THE EFFECTS OF pH, a_W, AND LACTIC ACID BACTERIA ON *LISTERIA MONOCYTOGENES* IN FERMENTED SAUSAGES

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Abstract

The survival of inoculated in fermented sausages *Listeria monocytogenes* strain was studied. The sausages were prepared with and without starter cultures. The survival limits of *L. monocytogenes* and lactic acid bacteria (LAB) were determined as colony forming units per gram (cfu g^{-1}) depending on water activity (a_w) and pH on 0, 1st, 3rd, 5th, 7th, 14th and 21st days of maturation. The decreasing water activity conditioned by moisture (weight) loss during ripening and pH decrease ensured negative polynomial growth rate of inoculated *L. monocytogenes* -0.27 lg (cfu g^{-1}) each day of ripening time, and -0.65 lg (cfu g^{-1}) on the first 7 days of maturation. A significant Pearson's correlation ($p<0.01$) was established between decreased values of *L. monocytogenes* count, a_w, salt concentration and LAB growth in sausages during the ripening period of 21 days. The main parameters, maintained negative exponential growth rate of *L. monocytogenes* in fermented sausages, are a_w value decrease and LAB (starter culture), which stopped *L. monocytogenes* growth at the beginning of fermented sausage maturation. If fermentation process went technically and hygienically correctly, the fermented (cold-smoked) sausages could be one of the safest meat products, because in real practice a low level contamination has been seen. The remaining count of *L. monocytogenes* in fermented sausage depends on the possible initial contamination level and could exceed the European Union regulation value 2.0 lg (CFU g^{-1}) for ready-to-eat products when contamination at first is more than lg 5.0.

Keywords: *Listeria monocytogenes*, lactic acid bacteria, fermented sausages, water activity, pH.

Introduction

Listeria monocytogenes is an ubiquitous bacterial pathogen that can be found in a large number of food products and can survive and multiply at refrigeration temperature (Lunden et al., 2003). Processed meat products such as fermented sausages are a part of major products associated with listeriosis (Thevenot et al., 2005). *L. monocytogenes* infection has a high mortality rate – 20–30% (Farber, 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 exceeded concentration of *L. monocytogenes* in ready-to-eat food to 100 colony forming units (CFU) per gram (Anonymous, 2005). Glass, Doyle (1989) found out that *L. monocytogenes* decreasing level in fermented sausages and ham would be 1–2 lg (sausages) in 14 days and 2–3 lg (ham) in 28 days. Because lactic acid bacteria (LAB) can grow under the same storage conditions as *Listeria* spp. (Bērziņš et al., 2007), many studies have been conducted to investigate if these gram-positive organisms can provide adequate competition against the pathogenic organisms that are also present.

The safety of fermented (cold smoked) sausages depends on the presence of factors such as concentration of sodium nitrite and salinity, relatively low water activity (a_w), low pH value, and application of probiotics (Lahti et al., 2001), like LAB used in fermented meat products (Bredholt et al., 2001). Liu et al. (2005) investigated that acid, alkali, and/or salt treatments, commonly used in food product processing, may not be sufficient to eliminate *L. monocytogenes*. Petran, Zottolla (1989) observed the growth of *L. monocytogenes* at the minimum a_w of 0.92. Below these minimum a_w levels, cell death is proportionate to water activity (Miller, 1992). According to literature sources available, some 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 ripening stages of fermented sausages when main bacteria growth factors changed in time. Therefore, the aim of the study was to determine the survival limits of *L. monocytogenes* inoculated in manufactured fermented sausages depending on the LAB, water activity (a_w), and pH value changes in ripening time.

Materials and Methods

Individual pieces of raw sausages, before smoking at 28 °C, in initial weight mean value of 0.394 kg, were inoculated internally with a cocktail of local (wild) strains of *L. monocytogenes* (serotypes 1/2a and 4b) originally isolated from surfaces and meat products of the mother factory (Bērziņš et al., 2007). The inoculated samples were labelled and subjected to smoking and maturating processes. All manipulations with samples were done in laboratory conditions (20 °C, 75–80% RH). The measurements and tests were done on 0, 1st, 3rd, 5th, 7th, 14th and 21st day of maturation. Three batches of fermented (cold smoked) sausages were investigated (a total of 60 samples) and the mean values of lg (CFU g^{-1}) were estimated between each other, and in addiction of pH, moisture content, and water activity (a_w) changes at ripening time.

