

## $\alpha$ -AMYLASE ACTIVITY IN FREEZE-DRIED AND SPRAY-DRIED HONEY

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### Abstract

Honey is a naturally supersaturated sugar solution, which tends to crystallize. The crystallization of honey can lead to unwanted fermentation that can have a negative impact to honey quality. The production of honey powder could be an alternative method to prevent honey from fermentation. Honey powder could be used as alternative substitute to liquid honey that would allow to use this product more widely in the food industry.  $\alpha$ -amylase activity is one of the most important parameters to evaluate the quality of honey. The aim of this study was to investigate the effect of freeze-drying and spray-drying on honey  $\alpha$ -amylase activity. Detection of  $\alpha$ -amylase activity was carried out by spectrophotometric method. High-performance liquid chromatography was used to determine the content of hydroxymethylfurfural in the powders. The obtained results showed that both drying methods had a negative impact to the enzyme activity in the samples. The lowest activity of  $\alpha$ -amylase (8.3 DN) was measured in the spray-dried honey powder. Concentration of hydroxymethylfurfural (HMF) in the powders did not exceed required concentration 40 mg kg<sup>-1</sup>.

**Key words:**  $\alpha$ -amylase activity, freeze drying, spray drying, honey, HMF.

### Introduction

Honey is well-known food product over the world due to its sweet taste, aroma, and health benefits. Moreover, honey is chemically complex food product, which consists of approximately 200 substances (Geana & Ciucure, 2020). Fructose and glucose are the main constituents of honey. There is also a wide range of minor components that are present in honey such as enzymes, organic acids, amino acids, phenolic compounds, etc. Honey quality and chemical composition depend on the floral and geographical origin, climate and processing (Sakač *et al.*, 2018).

As a supersaturated sugar solution, honey tends to spontaneously crystallize. The spontaneous crystallization of honey increases water activity, which can lead to unwanted fermentation (Tappi *et al.*, 2019). The unwanted fermentation can negatively affect the quality of honey. Thermal processing is often used to prevent the negative effect of crystallization (Tosi *et al.*, 2004; Ribeiro *et al.*, 2018). Thermal methods could be used to transform liquid honey into powdered honey by drying. This is an alternative method that could be used to preserve honey. Moreover, powdered honey has many advantages such as ease of packaging and handling, prolonged shelf life, expanded usage in the food industry (Kilinç & Demir, 2017). The production of honey in a powdered form is challenging due to its high concentration of sugars and organic acids. Sugar-rich products such as honey tends to form lumps or syrup during the drying process (Samborska, 2019). Different drying aids such as maltodextrin, whey protein isolate, Arabic gum are added to honey to increase the glass transition temperatures (Shi, Fang, & Bhandari, 2013; Muzaffar, 2015). The concentration of drying aids in honey powder cannot be lower than 50% of solids to successfully perform the drying of honey (Bhandari, Datta, & Howes, 1997). Samborska and co-workers (Samborska, Sokołowska, & Szulc,

2017a) reported that pre-treatment methods such as diafiltration can be used to obtain powder, which contains 75% of honey.

Dehydrated honey can be produced by vacuum drying, microwave vacuum drying, freeze-drying and spray drying (Cui *et al.*, 2008; Nurhadi & Roos, 2016; Sramek *et al.*, 2016). Spray drying is widely used to obtain honey powder. The method is very popular due to its cost effectiveness, short production time and high product quality (Jedlińska *et al.*, 2019; Samborska, 2019). Freeze-drying is an especially useful drying technique to preserve bioactive compounds. Unfortunately, due to the methods expensiveness and long production time, freeze-drying is rarely used to produce honey powder (Subramanian, Hebbar, & Rastogi, 2007).

Enzymatic activity is an important indicator to evaluate the quality of honey. Enzymes are very sensitive to thermal processing, which can lead to loss of enzymatic activity of honey (Kowalski & Lukaszewicz, 2017).  $\alpha$ -amylase, which is predominant enzyme in honey, can be inactivated by thermal treatments at 80 °C for 1.2 hours (Schade, Marsh, & Eckert, 1958; Tosi *et al.*, 2004).  $\alpha$ -amylase (diastase) activity is the main indicator to evaluate the quality of honey as well as authenticity. According to the Council of the European Union Directive relating to honey 2001/110/EC,  $\alpha$ -amylase activity is required not to be lower than 8 DN. Diastase number (DN) in Schade scale is defined as grams of starch hydrolysed during one hour at 40 °C (Kowalski *et al.*, 2012). Hydroxymethylfurfural is another important indicator that is used along with  $\alpha$ -amylase activity to evaluate the quality of honey (Pasiak, Kiriakou, & Proestos, 2017).

