ± 2 °C. Physical, chemical and sensory analyses were performed.

*Physical properties*

The mean diameter of one apple was determined by calibration method in 3 repetitions, 10 fruits were measured; the diapason of measured results was 30 – 90 mm.

The average mass of one apple (g) was determined by 3 recurrent weightings of each one from 10 selected fruits, using calibrated electronic scale 'Vibra', precision ± 0.5 g.

Flesh firmness (N) was determined by digital penetrometer TR 53205 according to LVS EN 1131:2001 nozzle diameter – 8 mm, penetration depth – 10 mm, speed of measurement – 600 mm $min^{-1}$. Pealed fruit flesh firmness was determined on the diametrically opposing sides of the fruit.

Color characteristics (Hunter L*, a* and b*) were measured by using a colorimeter ColorTec-PCM BenchTop Models-45 the method described by Mac Dougall D.B. (2002). The instrument was calibrated with a white standard plate immediately before each set of measurements (Y – 92.15, x – 0.3623 and y – 0.3434), b* – the yellow/blue coordinate (+ b* yellow, - b* blue). The colour parameters of flesh were measured after cutting of 1.5 cm thick upper slice from each apple. The measurements were repeated on 20 randomly selected locations on each sample. The colour of apple flesh was expressed as whiteness index (WI), which was calculated according to equation (1) as reported, by Albanese (Albanese et al., 2007):

$$WI = 100 - \sqrt{(100 - L^*)^2 + a^{*2} + b^{*2}} \quad (1)$$

*Chemical analyses*

Soluble solids content (°Brix) has been determined according to LVS EN 12147:2001 using refracometer ATAGO.

Content of total sugars was defined by high-performance liquid chromatography method using coefficient of refraction detector. Determination was made after the described method by Matiseks R. et al., 1998.

Titratable acidity was determined corresponding LVS EN 12147:2001.

pH was determined using a pH meter 3510 (Jenway) according to the standard LVS EN 1132 – pH Determination of Fruit and Vegetable Juice.

Sensory evaluation of apples was carried out by selected 20 previously trained panellists according to ISO 8586-1:1993. The intensity of sensory properties (taste, aroma, and consistency) of fruit flesh was evaluated by using a line scale, but the degree of liking was evaluated by 9-point hedonic scale (ISO 4121:2003).

*Analytic Hierarchy Process* was used to evaluate the apple sensory quality by results obtained with sensory descriptive method. Each sensory property: aroma, taste and consistency was accepted as basic criteria for valuation by relative significance scale – for intensity of importance 1 – 9. For calculation of the priority coordinates' vector, possible coherence coefficient 1.49 was used, corresponding to quantity of 10 evaluated apples. The priority coordinates' vectors were calculated according to equations (2) as reported, by (Saaty, 2008):

$$\left.\begin{aligned} a_1 &= \sqrt[3]{\left(\frac{w_1}{v_1}\times\frac{w_1}{v_2}\times\frac{w_1}{v_n}\times\frac{w_1}{v_{10}}\right)} \\ a_2 &= \sqrt[3]{\left(\frac{w_2}{v_1}\times\frac{w_2}{v_2}\times\frac{w_2}{v_n}\times\frac{w_2}{v_{10}}\right)} \\ a_3 &= \sqrt[3]{\left(\frac{w_3}{v_1}\times\frac{w_3}{v_2}\times\frac{w_3}{v_n}\times\frac{w_3}{v_{10}}\right)} \end{aligned}\right\} \quad (2)$$

$A_{1\text{-}3}$ priority coordinates vectors: $a_1$ – aroma, $a_2$ – taste, $a_3$ – consistency)

$W_{1\text{-}3}$ sensory properties ($w_1$ – aroma, $w_2$ – taste, $w_3$ – consistency)

$v_{1\text{-}n\text{-}10}$ – taster's evaluations.

*Statistical analysis*

Data processing was carried out by the following unvaried and multivariate methods of statistical analysis: analysis of variance, correlation analysis, principal component analysis and factor analysis using SPSS 15 software package. Sheffe criterion was used in the clarification of significant differences ($p<0.05$) among studied samples in the analysis of variance. Closeness of the relationship between the parameters was determined by analysis of Pearson correlation coefficient.

## Results and Discussion

The physical and chemical properties of apples depend on climatic conditions, growing geography, cultivar, harvesting time, as well as on storage conditions (Feliciano et al., 2010). The average mass and diameter of each apple are limited quality indices, their proportion is governed by regulation EEC 2081/92. When 10 selected commercial apple cultivars grown in Latvia were rated, it was established that average mass, diameter and firmness disparate among the cultivars ($p<0.05$, $n=10$). An average linkage exists between the mass and diameter of fruit ($r=0.687$), that substantially varied for cultivars 'Sinap Orlovskiy' (259.5 g) and 86.5 mm and for 'Orlik' (145.7 g and 72.5 mm) (Table 1). Scientists in Italy comparing commercial apple cultivars 'Golden Delicious' and 'Stark Delicious' (Rojas-Grau et al., 2007) have similar results. In Latvia widely recognised apple cultivar 'Auksis' was analyzed in Lithuania and obtained results indicate the average mass of one fruit only 173 g (Kvikliene et al., 2006), in the turn, in Latvia grown fruit average mass of the same cultivar was considerably higher – 204.2 g.

Apple flesh firmness which depends on harvesting

time and affects the apple application – freshly consumed, processed or stored is a very important quality indicator. The flesh firmness is substantially lower for apples of cultivar 'Kovalenkovskoe' – 8.7 N ($p<0.05$, n=10), their flesh firmness is soft and floury.

Table 1

**The mean indices of different cultivar apple physical characteristics**

| Cultivars | Diameter (mm) | Weight (g) | Firmness (N) | Flesh colour parameters | | | WI |
|---|---|---|---|---|---|---|---|
| | | | | L* | a* | b* | |
| 'Alesya' | 77.5$^{abc}$ | 177.7$^{b}$ | 17.1$^{cd}$ | 82.39$^{de}$ | -0.89$^{b}$ | 23.04$^{cd}$ | 70.99$^{de}$ |
| 'Antei' | 76.3$^{ab}$ | 176.3$^{b}$ | 19.5$^{e}$ | 79.14$^{b}$ | -0.78$^{b}$ | 23.72$^{d}$ | 68.39$^{bc}$ |
| 'Auksis' | 83.8$^{cd}$ | 204.2$^{bc}$ | 15.3$^{bc}$ | 76.62$^{a}$ | -2.71$^{a}$ | 31.58$^{g}$ | 60.6$^{a}$ |
| 'Belorusskoe 'Malinovoe' | 80.5$^{bcd}$ | 202.7$^{bc}$ | 15.8$^{bcd}$ | 80.64$^{c}$ | -2.08$^{a}$ | 26.80$^{f}$ | 66.86$^{b}$ |
| 'Korichnoe Novoe' | 81.8$^{bcd}$ | 195.0$^{bc}$ | 14.4$^{b}$ | 80.81$^{c}$ | 0.43$^{c}$ | 23.50$^{d}$ | 69.65$^{cd}$ |
| 'Kovalenkovskoe' | 80.8$^{bcd}$ | 183.7$^{bc}$ | 8.7$^{a}$ | 83.74$^{f}$ | -2.46$^{a}$ | 21.6$^{b}$ | 72.85$^{f}$ |
| 'Orlik' | 72.5$^{a}$ | 145.7$^{a}$ | 15.7$^{bc}$ | 83.58$^{ef}$ | -2.21$^{a}$ | 22.11$^{bc}$ | 72.37$^{ef}$ |
| 'Saltanat' | 81.8$^{bcd}$ | 202.5$^{bc}$ | 15.8$^{bcd}$ | 85.58$^{g}$ | -0.63$^{b}$ | 12.67$^{a}$ | 80.79$^{g}$ |
| 'Sinap Orlovskii' | 86.5$^{d}$ | 259.5$^{d}$ | 18.1$^{e}$ | 81.62$^{cd}$ | -2.94$^{a}$ | 25.34$^{e}$ | 68.56$^{c}$ |
| 'Zarya Alatau' | 79.5$^{bc}$ | 212.8$^{c}$ | 19.6$^{e}$ | 82.77d$^{ef}$ | -2.94$^{a}$ | 23.12$^{cd}$ | 70.97$^{de}$ |

$^{a,b,c,d,e,f,g,}$ – Values, marked with the same letter in a column, are not significantly different at $p<0.05$.

Apples are used as one of ingredients for the fresh cut fruit salad preparation. The colour is an important indicator of fresh cut fruit salads and substantially influences the consumers' choice. The colour indices of analysed various cultivar apples' flesh were different. The apple flesh colour of cultivar 'Auksis' was lighter – L* value 76.62, a* – 2.71 and b* – 31.58, in Lithuania at the Babtai institute the determined colour parameters of this cultivar were as follows: L* 66.6, a* 13, b*30.1 (Sasnauskas et al., 2008). A close negative correlation exists between colour parameters b*, L* and calculated whiteness index (WI) – r=-0.862, r=-0.987 respectively, while among colour parameter L* and WI a positive correlation exists – r=0.931. The apple flesh colour of cultivars ' Saltanat', 'Kovalenkovskoe' and 'Orlik' was lighter – L* values 85.58, 83.74, 83.58 respectively, and calculated WI value higher – 80.79, 72.85, 723.37 respectively, as a result that those apples got most ripe. The scientists in Spain concluded that the L* value are not essentially influenced by apple ripening stage (Rojas-Grau et al., 2007). Few scientists experimentally established a fact that the colour characteristics of fresh cut apple flesh obtained by instrumental analyser and by experts' sensory valuation correlates (Rocha and Morais, 2003).

The soluble solids content (SSC) and total sugars (TS) are inconsistent indicators of apples and more than any other quality parameters depend on growing year, weather conditions and harvesting time (Kvikliene et al., 2006). The SSC and sugar content of investigated apples substantially differs among cultivars ($p<0.05$, n=10). The sugar content in apples of cultivar 'Zarja Alatau', 'Alesya' and 'Auksis' were higher (Table 2), low sugar content has apples of cultivar 'Belorusskoe Malinovoe' – 8.17 g $100g^{-1}$. The SSC of analysed apples was 10.17 ('Korichnoe Novoe') till 13.97 Brix° ('Zarya Alatau'). Similar results have been obtained in Byelorussia analyzing apples of cultivars 'Sinap Orlovskii', 'Belorusskoe Malinovoe', 'Antei', 'Alesya', 'Kovalenkovkoe' when the SSC was 12.4, 12.4, 12.0, 12.0 Brix° respectively, and it is higher than for those in Latvia grown.

The titratable acid (TA) content substantially differs between the apples of different cultivars ($p<0.05$, n=10). The lowest TA content experimentally has been ascertained in apples cultivar of 'Kovalenkovskoe' – 0.13%, the highest of cultivar 'Sinap Orlovskii' apples – 0.75%. Scientists in Byelorussia defined the TA content in apples of 'Belorusskoe Malinovoe', 'Antei', 'Alesya', 'Auksis', 'Kovalenkovkoe' cultivars – 0.67%, 0.60%, 0.60%, 0.70% un 0.37% respectively, and it was higher than one established in Latvia, while in apples cultivar of 'Sinap Orlovskii' the TA content was the same as found in Latvia. The content of TA and pH value in apples essentially depends on cultivar and ripening stage (Максименко и Зуйкевич, 2010). The higher pH value has apples of cultivar 'Kovalenkovskoe', because those apples were the most ripe, therefore with less TA content.

Table 2

**The mean indices of chemical characteristics in apples of various cultivars**

| Cultivars | pH | TA (%) | SSC (Brix %) | Sugar (g $100g^{-1}$) | (SSC/TA) |
|---|---|---|---|---|---|
| 'Alesya' | $3.26^{b}$ | $0.49^{d}$ | $11.30^{bcd}$ | $11.86^{i}$ | 23.06 |
| 'Antei' | $3.25^{b}$ | $0.61^{e}$ | $11.42^{def}$ | $8.78^{b}$ | 18.87 |
| 'Auksis' | $3.42^{c}$ | $0.41^{c}$ | $11.60^{cd}$ | $10.56^{h}$ | 28.64 |
| 'Belorusskoe Malinovoe', | $3.17^{a}$ | $0.58^{e}$ | $10.32^{a}$ | $8.17^{a}$ | 17.94 |
| 'Korichnoe Novoe' | $3.42^{c}$ | $0.52^{d}$ | $10.17^{a}$ | $10.26^{f}$ | 19.74 |
| 'Kovalenkovskoe' | $4.59^{c}$ | $0.13^{a}$ | $11.50^{bcd}$ | $10.42^{g}$ | 92.00 |
| 'Orlik' | $3.68^{e}$ | $0.35^{b}$ | $11.92^{d}$ | $9.89^{e}$ | 34.05 |
| 'Saltanat' | $3.56^{d}$ | $0.35^{b}$ | $10.77^{ab}$ | $9.45^{c}$ | 30.76 |
| 'Sinap Orlovskii' | $3.14^{a}$ | $0.75^{g}$ | $10.87^{abc}$ | $9.53^{d}$ | 14.59 |
| 'Zarya Alatau' | $3.41^{c}$ | $0.69^{f}$ | $13.95^{e}$ | $12.31^{j}$ | 20.16 |

$^{a,b,c,d,e,f,g,h,i,j}$ – Values, marked with the same letter in a column, are not significantly different at $p<0.05$.

Numerous investigations declare the relationship between the quality of fruits and soluble solids and titratable acid ratio (SSC/TA) as consumers' acceptability of fruits (Harke et al., 2002). Scientists of Byelorussia defined the main importance of SSC/TA for the apple taste formation. They concluded that agreeable and integrated taste have apples of cultivars 'Alesya' un 'Auksis' 'Belorusskoe Malinovoe', 'Antei' and 'Sinap Orlovskij' with SSC/TA from 10 to 20 (Максименко и Зуйкевич, 2010). Our obtained results point out the cultivars 'Sinap Orlovskij' and 'Zarja Alatau' whose apple SSC/TA refers to this interval.

The influence of separate apple physical and chemical indices on their quality is problematically to interpret, because essentially different close correlations exist between them. Between indices WI and TA a close correlation exists (r=0.799), while an average close correlation exists among sugar content and SSC (r=0.598) and among consistency and sugar content (r=0.579). The factor analysis was used to select and combine more substation apple characteristic parameters into factors. After data processing it was concluded that in one factor could be accumulated the quality indices if close correlation exists among them. The factor analysis helped to obtain five factors from eight initially analyzed quality parameters (Table 3), while only three of them were selected with value >1. The first factor explains 39.88% of summary dispersion, the second – 33.24% and the third – 15.48%. The total sum of the research factors is 97.63%, and it is enough to ignore the fourth and fifth factor.

Table 3

**Total Variance Explained**

| Component | Initial EigenValues | | | Rotation Sums of Squared Loadings | | |
|---|---|---|---|---|---|---|
| | Total | % of variance | Cumulative % | Total | % of Variance | Cumulative % |
| 1 | 3.19 | 39.88 | 39.88 | 2.88 | 35.94 | 35.94 |
| 2 | 2.66 | 33.24 | 73.12 | 2.87 | 35.94 | 71.88 |
| 3 | 1.24 | 15.48 | 88.60 | 1.34 | 16.72 | 88.60 |
| 4 | 0.72 | 9.03 | 97.63 | | | |
| 5 | 0.19 | 2.37 | 100.00 | | | |

The values of factor components are shown in the Table 4. The first factor contains the main quality characterising indicators: SSC, sugar content, firmness and average weight of apple – the essential apple quantitative features connected with taste and consistency. The components of factor explain each other. Firstly, SSC and sugar content is connected with average apple weight – the higher SSC and sugar content, the heavier and sweeter the apples. Secondly, SSC content influences the apple flesh firmness: the higher SSC, the more compact the apple firmness. In the second factor following data processing quality parameters TA, WI, and apple diameter (D) are grouping, explaining apple flesh colour, as well as TA content. WI could depend on the diameter of apple (D) and flesh colour. In the turn, the third factor contains only one quality characteristic component – pH. The dispersion of factors is shown in the Fig. 1.

Table 4

**The apple quality indices' division into factors**

| Factor | TA | SSC | ph | Sugar | Firmness | WI | Diameter | Weight |
|---|---|---|---|---|---|---|---|---|
| 1 | | 0.897 | | 0.905 | 0.823 | | | -0.933 |
| 2 | 0.930 | | | | | 0.896 | -0.936 | |
| 3 | | | 0.951 | | | | | |

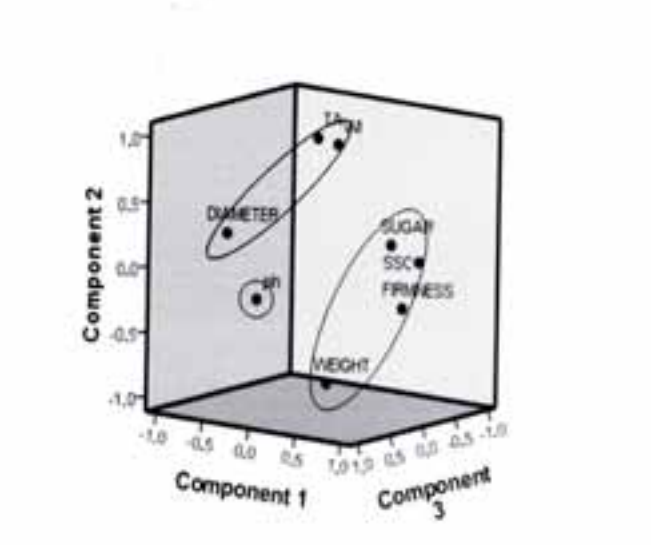



Figure1. The diagram of factors by rotation.

Sensory validation is considered as objective; reflecting experts' liking on sensory properties of apples (Strautniece, 2004). Panellists liking degree about each apple cultivar was applied. Experts in Hedonic 9-point scale (9 – extremely like upon 1 – extremely unpleasant) as more testy approved apples of cultivars 'Zarya Alatau' – 7.41 point, 'Sinap Orlovskii' points and 'Auksis' – 7.04 points. The apples of cultivar 'Kovalenkovskoe' were assessed to get the lowest scores – 4 points meaning slightly unpleasant. The apples of cultivars 'Orliks' and 'Saltanat' were evaluated by 5 points – don't like, don't dislike. Significant difference was found between evaluated apple samples in liking scale.

Descriptive sensory validation method (line scale) was used to select more appropriate apple cultivars for fresh cut salad production. The aroma, taste and firmness of apples were validated. Apples of cultivar 'Zarya Alatau' and 'Sinap Orlovkii', which have an average hard firmness, were stated as the best for fresh cut salad production by experts. Several experts have accepted as equally good apples of cultivar 'Antei', 'Auksis' and 'Belorusskoe Malinovoe' with mild consistency, panellists as more relished preferred apples of cultivar 'Zarya Alatau', 'Auksis' and 'Antei' with pleasant sweet and sour taste. In the turn, cultivars of 'Sinap Orlovkii', 'Korichnoe Novoe' and 'Antei' were recognized as the most aromatic by experts.

Analytic Hierarchy Process (AHP) was used for descriptive sensory analyses results analytical validation. The panellists preferred apple taste (priority vector 0.67) as prior sensory validation property. The second essential apple sensory property is consistency (priority vector 0.24), while the aroma has been considered as insignificant (priority vector 0.09). Figure 2 shows aroma, taste and consistency sensory evaluation of apple cultivars. Data from sensory analyses showed that apple cultivar 'Sinap Orlovkii' has been evaluated as the most aromatic (0.14) compared to other ones while 'Zarya Altau' was pointed as the most tasty (0.12) and with the best flesh consistency (0.14).

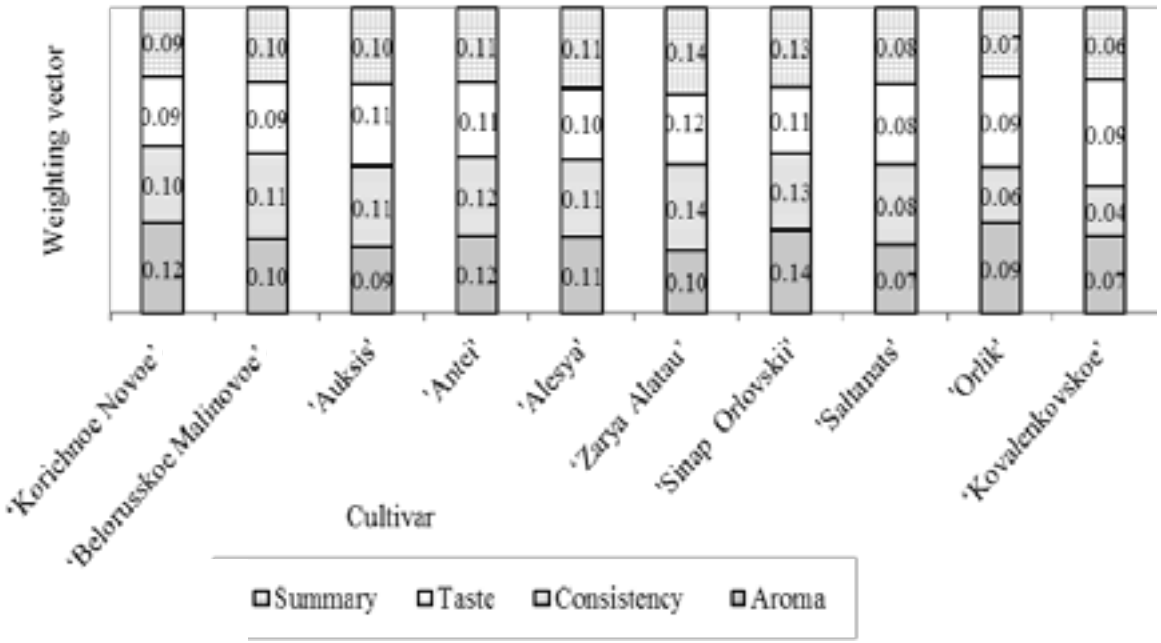



Figure 2. Aroma, consistency, taste and summary sensory evaluation of apples by Analytic AHP weighting vector.

To summarize, the highest weighting vector index in conformity with panellists validation according to AHP results was given to the apples of cultivar 'Zarya Alatau' (0.14) and 'Sinap Orlovkii' (0.13), the apples of cultivar 'Antei', 'Auksis' and 'Alesya' (0.11) were assessed slightly lower.

## Conclusions

Evaluation of apples on physical, chemical and sensory properties showed that the best cultivars for fresh cut fruit salad production are *'Zarya Alatau'* and *'Sinap Orlovskii'*. They had an average hard consistency and sweet-and-sour taste, their flash firmness was 14.5 – 20.0 N and soluble solids and titratable acid ratio varied from 14.5 to 25.0.

Apples of cultivars 'Antei', 'Auksis' and 'Alesya' with flash firmness 15 – 19 N and soluble solids and titratable acid ratio from 17 to 29 are the next concerning the most suitable stock for preparing fresh cut salads.

## Acknowledgements

This research and publication has been prepared within the framework of the ESF Project "Scientific capacity building in fruit-growing, forestry and information technology sectors, providing research on environmentally friendly growing strategies, product development and introduction aided by computer technologies" Contract No.2009/0228/1DP/1.1.1.2.0/09/APIA/VIAA/035

## References

1. Albanese D., Cinquanta L., Matteo M. (2007) Effects of an innovative dipping treatment on the cold storage of minimally processed Annurca apples. *Food Chemistry* 105, pp. 1054-1060.
2. Boyer J. and Liu R.H. (2004) Apple phytochemicals and their health benefits. Nutrition Journal 3, pp. 1-15.
3. Brookfield P.L., Nicoll S., Gunson F.A., Harker F.R., Wohlers M. (2011) Sensory evaluation by small postharvest teams and the relationship with instrumental measurements of apple texture. *Postharvest Biology and Technology* 59, pp. 179-186.
4. Feliciano R.P., Antunesa C., Ramos A., Serra A.T., Figueira M. E., Duartea C.M., Carvalhod A., Bronzea M.R. (2010) Characterization of traditional and exotic apple varieties from Portugal. Part 1 – Nutritional, phytochemical and sensory evaluation, *Journal of funkcional foods* 2, pp. 35-45.
5. Harker F.R., Marsh K.B., Young H., Murray S.H., Gunson F.A., Walker S.B. (2002) Sensory interpretation of instrumental measurements 2: sweet and acid taste of apple fruit. *Postharvest Biology and Technology* 24, pp. 241-250.
6. Kvikliene N., Kviklys D., Viškelis P. (2006) Changes in fruit quality during ripening and storage in the apple cultivar 'Auksis'. *Journal of Fruit and Ornamental Plant Research.* 14, pp. 195-202.
7. Lamikanra O. (2002) Fresh Cut Fruits and Vegetables. Science, Technology and Markets. CRC press Cambridge, UK, pp. 277-282.
8. Mac Dougall D.B. (2002) Colour in Food: Improving Quality. Edited by D MacDougall, Woodhead Publishing Limited, Abington Hall, Abington, Cambridge, CB21 6AH, UK, pp. 43.
9. Matiseks R., Šnēpels F.M., Šteinerte G. (1998) Pārtikas analītiskā ķīmija (Food Chemistry). Rīga Latvijas universitāte. pp. 122-126. (in Latvian).
10. Mehinagic E., Royera G., Symoneaux R., Bertrand D., Jourjon F. (2004) Prediction of the sensory quality of apples by physical measurements. *Postharvest Biology and Technology*, 34, pp. 257-269.

11. Peirs A., Tirry J., Verlinden B., Darius P., Bart M.N. (2003) Effect of biological variability on the robustness of NIR models for soluble solids content of apples. *Postharvest Biology and Technology,* 28(2), pp. 269-280.
12. Quevedo R., Jaramillo M., Diaz O., Pedreschi F., Aguilera J.M. (2009) Quantification of enzymatic browning in apple slices applying the fractal texture Fourier image, *Journal of Food Engineering* , 95, pp. 285-290.
13. Rocha C.N. and Morais M.B. (2003) Shelf life of minimally processed apple (cv. Jonagored) determined by colour changes. *Journal Food Control*, 14, pp. 13-20.
14. Rojas-Grau M.A., Grasa-Guillem R., Martin-Belloso O. (2007) Quality Changes in Fresh-Cut Fuji Apple as Affected by Ripeness Stage, Antibrowning Agents, and Storage Atmosphere , *Journal of Food Science,* 72 (1) pp. 32-43.
15. Saaty L.T. (2008) Decision making with the analytic hierarchy process *International Journal of Services Sciences*, 1 (1), pp. 83-98.
16. Sasnauskas A., Gelvonauskiene D., Gelvonauskis B., Viškelis P., Bobinas Č. (2008) Productivity and fruit quality of Lithuanian apple selections. Proceedings of international scientific conference 'Sustainable Fruit Growing: From Plant to Product', Latvia State Institute of Fruit -Growing, pp. 99-107.
17. Strautniece E. (2004) Pārtikas produktu sensorā vērtēšana. (Sensory Evaluation of Food Products). Jelgava: LLU PTF, 89. lpp. (in Latvian).
18. Suttirak W. and Supranee M. (2010) Potential Application of Ascorbic Acid, Citric Acid and Oxalic Acid for Browning Inhibition in Fresh-Cut Fruits and Vegetables. *Walailak Journal Science & Technology,* 7(1), pp. 5-14.
19. Valentines M.C., Vilaplana R., Torres R., Usall J., Larrigaudiere C. (2005) Specific roles of enzymatic browning and lignifications in apple disease resistance. Postharvest Biology and Technology, 36, pp. 227-234.
20. Varela P., Salvador A., Fiszman S. (2007) Changes in apple tissue with storage time: *Rheological, textural and microstructural analysesJournal of Food Engineering*, 78, pp. 622-629.
21. Максименко М.Г., Зуйкевич О.Г. (2010) Оценка раинированных сортов яблони на пригодность к выработке сока прямого отжима. (Evaluation of the Apple Cultivars Grown in Regions for Juice Production). Плодоводство: науч.тр./Руп "Ин-Т плодоводство"; редкол. В.А.Самусь (гл.ред.) (и др.). -Самохваловичи, Т.22, 267-274 с. (in Russian).

# INFLUENCE OF DIFFERENT YEAST STRAINS ON THE PRODUCTION OF VOLATILE COMPOUNDS IN FERMENTED APPLE JUICE

***Rita Riekstina-Dolge[1], Zanda Kruma[1], Daina Karklina[1], Dalija Seglina[2]***
*[1]Latvia University of Agriculture*
*[2]Latvia State Institute of Fruit Growing*
*rita.riekstina@llu.lv; dalija.seglina@lvai.lv*

## Abstract

Aroma forming volatiles are important components of fermented beverages. The aim of current research is to evaluate the influence of different yeast strains on the volatile compounds of fermented apple juice of the variety 'Lietuvas Pepins'. Apples were harvested in the Latvia State Institute of Fruit Growing. Apple variety 'Lietuvas Pepins' juice was fermented with four different commercial yeast strains - *Saccharomyces bayanis* yeasts 'EC-1118', 'Cider yeast', *Saccharomyces cerevisiae* yeasts '71B-1122' and 'K1V-1116'. Fermentation was performed in laboratories of Latvia University of Agriculture, Faculty of Food Technology. Volatile aroma compounds of apple juice, yeasts and fermented juice were determined. Extraction of aroma compounds was performed using solid phase microextraction (DVB/Car/PDMS fibre). Analysis of volatile aroma compounds was made using a Perkin Elmer Clarus 500 GC/MS. The data obtained in the present study shows the influence of the yeast strain on the final chemical and volatile composition. The main group of volatiles in juice was esters, whereas in fermented juices – alcohols. The highest percentage of esters was determined in juice fermented with 'Cider yeast' whereas the highest percentages of alcohols – in juice fermented with yeast K1V-1116 and also free terpenes, associated with the floral note. The 71B-1122 strain produced the highest amount of identified volatile compounds. The strains potentially producing a higher number of volatile compounds could contribute to a more complex aroma of the final product, due to their potential ability to utilize and transform numerous apple must precursors.
**Key words**: apple juice, yeast strains, fermentation, volatile compounds.

## Introduction

Fermented apple juice generally is regarded ascider. The quality of fermented drinks like cider is characteristic from presence of aroma compounds in product (Mangas et al., 1996), that are influenced by several factors, namely apple variety, yeast strains, fermentation conditions, the production process and fining treatments¯(Hidalgo et al., 2004; Martınez-Rodrıguez and Polo, 2003; Beech, 1993). Not all ciders are made from 'true' cider apples. Many modern ciders have a high proportion of dessert and culinary apple varieties (Lea, 1995). In this work, special attention is drawn to the use of culinary apples for cider production. The apple variety 'Lietuvas Pepins is one of the major commercially grown varieties in Latvia and is classified as autumn, early winter apple variety (stored until February), typically used for juice and wine production.

Cider flavour is composed by a wide range of compounds with different aromatic properties. Moreover, the main cider aroma holds a close relationship with the type and concentration of aromatic compounds derived from apples (varietal flavour), other compounds are produced by yeasts and bacteria during alcoholic and malolactic fermentation (fermentative flavour) and compounds that appear during the ageing process (post-fermentative flavour) (Rapp, 1987; Boulton et al., 1995) and it consists mainly of esters, higher alcohols, fatty acids, aldehydes, ketones, terpenes, lactones (Leguerinel et al., 1988; Leguerinel et al., 1989). The fermentation of apple must is a complex microbial reaction involving the sequential development of various strains of yeasts and bacteria (Duenas et al., 1994). Among these micro-organisms, yeasts are primarily responsible for alcoholic fermentation. The different yeast species developed during fermentation and their dynamics and frequency of appearance determine the taste and flavour characteristics of products (Cabranes et al., 1997; Beech and Davenport, 1970). Ethanol and glycerol are quantitatively the dominating alcohols, followed by higher alcohols and esters. The main ester produced during alcoholic fermentation is ethyl acetate, but other esters of fusel alcohols and medium chain fatty acids also appear. Two main species, *Saccharomyces cerevisiae* and *Saccharomyces bayanus*, are currently recognized among wine yeasts (Masneuf-Pomarède et al., 2007; Naumov et al., 2000). Different strains of *Saccharomyces cerevisiae* can produce significantly different flavour profiles when fermenting the same must. This is a consequence of both, the differential ability of wine yeast strains in releasing varied volatile compounds from grape precursors, as well as the differential capacity to synthesise new yeast-derived volatile compounds (Swiegers et al., 2006; Ugliano et al., 2006; Vilanova and Sieiro, 2006; Wondra and Boveric, 2001). *Saccharomyces cerevisiae* is the main species of yeast in wine making although many other yeasts may be present at the beginning of fermentation (Heard and Fleet, 1986).

Apples are the most cultivated fruit in Latvia, and fermented beverage production from culinary apples could be perspective. The aim of current research is to evaluate the influence of different yeast strains on the volatile compounds of fermented apple juice of the variety 'Lietuvas Pepins'.

## Materials and Methods

*Raw materials*

For analysis apples of the variety 'Lietuvas Pepins' ('LP') grown in the Latvia State Institute of Fruit Growing and harvested in October 2010 were used. Apple quality parameters were as follows: Streif index 0.18, starch index 8.6. Juice was obtained by the press Voran Basket Press 60K (voran Maschinen GmbH, Austria) and yield was 0.652 ± 0.004 L $kg^{-1}$. For conservation of juice Tannisol (Enartis, Italy) was added (concentration 10 g $L^{-1}$). Tannisol capsules consist of potassium metabisulphite (E 224) (9.5 g $L^{-1}$), ascorbic acid (0.3 g $L^{-1}$) and tannin (0.2 g $L^{-1}$). Sulphites have various permitted uses, their primary function is as a preservative or antioxidant to prevent or reduce spoilage (Fazio and Warner, 1990) and , they help to stabilize product colour and inhibit discolouration, thereby improving the appearance and flavour of many foods during preparation, storage and distribution (Adams, 1997). Quality parameters of the obtained apple juice are given in Table 1.

Table 1

**Quality parameters of 'Lietuvas Pepins' apple juice**

| Parameters | Amount in juice |
|---|---|
| pH | 3.22± 0.01 |
| Titratable acidity, g $L^{-1}$ | 8.31± 0.12 |
| Soluble solids, Brix % | 10.70±0.70 |
| Total sugars, g $L^{-1}$ | 100.12±2.85 |

*Fermentation conditions*

Fermentation was performed using four commercial yeasts - *Saccharomyces bayanis* yeast EC-1118 (Lalvin, Canada), *Saccharomyces bayanis* 'Cider yeast' (Youngs Home Brew., UK), *Saccharomyces cerevisiae* yeast 71B-1122 (Lalvin, Canada) and *Saccharomyces cerevisiae* yeast K1V-1116 (Lalvin, Canada). The EC-1118 strain is recommended for all types of wines, including sparkling, and cider. 'Cider yeast' is especially selected for its ability to produce exceptional crisp and refreshing ciders. 71B-1122 abilities are to produce isoamil acetate, reinforcing the aromatic profile of wine. According to the manufacturer, K1V-1116 under the right conditions of fermentations is one of the most floral ester (isoamyl acetate, hexyl acetate, phenyl ethyl acetate) producing yeasts. These esters bring fresh, floral aromas to neutral varieties or high yield grapes. Fermentation was carried out at 16 ± 1 °C for 28 days. The apple juice was fermented in a glass bottles (n=5) in the laboratories of Latvia University of Agriculture, Faculty of Food Technology. For analysis the average juice samples were combined from 5 bottles in equal proportions. All analysis of fermented drinks was performed immediately after 28 day fermentation. Chemical parameters of final fermented juices are presented in Table 2.

Table 2

**Fermentation conditions and fermented juice composition**

| Yeast variety | Abbreviation | Titratable acidity, g $L^{-1}$ | Soluble solids, g $L^{-1}$ | Alcohol, % vol. |
|---|---|---|---|---|
| EC-1118 | SB1 | 9.40±0.04 | 7.74±0.21 | 5.42±0.05 |
| Cider yeast | SB2 | 8.40±0.08 | 6.42±0.30 | 5.60±0.06 |
| 71B-1122 | SC3 | 8.02±0.06 | 5.16±0.14 | 5.91±0.03 |
| K1V-1116 | SC4 | 8.32±0.08 | 15.43±0.27 | 4.30±0.08 |

*Determination of volatile aroma compounds*

Volatiles from apple juice, activated yeasts and fermented drinks were extracted using solid phase microextraction (SPME). 5 g of sample were weighed in a 20 mL headspace vial and capped with a septum.

Adivinylbenzene/carboxen/polydimethylsiloxane (DVB /Car/PDMS) fiber (Supelco Inc., Bellefonte, PA, USA) was used for headspace SPME sampling. SPME parameters were: incubation time 30 min, extraction temperature 22 ± 2 °C, extraction duration 30 min, desorption 15 min, 250 °C. For the analysis of the SPME extracts, a Perkin Elmer Clarus 500 GC/MS and an Elite-Wax ETR (60 m × 0.25 mm i.d.; DF 0.25 μm) were used. Working conditions were the following: an injector 250 °C; transfer line to MSD 260 °C; oven temperature start 50 °C, hold 2 min, programmed from 50 to 100 °C at 5 °C $min^{-1}$ hold 5 min, and from 100 to 210 °C at 5 °C $min^{-1}$, hold 15 min; carrier gas (He) 1 mL $min^{-1}$; split ratio 2:1; ionization EI+; acquisition parameters in full scan mode: scanned m/z 50-300. Compounds were identified by comparison of their mass spectra with mass spectral libraries (Nist98), and by calculation of linear retention indexes and comparison with literature data. All analyses were performed in triplicate. As a quantitative measure, the share in the total GC peak area for each compound is given.

*Statistical analysis*

The differences in the volatile profiles during fermentation were analyzed using the analysis of variance (ANOVA) procedure of SPSS, Version 17.0. HSD Tukey's test was applied to compare the mean values of the volatile

compounds of different fermentation conditions. P-value at 0.05 was used to determine the significant differences in content of volatiles in fermented juice samples. Mean values with standard deviations are reported.

## Results and Discussions

Six volatile compounds with total peak area $4490.35\times10^5$ AU were detected in 'Lietuvas Pepins' apple juice (Tables 3). Each group of volatile compounds gives a typical odour characteristic to the apple juice. The main identified compound classes were esters forming 85% (total peak AU $3613.62\times10^5$) of total volatile compounds and alcohols 19.5% (total peak AU $876.73\times10^5$) (Fig. 1). Butyl acetate, hexyl acetate and 2-methylbutyl acetate were identified as characteristic volatile compounds of 'Lietuvas Pepins' apple juice (Table 3).

Table 3

**Volatile aroma compounds (AU $\times10^5\pm$ standard deviation) as measured by SPME-GC-MS in 'Lietuvas Pepins' apple juice and fermented juice**

| Compound | Juice 'Lietuvas Pepins' | SB1 | SB2 | SC3 | SC4 |
|---|---|---|---|---|---|
| ***Acids*** | | | | | |
| acetic acid | n.d. | 55.61 ±4.73$^{a}$ | 74.01±4.73$^{b}$ | 780.12±8.23$^{d}$ | 92.15±7.35$^{c}$ |
| octanoic acid | n.d. | 104.47±7.97 | 90.10±7.82 | n.d. | 49.64±2.91 |
| hexanoic acid | n.d. | 64.26±4.23 | n.d. | n.d. | 70.34±3.62 |
| *% from total volatile compounds* | n.d | *1.5* | *1.4* | *4.4* | *1.5* |
| ***Esters*** | | | | | |
| ethyl acetate | n.d. | 161.55±1182$^{ab}$ | 112.24±7.08$^{ab}$ | 110.18±95.45$^{a}$ | 240.38±18.55$^{b}$ |
| 3-methylbutyl acetate | n.d. | 314.32±744$^{a}$ | 312.02±20.80$^{a}$ | 659.24±13.44$^{c}$ | 486.43±22.44$^{b}$ |
| 2-methylbutyl acetate | 701.46±61.02 | 739.33±3852$^{b}$ | 798.23±60.73$^{b}$ | 1573.93±5.56$^{c}$ | 196.37±3.73$^{a}$ |
| butyl acetate | 1586.78±55.75 | n.d. | n.d. | n.d. | n.d. |
| hexyl acetate | 1086.51±94.12 | 1080.21±19.86$^{a}$ | 1136.93±77.48$^{a}$ | 1329.34±5.48$^{b}$ | 1495.67±107.77$^{b}$ |
| 2,2-dimethylpropyl butyrate | 238.87±23.16 | n.d | n.d. | n.d. | n.d. |
| octanoic acid ethyl ester | n.d. | 1107.61±19.15$^{b}$ | 1079.94±79.76$^{b}$ | 679.68±12.53$^{a}$ | 713.46±56.44$^{a}$ |
| decanoic acid ethyl ester | n.d. | 185.54±2.08$^{c}$ | 189.36±5.54$^{c}$ | 149.76±8.98$^{b}$ | 84.99±6.06$^{a}$ |
| hexanoic acid hexyl ester | n.d. | 1201.95±31.61$^{b}$ | 1260.47±110.40$^{b}$ | 1197.10±11.24$^{b}$ | 972.32±32.62$^{a}$ |
| *% from total volatile compounds* | n.d | *31.9* | *42.4* | *32.3* | *30.1* |
| ***Alcohols*** | | | | | |
| hex-2-en-1-ol | 368.55±7.61 | n.d. | n.d. | n.d. | n.d. |
| 2-hydroxy ethylhydrazine | n.d. | 5694.87±145.19$^{b}$ | 3561.01±238.47$^{a}$ | 6512.58±9.08$^{c}$ | 5107.14±396.29$^{b}$ |
| hexan-1-ol | 508.18±29.14 | 546.39±9.87$^{b}$ | 453.02±36.95$^{a}$ | 613.81±14.55$^{c}$ | 582.70±22.62$^{b}$ |
| 3-methylbutan-1-ol | n.d. | 1616.17±51.02$^{a}$ | 1262.10±90.17$^{a}$ | 1744.71±10.06$^{b}$ | 2665.78±71.31$^{c}$ |
| heptadecan-8-ol | n.d. | 1057.92±13.29$^{b}$ | 990.62±96.55$^{ab}$ | 850.65±6.65$^{a}$ | 883.02±79.33$^{a}$ |
| phenylethyl alcohol | n.d. | 233.85±11.04$^{a}$ | 221.67±11.46 | 385.08±14.55$^{c}$ | 303.95±9.14$^{b}$ |
| *% from total volatile compounds* | n.d | *61.0* | *56.2* | *57.3* | *68.4* |
| ***Terpenes*** | | | | | |
| limonene | n.d. | 662.87±53.23 | n.d. | 1054.76±14.77 | n.d. |
| γ-terpinene | n.d. | 180.46±14.59 | n.d. | n.d. | n.d. |
| *% from total volatile compounds* | n.d | *5.6* | n.d | *6.0* | n.d |

n.d. – not detected

Different letters in the same row indicate statistically significant differences (Tukey's test, $p<0.05$).

The esters and alcohols which are the products of fatty acid metabolism were the major groups in the apple juice, respectively esters, associated with 'fruity' attributes of fruit flavour, accounted up to 80 - 98% (Lopez et al., 1998).

Hex-2-en-1-ol and hexan-1-ol found in 'Lietuvas Pepins' apple juices were also reported in apples 'Pink Lady' as one of the characteristic aroma compounds (Elss et al., 2006). In literature, butanol was found as the most abundant alcohol in the apple juice (Karlsen et al., 1999), but it was not identified in 'Lietuvas Pepins' juice. Also 3- methylbutan-1-ol was not identified in 'Lietuvas pepins' apple juice, but other authors (Nikfardjam and Maier, 2011) reported that it plays the major role in the apple juice quality. This alcohol is not genuine constituent of the apple fruit, but it is inevitably formed during apple juice production, probably through transamination and decarboxylation of the amino acids leucine and isoleucine (Hey et al., 2007).