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 measured by optical densities (densitometer DEN-1B, UK) and prepared with sterile half Fraser broth (CM0895, SR0166E, Oxoid) to obtain approximately 8.0 lg (CFU mL^{-1}), and then samples of dry sausages inoculated portionally

(1 mL of inoculate in 100 g of sample) randomly leading to beginning concentration of 6.0 lg (CFU g^{-1}).
Ingredients of a 100 kg fermented sausage raw material were: pork – 30 kg, beef – 10 kg, bacon – 35 kg, structural emulsion – 25 kg. Salt and species summary was 3.25 kg and starter culture – 0.02 kg ('Optistart Plus', prepared by Raps GmbH and Co.KG, Germany).
The smoking, fermentation, and ripening process were carried out in climatic chambers (models HR-6000 and HR-9000 'Sorgo' Austria) at 28 °C with a relative humidity of 95% on first 3 days, down to 75% RH and 14–15 °C on 4^{th} to 21^{st} day.
The determination of *L. monocytogenes* count, CFU g^{-1}, was done according to Standard ISO 11290-2:1198 A:2005 'Microbiology of food and animal feeding stuffs. Horizontal method for the detection and enumeration of *L. monocytogenes*. Part 2: Enumeration method'. Each experimental batch was free of *L. monocytogenes* before culture inoculation, detected with standard method. The samples were analyzed by numbering *L. monocytogenes* on 0, 1^{st}, 3^{rd}, 5^{th}, 7^{th}, 14^{th} and 21^{st} days of maturation with the nine-tube most-probable-number (MPN) method. For analysis, 10 g of a carefully mixed fermented sausage sample 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 spread 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), were considered *L. monocytogenes* (McKellar, 1994). Total count (CFU g^{-1}) of *L. monocytogenes* in fermented sausage samples were calculated with classical formula given in Enumeration method standard.
The determination of LAB count, CFU g^{-1}, was done according to standard ISO 15214:1998 Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of mesophilic lactic acid bacteria - Colony-count technique at 30 °C.
pH was measured at the same time as other measurements. Three individual pieces of sausages were measured each time, 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 was measured with PawKit (Decagon) water activity meter. Calibration of device was done with saturated NaCl (sodium chloride) 6.0 molal standard solution (0.760 a_w at 20 °C). Samples for water activity measurement were collected in original polyethylene vessels with caps and measured immediately after collecting.
The digital salt-meter "PAL-ES2" was used for direct measurement of NaCl cocentration in fermented sausages. The device have been calibrated with 2.50% NaCl solution AB250 from manufacturer (Atago, Inc. US) directly before measuring.
The determination of nitrite content in fermented sausages by ISO 2918:1975 standard was used for measuring nitrite on photometry method with 538 nm wavelength.
Statistical analysis. All experiments were reiterated three times, and tests were triplicated. The results represent the mean ± standard deviations (SD). 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 appliances. To show the parameter changes in time, regression curves have been made for LAB, *L. monocytogenes*, a_w, and pH. The main factors, affecting the bacterial growth, have been calculated by correlation (SPSS, Factor analysis).

Results and Discussion

The results of the physicochemical parameters and bacterial analysis of the fermented sausages at the beginning and the end of the ripening time are reported in Table 1. The values of pH were about 4.6 in the final product - typical of medium acidity sausages, and this was the result of the classical trend of microbial growth in the fermented sausages, where LAB are increasing in numbers at the very beginning of the fermentations (Figure 1), producing acids and a decrease in the pH, followed in the phases of maturation by the activity of micrococci that are able to neutralize the acids produced (Comi et al., 2005). The value of water activity (a_w) showed a constant decrease during the maturation reaching final values of 0.80–0.82, and moisture of 251–258 g kg^{-1}. The final value of the salt content was around 40 g kg^{-1}, while the final nitrite about 9 mg kg^{-1} of NO_2^-. These changes of parameter were due to the effect of dehydration (Comi et al., 2005). Mean value of weight losses over 21 days of ripening, when relatively constant weight reached 75–76% of relative humidity of air (RH) in the climatic chamber, was approximately 110 and 117 g of samples with and without starter culture accordingly. Losses movement significantly ($p<0.001$) correlated with the mean value of moisture content.
Due to good adaptation of LAB to meat environment and their faster growth rates which were displayed during fermentation and ripening of sausages, they became the dominant microflora (Drosinos et al., 2005). The total count of LAB changes in fermented-cold-smoked sausages with and without starter culture is shown in Figure 1. Theoretically in sausage (A) by starter culture, calculated to 1 g sausage raw mass, lg 9.4 *Lactobacillus sakei* L110, lg 9.4 *Staphylococcus xylosus*, and lg 8.0 *Debaryomyces hansenii* were added.