The aim of this study was to investigate the effect of freeze-drying and spray-drying on honey  $\alpha$ -amylase activity.

Table 1

**Chemical composition of multifloral honey**

Free acidity, meq kg <sup>-1</sup>	pH	Moisture content, %	$\alpha$ -amylase activity, DN	Fructose, g 100 g <sup>-1</sup>	Glucose, g 100 g <sup>-1</sup>	HMF, mg kg <sup>-1</sup>
42.0±1.0	3.66±0.01	20.5±0.2	18.5±0.3	36.4±0.2	31.8±0.2	41.4±0.5

**Materials and Methods**

The research was carried out in the scientific laboratory of Natural compounds and the scientific laboratory of Food processing engineering at the Faculty of Food Technology, the Latvia University of Life Sciences and Technologies.

Multifloral honey sample was delivered by the Latvian beekeeper of southern Latvia. The production year of honey was 2018. The main characteristic properties of the honey sample are shown in Table 1.

Maltodextrin (STAR-DRI® 10 NG, TATE & LYLE) was supplied by a local company (BANG & BONSOMER LATVIA, Rīga, Latvia). Dextrose equivalent (DE) of maltodextrin was 10.4, and moisture content was 4.3%. Maltodextrin was used as a carrier.

*Obtaining freeze-dried and spray-dried honey*

20% aqueous solutions of honey with maltodextrin were prepared for freeze-drying and spray-drying experiments. The ratio of honey and maltodextrin in the solutions was 1:2.

Freeze-drying of honey solution: the prepared solution was poured into a plastic freezer box, where the thickness of solution was approximately one centimetre. The freezer box was placed in the freezer for 2 hours at -20±1 °C. After 2 hours, the frozen sample was transferred to a freeze-dryer ALPHA 1-2 LDplus (MARTIN CHRIST Gefriertrocknungsanlagen GmbH, Germany). The freeze-drying process was performed under following conditions: the temperature of condenser was set to -50.6±0.5 °C and the pressure was 0.036 mbar. The duration of lyophilisation was 72 hours.

Spray-drying of honey solution: the prepared honey solution was spray-dried in BÜCHI mini spray drier B-290 (Labortechnik AG, Switzerland). The spray-drying process was carried out under following condition: the inlet air temperature was set to 180 °C, outlet temperature was 80 °C, sample speed rate was 15 mL min<sup>-1</sup>.

After drying, the obtained powders were collected in polyethylene bags and stored in the dark and dry place at temperature of 20 °C until further analysis.

*Determination of moisture content*

One gram of obtained powders was weighted on glass fibre sheets and placed on the sample pan of moisture analyzer AND MX-50 (A&D Company, Limited, Japan). The moisture analysis was carried

out by heating up the samples at a drying temperature of 140 °C, the duration of analysis was 20 minutes. Moisture data was recorded using the software 'WinCT-Moisture'.

*Determination of  $\alpha$ -amylase activity*

$\alpha$ -amylase activity was evaluated in liquid honey sample and obtained honey powders. The determination of  $\alpha$ -amylase activity was performed by Amylzyme assay procedure (Megazyme, Ireland). The samples for analysis were prepared according to Amylzyme assay procedure. The absorbance of the analysed samples was measured by spectrophotometer Jenway 6405 UV/Vis (JENWAY, the U.K.) at 590 nm wavelength. The activity of the enzyme was calculated using the following formula:

$$\text{Schadeunits} = 20.0 \times \Delta \text{Abs} \quad (1)$$

where: is the absorbance of the sample at 590 nm.