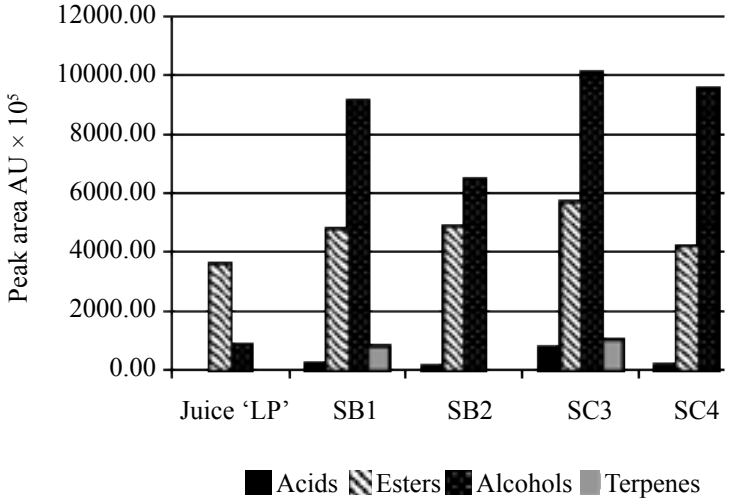



Figure 1. Changes in the peak area (AU × $10^5$) of the main volatile compound chemical groups in apple 'Lietuvas Pepins' juice and fermented juice.

Yeast metabolism makes an important contribution to the flavour of the fermented juices. In addition to the reduction of sugars (glucose and fructose) to ethanol and carbon dioxide during alcoholic fermentation, the use of wine yeasts produces a number of intermediate products like acetaldehyde and several organic acids. Ten different volatile compounds were detected in yeasts representing two main volatile compound classes - alcohols 69.3 - 97.3% and acids 2.5 - 29.4% (Table 4). Additionally, ketone 3-hydroxybuta -2-none were identified in the headspace of yeasts K1V-1116 and ester pentafluoropropionic acid hexyl ester in yeasts K1V-1116 and 71B-1122.

A total of 17 volatile compounds were detected in fermented juice of apples 'Lietuvas Pepins' (Table 3). Three sources of aroma compounds of fermented juices - apple juice, yeast, and yeast metabolites - were analysed. It was not possible to identify such esters as butyl acetate, 2,2-dimethylpropyl butyrate and alcohol hex-2-en-1-ol found in apple juice and in fermented apple juices. In all fermented samples it is possible to identify 3 compounds previously detected in apples, namely, 2-methylbutyl acetate, hexyl acetate and hexan-1-ol. In the headspace of fermented drinks four yeast volatile compounds were identified: acetic acid, 2-hydroxyethylhydrazine, phenylethyl alcohol and 3-methylbutyl acetate. Literature data showed that different strains produce 3-methyl-1-butanol and it is the major higher alcohol in wine (Romano et al., 2008; Garde-Cerdan and Ancżn-Azpilicueta, 2007)

The main groups of compounds formed during the fermentation are alcohols and esters. Literature data showed that the main groups of compounds that form the fermentation bouquet are the acids, alcohols and esters and, to a lesser extent, aldehydes and ketones (Lambrechts and Pretorius, 2000). In analysed fermented juice samples ketones and aldehydes were not identified. Alcohols were the most abundant group in the tested fermented juice and represented more than 56 - 68% of total volatile compounds. Main alcohols were 2-hydroxyethylhydrazine, hexan-1-ol and phenylethyl alcohol. Juices inoculated with *S.cerevisiae* show higher content of alcohols, and the same tendency was observed for wines (Mateo et al., 2001). In fermented products, esters can be classified into two groups, those formed enzymatically and those formed during ageing, by chemical esterification between alcohol and acids at low pH (Margalit, 1997). Esters represented more 30 - 42% of aroma compounds. The main esters found in fermented samples are hexyl acetate, ethyl octanoate, hexyl hexanoate, 2-methylbutyl acetate. The $C_4$-$C_{10}$ ethyl esters of organic acids, ethyl esters of straight chain fatty acids and acetates of higher alcohols are largely responsible for the fruity aroma of wine (Ebeler, 2001). The fatty acids ethyl esters (ethyl hexanoate, octanoate and decanoate) and fusel alcohols are also important aroma compounds in white wines (Mateo et al., 2001). Ethyl acetate that intensifies the

acetified cider perception (Campo et al., 2008) was identified in all fermented juice samples, with the highest peak area in the sample SC4. Three acids - acetic acid, octanoic acid and hexanoic acid- were detected in fermented samples. Production of acetic acid is frequently accompanied by a greater production of ethyl acetate (Campo et al., 2008), but in our experiment such a tendency was not observed. Hexanoic acid was found in two samples (SB1 and SC4) in small concentrations. Synthesis of this compound mainly is associated with spontaneous fermentation, and its concentration decreases when concentration of inoculated yeast increases (Mateo et al., 2001).

Table 4

**Volatile aroma compounds (AU ×10$^5$ ± standard deviation) as measured by SPME-GC-MS in yeasts**

| Compound | EC-1118 | Cider yeast | 71B-1122 | K1V-1116 |
|---|---|---|---|---|
| ***Acids*** | | | | |
| acetic acid | 151.71±1.78 | 61.94±4.03 | 10.86±0.86 | 80.04±5.27 |
| 2-methylpropanoic acid | 51.63±1.39 | 38.77±2.82 | 5.63±0.23 | 90.29±6.03 |
| 4-methylpentanoic acid | 49.46±0.78 | 42.60±2.54 | 3.61±0.01 | 116.75±6.09 |
| butanoic acid | n.d. | 5.35±0.40 | n.d. | 8.79±0.40 |
| *% from total volatile compounds* | *27.4* | *17.9* | *2.5* | *29.4* |
| ***Esters*** | | | | |
| pentafluoropropionic acid hexyl ester | n.d. | n.d. | 1.33±0.13 | 6.29±0.60 |
| *% from total volatile compounds* | n.d. | n.d. | *0.2* | *0.6* |
| ***Ketones*** | | | | |
| 3-hydroxybuta-2-none | n.d. | n.d. | n.d. | 6.37±0.25 |
| *% from total volatile compounds* | n.d | n.d | n.d | *0.6* |
| ***Alcohols*** | | | | |
| 2-hydroxyethylhydrazine/ 4-penten-2-ol | 138.75±2.32 | 229.49±0.73 | 259.05±10.92 | 285.96±8.96 |
| 2-methylpropan-1-ol | 41.12±0.56 | 22.77±0.07 | 46.80±0.45 | 27.33±0.21 |
| 2-ethylhexan-1-ol | 50.70±1.28 | n.d. | n.d. | n.d. |
| 3-methylbutan-1-ol | 381.51±11.35 | 411.26±4.81 | 445.03±3.76 | 335.58±16.61 |
| phenylethyl alcohol | 58.73±0.72 | 19.03±1.78 | 32.70±3.08 | 47.82±4.28 |
| *% from total volatile compounds* | *72.6* | *82.1* | *97.3* | *69.3* |

n.d. – not detected

In general, peak area of total and individual compounds in fermented juices were influenced by used yeast strain, and it is not possible to distinguish differences between *S. cerevisiae* and *S.bayanus* yeasts. In wine *S. cerevisiae* strain represents one of the primary parameters affecting wine fermentative volatile composition, the strain-specific aromatic potentiality becomes a selective tool in the choice of the starter that will drive the alcoholic fermentation (Mauriello et al., 2009).

## Conclusions

The data obtained in the present study showed the effect of yeast strain on the final chemical and volatile composition. Yeast strains represent one of the primary parameters affecting wine fermentative volatile composition; the strain-specific aromatic potentiality becomes a selective tool in the choice of the starter that will drive the alcoholic fermentation. The main group of volatiles in juice was esters, whereas in fermented juice – alcohols. The highest percentage of esters was determined in juice fermented with 'Cider yeast' whereas the highest percentages of alcohols in juice fermented with yeast K1V-1116. The 71B-1122 strain produced the highest amount of identified volatile compounds and also free terpenes, associated with the floral note. The 71B-1122 strain also produced the highest amounts of alcohols, acetates and esters. The strains potentially producing a higher number of volatile compounds could contribute to a more complex aroma of the final product, due to their potential capability to utilize and transform numerous apple must precursors.

**Acknowledgement**

The research has been done within the National Research Programme "Sustainable use of local resources (earth, food, and transport) – new products and technologies (Nat Res)" (2010 - 2013) Project no. 3. „Sustainable use of local agricultural resources for development of high nutritive value food products (Food)"

**References**

1. Adams J.B. (1997) Food additive –additive interactions involving sulphur dioxide and ascorbic and nitrous acids. *Food Chemistry,* 59, pp. 401-409.
2. Beech F.W., Davenport R.R. (1970) The role of yeast in cider making. In: Rose A.H., Harrison I.S. (eds), *Yeast,* Academic Press, London UK, pp. 73-146.
3. Beech F.W. (1993) Cider making and cider research. *Journal of the Science of Food and Agriculture*, 6, pp. 259-270.
4. Boulton R.B., Singleton V.L., Bisson L.F., Kunkee R.E. (1995) *Principles and Practices of Winemaking*, Chapman & Hall, New York, 455 p.
5. Cabranes C., Mangas J.J., Blanco D. (1997) Selection and biochemical characterisation of *Saccharomyces cerevisiae* and *Kloeckera apiculata* strains isolated from Spanish cider. *Journal of Institute of Brewing*, 103, pp. 165-169.
6. Campo G., Santos J.I., Munduate A. (2008) Differentiation of Basque cider apple juices from different cultivars by means of chemometric techniques. *Food Control*, 6, pp. 549-555.
7. Duenas M., Irastorka A., Fernandez K., Bilbao A., Huerta A. (1994) Microbial population and malolactic fermentation of apple cider using traditional and modified methods. *Journal of Food Science*, 59, pp. 1060-1064.
8. Ebeler S.E. (2001) Analytical chemistry, unlocking the secrets of wine flavour. *Food Review International*, 17, pp. 45-64.
9. Elss S., Preston C., Appel M., Heckel F., Schreier P. (2006) Influence of technological processing on apple aroma analysed by high resolution gas chromatography–mass spectrometry andon-line gas chromatography-combustion/pyrolysis-isotoperatio mass spectrometry. *Food Chemistry*, 2, pp. 269-276.
10. Fazio T. and Warner C.R. (1990) A review of sulphites in foods: Analytical methodology and reported finding. *Food Additives and Contaminants*, 7, pp. 433-454.
11. Garde-Cerdan T., Ancżn-Azpilicueta C. (2007) Effect of $SO_2$ on the formation and evolution of volatile compounds in wines. *Food Control*, 18, pp. 1501-1506.
12. Heard G.M., Fleet G.H. (1986) Occurrence and growth of yeast species during the fermentation of some Australian wines. *Food Technology in Australia*, 38(1), pp. 22-25.
13. Hey M., Kurbel P., Hopf I., Dietrich H. (2007) Untersuchung sortenreiner Apfelsaftaromen. (Investigation varietal aromas of apple juice). *Flüssiges Obst*, 2, S. 62-67. (in German).
14. Hidalgo P., Pueyo E., Pozo-Bayo´n M.A., Martınez-Rodrıguez A.J., Martın- Alvarez P., Polo M.C. (2004) Sensory and analytical study of rose´sparkling wines manufactured by second fermentation in the bottle. *Journal of Agricultural and Food Chemistry*, 52, pp. 6640-6645.
15. Karlsen A.M., Aaby K., Sivertsen H., Baardseth P., Ellekjar M.R. (1999) Instrumental and sensory analysis of fresh Norwegian and imported apples. *Food Quality and Preference*, 10, pp. 305-314.
16. Lambrechts M.G., Pretorius I.S. (2000) Yeast and its importance to wine aroma. *South African Journal of Enology and Viticulture*, 21, pp. 97-129.
17. Lea A.G.H. (1995) (edited by A.G.H. Lea and J.R. Piggott). Cider making. In: *Fermented Beverage Production*. Chapman & Hall, London, pp. 66-96.
18. Leguerinel I., Cleret J.J., Bourgeois C., Mafart P. (1988) Yeast strain and the formation of flavor components in cider. *Journal of the Institute of Brewing*, 96, pp. 391-395.
19. Leguerinel I., Mafart P., Cleret J.J., Bourgeois C. (1989) Yeast strain and kinetic aspects of the formation of flavor components in cider. *Journal of the Institute of Brewing*, 95, pp. 405-409.
20. Lopez M.L., Lavilla T., Recasens I., Riba M., Vendrell M. (1998) Influence of different oxygen and carbon dioxide concentrations during storage on production of volatile compounds by Starking Delicious apples. *Journal of Agriculture and Food Chemistry*, 46, pp. 634-643.
21. Mangas J.J., Gonzalez M.P., Rodrıguez R., Blanco D. (1996) Solid phase extraction and determination of trace aroma and flavour components in cider by GC-MS. *Chromatographia*, 42, pp. 101-105.
22. Margalit Y. (1997) *Concepts in wine chemistry*. The Wine Appreciation Guild, San Francisco, 346 p.
23. Martınez-Rodrıguez A.J., Polo M.C. (2003) Effect of the addition of bentonite to the tirage solution on the nitrogen composition and sensory quality of sparkling wines. *Food Chemistry*, 81, pp. 383-388.
24. Masneuf-Pomarède I., Jeune C. Le Durrens P., Lollier M., Aigle M., Dubourdieu D. (2007) Molecular typing of wine yeast strains *Saccharomyces bayanus* var. *uvarum* using microsatellite markers. *Systematic and Applied Microbiology*, 30, pp. 75-82.
25. Mateo J.J., Jiménez A., Pastor A., Huerta T. (2001) Yeast starter cultures affecting wine fermentation and volatiles. *Food Research International*, 34, pp. 307-314.
26. Mauriello G., Capece A., D'Auria M., Garde-Cerdán T., Romano P. (2009) SPME–GC method as a tool to differentiate VOC profiles in Saccharomyces cerevisiae wine yeasts. *Food Microbiology*, 26, pp. 246-252.
27. Naumov G.I., Masneuf I., Naumova E.S., Aigle M.,

Dubordieu D. (2000) Association of *S. bayanus* var. uvarum with some French wines: genetic analysis of yeast populations. *Research in Microbiology*, 151, pp. 683-691.
28. Nikfardjam M.P., Maier D. (2011) Development of a headspace trap HRGC/MS method for the assessment of the relevance of certain aroma compounds on the sensorial characteristics of commercial apple juice. *Food Chemistry*, 126, pp. 1926-1933.
29. Rapp A. (1987) Methods for the chemical analysis of the aroma in grapes and wines: Current experiences and future outlook. In: *Procceding of the International Symposium on the Aromatic Substances in Grapes and Wines,* S. Michele all'Adige, Italy, pp. 243-247.
30. Romano P., Capece A., Serafino V., Romaniello R., Poeta C. (2008) Biodiversity of wild strains of *Saccharomyces cerevisiae* as tool to complement and optimize wine quality. *World Journal of Microbiology and Biotechnology*, 24, pp. 1797-1802.
31. Swiegers J.H., Francis I.L., Herderich M.J., Pretorius I.S. (2006) Meeting consumer expectations through management in vineyard and winery: the choice of yeast for fermentation offers great potential to adjust the aroma of Sauvignon Blanc wine. *Australian and New Zealand Wine Industry Journal*, 21, pp. 34-42.
32. Ugliano M., Bartowsky E.J., McCarthy J., Moio L., Henschke P.A. (2006) Hydrolysis and transformation of grape glycosidically bound volatile compounds during fermentation with three *Saccharomyces* yeast strains. *Journal of Agriculture and Food Chemistry*, 54, pp. 6322-6331.
33. Vilanova M., Sieiro C. (2006) Contribution by *Saccharomyces cerevisiae* yeast to fermentative flavour compounds in wines from cv. Albariño. *Journal of Industrial Microbiology and Biotechnology*, 33, pp. 929-933.
34. Wondra M., Boveric M. (2001) Analyses of aroma components of Chardonnay wine fermented by different yeast strains. *Food Technology and Biotechnology*, 39, pp. 141-148.

# THE COMPOSITION OF SUGARS AND SUGAR-ACID RATIO OF HIGHBUSH BLUEBERRY VARIETIES GROWN IN LATVIA

***Elga Šnē, Solvita Kampuse, Elga Berņa***
*Latvia University of Agriculture*
*elga.sne@gmail.com*

**Abstract**

Highbush blueberries are thought to provide protection against oxidative damage of free radicals and contribute to positive health benefits. They have been studied little in Latvia; therefore, the aim of study was to evaluate the soluble solids content, titratable acidity and sugar content of berries harvested in 2010.

Seven varieties of cultivated highbush blueberries (*Vaccinium corymbosum* L.) grown in Latvia were analysed.

Analyses were done for frozen berries of varieties 'Bluecrop', 'Chandler', 'Chippewa', 'Duke', 'Northblue', 'Patriot' and 'Polaris'. All experiments were performed at the Latvia University of Agriculture, Faculty of Food Technology.

The content of titratable acids (TA) was detected using standard method ISO 750:1998 by titration with 0.1N NaOH. Soluble solids content (SSC) was analysed using standard method ISO 2173:2003 by hand refractometer. Sugars (fructose, glucose and sucrose) were analysed by high-pressure liquid chromatography HPLC analysis.

Results showed that there are significant differences between varieties ($p<0.05$) for all parameters. TA content varied from 0.4 – 1.2%, SSC - 8.5 – 10.8 °Brix and total sugars from 7.6 to 9.3 g 100 $g^{-1}$. The highest ratio between sugars and titratable acids was for 'Polaris' while the lowest – 'Chandler' and 'Bluecrop'.

**Key words**: blueberries, sugars, titratable acids, soluble solids.

## Introduction

Blueberries (*Vaccinium corymbosum* L.) are known as berries having great potential health benefits – reduce the risk of cancer and heart diseases, prevent ageing related illnesses and problems, improve brain function, improve action of digestive system and have positive effect to immune system overall (Howell, 2009; Freitas et al., 2008). It is also known that blueberry's 'cousin' - bilberry is popular because of its ability to preserve the vision, enhance it at night time, reduce eyestrain and improve eye health in general, therefore these qualities is attributed to blueberries as well (Girard and Sinha, 2006).

Blueberries are rich of antioxidants (flavonoids, phenolic acids, and vitamin C), minerals (manganese, copper, potassium) and other valuable compounds (carotenoids, fiber and vitamin E). They are native in North America and growing plantations in USA reaching approximately 20.8 thousands ha in 2007 (Pormale and Osvalde, 2010). In Latvia this number is gradually growing, and in 2008 it reached 222 ha while harvest is collected from 11 ha (Skrīvele et al., 2008).

Chemical composition of berries is affected not only by climatic conditions, cultivating, harvesting and storing practise, but also by genotype and maturity level (Belitz et al., 2009; Sinelli et al., 2008). During the ripening physiological, biochemical and structural changes occur (Sinelli et al., 2008) and parameters like soluble solids content and titratable acids are used as quality assessments (Yoon et al., 2006).

The soluble solids content consists mainly of sugars, polysaccharides and organic acids (Belitz et al., 2009). When berries are mature for 100%, they contain the highest soluble solids content meanwhile amount of titratable acids is low (Wang et al., 2009).

Main sugars in blueberries are fructose and glucose (Girard and Sinha, 2006), and small amount of sucrose. Low level of sucrose could be explained by hydrolysis that happens after this sugar translocation from leaves to the berries (Wang et al., 2009). During the ripening process the level of sucrose is also decreasing which is replaced by growing amount of simple sugars like fructose and glucose.

Polysaccharide starch is more found in immature berries. Its content is decreasing as a result of hydrolysis during ripening, and amount of fructose, glucose and sucrose is rising (Belitz et al., 2009).

Organic acids in berries are distributed unequally – in skin and seeds their content is smaller compared to the pulp. During the ripening and storage amount of acids is decreasing due to synthesis of sugars (Hohn et al., 2005) and formation of polyphenols carbon structure that enhances antioxidant activity of berries (Wang et al., 2009). The main acids in blueberries are citric acid and malic acid (Belitz et al., 2009). Organic acids provide vitamin C and anthocyanins stability (Wang et al., 2009) and together with sugars ensure a taste and aroma development (Belitz et al., 2009; Wang et al., 2009; Zagory and Kader, 1989).

The ratio between sugars and titratable acids is considered to contribute berry flavour, and estimate the best time of harvesting (Wang et al., 2009; Kafkas et al., 2006). And flavour provided parameters the best predict consumer preference of blueberries in sensory evaluation (Saftner et al., 2008). Optimum is when berries contain high sugar and high acid content which together provide a good flavour. Meanwhile high acid content and low sugar content make tart taste, in opposite case – high sugar and low acid content

shows delicate taste but when both of constituents are low, the fruit is tasteless (Wang et al., 2009).

Highbush blueberries due to their dormant health benefits have been studied widely for last decades, while in Latvia detailed research on these berries started a few years ago. Accordingly, there is little data on chemical composition of highbush blueberry varieties grown in this region. Therefore, the purpose of the present study was to evaluate the soluble solids content, titratable acidity and sugar content of berries harvested in 2010.

## Materials and Methods

*Plant material*

The research object was berries from seven different varieties of highbush blueberries – 'Bluecrop', 'Chandler', 'Northblue', 'Polaris', 'Duke', 'Chippewa', 'Patriot'- collected in August 2010. All varieties were grown in the same field under same conditions. Berries were frozen and stored for 4 months at temperature - 20 ± 2 °C. Before analyses blueberry samples were prepared by homogenisation of frozen berries in a manual blender (Philips) for 1 minute.

Soluble solids content (SSC)

SSC (°Brix) was detected by standard method (ISO 2173:2003) using a hand refractometer (Krüss) squeezing sample from blueberry puree through the two layers of cheesecloth.

Titratable acids (TA)

TA (%) of berries was analysed by standard method ISO 750:1998. Where 25 g of sample doused with 150 mL of water, heated 30 min at 80 °C in a water bath, cooled till ambient temperature, 100 mL of water added and filtered. To the 25 mL of filtrate 25 mL of water added, 1 – 2 drops of 1% phenolphthalein was used as an indicator and titrated with 0.1M NaOH till colour changes were stable for 30 seconds. Results were expressed as citric acid.

Analyses of sugars

The content and amount of sugars were detected using high-pressure liquid chromatography (HPLC) analysis. To 5 g of sample 20 mL of water was added into a 50 mL volumetric flask. Heated for 20 min at 60 °C in a water bath and cooled to ambient temperature (20 ± 2 °C).

Then, 1 mL of Carrez I and 1mL of Carrez II solutions were added and shaken. A volumetric flask was filled up with water till the mark and shaken well. First, solution was filtered through the paper filter. The obtained extract was filtered through a membrane filter with pore size of 0.2 μm. Second, extract was placed in a vial and tested by HPLC Prominence (Shimadzu, Japan) equipped with Sypelcosil™ LC-$NH_2$ column (250 x 4.6 mm, particle size – 5 μm) and autosampler SIL-20A. Sugars were detected with a refractive index detector RID-10A (Shimadzu); acquired data were processed using Shimadzu LabSolutions software (LCsolution Version 1.21 SP1). Acetonitrile: water (80:20 v/v) was used as eluent while column temperature was held at 30 °C. The flow rate was 1.0 mL $min^{-1}$. Injection volume of samples was 10 μL. Calibration curve was acquired after two repeated HPLC runs of seven standard solutions of reference compounds (Table 1).

Table 1

**Concentration ranges and calibration equations of reference compounds used for the HPLC analysis**

| HPLC parameters | Fructose | Glucose | Sucrose |
|---|---|---|---|
| Concentration range, g $L^{-1}$ | 0.11-22.67 | 0.10-20.31 | 0.11-20.15 |
| Calibration equation | $y=1.21918\cdot10^{-5}\cdot x+0$ | $y=1.19809\cdot10^{-5}\cdot x+0$ | $y=1.215\cdot10^{-5}\cdot x+0$ |
| $R^2$ | 0.9999894 | 0.9999794 | 0.9999321 |

Amount and content of sugars (fructose, glucose and sucrose) were analysed using peak area measurement and calculated by formula (1):

$$W = \frac{C \times V}{m} \times 100 \quad (1)$$

where W – sugar content, g 100 $g^{-1}$;

C – sugar concentration range from HPLC graph, g $L^{-1}$;

V – volume of extract, L;

m – sample weight, g.

Total sugar content was detected by sum of glucose, fructose and sucrose.

*Statistical analyses*

Data was processed by SPSS software version 11.0. Data was analysed using descriptive statistics and processed by one-way analysis of variance (Anova) where factor was blueberry cultivar (7) and dependent parameters- performed analyses (5). Duncan's criterion was used for individual variety characterization by a parameter. Statistical differences were considered significant at $p<0.05$.

## Results and Discussion

The soluble solids content (SSC) among studied blueberry varieties differed significantly ($p<0.05$). SSC ranged from 8.5 °Brix to 10.8 °Brix (Table 2). In research of N. Sinelli et al. (2008) soluble solids content varied even more - from 5.0 °Brix to 12.35 °Brix. K. Skupień (2006) reported SSC of cultivar 'Bluecrop' in different years. Results showed that soluble solids content is higher in Poland grown berries (12.55 °Brix, 13.55 °Brix and 13.85 °Brix in 2001, 2002 and 2003 respectively) compared to

Latvia grown- 9.5 °Brix.

Low content of soluble solids could be explained by climatic conditions. Summer 2010 was quite hot, reaching temperature about 30 °C at day time and more, what is believed to affect photosynthesis in plants and inhibit formation of soluble solids according to Poudel et al. (2009).

Table 2

**Soluble solids content and titratable acids in highbush blueberry varieties***

| Variety | Soluble solids content, °Brix | Titratable acids, % |
|---|---|---|
| 'Bluecrop' | 9.5 ± 0.05$^{c}$ | 1.0 ± 0.04$^{b}$ |
| 'Chandler' | 10.8 ± 0.05$^{a}$ | 1.2 ± 0.04$^{a}$ |
| 'Chippewa' | 9.1 ± 0.04$^{e}$ | 0.7 ± 0.04$^{d}$ |
| 'Duke' | 8.5 ± 0.08$^{g}$ | 0.5 ± 0.04$^{f}$ |
| 'Northblue' | 9.4 ± 0.05$^{d}$ | 0.8 ± 0.00$^{c}$ |
| 'Patriot' | 9.0 ± 0.07$^{f}$ | 0.6 ± 0.04$^{e}$ |
| 'Polaris' | 10.1 ± 0.05$^{b}$ | 0.4 ± 0.00$^{g}$ |

* – Data expressed as a mean ± standard deviation; n=6 (soluble solids content), n=5 (titratable acids)

a, b, c, d, e, f, g, – Values, marked with the same letter in a column, are not significantly different at p<0.05

*There were significant differences (p<0.05) in titratable acids amount among seven studied varieties* (Table 2). TA was 3 times higher in 'Chandler' (1.2%) compared to 'Polaris' (0.4%). While mean value of titratable acid content among the studied highbush blueberry varieties was 0.8% and it is the same as K.K. Girard and N. Sinha (2006) described in their study. In Poland, cultivated blueberries titratable acid content varied from 0.27 to 1.13% during different years (Skupień, 2006). The same author examinated 'Bluecrop' TA content at three harvest seasons – the years 2001, 2002 and 2003 – and obtained quite diverse results: 1.13%, 0.54% and 0.73%, accordingly.

Table 3

**Sugar content in highbush blueberry varieties***

| Variety | Fructose, g 100 g$^{-1}$ | Glucose, g 100 g$^{-1}$ | Total sugars, g 100 g$^{-1}$ |
|---|---|---|---|
| 'Bluecrop' | 3.9 ± 0.53$^{b}$ | 4.0 ± 0.39$^{bc}$ | 7.9 ± 0.91$^{bc}$ |
| 'Chandler' | 4.7 ± 0.24$^{a}$ | 4.6 ± 0.18$^{a}$ | 9.3 ± 0.42$^{a}$ |
| 'Chippewa' | 3.9 ± 0.09$^{b}$ | 3.7 ± 0.06$^{c}$ | 7.6 ± 0.15$^{c}$ |
| 'Duke' | 4.0 ± 0.19$^{b}$ | 3.9 ± 0.26$^{c}$ | 7.9 ± 0.45$^{bc}$ |
| 'Northblue' | 4.3 ± 0.21$^{ab}$ | 4.3 ± 0.18$^{ab}$ | 8.6 ± 0.38$^{ab}$ |
| 'Patriot' | 3.9 ± 0.08$^{b}$ | 3.7 ± 0.02$^{c}$ | 7.6 ± 0.09$^{c}$ |
| 'Polaris' | 4.6 ± 0.19$^{a}$ | 4.6 ± 0.20$^{a}$ | 9.2 ± 0.39$^{a}$ |

* – Data expressed as a mean ± standard deviation; n=3

ab, c – Values, marked with the same letter in column, are not significantly different at p<0.05

Blueberries could be used for juice and jam processing, for bakery, dairy and convenience foods, but it is in the best for fresh consumption as a dessert fruit. Factor which determines exactly the quality of the berries is sugar accumulation, especially fructose (Kafkas et al., 2006). It has higher sweetness level compared to glucose and sucrose, therefore more acceptable to consumers who mostly prefer sweet taste (Wang et al., 2009).

One-way Anova showed that for fructose, glucose and total sugar level in berries (Table 3) there are significant differences between samples (p<0.05). The fructose content varied from 3.9 g 100 g$^{-1}$ ('Bluecrop', 'Chippewa', 'Patriot') to 4.7 g 100 g$^{-1}$ ('Chandler') while mean content of fructose among studied blueberry varieties was 4.2 g 100 g$^{-1}$. According to United States Department of Agriculture (USDA..., 2010) database, fresh blueberries contain 4.97 g 100 g$^{-1}$ of fructose. Much higher fructose content in blueberry samples detected Y.S.Wang et al. (2008) exploring organically and conventionally cultivated berries in the United States of America. The content was approximately 2 times higher – 9.71 g 100 g$^{-1}$ in organically and 7.93 g 100 g$^{-1}$ in conventionally grown berries comparing to studied berries.

Glucose content in the present study differed from

3.7 g 100 $g^{-1}$ ('Chippewa', 'Patriot') to 4.6 g 100 $g^{-1}$ ('Chandler', 'Polaris'). Mean value was 4.1 g 100 $g^{-1}$ which is lower compared to the United States Department of Agriculture (USDA ..., 2010) determined 4.88 g 100 $g^{-1}$. Data are similar that Y.S. Wang et al. (2008) obtained: 4.55 g 100 $g^{-1}$ of glucose for organically and 2.97 g 100 $g^{-1}$ of glucose for conventionally grown highbush blueberries.

Sucrose was not detected in the analysed samples (HPLC data showed less than 0.01 g 100 $g^{-1}$). E. Kafkas et al. (2006) in their study of sugar content in blackberry varieties detected very small amount of sucrose in berry samples and explained that by ripening process and sucrose transformations to invert sugars.

According to US Highbush blueberry council (2011) main sugars in blueberries are fructose (49%), glucose (48%) and sucrose (3%); therefore, total sugar content is expressed as a sum of these sugars. The highest total sugar content was detected in 'Chandler' – 9.3 g 100 $g^{-1}$ but the lowest one in 'Patriot' and 'Chippewa'- both contained 7.6 g 100 $g^{-1}$. USDA (2010) reported that total sugar content of frozen berries was 8.45 g 100 $g^{-1}$ which is close to mean value of total sugars in varieties examined in this study – 8.3 g 100 $g^{-1}$.

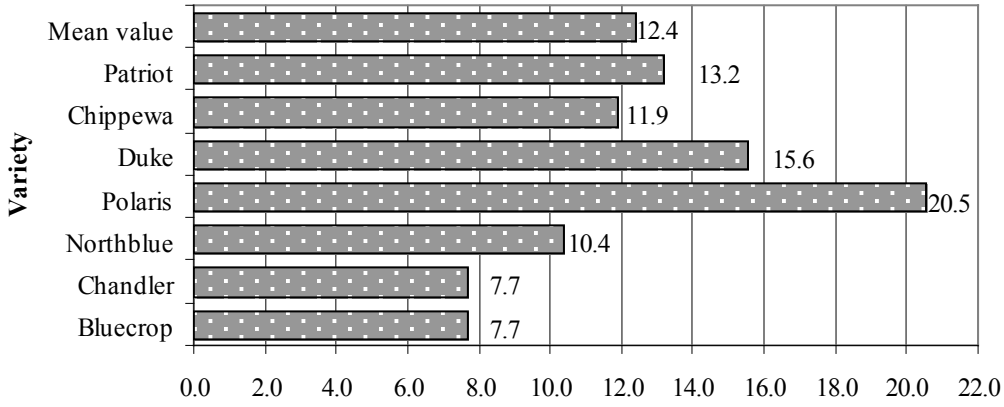



Figure 1. Sugar and titratable acid ratio (S/TA).

The best harvesting time and flavour characteristics in fruit industry frequently is explained by sugar or soluble solids content ratio to titratable acid- S/TA or SSC/TA respectively. Both ratios could be used to describe berry parameters since largest part of soluble solids is sugars. This study showed large differences in S/TA ratio among varieties (Figure 1). The lowest ratio was detected in 'Chandler' and 'Bluecrop' – 7.7 due to the high content of acids (Table 2). Variety containing more sugars and less acid had the highest S/TA ratio – 'Polaris' (20.5). Sugar and acid ratio in highbush blueberries during maturing have been reported: 3.8 for unripe, 9.5 for ripe and 25.8 for overripe berries (deMan, 1999). There are still no scientific data about the best values of S/TA ratio in highbush blueberries, while it is stated that SSC/TA should be in a range from 10 to 33 (Saftner et al., 2008). However, no instrumental measurement can adequately predict consumer acceptance and chemical analyses should be carried on together with sensory evaluation.

Although in this study frozen berries were used as a research object, it is believed that berry freezing and preserving for 3 months has not affected chemical constituents significantly. S. Garcia et al. (2011) reported that in grapes sugar concentration and soluble solids content remained unchanged after 3 months storage in frozen state.

Similar results of raspberries, black, red and white currants S. Kampuse (2006) notified, where soluble solids content after freezing and frozen storage was significantly changed only in black currant berries while acid content was constant.

## Conclusions

Results obtained in this study showed that there are significant differences in the soluble solids content, titratable acids and sugar content between different highbush blueberry berry varieties grown in Latvia in 2010.

The highest content of the soluble solids was in varieties 'Chandler' and 'Polaris' – 10.8 °Brix and 10.1 °Brix, accordingly.

The titratable acids of highbush blueberry varieties differed even 3 times (0.4% – 1.2%).

The main sugars in blueberries were fructose (3.9 – 4.6 g 100 $g^{-1}$) and glucose (3.7 – 4.6 g 100 $g^{-1}$) while sucrose was not detected.

Sugar and titratable acid ratio also varied significantly: the highest ratio was observed in 'Polaris' (20.5) while the lowest ratio (7.7) was in 'Chandler' and 'Bluecrop'.

## Acknowledgements

The authors thank the farm 'Strēlnieki' for blueberry samples, and Customs laboratory for support to perform analyses.

This publication has been prepared within the framework of the ESF Project „Formation of the research group in food science", Contract No. 2009/0232/1DP/1.1.1.2.0/09/APIA/VIAA/122".

**References**

1. Belitz H.D., Grosch W. and Schieberle P. (2009) *Food Chemistry. 4th revised and extended edition*, Springer - Verlag, Berlin, Heidelberg, pp. 1070.
2. deMan J.M. (1999) *Principles of Food Chemistry, 3rd edition*, Aspen Publishers, Maryland, pp. 520.
3. Freitas P.S., de Andrade S.F. and Maistro E.L. (2008) Evaluation of the in vivo mutagenic potential of hydroalcoholic extracts of the northern highbush blueberry (Vaccinium corymbosum L. Ericales, Ericaceae) on peripheral blood cells of Swiss mice (Mus musculus Rodentia, Muridae). *Genetics and Molecular Biology*, 31, 2, pp. 555-560.
4. Garcia S., Santesteban L., Miranda C. and Royo J. (2011) Variety and storage time affect the compositional changes that occur in grape samples after frozen storage. *Australian Journal of Grape and Wine Research*, 17, pp. 162-168.
5. Girard K.K. and Sinha N. (2006) Cranberry, Blueberry, Currant, and Gooseberry. In: Hui Y.H. (eds) *Handbook of fruits and fruit processing*, Blackwell Publishing, Ames, IA, USA, pp. 369-390.
6. Hohn A., Sun D. and Nolle F. (2005) Enzymes in the fruit juice and wine. In: Barett D.M., Somogyi L., Ramaswamy H. (eds) *Processing fruits, Science and Technology. 2nd edition,* CRC Press, Boca, Raton, London, New York, Washington, D.C., pp. 97-112.
7. Howell A.B. (2009) Update on health benefits of cranberry and blueberry. *Acta Horticulturae (ISHS)* 810, pp. 779-785. Available at: http://www.actahort.org/books/810/810_104.htm, 8 August 2011.
8. ISO 2173:2003 Determination of soluble solids – Refractometric method. Technical comunity/ subcommittee TC 34/SC 3; ISO standarts ICS: 67. 080.01.
9. ISO 750:1998 Fruit and vegetable products – determination of titrable acidity. Technical comunity/ subcommittee TC 34/SC 3; ISO standard 2003-07-15.
10. Kafkas E., Koşar M., Türemiş N. and Başer K.H.C. (2006) Analysis of sugars, organic acids and vitamin C contents of blackberry genotypes from Turkey. *Food Chemistry*, 97, pp. 732-736.
11. Kampuse S. (2006) Suitability to Freezing of Berries from Different Raspberry, Black, Red and White Currant Cultivars. Grown in Latvia. Summary of Promotion work for acquiring the Doctor's degree of Engineering Sciences in Food Science. Latvia University of Agriculture, Faculty of Food Technology, Jelgava, pp. 44. Available at: http://llufb.llu.lv/dissertation-summary/food/kopsav-Solvita-Kampuse.pdf, 5 August 2011.
12. Pormale J. and Osvalde A. (2010) Krūmmellenes vai savvaļas mellenes? (Highbush blueberries or bilberries?). *Dārzs un Drava.* Available at: http://www.darzsundrava.lv/?article=7880/, 13 March 2011. (in Latvian).
13. Poudel P.R., Mochioka R., Beppu K. and Kataoka I. (2009) Influence of Temperature on Berry Composition of Interspecific Hybrid Wine Grape 'Kadainou R-1' (*Vitis ficifolia* var. *ganebu* × *V. vinifera* 'Muscat of Alexandria'). *Journal of the Japanese Society for Horticultural Science* ,78 (2), pp. 169-174. Available at: http://www.lib.kagawa-u.ac.jp/metadb/upAA12177046/AA12177046_78_2_169.pdf, 17 March 2011.
14. Saftner R., Polashock J., Ehlenfeldt M. and Vinyard B. (2008) Instrumental and sensory quality characteristics of blueberry fruit from twelve cultivars. *Postharvest Biology and Technology*, 49, pp. 19-26.
15. Sinelli N., Spinardi A., Eigidio D.V., Mignani I. and Casiraghi E. (2008) Evaluation of quality and nutraceutical content of blueberries (*Vaccinium corymbosum* L.*)* by near and mid-infrared spectroscopy. *Postharvest Biology and Technology*, 50, pp. 31-36.
16. Skrīvele M., Kaufmane E., Strautiņa S., Ikase L., Ruisa S., Rubauskis E., Blukmanis M. and Segliņa D. (2008) Fruit and berry growing in Latvia. In: *Sustainable Fruit Growing: From Plant to Product. Proceedings of international scientific conference*, Jūrmala - Dobele, Latvia, pp. 5-14.
17. Skupień K. (2006) Chemical composition of selected cultivars of highbush blueberry fruit (*Vaccinium corymbosum* L.). *Folia Horticultura*, 18 (2), pp. 47-56.
18. USDA National Nutrient Database for Standard Reference, Release 23 (2010) Available at: http://www.nal.usda.gov/fnic/foodcomp/search/, 11 March 2011.
19. US Highbush blueberry council (2011) Tehnical brochure. Available at: http://www.blueberry.org/publications/technical/tech%20brochure-sm.pdf, 3 August 2011.
20. Yoon K.Y., Woodams E.E. and Hang Y.D. (2006) Relationship of acid phosphatase activity and Brix/acid ratio in cherries. LWT - Food Science and Technology, 39 (3), pp. 316-320.
21. Wang Y.S., Chen T.S., Sciarappa W., Wang C.Y. and Camp M.Y. (2008) Fruit Quality, Antioxidant Capacity and Flavonoid Content of Organically and Conventionally Grown Blueberries. *Journal of Agriculture and Food Chemistry*, 56, pp. 5788-5794.
22. Wang Y.S., Chen C.T. and Wang Y.C. (2009) The influence of light and maturity on fruit quality and flavonoid content of red raspberries. *Food Chemistry*, 112, pp. 676-684.
23. Zagory D. and Kader A.A. (1989) Quality maintenance in fresh fruits and vegetables by controlled atmospheres. In: Jen J.J. (eds) *Ouality Factors of Fruits and Vegetables - Chemistry and Technology*, American Chemical Society, Washington D.C., pp. 174-188.

# CHANGES OF JELLY STRUCTURAL PROPERTIES DEPENDING ON DIFFERENT SWEET MATTERS

***Maija Kronberga, Daina Karklina***
*Latvia University of Agriculture*
*maija.kronberga@inbox.lv*

**Abstract**

There is growing Interest in products without added sugar; thus, sugar consumption is directly related to diabetes and other illnesses such as obesity. A change in the type of sugar and content may therefore both change the perception of sweetness and texture of products. The aim of the research work was to evaluate properties of agar-agar jellies prepared with inulin syrup, galactose syrup and malt extract to replace sugar. Agar gels were prepared to substitute sugar with inulin syrup, galactose syrup and malt extract.

Texture of experimental samples was determined by using a Texture Analyser (Model TA.XT Plus; Stable Micro Systems). Colour of jellies was evaluated by using Colour Tec-PCM and Jenway 3510 was used for pH measurements.

Obtained results showed that different kinds of sugar containing syrups could be used as sugar substitute for production of a new type jellies. Hardness of the experimental samples is influenced by the sugar type containing syrup. The hardness is determined in the experimental samples by replacement of sugar by increasing concentration of inulin syrup. Decreasing hardness values are observed by increase of added malt extract (maltose) and galactose syrup concentration in samples. The pH values of experimental samples ranged between 3.11 and 4.45. Higher L* value of experimental samples are with galactose syrup. Lightness "L" changed between 20.92 and 18.11 increasing inulin syrup concentration. The same situation can be observed using malt extract as sugar substitute.

**Key words**: inulin syrup, galactose syrup, malt extract, jellies.

## Introduction

During the last 30 years, caloric intake of people in Latvia has risen from 5% to 15%. Obesity affects one out of every three adults, one out of every six children, and the prevalence continues to rise. (Mintāle et al., 2010). A lot of overweight people and those having a tendency to develop diabetes often have a craving for sweet candies and find it difficult to abstain from eating high calorie products (Pudule et al., 2007).

Since the principal ingredient of most candies is sugar (sucrose), attempts at reducing the caloric content have been made by reducing the amount of sugar used in preparing candies. Sugar is often used as a bulk chemical, i.e. added in large amounts to food and contributes to sweetness and texture properties. A change in sugar content may therefore both change the perception of sweetness and texture (Bayarri et al., 2004; Kaur and Gupta, 2002).

There is an increasing interest in products without added sugar; thus, sugar consumption is directly related to diabetes and other illnesses such as obesity. From a chemical point of view, there is a substantial number of sugars, and they taste very differently (Figuerola, 2007; Gedrovica and Karklina, 2009).