Table 1

The measured parameters values on start 0th day (S) and finishing 21st day (F)

Parameters	Time	Samples with (A) starter culture	Samples without (B) starter culture
L.monocytogenes, lg (CFU g^{-1})	S	6.57±0.26	6.83 ± 0.12
	F	1.42±0.43	2.86±0.36
Total count of lactic bacteria, lg (CFU g^{-1})	S	5.72±0.18	3.35±0.15
	F	9.41±0.32	6.95±0.27
Mean sausage weight, kg	S	0.39±0.05	0.39±0.05
	F	0.28±0.05	0.28±0.05
Moisture content, %	S	34.78±0.40	39.43±0.38
	F	25.12±0.38	25.88±0.36
pH value	S	5.80±0.02	5.76±0.016
	F	4.67±0.016	4.59±0.015
a_w value	S	0.96±0.002	0.95±0.002
	F	0.82±0.003	0.80±0.003
Salt (NaCl) content, %	S	2.94±0.85	28.40±0.64
	F	3.98±0.75	40.20±0.82
Nitrite (NO_2^-) concentration, mg kg^{-1}	S	14.00±0.40	14.00±0.40
	F	9.00±0.30	9.00±0.30

The difference of detected LAB count between sausage A and B variants on 0th day was approximately lg 2.37, that evident of artificially increasing count of LAB in sausage A by approximately 100 times in comparison to sausage B.

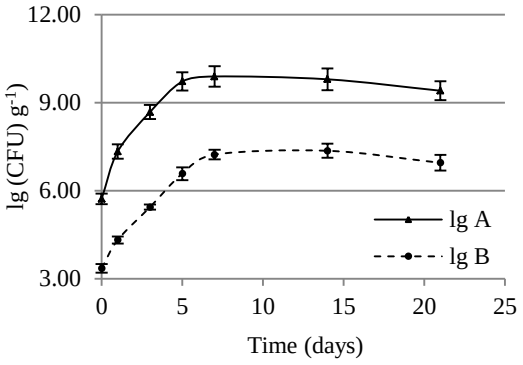

Figure 1. The polynomial changes of lactic acid bacteria count (lg values and SD values as±bars) in fermented sausages with (A) and without (B) starter culture during ripening (p<0.01)

The most significant regression of bacteria count during all time investigated has been shown by a polynomial graph curve (Figure 1). In the bacterial growth period on first 7 days the best conformity (R^2=0.98) to time, temperature, and interior factors are represented by linear regression in variant B (Figure 2).

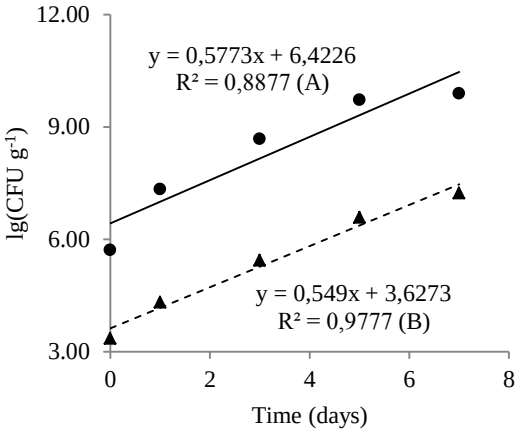

Figure 2. The linear regression trends of lactic acid bacteria count - lg (CFU g^{-1}) values in fermented sausages with ── (A) and without ····· (B) starter culture on first 7 days of ripening (p<0.05)

It can be seen in Figure 1 and Figure 2 that exponential phase of lactic bacteria growth stops on the 7th day of ripening, when a_w decreases below 0.92–0.90, and moves to stationary phase for next 7 days. The main species of LAB, detected before in the meat products prepared at the mother factory, and its minimal a_w value by Vermeiren, Debevere (2004), were 0.94 for *Lactobacillus brevis* and 0.92 for *Lactobacillus plantarum*, which have been detected as the main LAB species in experimental sausage samples too.