*Determination of hydroxymethylfurfural*

The concentration of hydroxymethylfurfural was detected by high-performance liquid chromatography (HPLC). Honey and honey powder solutions were prepared at a concentration of 50 g L<sup>-1</sup>. The analysis of the samples was performed using an analytical column PerkinElmer C18 (4.6 mm × 250 mm I.D., particle size 5 mm). The temperature of column and detector for the analysis was set to 25 °C. The mixture of acetonitrile (HPLC grade, Sigma-Aldrich) and deionized water (HPLC grade) was used as a mobile phase. The ratio of acetonitrile and deionized water was 10:90 (v/v). The analysis of the samples was carried out under isocratic conditions. Flow rate was 1.3 mL min<sup>-1</sup>. The volume of the sample injection was 10  $\mu$ L, which was performed by autosampler SIL-20A. Detection of hydroxymethylfurfural carried out at 280 nm wavelength. The analysis was performed on Shimadzu LC-20 Prominence liquid chromatograph (Shimadzu USA Manufacturing Inc, Canby, USA) with a Shimadzu DAD SPD-M20A detector.

*Determination of fructose and glucose content*

Honey and honey powder solutions were prepared at a concentration of 50 g L<sup>-1</sup> to evaluate the content of fructose and glucose. The analysis of the sample was performed on Shimadzu LC-20 Prominence liquid chromatograph. The analytical column SUPELCOSIL™ LC-NH<sub>2</sub> (4.6 mm × 250 mm I.D., particle size 5mm). The temperature of column and detector was set to 30 °C. The mixture of acetonitrile

(HPLC grade, Sigma-Aldrich) and deionized water (HPLC grade) was used as a mobile phase. The ratio of acetonitrile and deionized water was 80:20 (v/v). The detection of glucose and fructose was performed under isocratic conditions. Flow rate was 1 mL min<sup>-1</sup>. Injection volume of 10  $\mu$ L was performed using an autosampler SIL-20A. Shimadzu RID 10A Refractive Index detector was used to detect the presence of fructose and glucose in the samples.

#### Statistical analysis

All experiments were repeated in three replications. The obtained data of the research was expressed as the mean $\pm$ standard deviation. The data was assessed by analysis of variance (ANOVA) and compared by Tukey comparison test ( $p < 0.05$ ). The statistical analysis was carried out using Microsoft Office Excel 2016.

### Results and Discussion

In this research, dehydration of honey was performed by two methods. One of the drying techniques was freeze-drying, which is widely used in pharmaceuticals due to its ability to preserve bioactive compounds (Nascimento *et al.*, 2015). Another drying method, which was used to obtain dehydrated honey, was spray-drying. Spray-drying is one of the most common techniques to obtain honey-rich powders (Samborska, Gajek, & Kamińska-Dwórznička, 2015). In this study, the drying experiments of 20% honey solutions with maltodextrin resulted in honey-rich powders. The moisture content in the honey powder,

which was obtained by freeze-drying, was 5.3% and in the honey powder, which was obtained by spray-drying, was 4.8%. Maltodextrin is mainly used for drying sugar and acid rich food products (Umesh Hebbar, Rastogi, & Subramanian, 2008). It has a high molecular weight, which helps to increase the glass temperature of drying particles and reduces hygroscopicity. Nurhardi and co-workers also used maltodextrin (1:1) in their study of obtaining honey powder by spray-drying and vacuum drying methods. The moisture content in the spray-dried honey powder was 2.3%, but in the vacuum-dried powder it was 1.0% (Nurhadi *et al.*, 2012). Sramek and co-authors obtained honey-rich powder by the freeze-drying method. In this study, honey was mixed with glucose syrup to prepare the powder. The moisture content in the final product was 3.1% (Sramek *et al.*, 2016). Our results and other researchers' studies showed that moisture content could be reduced by choosing different drying aids and changing the ratio of honey and drying aids in solutions.

$\alpha$ -amylase activity was detected to evaluate the quality of obtained powders. The value of diastase number in both dehydrated honey samples was not lower than 8 (Figure 1).

