

SURVIVAL OF PATHOGENS IN HIGH PRESSURE PROCESSED MILK

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Abstract

The aim of the study was to assess the ability of pathogens metabolic repair from injury within 10 days of refrigerated storage of milk after high pressure treatment. Two pathogenic strains – *Listeria monocytogenes* ATCC 7644 (LM) and *Escherichia coli* ATCC 25922 (EC) were inoculated in ultrahigh-temperature treated (UHT) milk at concentration of about 10^7 CFU mL⁻¹ and treated at 400, 500, 550, and 600 MPa for 15 min with inlet temperatures 20 °C, and then stored at 4 ± 2 °C to evaluate survival and growth of pathogens. By increasing the applied pressure, an increased rate of the pathogens' inactivation was achieved. After 10 days of storage, milk treated at 400 MPa showed growth over 3.5 log CFU mL⁻¹ of *L. monocytogenes* and 1.7 log CFU mL⁻¹ of *E. coli*. In 550 MPa and 600 MPa treated milk samples after 8 and 10 days of storage colony formation occurred (3 CFU mL⁻¹ (550 LM) and 2 CFU mL⁻¹ (550 EC, 600 LM and 600 EC)). Although high pressure treatment is effective method for reducing of pathogenic bacteria, the metabolic repair from injury of bacterial cells in milk during storage should be considered.

Key words: high pressure, *Listeria monocytogenes*, *Escherichia coli*, metabolic repair, storage.

Introduction

A major function of the high pressure processing (HPP) of food is the inactivation of microorganisms (Mandal & Kant, 2017). Several researchers have investigated the efficacy of high pressure to inactivate the important foodborne pathogens such as *Listeria monocytogenes* (Possas *et al.*, 2017), *Cronobacter sakazakii* (Jiao *et al.*, 2016), psychrotrophic *Bacillus cereus* spores in reconstituted milk (Evelyn & Silva, 2015), *Listeria innocua* in skimmed milk (Pinho *et al.*, 2015), stationary-phase cultures of *Escherichia coli* E218/02 and *Escherichia coli* C-600 (Prieto-Calvo *et al.*, 2014).

High pressure processing could induce a number of changes: biochemical reactions, and genetic mechanisms, and to cell membrane and the wall of microorganisms (De Lamo-Castellví *et al.*, 2005) injured or inactivated bacteria. When high pressure damages the cell membrane, the absorption of nutrients is affected, elimination of the waste accumulated inside the cell is hindered, and normal metabolic pathway is disrupted (Torres & Velazquez, 2008). In addition, microorganisms are more likely to be stressed or injured than killed under the HPP of alternative preservation technologies (Huang *et al.*, 2014).

Some researchers have demonstrated the metabolic repair of injured cells during storage of pressure-treated products (Bozoglu, Alpas, & Kaletunç, 2004; De Lamo-Castellví *et al.*, 2005; Syed *et al.*, 2013). Microorganisms metabolic repair from injury during storage is a critical issue from the viewpoint of food safety. This phenomenon depends on a treated product (pH, water activity, nutrients), microorganisms variation and growth stage, treatment regime and storage conditions.

Our previous studies showed inactivation of *Escherichia coli* and *Listeria monocytogenes* strains

at a pressure of 550 MPa (15 min) or above at different inoculation rates of 10^4 and 10^7 CFU mL⁻¹ (Liepa *et al.*, 2018b, in press), but for better understanding how pressure can influence the ability of pathogens to metabolic repair from injury during storage, the survival and growth of target microorganisms in ultrahigh-temperature treated milk were evaluated within 10 days of refrigerated storage.

Materials and Methods

Cultures and media

The cultures of *Escherichia coli* ATCC 25922 and *Listeria monocytogenes* ATCC 7644 were obtained from the ATCC (American Type Culture Collection) of MicroBioLogics, Inc (Minnesota, USA). Prior to the experiments, bacteria's were reactivated in TSA (*Tryptic Soy Agar*; Biolife Italiana, Italy) at 37 °C for 24 h to follow the standard method LVS EN ISO 11133:2014 'Microbiology of food, animal feed and water – Preparation, production, storage and performance testing of culture media'. A volume of 1 µL of bacteria culture was cultivated in non-selective tryptic soy broth (TSB; Biolife Italiana, Italy) at 37 °C for 18 – 24 h.