The main bacterial growth factor a_w minimal values for starter culture components are: 0.91 *L. sakei* (Leroy, de Vuyst, 1999), 0.86 *S. xylosus* (Terra et al., 2007), and 0.81 *D. hansenii* (Aggarwal, Mondal, 2009). These different requests of minimal a_w guaranty a constant level of pH during necessary ripening time.

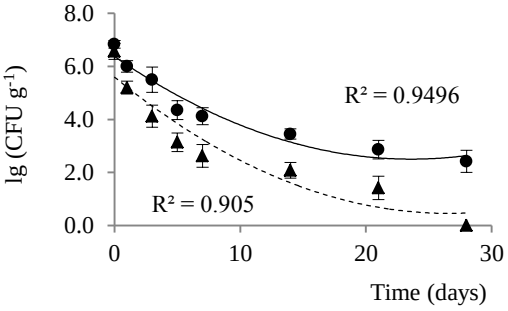

Figure 3. The polynomial regression trends of *L. monocytogenes* count - lg (CFU g^{-1})····· (A) with and ── (B) without starter culture in fermented sausages during ripening. ± SD values (p > 0.05)

Due to its water binding and ionic characteristics, salt affects the metabolism of a starter culture. The growth of lactic acid bacteria is sometimes enhanced in the presence of low content of sodium chloride (1 to 2%, 10–20 g kg^{-1}), but growth is clearly inhibited in the presence of NaCl content greater than 3% (30 g kg^{-1}) (Korkeala et al., 1992; Samapundo et al., 2010).

Homofermentative LAB is more resistant to sodium chloride than heterofermentative LAB are, and strains resembling *L. sakei* have been shown to be more resistant than other strains.

The initial *L. monocytogenes* inoculation concentration averaged 6.6–6.8 lg (CFU g^{-1}) was significantly ($p<0.01$) reduced at any ripening stage in both (A and B) sample variants (Figure 3). The sausage samples from this study had finally pH< 4.7 and a_w < 0.82. Such values guarantee no growth of *L. monocytogenes* (Vermeulen et al., 2007) and the rest count possibly depends on initial count.

In both batches (A and B) the decrease of detected *L. zmonocytogenes* count showed a negative linear regression curve during the first 7 days (Figure 4) with lg (CFU g^{-1}) decreasing rate lg -0.54 (A) and lg -0.39 (B).

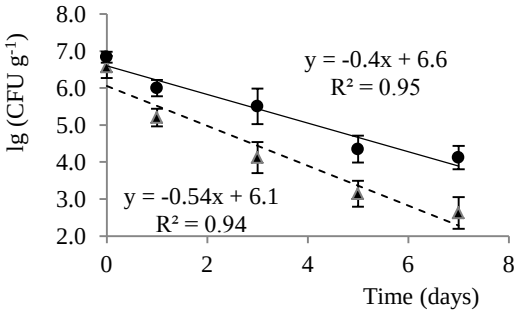

Figure 4. The linear regression trends of *L. monocytogenes* count - lg (CFU g^{-1})····· (A) with and —— (B) without starter culture in fermented sausages during the first 7 ripening days (p>0.05)

However, it can be said that *L. monocytogenes* were inhibited and did not exceed the growth in all observed ripening time (21 day). As it can be seen in Table 1, and Figure 4, the addition of starter culture hastened *L. monocytogenes* live cells, and detected count of *L. monocytogenes* decreased two times. All changes of the physic-chemical parameters, except salt content, were decreased, but all of them did not support *L. monocytogenes* growth. All parameter changes more or less correlated (Table 2) between each other, but water activity is the parameter which summarizes these changes, and that is why it can be conferred as the main factor which limited pathogen growth in food products.

The latest 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, such as lactic acid bacteria, entered stationary phase (Al-Zeyara et al., 2011).

pH, a_w, NaCl (g kg^{-1}), and NO_2^- (mg kg^{-1}) values changes are shown in double graph in Figures 5 and 6. The measurements of water activity show that *L. monocytogenes* growth would have been theoretically stopped on the 7th day of ripening when a_w decreased to 0.90 according to Vermeulen et al. (2007), but the observed results of bacterial count decrease made an idea of importance of a_w motion as the most significant factor against *L. monocytogenes* growing and survival in meat products.