The lowest enzymatic activity was presented in the spray-dried honey, where the value of diastase number was 8.3 $\pm$ 0.2. During the spray-drying, the water of the solution was removed, which resulted in degradation of molecules and loss of enzymatic activity. Samborska and co-workers (Samborska *et*

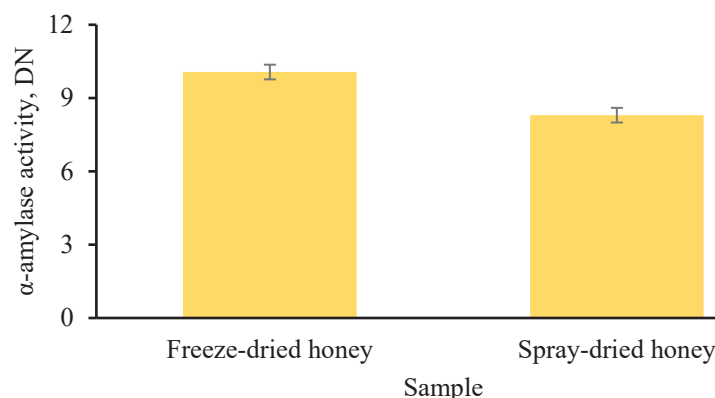


Figure 1.  $\alpha$ -amylase activity in freeze-dried and spray-dried honey samples.

Table 2

#### Content of HMF, fructose, and glucose in dehydrated honey

Sample	HMF, mg kg <sup>-1</sup>	Fructose, g 100 g <sup>-1</sup>	Glucose, g 100 g <sup>-1</sup>
Freeze-dried honey	28.1 $\pm$ 0.2	16.7 $\pm$ 0.3	9.2 $\pm$ 0.4
Spray-dried honey	36.5 $\pm$ 0.3	16.5 $\pm$ 0.2	9.5 $\pm$ 0.5

*al.*, 2017b), who obtained honey powder using spray drying method, reported that  $\alpha$ -amylase (diastase) activity in their product was  $10.9 \pm 0.2$  DN. They also pointed out that the enzymatic activity in the honey powder was lower than it was in fresh honey ( $14.4 \pm 0.2$  DN). Freeze-dried honey sample showed higher  $\alpha$ -amylase activity than the spray-dried sample. The value of  $\alpha$ -amylase activity in the freeze-dried sample was  $10.0 \pm 0.3$  DN. The obtained results on  $\alpha$ -amylase activity showed that freeze-drying is more gentle drying technique than spray-drying. Unfortunately, there are lack of literature data on  $\alpha$ -amylase activity changes during the freeze-drying process that could be used to compare our obtained results. The concentration of hydroxymethylfurfural (HMF) was detected in obtained dehydrated honey samples (Table 2).

The powder, which was produced using a spray dryer, had higher concentration of HMF than the powder that was obtained by freeze-drying. The concentration of HMF in the spray-dried honey was  $36.5 \pm 0.3$  mg kg<sup>-1</sup> and in the freeze-dried honey it was  $28.1 \pm 0.2$  mg kg<sup>-1</sup>. The obtained results showed that the used drying method significantly ( $p < 0.05$ ) impacts the concentration of HMF in the product. Hydroxymethylfurfural is formed during dehydration of monosaccharides (fructose and glucose) and/or during the Maillard reaction (Kowalski & Lukasiewicz, 2017; Shapla *et al.*, 2018). The formation of hydroxymethylfurfural also depends on the factors such as pH, free acidity, temperature etc (Kowalski *et al.*, 2013). As high temperatures (inlet air temperature: 180 °C; outlet air temperature: 80 °C) were used during the spray-drying process, it caused the increase of the content of hydroxymethylfurfural in the final product. The high temperature in the spray drying process and low pH of multifloral honey (pH=3.66±0.01) were the

catalysts of the formation of hydroxymethylfurfural (Shapla *et al.*, 2018). The statistical analysis of the obtained data showed that the chosen drying methods did not impact the content of fructose ( $p > 0.05$ ) and glucose ( $p > 0.05$ ) in the honey powder.

The further studies should be focused on the obtaining of honey powder by freeze-drying. Freeze-drying is a gentle drying method, which preserves bioactive compounds and allows to produce high-quality food products (Karam *et al.*, 2016). The method should be modified to improve the quality of honey powders. The method could be used as an alternative method to obtain high quality honey-rich powder in the food industry. The obtained results showed that the used drying method impacts two important quality parameters  $\alpha$ -amylase activity and the concentration of hydroxymethylfurfural in the honey-rich powder.

### Conclusions

The obtained results showed that freeze-drying and spray drying can be used to obtain honey-rich powders.  $\alpha$ -amylase activity in both powders decreased during the drying process. Freeze-dried honey powder had better quality as  $\alpha$ -amylase activity was higher and the content of hydroxymethylfurfural was lower than spray-dried honey powder.

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