Sucrose can be replaced by fructose, galactose, maltose or different kinds of syrups prepared from separate sugars. Inulin, polysaccharide composed of fructose, is legally classified as food ingredients, as a low-calorie sweetener (Roberfroid, 2005; Glibowski et al., 2008) in all countries where it is used. Jerusalem artichoke (*Helianthus tuberous* L.) is one of the raw materials used for production of high fructose syrups. Galactose, one of natural monosaccharides in milk sugar, has a high solubility and a relatively less sweetness than sucrose. The stability of galactose in acidic and high-temperature conditions enables it to be applied without decomposition in wide varieties of foods as low calorie sweetener (Bayarri et al., 2004). This is especially useful for diabetics and obese patients, since these sugars are not digested but taste and sweetness are not sacrificed. Malt extracts from starch hydrolysis containing disaccharide maltose are prepared from wort. Malt extract products may improve glycemic control in diabetic populations and is used in desserts, soft drinks, ice cream and other foods that are high in carbohydrates as substitute of sugar.

Replacement of sucrose by other sweet ingredients in jellies that are high-energy products causes technological problems since desired sweet taste in jellies sucrose is essential to form the product structure given the gels with gelling material. As a result different quality jellies are obtained (Figuerola, 2007).

Therefore the aim of the research work was to evaluate properties of agar-agar jellies prepared with inulin syrup, galactose syrup and malt extracts to replace sugar.

## Materials and Methods

The research was done at Latvia University of Agriculture, Faculty of Food Technology, in 2011.

In jellies to replace sucrose Jerusalem artichoke juice concentrate produced by Topina, Diät Rohstoff Gmb, (Germany) as inulin syrup, galactose syrup produced by Smiltenes Dairy (Latvia) and malt extracts syrup produced by Ilgezeem brewery (Latvia) were used. Glucose syrup

was obtained from confectionary factory 'Laima' (Latvia). Dansukker (Denmark) sugar was purchased in local retailing.

As gelling material for preparing jellies NordAgar S (E 406) by Nordingredients (Estonia) as low calorie material (not digested by humans) was used.

The Standard formulation for jelly is: agar- agar (2 g), glucose syrup (62 g), sugar (104 g), citric acid (2 g of 50% citric acid solution), water (100 g).

Agar-agar was dissolved in cold water, and then the mixture was heated until desired structure of agar-agar/ water was achieved and boiled 5 min at temperature 105 °C. Then sucrose and glucose syrup were added and the mixture refrigerated up to 65 °C. Agar-agar gels were prepared substituted sugar by inulin syrup as well the same proportions of galactose syrup and malt extract (Table 1). Sugar and replacement ratios in the samples are 100:0; 80:20; 60:40; 40:60; 20:80; 0:100. The product was manually mixed with desirable concentration of experimental syrups (inulin or galactose or malt extract) and hot – filled in polystyrene containers (150 ml), which were sealed with covers and refrigerated at 18 °C. The experiments were carried out in triplicate.

Table 1

**Amount of inulin syrup, galactose syrup and malt extract substituted sugar in jellies**

| Samples No | Amount of sugar, g $kg^{-1}$ | Substitution of sugar by inulin syrup or galactose syrup or malt extract g $kg^{-1}$ |
|---|---|---|
| Control | 520 | 0 |
| I | 416 | 104 |
| II | 312 | 208 |
| III | 208 | 312 |
| IV | 104 | 416 |
| V | 0 | 520 |

Hardness of jelly was characterised by texture profile analysis. Texture was determined by using a Texture Analyser (Model TA.XT Plus; Stable Micro Systems). Wire cutter (A/BC) was used to slice the jelly samples. Measurements were carried out on room tempered samples. The colour of the jellies was measured in the system of CIE L* a* b* by using Colour Tec-PCM. Light penetration was measured and expressed as *L*a*b** values.

pH was determined by – pH-meter (Jenway 3510) with a combined glass electrode was used based on ГОСТ 5898-87.

Microsoft Excel software was used for the research purpose to calculate mean values and standard deviations of the mathematical data used in research.

## Results and Discussion

Jellies' products for desirable sweetness and texture require the correct combination of agar, sugar and glucose syrup (Tabata, 1999; Armisen and Galatas, 2000; Barrangou et al., 2006; Bayarri et al., 2004). Even very minor changes in composition or processing variables can influence the gelation properties (Kim et al., 2001; Matsuhashi, 1990; Figuerola, 2007; Panouille and Larreta-Garde, 2009) as well as texture of jellies.

The hardness values of experimental samples are shown in Fig.1. Hardness of jellies where sugar is substituted by inulin syrup increases from 7.406 till 10.001 N. The hardness increasing the concentration of inulin syrup in the experimental samples could explain that agar-agar and inulin are food polysaccharides from various natural sources. Agar is linear polymer based on a disaccharide repeat structure of 3 - linked β-D-galactopyranosyl and 4 - linked 3,6 anhydro - α -L- galactopyranosyl units (Norziah et al., 2006). Inulin consists of a long chain made up of 22 – 60 fructose molecules and one glucose molecule at one end. The fructose molecules are connected by β - (2-1) bonds. The last fructose is linked with glucose by an α - (1-2) bound as sucrose. The average molecular weight and degree of inulin polymerization depend on the source of inulin. The degree of native Jerusalem Artichoke inulin polymerization is average 10 (Roberfroid, 2005; Kim et al., 2001). Gelation mechanism of agar and inulin in aqueous solutions is different for both polysaccharides and depends also on temperature and water amount in samples. The elasticity of gels arises from stretching, bending, association, aggregation of polysaccharide molecules (Glibowski and Wasko, 2008; Kim et al., 2001). Gel formation from carbohydrates is affected by many factors such as the heating temperature, and pH.

Opposite situation is observed by increasing the concentration of malt extract and galactose syrup concentration, whose main sugars are monosaccharides and disaccharides. The hardness of samples decreases from 9.320 to 4.093 substitute sugar by galactose and from 8.182 to 5.881 N substitute sugar by malt extract syrup. The disaccharide maltose and the monosaccharide galactose do not have gel formation properties.

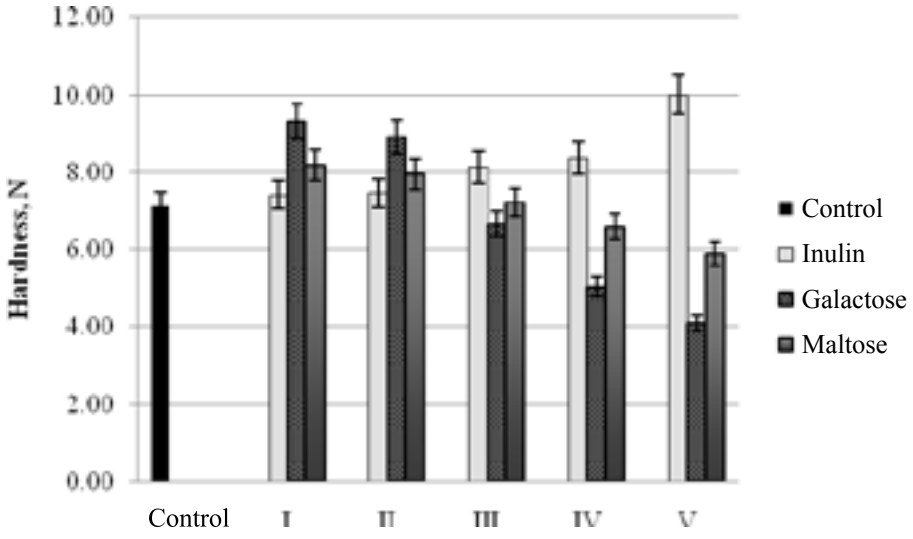



Figure 1. Changes of hardness of experimental jellies samples substituted sugar by inulin syrup, galactose syrup and malt extract (N).

From technological and safety point changes of pH value are not very good because it be could promote the growth of moulds on surface of jellies.

The prosper level of acidity is critical to gel formation. If there is too little acid, the gel never sets; if there is too much acid, the gel will look liquid. The pH of experimental jellies changed little by the addition of different kind and concentrations of syrups. The pH values of experimental samples (Fig. 2) ranged between 3.11 (sample I with inulin syrup) and 4.45 (sample V with galactose syrup).

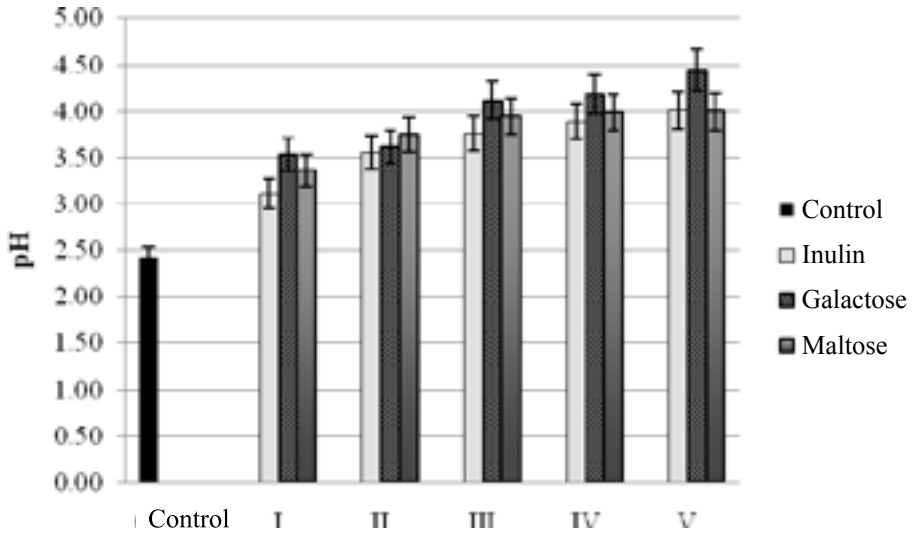



Figure 2. Changes of pH experimental jellies samples substituted sugar by inulin syrup, galactose syrup and malt extract.

The differences of pH value between control sample and experimental samples could be explained by increasing syrup concentration in samples. The pH value of inulin syrup is 5.0, malt extract is 5.23 and galactose is 5.58. Changes of pH values could affect the gel strengths of samples.

While replacing sugar with syrups different trends were observed. At the moment compared with control when the inulin syrup has been added to the jelly samples, the level of pH increases by obtaining higher hardness of the samples. Correlation between hardness and pH in samples with inulin syrup is showed in Fig. 3. The gel hardness goes up (Fig. 3) increasing the pH value in samples (r=0.747). In case of galactose the negative correlation was observed (r=-0.528) but added amount of malt extract did not influence the hardness of gel.

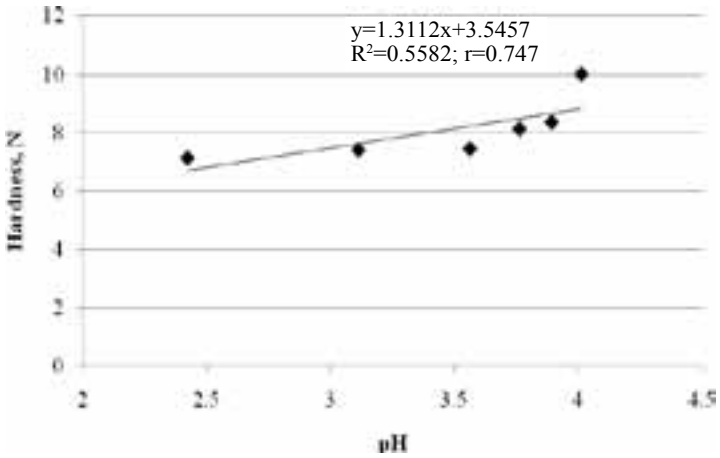



Figure 3. Correlation between pH and hardness of experimental jellies samples with inulin syrup.

Food colour has a significant impact by being one of the major factors used by consumers to take the purchasing decision because it influences human behaviour. Generally, colour change was the most evident quality changewith addition to other kind of sweet matters. Results of surface colour measurement of experimental jellies are presented in Fig. 4 and Fig. 5

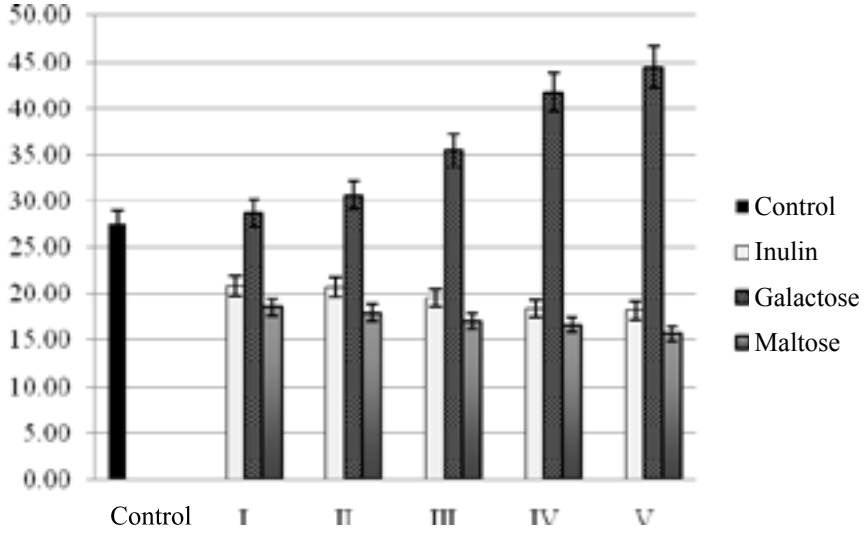



Figure 4. Lightness "L" of experimental jellies samples substituted sugar by inulin syrup, galactose syrup and malt extract.

A higher L* value of experimental samples with galactose syrup compared with other samples indicates a lighter colour which is desirable in order to prepare commercial samples and to ensure that new products will have high consumer acceptance. As obtained results show, the added amount of inulin syrup and maltose syrup in result gives darker colour for experimental jellies (Fig. 4).

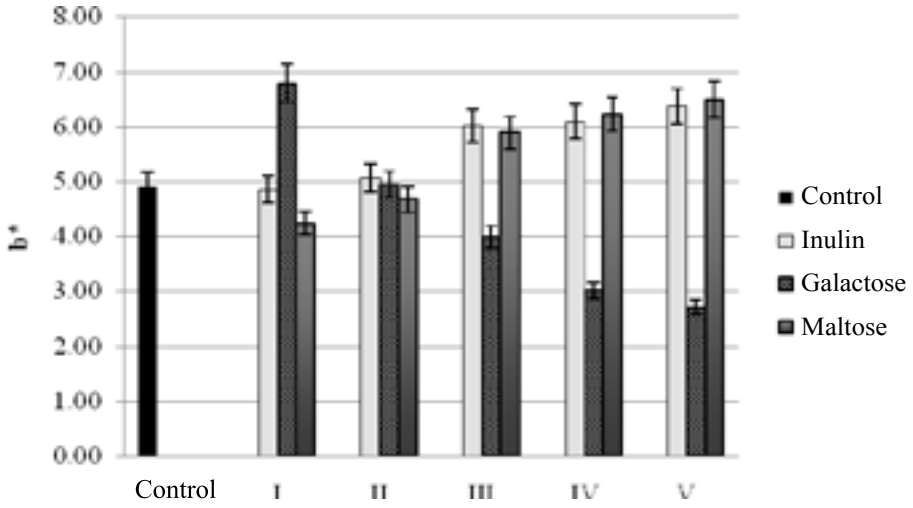



Figure 5. Yellowness b* of experimental jellies samples substituted sugar by inulin syrup, galactose syrup and malt extract.

Lightness "L" changed between 20.92 and 18.11 by increasing inulin syrup concentration. The same situation can be observed using malt extract as sugar substitute.

The blue-yellow chromatically (b*) values were also significantly higher for the samples prepared with inulin syrup and with malt extract in direct concentrations than for samples with galactose syrup and control (Fig. 5).

No changes in a* values in all prepared samples produced by different amount of syrup were observed.

Aspects that require further investigation include the formulation of sensory properties of new jellies and identification of optimal content and processing conditions to ensure safety of products. More extensive studies would be needed to obtain clearer picture of gelation process as function of sugar substitute, especially with inulin syrup.

## Conclusions

1. Obtained results showed that different kinds of sugar containing syrups could be used as sugar substitute for production of a new type jellies.
2. Hardness of the experimental samples is influenced by the sugar type and syrup concentration. The higher values of hardness are determined in the experimental samples replacement of sugar by increasing concentration of inulin syrup. Decreasing hardness values are observed by increasing the concentration of malt extract and galactose syrup in samples.
3. The pH values of experimental samples increase as the syrup concentration is increased in samples.
4. The gel hardness rises increasing the pH value in samples with inulin syrup. In case of galactose the negative correlation was observed but added amount of malt extract did not influence the hardness of gel.
5. Higher L* value of experimental samples are with galactose syrup. Lightness L* changed between 20.92 and 18.11 increasing inulin syrup concentration. The same situation can be observed using malt extract as sugar substitute.
6. The blue-yellow chromatically (b*) values were higher for the samples prepared with inulin syrup and with malt extract than for samples with galactose syrup and control.

## Acknowledgements

The research has been done in the frame of the National Research Programme "Sustainable use of local resources (earth, food, and transport) – new products and technologies (Nat Res)" (2010 - 2013) Project no. 3. "Sustainable use of local agricultural resources for development of high nutritive value food products (Food)"

## References

1. Armisen R., Galatas F. (2000) Agar. In: Phillips G.O. and Williams P.A. (eds) *Handbook of Hydrocolloids*, Woodhead Publishing co., Cambridge, U.K, pp. 21-34.
2. Barrangou L.M., Daubert C.R., Foegeding E.A. (2006) Textural properties of agarose gels. I. Rheological and fracture properties. *Food Hydrocolloids*, 20, pp. 184-195.
3. Bayarri S., Duran L., Costello E. (2004) Influence of sweeteners on the viscoelasticity of hydrocolloids gelled systems. *Food Hydrocolloids*, 18, pp. 611-619.
4. Figuerola F.E. (2007) Berry jams and jellies. In: Zhao Y. (eds) *Berry Fruit.* CRC Press, New York, pp. 367-386.
5. Frack A. (2002) Technological functionality of inulin and oligofructose. *British Journal of Nutrition*, 87, pp. 287-291.
6. Gedrovica I., Karklina D. (2009) Characteristics of cakes enriched with Jerusalem artichoke powder. *Chemine Technologija*, 3, pp. 50-55.
7. Glibowski P., Wasko A. (2008) Effect of thermochemical treatment on the structure of inulin and its gelling properties. *International Journal of Food Science and Technology*, 43, pp. 2075-2082.
8. Kaur N., Gupta A.K. (2002) Applications of inulin and oligofructose in health and nutrition. *Journal of*

*Bioscience*, 27, pp. 703-714.
9. Kim Y., Faqih M.N., Wang S.S. (2001) Factors affecting gel formation of inulin. *Carbohydrate Polymers*, 46, pp. 135-145.
10. Matsuhashi T. (1990) Agar. In: Harris P. (eds) *Food Gels.* Elsevier Applied Science, London, pp. 1-51.
11. Mintāle I., Ērglis A., Dinne A., Bajāre I., Kalvelis A., Latkovskis G., Jēgere S., Taluts-Dzērve V., Zakke I. (2010) *Sirds un virtuve. (Heart and kitchen)*. The Centre of innovative medicine, Rīga, 141 lpp. (in Latvian).
12. Norziah M.H., Foo S.L., Karim A.A. (2006) Rheological studies on mixtures of agar (Gracilaria changii) and k-carrageenan. *Food Hydrocolloids*, 20, pp. 204-217.
13. Panouille M., Larreta-Garde V. (2009) Gelation behavior of gelatin and alginate mixtures. *Food Hydrocolloids*, 23, pp. 1074-1080.
14. Pudule I., Villerša A., Grīnberga D., Velika B., Tilgale N., Dzērve V., Zīle S., Konttinen H., Prattala R. (2007) *Health Behaviour among Latvian Adult Population, 2006*. Julkaisija-Utgivare-Publisher, Helsinki, 164 p.
15. Roberfroid M. (2005) *Inulin –Type Fructans. Functional Food Ingredients*. CRC Press, Boca Raton, Florida, 359 p.
16. Stanley N.F. (2006) Agars. In: Stephen A.M., Phillips G.O., Williams P.A. (eds) *Food Polysaccharides and Their Applications*, CRC Press, New York, pp. 217-238.
17. Tabata K. (1999) Functions and applications of agar jelly. *Chem. Abstr*., 130, pp. 295-652.
18. ГОСТ 5898 (1987) Изделия кондитерские. Методы определения кислотности и щелочности. (Confectionery. Methods for determination of acidity and alkalinity). ВНИИКП, Москва, 9 с. (in Russian).

# CONSUMERS' ATTITUDE TO MILK POMADE SWEET – SHERBET CONSUMPTION AND ITS QUALITY ON THE SALES NETWORK OF LATVIA

***Eva Ungure, Sandra Muizniece-Brasava, Lija Dukalska***
*Latvia University of Agriculture*
*eva.ungure@gmail.com*

**Abstract**

Sherbet with crunchy peanut chips could be classified as milk pomade. It is one of popular sweets in Latvia produced by Joint-stock Company *Laima*, which is one of the oldest producers of sweets in the Baltic States. Freshly made sherbet is soft and savoury but after several days' storage at the open air gradually hardens, the product loses eye appeal, taste and becomes unmarketable. This problem limits the shelf life, so sherbet with crunchy peanut chips can be marketed only at the local market. The target of this study was to clarify the situation on the market in Latvia and to examine an issue of Latvian consumers' awareness of milk pomade sweets – sherbet quality indicators, packaging and its presentation to consumers, as well as the sherbet market expandability. A questionnaire was developed – 800 respondents answered on the 14 questions – how well-recognized the milk pomade sweet – sherbet is, what the main features for this kind of sweets selection by consumers are, which quality indices are important for consumers. One of most important questions was to get know the consumers opinion about sweets, mainly sherbet possible packaging kind and the market turnover.

Summarizing the questionnaire data, the response from consumers in Latvia was heartening – they like milk pomade candies. As a primary quality defect the hardness of sherbet was mentioned. Eliminating this main failing of quality, the demand of sherbet on the market could rise, as well the product marketing opportunities will grow.

**Key words**: sherbet quality, consumers, consumption.

## Introduction

The confectionery industry is enormous ranging from small shops to branches of the largest companies in the food industry. The sweets are divided into three classes: chocolate, flour and sugar confectionery. Manufactures of most modes of confectioneries are not science-based professionals an exception to this is development of products resembling sugar confectionery but free of sugar, where more scientific efforts have been required (Quinton and Kennedy, 2002; Manley, 1998). Milk pomade sweets are one of the sugar confectionery products and usually contain sugar, glucose syrup, water, condensed milk; it may also contain nuts depending on product category. The shelf life of milk pomade sweets depend on several parameters including: storage temperature and humidity, availability of oxygen in the immediate environment, directly related to packaging material used, as well as the addition of other ingredients such as fats, nuts etc. (Raisi and Aroujalian, 2007; Labuza et al., 2004).

The formulation and marketing of consumer products today have become a very complex operation in which both sensory testing and market research have important parts to play. Both disciplines are united by a common goal to produce a product with an optimum combination of product attributes, compatible with manufacturing costs that will sell successfully and profitably in the market place. Yet despite this common goal, the different requirements of technical and marketing personnel have led to the separation of and too little interaction between the two disciplines. There are, however, techniques and approaches used by both the sensory and market researcher which, combined, can make for improved product testing (Wilton and Greenhoff, 1988).

Food choice is not determined solely by the perceived properties of a foodstuff. It depends on personal attitude towards the attributes a product possesses. Consumer responses are vital in the development of a product if it is to compete successfully in the market place (Frances and Piggott, 1991-1992). There are wide varieties of market research procedures which could be used to elicit information about product attributes, and to measure attitudes and beliefs. These include unstructured spontaneous techniques, such as interviews and projective methods, through to highly structured methods, such as dissimilarity scaling where appropriate statistical procedures are used to obtain product spaces (maps) and identify salient product dimensions (McEwan and Thomson, 1989).

Sugar confectionery has been developed over the centuries with increasing sophistication, and it exists in countless formats with different degrees of sweetness, flavours and aromas, textures and mouthfeel. Confectionery serves a very simple purpose; the rush of sweetness coupled with pleasant flavours, aromas and mouthfeel provokes an almost instantaneous feeling of well-being and happiness. Sugar confectionery by definition is meant to include products that contain predominantly one form or another of the following sugars: sucrose (usually cane or beet sugar); dextrose (otherwise known as glucose, usually corn sugar); fructose (often referred to as fruit sugar) or lactose (otherwise known as milk sugar) (Zumbé et al., 2001).

The raw materials for food production are biological systems, therefore serious difficulties originate for the stockholder Laima, Latvia concerning to provide the texture of finished products (Blija and Galoburda, 2008) that are changeable. The texture changes of milk pomade

sweets (moisture content increase and corresponding hardening) are observed during the storage time; therefore, development of methods to estimate objective information regarding raw materials' and finished product structural features is a vital issue (Blija and Galoburda, 2008).

Freshly made sherbet is soft and savoury but after several days' storage at the open air gradually hardens, as it has been observed at the market place and laboratories, the product loses eye appeal, taste and becomes unmarketable. This problem limits the shelf life, so sherbet with crunchy peanut chips can be marketed only at the local market (Vorma et al., 2010). On the market place peanut sherbet for the time being could be found only in bulk carton transport packaging boxes by 5 to 10 kg in each. In this case the product is in contact with oxygen promoting the hardening and possible fat oxidation. Sherbet is recommended to keep + 18 ± 3 °C. The development of attractive small amount consumer packaging should be necessary to be implemented on the market.

*The target of this study was* to clarify the situation on the market in Latvia and examine an issue of Latvian consumers' awareness of milk pomade sweets – sherbet quality indicators, packaging and its presentation to consumers, as well as the sherbet market expandability.

Consumers' evaluation is based upon their own individual experience and particular liking and disliking of sherbet (Wilton and Greenhoff, 1988).

## Materials and Methods

To analyze the situation on the market of Latvia and to study a Latvian consumer's attitude to milk pomade sweet – sherbet consumption and its quality on the sales network of Latvia, a questionnaire was developed – 800 respondents (32% men and 68% women) answered 14 questions – how well-recognized the milk pomade sweet – sherbet is, what the main features for this kind of sweets selection by consumers are, which quality indices are important for consumers. One of most important questions was to get to know the consumers opinion about sweets, mainly possible kind of sherbet packaging and the market turnover. Respondents were asked to evaluate the quality of sherbet, likeness, consumer demand and the packaging options. 11 questions were related to the product (sherbet); three questions of all the questions were related to obtaining basic information about the self. Seven of the questions were formulated in yes - no ones, or given the opportunity to give respondent's own answer. Other issues were presented so that the respondent can easily comment, noting some of the given multiple-choice or by assigning the preferable view. Data collections were used by all 800 respondents who completed questionnaires.

The results were processed by mathematical and statistical methods. Statistics on completely randomized design were determined by using the General Linear Model procedure SPSS 16.00.

## Results and Discussion

Division of respondents according to their age is presented on the Fig. 1.

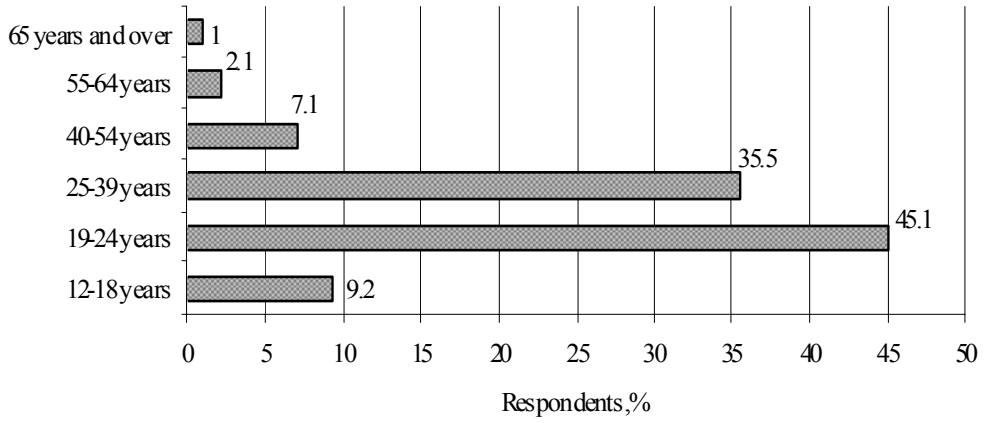



Figure 1. Respondents' division according to their age, %.

Generally, the respondents were in the age up to 40, who are also considered as the main consumers of milk pomade sweets. The top questions of inquire were to get information from consumers regarding sherbet identification on the market of Latvia, about its liking/disliking as well as its purchasing frequency. A fact was established that 96.4% of respondents recognize the milk pomade sweet – sherbet, only 3.6% are not familiar with this kind of sweets. Majority of consumers (80.8%) admit the sherbet as tasty sweet, while 19.2% of respondents do not enjoy it because it is too sweet, some people do not like the main ingredient of sherbet – nuts. Analyzing the consumption frequency of sherbet it has been ascertained that 69% of enquired persons seldom purchase the sherbet, 8.2% – at least once a month, but nobody purchases more frequently (Fig. 2). An interesting answer is that 22.8% of inquired consumers never purchase sherbet themselves; nevertheless, when feasted, willingly have eaten it.

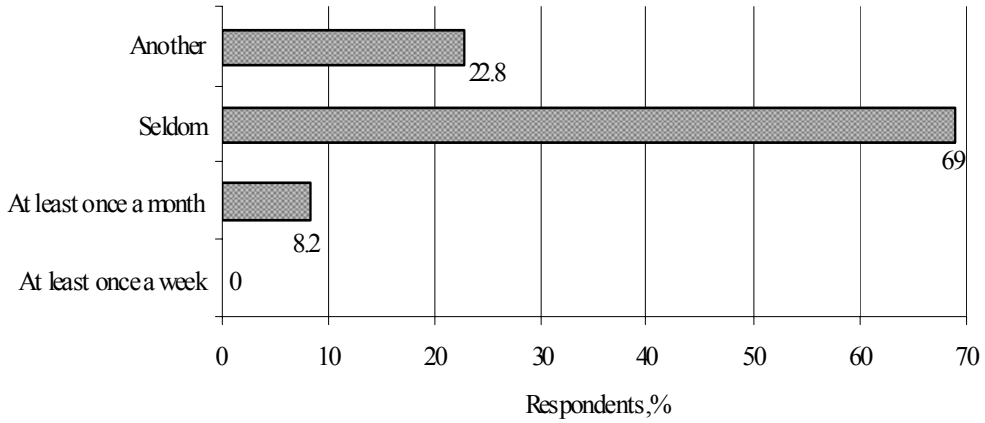



Figure 2. Purchasing frequency of sherbets, %.

The major fixed quality indices of sherbet stated in laboratory examination were as follows: shape and looks like place, color, cut and breach place, hardness, taste and smell. The significance of indicated indices respondents have noted as insignificant, important, very important (Fig. 3).

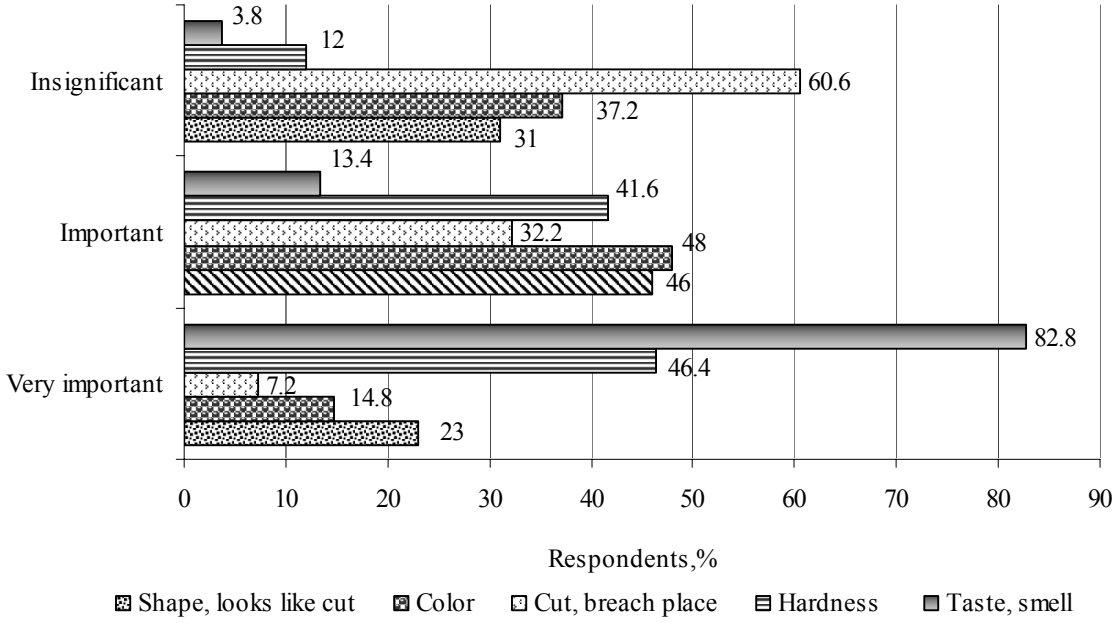



Figure 3. Significance of the sherbet main quality indicators.

Majority of respondents (82.8%) taste and aroma considered as important properties of quality, hardness of sherbet – 46.4% and 41.6% respectively considered as very important and important. Looks like cut, cut and breach place seem insignificant characteristics for consumers. The respondents were asked to evaluate the most frequently observed defects of sherbet (Fig. 4).

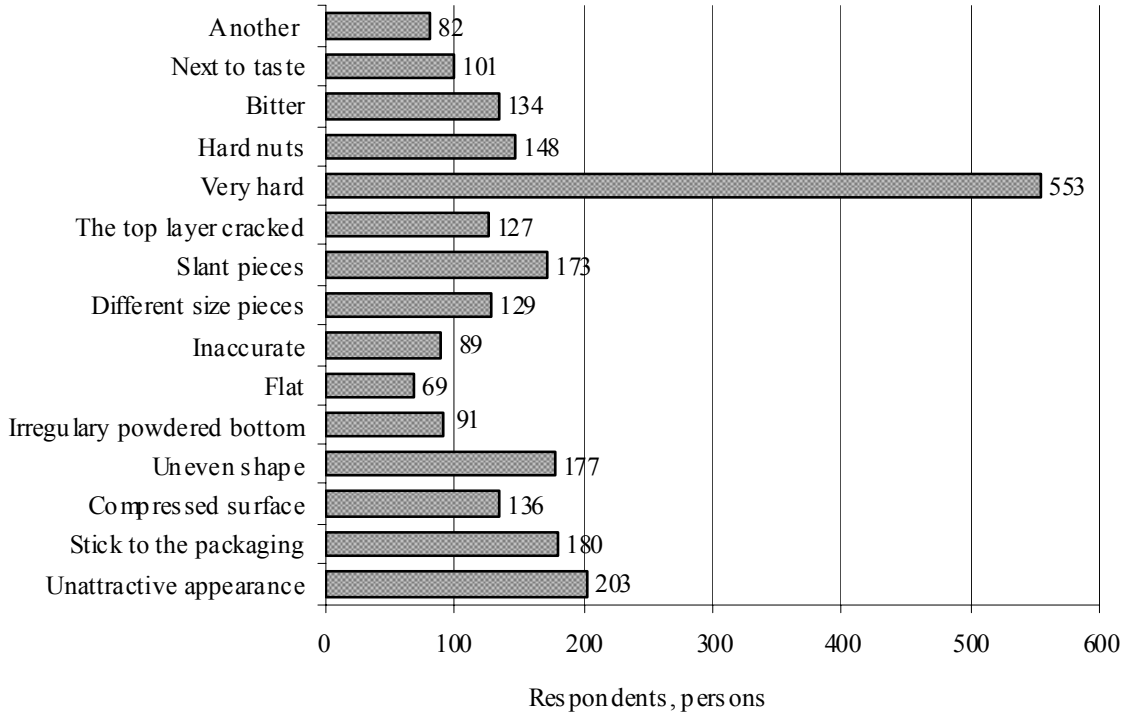



Figure 4. Consumers' most frequently observed defects of sherbet.

Hardness was mentioned (553 people) as consumers' most frequently observed defect of sherbet. This defect could be explained because of the sherbet availability on the market at this moment marginally as good sold by weight; therefore, the moister content has been lost and sherbet hardens very fast. The authors have done a study on sherbet hardness changes during storage and found out that sherbet offered on the local market rapidly solidifies after two weeks of storage (Vorma et al., 2010). This is the main reason why consumers often choose other sweets available on the market instead of sherbet. Defects on other kind mentioned by respondents were crannied upper layer, indistinctive taste, and different sizes of pieces, unattractive appearance, which also could be connected with the way of product distribution. This defect could be avoided by appropriate small size packaging. One of sometimes mentioned defects was irregular pieces of product, which could be explained by heterogeneous nuts consisting mass of sherbet. Sometimes the pieces of sherbet are flat compressed. This defect is caused by bulk packaging of 5 kg in carton boxes when the freshly made product pieces could be mechanically compressed.

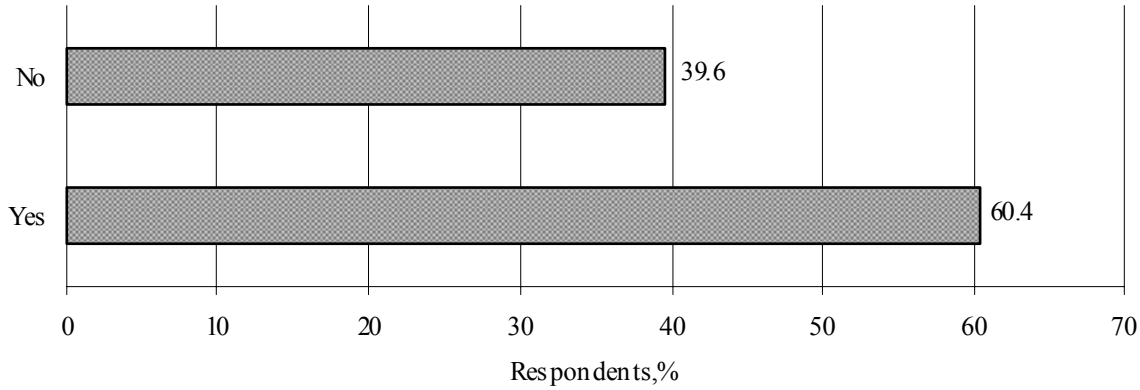



Figure 5. Does the packaging material and design affect the consumers' choice of product when purchasing it?

Some questions were asked to respondents to specify their attitude to packaging design, as well as how those features affect the consumers' choice of product on the market. Majority of respondents (60.4%) admit the effect of packaging material and design on the choice of product when purchasing it (Fig. 5). The rest of respondents (Fig. 6)

(48%) have not been influenced by packaging design. A small part of consumers (1.8%) answered that they are not sure about any packaging material significance. Presumably, the demand of sherbet could be increased by successful packaging material option with attractive design. (Fig. 5**)**

A couple of questions were asked regarding significant packaging influence on sherbet quality during the storage time. The greatest part of respondents (50.2%) considers their knowledge about packaging influence on the product quality during the storage time (Fig. 6.), while 48% of respondents generally were not informed about this phenomena. Only few of respondents (1.8%) had another points of view – they were not sure about the influence or admit that there might be one, still they have never had a chance to get informed about it and check it themselves.

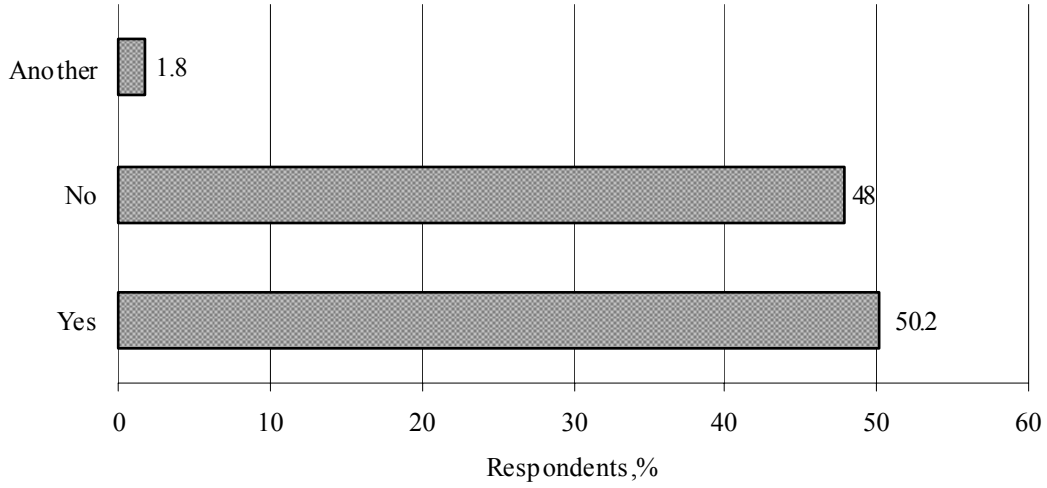



Figure 6. Consumers' information on the packaging influence on sherbet quality during storage time.

The majority of respondents' view (47.2%) regarding the desiderate weight was that there should be 180 g or 6 pieces sherbet in one small packaging (Fig.7.).

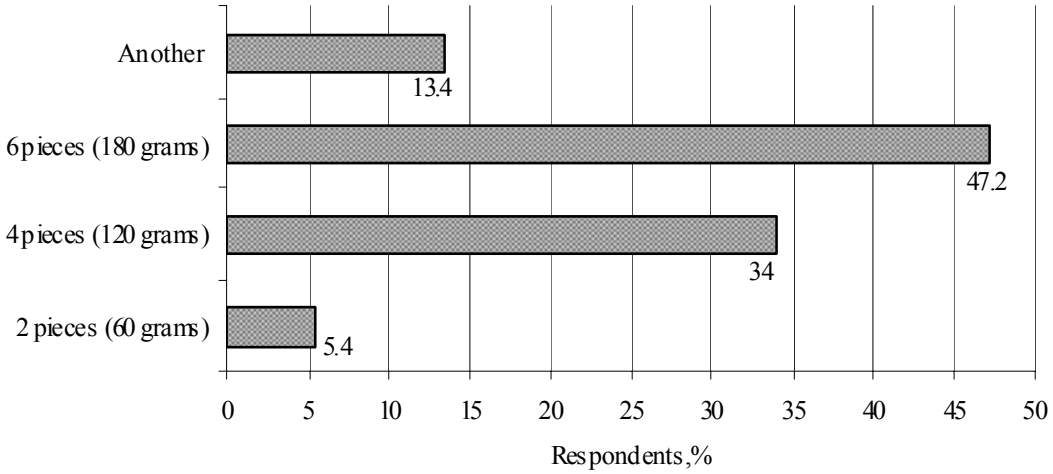



Figure 7. Respondents' view regarding desiderate weight of sherbet in one packaging.

Respondents' view regarding selling places of milk pomade sweet – sherbet in small packaging is that the most suitable for this kind of product could be shops (39.4%); however, 31.7% of respondents would be willing to purchase this product in vending machines, 22.9% – don't raise an objection to sell sherbet in the coffee-bars of educational establishments.