Inoculation of milk samples for high pressure (HP) treatment

UHT milk of 2% fat content (Mlekpól, Poland) was obtained from a local supermarket, and was used in this study to evaluate survival and growth of pathogens during storage without the presence of other microorganisms, thus avoiding the interactions among various microflora. After incubation, the cultures were separately added to milk at concentration of about 10^7 CFU mL⁻¹. For obtaining uniform samples, milk was mixed for 2 min and filled (120 ± 10 mL) in sterile polyethylene terephthalate (PET) plastic bottles

(NF2–Æ28 mm), avoiding any head space. Samples were vacuum sealed in polyethylene pouches (70×200 mm sized, with 65 µm thickness) using a chamber type vacuum packaging machine Multivac C350 (MULTIVAC Sepp Haggenmüller SE & Co. KG, Germany) before being pressurized (Liepa, Zagorska, & Galoburda, 2017).

High pressure treatment

During the current research each milk sample was inoculated with a single pathogen strain and exposed to 400, 500, 550 and 600 MPa for 15 min (Table 1). Milk samples were pressurized in the Iso-Lab High Pressure Pilot Food Processor S-FL-100-250-09-W (STANSTED fluid power LTD, UK) with a pressure chamber of about 2 litres. Pressure transmitting liquid was a mixture of water and isopropanol (2 : 1, v/v). The pressurization was completed at room temperature. Product temperature increased during pressurization up to 30 °C and dropped during pressure release to about 17 °C. Pressurising rate was 600 MPa min⁻¹, depressurising rate – 3000 MPa min⁻¹. The treatment time did not include the compression (about 1.5 min for 600 MPa) and decompression (less than 0.5 min). After pressurization, the two outer polyethylene pouches were removed. Pressurized milk was stored at 4 ± 2 °C over a period of 10 days, which was indicated by our previous studies, when the current treatment regimes showed maximal storage time – 10 days (Liepa *et al.*, 2018a, in press). Milk samples were analyzed immediately after pressurization and on 1, 4, 6, 8 and 10 day of storage. Totally, 288 milk samples were analysed.

Microbiological analysis

Milk samples were serially decimal diluted with Maximum Recovery Diluent (Oxoid, England) according to the standard LVS EN ISO 6887-5:2011 'Microbiology of food and animal feeding stuffs -

Preparation of test samples, initial suspension and decimal dilutions for microbiological examination – Part 5: Specific rules for the preparation of milk and milk products' (ISO 6887-5:2010) and appropriate dilutions were plated on agar medium.

The method for enumeration of *Listeria monocytogenes* ATCC 7644 was on ALOA (Agar *Listeria* acc. to Ottaviani & Agosti) agar (Biolife Italiana, Italy) with aerobic incubation (24 ± 3 h, 37 °C) (LVS EN ISO 11290-2+A1:2007 'Microbiology of food and animal feeding stuffs – Horizontal method for the detection and enumeration of *Listeria monocytogenes* – Part 2: Enumeration method', LVS EN ISO 11290-1 + A1:2007 'Microbiology of food and animal feeding stuffs – Horizontal method for the detection and enumeration of *Listeria monocytogenes* – Part 1: Detection method').

Escherichia coli ATCC 25922 was enumerated in depth seeding in TBX (Tryptone Bile X–Glucuronide) medium (Biolife Italiana, Italy) and the characteristic colonies were subsequently counted in a chromogenic selective medium after incubation at 44 °C for 18 – 24 h according to LVS ISO 16649-2:2007 'Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of betagluconidase-positive *Escherichia coli* – Part 2: Colony-count technique at 44 °C using 5-bromo4-chloro-3-indolyl-beta-D-glucuronide'. For the detection and enumeration of presumptive *Escherichia coli* by means of the liquid-medium culture technique and calculation of the most probable number after incubation at 37 °C, then at 44 °C LVS ISO 7251:2006 'Microbiology of food and animal feeding stuffs – Horizontal method for the detection and enumeration of presumptive *Escherichia coli* - Most probable number technique' was used.

The number of surviving cells of pathogen bacteria was determined by plate counting method. Total plate

Table 1

Inoculants and treatment parameters for the experimental samples

Sample abbreviation	<i>Escherichia coli</i>	<i>Listeria monocytogenes</i>	Treatment applied to milk
400 EC	+	-	400 MPa, 15 min
400 LM	-	+	
500 EC	+	-	500 MPa, 15 min
500 LM	-	+	
550 EC	+	-	550 MPa, 15 min
550 LM	-	+	
600 EC	+	-	600 MPa, 15 min
600 LM	-	+	

count was investigated according to the standard method LVS EN ISO 4833:2013 ‘Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of microorganisms – Colony-count technique at 30 degrees C’. Counting of colonies was accomplished by automatic colony counter Acolyte (Synbiosis UK, Cambridge, UK). Colony forming units from replicate plates were counted and the number of CFU mL⁻¹ were averaged and converted into logarithmic units.

Data analysis

The obtained data were processed using Microsoft Excel (Microsoft Office Enterprise 2007, License: Shareware N/A); differences among results were considered significant if p-value < 0.05. For the interpretation of the results it was assumed that α=0.05 with 95% confidence.

Results and Discussion

From the food safety aspect during treatment (thermal or pressure) it is very important to decrease total and completely inactivated pathogen bacteria in the product, as well as eliminate metabolic repair from injury of microorganisms during storage. After HPP bacterial cells can present at 3 possible states: active cells (colonies are formed on both non-selective and selective agar); primary injury (structural damages like cell wall and/or cytoplasmic membrane injury; colonies are formed on non-selective agar but not on selective agar); and secondary injury (metabolic injuries; no colonies are formed in either non-selective and selective agar, however, colonies might form metabolic repair injury after a long period of storage first on non-selective agar and later on selective agar) (Bozoglu, Alpas, & Kaletunç, 2004).

Behaviour of Listeria monocytogenes ATCC 7644 after high pressure treatment

Although there was no significant difference (p>0.05) between all LM milk samples, a few viable cells were still observed after 400 MPa and 500 MPa treatments. The number of cells was 2 CFU mL⁻¹ (Figure 1) for 400 LM and 500 LM samples. Treatment at 550 MPa or above proved to be effective for inactivation of target Gram-positive bacteria strain. Even 550 LM and 600 LM samples showed 7 log reduction in the number of surviving cells for *L. monocytogenes*.

These results are in agreement with the findings of Bozoglu and co-authors (2004). In this study after pressure treatment at 550 MPa, *L. monocytogenes* CA was not observed in selective or non-selective agar. Buzrul *et al.* (2008) noted that, 20 min of pressurization was necessary to achieve more than 8 log reduction at 600 MPa for *L. innocua* ATCC 33090 in whole milk. *L. monocytogenes* ATCC 19115 in human milk was inactivated by 8 log after 4 min of pressure treatment at 400 MPa (Viazis, Farkas, & Jaykus, 2008). Previous studies about HPP influence on microorganisms showed that the pressure susceptibility of *L. monocytogenes* varies between different strains. For example, Tay *et al.* (2003) compared the sensitivity of *L. innocua* and 9 strains of *L. monocytogenes* in tryptose broth, and reported that *L. monocytogenes* OSY-8578 and Scott A were the most resistant and labile to pressures, and their death inactivation kinetics were evaluated at pressures varying from 350 to 800 MPa during 1 – 20 min at 30 °C.

As it was mentioned earlier, microorganisms ability to metabolic repair from injury during storage after pressure treatment is extremely important

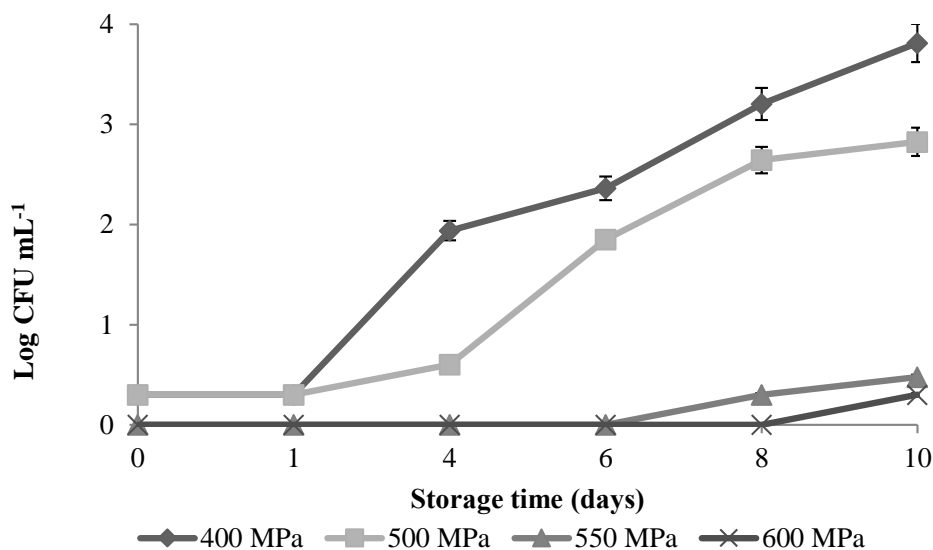


Figure 1. The growth curves of *Listeria monocytogenes* in UHT milk after HP treatment during storage.