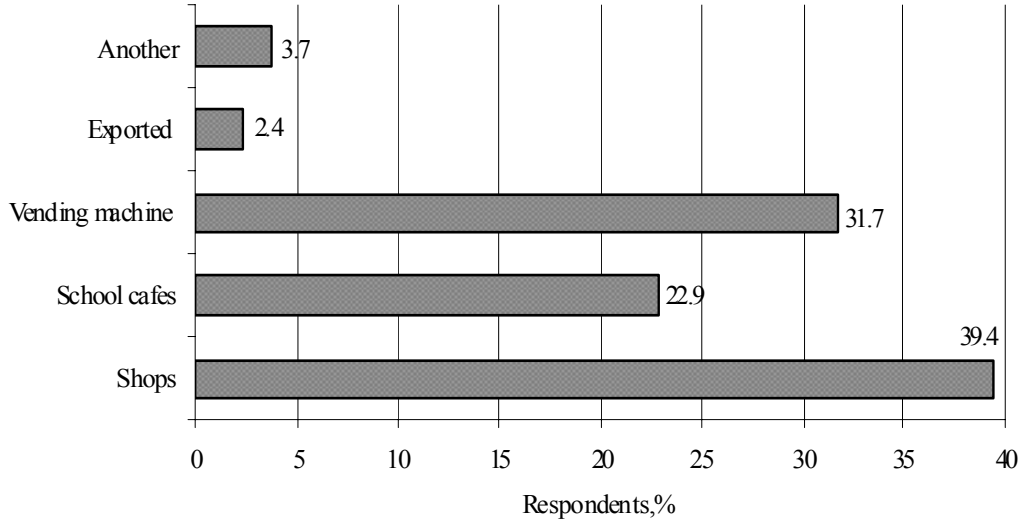



Figure 8. The possible selling places of sherbet in small packaging.

Other proposals mentioned for selling of sherbet in small packaging were petrol stations, theatre coffee-bars, news-stands, sporting activities, and fairs. The weight of sherbet in one small packaging might depend on some of already mentioned selling arrangements.

Part of respondents was school children; therefore, the question was raised whether sherbet is considered to be healthy sweet. Less than half of respondents (41%) were not sure about it (Fig. 8), 25% of respondents were of the opinion that sherbet is healthy and they could willingly purchase this sweet.

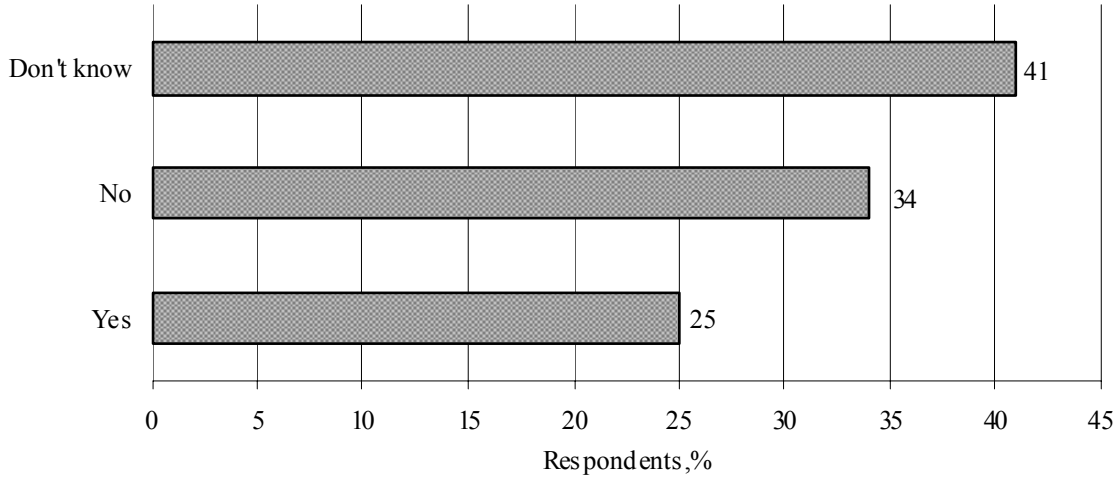



Figure 9. Respondent's view whether: sherbet is healthy sweet.

Parents support the idea that if there were an opportunity to buy sherbet in school's coffee-bars and vending machines instead of imported production, pupils would gladly do it and purchase locally produced sweets. In regulations issued by the Cabinet of Ministers Nr. 610 "The hygiene requirements for basic, all-round and professional educational establishments" ("LV", 2 (2767), 07.01.2003.) section of catering in schools there is a regulation that food additives sold in schools must not contain sugar confectionery. Given that sherbet does not contain any of food additives that are on the regulation lists, it is recommended for selling in schools.

## Conclusions

The consumers in Latvia are willing to purchase milk pomade sweet – sherbet on the local market. The main quality defect of sherbet is its fast hardening for which consumers do not like it. The small packaging and quality assurance of sherbet could increase te level of its consumption. Respondents' opinion regarding the weight of sherbet in one small packaging differs, but mainly it is mentioned as 2 – 6 pieces in one unit of packaging.

**Acknowledgements**

The research was supported by the ESF "Support for doctoral studies in LLU", Contract No.2009/0180/1DP/1.1.2.1.2./09/IPIA/VIAA/017

**References**

1. Blija A., Galoburda R. (2008) Pārtikas produktu struktūrmehāniskās īpašības. (Texture and mechanical properties of foods). LLU, Jelgava, pp. 118-171. (in Latvian).
2. Frances R.J., Piggott J.R. (1991-1992) Free choice profiling in consumer research. *Food Quality and Preference,* vol. 3, 3, pp. 129-134.
3. Labuza T., Roe K., Payne C., Panda F., Labuza T.J., Labuza P.S., Krusch L. (2004) Storage Stability of Food Systems: Influence of State Changes during Drying and Storage. In: *Proceedings of the 14th International Drying Symposium (IDS 2004)*, São Paulo, Brazil, vol. A, pp. 48-68.
4. Laima LTD (2006-2007) Product catalogue. Available at: http://www.laima.lv/files/catalogue2006-2007.pdf, 19 December 2010.
5. Manley D. (1998) *Biscuit, Cookie and Cracker Manufacturing: Manual 1 – Ingredients.* D. Manley, Ed. Cambridge, UK: Woodhad Publishing Limited, 82 p.
6. McEwan J.A., Thomson D.M.H. (1989) The repertory grid method and preference mapping in market research: A case study on chocolate confectionery. *Food Quality and Preference,* vol. 1, 2, pp. 59-68.
7. MK noteikumi Nr.610 (2003) Higiēnas prasības vispārējās pamatizglītības, vispārējās vidējās izglītības un profesionālās izglītības iestādēm (The hygiene requirements for basic, secondary and vocational education establishments). Available at: http://www.likumi.lv/doc.php?id=69952, 21 February 2011. (in Latvian).
8. Quinton L.A., Kennedy J.F. (2002) Book review: The Science of Sugar Confectionery: W.P. Edwards, The Royal Society of Chemistry, 2000. *Carbohydrate Polymers*, 47, 1, pp. 88-89.
9. Raisi A., Aroujalian A. (2007) Reduction of the glucose syrup browning rate by the use of modified atmosphere packaging. *Journal of Food Engineering*, vol.80, 1, pp. 370-373.
10. Vorma E., Muizniece-Brasava S., Dukalska L. (2010) Shelf life extension of milk pomade sweet – sherbet with crunchy peanut chip's by MAP in various packaging materials. In: *ICAFE 2010: "International Conference on Agricultural and Food Engineering"*, Tokyo, Japan. World Academy of Science, Engineering and Technology, Volume 65, Part II, pp. 298-304.
11. Vorma E., Muizniece-Brasava S., Dukalska L. (2010) The studies of vacuum packed milk pomade sweet hardness at the storage time. In: *Proceedings of the International Conference of Food Innovation "Food Innova 2010"*, Valencia, Spain, Number 54, pp. 1-4.
12. Wilton V., Greenhoff K. (1988) Integration of sensory techniques into market research. *Food Quality and Preference,* vol. 1, 1, pp. 33-35.
13. Zumbé A., Lee A., Storey D. (2001) Polyols in confectionery: the route to sugar-free, reduced sugar and reduced calorie confectionery. *British Journal of Nutrition*, 85, pp. 31-45.

# OCCURRENCE OF *CAMPYLOBACTER* SPP. ON FRESH BROILER CHICKEN CARCASSES AT RETAIL LEVEL IN LATVIA

***Kaspars Kovaļenko[1], Mati Roasto[2], Aija Ruzaiķe[1], Edgars Liepiņš[1]***
*[1]Latvia University of Agriculture*
*[2]Estonian University of Life Sciences*
*kkovalenko@inbox.lv*

**Abstract**

*Campylobacter jejuni* and *Campylobacter coli* are the most commonly registered cause of human campylobacteriosis. Mainly the source of these bacteria is from the contaminated foods of animal origin and especially broiler chicken (*Gallus gallus domesticus)* meat.

The aim of the present study was to determine the occurrence of *Campylobacter* spp. in fresh broiler chicken carcasses at the retail level in Latvia. Sampled broiler chicken carcasses originated from two biggest Latvian chicken companies/slaughterhouses and samples were taken during the year 2010. A total of 56.7% of the fresh broiler chicken carcass samples were positive for *Campylobacter*. There was no distinct seasonal variation in *Campylobacter* contamination in Latvia. Additionally, only slight differences between the proportions of *Campylobacter*-positive broiler chicken meat samples of the studied companies were determined.
**Key words**: *Campylobacter* occurrence, fresh broiler chicken carcasses, slaughterhouses.

## Introduction

*Campylobacter coli* and *Campylobacter jejuni* are mostly slender, spirally curved, gram-negative rods with a characteristic corkscrew-like darting motility. Compared to other food-borne bacterial pathogens, *Campylobacter* are more fragile and require microaerobic conditions for multiplication (Park, 2002). Nowadays, *Campylobacter jejuni* and *C. coli* are the most common registered bacterial causes of human intestinal infections in European Union (EFSA, 2010). The most important sources of this bacterium are the foods of animal origin, especially poultry meat. Therefore, control of *Campylobacter* in poultry meat is a major public health strategy for the prevention of human campylobacteriosis (Friedman et al., 2004) In 2008, the proportions of *Campylobacter*-positive broiler meat samples varied widely between European Union member states (EU), from 3.0% to 86.2% (EFSA, 2009 and 2010). For fresh broiler chicken carcasses the average *Campylobacter* prevalence rate in EU was 75.8% (Rantsiou et al., 2010; EFSA, 2010).

First poultry exposure to the *Campylobacter* usually is at farm level and directly related with insufficient biosecurity measures in and around poultry farm (Newell et al., 2003; Ellis-Iversen et al., 2009). In a flock with 20,000 broilers prevalence of *Campylobacter* can increase from 5% to 95% within 6 days after initial *Campylobacter* introduction (Van Gerwe et al., 2005). The cross-contamination of the chicken meat has been observed at scalding, evisceration and water chilling stages following by the transmission of the *Campylobacter* contamination to the retail level (Hue et al., 2010; Jacobs-Reitsma, 2000). Studies in neighboring Baltic countries Estonia and Lithuania showed seasonal peak of *Campylobacter* occurrence to be in winter and spring season in Lithuania and summer and early autumn in Estonia (Pieskus et al., 2008; Meremae et al., 2010).

The aim of present study was to determine *Campylobacter* spp. occurrence in broiler chicken (*Gallus gallus domesticus)* carcasses at retail level in Latvia.

## Materials and Methods

*Collection of samples*

Totally 240 fresh broiler chicken carcasses were collected during the year 2010. Sampling was performed on a monthly basis, and 10 samples from each of two biggest broiler chicken meat producer in Latvia were collected randomly at retail level in each month. Broiler chicken carcasses from slaughterhouse 'A' were sold in tight, sealed plastic bags opposite to the slaughterhouse 'B' where production of broiler chicken carcasses was sold in loose, unsealed plastic bags.

*Campylobacter* spp. *isolation*

One to two hours after sampling, 10 grams of skin material from the backs of the broiler chicken carcasses were aseptically taken and placed into sterile plastic bags for enrichment. Plastic bags were prefilled with 90 mL of sterile Bolton broth (Oxoid; Basingstoke, Hampshire, UK), and the samples were processed for one minute in a stomacher and then incubated in microaerobic atmosphere at 37 °C for 4 h to 6 h, followed by 41.5 °C for 44 ± 4 h. After enrichment, detection method of *Campylobacter* spp. described by ISO 10272-1:2006 was followed. Briefly, 10 µL of the enrichment broth were plated on modified charcoal cefoperazonedeoxycholate agar (Oxoid; Basingstoke, Hampshire, UK) and incubated for 48 h at 42 ± 0.5 °C under microaerobic conditions. Typical *Campylobacter* colonies on mCCDA plates were streaked on Columbia blood agar (Oxoid) plates, which were incubated for 24 h at 41.5 °C in microaerobic conditions. Isolation of *Campylobacter* spp. from broiler chicken carcass samples was carried out at the Latvia University of Agriculture, Faculty of Veterinary medicine, in laboratory of Food Hygiene of the Institute of Food and Environmental Hygiene (Jelgava, Latvia).

*Campylobacter identification*

Gram staining, motility analysis, oxidase and catalase tests were performed for the identification of *Campylobacter* species according to the instructions of the international standard ISO 10272-1:2006.

*Statistical methods*

The *Campylobacter* spp. occurrence data was analyzed by Microsoft Excel 2010 in order to determine confidence interval.

## Results and Discussion

The bacteria isolated from broiler chicken carcasses that showed typical growth on mCCDA, were gram negative, had corkscrew-like darting motility and were catalase and oxidase positive were considered as *Campylobacter* spp. The occurrence of *Campylobacter* spp. on broiler chicken carcasses sampled at retail level during the year 2010 is shown in Table 1.

Table 1

**Campylobacter spp. positive samples on raw broiler chicken carcasses at the retail level in Latviain 2010**

| Month | Slaughterhouse 'A'[1] | Slaughterhouse 'B'[1] |
|---|---|---|
| January | 8/10 | 0/10 |
| February | 9/10 | 6/10 |
| March | 10/10 | 2/10 |
| April | 8/10 | 6/10 |
| May | 7/10 | 8/10 |
| June | 9/10 | 8/10 |
| July | 10/10 | 6/10 |
| August | 6/10 | 4/10 |
| September | 10/10 | 10/10 |
| October | 5/10 | 5/10 |
| November | 0/10 | 0/10 |
| December | 4/10 | 1/10 |
| **Total** | **86/120 (66.7%)** | **56/120 (46.7%)** |

[1] – number of positive samples/total samples taken

The average proportion of *Campylobacter* positive broiler chicken carcasses at retail level was 59.2% (95% confidence interval: 52.9 to 65.4%) which is 17% lower compared with average European union member state level where broiler chicken carcasses at slaughterhouse level were investigated (EFSA, 2010). However, the contamination in Latvia was higher than in neighboring country Estonia where the occurrence of broiler chicken carcasses at retail level was 12.3% (Meremae et al., 2010). There is no up-to-date research data available about *Campylobacter* spp. on broiler chicken carcasses at retail level in Lithuania. Previous *Campylobacter* occurrence studies in Lithuania showed that chicken wings and drumsticks at the retail level were contaminated up to 46.5%, and broiler chicken carcasses at slaughterhouse level – up to 45.8%, respectively (Bunevičienė et al., 2010; EFSA, 2010). In the present study the higher occurrence of *Campylobacter* spp. was determined in the products originated from slaughterhouse 'A' compared with products of slaughterhouse 'B'. Small differences in *Campylobacter* occurrence on broiler chicken carcasses of two biggest Latvian broiler chicken meat producers could be associated with the differences in chicken carcass packaging methods (Kovaļenko et al., 2010).

In 2010 only slight seasonal variation in *Campylobacter* contamination levels was observed in Latvia (Table 1). The only months with no *Campylobacter* contamination was November for slaughterhouse 'A' broiler chicken carcasses and November and January for slaughterhouse 'B' broiler chicken carcasses.

We may conclude that *Campylobacter* occurrence in broiler chicken carcasses of both slaughterhouses was relatively high in the year 2010. There is need for further investigation to determine the reason of differences in *Campylobacter* contamination levels compared to Estonia where distinct seasonal variation was observed and very low *Campylobacter* contamination of broiler chicken meat in 2010 was determined (Meremae et al., 2010). Estonia, Latvia and Lithuania are considered to be in the same geographic region where there are still considerable differences in occurrence of *Campylobacter* spp. contamination in broiler chicken production chain. Biosecurity measures of both Latvian broiler chicken meat companies at farm level should be strengthened and proper epidemiological investigation applied to determine *Campylobacter* source attribution and possible contamination routes.

## Conclusions

1. According to the study it can be concluded that at Latvian retail level on average 59.2% of broiler chicken carcasses were contaminated with *Campylobacter* spp.
2. There is only slight seasonal variation in *Campylobacter* spp. occurrence on broiler chicken carcasses at retail level in Latvia.

## Acknowledgements

Academic study and publication is financed by the Project 'Support for doctoral studies in LLU' /2009/0180/1DP/1.1.2.1.2/09/IPIA/VIAA/017/ agreement No.044-08/EF2.D4.01.

**References**

1. Bunevičienė J., Kudirkienė E., Ramonaitė S., Malakauskas M. (2010) Occurrence and numbers of *Campylobacter* spp. on wings and drumsticks of broiler chickens at the retail level in Lithuania. *Veterinarija ir Zootechnika (Vet Med Zoot)*, 72, pp. 9-14.
2. Ellis-Iversen J., Jorgensen F., Bull S., Powell L., Cook A.J., Humphrey T.J. (2009) Risk factors for *Campylobacter* colonisation during rearing of broiler flocks in Great Britain. *Preventive Veterinary Medicine*, 89, pp. 178-184.
3. European Food Safety Authority (2010) Analysis of the baseline survey on the prevalence of *Campylobacter* in broiler batches and of *Campylobacter* and *Salmonella* on broiler carcasses in the EU, 2008 - Part A: *Campylobacter* and *Salmonella* prevalence estimates. *EFSA Journal 2010, 8(03):1503*, pp. 1-99.
4. EFSA, European Food Safety Authority (2009) EFSA Scientific Colloquium Summary Report. Assessing Health Benefits of Controlling *Campylobacter* in the Food Chain, 4-5 December 2008, Rome, Italy, pp. 1-180.
5. Friedman C.R., Hoekstra R.M., Samuel M., Marcus R., Bender J., Shiferaw B., Reddy S., Ahuja S.D., Helfrick D.L., Hardnett F., Carter M., Anderson B., Tauxe R.V. (2004) Group, Emerging Infections Program FoodNet Working Risk factors for sporadic *Campylobacter* infection in the United States: A case-control study in FoodNet sites. *Clinical Infectious Diseases*, 38 (Suppl. 3), pp. 285-296
6. Hue O., Le Bouquin S., Laisney M.J., Allain V., Lalande F., Petetin I., Rouxel S., Quesne S., Gloaguen P.Y., Picherot M., Santolini J., Salvat G., Bougeard S., Chemaly M. (2010) Prevalence of and risk factors for *Campylobacter* spp. contamination. *Food Microbiology*, 27, pp. 992-999.
7. Jacobs-Reitsma W. (2000) *Campylobacter* in the food supply. In: Nachamkin I. and Blaser M.J., Jacobs-Reitsma W. *Campylobacter, 2nd ed*. Washington, DC, USA, American Society for Microbiology Press, pp. 467-481.
8. Kovaļenko K., Ruzaiķe A., Roasto M., Liepiņš E. (2010) Dynamic of *Campylobacter jejuni* in raw poultry meat depending on packaging atmosphere. *Veterinārmedicīnas raksti 2010*, 165 p.
9. Meremae K., Elias P., Tamme T., Kramarenko T., Lillenberg M., Karus A., Hanninen M.L., Roasto M. (2010) The occurrence of *Campylobacter* spp. in Estonian broiler chicken production in 2002-2007. *Food Control*, 21 (3), pp. 272-275.
10. Newell L.C., Fearnley D.G. (2003) Sources of *Campylobacter* Colonization in Broiler Chickens. *Applied and Environmental Microbiology*, 69, pp. 4343-4351.
11. Park S.F. (2002) The physiology of *Campylobacter* species and its relevance to their role as foodborne pathogens. *International Journal of Food Microbiology*, 74, pp. 177-188.
12. Pieskus J., Butrimaite-Ambrazeviciene C., Kazeniauskas E. (2008) Risk Factors for the Presence of *Campylobacter* Sp. in Lithuanian Broiler Flocks. *International Journal of Poultry Science*, 7 (12), pp. 1242-1246.
13. Rantsiou K., Lambert C., Cocoli L. (2010) Survey of *Campylobacter jejuni* in retail chicken meat products by application of a quantitative PCR protocol. *International Journal of Food Microbiology*, 141, pp. 75-79.
14. Van Gerwe T.J.W.M., Bouma A., Jacobs-Reitsma W.F., van den Broek J., Klinkenberg D., Stegeman J.A., Heesterbeek J.A.P. (2005) Quantifying Transmission of *Campylobacter* spp. among Broilers. *Applied and Environmental Microbiology*, 7, pp. 5765-5770.

# HISTOLOGICAL STATUS OF LYMPH NODES FOR CIRCOVIRUS-2 SEROPOSITIVE AND SERONEGATIVE PIGS

***Inga Pigiņka[1,2], Edīte Birģele[1]***
*[1] Latvia University of Agriculture*
[2] Institute of Food Safety, Animal Health and Environment „BIOR"
*piginka@inbox.lv; edite.birgele@llu.lv*

## Abstract

The aim of the present study was to investigate the histological status of lymph nodes *lnn. inguinales superficiales sinister et dexter, lnn. mesenterici cranial,* and *lnn. tracheobronchales* for porcine circovirus-2 (PCV2) seropositive and seronegative pigs from two Latvian farms. The research was carried out in 2009. Two Latvian farms were investigated – conditional farm A in Latgale, and farm B in Kurzeme. Pigs from farm A had never been vaccinated against PCV2, but on farm B the PCV2 vaccination programme had been stopped six months ago. Ten pigs, 5-15 weeks old, were selected from each farm for investigation. Generally, four lymph nodes were taken from each animal. The blood serum of all 20 pigs was serologically tested for the PCV2 antibody of ELISA Synbiotics, Serelisa PCV2 Ab Mono Blocking kits. Four structural parameters of a lymph node were detected on histological slides: visualization of follicle structure, lymphocyte depletion, amount of histiocytic cell infiltration, and amount of multinucleate giant cell infiltration and localization in the lymph node. Only in lymph nodes of PCV2 seropositive pigs multinucleate giant cells were detected, which is an important additional parameter for serological test and is suggestive of PCV2 infection. As a result, an interrelation between the amount of histiocytic cells and the follicle structure was detected in pigs' lymph nodes: the more histiocytic cells in the lymph node, the greater the loss of the lymphoid follicle structure.

**Key words**: pig, lymph node, multinucleate giant cells, histiocytic cells.

## Introduction

Porcine circovirus-2 (PCV2) infection is considered worldwide distributed throughout domestic pig population, independently of pig health status and clinical condition (Allan and Ellis, 2000; Segales et al., 2005). Many countries of the world have detected that the number of PCV2 seropositive pigs is close to 100% in their slaughter age; moreover, almost no PCV2 antibody free farms were found in epidemiological studies around the world (Larochelle et al., 2003; Rose et al., 2003; Lopez-Soria et al., 2005). In Latvia no investigations about PCV2 distribution in pig population have been made yet.

It is considered that PCV2 is the primary agent of Postweanig Multisystemic Wasting Syndrome (PMWS). This syndrome was first described in 1991 in Canada. Etiology for PMWS was confirmed only in 1998 when PCV2 was isolated from pigs affected by PMWS (Mankertz et al., 1998; Meehan et al., 1998). First data about PVC2 infection were demonstrated in Europe in the mid-1990s. At present PCV2 infection is recognized in all European countries. The first report about PCV2 infection in Lithuania and Latvia was made in 2007 (Stankevicius et al., 2007).

PMWS clinical signs and gross lesions are variable and non-specific (Harding, 2004). However, microscopic lesions in lymphoid tissues are almost unique and constitute the basis of PMWS diagnosis (Segales et al., 2004a). According to the data provided in many papers, PCV2 infected pigs have a systemic lymphadenopathy (Allan and Ellis, 2000; Sorden, 2000), which means different lymph node reactions – for example, enlargement of the inguinal, mesenteric and bronchial lymph nodes (Segales et al., 1997; Harding, 2004). PCV2 specific lymphoid tissue reaction could be detected only by means of the histological test. It is known that lesions of lymph nodes in PCV2 infected pigs are as follows: lymphocyte depletion, hystiocytic cell proliferation, sometimes multinucleate giant cell infiltration, as well as multiple, sharply demarcated, spherical, basophilic cytoplasmic inclusions of bodies in histiocytic cells (Harding et al., 1998; Rosell et al., 1999; Segales et al., 2004b). Moreover, different reactions of lymphoid tissues between different pig species are possible (Opriessnig et al., 2009). Summarizing the above stated, a conclusion is made that it is important to start investigating pig lymph node reaction to the PCV2 in Latvia. **The aim of the study** was to investigate the histological status of lymph nodes *lnn. inguinales superficiales sinister et dexter, lnn. mesenterici cranial,* and *lnn. tracheobronchales* for PCV2 seropositive and seronegative pigs from two Latvian farms.

**The tasks of the study:**

1) to investigate the histological status of lymph nodes for PCV2 seropositive and seronegative pigs;
2) to detect a difference in lymph node reactivity (in separate parameters) in pigs from the farm where pigs had been vaccinated against PCV2, and from the farm where pigs had never been vaccinated against PCV2 infection.

## Materials and Methods

The research was carried out in 2009. The investigations were done in two Latvian farms – conditional farm A in Latgale, and farm B in Kurzeme. Pigs from farm A had never been vaccinated against PCV2, but in farm B the

PCV2 vaccination programme had been stopped six months ago before the research. On both farms pigs with clinical signs of PMWS were observed, and pig mortality was registered. Before death, pigs had progressive weight loss or wasting, diarrhoea or respiratory disease, pallor or jaundice (Harding et al., 1998; Rosell et al., 1999; Segales et al., 2004a). For research purposes ten pigs of the age of 5-15 weeks were selected from each farm.

Pig necropsy was performed during 12 hours after pigs' death, and samples of blood for serological test and lymph nodes *lnn. inguinales superficiales sinister et dexter, lnn. mesenterici cranial,* and *lnn. tracheobronchales* were collected for the histological test. In total, four lymph nodes were taken from each animal.

Blood serums of all 20 pigs were investigated serologically for the PCV2 antibody detection using ELISA Synbiotics, Serelisa PCV2 Ab Mono Blocking kits.

Lymph nodes were fixed by immersion in 10% neutral buffered formalin. Fixed samples were dehydrated, embedded in paraffin wax, sectioned at 5 μm, and stained with hematoxylin and eosin. Histological slides were investigated by Zeiss light microscope.

For each histological slide, four structural parameters of lymph nodes were detected. Their parameters were evaluated by score ranges from 0 to 3:

1) visualization of the follicle structure (0 – normal, no changes, 1 – visualization of the follicle structure a little deplete, 2 – the follicle structure poorly visualized, 3 – loss of the lymphoid follicle structure);
2) the amount of lymphocyte depletion (0 – normal, no changes, 1 – a mild amount of lymphocyte depletion, 2 – a moderate amount of lymphocyte depletion, 3 – a severe amount of lymphocyte depletion);
3) the amount of histiocytic cells (0 – histiocytic cells not detected in tissues, 1 – a mild amount of histiocytic cells, 2 – a moderate amount of histiocytic cells, 3 – a severe amount of histiocytic cells) (Opriessing et al., 2004);
4) the amount of multinucleate giant cells and localization in lymph nodes (0 – multinucleate giant cells not detected in tissues, 1 – a mild amount of these cells, 2 – a moderate amount of multinucleate giant cells, 3 – a severe amount of these cells).

The results were statistically calculated by Microsoft Excel t-test: Two-Sample Assuming Unequal Variance for comparing the structural parameters of the lymph node between PCV2 seropositive and seronegative pigs ($p<0.05$).

## Results and Discussion

The investigation of ten pigs from farm A and farm B revealed similar results: eight pigs from each farm were serologically positive for PCV2 antibody, and two pigs from each farm were serologically negative for PCV2 antibody. It should be noted that the histological investigation of all lymph nodes of each pig had similar microscopic changes, independently of the location of lymph nodes. Therefore all lymph nodes were investigated together and the results are shown in the tables.

The summarized data of the structural parameters of 64 lymph nodes for PCV2 seropositive pigs (32 lymph nodes of pigs from each farm) are shown in Table 1. In this table the quantitative parameters of lymph nodes are summarised according to the score system.

Table 1

**Evaluation of lymph node microscopic lesions of PCV2 seropositive pigs from farm A and B***

| Structural parameters of lymph node | Farm | The amount of lymph nodes according to score ranges: | | | |
|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 |
| Visualization of the follicle structure | A | - | - | 12 (37.5%) | 20 (62.5%) |
| | B | - | - | 6 (18.75%) | 26 (81.25%) |
| The amount of lymphocyte depletion | A | - | 2 (6.3%) | 17 (53.1%) | 13 (40.6%) |
| | B | - | - | 19 (59.4%) | 13 (40.6%) |
| The amount of histiocytic cells | A | - | - | 17 (53.0%) | 15 (47.0%) |
| | B | - | - | 24 (75.0%) | 8 (25.0%) |
| The amount of multinucleate giant cells | A | 18 (56.3%) | 8 (25.0%) | 5 (15.6%) | 1 (3.1%) |
| | B | 28 (87.5%) | 4 (12.5%) | - | - |

* n=32 on both farms

Visualization of the follicle structure of pigs' lymph nodes from both farms was scored from 2 to 3 ranges: from farm A pigs, 12 (37.50%) lymph nodes had poor visualization of the follicle structure and 20 lymph nodes (62.5%) – the loss of the lymphoid follicle structure; from farm B pigs, only six (18.75%) out of the 32 lymph nodes had poor visualization of the follicle structure, and 26 lymph nodes (81.25%) – the loss of the lymphoid follicle structure. It is known that lymphoid follicle basically contain B lymphocytes (Brūveris, 1993), therefore B lymphocyte population in PCV2 positive pigs could change and pigs could be subject to disorder of humoral immunity (Allan et al., 1999; Bolin et al., 2001; Krakowka et al., 2001).

Next estimated structural parameter of lymph nodes was the amount of lymphocyte depletion. Changes in this parameter in pigs' lymph nodes from both farms were quite similar: a mild amount of lymphocyte depletion was registered in 17 lymph nodes (from pigs of farm A) and in 19 lymph nodes (from pigs of farm B), but a severe amount of lymphocyte depletion was noted in 13 lymph nodes

(from pigs of both farms); only two lymph nodes (from pigs of farm A) had a mild amount of lymphocyte depletion.

Changes in the amount of histiocytic cells in pigs' lymph nodes from both farms were also similar and had a score range from 2 to 3 (Table 1). This means that lymph nodes had a loss of lymphocytes and that were replaced with histiocytic cells (Harding et al., 1998; Rosell et al., 1999; Segales et al., 2004a).

However, the amount of multinucleate giant cells detected in some lymph nodes of pigs from both farms was significantly different. A varying amount of multinucleate giant cells was detected in 14 lymph nodes in pigs from farm A: few multinucleate giant cells were recognized in eight (25.0%) lymph nodes, a moderate amount of these cells – in five (15.6%) lymph nodes, but a severe amount – in one lymph node. Whereas four (12.5%) lymph nodes were detected with multinucleate giant cells in pigs from farm B, but the amount of these cells was insignificant (score 1) (Table 1).

Consequently, 43.7% of lymph nodes with multinucleate giant cells were detected in pigs from farm A and 12.5% of lymph nodes – in pigs from farm B. There are data in many papers that multinucleate giant cells could be present in lymph nodes of PCV2 infected pigs and it is one of the histological parameters for PCV2 specific lesion in lymphoid tissues (Harding et al., 1998; Rosell et al., 1999; Segales et al., 2004b). The localization of these cells in lymph nodes has not been clarified up to now. The data were summarized in Table 2 concerning multinucleate giant cell localization in lymph nodes for PCV2 seropositive pigs.

Table 2

**Multinucleate giant cell localization in lymph nodes of PCV2 seropositive pigs from farm A and B***

| Localization in lymph node | Farm | The amount of lymph nodes according to the score ranges: | | | |
|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 |
| Sinusoid, follicle and paracortex | A | - | - | 2 (14.3%) | 1 (7.1%) |
| | B | - | - | - | - |
| Follicle and paracortex | A | - | 1 (7.1%) | 2 (14.3%) | - |
| | B | - | - | - | - |
| Follicle | A | - | 4 (28.6%) | 1 (7.1%) | - |
| | B | - | - | - | - |
| Paracortex | A | - | 3 (21.5%) | - | - |
| | B | - | 4 (100%) | - | - |

* n=14 on farm A
n=4 on farm B

Different amounts of multinucleate giant cells were detected in sinusoid, follicles and paracortex of pigs' lymph nodes from farm A. However, relatively frequently these cells were located in lymphoid follicles (Table 2). In 12.5% of pigs' lymph nodes from farm B, a mild amount of multinucleate giant cells was registered (score 1), although these cells were located mostly in paracortex.

The reason of difference in multinucleate giant cells' localization and the amount in lymph nodes between pigs from different farms is not determined, because the study is still in progress.

The summarized data of the structural parameters of lymph nodes for PCV2 seronegative pigs from either farm are shown in Table 3.

Table 3

**Evaluation of lymph node microscopic lesions of PCV2 seronegative pigs from farm A and B***

| The structural parameters of lymph node | Farm | The amount of lymph nodes according to the score ranges: | | | |
|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 |
| Visualization of follicle structure | A | 4 (50%) | 4 (50%) | - | - |
| | B | - | 5 (62.5%) | 3 (37.5%) | - |
| The amount of lymphocyte depletion | A | - | 4 (50%) | 4 (50%) | - |
| | B | - | 4 (50%) | 4 (50%) | - |
| The amount of histiocytic cells | A | 4 (50%) | 4 (50%) | - | - |
| | B | - | 4 (50%) | 4 (50%) | - |
| The amount of multinucleate giant cells | A | 8 (100%) | - | - | - |
| | B | 8 (100%) | - | - | - |

* n=8 on both farms

It should be pointed out that there were two PCV2 seronegative pigs out of 10 investigated animals; and from each pig four lymph nodes were tested. In total, 16 lymph nodes were investigated – eight from each farm.

Visualization of the follicle structure of pigs' lymph nodes from both farms revealed relatively small changes (score from 0 to 2): pigs' lymph nodes from farm A had smaller changes (score 0-1) than from farm B (score 1-2).

Changes in lymphocyte depletion in pigs' lymph nodes from both farms were comparatively similar (score 1-2) (Table 3).

The amount of histiocytic cells in pigs' lymph nodes from farm B was higher (score 1-2) than from farm A (score 0-1).

This trend of the increasing amount of histiocytic cells in pigs' lymph nodes from farm B could be related to the follicle structure: the more histiocytic cells in lymph node, the greater the loss of the lymphoid follicle structure.

It is important that multinucleate giant cells were not detected in the lymph nodes of PCV2 seronegative pigs (Table 3).

In general, each structural parameter of lymph nodes of PCV2 seropositive pigs had more changes (score 2-3) than the respective parameters of lymph nodes of PCV2 seronegative pigs (score 0-2). Statistically significant differences were confirmed in the structural parameters of lymph nodes between PCV2 seropositive and seronegative pigs ($p<0.05$) (Table 4).

Table 4

**Evaluation of lymph node microscopic lesions of PCV2 seropositive* and seronegative** pigs from both farms**

| Structural parameters of lymph node | Results of serological investigation for PCV2 antibody | The amount of lymph nodes according to the score ranges: | | | | Mean of the score | P value |
|---|---|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 | | |
| Visualization of the follicle structure | seropositive | 0 | 0 | 18 | 46 | 2.719 | $9.80 \times 10^{-9}$ |
| | seronegative | 4 | 9 | 3 | 0 | 0.938 | |
| The amount of lymphocyte depletion | seropositive | 0 | 2 | 36 | 26 | 2.375 | $3.54 \times 10^{-6}$ |
| | seronegative | 0 | 8 | 8 | 0 | 1.500 | |
| The amount of histiocytic cells | seropositive | 0 | 0 | 41 | 23 | 2.359 | $1.36 \times 10^{-6}$ |
| | seronegative | 4 | 8 | 4 | 0 | 1.000 | |
| The amount of multinucleate giant cells | seropositive | 46 | 12 | 5 | 1 | 0.391 | $3.72 \times 10^{-5}$ |
| | seronegative | 16 | 0 | 0 | 0 | 0.000 | |

* – Lymph nodes of PCV2 seropositive pigs – n=64
** – Lymph nodes of PCV2 seronegative pigs – n=16

## Conclusions

1. The lymphoid follicle structure of PCV2 seropositive pigs was fully lost or poorly visible. However, the PCV2 seronegative pigs showed no changes in the lymphoid follicle structure or the follicle structure was slightly depleted.
2. Multinucleate giant cells were detected only in lymph nodes of PCV2 seropositive pigs, which is an important additional parameter for serological test and is suggestive of PCV2 infection.
3. The amount of histiocytic cells in lymph nodes of PCV2 seropositive pigs was higher than in lymph nodes of PCV2 seronegative pigs. An interrelation was detected between the amount of histiocytic cells and the follicle structure in pigs' lymph nodes: the more histiocytic cells in lymph node, the grater the loss of the lymphoid follicle structure.

## References

1. Allan G.M., Ellis J.A. (2000) Porcine circovirus: a review. *Journal* of *Veterinary Diagnostic Investigation*, 12, pp. 3-14.
2. Allan G.M., Kennedy S., McNelly F., Foster J.C., Ellis J.A., Krakowka S.J., Meehan B.M., Adair B.M. (1999) Experimental reproduction of severe wasting disease by co-infection of pigs with porcine circovirus and porcine parvovirus. *Journal of Comparative Pathology*, 121, pp. 1-11.
3. Bolin S.R., Stoffregen W.C., Nayar G.P., Hamel A.L. (2001) Postweaning multisystemic wasting syndrome induced after experimental inoculation of caesarean-derived, colostrums-deprived piglets with type 2 porcine circovirus. *Journal* of *Veterinary Diagnostic Investigation*, 13, pp. 185-194.
4. Brūveris Z. (1993) *Mājdzīvnieku speciālā histoloģija (Special histology of domestic animals)*, Zvaigzne, Rīga, Latvija, 270 lpp. (in Latvian).

5. Harding J.C., Clark E.G., Strokappe J., Willson P., Ellis J. (1998) Postweaning multisystemic wasting syndrome: Epidemiology and clinical presentation. *Journal of Swine Health and Production*, 6, pp. 249-254.
6. Harding J.C. (2004) The clinical expression and emergence of porcine circovirus 2. *Veterinary Microbiology*, 98, pp. 131-135.
7. Krakowka S., Ellis J.A., McNeilly F., Ringler S., Rings D.M., Allan G. (2001) Activation of the immune system is the pivotal event in the production of wasting disease in pigs infected with porcine circovirus-2. *Veterinary Pathology*, 38, pp. 31-42.
8. Larochelle R., Magan R., D'Allaire S. (2003) Comparative serologic and virologic study of commercial swine herds with and without PMWS. *The Canadian Journal of Veterinary Research,* 67, pp. 114-120.
9. Lopez-Soria S., Segales J., Rose N., Vinas M.J., Blanchard P., Madec F., Jestin A., Casal J., Domingo M. (2005) An exploratory study on risk factors for PMWS in Spain. *Preventive Veterinary Medicine*, 69, pp. 97-107.
10. Mankertz A., Mankertz J., Wolf K., Buhk H.J. (1998) Identification of protein essential for replication of porcine circovirus. *Journal of General Virology,* 79, pp. 381-384.
11. Meehan B.M., McNelly F., Todd D., Kennedy S., Jewhurst V.A., Ellis J.A., Haines D.M., Adair B.M., Allan G.M. (1998) Characterization of novel circovirus DNAs associated with wasting syndrome in pigs. *Journal of General Virology*, 79, pp. 2171-2179.
12. Opriessnig T., Patterson A.R., Madson D.M., Pal N., Rothschild M., Kuhar D., Lunney J.K., Juhan N. M., Meng X.J., Halbur P.G. (2009) Difference in severity of porcine circovirus type two-induced pathological lesions between Landrace and Pietrain pigs. *Journal of Animal Science*, 87, pp. 1582-1590.
13. Opriessnig T., Thacker E.L., Yu S., Fenaux M., Meng X.-J., Halbur P.G. (2004) Experimental reproduction of postweaning multisystemic wasting syndrome in pigs by dual infection with *Mycoplasma hyopneumoniae* and porcine circovirus type 2. *Veterinary Pathology*, 41, pp. 624-640.
14. Rose N., Larour G., Le Diguerher G., Eveno E., Jolly J.P., Blanchard P., Oger A., Le Dimna M., Jestin A., Madec F. (2003) Risk factors for PMWS in 149 French farrow-to finish herds. *Preventive Veterinary Medicine*, 61, pp. 209-225.
15. Rosell C., Segales J., Plana-Dura´n J., Balasch M., Rodrı´guez-Arrioja G.M., Kennedy S., Allan G.M., McNeilly F., Latimer K.S., Domingo M. (1999) Pathological, immunohistochemical, and *in situ* hybridization studies of natural cases of postweaning multisystemic wasting syndrome (PMWS) in pigs. *Journal of Comparative Pathology*, 120, pp. 59-78.
16. Segales J. (2002) Update on postweaning multisystemic wasting syndrome and porcine dermatitis and nephropathy syndrome diagnostics. *Journal of Swine Health and Production*, 10, pp. 277-281.
17. Segales J., Allan G.M., Domingo M. (2005) Porcine circovirus diseases. *Animal Health Research Reviews*, 6, pp. 119-142.
18. Segales J., Domingo M., Chianini F., Majo N., Dominguez J., Darwich L., Mateu E. (2004a) Immunosuppression in post-wearning multisystemic syndrome affected pigs. *Veterinary Microbiology*, 98, pp. 151-158.
19. Segales J., Rosell C., Domingo M. (2004b) Pathological findings associated with naturally acquired porcine circovirus type 2 associated disease. *Veterinary Microbiology*, 98, pp. 137-149.
20. Segales J., Sitjar M., Domingo M., Dee S., DelPozo M., Noval R., Sacristan C., DeLasHeras A., Ferro A., Latimer K.S. (1997) First report of post-weaning multisystemic syndrome in pigs in Spain. *Veterinary Record*, 141, pp. 600-601.
21. Sorden S.D. (2000) Update on porcine circovirus and postweaning multisystemic wasting syndrome. *Journal of Swine Health and Production,* 8, pp. 133-136.
22. Stankevicius A., Sibila M., Čepulis R., Segales J. (2007) First description of porcine circovirus type 2 associated diseases in Lithuania and Latvia. In: Markowska-Daniel I. (eds) PMWS, PRRS, Swine influenza and associated diseases. *Proceedings of 5th international symposium on emerging and re-emerging pig disease*, PHU Kopiorama, Kraków, Poland, pp. 82.

# EFFICACY OF THE FIRST LARGE-SCALE RABIES ORAL VACCINATION CAMPAIGNS IN LATVIA DURING 2005

***Edvīns Oļševskis[1,2,3], Edgars Liepiņš[1], Kristīne Lamberga[2], Ieva Rodze[3]***
[1]*Latvia University of Agriculture*
[2]*Food and Veterinary Service of the Republic of Latvia*
[3]Institute of Food Safety, Animal Health and Environment „BIOR"
*edvins.olsevskis@pvd.gov.lv; edgars.liepins@llu.lv;*
*kristine.lamberga@pvd.gov.lv; ieva.rodze@bior.gov.lv*

**Abstract**

Rabies is present in the entire territory of Latvia and as a zoonosis poses risk to both human and animal health. The disease is also endemic in countries bordering with Latvia**,** namely Lithuania, Estonia, Russia and Belarus. Reservoirs for the rabies virus (RABV), the causative agent of the disease in Latvia, are the red fox *(Vulpes vulpes)* and the raccoon dog *(Nyctereutes procyonoides)*. The first large-scale oral rabies vaccination campaigns using aircrafts were carried out in spring and autumn 2005 using Fuchsoral® SAD B19 oral vaccine baits. In total, 1,894 wild animals (1,366 foxes and 528 raccoon dogs) were hunted in vaccinated areas to evaluate the efficacy of the campaign. After spring and autumn vaccination campaigns**,** 51.6% of the fox and raccoon dogs were positive for tetracycline. Herd immunity was found in 50.5% of foxes and raccoon dogs using FAVN test and only in 14.8% using ELISA test. After oral vaccination campaigns**,** rabies incidence decreased in vaccinated area, however, an increase of the incidence was observed in unvaccinated area. Despite the slight increase in rabies incidence in Latvia in 2006 compared to 2005, in 2005 only 141 rabies cases (30%) were found in the vaccinated area whereas 330 cases were detected in the unvaccinated area.
**Key words**: rabies, oral vaccination, red fox, raccoon dog, Latvia**.**

## Introduction

Rabies is endemic in Latvia for centuries. The first written report on rabies in Latvia dates from 1822. At the end of the 19th and the beginning of the 20th century**,** dog-mediated (synonym 'urban') rabies had been predominantly occurring in the country. In the 1920s a drastic increase of dog-mediated rabies was observed from 96 cases to 403 cases reported in 1921 and 1927, respectively. For reasons unknown, rabies cases decreased to 68 cases in 1939. In this period, 62% of the rabies cases in Latvia were reported in dogs (Westerling et al., 2004).

Fox-mediated rabies (sylvatic rabies) had been spreading through Europe since the 1940s (Niin et al., 2008) with a speed of approximately 25 – 60 km per year (Anonymous, 2005). After Second World War, in 1951, the first cases of rabies in wildlife foxes *(Vulpes vulpes)* and wolves *(Canis lupus)* were observed in three districts of eastern Latvia. From 1955 onwards, wildlife rabies was reported annually**,** and the first case in a raccoon dog *(Nyctereutes procyonoides)* was observed in 1958. During the 1960s and 1970s**,** a total of 807 and 1,054 rabies cases were reported. Of these rabies cases, 64.9% were detected in wild animals (47.2% – in foxes, 12.6% – in raccoon dogs**,** and 5.1% in – others). Between 1980 and 1999, the number of rabies cases in Latvia varied between 108 and 306 (Westerling et al., 2004).

Whilst in the interval between the two World wars, 10 human rabies cases were reported, mostly as a result of dog bites, after the Second World War the number of human rabies cases increased initially from 55 in 1946 to 149 in 1950. In the following years the number of human rabies cases drastically decreased to 18 in 1959 due to the compulsory vaccination of dogs against rabies. Nevertheless, from 1960 to 1996**,** a further 13 human rabies cases were reported after exposure to rabies infected foxes, raccoon dogs and cats (*Felis silvestris catus)* (Westerling et al., 2004). Since 2003 no human rabies cases have been reported in Latvia.

Historically, destruction of the fox population including fox culling was the method of choice in an effort to control rabies throughout Europe (Matouch and Polak, 1982), however, those methods completely failed. In 1977, a first field trial using baits containing oral rabies vaccine was initiated in Switzerland, which offered new perspectives in rabies control in wildlife. Later, oral rabies vaccination (ORV) field trials of foxes were initiated in West Germany in 1983. In the mid to late 1980s and early 1990s, ORV programs were initiated in Austria, Belgium, Czechoslovakia, East Germany, France, Hungary, Italy, Luxemburg, the Netherlands and Slovenia (Jackson and Wunner, 2007). As a result of ORV campaigns, the rabies situation in European countries has greatly improved since 1989 (Müller, 2000).

The first ORV field trials in Latvia were conducted in 1991 using chicken head or fish baits containing a Russian oral rabies virus vaccine. At the time, baits were distributed manually near fox dens by specialists of the State Veterinary Service and Hunters association in one district where the rabies incidence was highest. As the baits did not contain a biomarker at the time, it was not possible to evaluate bait uptake after vaccination campaign. In 1992, the field

trials were extended to a further 11 districts. In 1995 and 1996, in 8 and 4 districts ORV was conducted using the same approach. Since 1998, industrially produced baits containing vaccine had been used in ORV campaigns. From 1999 to 2003, two ORV campaigns were carried out annually with the entire territory of Latvia covered between 2001 and 2003 using manual distribution of baits. Due to financial constrains, ORV was not carried out in 2004. As a result a PHARE Twinning Light project 'Eradication of rabies among wildlife animals in Latvia', in 2005, for the first time ORV in Latvia was carried out using SAD B19 vaccine using aerial distribution.

The aim of our study was to evaluate the efficacy of the first large-scale wildlife ORV campaign implemented in Latvia during 2005 using aerial distribution by assessing different parameters, i.e. the incidence of rabies before and after 2005 in vaccinated and unvaccinated areas, the bait-uptake, and the herd immunity in target animals.

## Materials and Methods

In 2005 and 2006, Latvia was divided into 26 administrative districts comprising 64,589 $km^2$ with forests covering 44.1% of the territory of the country. Latvia borders with Lithuania in the South, Estonia in the North, Russia and Belarus in the East.

Organization of the ORV campaign, public awareness campaigns and informating veterinary authorities of the neighbouring countries was performed under supervision of the Food and Veterinary Service (FVS) in collaboration with the State Air Force (SAF). In 2005, two vaccination campaigns were conducted with the spring and autumn campaign carried out between 18 – 29 April and 19 September – 5 October, respectively.

During the spring and autumn ORV campaigns in 2005, a total of 628,800 and 618,400 Fuchsoral® vaccine baits (Impstoffwerk Dessau-Tornau GmbH, Germany) containing the attenuated rabies vaccine virus SAD B19 (Neubert et al., 2001) were distributed in a vaccination area comprising 27,274 $km^2$, respectively, using a bait density of 23 baits per $km^2$. The vaccination area included 15 administrative districts of Latvia (five of them partly), ranging from the Baltic Sea in the West to and the river Daugava in the East. Vaccine batch titration before delivery revealed all titres were over $10^{6.0}$ FFU $ml^{-1}$ (foci forming units (FFU) in ml). Vaccine baits were delivered by supplier shortly before the start of the vaccination campaign, stored at -20 °C before distribution and delivered to the airport at the day of distribution.

Aerial distribution was conducted using helicopters and fixed-wing aircraft (Antonov 2) flying with ground speed of 150 - 180 km $h^{-1}$ at an altitude of 100-150 m using a flight line distance of 1000 meters. Vaccine baits were manually dropped from aircrafts by trained veterinary inspectors of the FVS. Dropping of vaccine baits was interrupted over rivers, lakes, cities, villages and main roads.

Epidemiological data before and after the ORV campaigns in 2005 was analysed using data provided by the FVS of Latvia. For the monitoring of the ORV campaign, approximately 8 wildlife animals (foxes and raccoon dogs) per 100 $km^2$ were collected from vaccinated areas and submitted to the National Reference Laboratory (NRL) for rabies (State Veterinary Medicine Diagnostic centre of FVS) in Riga. Detection of rabies virus antigen in brain samples was performed using the Fluorescent antibody test (FAT) as the gold standard test. FAT negative results were confirmed using the rabies tissue-culture infection test (RTCIT) as described by F.Cliquet and J.Barrat (2008).

For monitoring of ORV campaigns, the bait-uptake and herd immunity of the target population (fox and racoon dog) was determined. The presence of the biomarker (bait-uptake) in adult foxes and raccoon dogs collected was detected by demonstration of Tetracycline (TC)-induced fluorescence in the teeth and bones (mandibula) of the animals using a method described elsewhere (Linhart and Kenelly, 1967; Johnston et al., 1987).

The immunity level (anti-rabies antibodies) in blood samples of foxes and racoon dogs was evaluated using the fluorescent antibody virus neutralisation test (FAVN) (Cliquet et al., 1998) or a commercially available indirect enzyme-linked immunosorbent assay (ELISA, Platelia I, Biorad) (Cliquet et al., 2003).

The 95% (P=0.05) confidence interval (CI) limits for the results obtained on TC presence and herd immunity in target animals were calculated using the online software package (http://statpages.org/confint.html). The Microsoft Office Excel 2007 was used to perform correlation analysis and F-Test Two-Sample for Variances.

In total, 1,894 wild animals (1,366 foxes and 528 raccoon dogs) were hunted in a period from June 2005 to February 2006 and submitted for testing to NRL. Results of the tests were regularly reported to FVS.

## Results and Discussion

Numerous European countries are free of rabies in terrestrial animals either because they were never affected by wildlife rabies, e.g. Ireland, the United Kingdom, Sweden, Norway, Denmark, Spain and Portugal, or due to successful ORV campaigns, e.g. Finland, the Netherlands, Luxembourg, Belgium, France, Switzerland, the Czech Republic (Bourhy et al., 2005). In 2008, Germany and Austria declared themselves free from terrestrial rabies and ceased ORV campaigns on their territories. In contrast, however, wildlife rabies still predominates in Baltic States (Anonymous, 2008).

In Latvia, since 1990 about 200 cases of rabies had been reported annually showing a wave-like pattern observed in the rabies incidence with a frequency of 3-4 years. The number of rabies cases increased to 516 in 2000 and despite ORV campaigns conducted twice a year in the entire territory using manual distribution in the following years peaked in 2003 (Table 1). Most of the affected animals in 2003 were foxes (48.9%) and raccoon dogs (29.6%). On the one hand, this development could have been influenced by an increase of the fox and raccoon dog population.

According to data from the State Forest Service of Latvia, fox population in 2003 has been estimated at 30,000 (26,000 in 2000) and raccoon dog population at 15,000 (11,700 in 2000) animals (personal communication). On the other hand, however, it clearly showed that the old ORV strategy implemented at the time, which exclusively focussed on manual distribution of baits, was in fact ineffective. For reasons unknown, after a peak in 2003, rabies incidence decreased in 2004 and 2005 (Table 1).

Table 1

**Number of rabies cases registered in wildlife and domestic animals in Latvia from 2000 to 2006**

| Species | Year | | | | | | | Total | % |
|---|---|---|---|---|---|---|---|---|---|
| | 2000 | 2001 | 2002 | 2003 | 2004 | 2005 | 2006 | | |
| Dog | 49 | 33 | 31 | 62 | 33 | 20 | 31 | 259 | 6.8 |
| Cat | 41 | 37 | 32 | 52 | 35 | 29 | 44 | 270 | 7.1 |
| Cattle | 25 | 12 | 22 | 21 | 25 | 17 | 13 | 135 | 3.6 |
| Other domestic animals | 3 | 2 | 4 | 0 | 0 | 2 | 0 | 11 | 0.3 |
| Domestic animals (total) | 118 | 84 | 89 | 135 | 93 | 68 | 88 | 675 | 17.8 |
| Fox | 239 | 241 | 247 | 470 | 186 | 177 | 187 | 1,747 | 46.1 |
| Racoon dog | 121 | 126 | 134 | 285 | 138 | 136 | 153 | 1,093 | 28.8 |
| Badger | 15 | 7 | 11 | 33 | 10 | 13 | 8 | 97 | 2. 6 |
| Marten | 0 | 0 | 3 | 14 | 3 | 10 | 6 | 36 | 0.9 |
| Other wildlife | 23 | 19 | 16 | 26 | 13 | 17 | 29 | 143 | 3.8 |
| Wildlife (total) | 398 | 393 | 411 | 828 | 350 | 353 | 383 | 3,116 | 82.2 |
| Total | 516 | 477 | 500 | 963 | 443 | 421 | 471 | 3,791 | 100 |

A similar trend was also observed in Estonia where the number of rabies cases increased within three years from 167 cases in 2001 to 814 cases in 2003 (Niin et al., 2006), and in Lithuania where the rabies incidence increased from 850 in 2000 to 1,108 cases in 2003 (Maciulskis et al., 2006). The highest incidence of rabies in Baltic States so far was observed in 2003 in Lithuania. In all Baltic countries alike, the majority of rabies cases from 2000 to 2006 were registered in wild animals (82.19%). The main vectors and rabies virus reservoirs in Baltic countries are red fox and raccoon dog (Vanaga et al., 2003; Maciulskis et al., 2006; Singer et al., 2009). While the raccoon dog had become the major wildlife species affected by rabies in Estonia (Niin et al., 2008) and in Lithuania (Milius et al., 2004; Maciulskis et al., 2006), such trend was not observed in Latvia, where the red fox remained the main reservoir for rabies virus (46.08%). The raccoon dog is second most affected wildlife species and appears to play an important role in rabies epidemiology as well.

After the first two aerial large-scale ORV campaigns carried out in 2005, the rabies incidence in vaccinated and unvaccinated areas showed divergent developments. During the first six months of 2005, majority of rabies cases originated from vaccinated area; however**,** the situation changed after the first ORV campaign carried out in spring 2005. Although a slight increase of rabies incidence in 2006 (471 cases compared to 421 in 2005) was observed, 70% of all rabies cases originated from areas that were not vaccinated in 2005. Extension of ORV programme to whole country in 2006 resulted in a decrease of rabies cases in the formerly unvaccinated area during the second half of 2006 too (Figure 1). This indicates that the new ORV strategy using large-scale aerial distribution is much more effective compared to the old approach of manual distribution of baits.

The efficacy of ORV campaigns in Latvia using SAD B 19 vaccine can be compared to results obtained in Estonia. The similar approach, performing one large-scale ORV campaign in a part of the country was applied in autumn 2005 using SAG2 vaccine. The number of rabies cases in the vaccinated area decreased from 56 cases in 2005 to 16 cases in the first five months of 2006. In contrast, the non-vaccinated area reported 81 cases in 2006 versus 41 cases in 2005 for the same period. After the spring campaign in 2006, performed throughout Estonia, the number of new rabies cases dropped dramatically to 17 from June to December 2006, versus 169 cases during the same period in 2005 (Niin et al., 2008).

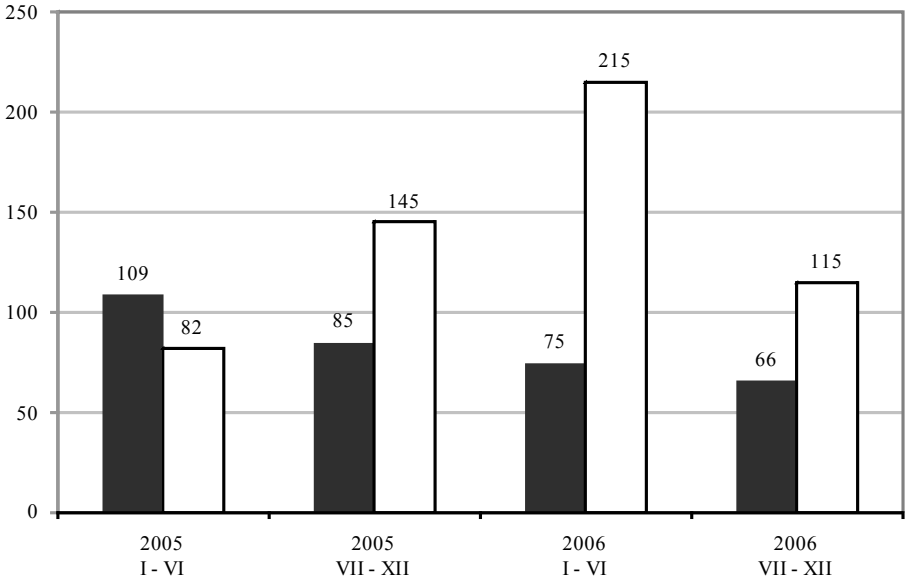



Figure 1. Number of rabies cases registered in Latvia between 2005 and 2006 in areas vaccinated and unvaccinated in 2005: black – vaccinated area, white – unvaccinated area.

Brain samples of target animals hunted in West Latvia (1,360 foxes and 525 raccoon dogs) for monitoring of the 2005 ORV campaigns revealed that only 1% of foxes and 2.1% of the raccoon dogs were positive for rabies confirming that animals from the hunting bag are not a reliable source for rabies surveillance. Investigating indicator animals (suspect animals, animals showing abnormal behaviour**,** road kills and animals found dead) should be given priority (Cliquet et al., 2010).

Determination of TC, a post-mortem biomarker, provides information on bait uptake in the target population and can be used to identify failures in ORV campaigns (Anonymous, 2002). Vaccination coverage of 70% of population in a given area is supposed to be efficient in breaking the transmission cycle of rabies among foxes (Anonymous, 2005). However, there is evidence that even lower vaccination coverages can result in rabies elimination (Thulke and Eisinger, 2008). After the 2005 ORV campaigns, 51.6% of 1,892 animals (1,364 foxes and 528 raccoon dogs) were positive for TC. Based on our results, foxes showed a higher bait uptake rate (56.2%) than raccoon dogs (47.0%) (Tables 2 and 3). However, differences in bait uptake and herd immunity observed between districts bordering with unvaccinated area and others were not statistically significant. Since the age of the animals submitted for testing was not analysed, the results could be influenced by unknown proportion of cubs tested. Especially during spring vaccination campaign in 2005, it is most likely that juvenile animals were still too young to consume vaccine baits. Although additional vaccination campaigns aimed at juvenile foxes during summer months are a promising alternative (Selhorst et al., 2001), they can get very cost-ineffective as the home ranges of the juveniles are still too small at this time of the year compared to adult animals (Vos et al., 2008). In any case, the age of the animals should be taken into consideration in future analysis to obtain more precise results on bait uptake and herd immunity. The results of bait uptake obtained during this study can be also influenced from ORV campaigns carried out in previous years.

Table 2

**Bait uptake and herd immunity results with confidence intervals (CI) in red foxes**

| | TC | | | | FAVN | | | | ELISA | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| District | n | % positive | P=0.05 CI | | n | % positive | P=0.05 CI | | n | % positive | P=0.05 CI | |
| Aizkraukle | 37 | 43.2 | 0.27 | 0.61 | 9 | 44.4 | 0.14 | 0.79 | 28 | 17.9 | 0.06 | 0.37 |
| Bauska | 114 | 57.0 | 0.47 | 0.66 | 21 | 42.9 | 0.22 | 0.66 | 90 | 14.4 | 0.08 | 0.23 |
| Daugavpils | 81 | 59.3 | 0.47 | 0.70 | 7 | 14.3 | 0.00 | 0.58 | 74 | 12.2 | 0.06 | 0.22 |
| Dobele | 114 | 46.5 | 0.37 | 0.56 | 37 | 32.4 | 0.18 | 0.50 | 74 | 10.8 | 0.05 | 0.20 |
| Jēkabpils | 74 | 60.8 | 0.49 | 0.72 | 12 | 66.7 | 0.35 | 0.90 | 62 | 21.0 | 0.12 | 0.33 |
| Jelgava | 104 | 55.8 | 0.46 | 0.66 | 22 | 63.6 | 0.40 | 0.82 | 79 | 8.9 | 0.04 | 0.17 |
| Krāslava | 10 | 40.0 | 0.12 | 0.74 | 2 | 50.0 | 0.01 | 0.99 | 8 | 12.5 | 0.00 | 0.53 |
| Kuldīga | 128 | 59.4 | 0.50 | 0.68 | 34 | 41.2 | 0.25 | 0.59 | 90 | 24.4 | 0.16 | 0.35 |
| Liepāja | 132 | 61.4 | 0.52 | 0.70 | 31 | 64.5 | 0.45 | 0.81 | 98 | 16.3 | 0.10 | 0.25 |
| Ogre | 10 | 60.0 | 0.26 | 0.88 | 0 | 0.0 | 0.00 | 0.00 | 10 | 20.0 | 0.03 | 0.56 |
| Rīga | 32 | 31.3 | 0.16 | 0.50 | 0 | 0.0 | 0.00 | 0.00 | 32 | 12.5 | 0.04 | 0.29 |
| Saldus | 131 | 52.7 | 0.43 | 0.61 | 21 | 42.9 | 2.33 | 1.67 | 110 | 11.8 | 0.06 | 0.19 |
| Talsi | 154 | 57.1 | 0.49 | 0.65 | 26 | 50.0 | 0.30 | 0.70 | 126 | 15.9 | 0.10 | 0.23 |
| Tukums | 126 | 61.9 | 0.53 | 0.70 | 49 | 63.3 | 0.48 | 0.76 | 73 | 13.7 | 0.07 | 0.24 |
| Ventspils | 117 | 59.0 | 0.50 | 0.68 | 21 | 38.1 | 0.18 | 0.62 | 96 | 19.8 | 0.12 | 0.29 |
| Total | 1364 | 56.2 | 0.53 | 0.59 | 292 | 49.3 | 0.43 | 0.56 | 1050 | 15.4 | 0.13 | 0.18 |

For the evaluation of herd immunity, blood samples from 1,858 hunted target animals (1,342 foxes and 516 raccoon dogs) were tested. Two laboratory diagnostic tests were used – FAVN and ELISA. On average, 50.6% (n=216) of the animals analysed using FAVN test showed presence of antibodies. However, only 14.8% (n= 215) of the animals analysed using BioRad ELISA test had seroconverted. On average, seroconversion rates were similar in fox and raccoon dog; however, differences were observed in various administrative districts (Tables 2 and 3).

Table 3

**Bait uptake and herd immunity results with confidence intervals (CI) in raccoon dogs**

| | TC | | | | FAVN | | | | ELISA | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| District | n | % positive | P=0.05 CI | | n | % positive | P=0.05 CI | | n | % positive | P=0.05 CI | |
| Aizkraukle | 10 | 60.0 | 0.26 | 0.88 | 4 | 50.0 | 0.07 | 0.93 | 6 | 16.7 | 0.00 | 0.64 |
| Bauska | 33 | 36.4 | 0.20 | 0.55 | 8 | 75.0 | 0.35 | 0.97 | 25 | 8.0 | 0.00 | 0.26 |
| Daugavpils | 55 | 32.7 | 0.20 | 0.47 | 2 | 50.0 | 0.01 | 0.99 | 53 | 7.5 | 0.02 | 0.18 |
| Dobele | 71 | 39.4 | 0.28 | 0.52 | 36 | 33.3 | 0.19 | 0.51 | 35 | 14.3 | 0.05 | 0.30 |
| Jēkabpils | 56 | 53.6 | 0.39 | 0.67 | 12 | 25.0 | 0.05 | 0.57 | 44 | 15.9 | 0.07 | 0.30 |
| Jelgava | 21 | 28.6 | 0.11 | 0.52 | 2 | 0.0 | 0.00 | 0.84 | 18 | 16.7 | 0.04 | 0.41 |
| Krāslava | 6 | 50.0 | 0.12 | 0.88 | 2 | 50.0 | 0.01 | 0.99 | 4 | 0.0 | 0.00 | 0.60 |
| Kuldīga | 49 | 53.1 | 0.38 | 0.67 | 27 | 59.3 | 0.39 | 0.78 | 21 | 19.0 | 0.05 | 0.42 |
| Liepāja | 30 | 63.3 | 0.44 | 0.80 | 11 | 54.5 | 0.23 | 0.83 | 17 | 23.5 | 0.07 | 0.50 |
| Ogre | 6 | 16.7 | 0.00 | 0.64 | 0 | 0.0 | 0.00 | 0.00 | 6 | 0.0 | 0.00 | 0.50 |
| Rīga | 2 | 50.0 | 0.01 | 0.99 | 0 | 0.0 | 0.00 | 0.00 | 1 | 0.0 | 0.00 | 0.98 |
| Saldus | 71 | 50.7 | 0.39 | 0.63 | 7 | 85.7 | 0.42 | 0.10 | 63 | 20.6 | 0.11 | 0.33 |
| Talsi | 33 | 42.4 | 0.25 | 0.60 | 7 | 57.1 | 0.18 | 0.90 | 24 | 12.5 | 0.03 | 0.32 |
| Tukums | 31 | 58.1 | 0.39 | 0.75 | 15 | 66.7 | 0.38 | 0.88 | 14 | 7.1 | 0.00 | 0.34 |
| Ventspils | 54 | 55.6 | 0.41 | 0.69 | 6 | 83.3 | 0.36 | 0.10 | 46 | 13.0 | 0.05 | 0.26 |
| Total | 528 | 47.0 | 0.43 | 0.51 | 139 | 51.8 | 0.43 | 0.60 | 377 | 14.1 | 0.11 | 0.18 |

The discrepancy of immunity level found with FAVN (49.3% positive in foxes; 51.8% positive in raccoon dogs) and ELISA (15.4% positive in foxes; 14.1% positive in raccoon dogs) is very high. This is in accord with other studies which found similar low seroconversion with the same test confirming assumptions that this particular

commercial serological ELISA test has an insufficient sensitivity for wildlife sera and therefore should not be used for wildlife (Knoop et al., 2010).

## Conclusions

The results of this study clearly demonstrate that in contrast to previous years the first implementation of aerial large-scale ORV campaigns using SAD B19 oral rabies virus vaccine in 2005 substantially improved the epidemiological rabies situation in vaccinated areas in Latvia. This strongly suggests large-scale aerial distribution of vaccine baits to be the method of choice for future ORV campaigns. Nevertheless, complementary manual distribution of vaccine baits should be considered in urban and suburban settlements or other non-flying zones.

Adequate rabies surveillance and continuous monitoring of ORV campaigns should be guaranteed to analyse the success of ORV campaigns and to allow flexible adaptation of the vaccination strategy in the future.

## Acknowledgements

Academic study and publication was financed by the Project 'Support for doctoral studies in LUA' Contract No. 2009/0180/1DP/1.1.2.1.2/09/IPIA/VIAA/017/, agreement No. 04.4-08/EF2.D1.23.

The authors would like to thank Emīls Jēgers for consultation and historical data.

Special thanks to Dr. Thomas Müller and Dr. Conrad Freuling from WHO Collaborating Centre for Rabies Surveillance and Research (Germany) for technical assistance and advice.

## References

1. Anonymous (2002) The oral vaccination of foxes against rabies. Report of the scientific committee on animal health and animal welfare, European Commission, 55 p. Available at: http://ec.europa.eu/food/fs/sc/scah/out80_en.pdf, 11 January 2011.
2. Anonymous (2005) WHO Expert Consultation on Rabies. *WHO Technical Report Series;* 931, First report, Geneva, Switzerland, 121 p. Available at: http://www.who.int/rabies/trs931_%2006_05.pdf, 15 December 2010.
3. Anonymous (2008) Austria and Germany declared 'free from terrestrial rabies'. *Rabies Bulletin Europe*, Vol. 32 (2), 6 p.
4. Bourhy H., Dacheux L., Strady C. and Mailles A. (2005) Rabies in Europe in 2005. *Euro Surveillance*, 10(11), p. 575. Available at: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=575, 6 December 2010.
5. Cliquet F., Aubert M.F., Sagn´e L. (1998) Development of a fluorescent antibody virus neutralizing test (FAVN test) for the quantitation of rabies-neutralising antibody. *Journal of Immunological Methods,* 212, pp. 79-87.
6. Cliquet F. and Barrat J. (2008) Rabies (chapter 2.1.13). *Manual of diagnostic tests and vaccines for terrestrial animals*, OIE Publications, Paris, pp. 304-322.
7. Cliquet F., Freuling C., Smreczak M., Van der Poel W.H.M., Horton D., Fooks A.R., Robardet E., Picard-Meyer E., Müller T. (2010) Development of harmonised schemes for monitoring and reporting of rabies in animals in the European Union. Available at: http://www.efsa.europa.eu/en/scdocs/scdoc/67e.htm, 22 February 2011.
8. Cliquet F., Müller T., Mutinelli F., Geronutti S., Brochier B., Selhorst T., Schereffer J.L., Krafft N., Burow J., Schameitat A., Schlüter H., Aubert M. (2003) Standardisation and establishment of a rabies ELISA test in European laboratories for assessing the efficacy of oral fox vaccination campaigns. *Vaccine,* 21, pp. 2986-2993.
9. Jackson A.C. and Wunner W.H. (2007) *Rabies.* Second edition, Elsvier, London, UK, 660 p.
10. Johnston D.H., Joachim D.G., Bachmann P., Kardon K.V., Stewart R.F.A., Dix L.M., Strickland M.A. and Watt F.O. (1987) Aging furbearers using tooth structure and biomarkers. In: Novak M.J., Baker J.A., Obbard M.E. and Malloch B. (eds). *Wild furbearer management and conservation in North America*, Trappers Association, North Bay, Ontario, Canada, pp. 228-243.
11. Knoop E.V., Freuling C.M., Kliemt J., Selhorst T., Conraths F.J., Müller T. (2010) Evaluation of a commercial rabies ELISA as a replacement for serum neutralization assays as part of the pet travel scheme and oral vaccination campaigns. *Berliner Münchener Tierärztliche Wochenschrift* 123, 7/8, pp. 278-285.
12. Linhart S. and Kennelly J.J. (1967) Fluorescent bone labelling of coyotes with demethyl-chlortetracycline. *Journal of Wildlife Management* 31, pp. 317-321.
13. Maciulskis P., Lukauskas K., Sederevicius A., Kiudulas V., Pockevicius A. (2006) Epidemiology of enzootic rabies in Lithuania. *Medycyna Wet,* 62(7), pp. 769-772.
14. Matouch O. and Polak L. (1982) Rabies epizootiology and control in Czechoslovakia. *Comparative Immunology, Microbiology and Infectious Diseases* 5, pp. 303-307.
15. Milius J., Jacevicius E., Tamasiunas V., Paulauskas V. and Lukauskas K. (2004) Rabies among wild and domestic animals in Lithuania in 1993-2002: epizootiology, diagnosis and vaccination. *The Bulletin of the Veterinary Institute in Pulawy,* 48, pp. 195-200.
16. Müller W.W. (2000) Review of rabies case data in Europe to the WHO Collaborating Centre Tübingen from 1977 to 2000. *Rabies Bulletin Europe*, 4, pp. 11-19.
17. Neubert A., Schuster P., Müller T., Vos A. and Pommerening E. (2001) Immunogenicity and efficacy of the oral rabies vaccine SAD B19 in foxes. *Journal of Veterinary Medicine* B 48, pp. 179-183.
18. Niin E., Barrat J., Kristian M., Demerson J.M., Cliquet F. (2006) First oral vaccination of wildlife against rabies in Estonia. In: Dodet B., Schudel A.,

Pastoret P.P., Lombard M. (eds) Rabies in Europe, *Developmental Biology* (Basel) Karger, vol., 125 pp. 145-147.

19. Niin E., Laine M., Guiot A.L., Demerson J.M., Cliquet F. (2008) Rabies in Estonia: Situation before and after the first campaigns of oral vaccination of wildlife with SAG2 vaccine bait. *Vaccine* 26, pp. 3556-3565.
20. Selhorst T., Thulke H.H., Müller T. (2001) Cost-efficient vaccination of foxes *(Vulpes vulpes)* against rabies and need for a new baiting strategy. *Preventive Veterinary Medicine* 51, pp. 95-109.
21. Singer A., Kauhala K., Holmala K., Smith G.C. (2009) Rabies in north-eastern Europe – the threat from invasive raccoon dogs. *Journal of wildlife diseases,* 45 (4), pp. 1121-1137.
22. Thulke H.H., Eisinger D. (2008) The strength of 70%: revision of a standard threshold of rabies control. *Developmental Biology* (Basel), 131, pp. 291-298.
23. Vanaga S., Van der Heide R., Joffe R., Van der Poel W.H. (2003) Rabies in wildlife in Latvia. *Vector-Borne and Zoonotic Diseases* Vol., 3, No. 3, pp. 117-124.
24. Vos A., Selhorst T., Schröder R. and Mulder J. (2008) Feasibility of oral rabies vaccination campaigns of young foxes (*Vulpes vulpes*) against rabies in summer. *European Journal of Wildlife Research* 54, 4, pp. 763-766.
25. Westerling B., Andersons Z., Rimeicans J., Lukauskas K. and Dranseika A. (2004) Rabies in Baltics. In: King A.A., Fooks A.R., Aubert M., Wandeler A.I. (eds). *Historical perspective of rabies in Europe and Mediterranean Basin*, Paris, OIE Publications, pp. 33-46.

# DEVELOPMENT OF EXPERIMENTAL EQUIPMENT FOR VEGETABLE OIL FUEL RESEARCH

***Aivars Birkavs, Ilmars Dukulis***
*Latvia University of Agriculture*
*aivars.birkavs@llu.lv; ilmars.dukulis@llu.lv*

## Abstract

The European Parliament and Council Directive 2003/30/EK 'On the promotion of the use of biofuels and other renewable fuels for transport' determines that pure or straight vegetable oil, produced from oil plants by pressing, extracting or comparable procedures, crude or refined but chemically unmodified, compatible with common engines, and corresponding to emission requirements, is also considered as biofuel. The biggest problems imposed by these conditions are directly associated with the carrying-out of the emission requirements, because when using vegetable oil as a fuel, usually increases the composition of the solid particles and nitrogen oxides in exhaust gases, that not only adversely affect the environment, but also is a serious threat to human health, and as a result trying to save the world from the global warming, human health continues to deteriorate. It is therefore necessary to carry out studies and find solutions to reduce harmful emissions from diesel engines when using vegetable oil fuel. For more qualitative and effective research on vegetable oil fuel emissions, the equipment for vegetable oil fuel testing has been developed. This equipment allows fast checking of theoretically proposed hypotheses and detailed calculations for vegetable oil fuel combustion processes and objective data acquisition. The equipment consists of the classic diesel engine adapted for work with vegetable oil and is equipped with several high-precision devices to get and store the measuring data. During pilot tests the optimal measuring modes (engine rotation frequencies, number and duration of repetitions) for further research are estimated.

**Key words**: straight vegetable oil (SVO), two-tank system, exhaust emissions.

## Introduction

As technologies evolve, vegetable oil in pure form or as a blend with fossil diesel is increasingly used as a diesel engine fuel. Based on the results of previous studies, it may be concluded that the vegetable oil which corresponds to EU standards can be used in diesel engines for a long time, and its application does not cause the engine or the system damage. Investigations on vegetable oil fuel emissions composition prove that the content of the toxic substances in the exhaust gases, similar to biodiesel, is reduced in comparison with the fossil diesel, and the quantity of the certain toxic emissions is even less than using biodiesel. However, the vegetable oil as fuel does not display very significant reduction of the harmful emissions in the exhaust gases. Particularly, the level of the mechanical particles and nitrogen oxide either increases or slightly decreases depending on the type of the engine, method of conversion, and fuel injection in a diesel engine cylinder (Dukulis et al., 2010).

The exhaust gases contain such harmful substances as nitrogen oxides, carbon oxides, hydrocarbons, carbon monoxide, soot, particulate matter, and sulphur dioxide. The most harmful exhaust gases arise from the engine warming up and idling.

In the previous studies performed at the Scientific Laboratory of Biofuels (Latvia University of Agriculture) generally were used vehicles as a whole (cars) that had been tested on power bench (Dukulis et al., 2009, 2009a, 2009b, 2010). Investigating such vehicles, the original car fuel tank was used also for vegetable oil, and thereby it was difficult to carry out experiments on various vegetable oil blends. Therefore, only 'pure' fuels, i.e., fossil diesel, biodiesel and rapeseed oil were used in these experiments. Pilot tests on different fuel blends were labour and time consuming, because a large amount of fuel was needed for testing, and it was practically impossible to empty fuel tank when changing the fuel. So, when carrying out experiments on a car with diesel engine running on vegetable oil, it is impossible to draw general conclusions about the change tendencies of the exhaust gases, using a variety of fuel or fuel blends, because the switching from one fuel to another creates complications, for example, it is not possible to determine the time taken for the new fuel to fill the entire fuel system, as well as it's difficult to determine the fuel consumption in different engine operating modes.

Another problem is that the vegetable oil usually is not compatible with the modern exhaust gas purification systems, such as particulate filters or SCR (*Selective Catalytic Reduction*) system. Using vegetable oil as a fuel, it is impossible to make full use of the vehicle potential to reduce the harmful emissions (Alternative Fuels, 2007).

Taking into account these arguments and in order to successfully carry out experiments for the purpose of determining the exhaust gas and fuel consumption changes of the vegetable oil and blends, the following tasks were set for this investigation:

- to build a diesel engine test bench;
- to equip diesel engine with the 'two-tank system' to run on vegetable oil;
- to provide a free exhaust system, i.e., not to use catalytic neutralisation and exhaust gas recirculation;
- to supplement diesel control measuring equipment with the engine rotation frequency meter;
- to work out and connect the computerized fuel consumption measuring system;

- to estimate the optimal measuring modes (speed, time, number of repetitions) for further studies.

## Materials and Methods

The equipment for determination of vegetable oil fuel emissions from diesel engines and for measuring of fuel consumption operated in the laboratory, where temperature of the surrounding air was 20 – 22 °C. The exhaust gas absorption system was added to the engine exhaust system to prevent the harmful effect of the emissions during the experiment.

The equipment consisted of *Opel 16 DA* diesel engine, *Elsbett* 'two-tank system' to run engine on vegetable oil, and *KERN 440-49A* weighing system. To determine the composition of the exhaust gases during the experiment, *AVL SESAM FTIR* multicomponent exhaust gas measurement system was additionally connected. For the engine rotation frequency control, the stroboscope *DG 85* was used, receiving impulses from the first cylinder high-pressure pipe.

*Opel 16 DA* diesel engine is single line *4/OHC* engine having a capacity of 1598 $cm^3$, compression ratio – 23, and the power of 40 kW. Diesel fuel system is powered by fuel pump *Bosch VE 4/9R215* providing a nozzle opening pressure to 135 bar.

To run the diesel engine on vegetable oil, *Elsbett* 'two-tank system' was installed and consisted of the following components:

- additional 20 litre fuel tank;
- additional fuel filter;
- electric fuel pumps;
- heat exchanger for fuel heating;
- thermo switch;
- electromagnetic valve;
- back-pressure valve;
- control unit;
- switches and sockets;
- fuel and cooling liquid pipelines.

'Two-tank system' included the following subsystems:

- fuel supply and heating system (Figure 1);
- electronic control system (Figure 2).

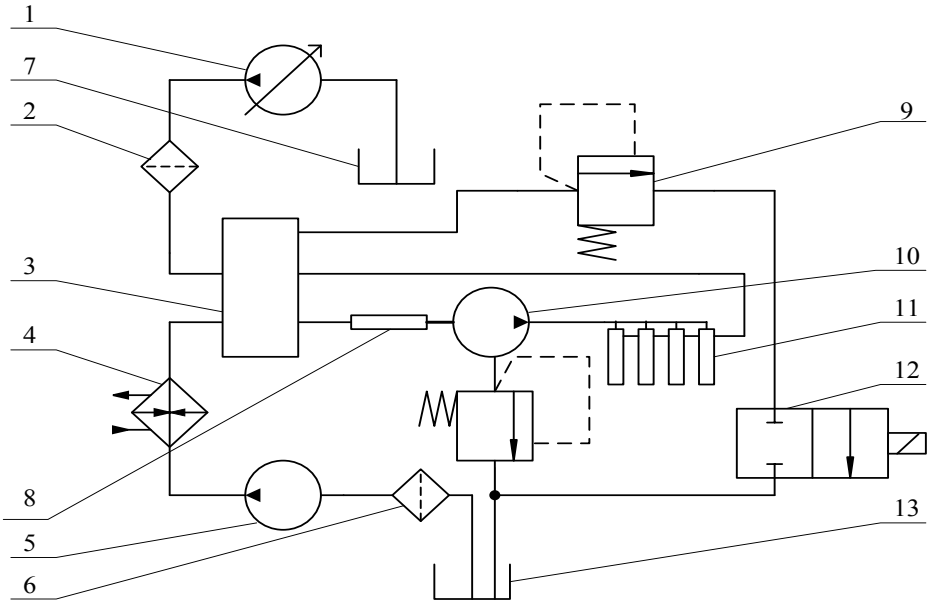



Figure 1. Fuel supply and heating system: 1, 5 – electric pumps; 2 – fossil fuel filter; 3 – distributor; 4 – heat exchanger; 6 – vegetable oil filter; 7 – fossil fuel tank; 8 – transparent glass tube; 9 – one-way valve; 10 – high-pressure fuel pump; 11 – nozzles; 12 – electromagnetic valve; 13 – vegetable oil tank.

The fuel supply and heating system was based on fuel pumps which were intended for the vegetable oil and fossil diesel fuel supply to a diesel engine high-pressure pump, as well as for providing the low-pressure system with the required fuel pressure. To guarantee the vegetable oil with the necessary viscosity, served a heat exchanger that provided heat transfer between the cooling system and the vegetable oil supply system. An additional fuel tank served for the fossil diesel fuel storage, required for the diesel engine warm-up process, as well as before fuel supply system to be filled. Electromagnetic valve provided vegetable oil flow to the vegetable oil fuel tank. The valve could be controlled also manually.

The electronic control system was based on the control unit which task was to ensure a proper function of all components and to maintain stable running of the engine with fossil diesel and vegetable oil or blends. One of the most important elements of the electrical system was thermo switch that allowed switching the diesel engine running on vegetable oil only after the engine has reached 70 – 80 °C temperature. If the engine temperature dropped below 70 °C, the system automatically switched to powering by fossil diesel fuel.

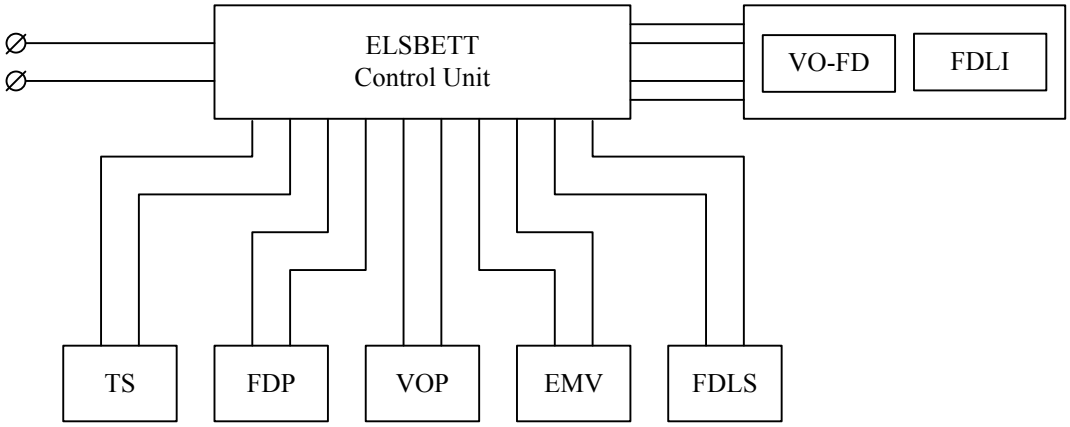



Figure 2. Simplified electronic control system: TS – thermo switch; FDP – fossil diesel fuel pump; VOP – vegetable oil pump; EMV – electromagnetic valve; FDLS – fossil diesel fuel level sensor; VO-FD – vegetable oil to fossil diesel fuel switch; FDLI – fossil diesel fuel level indicator.

The fuel consumption measurement system was developed on the basis of *KERN 440-49A* electronic weighing system which provides measurements with an accuracy of 0.1 g at intervals of 1 second. Weighing system management and data registration is computer controlled.

Before starting the measurements, diesel exhaust pipe was connected to the *AVL SESAM FTIR* multicomponent exhaust gas measurement system, and the following operations were done:

- connect the exhaust gas extraction system;
- connect the engine revolution frequency meter (stroboscope);
- inspect engine technical condition, paying particular attention to engine oil and coolant levels;
- turn on electricity supply;
- connect and adjust electronic weighing system;
- place the fuel storage container on the weighing platform and fix the fuel hoses so that they do not touch the container edges;
- fill the fuel container with tested fuel;
- check whether diesel engine is switched to powering by fossil diesel fuel;
- start the diesel engine;
- check the stroboscope operation;
- warm the engine up to 70 – 80 °C;
- switch the engine to work on the tested fuel.