for the product safety. In our study, significant microorganism growth started after 4 days of storage in 400 LM sample, the number of bacteria increased by 1.64 log CFU mL⁻¹ (p<0.05) (from 0.3 to 1.94). The increase of microorganism counts strongly correlated to storage time, as a result, the growth over 3.5 log units after approximately 10 days of storage at 4 °C was observed.

Similar results were observed by Mussa & Ramaswamy (1997), in pressure treated (at 350 MPa) raw milk an increase of 4 log CFU mL⁻¹ of *L. monocytogenes* after 12, 18 and 25 days of storage at 10, 5 and 0 °C, respectively. Recommended refrigerated storage (4 ± 2 °C) after pressurization to inhibit the rapid recovery of microorganisms was used in the current research.

In 500 LM sample the increase by more than 1.5 log CFU mL⁻¹ was detected after 6 days of storage, and reached the level of 2.83 log CFU mL⁻¹ after 10 days of storage. Pressure treatment at 550 MPa and 600 MPa for 15 min had more destructive effect on bacteria. In 550 LM and 600 LM milk samples initially colony formation was not observed, suggesting that all cells were inactivated. However, after 8 days of storage colony formation occurred in 550 LM milk samples – 0.3 log CFU mL⁻¹. After 10 days of storage, the number of cells was 3 CFU mL⁻¹ in 550 LM sample and 2 CFU mL⁻¹ in 600 ML sample, respectively. It can be explained with increases the expression of genes related to the repair mechanisms of DNA, protein complexes of transcription and translation, the septal ring, the system of general translocase system, flagella assemblage and chemotaxis, and lipid and peptidoglycan biosynthetic pathways (Bowman, Bittencourt, & Ross, 2008).

Similar results, even raising treatment temperature, were found in studies carried out by Bozoglu, Alpas, & Kaletunç (2004). After pressure treatment at 550 MPa at 45 °C for 10 min of UHT 1% low fat milk, at 4 °C after 6 days colony formation of *L. monocytogenes* CA occurred in selective and non-selective agar. Koseki, Mizuno, & Yamamoto (2008) reported that the number of *L. monocytogenes* cells in milk treated at 550 MPa for 5 min increased by > 8 log CFU mL⁻¹ regardless of the inoculum levels (3, 5, and 7 log CFU mL⁻¹) after 28 days of storage at 4 °C. The authors proposed a technique to inhibit the bacterial metabolic repair from injury during storage after HP processing (550 MPa for 5 min) using a mild-heat treatment (30 – 50 °C), thereby to enhance the safety of HP treated milk.

Behaviour of Escherichia coli ATCC 25922 after high pressure treatment

Immediately after pressure treatment, at 400 MPa, *E. coli* strains was reduced significantly (p<0.05) by 6.7 log cycles (Figure 2). At other pressure regimes (500, 550, and 600 MPa) the counts of target Gram-negative bacteria decreased by about 7 log cycles, so it seems that immediately after pressurization no cells that were able to form colonies on selective agar were found in 1 mL of milk (< 1 CFU mL⁻¹). Comparing pressure regimes, there were no statistically significant differences (p>0.05) in the number of surviving cells after inactivation of *E. coli*.