To make it easier to determine what fuel is in the fuel supply system, a transparent glass tube was built in the system. If it is not possible to visually determine what fuel there is in the supply system, starting another fuel testing it is necessary to undertake the following actions:

- pour out the previous test fuel from container;
- fill the new test fuel into a fuel container;
- remove the back-flow pipe from the fuel container and place in a measuring cup;
- check whether diesel engine is switched to work with vegetable oil and start the engine;
- ensure that the system works with the new fuel;
- stop the engine and put the back-flow pipe into the fuel container;
- adjust electronic weighing system;
- start the engine and the measurement.

The results of the fuel consumption and exhaust gas content were fixed by the computerized measurement systems, and then processed with a spreadsheet application tools.

## Results and Discussion

The investigation resulted in a fully functioning diesel engine test bench, equipped with a ‘two-tank system’ to run on rapeseed oil, supplemented by the engine rotation frequency meter and connected to a computerized fuel consumption measurement system (Figure 3).

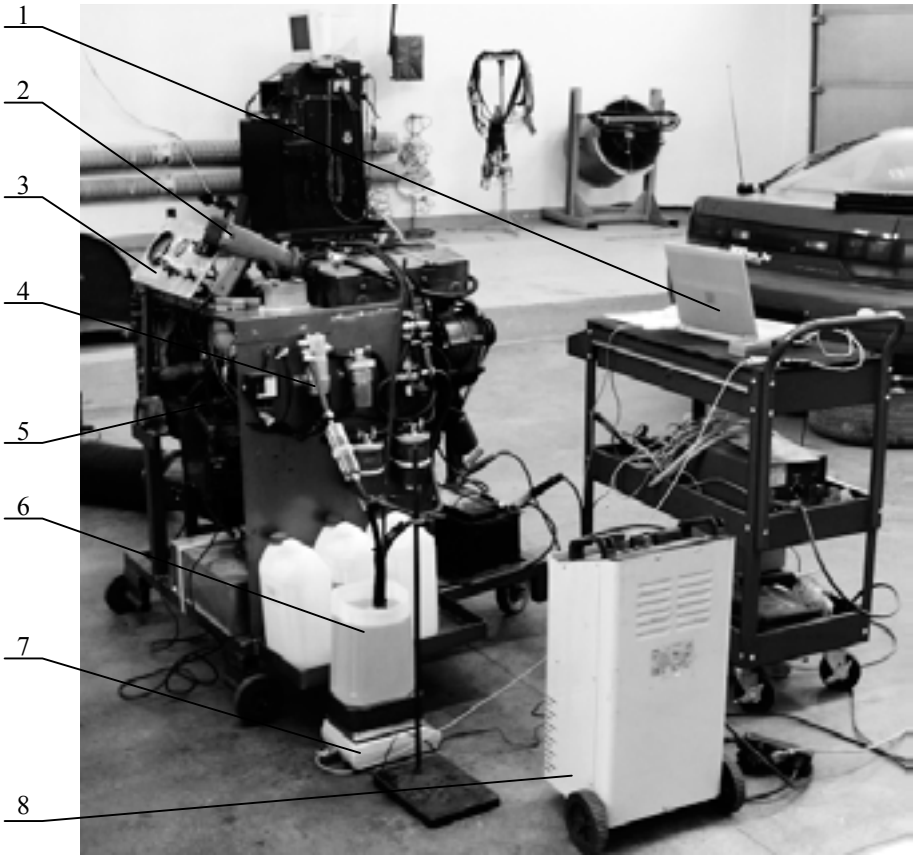



Figure 3. Diesel engine test bench: 1 – PC with special *KERN 440-49A* software; 2 – stroboscope *DG 85*; 3 – *ELSBETT* control unit; 4 – 'two-tank system' components; 5 – *Opel 16 DA* diesel engine; 6 – container with tested fuel; 7 – *KERN 440-49A* weighing system; 8 – additional electric power source.

To get reliable and verifiable results of different fuel or fuel blends experiments, the testing modes were determined, as well as the fuel system volume, timing for each test, and the number of repetitions.

Engine rotation frequency increase contributes to oxides ($NO_x$, NO and CO) increase, while the engine working at nominal speed significantly reduces the carbon monoxide content of exhaust gases (Smigins, 2010). Consequently, three speed regimes were selected to determine the emission content changes – 850 to 900 rpm, 1500 rpm and 2500 rpm.

Fuel system volume was determined experimentally, using two fuels with different colours. The fuel system was switched to a new fuel, but the back-flow fuel pipe was placed into a separate measuring cup. By visual observation (transparent glass tube in the system) filling up of the fuel supply system with the new fuel was stated. According to the measurement it was established that the fuel supply system volume was 1.5 litres. It means that this volume has to flow out of the system before the experiments with the new fuel can be started.

To determine the optimal duration for each experiment and the number of repetitions, pilot tests were carried out. Taking into account the fuel consumption absolute values for each test mode, at the lowest engine speeds, i.e., 850 rpm, the duration for each experiment initially was chosen longer (5 minutes) than at higher speeds, i.e., 1500 and 2500 rpm (3 minutes).

Whereas the *KERN 440-49A* weights can give a signal to a computerized data recording system only when the weights are at equilibrium, but flowing out fuel from the container does not always provide such a balance then simple reading of the values of fuel mass at the beginning and the end of the experiment by default can cause errors in research results. Therefore fuel consumption graphs for each test mode were created, but the regression function and $R^2$ value characterizing the closeness between experimental data and the regression function were determined for the first minute data, the first two minutes data, the first three minutes data, and so on.

As an example Figure 4 shows such pilot test fuel consumption graphs at 2500 rpm.

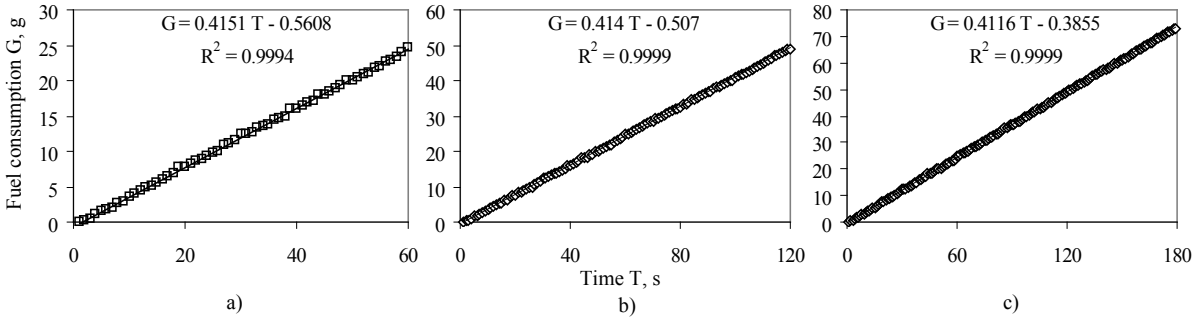



Figure 4. Pilot test fuel consumption graphs at 2500 rpm: a – 1st minute of test; b – 1st and 2nd minute of test; c – 1st, 2nd and 3rd minute of test.

As can be seen from the graphs, after the second measuring minute the $R^2$ value stopped to increase, so at 2500 rpm for further research the duration for each measurement can be estimated to 2 minutes, but the regression function can be used for calculating the hourly fuel consumption in this mode, for example:

$$G_{hour} = 0.414 \cdot T_{hour} - 0.507 = 0.414 \cdot 3600 - 0.507 = 1489.9\ g\ h^{-1}$$

By analogy, it was determined that at the lowest engine speeds, i.e., 850 – 900 rpm, the optimal duration of each measurement was 3 minutes, but at 1500 rpm – 2 minutes.

The correlation between the measurement results of individual repetitions was calculated. As an example Table 1 shows the fuel consumption data fragment from three repetitions (tests) at 850 rpm.

Table 1

**Correlation calculations between the fuel consumption measurement results of individual repetitions**

| Time, s | Fuel consumption, g | | |
|---|---|---|---|
| | Test No 1 | Test No 2 | Test No 3 |
| 1 | 0 | 0 | 0 |
| 2 | 0.3 | 0.3 | 0.6 |
| 3 | 0.6 | 0.3 | 0.6 |
| 4 | 0.6 | 0.9 | 0.6 |
| 5 | 0.9 | 0.9 | 0.9 |
| 6 | 0.9 | 0.9 | 0.9 |
| 7 | 1.2 | 1.4 | 0.9 |
| 8 | 1.2 | 1.4 | 1.2 |
| 9 | 1.4 | 1.7 | 1.4 |
| 10 | 1.7 | 1.7 | 1.4 |
| … | … | … | … |
| … | … | … | … |
| … | … | … | … |
| 178 | 30.2 | 29.5 | 28.4 |
| 179 | 30.4 | 29.9 | 28.7 |
| 180 | 30.4 | 29.9 | 29.2 |
| **Correlation** | | | |
| **Test No 1** | N/A | 0.999005 | 0.999246 |
| **Test No 2** | 0.999005 | N/A | 0.999524 |
| **Test No 3** | 0.999246 | 0.999524 | N/A |

As can be seen from the data of Table 1, correlation between the individual data points of three measurement repetitions was above 99.9%. These results qualify as a high rating, therefore more than three repetitions at each of the modes are not needed.

Finally, it was verified if at a chosen number of repetitions and duration of each experiment there is sufficiently close dispersion of exhaust gas components, that, taking into account the fact that the exhaust gases are not 'very homogeneous substances', is common in experiments of many researchers. As an example Table 2 shows the exhaust emission measurement data from three repetitions (tests) at 850 rpm comparing the components most often analyzed in studies around the world.

Table 2

**Exhaust emission measurement results of individual repetitions**

| Test No | Content in exhaust emissions, ppm | | | | | |
|---|---|---|---|---|---|---|
| | $NO_x$ | Mechanical particles | $SO_2$ | Unburned hydrocarbons | $CO_2$ | CO |
| 1 | 167.72 | 5.50 | 0.28 | 141.36 | 23700.37 | 354.46 |
| 2 | 169.80 | 5.47 | 0.29 | 144.17 | 23422.70 | 349.72 |
| 3 | 168.38 | 5.27 | 0.29 | 141.01 | 23763.61 | 354.03 |
| Average | 168.64 | 5.41 | 0.28 | 142.18 | 23628.89 | 352.74 |
| Difference, (Max/Min) % | 1.25% | 4.24% | 2.57% | 2.24% | 1.46% | 1.36% |

The differences between individual repetition maximum and minimum values did not exceed 5%, which for exhaust emission studies was assessed as a very good result, therefore all selected modes were considered optimal for future studies.

## Conclusions

1. Developed diesel engine test bench effectively allows carrying out research on vegetable oil fuel and fossil fuel blend combustion process.
2. The main advantage of the installed 'two-tank system' is that experiments with vegetable oil fuel can be started or stopped at any engine temperatures, without any engine or fuel system damage.
3. Free exhaust system enables to determine the emission content changes directly after fuel combustion process.
4. The use of the stroboscope for rotation frequency measurement is the most accurate from analogue systems because it gets impulse from high pressure fuel pipe at the moment of the fuel injection.
5. Computerized fuel consumption measurement system, worked out on the basis of *KERN 440-49A* weighing system, can be used for high-accuracy dynamic measurements on the fuel consumption changes.
6. During pilot tests the optimal measuring modes (engine rotation frequencies, number and duration of repetitions) for further studies were estimated. At the lowest engine speeds, i.e., 850 – 900 rpm, the optimal duration of each measurement was 3 minutes, but at 1500 rpm and 2500 rpm – 2 minutes. Three repetitions at each of the modes are enough to get valid results.

## Acknowledgements

The authors gratefully acknowledge the funding from Latvian Council of Science (project No. 09.1602 'Investigations of biofuel and biomass technologies') and the European Union and European Structural Fund (grant No. 2009/0180/1DP/1.1.2.1.2/09/IPIA/VIAA/017 'Support for Doctoral Study Programs').

## References

1. Alternative Fuels (2007) Vol. 1. Teaching and Learning Material. Supported within the 6th EU Framework Programme, 108 p. Available at: http://www.infolizer.com/5eltisa1or7g/Alternative-fuels.html, 15 March 2011.
2. Dukulis I., Birkavs A., Birzietis G., Pirs V. (2009) Adapting of Automobile VW Golf for Using Pure Rape Oil As a Fuel. *Proceedings of the 8th International Scientific Conference 'Engineering for Rural Development'*. Latvia University of Agriculture, Jelgava, pp. 141-147.
3. Dukulis I., Pirs V., Jesko Z., Birkavs A., Birzietis G. (2009a) Development of Methodics for Testing Automobiles Operating on Biofuels. *Proceedings of the 8th International Scientific Conference 'Engineering for Rural Development'*. Latvia University of Agriculture, Jelgava, pp. 148-155.
4. Dukulis I., Pirs V., Jesko Z., Birkavs A., Birzietis G. (2009b) Testing of Automobile VW Golf Operating on Three Different Fuels. *Proceedings of the 8th International Scientific Conference 'Engineering for Rural Development'*. Latvia University of Agriculture, Jelgava, pp. 7-13.
5. Dukulis I., Birzietis G., Birkavs A., Pirs V., Jesko Z. (2010) Exhaust Emissions from the Vehicles Operating on Rapeseed Oil Fuel. *Agronomy Research*, Volume 8, Special Issue 1. Estonian University of Life Sciences, Tartu, pp. 39-46.
6. Smigins R. (2010) Impact of Biodiesel on Working Parameters of the Engine. Promotion Paper Summary for Acquaring the Doctor's Degree in Engineering Sciences. Latvia University of Agriculture, Jelgava, 54 p.

# EVALUATION OF STUDY PROGRAMME EXTERNAL QUALITY

***Sandra Sproģe***
*Latvia University of Agriculture*
*Sandra.Sproge@llu.lv*

**Abstract**

Quality assessment of a study programme is a topical issue in the single education area. Quality is not a unequivocal term in higher education area, which lends itself to many understandings and interpretations according to different criteria. The best practice of software engineering may be applied for the study programme evaluation if by analogy it is viewed as software product. Study programme similar to software product has internal and external quality. Students as direct users of the study programme may be engaged in the evaluation of its external quality, in case the evaluation of internal quality of the study programme is mainly based on internal resources of a higher education institution. The paper provides the methodology for evaluation of external quality of a study programme based on software product quality model and quality assurance standards. Approbation of the methodology was started in 2009 at the Faculty of Information Technologies, where students evaluated external quality of undergraduate study programmes. Results obtained during the approbation lead to the conclusion that the chosen methodology ensures significant information for the enhancement of quality of a study course and simultaneously the entire study programme.

**Key words**: study programme external quality, quality requirements, measurement, quality evaluation.

## Introduction

Quality enhancements of the study process and study programmes are issues that have always been more or less topical for higher education institutions. Currently this topicality is especially explicit when European countries collaborate on the establishment of a single Higher Education Area. In compliance with the decisions (Standards and Guidelines…, 2005) passed in the framework of the Bologna Process, the primary responsibility for the study process quality assurance in higher education lies with each institution itself. In the sphere of education, external assessment of the study programme relates to an audit performed by an external organisation and traditionally it is accreditation. Accreditation ensures the state guarantee for the study programme quality. However, internal quality assurance of a study programme includes measures regularly undertaken by higher education institutions themselves. Annual self-evaluation of study programmes is one of these measures. Self-evaluation is a vision of a higher education institution on the internal and external quality of the study programme. Consideration of the data included into self-evaluation reports of the study programmes leads to the conclusion that the valuation in compliance with the quality definition (Procedure for Accreditation…, 2006) is performed with regard to certain defined requirements. For example, Item "Evaluation of Study Programme" of the Cabinet Regulations "*Procedure for Accreditation of Higher Education Institutions, Colleges and Higher Education Study Programmes*" covers such information as "valuation system" (justification for the choice of valuation methods and analysis of results). Self-evaluation reports are also one of the sources of information used by external evaluation organisations. Hence, self-audits and self-evaluation reports prepared by an education institution are essential tools for enhancement of the study process. Criteria according to which a study programme is to be evaluated shall be defined prior to the performance of self-audit or self-evaluation of a study programme. Self-evaluation report shall include the following information: development of the study programme, its practical implementation, valuation system of study results, information on students, qualification of the academic staff, sources of financing and provision of infrastructure, recommendations for the enhancement of work quality, and programme development plan (Procedure for Accreditation, 2006). Nevertheless, this information is detailed, mainly it is descriptive information, and it does not reflect a detailed quality evaluation of the study programme content. It is difficult to make inter-comparison of study programmes or qualitative evaluation of their content based only on the self-evaluation reports of these study programmes. Literature suggests possibilities for the evaluation of study programmes. A. Briška (Briška, 2008) proposes to determine quantitative indicators, for example, number of applicants, financing, resources, number of students, study environment, academic staff, internationalisation, and alumnae, for the evaluation of the study programme. The higher the quantitative indicators, the more qualitative is the programme. I. Gvaramadze (Gvaramadze, 2008) suggests reorienting the focus from quality assurance towards quality culture, thus displaying two significant emphases – quality transformation on the level of academic and administrative staff of the education institution and quality enhancement on the administrative level of the education institution. According to I. Gvaramadze, periodic quality assurance mechanisms are not enough to deliver and maintain the quality level of a study programme but they need a permanent updating, and developing of means and facilities for programme delivery, educational processes and educational outcomes. H. Coates (Coates, 2005) in his researches denotes the increasing importance of student engagement in the study programme quality assurance and

enhancement. Students' evaluation may display the most essential drawbacks in the quality of a study programme as well as positive trends of the study programme quality.

## Materials and Methods

The author of the present paper would like to offer a method for the evaluation of external quality of the study programme based on the student engagement in the particular process. Quality evaluation methodology is based on the analogy with quality model of software product ISO 9126 and quality assurance standards. Internal and external quality of a study programme may be viewed similar to the quality of software products (Čevere and Sproģe, 2010). *External quality* – degree up to which programme satisfies a user's defined and imagined needs applying it on certain conditions. *Internal quality* – a set of programme attributes, which determines its ability to satisfy defined or indirectly specified needs, when applying it on certain conditions. Figure 1 reflects a schematic description of a study programme quality.

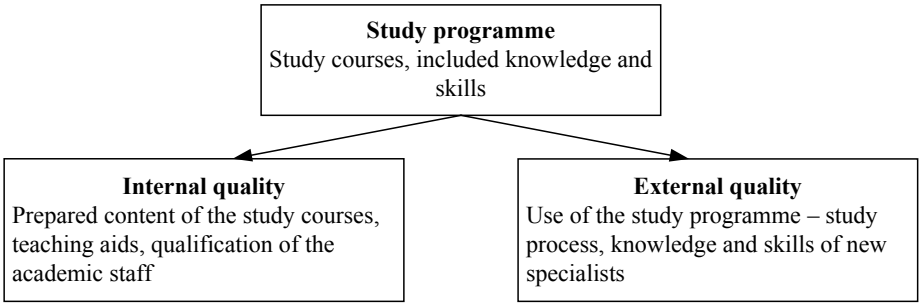



Figure 1. Classification of quality of a study programme.

Software product quality is determined by:

- internal quality – internal characteristics of software product;
- external quality – external characteristics of software product defined during its performance;
- quality in use – degree up to which a user may achieve its goal in a certain environment.

All software product characteristics originate during the development process. Quality of development process promotes assurance of product internal and external quality; while product quality structured by internal and external quality, promotes the quality of application (use). Feedback for the evaluation of application quality shall be established to achieve the enhancement of quality to be able to improve the product; while feedback for the evaluation of the development process shall be established for the evaluation of the product.

The software product quality model ISO 9126 (ISO/IEC 9126-1, 2001) earmarks 6 internal and external quality characteristics and 27 sub-characteristics (Figure 2), which generally determine the quality of a product.

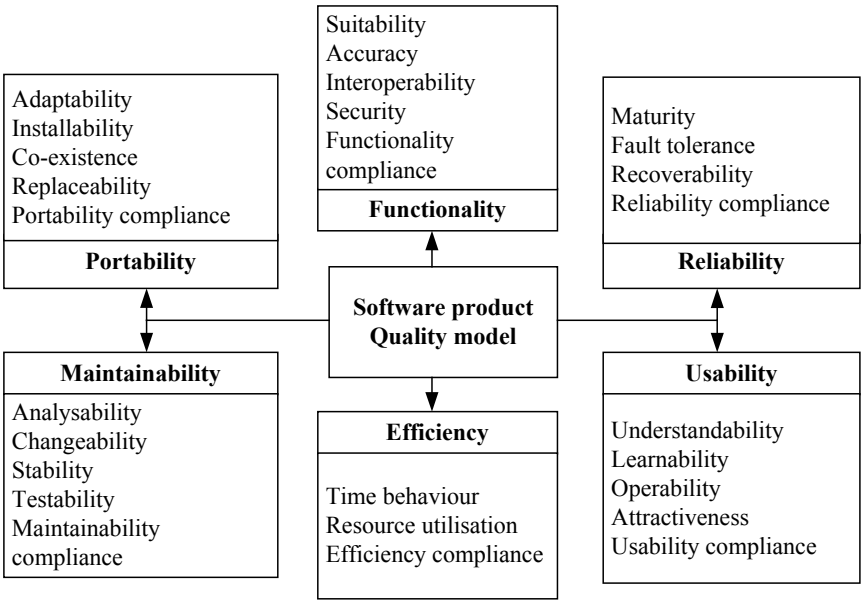



Figure 2. Software product quality model ISO 9126.

Quality characteristics of a model are related both to internal and external quality of software product. The same characteristics may be used, since the differences appear during measurement and type of applied metrics. Software product quality is defined by measuring its characteristics. The following notions are earmarked in the quality assurance of software product (ISO/IEC 15939, 2007):

- metrics – a definite method of measurement and scale of measures (metrics may be internal and external, direct and indirect; metrics include methods for categorisation of qualitative data);
- measurement – application of metrics to assign a value to an attribute of software product item from a definite scale (value may include either number or category);
- measure – value assigned to an attribute of an item during measurement;
- to measure – to define what amount of what specified quantitative unit exists.

Schematically a model for measurement of the software product quality is shown in Figure 3. The function of measurement ensures interpretation of quality characteristics, i.e. it assigns a definite value to an appropriate characteristic.

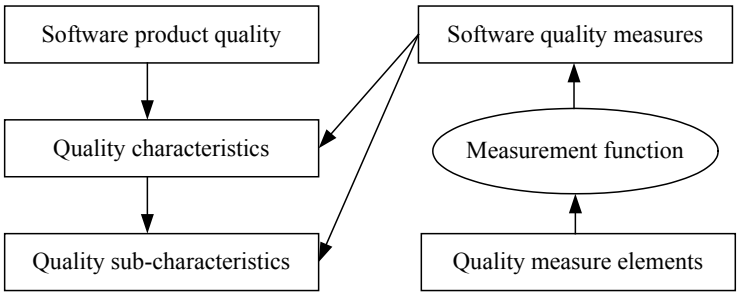



Figure 3. Software product quality measurement model (ISO/IEC 25030, 2007).

The author in her previous researches has demonstrated that ISO 9126 quality model and measurement process recommended by the standards may be applied in quality evaluation of a study programme (Sproge and Cevere, 2010). Similarly, as software product consists of various functions, a study programme consists of study courses. Every study course shall be evaluated to evaluate the whole programme. The characteristics of a quality model related to the study course are as follows:

- functionality – are all the required topics included into the study course;
- usability – is the study course easy to teach and to learn;
- efficiency – is the study course efficient, are the expected study results achieved;
- maintainability – is it easy to maintain the study course, are the necessary resources available;
- portability – is it easy to adapt the study course to another audience, is it easy to be modified.

Each higher education institution itself may choose content quality criteria for the programme evaluation. Metrics and quality characteristics for the external quality evaluation of a study course offered by the author are shown in Table 1.

Table 1

**Metrics and quality characteristics for the evaluation of a study course**

| Metrics | Quality characteristics |
|---|---|
| Conformity of the delivered information to the defined content of the course | Functionality (functional suitability) |
| Conformity of the delivered information to a student's expectations | Usability (suitability) |
| Understandability of the study course | Usability (understandability) |
| Quality of lectures | Functionality (suitability) |
| Quality of practical/ laboratory work | Functionality (suitability) |
| Proportion of lectures and practical/ laboratory work in the study course | Usability (suitability) |
| Quality of teaching aids of lectures/ practical/ laboratory work | Usability (learnability) |
| Availability and adequacy of the necessary literature to the study course requirements | Usability (learnability) |
| Understandability on the significance of the course | Usability (learnability) |
| Proportion of the necessary unassisted work for the particular course | Efficiency (time behaviour) |
| Relation of the content to another courses | Portability (co-existence) |
| Overlapping of the course information with other courses | Portability (co-existence) |

The standards (ISO/IEC 9126-2, 2003) for the evaluation of software product quality characteristics provide various metrics according to internal and external characteristics of the quality model. By analogy, the author used a single outline for the definition of metrics and interpretation of values for the evaluation of internal quality indicators of study courses. One example of metrics is described in Table 2.

Table 2

**Outline for definition and evaluation of metrics**

| | |
|---|---|
| Purpose of the metrics | Does the content of study course conform to the initially defined? |
| Method of application | Count the number of negative estimations (very bad, bad, average) given in the questionnaire and compare it with the total number of estimations |
| Formula and data element computation | X=1-A/B<br>A – number of negative estimations<br>B – total number of estimations |
| Interpretation of value | 0<=X<=1<br>The closer to 1.0, the better |
| Metric scale type | Absolute |
| Measure type | A, B – count<br>X – count/ count |

## Results and Discussion

From the study year 2009/2010, the Faculty of Information Technologies has started a profound evaluation of external quality of study courses. The evaluation is undertaken by students as direct course users after each mastered course. Directly students are engaged in the programme implementation; hence, they are able to estimate a degree to which study courses delivered within the study programme satisfy their defined needs and expectations. The polls of undergraduate students are carried out at the end of each term and students are asked to evaluate each mastered study course according to 10 criteria (metrics) providing a value from 1 to 5 (1 – shocking bad, 2 – bad, 3 – average, 4 – good, 5 – very well). The questionnaire is carried out electronically. Table 3 includes the information on the number of respondents and evaluated study courses.

Table 3

**Data on students' polls**

| Academic year | Terms when students were polled | Total number of students | Number of respondents, % | Number of evaluated study courses |
|---|---|---|---|---|
| Autumn 2009 | Term 1, Term 3 | 120 | 41 (34%) | 16 |
| Spring 2010 | Term 2, Term 4 | 110 | 27 (25%) | 21 |
| Autumn 2010 | Term 1, Term 3, Term 5 | 179 | 67 (37%) | 35 |

Applying the sample of metrics described in Table 2, the obtained results on students' evaluations in each measurement process are depicted in Table 4. Total evaluation of study courses according to each metrics ranges between 0.60 and 0.84. If the author's proposed outline for interpreting of the measurement results is - the closer to 1, the better (0<=X<=1), then the obtained results show that the external quality of study courses shall be enhanced according to all mentioned characteristics. The highest evaluation is gained according to the first metrics – the delivered content of the course conforms to the defined content of the course, while the lowest evaluation is gained according to the eight metrics – adequacy and availability of the necessary literature to the study course requirements.

Table 4

**Total evaluation of the study courses**

| No. | Metrics | Autumn 2009 | Spring 2010 | Autumn 2010 |
|---|---|---|---|---|
| 1 | Conformity of the delivered information to the defined content of the course | 0.80 | 0.84 | 0.81 |
| 2 | Conformity of the delivered information to a student's expectations | 0.71 | 0.78 | 0.74 |
| 3 | Understandability of the study course | 0.56 | 0.75 | 0.75 |
| 4 | Quality of lectures | 0.72 | 0.79 | 0.76 |
| 5 | Quality of practical/ laboratory work | 0.76 | 0.80 | 0.72 |
| 6 | Proportion of lectures and practical/ laboratory work | 0.73 | 0.81 | 0.84 |
| 7 | Quality of teaching aids used in lectures/ practical/ laboratory work | 0.71 | 0.74 | 0.72 |
| 8 | Adequacy and availability of the necessary literature to the study course requirements | 0.60 | 0.73 | 0.72 |
| 9 | Understandability on the significance of the course | 0.70 | 0.74 | 0.77 |
| 10 | Proportion of the necessary unassisted work for the particular course | 0.66 | 0.76 | 0.76 |

The target and quality characteristics for the evaluation shall be defined before starting a particular process of measurement. It is recommended to determine the proportion of these characteristics. None process of measurement simultaneously includes the evaluation of entire quality characteristics and sub-characteristics. External quality evaluation of study courses carried out at the Faculty of Information Technologies is based on 3 characteristics and 6 sub-characteristics of the quality model (Table 5). Evaluation of characteristics ranges between 0.71 and 0.82. Evaluations have differed in every study year; however, a general tendency shows that the evaluation of quality characteristics increases with every next time of measurement.

Table 5

**Total evaluation of quality characteristics of the study courses**

| Quality characteristics | Autumn 2009 | Spring 2010 | Autumn 2010 | Total |
|---|---|---|---|---|
| Functionality (functional suitability) | 0.80 | 0.84 | 0.81 | 0.82 |
| Functionality (suitability) | 0.74 | 0.79 | 0.74 | 0.76 |
| Usability (suitability) | 0.72 | 0.79 | 0.79 | 0.77 |
| Usability (understandability) | 0.63 | 0.74 | 0.76 | 0.71 |
| Usability (learnability) | 0.66 | 0.74 | 0.72 | 0.71 |
| Efficiency (time behaviour) | 0.66 | 0.76 | 0.72 | 0.71 |

The analysis of evaluation provided for the study courses of Autumn terms 2009 and 2010 (Figure 4) shows that the number of positive evaluations marked by students (4 – good, 5 – very well) is growing. It may be explained by the fact that after each process of measurement, the academic staff receives a feedback on their study courses and it promotes the increase of the course quality.

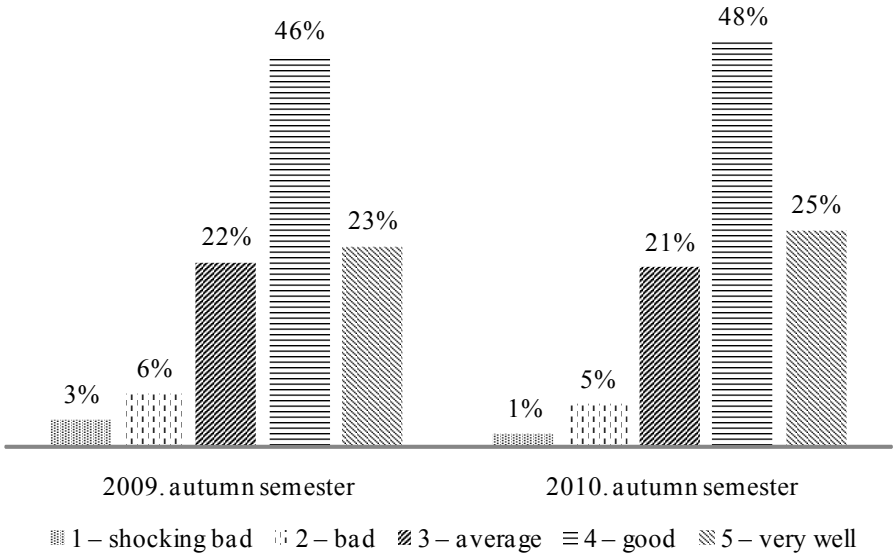



Figure 4. Evaluation of study courses by study years.

## Conclusions

1. Software product quality models and evaluation methods may be applied for the quality evaluation of a study programme, since by analogy software product and study programme are similar. Both products have internal and external quality.
2. Quality assurance of a study programme is under the competence of higher education institution itself. Students may be engaged in the evaluation of external quality of a study programme, since they participate in the implementation of the study programme and the content of the study programme facilitates the achievement of desired study results.
3. Engagement of students in the evaluation of external quality of a study programme shall be established as tradition of a higher education institution. Thus, students are involved in the improvement and strengthening of quality. Evaluation of study courses shall become an integral part of the study process.
4. Judgement on the general quality of study programme may be made after receiving the evaluation of completely all study courses included into the particular study programme. Currently the Faculty of Information Technologies has summarised evaluations for the study courses of the two first study years.
5. Feedback shall be established after each evaluation process – both the academic staff and students shall have the possibility to be acquainted with the course evaluation.
6. Evaluation of internal quality of a study programme shall be carried out along with the evaluation of its external quality, thus forming the quality culture of a higher education institution.

## Acknowledgements

This research has been supported by the European Social Fund within the project "Support for the Implementation of Doctoral Studies at Latvia University of Agriculture". Agreement No. 2009/0180/1DP/1.1.2.1.2/09/IPIA/VIAA/017

## References

1. Briška A. (2008) Studiju programmu kvalitātes analīze un to ranžēšanas kritēriji (Study Programme Quality Analysis: Ranking Criteria in Higher Education Institutions in Latvia). Latvijas Universitāte, 721, pp. 217-234. (in Latvian).
2. Coates H. (2005) The Value of Student Engagement for Higher Education Quality Assurance. *Quality in Higher Education*, 11, 1, pp. 25-36.
3. Čevere R., Sproģe S. (2010) Application of Software Quality Models in Evaluation of Study Quality. *Problems of Education in the 21st Century,* 21, pp. 37-48.
4. Gvaramadze I. (2008) From Quality Assurance to Quality Enhancement in the European Higher Education Area. *European Journal of Education,* 43, 4, pp. 443-455.
5. ISO/IEC 15939 (2007) Systems and Software Engineering – Measurement Process – International Organisation for Standardisation, pp. 7-18.
6. ISO/IEC 25030 (2007) Software Engineering – Software Product Quality Requirements and Evaluation (SQuaRE) – Quality Requirements - International Organisation for Standardisation, pp. 7-8.
7. ISO/IEC 9126-1 (2001) Software Engineering – Product Quality – Part 1: Quality model – International Organisation for Standardisation, pp. 7-11.
8. ISO/IEC 9126-2 (2003) Software Engineering – Product Quality – Part 2: External Metrics – International Organisation for Standardisation, pp. 7-67.

9. ISO/IEC 9126-4 (2004) Software Engineering – Product Quality – Part 4: Quality in Use Metrics - International Organisation for Standardisation, pp. 4-11.
10. *Procedure for Accreditation of Higher Education Institutions. Colleges and Higher Education Study Programmes*. Cabinet Regulations (2006) Available at: http://www.likumi.lv/doc.php?id=145125, 11 January 2011 (in Latvian).
11. Sproge S., Cevere R. (2010) Quality Model of the Curricula of Information Technology Studies. In: *Proceedings of the 4th International Scientific Conference on the Applied Information and Communication Technologies* [CD-ROM], Jelgava: LLU (Latvia University of Agriculture), pp. 148-157.
12. *Standards and Guidelines for Quality Assurance in the European Higher Education Area* (2005) Helsinki: European Association for Quality Assurance in Higher Education. Available at: http://www.eqar.eu/uploads/media/ 050221_ENQA_report_01.pdf, 12 February 2011.

# THE USE OF BLACK-BOX MODELLING IN BIOPROCESS SCALE-UP

***Martins Mednis***
*Latvia University of Agriculture*
*martins.mednis@llu.lv*

## Abstract

Computer models of bioprocesses have become an essential tool in biotechnology. This paper describes various bioprocess scale-up releated problems. The problems at different scales are explained. In this paper the use of computer model in *E.coli* fermentation scale-up from shake flask to laboratory scale bioreactor is discussed. A black box modelling approach was used. Fermentation results have been visualized and discussed. The computer model created in Matlab environment was used for bioprocess behaviour prediction. Possible bioprocess scale-up software improvements and bioprocess optimization are discussed.
**Key words**: bioprocess scale-up, computer model, fed-batch fermentation, Matlab.

## Introduction

The scale-up of fermentation process as a central problem in biotechnology was first recognized and described during industrial penicilin production at the beginning of the 1940s (Humphrey, 1998). Bioprocess scale-up is aimed at the manufacture of larger product quantities with simultaneous increase or at least consistency of specific yields and product quality (Schmidt, 2005). Bioprocess scale-up is a process of transfering a biological cultivation from smaller volume to larger one. Trying to run the same bioprocess in a larger vessel involves a number of changes which lead to following problems: larger volume – longer mixing time; changed vessel geometry – changed mass transfer dynamics. Scale-up of a bioprocess means different environment control mechanisms. During scale-up from a shake flask to a laboratory-scale bioreactor, the temperature, pH, $pO_2$ control and agitation is changed. Temperature control in a shake flask (often only heating is needed) is done through shake flask's surface in a thermostat device. Bioreactors use heating and cooling coils instead. Shake flask agitation is done by shaker – the whole flask is shaken. However, bioreactors has stirrers inside the vessel. In a typical shake flask cultivation it is not possible to control $pO_2$ and pH. A typical bioreactor offers wider on-line measurement and control possibilities than a typical shake flask cultivation. In a bioreactor it is possible to monitor $pO_2$, pH, temperature and concentrations of outgases and much more. The online connection of mass spectrometer, NIR (Near Infrared Spectroscopy), electrochemical and multi-array gas sensors by the neuronal network module NeurOnline capable of processing 1800 signals simultaneously for the calculation of the process state and adaptive control as well as for the development of appropriate process models and its connection to a real-time expert system based on *Gensym G2* system has been described by Cimander et al., (Cimander, 2003). The phenomenon is that in a bioreactor with much more monitoring and control abilities than a shake flask one cannot repeat the same result just with larger volumes. The changed geometric and physical conditions in larger scales lead to impaired physiological reaction conditions which in turn may lead to a decreased process constance and reproducibility, to reduced specific yields and to an increase in unwanted side products and thus ultimately to a diminished batch-to-batch consistency and product quality (Schmidt, 2005). Bioprocesses in industrial scale also involves a reduced mixing quality. A result of conventional bioreactor design leads to the opposite substrate and oxygen gradients along the vessel height. Optimization of operational conditions for production processes in biochemical industry is of considerable importance. Such optimal control strategies are necessary in order to assure high performance of these processes. In the face of increased competition on the market, model-based process optimization is a natural and straightforward choice for reducing production costs, fulfilling safety requirements, increasing process quality and reducing variability (Bonvin, 1998). The aim of this article is to check how well a bioprocess computer-model performs with changed process volume and to compare results with actual measurements.

## Materials and Methods

There are a number of things that change during bioprocess scale-up. A. Humprey (Humphrey, 1998) lists 8 factors:

- heat transfer surface-to-volume ratio;
- quality of mixing;
- shear (agitator or impeller tip speed);
- superficial air velocity (flooding tendency);
- time of inoculum transfer;
- time to set the fermentation;
- age and stability of culture (number of generations);
- selection of cheaper media resulting in expression of hidden auxotrophy.

The computer model inspected in this investigation takes into account superficial air velocity, time of inoculum transfer, quality of mixing, and number of generations.

In order to gather data for computer model one needs to regularly take samples and analyze them.

Sampling from shake flask and bioreactor is different.

The purpose of sampling is to obtain a representative sample, i.e. a sample that corresponds to the overall state of the process. Sampling must not compromise microbiological purity of the process; therefore, it must be performed over a sterile barrier (Vojinović, 2006). Sterile sampling from shake flask is problematic and therefore avoided during fermentations. However, a reference flask was prepared for sampling during fermentation. Two shake flasks for inoculation in bioreactor and one flask for reference purposes – online pH measurements and sampling.

Computer model was created in Matlab environment. The solver chosen for balance equations was Matlab built-in solver ode45(). The model can receive biomass, glycerol and acetate amount as an input data. In our experiments only biomass was measured. At the beginning, new samples were taken every one hour. After 7th hour, samples were taken every 30 minutes. The bioreactor used in *E.coli* fermentations was BTC Bio3 conventional stirred tank reactor.

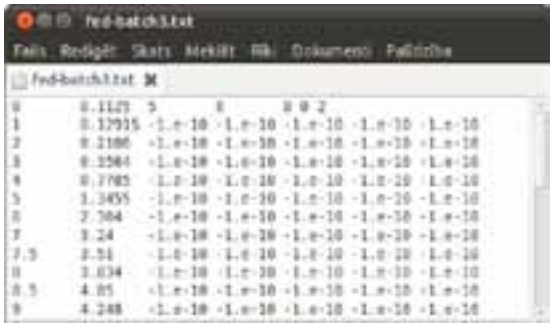


Figure 1. Measurements file.

Source: created by the author.

All measured values were entered in a simple text file and sepparated by 'tab'. The measurement file is illustrated in figure 1. Since glycerol and acetate concentrations were not measured, negative values were given.

## Results and Discussion

The results of computer model calculations are illustrated in figures 2 and 3. Computer model predicts biomass, glycerol and acetate concentration. The model can also calculate specific biomass growth rate, specific glycerol consumption rate, specific acetate consumption rate, and recommended feeding rate.

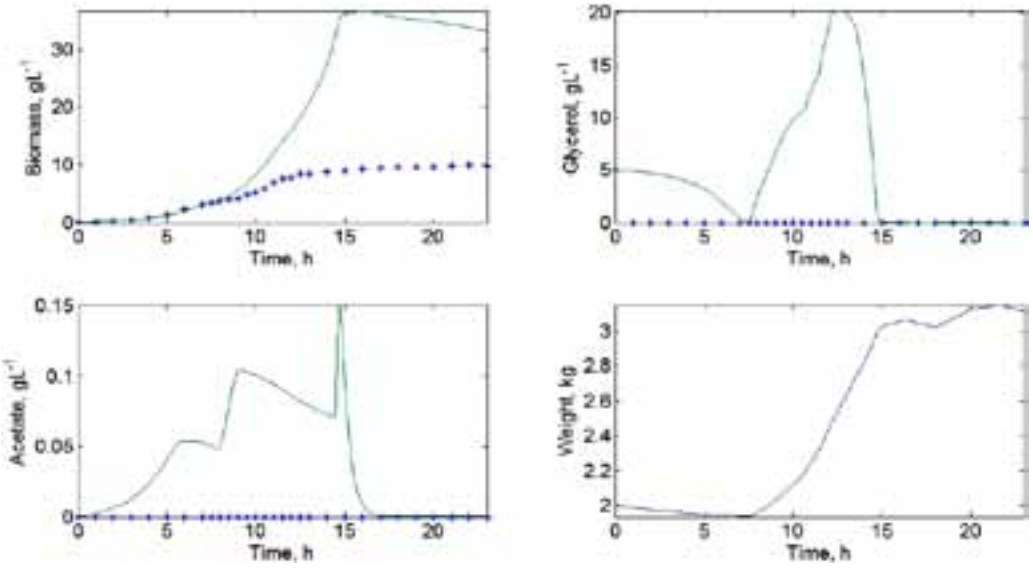



Figure 2. Model calculations: biomass, glycerol, acetate, weight.

Source: created by the author using computer model by V. Galvanauskas.

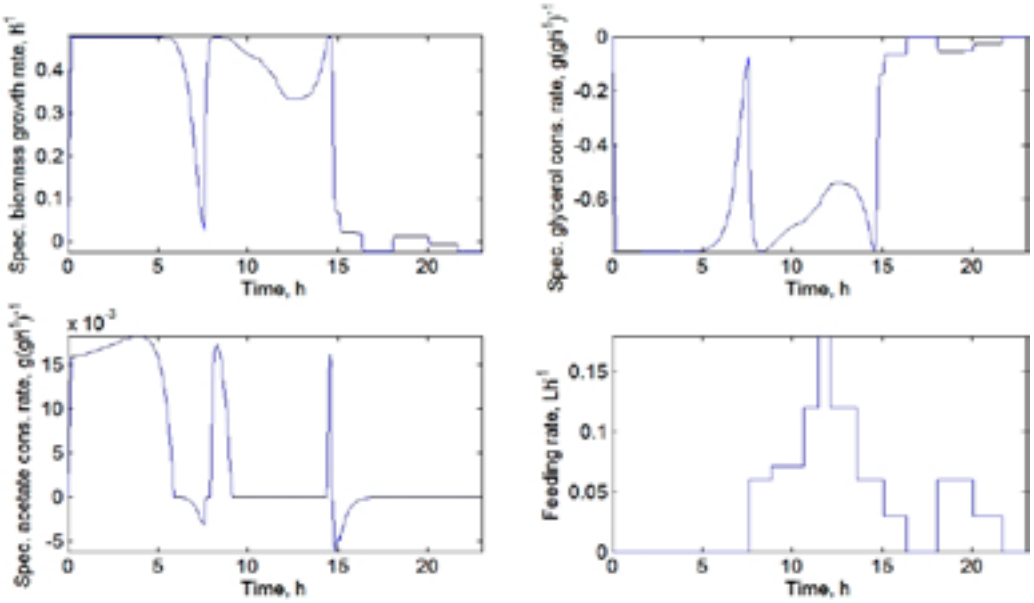



Figure 3. Model calculations: specific rates and feeding rate.
Source: created by the author using computer model by V. Galvanauskas.

Computer model predictions are accurate only for the beginning of the fermentation. Lines represent modelled value, stars represent measured values. During the experiment only biomass concentration was measured. In order to allow computer model still work with missing values, a negative concentration was introduced. Computer model code was changed to automatically ignore negative values. According to the computer model, the microorganism (*E.coli*) should go into exponential growth phase after the 7th hour of fermentation. Biological reasons why organism has extended lagphase can be various: non-optimal medium, weakened strain, contamination or hidden auxotrophy. Sort of metabolic shifts are also possible. One of the most important consequences of stress-induced metabolic shifts affecting product quality is the misincorporation of amino acids into native and recombinant proteins, the reasons for which are complex and not yet fully clarified: according to current knowledge, stress-triggered changes in glycolysis, citrate cycle and of pathways involved in anaerobic and mixed acid fermentation lead to shifts in the coupled amino acid biosynthetic pathways (Fenton, 1997). Assuming that fermentation itself has no deviations, there can be computer model releated problems, for instance, inaccurate model parameters. At this moment author have no certain explanation why computer model alters from bioprocess at 7th hour. Possibly an advanced parameter estimation could help to make computer model closer to real bioprocess. In any case, process optimization is necessary. From engineering point of view, such optimization includes elaboration of optimal starting conditions and profiles for control variables (Galvanauskas, 2007).

## Conclusions

The figure 4 roughly illustrating the theoretical steps and stages of fermentation process optimization and scale up, is characterised by gliding transitions. Process development usually starts with a detailed process characterisation through elucidation of the mutual influences of physiological parameters (e.g. productivity, metabolite and substrate concentrations, cell mass and viability, respiration) and of physical process parameters (e.g. aeration, agitation, pressure, temperature, fluid dynamic conditions), eventually supported by neural networks and integrated fluid dynamics. Employable analytical tools for physiological parameter characterisation are the established physical and chemical procedures like flow cytometry, spectroscopy, chromatography, etc. as well as techniques yet in development for biotechnological application like electric tongues, artificial noses or the lab-on-a-chip technology.

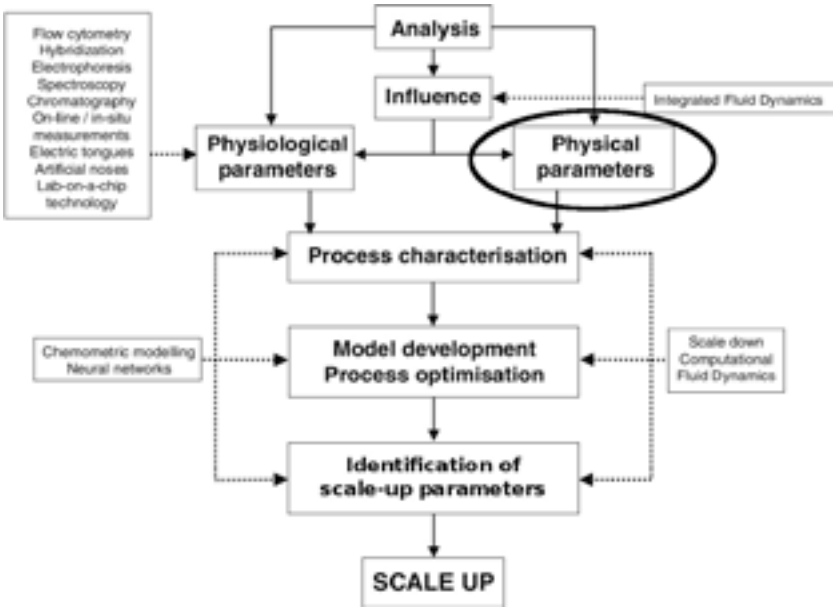



Figure 4. Bioprocess scale-up.

Source: from the article of F.R.Schmidt, 2005 with author's additions.

Process characterisation as well as the subsequent process optimisation, which aim at the improvement physiological reaction conditions and reduction of stress exposure and which enable the identification of suitable scale-up parameters and strategies, are usually performed on the basis of experimentally developed data and knowledge-driven assumptions and models. Supportive approaches and tools for model development are scale-down experiments, (neural network based) chemometric modelling, and computational fluid dynamics (Schmidt, 2005). The model described in this article deals only with physical parameters of fermentation. The model uses black-box approach – modelling bioprocess behaviour without simulation of metabolic reactions. The conclusion can be made that computer model that fits small-scale fermentation does not fit perfectly a larger-scale fermentation. Figure 2 clearly shows it. The specialized scale-up algorithm (software) is needed. The future plans involve model inclusion in a larger software system aimed at bioprocess scale-up. The software is based on fact that knowing shake flask and bioreactor physical properties and bioprocess conditions in a shake flask it is possible to pre-calculate profiles for bioreactor's control mechanisms. With given data, software (Fig. 5) could tell: 1) if it is possibble at all to ensure similar conditions on the new-scale bioreator; 2) if possible, what are profiles for control mechanisms; 3) if not possible, what should be improved to reach minimal requirements for successful fermentation.

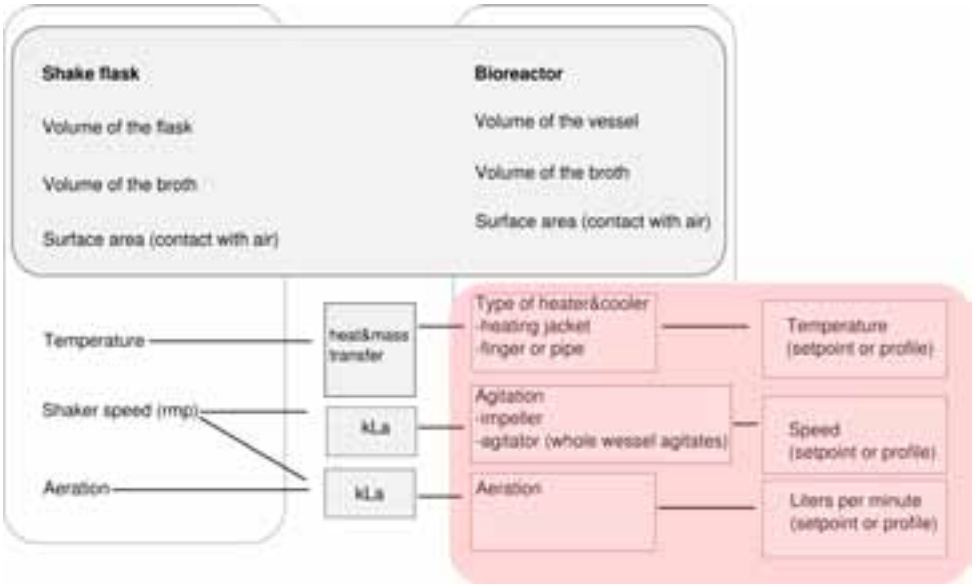



Figure 5. Scale-up paramaters calculation software.

Source: created by the author.

The advantages of such software are that if there is not a confidence how to repeat bioprocess on a larger scale, user gets a calculated proof that it is possible or not and why. At this moment computer model uses black-box approach, but in future versions also intracellular metabolic fluxes should be taken into account. The overall progress in the metabolic modelling has allowed a deeper knowledge on many biological systems with industrial interest. This knowledge is still rarely used for advanced bioprocess monitoring and control at the bioreactor level. The use of metabolic model to calculate optimal feeding rate has been described by A.P. Teixeira (Teixeira, 2007).

**Acknowledgements**

The investigation was funded from ESF project "Establishment of Latvian interdisciplinary inter-university scientific group of systems biology" (ref. no. 2009/0207/1DP/1.1.1.2.0/09/APIA/VIAA/128). The author gratefully acknowledges Dr.sc.ing. V. Galvanauskas from Kaunas University of Technology for the incredible work with computer model creation. The author also acknowledges M.chem. O. Grīgs from Riga Technical University who helped to cary out fermentations with *E.coli*.

**References**

1. Cimander C., Bachinger T., Mandenius C. (2003) Integration of distributed multi-analyzer monitoring and control in bioprocessing based on a real-time expert system. *Journal of Biotechnology*, 103(3), pp. 237-248.
2. Bonvin D. (1998) Optimal operation of batch reactors - a personal view. *Journal of Process Control*, 8, pp. 355-368.
3. Fenton D., Lai H., Lu H., Mann M., Tsai L. (1997) Control of norleucine incorporation into recombinant proteins. US Patent 5599690. Available at: http://www.freepatentsonline.com/5599690.pdf, 21 March 2011.
4. Galvanauskas V., Simutis R. (2007) Software tool for efficient hybrid model-based design of biochemical processes. *WSEAS Transactions on Biology and Biomedicine*, 4(9), pp. 136-144.
5. Humphrey A. (1998) Shake flask to fermentor: What have we learned? *Biotechnology Progress*, 14(1), pp. 3-7.
6. Schmidt F.R. (2005) Optimization and scale up of industrial fermentation processes. *Applied microbiology and biotechnology*, 68(4), pp. 425-35.
7. Teixeira A., Alves C., Alves P., Carrondo M., Oliveira R. (2007) Hybrid elementary flux analysis/nonparametric modeling: application for bioprocess control. *BMC bioinformatics*, 8, 30 p.
8. Vojinović V., Cabral J.M.S., Fonseca L.P. (2006) Real-time bioprocess monitoring Part I: In situ sensors. *Sensors and Actuators B: Chemical*, 114(2), pp. 1083-1091.

# AN AGENT-BASED HYBRID INTRUSION DETECTION SYSTEM

***Nauris Paulins***
*Latvia University of Agriculture*
*nauris.paulins@llu.lv*

## Abstract

Intrusion Detection Systems is defined as a component that analyses system and user operations in computer and network system to protect it from possible intrusions. Current intrusion detection technologies have several shortcomings. Applying mobile agents to intrusion detection design is step forward on better intrusion detection. Mobile-agent based distributed intrusion detection systems are very promising for the following reasons: reduction of data movement, load-balance, flexibility, fault-tolerance, detection of distributed attacks. Hybrid intrusion detection is defined by both the method used to detect attacks and the placement of the system on the network. Intrusion detection system may perform either misuse detection or anomaly detection and may be deployed as network-based or host-based system. This paper proposes to distribute classical intrusion detection model with mobile agents making an agent-based hybrid intrusion detection system. The proposed model can help detect simple intrusions in early stage and also distributed intrusions by monitoring several subjects installed on network. Main benefit from mobile agents in such system is ability to generate separate services for specific tasks and analyze unknown user patterns with several methods of artificial intelligence.
**Key words**: intrusion detection systems, mobile agents, hybrid intrusion detection, network security.

## Introduction

Nowadays technology, internet and spreading of online procedures have become an integral part of regular communication. Benefiting from the online services comes at the price of high security risk. There are various threat sources like software bugs, hackers, viruses, network complexity and so on. Traditional intrusion detection techniques, such as firewalls, access control or encryption, have failed to protect computer networks and systems. As result, intrusion detection systems have become an important component of network security, to detect threats before they cause some damage. An intrusion detection system (IDS) dynamically monitors events which are symptomatic of an attack or constitute a legitimate use of the system (Debar, 1999).

Intrusion to computer networks is called "attacks", and these attacks threaten security of networks by violating privacy, integrity and accessibility mechanisms. Attacks can be originated from users who login to the computer using the Internet trying to gain administrator rights and other users who misuse the rights they have (Scarfone and Mell, 2007). IDS are software that automates the intrusion detection process. Intrusion detection techniques can be categorized into misuse detection, anomaly detection, and protocol inspection detection. Misuse detection identifies intrusion by matching observed data with pre-defined description of intrusive behavior. Therefore, well-known intrusion can be detected efficiently with a very low false alarm rate. For this reason, the approach is widely adopted in the majority of commercial systems (Wu and Banzhaf, 2010). Misuse detection will fail easily when facing unknown intrusion. To solve this problem is used anomaly detection and stateful protocol inspection. Anomaly detectors detect behaviors on a computer or computer network that are not "normal". According to this approach, behaviors deviating from behaviors assumed as ''normal" are thought to be attacks, and anomaly detectors compute the deviation in order to detect these attacks. Anomaly detectors construct profiles of users, servers and network connections using their normal behaviors. These profiles are produced using the data that is accepted as normal (Aydin et al., 2009). Stateful protocol inspection is similar to anomaly-based detection, but it can also analyze traffic at the network and transport layer and vender-specific traffic at the application layer, which anomaly-based detection cannot do (Tzeyoung, 2009). But, currently, IDS are often designed using centralized method, and they are prone to design and implementation errors (Wang et al., 2006). Such design is faced also with some shortcomings like delay of time, single point of failure, hard communicate mutually between different IDS, hard computational requirements, network flow overload in single segment. IDS implemented using mobile agents (MA) is one of new paradigms for intrusion detection. As MA, the IDS components can hide themselves in a network, evade attackers, and recover themselves if killed. The agents randomly move around the network and thus it is difficult for an attacker to pinpoint an important agent's location (Mell and Mclarnon, 1999). Mobile-agent based distributed IDS are very promising for the following reasons: reduction of data movement, load-balance, flexibility, fault-tolerance, detection of distributed attacks. The idea of distribution the intrusion detection system using agent software is not entirely new, but most of the related works emphasize static agents of mobile ones. The purpose of this paper is to describe classic IDS architecture distributed by MA. Accordingly, to achieve the research aim, the following research tasks were set forth – to investigate the nowadays intrusion detection methods, to characterize IDS architecture and hybrid detection model, distribute centralized IDS architecture with static and mobile agents, and finally discuss their advantages and disadvantages.

**Materials and Methods**

To examine existing IDS methods and MA solutions for intrusion detection, a theoretical research has been done, scientific literature has been analyzed like publications and protocols, and leading network security companies' reports have been studied. Several previous works have been concerned with mobile agent solutions and IDS of active network security. Here, two approaches are discussed:

1. Hybrid intrusion detection – IDS are defined by both the method used to detect attacks and the placement of the IDS on the network. IDS may perform either misuse detection or anomaly detection and may be deployed as network-based or host-based system. Systems such as EMERALD (Neumann and Porras, 1999) may use two or more detection techniques and placement methods in parallel to achieve higher detection accuracy. Mostly are proposed misuse-detection and anomaly-based detection combination (Onashoga et al., 2009; Verwoerd and Hunt, 2002), because stateful protocol inspection is a new detection method. The primary drawback to stateful protocol analysis methods is that they are very resource-intensive because of the complexity of the analysis and the overhead involved in performing state tracking for many simultaneous sessions. Another serious problem is that stateful protocol analysis methods cannot detect attacks that do not violate the characteristics of generally acceptable protocol behavior, such as performing many benign actions in a short period of time to cause a denial of service. Yet another problem is that the protocol model used by an IDPS might conflict with the way the protocol is implemented in particular versions of specific applications and operating systems, or how different client and server implementations of the protocol interact (Scarfone and Mell, 2007). Denning generic hybrid ID model (Denning, 1987) as base model for hybrid intrusion detection was accepted (Fig. 1).

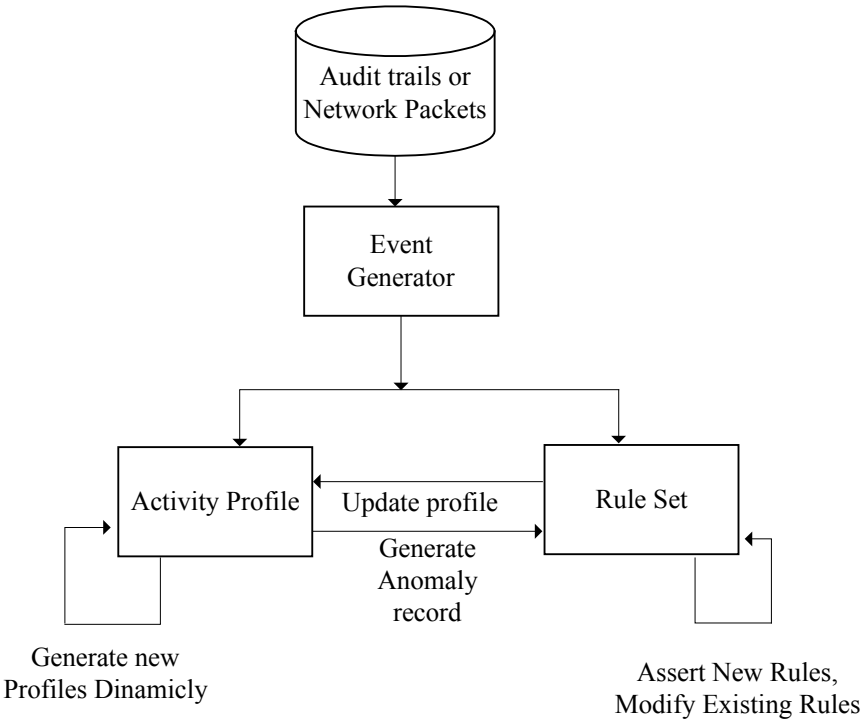



Figure 1. Denning's generic hybrid intrusion detection model.

The model contains a set of profiles for subjects, which are the initiators of actions in the monitored system. Subjects are users, system or system processes, but objects of system are receptors of actions as files, programs and records. The activity profiles contain variables that represent behavior of the system using predefined statistical measures. The activity profile module detects anomalies, while the rule-set module performs misuse detection. Denning's model has eight components: subjects, objects, audit records, statistical metrics, statistical models, profiles and profile templates, anomaly records and activity rules.

2. An agent-based intrusion detection system – as mentioned before, current commercial technologies cannot cover all possible attacks on the network and have several disadvantages. The idea is that instead of single large IDS defending the network, several independent and intelligent software agents cooperate in securing the network. The major benefits of such approach include detection of distributed attacks, distribution of computations, reduction in the amount of information sent over the network, platform independence (Wang et al., 2006). The major drawback of agent systems is the lack of viable research in understanding and addressing agent-based systems security problems. Most of research works focused on

distribution of data collection and analysis tasks as well as communication and cooperation among agents. In this paper author concentrates on system distribution also in event detection and profile updates using agents. Agent system based on Belief-Desire-Intention (BDI) architecture (Fig. 2)

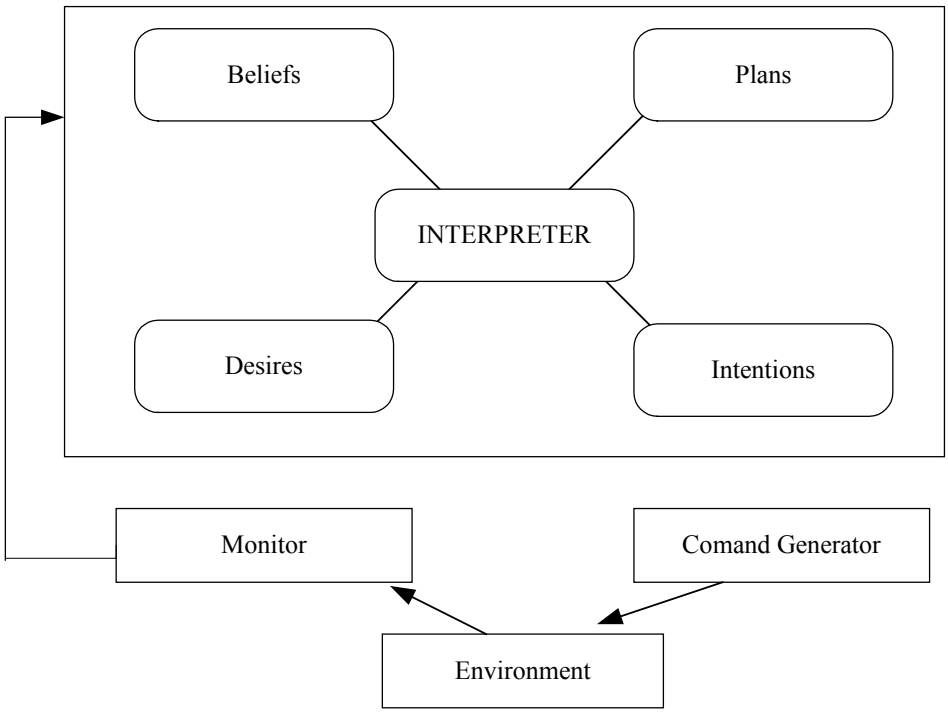



Figure 2. Belief-Desire-Intention Agent Architecture (Michael, 2005).

A BDI agent is able to continuously reason about beliefs, goals, and intentions and act accordingly. There are four major concepts in BID architecture (Fig. 2):

- Beliefs of an agent are information about the environment. They are subject to uncertainty and error.
- Desires are goals assigned to the agent.
- Intentions are commitments by an agent to achieve particular goals. In other words, they are plans that are currently being executed.
- Plans are choices available to the agent at any moment of time to achieve its goals.

**Results and Discussion**

According to our research, following model is proposed in this section. Intention here is to demonstrate agent- based detection model rather than present a complete framework. The model contains various agents and knowledge base to do specified tasks (Fig. 3):

- Monitoring agents. Each system node (object in Denning's model) contains static monitoring agent for data collection and analysis. Monitoring agent is stationary and uses its own knowledge base to monitor network traffic and system events, to communicate with other agents in the network, and to handle request services. The basic idea is that agent monitoring system using their knowledge about environment to lookup system events. If agent detects possible intrusion pattern or unrecognized event initiated by the subject it sends information to analyzer.
- Analyzer is the main knowlge-base attached agent which consists of rule sets and activity profiles. Latest events are stored in buffer zone and are analyzed not just individualy but also by artificial immune pattern recognition algoritms, data pattern feature vectors, immune network computional models. It recives messages form all monitoring agents. Analyzer's interpreter checks whether this event profile is intrusion or a new unique event profile. If it is intrusion, analyzer sends alarm and request to mobile agent generator.
- Mobile agent generator. If analyzer confirms alert it can send request to the generator to genterate mobile agents to solve additional problem or prevent intrusion progress. A mobile agent is software agent that is capable of migrating from one host to another in a network and resumes the execution in new host. Different types of mobile agents can be created and dispatched to sensor nodes as needed. For example, the mobile agents can be dispatched to analyze specified event. Each agent has its own ID and limited life time. The intermediate results from each task must be sent to analyzer, if analyzer not recieve message from agen duaring its lifetime, it's sendinga alarm.
- Knowledge base. It contains information about current event profiles and security rules and collects accepted events from users. Each user action profile is stored in knowledge base, also new event profile templates are sent by analyzer to knowledge base.

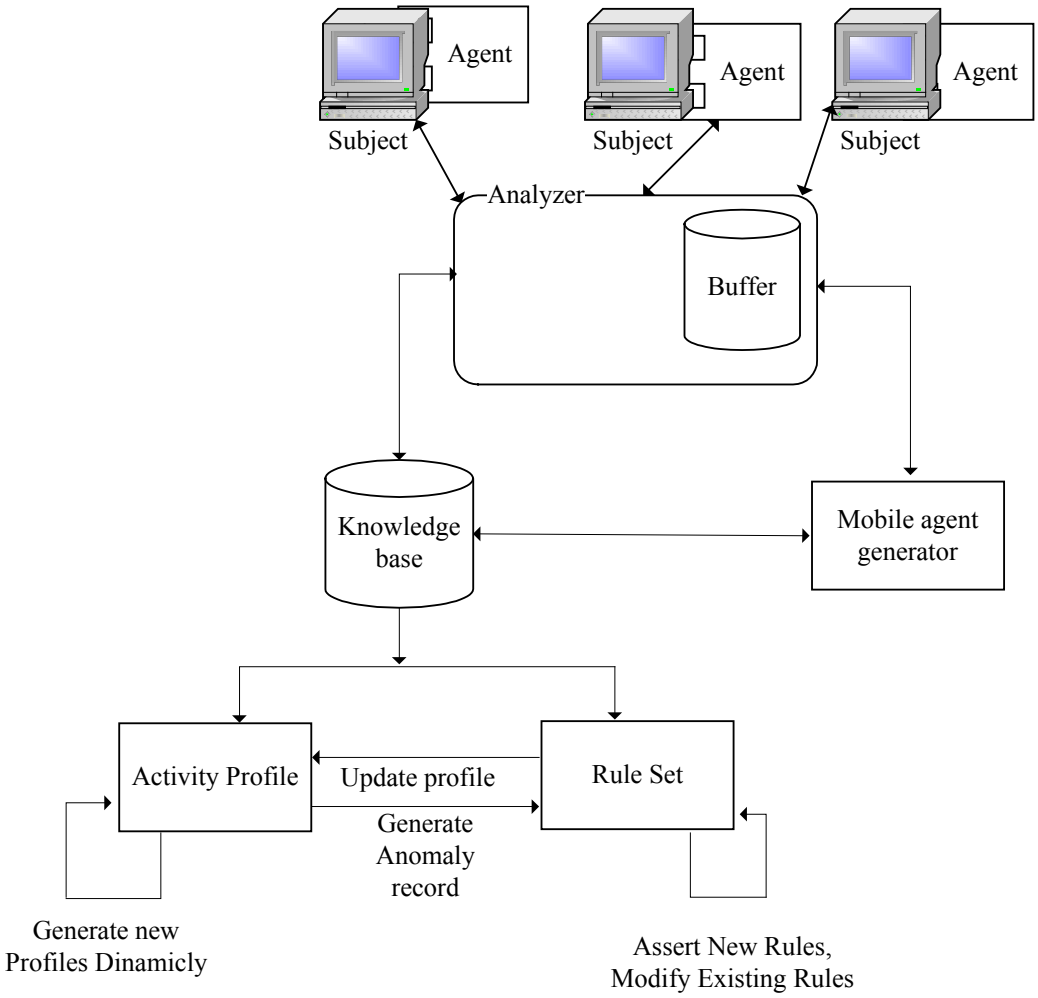



Figure 3. Agent based hybrid intrusion detection model.

Related architecture proposed by Mehdi Shajari and Ali Ghorbani called Fuzzy Adaptive Survivabilty Model (FASM) and Boudaoud and Guessoum called Multi-agents System-based Network security Management Architecture (MANSMA), these are the researches that has pointed to the use of BDI agents in intrusion detection. MANSMA have two agent layers in their model: Manager Layer and Local Layer, where manager layer manages global security of large network, while the Local Layer manages security of local segment. FASM has similar architecture where monitor box manages local operational variables and sending information to detection box to detect intrusion and send information to decision box to accommodate the uncertainties of decision process. These models allow detecting of distributed attacks, but these prototypes allow detection of well-known attacks, but there is not defined agent's behavior to new kind of attacks. In the current research, mobile agents are used not just for information collection, but also for specific task calculation and inspection. Such agents allow using several kinds of methods of artificial intelligence and obtain different kinds of information about the same situation. Also important feature is mobile agent generator which generates mobile agents for a particular problem, not just for network monitoring.

## Conclusions

Agent-based intrusion detection is discussed in this paper. Mobile agents can be applied to intrusion detection to evolve new designs that are more efficient and scalable, to optimize distributed computing over the network. Proposed model is the main concept for future research and experiments. Greater detail as well as results from experiments will be made available in later stage of this research. Agent solution can help detect simple intrusions in early stage and also distributed intrusions by monitoring several subjects installed on network. Each agent used in the system performs separate task and cooperates with each other to solve complicated tasks. To exploit the potential of multi-agent systems, this paper proposed combine static agents with mobile agents and discussed their usages, advantages and disadvantages. The main benefit from mobile agents in such system is ability to generate separate services for specific tasks and to analyze

unknown user patterns with several methods of artificial intelligence. Mobile agents can provide benefits not only in detection side, but also in the response side. An expansion of the distributed systems seems to be possible to prevent distributed attacks. Future experimental and testing work needs to be done for the current model.

**Acknowledgements**

Academic study was financed by the project "Support for doctoral studies in LUA", /2009/0180/1DP/1.1.2.1.2/09/IPIA/VIAA/017/ agreement No. 04.4-08/EF2.D2.28

**References**

1. Aydin A., Zaim A., Ceylan K. (2009) A hybrid intrusion detection system design for computer network security. Computer&Electrical Engineering, 35, pp. 517-526.
2. Debar H. (1999) Towards a taxonomy of intrusion-detection systems. Computer Networks, 31, pp. 805-822.
3. Denning D. (1987) An Intrusion-Detection Model. IEEE Transactions on Software Engineering, SE-13, pp. 222-232.
4. Mell P., Mclarnon M. (1999) Mobile Agent Attack Resistant Distributed Hierarchical Intrusion Detection Systems. Information Systems Security, 8 p.
5. Neumann P., Porras P. (1999) Experience with EMERALD to Date. Proceedings of the 1st USENIX Workshop on Intrusion detection and Network Monitoring, pp. 73-80.
6. Onashoga S., Adebayo D., Sodiya S. (2009) A Strategic Review of Existing Mobile Agent-Based Intrusion Detection Systems. Informing Science, 6, pp. 669-682.
7. Scarfone K., Mell P. (2007) Guide to Intrusion Detection and Prevention Systems (IDPS). Recommendations of the National Institute of Standards and Technology, 127 p.
8. Tzeyoung T. (2009) Intrusion Detection Systems. Information Security Technical Report. 10, 93 p.
9. Verword T., Hunt R. (2002) Intrusion detection techniques and approaches. Computer Communications, 25, pp. 1356-1365.
10. Wang Y., Behera S., Wong J., Helmer G., Honavar V., Miller L., Lutz R., Slagell M. (2006) Towards the automatic generation of mobile agents for distributed intrusion detection system. Journal of Systems and Software, 79, pp. 1-14.
11. Winikoff M. (2005) Simplifying Agent Concepts (SAC): Survey of Agent Systems. Available at: http://www.cs.rmit.edu.au/agents/SAC/survey.html, 15 March 2011.
12. Wu X.S., Banzhav W. (2010) The use of computational intelligence in intrusion detection systems: A review. Applied Soft Computing, 10, pp. 1-35.

# IN SILICO ANALYSIS OF STEADY STATE MECHANISMS OF METABOLIC NETWORKS IN COBRA TOOLBOX AND FBA–SIMVIS

***Ilona Odzina, Agris Pentjušs***
*Latvia University of Agriculture*
*ilona.odzina@llu.lv; agris.pentjuss@gmail.com*

**Abstract**

Metabolic analysis is one of the research focuses of systems biology. Two aspects of metabolic networks -network topology and stoichiometry - are what current researchers are most interested in, and both studies have revealed significant information. The research of the stoichiometric matrix of metabolic network has generated a series of powerful methodologies such as flux balance analysis (FBA). For FBA different methods execution are used different software like COBRA Toolbox and FBA-SimVis. The aim of this paper is to compare and analyze functionality of these two toolboxes, metabolic network data conformation conditions, and to compare all available FBA methods comparison in calculation possibilities and visual interpretation way. FBA, when analyzing all fluxes using different options, gives results ints metabolic network flow chart, although COBRA Toolbox returns the results in the matrix in number formats. FBA-SimVis for Steady state metabolic network models analysis is provided for a small metabolic network, because making some FBA analysis there are a ten possibilities to change an unlimited count of variables, to choose or change or optimize reactions as variables. COBRA Toolbox for Steady state metabolic network models analysis is provided for greater metabolic networks with hundreds or thousands of reactions. It allows changing an unlimited count manipulating and optimizing reactions fluxes.
**Key words**: Flux balance analysis, COBRA Toolbox, FBA-SimVis.

## Introduction

Systems biology is a rapidly growing science field that is based on building and validating *in silico* models of biological systems. Under different environmental conditions and genetic backgrounds, the mathematical constraint–based modeling approach of metabolism is used to predict an optimal metabolic yield and steady state flux distributions (Varma and Palson, 1994).

A variety of tools have been developed to facilitate simulations and to handle the models like COBRA toolbox *(http://gcrg.ucsd.edu/Downloads/Cobra_Toolbox*), FBA-SimVis (*fbasimvis.ipk-gatersleben.de/installation.html*). Tools called COBRA toolbox (Kauffman et al., 2003; Reed and Palsson, 2003) and FBA-SimVis (Belau et al., 2009) include implementations of many of the commonly used forms of constraint-based analysis such as flux balance analysis (FBA), gene deletions, flux variability analysis, sampling, and batch simulations together with tools to read in and manipulate constraint-based models (Rahmanian et al., 2009*)*.

The methods provided in the COBRA Toolbox and FBA-SimsVis can be used, in principle, on any metabolic network, but the more computationally intensive calculations may require extensive computer processing time. The metabolic network models should be represented in Systems Biology Markup Language (SBML) (Becker et al., 2009). Mathematical constraint–based modeling approach of metabolism in these tools offers a lot of potential steady state solutions of metabolic networks. These solution mechanisms are difficult to analyze and understand without additional data representation. To make different background of scientists better understand and interpret enormous metabolic network data, there is a need for additional metabolism data representation mechanism like much more visualizations, less calculations. The aim of this paper is to compare COBRA toolbox and FBA-SimVis models output types, buil-in metabolic network analyses functions, calculation resources, data representation type, results analysis methods and experimental data input-output possibilities.

## Materials and Methods

The experimental data for comparison and analyze opportunities in COBRA and FBA–SimVis Toolboxes has been used steady-state metabolic network of bacteria *Zymomonas mobilis* adaptation for glycerol conversion into bioethanol. The *Zymomonas mobilis* parameter values were conducted in Vitro at the temperature of 30 °C, pH was about 6.5. These in vitro parameters were taken into account making *in Silico* steady state metabolic network models using COBRA and FBA–SimVis Toolbox.

To better understand and interpret enormous metabolic network data, authors use following methods: COBRA Toolbox and FBA-SimVis functionality, and constraint–based model analysis.

*COBRA Toolbox and FBA-SimVis functionality* (metabolic network model formats, mathematical calculation resources, metabolic network model visualization)

*Metabolic network model formats*. Although the COBRA Toolbox (http://sbml.org/software/sbmltoolbox/) could (with some modification) handle any reasonable input format like XLS file format for the models, we describe the metabolic network model input as using the Systems Biology Markup Language (SBML) format (Hucka, 2003).

Compared with COBRA Toolbox, FBA-SimVis can use only SBML metabolic network model format (Belau

et al., 2009) integrated in open source software VANTED through import function.

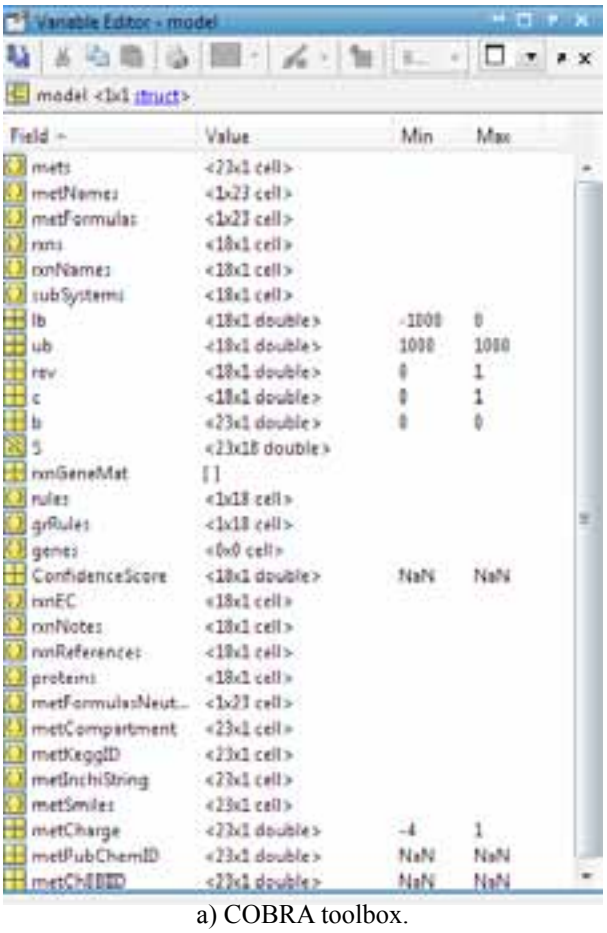
*Mathematical calculation resources.* COBRA Toolbox Version 6.0 or above of Mathlab (Mathworks Inc.) numerical computation and visualization software (http://www.mathworks.com). COBRA Toolbox uses few different kinds of solvers like:

- lp_solve: (https://sourceforge.net/projects/lpsolve),
- glpk: http:(//www.gnu.org/software/glpk/),
- LINDO (LINDO Systems Inc.) Matlab API: (http://www.lindo.com),
- CPLEX (ILOG Inc.) through the Tomlab (Tomlab Optimization Inc.) optimization environment: (http://tomopt.com/),
- Mosek (MOSEK ApS): (http://www.mosek.com).

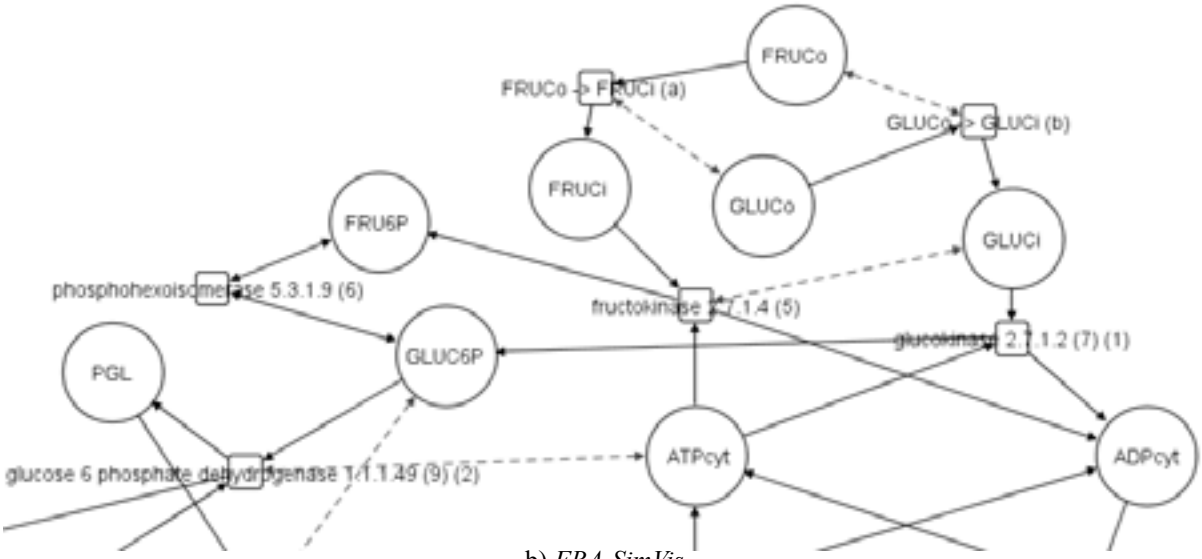
FBA-SimVis is a user-friendly tool providing visual interpretation and analysis of constraint–based metabolic models. FBA-SimVis is implemented in Java-based open source software VANTED as a toolbox. FBA-SimVis uses in Mathlab integrated mathematical approaches for constraint–based model analysis and for user friendly interactive flux visualization way use Java-based VANTED software.

*Metabolic network model visualization.* COBRA Toolbox metabolic network visualization returns various forms of matrix, which interpretation and data examination for a scientist without specific software resources is a time-consuming process (Fig. 1. a), because all data is displayed as various kinds of matrices in Mathlab simulation environment. Model each data analyses function gained data is represented in own matrix file. This data visualization method for checking data examination and interpretation is based on many number comparing between different matrices without additional usage of comparable software is time consuming process.

FBA–SimVis framework Tool for metabolic network visualization is based on graphical data examination and interpretation. FBA–SimVis tool uses open source software VANTED opportunities, and visualization options for biological networks containing experimental data (Fig. 1. b). To reconstruct a metabolic network model, the user can either create a new model (using the graphical model editor to edit existing or dropping new elements onto the canvas), or import a metabolic network model via SBML from files (by dropping the file onto the canvas) or model databases, and layout the corresponding network map by using the different layout algorithms provided by VANTED (Belau et al., 2009).




a) COBRA toolbox.




b) *FBA-SimVis*.
Figure 1. Metabolic network model visualization.

*Metabolic network data conformation.* FBA–SimVis and COBRA Toolbox are software for in silico metabolic network reconstruction creation and usage different analysis methods. All metabolites and reactions data must satisfy each specific requirement for software analysis methods. FBA–SimVis and COBRA toolbox has own metabolic network data different requirements and prepared file structure.

COBRA Toolbox uses XLS format file to store additional information into each metabolite and each reaction sheet (Thiele and Palsson, 2010). All this information for metabolites and reactions must be taken from databases and tools on the internet (Klipp et al., 2006) and completed in XLS file without disturbing the file structure. The completed file is loaded with COBRA Toolbox commands in Mathlab, where it is tested for structural or syntactical errors. One of the main conditions is that all reactions must be balanced and all metabolites must have correctly assigned chemical formula. When above motioned steps are reached and metabolic network data are correctly conformed, only then we can make metabolic network model analysis.

FBA–SimVis toolbox integrated in VANTED software uses its possibility to import already made SBML file format metabolic network model or to create a new one with built-in VANTED software possibilities, and then creates several files before FBA functions can be made. From imported SBML model, FBA–SimVis toolbox creates several files:

- reaction file – stores SBML metabolic network reactions information ;
- C-matrix file – stores each metabolite carbon atoms count (if VANTED software cannot find any metabolite with additional name, then this count manually must be tilled in);
- SBML file – stores metabolic network model;
- metabolite file – stores all metabolites information.

Before make metabolic network model analysis can be made, the imported metabolic network model must be conformed to FBA–SimVis standard: all metabolites must be redrawn like circle shape and all reactions – like rectangle shape, because imported metabolic network model is in SBGN standard (Systems Biology Graphical Notation), but FBA–SimVis uses its own standard. As VANTED software has an opportunity to connect to Kyoto Encyclopedia of Genes and Genomes (KEGG) database (http://www.genome.jp/kegg/), then all metabolites and reaction abbreviations must be correctly tilled in like abbreviations into KEGG database in order to automatically balance all reactions.

*Constraint–based model analysis*

*Flux balance analysis.* Flux balance analysis is based on the optimization of an objective function, which is used as an evaluation criterion to identify an optimal flux distribution among all possible steady state flux distributions that meet the objective (Belau et al., 2009).

Flux balance analysis can be made in Mathlab with COBRA Toolbox, where the models are structures with fields, such as ‘rxns’ (a list of all reaction names), ‘mets’ (a list of metabolite names), and ‘S’ (stoichometric matrix). The function ‘optimizeCBModel’ is used to perform FBA. To change the bounds on reactions, use a function ‘changeRxnBounds’. Flux balance analysis can be performed to optimize one reaction by changing more than one reaction setting for each upper and lower flux flow amplitude limits, but then it must be noticed which of these reactions is the primary balanced reaction. Flux flow amplitude limits can be changed using COBRA Toolbox specific commands or changing them into XLS format file. Also the whole model must be reloaded into the Mathlab environment.