The level of inactivation achieved was comparable to other studies. In comparison, Viazis *et al.* (2008) observed, that same bacteria strain was inactivated by 8 log at 400 MPa at ambient temperature after 10 min in peptone solution and by 2 log and 6 log after

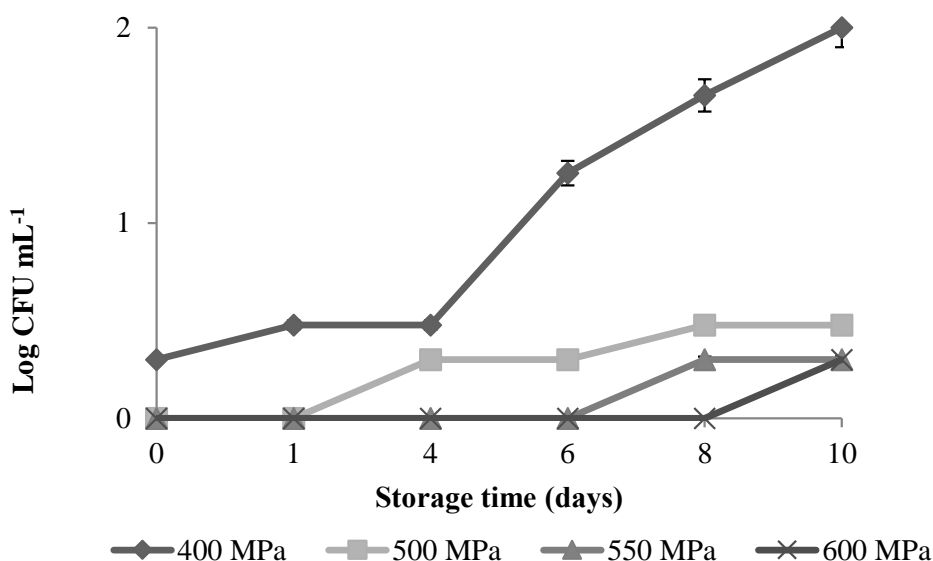


Figure 2. The growth curves of *Escherichia coli* in UHT milk after HP treatment during storage.

10 and 30 min in human milk, respectively. Large strain variations in pressure resistance for target pathogen have been reported by other authors. Two enterohemorrhagic *Escherichia coli* strains, no. 94 and 402 (serotype O157:H7), and a saprophytic *E. coli* 1 strain were pressure treated in skim milk at 400 MPa for 20 min at 20 °C and were reduced by 1.5, 2.7, and 2.1 log, respectively (Usajewicz & Nalepa, 2006).

According to our results the number of *E. coli* ATCC 25922 cells increased by 1.7 log CFU mL⁻¹ in 400 EC sample during storage at 4 °C and reached the level of 2.0 log CFU mL⁻¹ on the 10 day of storage. In the case of 500 EC sample, an increase of bacteria was significant ($p < 0.05$). First, pathogen metabolic repair signs were observed after 4 days of storage, the amount of target microorganism increased by 0.3 log CFU mL⁻¹. Then, after 6 days level of bacteria was the same, and the next rise (by 0.48 log CFU mL⁻¹) was noticed after 8 days of storage. The same count of bacteria after 10 days of storage can be mentioned as a positive tendency.

Similarly, to *L. monocytogenes*, in 550 EC and 600 EC milk samples metabolic repair from injury of bacteria was observed later. After 8 days of storage an increase by 0.3 log CFU mL⁻¹ was noticed in 550 EC sample. Better results were obtained in the case with the highest pressure used in the current research, colony formation occurred only after 10 days of storage. After 10 days of storage, the number of cells was 2 CFU mL⁻¹ in both samples – 550 EC and 600 EC.

In comparison, Yamamoto *et al.* (2017) reported, that the HP treated (at 500, 550, and 600 MPa) and injured *Escherichia coli* ATCC 25922 recovered close to 10⁸ CFU mL⁻¹ when the cell suspensions (in phosphate-buffered saline with inoculation rate 8 log CFU mL⁻¹) were stored at 4 °C for 5 days and thereafter moved to an environment at 25 °C. Syed *et al.* (2013) investigated the effect of compression and decompression rates of high hydrostatic pressure (600 MPa for 3 min) on *Escherichia coli* O157:H7. The authors observed, that after 15 days at 4 °C, *E. coli* cells in skimmed milk recovered significantly by 1.93 log CFU mL⁻¹. The microbial recovery trend in skimmed milk can be justified by favorable pH conditions for *E. coli* cells where they were able to

repair the minor cellular injuries and pressure shock effects (Syed *et al.*, 2013).