Flux balance analysis in VANTED software using FBA–SimVis toolbox can be performed to optimize only one reaction by changing up to three different reactions setting for each upper and lower flux flow amplitude limits in user friendly graphical interface. Each reaction is chosen from drop list of all metabolic network model reactions.

*Knock-out analysis.* Knock-out analysis (the deletion of given enzyme or gene) is performed by setting the flux trough a particle reaction to zero and calculating objective function. *In silico* knock-out analysis provides an efficient method to study the essentiality of a reaction in a metabolic network and to gain insight into metabolic changes caused by the deletion (Belau et al., 2009).

Knock-out analysis function in Mathlab environment with COBRA Toolbox is performed to delete one or two enzymes or genes with additional command '*singleGene Deletion(model,method)'*, '*doubleGeneDeletion(model,method)'*.

FBA-SimVis knock–out analysis function can be performed by two different methods.

- Complete – in this scenario we optimize the chosen function by disabling one by one all reactions and seeking from chosen settings the best solution for metabolic network model flux flow. The settings are an optimized reaction, the mode of optimization as maximization or minimization, and the type of optimization linear or non-linear.
- Specific – before call knock-out specific function the user must select reaction on canvas which will be knocked out, then call knock–out function from FBA menu list. In this scenario we optimize the chosen function by disabling selected reactions and seeking from chosen settings the best solution for metabolic network model flux flow. The settings are an optimized reaction, the mode of optimization as maximization or minimization, and the type of optimization linear or non-linear.

*Robustness analysis.* Robustness analysis is performed by varying a particular flux over a specified range of values and by recalculating the objective function. As the resulting curve depicts the sensitivity of the objective function to that particular flux, robustness analysis can be used to assess the effect of reducing flux trough particular reaction on a given objective (Belau et al., 2009).

COBRA Toolbox robustness analysis can be made in Mathlab with COBRA Toolbox by calling '*robustness analysis(model,controlRxn,nPoints)'* command, which is used to compute and plot the value of the model objective function as a function of flux values for a reaction of interest (*control Rxn*) as a means to analyze the network robustness with respect to that reaction. A plot with a defined number of points (*nPoints*) can be generated to visually assess how the objective function changes as the flux through the control reaction varies.

FBA–SimVis robustness analysis function can be performed by two different methods.

- Complete – in this scenario we optimize the chosen function by changing one by one all reactions flux flow and seeking from chosen settings the best solution for metabolic network model flux flow. The settings are an optimized reaction, the mode of optimization as maximization or minimization, and the type of optimization linear or non-linear;
- Specific – the user calls robustness analysis from FBA menu list. In this scenario we optimize the chosen function flux flow by changing selected reactions flux flow and seeking from chosen settings the best solution. The settings are an optimized reaction, the mode of optimization as maximization or minimization, and the type of optimization linear or non-linear and reaction which will be varied.

*Flux variability analysis* is performed by constraining the objective function to the optimal value and computing the minimal and maximal flux through every reaction in the network (range of fluxes). Flux variability analysis can be used to study the redundancies of the metabolic model under investigation (Belau et al., 2009).

COBRA toolbox determines the minimum and maximum flux value that each reaction in the model can possess while satisfying the steady-state assumption of FBA and the constraints on the system using the function: *'[minFlux,maxFlux]= fluxVariability(model,optPercentage)'*.

FBA-SimVis Simulation results of flux variability analysis are provided by displaying the minimal and maximal flux for each of the network reactions within the respective reaction nodes.

## Results and Discussion

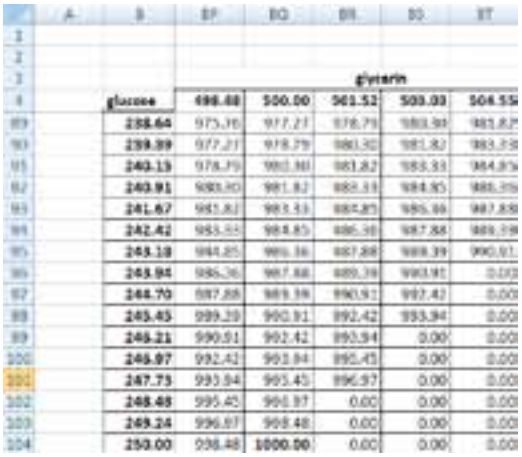
FBA, when analyzing all fluxes using different options, gives results into metabolic network flow chart, although COBRA Toolbox returns the results in the matrix in number formats. This information using COBRA Toolbox on the metabolic network is given into several different matrices. To better analyze these multiple matrices with single software there is need for other software like MS Excel, SPSS, Open Office CALC sheets technology. Each time you need to compare several data from different matrices changing focus from one to another.

FBA flow can be represented in flow chart, but this tool for flow chart creation for researchers is not available for experiments. COBRA Toolbox provides many visualization maps for FBA flow rate, and can show graphical user friendly maps, but free maps versions is difficult to create, because creation tool SimPheny (http://www.gtlifesciences.com/technology/Simpheny.html) is a commercial toolbox for FBA simulation creation. An alternative way to analyze FBA data from COBRA Toolbox is to create built in Mathlab 2D or 3D charts. For example, robustness analysis has been made for *Zymomonas mobilis* bacteria steady state metabolic network adaptation for glycerol conversion into bioethanol. First of all, S matrix has been created and correctly filled, then robustness analysis function has been executed with COBRA toolbox, and afterwards the obtained information can be analyzed numerically through matrices switching between them. A different method is to call 2D or 3D graph commands and analyze these graphs. For example, glucose flux constrain is +250 Unit - mmol $gDW^{-1}$ $h^{-1}$ (mill moles per gram dry cell weight per hour) and maximized Ethanol transport flux (objective). When

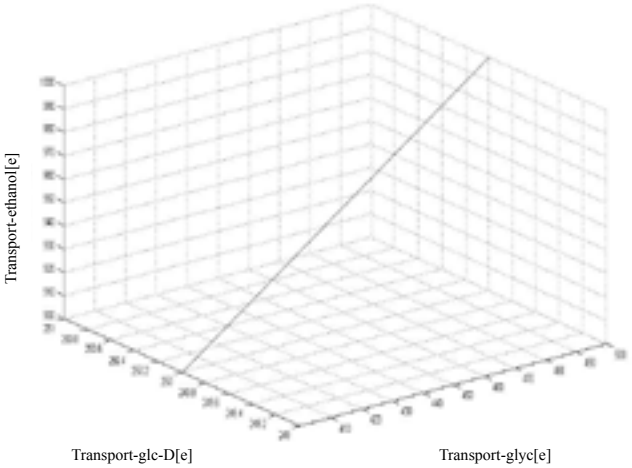
Transport Glycerol flux constrains reaches +500 Unit - mmol $gDW^{-1}$ $h^{-1}$, then is reached maximal flux of Ethanol in steady state + 1000 Unit - mmol $gDW^{-1}$ $h^{-1}$. In case when Transport Glycerol flux overruns +500 Unit - mmol $gDW^{-1}$ $h^{-1}$, then starts inhibition. The results are given in matrix form (Fig. 2. a) and the results can then be plotted as a 3D surface (Fig. 2. b). The chart is easier to analyze and perceive than the numeric value.

FBA-SimVis the visualization of simulation results obtained from robustness analysis is offered in two ways, either displaying the sensitivity curve for each of the network reactions within the respective reaction nodes (Fig. 2. c), or by displaying the flux distribution of a particular enzymatic reaction, which can be varied by slider interactions (Fig. 2. d). While the first option provides a rapid and comparative overview of all reactions included in the network, the second option offers the user the possibility to obtain detailed insights into specific enzyme/objective function dependencies.

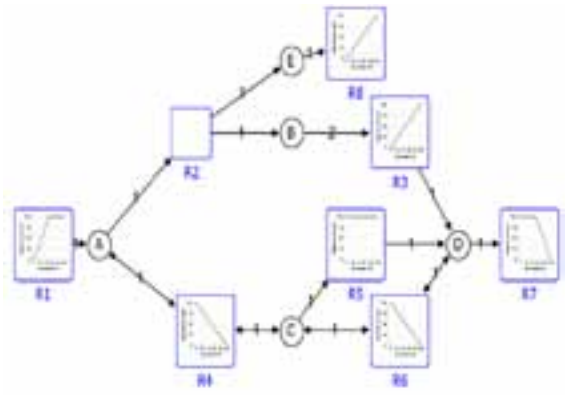
In FBA–SimVis for steady state *Zymomonas mobilis* bacterial metabolic network adaptation for glycerol conversion into bioethanol model was executed robustness analysis function with default constraint, where FBA-SimVis, unlike the COBRA Toolbox, results was represented in user friendly graphical interface as dynamically visually changing thickness of arrows with additional numerical values. FBA–SimVis visually on canvas represents steady state metabolic network each reaction flow rate, direction and numeric rate value, but it offers to vary with only 3 reactions to optimize only one function, unlike the COBRA Toolbox which analyzes all the reaction flows at the same time and optimized can be more than one function. COBRA Toolbox is a powerful FBA calculation tool for metabolic networks, but the FBA-SimVis is a great FBA calculation tool with limited conditions opportunities.



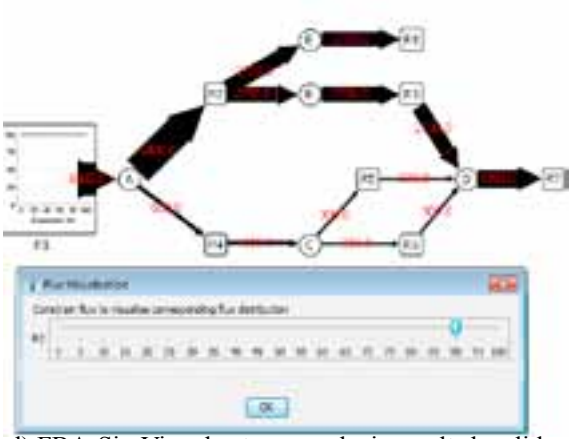

a) COBRA toolbox Glycerol robustness analysis results matrix 100×100.




b) COBRA toolbox Glycerol robustness analysis results chart.



c) FBA-SimVis robustness analysis results.



d) FBA-SimVis robustness analysis results by slider interactions.

Figure 2. Robustness analysis results visualization.

## Conclusions

FBA-SimVis for Steady state metabolic network models analysis is provided for a small metabolic network, because making some FBA analysis there are small possibilities to change an unlimited count of variables, to choose or change or optimize reactions as variables.

FBA-SimVis does support SBGN standard to importing SBML files. It would be great advantage to improve FBA-SimVis with a supported SBGN standard automatically change metabolites and reactions shapes to FBA-SimVis eligible shapes. FBA analyses need to extend possibilities to change an unlimited count of variables, to choose change and optimize reactions as variables.

COBRA Toolbox for Steady state metabolic network models analysis is provided for greater metabolic networks with hundreds or thousands of reactions. It allows changing an unlimited count manipulating and optimizing reactions fluxes. To better analyze the obtained data from FBA analysis we can call maps which are made by freeware SimPheny and can get results in user friendly graphical interface in Internet browser. For example, has been free available example for *Escherichia coli* bacteria with full genome scale metabolic network, where, with the help of COBRA toolbox available commands, it is possible to experiment and obtain data but with no full functionality. Our proposal is to release map making software on next nearest COBRA toolbox versions with easy, user friendly graphical interface and functionality between the maps and matrices. Including a freeware map making tool on next releases, COBRA Toolbox would attract much more researchers, researchers' teams in silico analysis of steady state mechanisms of metabolic networks research. COBRA Toolbox is a calculation tool for an enormous amount of data, but FBA - SimVis Tool is at FBA flux visualization at user friendly graphical interface.

**Acknowledgements**

This study is supported by ESF project 'Latvian Interdisciplinary Interuniversity Scientific Group of Systems Biology' /2009/0207/1DP/1.1.1.2.0/09/APIA/VIAA/128.

**References**

1. Becker S.A., Feist A.M., Mo M.L., Hannum G., Palsson B.Ø., Herrgard M.J. (2007) Quantitative prediction of cellular metabolism with constraint-based models: the COBRA Toolbox. *Nature Protocols,* **2**, pp. 727-738.
2. Belau E.G., Klukas C., Junker B.H., Schreiber F. (2009) FBA-SimVis: interactive visualization of constraint-based metabolic models. *Systems biology*, 25, pp. 2755-2757.
3. Hucka M. (2003) The systems biology markup language (SBML): a medium for representation and exchange of biochemical etwork models. *Bioinformatics,* 19, pp. 524-531.
4. Kauffman J.L., Prakash P., Edwards J.S. (2003) Advances in flux balance analysis. *Current Opinion in Biotechnology*, 14, pp. 491-496.
5. Klipp E., Herwig R., Kowald A., Wierling C., Lehrach H. (2006) Systems Biology in Practice. *Weinheim*, *Wiley-Vch Verlag GmbH&Co.KGaA*, 465 p., pp. 399-419.
6. Rahmanian S., Schellenberger J., Palsson B.Ø. (2009) Customized Visualization of Metabolic Maps in the Cobra Toolbox. Available at: http://ucsdstudents.calit2.net/files/posters/2009/Sorena%20Rahmaniyan.pdf, 15. February 2011.
7. Reed J.L., Palsson B.Ø. (2003) Thirteen years of building constraint-based in silico models of *Escherichia coli*. *Bacteriology Journal,* 185, pp. 2692-2699.
8. Thiele I., Palsson B.Ø. (2010) A protocol for generating a high-quality genome-scale metabolic reconstruction. *Nature Protocols,* 5, pp. 93-12.
9. Varma A., Palsson B.Ø. (1994) Review: metabolic flux balancing: basic concepts, scientific and practical. *Nature Protocols,* 12, pp. 994-998.

# LOW-COST ELECTRICAL ENERGY MONITORING METHOD WITH WIRELESS ICT

***Peteris Apse-Apsitis, Ansis Avotins, Leonids Ribickis***
*Riga Technical University*
*peter.apse@gmail.com; ansis@eef.rtu.lv; leonids.ribickis@rtu.lv*

**Abstract**

The electricity consuming end-user profile has changed in latest years, due to development of new electrical devices with increased functionality and complexity. Also increasing number of electricity consuming equipment that is available to end-user, makes very hard to identify real electricity consumption of each device. The existing wall plug digital power meters are too expensive for long-term power metering of each consuming device. The article describes a different approach of power metering system that lowers the costs and price of needed metering equipment. A new concept of low-cost energy monitoring system with wireless communication is developed.

**Key words**: energy efficiency, energy monitoring, wireless communication.

## Introduction

It is obvious that in order to achieve global goals of energy efficiency, an additional economic and political stimulators will be needed to change consumer habits of conservative household, residential building in cities or rural region end-user. One of such economical stimulator is natural continuous rise of price for electricity, as it is connected with limited availability of fossil fuels. Other economical stimulators are different discounts or economical bonuses for community, if using Green Energy tariff, like it is proposed in Green Energy Supply Certification Scheme by OFGEM – the government regulator of gas and electricity markets in the UK. The political stimulators are enforced with law or government rules, like restrictions for incandescent bulb in EU, or Green energy Tariff in Ukraine, and other countries, to stimulate alternative energy development. European Union at year 2008 adopted an integrated energy and climate change limitation policies to be implemented by year 2020, where such events as greenhouse gas reduction by 20%, energy consumption reduction by 20% (or improvement of energy efficiency), 20% of EU energy obtain from renewable sources, should be implemented.

Moreover, 40% of energy consumption in Europe is related to buildings (residential, public, commercial and industrial buildings) (EC, 2002, Bordeau, 2009). Energy Efficiency Action Plan predicted that the biggest cost-effective energy savings potential is found in residential buildings (around 27%) and commercial buildings sector (around 30%), therefore, a special emphasis on energy efficiency and need of integration of alternative energy sources, must be made (Bordeau, 2009), as it is introduced also in SmartGrid concept (availability of two way energy flow).

At the same time, load growth forecast for the housing sector shows that, due to raising the quality of life, households are becoming increasingly available to a wider range of household electrical appliances, thus contributing to the electrical load increase, actualizing the problem. For example in Latvia, after local electrical energy supplier A/S Latvenergo and Riga Energy Agency data (Golunovs, 2008), comparing the year 2007 with year 2003, the electrical energy consumption has increased by 11%, and is expected to continue, as well as the global trend is showing the same tendency all over the world (see Fig. 1.) (EIA, 2008).

Existing dwellings consume about 3 times more energy than it is prescribed in the current Latvian building regulations, which were developed before households became available with wide range of electrical appliances, as well as the classification of average consumption per device type has been changed.

As the prices for electrical energy are increasing, the idea of SmartMetering systems and SmartPlugs in recent years got attention from both sides – energy supplier and consumer, as it could greatly contribute to energy consumption reduction, by changing the habits of consumer, and thus creating more stable power grid in future.

It can be concluded that one of the fundamental problems in electrical household is non-saving energy consumer. In order to solve this problem, the end-user must be informed about his possibilities to save energy, which could be reached by implementing smart metering systems with graphical indicators on screen, or visualization on PC with help and tips for possible solutions of energy consumption reduction possibilities of each consuming device. Thus the main task for authors is to develop concept of low-cost monitoring system with possibility to transmit gathered data to one or more central processing units (CPU). The second task is to develop precise and low-cost current measurement element to replace Hall sensors, which are very expensive.

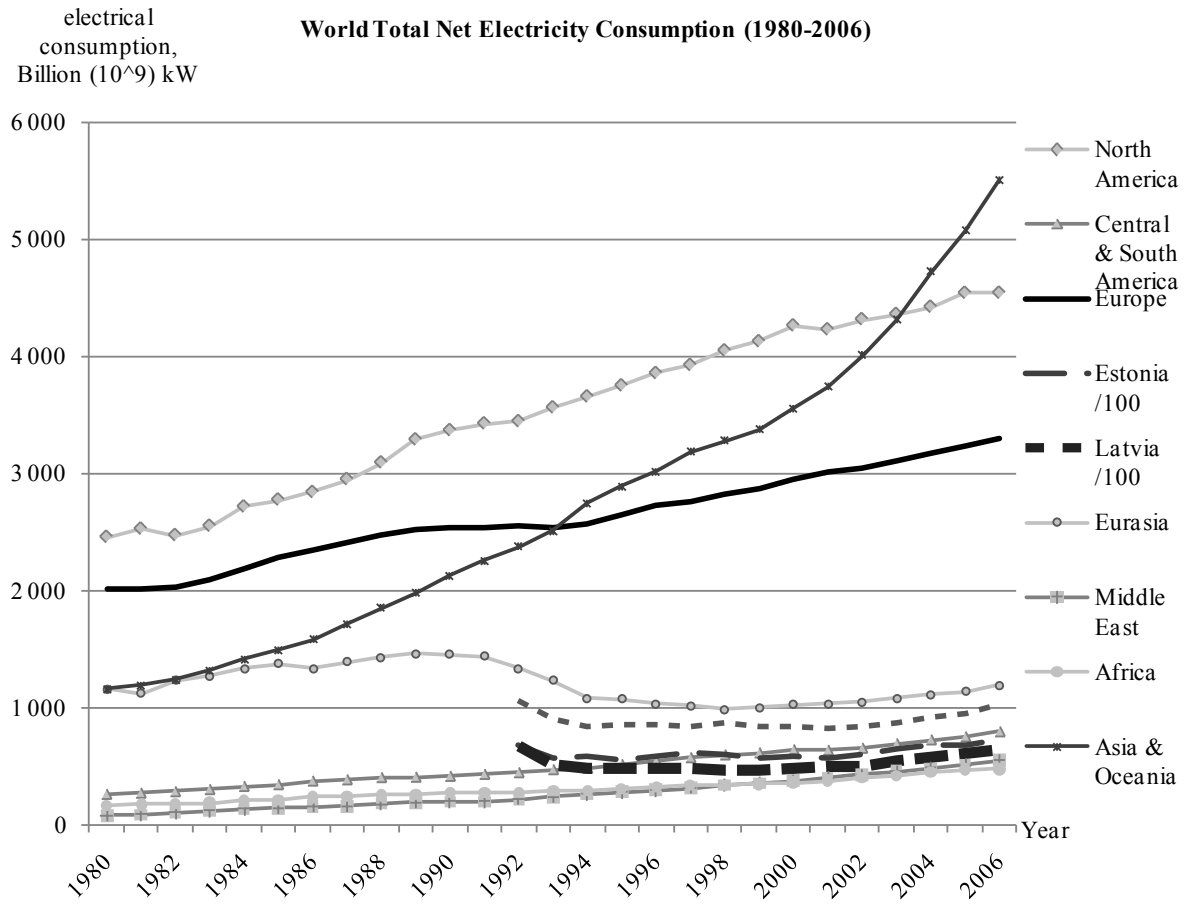



Figure1. World total electricity consumption 1980-2006, according to (EIA, 2008).

## Materials and Methods

Existing metering systems (see Fig. 2.), in office or household applications, has just one electrical energy measuring device, regardless that there are many energy consumer types ($P_1$...$P_n$) – TV's, music, fridges, microwaves, washing machines, heaters, boilers, computers, lighting ($P_2$) etc, and without additional metering devices or special calculations, proper energy consumption of each consumer device can't be obtained.

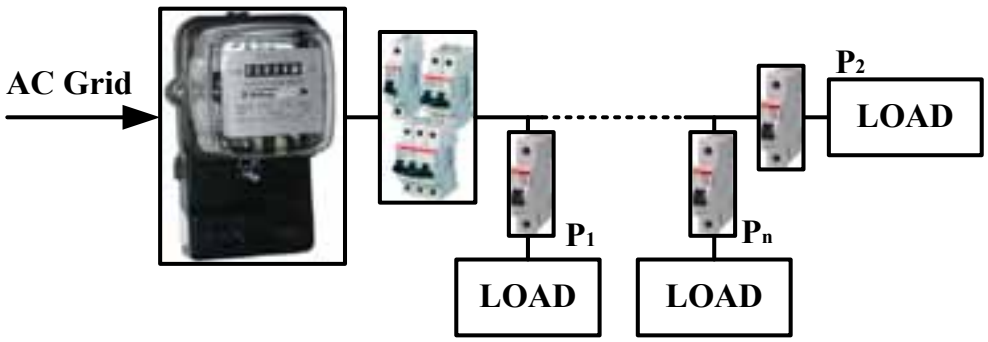



Figure 2. Block diagram of energy metering method for existing system.

For end-user, a digital metering devices, that can be plugged into electrical socket for additional electrical consumption metering (see Fig. 3.), already are available on market, but they are very expensive to determine energy consumption for each consumer type, as the average household has at least 8 – 10 regular electricity consuming devices, and as the digital metering devices, that can be plugged into electrical socket cost around 18-30 EUR (WEB catalogues, 2011). Thus to monitor and understand electrical consumption of each device, an investment of 180 – 300 EUR must be made, which is too high for typical end-user.

This method also doesn't allow monitoring consumption of lighting system, which possibly has different light sources, like incandescent bulbs or Compact Fluorescent, and installation of digital socket metering devices is not possible. Another problem is that typical end-user doesn't have educational background to properly understand meanings of W, VA, VAr, kW, kWh, A, V and cosφ values visualised on the digital socket metering device display.

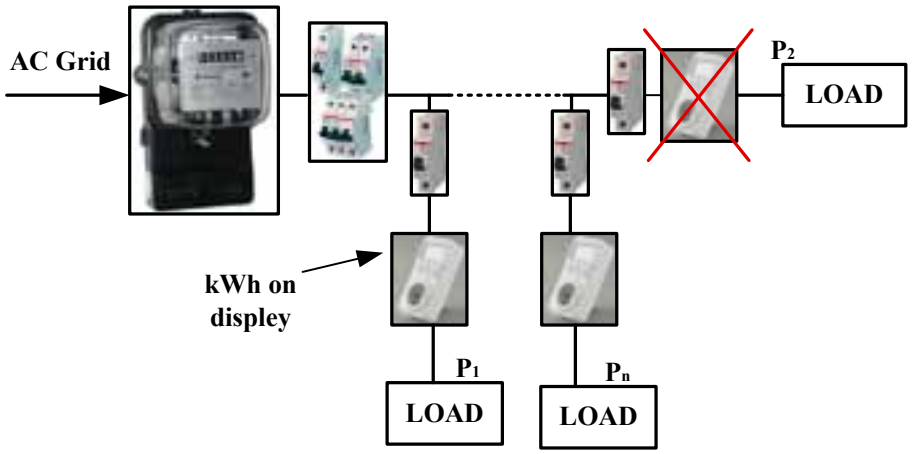



Figure 3. Block diagram of energy metering method with digital socket-plugged energy meters.

This clearly indicates the need for much cheaper solution with much direct and understandable message to end-user. Also the metering device should have small dimensions, so that it could be integrated into back of socket or into luminary chuck (typically E27) (see Fig. 4. $P_2$ load). The main task is to provide energy consumption apportionment between consumers instead of precise energy consumption metering for each consumer type, as the distance between central measurement point, and monitoring point is less than 100m, the voltage u(t) practically is the same for each consumer and energy consumption can be characterized by just monitoring each consumer current i(t), and central measurement point makes precise measurements of P, u(t), i(t), receives relative current values from monitoring device, via wireless or power line communications, and makes indicative visualisation of energy consumption per consumer on display or sends it to PC. 2.5% – 5% precision is enough for monitoring task and such precision corresponds to 80 sec or 180 sec consumer state "ON". Energy consumer power typically is within range from 10W up to 2250W (typical 10A wall plug), consumers have R or RL (also RC) load characteristics, current and voltage graphs are sinusoidal under normal conditions.

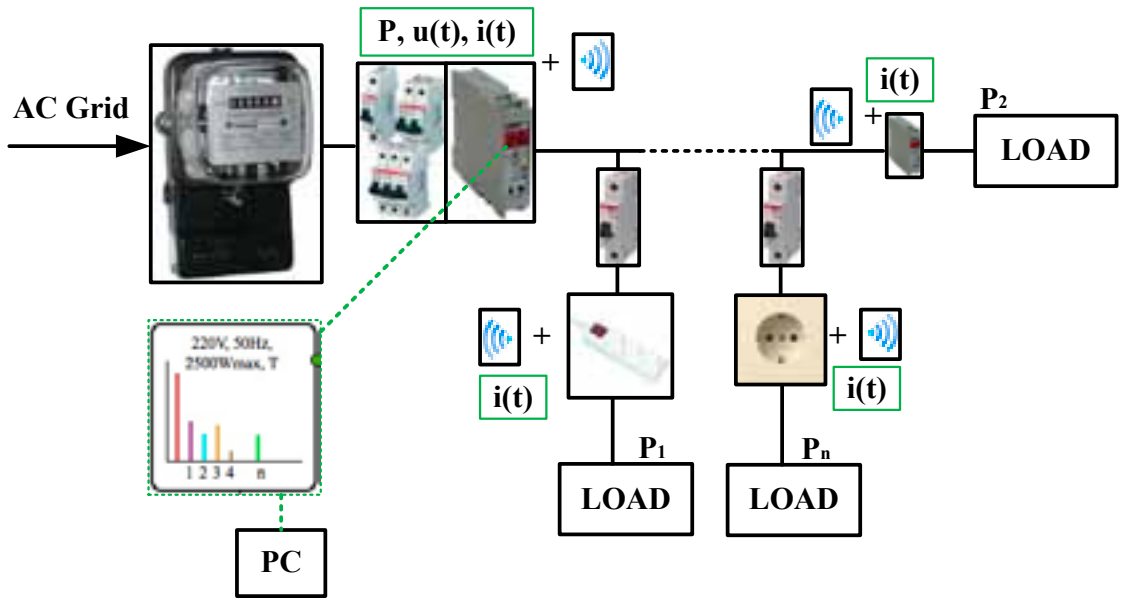



Figure 4. Block diagram of energy metering method of proposed solution.

Electrical power contains active and reactive components and they correspond to each other via cosφ. Energy supplier place bill, in most cases of individuals and legal persons, just for active component consumption, for example in Latvia charges for reactive energy, when tg φ is greater than 0.4 (cos φ <0.929) and allowed load 100 kW and more, is additional 0.003 Ls $kV^{-1}$ Arh.

$$P_m = I_m \times \sin(\omega t) \times U_m \times \sin(\omega t) = \frac{I_m \times U_m \times [1 - \cos(2\omega t)]}{2}$$

It is known that for sinusoidal waveforms AC circuit is characterized by equation (1, 2) (Grow, 1997):

$$P = I \times U \times \cos\varphi \quad (1)$$

or

$$P = I \times U \times \sin\left(90^{o} \pm \varphi\right) \quad (2)$$

Voltage, active and reactive current graphs under different cosφ values are shown on Fig. 5.

From here it is possible to determine that active current max value (amplitude) is full current value measured at 1/4 period (90°) or 3/4 period (270°) and reactive current max value is full current value measured at 1/2 period (180°) or period (0° or 360°).

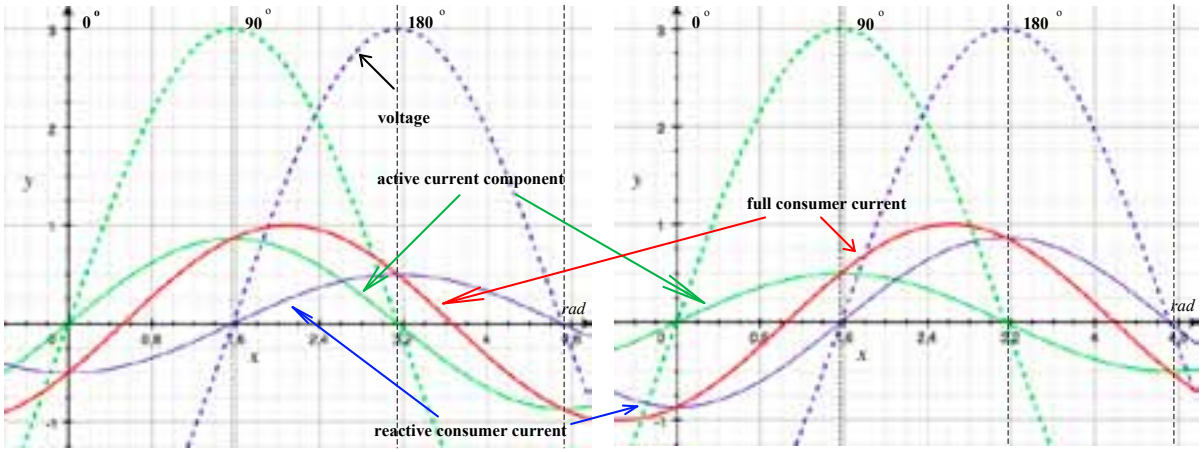



Figure 5. Voltage, active, reactive and full current graphs, where a) cosφ=0.5; φ=60; b) cosφ=0.866; φ=30.

**Results and Discussion**

In order to get precise measurements and relative current value distribution in total energy consumption, the central measurement point, measuring both voltage and current values, must be synchronized with monitoring measurement point, which measures only current value, at the exact point.

The key element is wireless signal transceiver, where to fulfil the low-cost problem, a radio frequency transceiver with SC2262 encoder and SC2272 decoder is chosen, both operating at 400 MHz frequency, each integrated circuit (IC) costs around 0.154 USD, ready PBC mounted solution costs 1.08 USD (FOB China). Also other solutions are available, like radio frequency Wireless receiver ZR7AZ which operates at frequency of 315/433.92MHZ, voltage DC 5V+/-0.5V and quiescent current is 2.7mA (1.1-1.5 USD), but SC2262 has wider input voltage range – 1.8-12V and together with SC2272 – wider temperature range (-30-70 °C).

All ICs works with amplitude modulation, has 8-12 tri-state code address pins and 2-6 info pins, which gives totally 50000 addresses, and that is more than enough for this type of application. Each monitoring device measurement point has his unique address, at which receiver of monitoring device receives synchronization impulse. While device is measuring, counter counts 100 Hz, when measurement is finished, measurement device sends STOP signal to counter. Active and reactive current sampling and timing circuits are shown on Fig. 6. and operation diagrams are shown on Fig. 7.

Each of consumers has his own address for data communication. Wireless data exchange as well as current measurement technical design can be made in very different ways. Mentioned solution use the lowest price design. Price reduction reduces information acquiring speed and increase accuracy.

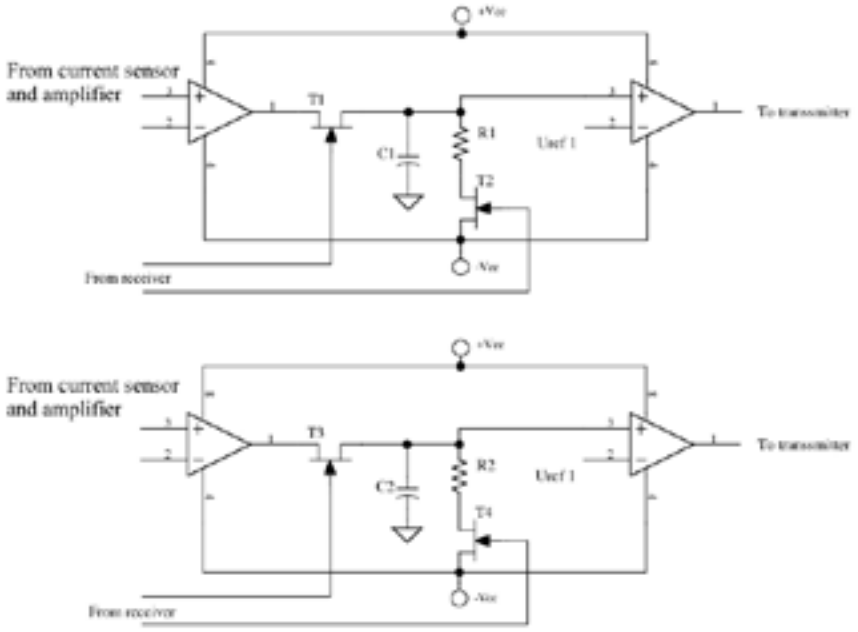



Figure 6. Active and reactive current sampling and timing circuits.

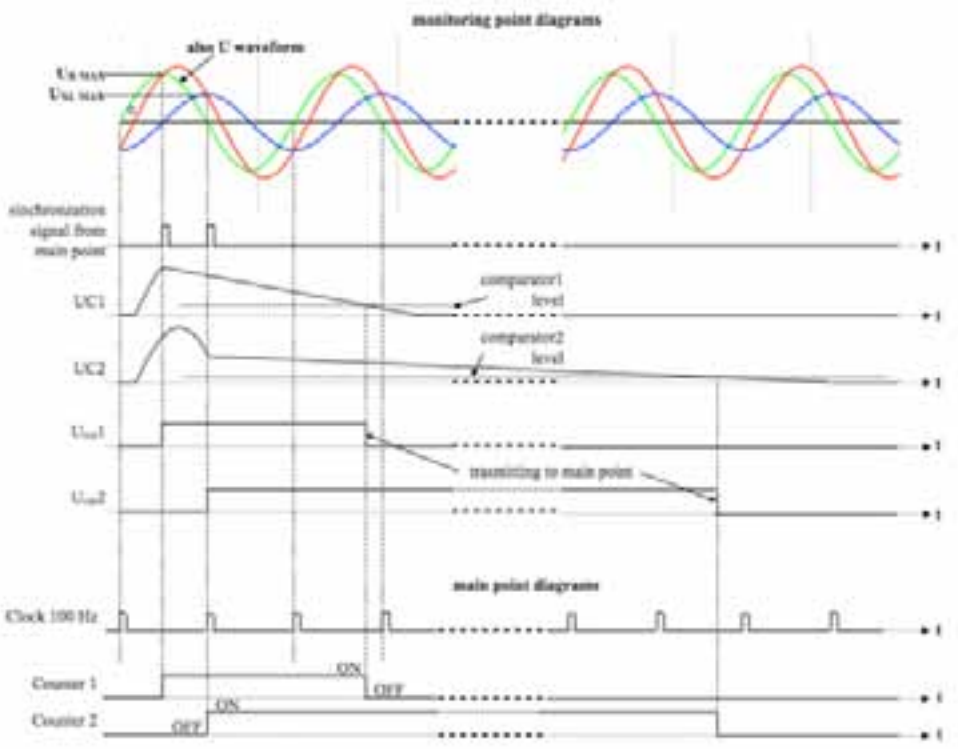



Figure 7. Operation diagrams electrical consumption monitoring device.

Main point sends synchronization signal to measuring point determined by its 12-bit address at period 90°. Switch T1 opens to charge capacitor C1 for a short while. Located in the main point, Counter1 starts to count mains half periods at 100Hz rate, where clock signal is generated by means of zero-crossing detection of the grid voltage. Switch T1 is closed after C1 is charged to level and switch T2 is opened. Capacitor C1 is discharged through resistance R1. Discharge is characterized by following time constant $\tau$=R1C1:

$$U_{C1} = \left(U_{C1}max + Vcc-\right) \times e^{-t/\tau} \quad (3)$$

Capacitor is discharged against Vcc- level to achieve better results – to avoid capacitor exponential discharge end phase. For UC1max=4V, -Vcc=-5V, τ=4.4, T=250/100=2.5 sec calculated τ is 4.4sec – time to discharge C1 from UC1max to 0. Signal from measuring point is send to main point when UC1 < Uref1 and Counter1 stops. Counter1 counted number represent consumer active current amplitude value. Reactive current amplitude is determined in to the similar way via T3, T4, C2, R2 and Counter2. The typical household consumes also reactive (non-sinusoidal) current, thus for implementing proposed measuring system, a filtering element, like operation amplifier, for measured signal will be implemented.

Readings from measuring points are provided sequentially and corresponding counter values are shown as bar graph at the central measurement point device display. There are no power or energy calculations in-between, so it is possible to use spare clock frequency for other matters. Bar graphs represent energy consumption apportionment between measuring points.

Shunt was used as current sensor, and dissipated power in shunt must be taken in to account as shown in Table.1 and this fact reduce shunt value and make more difficult to read voltage drop on it for low consumer energy (power). Unfortunately other current sensors like Hall effect (LEM FHS 40-P/SP600) etc. are more expensive, 3 – 7 EUR (WEB catalogues, 2011) and cannot be applied in this case, as the goal is to get low-cost measurement device, the shunt resistors like ERJ-M1WSJ8M0U, PMR50HZPJU8L0, or similar, has price from 0.4 – 1.00 EUR, which is significant price.

Table 1

**Measurements of shunt power**

| P, W | I, A | Imax, A | Rshnt, Ω | Pshunt, W |
|---|---|---|---|---|
| 10 | 0.045 | 0.065 | 0.0084 | 0.000017 |
| 15 | 0.068 | 0.097 | 0.0084 | 0.000039 |
| 20 | 0.091 | 0.129 | 0.0084 | 0.00007 |
| 30 | 0.136 | 0.194 | 0.0084 | 0.00016 |
| 40 | 0.182 | 0.258 | 0.0084 | 0.00028 |
| 50 | 0.227 | 0.323 | 0.0084 | 0.00043 |
| 100 | 0.455 | 0.645 | 0.0084 | 0.0017 |
| 200 | 0.909 | 1.291 | 0.0084 | 0.0069 |
| 400 | 1.818 | 2.582 | 0.0084 | 0.0278 |
| 500 | 2.273 | 3.227 | 0.0084 | 0.0434 |
| 600 | 2.727 | 3.873 | 0.0084 | 0.0625 |
| 800 | 3.636 | 5.164 | 0.0084 | 0.1111 |
| 1000 | 4.545 | 6.455 | 0.0084 | 0.1736 |
| 1200 | 5.455 | 7.745 | 0.0084 | 0.2499 |
| 1400 | 6.364 | 9.036 | 0.0084 | 0.3402 |
| 1600 | 7.273 | 10.327 | 0.0084 | 0.4443 |
| 1800 | 8.182 | 11.618 | 0.0084 | 0.5623 |
| 2000 | 9.091 | 12.909 | 0.0084 | 0.6942 |
| 2250 | 10.227 | 14.523 | 0.0084 | 0.8786 |

For further developments, the data exchange between central measurement point (also data concentrator can be in middle for larger systems) can be provided via different communication methods. Due to SMART GRID concept, a Power Line Communication and wireless communications (ZigBee supporting protocol) are tended to be used in near future for various electrical grid and data network. There are various examples of Power Line Communication (PLC) modems for this purpose, which differs by functionality, availability of internal peripherals, implemented coding algorithm, as well as integrated/supported protocol. These ICs of PLC or wireless communication, significantly increases total costs of metering or monitoring device, approximately by 7 - 9 EUR, thus this won't be a low-cost application, unless the prices for those ICs would drop-down, in that case the functionality of metering device could be increased.

In Smart Grid context, new technologies for smart metering are uprising, for example a Philips Smart Switch, which monitors power consumption of plugged in devices, and sends the data to home desktop PC via wireless communication, and with built in triac, can switch ON/OFF the consuming device from computer.

For energy consumption monitoring system, a communication network with MESH topology also can be used, where each monitoring point is connected to all closest monitoring points, in case of low signal strengths or other similar problems. The ZigBee protocol is supporting

MESH topology. ZigBee is broadly categorized as a low rate WPAN (Wireless Personal Area Network), where with WPAN is possible to identify and with one command switch on/off lots of measuring point devices, because max number of PAN is 216=65535 ("0" doesn't counts), also a PAN can consist of 65535 objects. It is more sufficient for large systems, including all possible sensors, and other future objects. ZigBee uses low data rate (20 - 250 kbps), low power consumption, and works with small packet devices. ZigBee - set of high level communication protocols based upon the specification produced by IEEE 802.15.4.

## Conclusions

A new concept of low-cost energy monitoring system with wireless communication is developed. This method allows designing and implementing low cost consumed energy monitoring for several consumers. For current measurements a shunt is selected, still power dissipation values will be various, if using different copper suppliers/ materials, thus a additional compensating scheme is needed. Achieved price level is 4.55 – 4.70 EUR per measuring point and 29.88 – 32.77 EUR per main point unit, not including assembly costs. Overall costs are less than 71 EUR and it is acceptable for average household or small office. A data communication between two MRF24J40MA and two RFM12B based transceivers were established and can be used for electrical energy monitoring device communication purposes.

## References

1. Bourdeau Luc (2009) Energy Efficient Buildings European Initiative, ECTP & E2BA Secretary General. 29.04.2009. Available at: http://www.ectp.org/cws/params/ectp/download_files/ 36D928v2_E2BA_Brochure.pdf, 22 March 2011.
2. EIA (2008) Energy Information Administration (U.S.) International Energy Annual 2006, Posted: 8 December 2008. Available at: www.eia.doe.gov/iea/, 22 March 2011.
3. EC-European Commission (2002) Building energy efficiency directives 2002/91/EK 6. Available at: http://ec.europa.eu/energy/efficiency/buildings/buildings_en.htm, 22 March 2011.
4. Golunovs J. (2008) Riga household specific electrical loads – planning instrument for improvement of energy efficiency. *Energy and Automation.* 10/2008 (publicity of research results of J.Golunovs), pp. 32-34.
5. Grob B. (1997) *Basic Electronics, eight edition*, The McGraw-Hill Companies, 936 Eastwind Drive Westerville, Ohio 43081, pp. 416-456.
6. Greivulis J., Raņķis I. (1992) Iekārtu vadības elektroniskie elementi un mezgli (Electronic elements and nodes for device control). Rīga: *Izdevniecība «Avots»*. 288 lpp. (in Latvian).
7. Online WEB electronic format catalogues (2011) Electronic component and device catalogues 2011 of - ELFA, FARNELL, DIGI-KEY, Available at: www.elfa.com, 22 March 2011.