In our study, the large number of bacteria cells of *L. monocytogenes* after 10 days of storage indicated that Gram-positive bacteria metabolic repair is more pressure resistant than Gram-negative bacteria – *E. coli* ATCC 25922. The obtained results are in an agreement with the conclusions of Vachon and co-authors, that *L. monocytogenes* LSD 105-1 was found to be less sensitive to high pressure than *E. coli* O157:H7. The resistance of *L. monocytogenes* to pressurization can be explained by its wall structure, which is made up of a large number of peptidoglycan layers (Vachon *et al.*, 2002).

Although high pressure treatment reduces pathogen strain levels, the metabolic repair of injured cells was established during storage in milk, speed of this process differs both for pressure regimes and for microorganism strains. Further research is needed to establish recommended processing regimes (temperature, pressure, time) that results in a reliable inactivation and unrecovery of pathogens in milk. These parameters help effectively control microorganism safety risks in products.

Conclusions

During subsequent storage of high pressure treated milk at 4 ± 2 °C, metabolic repair of pathogen cells from injury was significantly different ($p < 0.05$) among pressures used in the research. Lower ability to repair was detected in milk samples 600 EC and 600 LM, after 10 days of storage 2 CFU mL⁻¹ of *Escherichia coli* ATCC 25922 and *Listeria monocytogenes* ATCC 7644 was detected in analysed samples.

Comparing analysed bacteria, a significant resistance ($p < 0.05$) to HPP demonstrated *Listeria monocytogenes* ATCC 7644, although its ability to metabolic repair still was noticed after 10 days of storage.

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References

1. Bowman, J.P., Bittencourt, C.R., & Ross, T. (2008). Differential gene expression of *Listeria monocytogenes* during high hydrostatic pressure processing. *Microbiology*, 154(2), 462–475. DOI: 10.1099/mic.0.2007/010314-0.
2. Bozoglu, F., Alpas, H., & Kaletunç, G. (2004). Injury recovery of foodborne pathogens in high hydrostatic pressure treated milk during storage. *FEMS Immunology and Medical Microbiology*, 40(3), 243–247. DOI: 10.1016/S0928-8244(04)00002-1.
3. Buzrul, S., Alpas, H., Largeteau, A., & Demazeau, G. (2008). Modeling high pressure inactivation of *Escherichia coli* and *Listeria innocua* in whole milk. *European Food Research and Technology*, 227(2), 443–448. DOI: 10.1007/s00217-007-0740-7.

4. De Lamo-Castellví, S., Roig-Sagués, A.X., Capellas, M., Hernández-Herrero, M., & Guamis, B. (2005). Survival and growth of *Yersinia enterocolitica* strains inoculated in skimmed milk treated with high hydrostatic pressure. *International Journal of Food Microbiology*, 102(3), 337–342. DOI: 10.1016/j.ijfoodmicro.2004.11.025.
5. Evelyn, E., & Silva, F.V.M. (2015). High pressure processing of milk: Modeling the inactivation of psychrotrophic *Bacillus cereus* spores at 38–70 °C. *Journal of Food Engineering*, 165, 141–148. DOI: 10.1016/j.jfoodeng.2015.06.017.
6. Huang, H.-W., Lung, H.-M., Yang, B.B., & Wang, C.-Y. (2014). Responses of microorganisms to high hydrostatic pressure processing. *Food Control*, 40, 250–259. DOI: 10.1016/J.FOODCONT.2013.12.007.
7. Jiao, R., Gao, J., Li, Y., Zhang, X., Zhang, M., Ye, Y., ... Fan, H. (2016). Short communication: Effects of high-pressure processing on the inactivity of *Cronobacter sakazakii* in whole milk and skim milk samples. *Journal of Dairy Science*, 99(10), 7881–7885. DOI: 10.3168/jds.2016-11418.
8. Koseki, S., Mizuno, Y., & Yamamoto, K. (2008). Use of mild-heat treatment following high-pressure processing to prevent recovery of pressure-injured *Listeria monocytogenes* in milk. *Food Microbiology*, 25(2), 288–93. DOI: 10.1016/j.fm.2007.10.009.
9. Liepa, M., Zagorska, J., & Galoburda, R. (2017). Effect of high pressure processing on milk coagulation properties. *Proceedings of the 23rd Annual International Scientific Conference "Research for Rural Development 2017," 1*, 223–229. DOI: 10.22616/trd.23.2017.033.
10. Liepa, M., Zagorska, J., Galoburda, R., & Kostascuka, S. (2018a). Effect of high-pressure processing on microbial quality of skimmed milk. *Proceedings of the Latvian Academy of Sciences. Section B. Natural, Exact, and Applied Sciences 72 (2)*, 118–122. DOI: 10.2478/prolas-2018-0019.
11. Liepa, M., Zagorska, J., Galoburda, R., & Baltrukova, S. (2018b). Inactivation of pathogens in milk by high pressure processing. In press.
12. Mandal, R., & Kant, R. (2017). High-Pressure Processing and Its Applications in the Dairy Industry. *Food Science and Technology: An International Journal (FSTJ)*, 1(1), 33–45. Retrieved January 11, 2018, from: https://www.researchgate.net/publication/321724626_HighPressure_Processing_and_Its_Applications_in_the_Dairy_Industry.
13. Mussa, D.M., & Ramaswamy, H.S. (1997). Ultra High Pressure Pasteurization of Milk: Kinetics of Microbial Destruction and Changes in Physico-chemical Characteristics. *LWT – Food Science and Technology*, 30(6), 551–557. DOI: 10.1006/FSTL.1996.0223.
14. Pinho, C.R.G., Oliveira, M.M., da Castro Leite Júnior, B.R., Atribst, A.A.L., & Cristianini, M. (2015). Inactivation of *Pseudomonas fluorescens*, *Listeria innocua* and *Lactobacillus helveticus* in skimmed milk processed by high pressure homogenization. *International Food Research Journal*, 22(4), 1687–1691. Retrieved January 11, 2018, from: https://www.researchgate.net/publication/281148487_Inactivation_of_Pseudomonas_fluorescens_Listeria_innocua_and_Lactobacillus_helveticus_in_skimmed_milk_processed_by_high_pressure_homogenization.
15. Possas, A., Pérez-Rodríguez, F., Valero, A., & García-Gimeno, R.M. (2017). Modelling the inactivation of *Listeria monocytogenes* by high hydrostatic pressure processing in foods: A review. *Trends in Food Science and Technology*, 70(December), 45–55. DOI: 10.1016/j.tifs.2017.10.006.
16. Prieto-Calvo, M., Prieto, M., López, M., & Alvarez-Ordóñez, A. (2014). Effects of high hydrostatic pressure on *Escherichia coli* ultrastructure, membrane integrity and molecular composition as assessed by ftir spectroscopy and microscopic imaging techniques. *Molecules*, 19(12), 21310–21323. DOI: 10.3390/molecules191221310.
17. Syed, Q.A., Buffa, M., Guamis, B., & Saldo, J. (2013). Lethality and injuring the effect of compression and decompression rates of high hydrostatic pressure on *Escherichia coli* O157:H7 in different matrices. *High Pressure Research*, 33(1), 64–72. DOI: 10.1080/08957959.2013.767898.
18. Tay, A., Shellhammer, T.H., Yousef, A.E., & Chism, G.W. (2003). Pressure death and tailing behavior of *Listeria monocytogenes* strains having different barotolerances. *Journal of Food Protection*, 66(11), 2057–2061. DOI: 10.4315/0362-028X-66.11.2057.
19. Torres, J.A., & Velazquez, G. (2008). Hydrostatic pressure processing of foods. In: *Food Processing Operations Modeling: Design and Analysis*, 2nd ed.; (pp. 173–212). CRC Press: Boca Raton, FL, USA.
20. Usajewicz, I., & Nalepa, B. (2006). Survival of *Escherichia coli* O157 : H7 in Milk Exposed to High Temperatures and High Pressure. *Food Technology and Biotechnology*, 44(1), 33–39.
21. Vachon, J.F., Kheadr, E.E., Giasson, J., Paquin, P., & Fliss, I. (2002). Inactivation of foodborne pathogens in milk using dynamic high pressure. *Journal Food Protection*, 65(2), 345–352. DOI: 10.4315/0362-028X-65.2.345.

22. Viazis, S., Farkas, B.E., & Jaykus, L.A. (2008). Inactivation of bacterial pathogens in human milk by high-pressure processing. *Journal of Food Protection*, 71(1), 109–118. DOI: 10.4315/0362-028X-71.1.109.
23. Yamamoto, K., Morimatsu, K., Nakaura, Y., Takashi, I., & Keitarou, K. (2017). Microbial cannibalism and recovery of bacteria injured by high hydrostatic pressure. *Journal of Physics: Conference Series*, 950. DOI: 10.1088/1742-6596/950/3/032012.