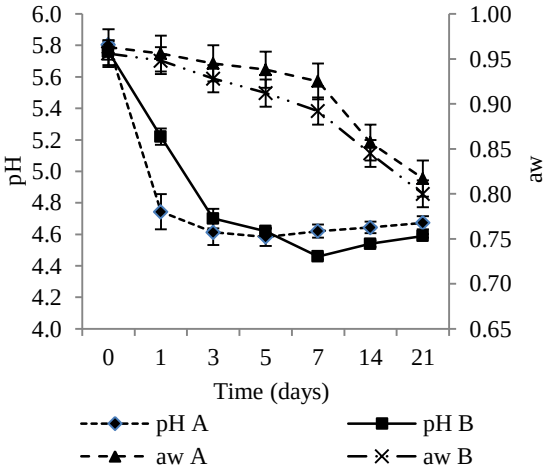

Figure 5. The changes of physico-chemical parameters: pH and a_w in fermented sausages with (A) and without (B) starter culture during 21 ripening days

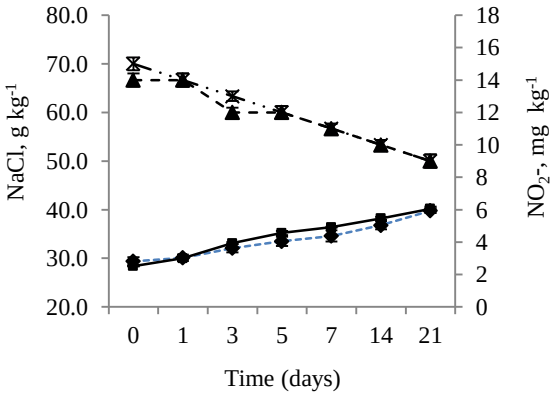

Figure 6. The changes of physic-chemical parameters: NaCl and NO_2^- in fermented sausages with (A) and without (B) starter culture during 21 ripening days

.....♦.... NaCl A, ----■--- NaCl B, - - ▲ - - NO_2^- A, -..-..x-..-.. NO_2^- B.

The samples of fermented sausages had a mean initial pH value of 5.80±0.02, which agrees with the results found by Paleari et al. (2003). A rapid decrease in pH was observed during the first three days of fermentation. The final pH of the fermented sausages had a mean value of 4.67–4.56±0.02; this drop in pH was due to lactic acid production by the starter culture used for fermentation (Vermeiren, Debevere, 2004). *Lactobacilli* are the major producers of lactic acid responsible for the decrease in pH and the increase in acidity during fermentation (Schillinger et al., 1991). Lactic and acetic acids are often suggested to be major contributors to the acid aromas and tastes and the

development of the texture of fermented sausage (Visessanguan et al., 2005).

Table 2

The correlation (r) values and their significance (p) levels between measured physic-chemical parameters and changes of inoculated *L. monocytogenes* count

Parameters	A		B	
	r	p	r	p
Time (days)	-0.896	0.003	-0.963	0.000
lg LAB	-0.865	0.006	-0.873	0.005
pH	0.609	0.073*	0.765	0.023*
a_w	0.867	0.006	0.980	0.000
NaCl, %	-0.954	0.000	-0.981	0.000

*Correlation is not significant

Under the fermentation and maturation conditions in this work, the decrease of *L. monocytogenes* count in fermented sausage was less intense than reported by other studies (Työppönen et al., 2003; Tolvanen et al., 2008) where an expressive decrease was observed at the beginning of ripening process. This is probably due to a lower pH and water activity in the first days of maturation noted in other studies, and higher initial *L. monocytogenes* concentration in our experiments.

The mean values of the decrease rate in *L. monocytogenes* count in batch B are bigger than those Glass, Doyle (1989) found in sausages without added lactobacilli cultures.

No significant correlation was calculated in both experimental batches with and without starter culture between *L. monocytogenes* count and pH value. That could be explained by a relatively short time when pH value decreased to constant level and long stationary phase of pH value.

Conclusions

The main parameters, maintained negative exponential growth rate of *L. monocytogenes* in fermented sausages are a_w value decrease and lactic acid bacteria, which stopped *L. monocytogenes* growing at the beginning of fermented sausage maturation. If fermentation process goes technically and hygienically correctly, the fermented (cold-smoked) sausages could be one of the safest meat products, because in real practice we observed a low level of contamination.

The remaining count of *L. monocytogenes* in fermented sausage depends on the possible initial contamination level and could exceed the European Union regulation value 2.0 lg (CFU g^{-1}) for ready-to-eat products when contamination at first is more than lg 5.0.

References

1. Aggarwal M., Mondal A.K. (2008) *Debaryomyces hansenii*: An Osmotolerant and Halotolerant Yeast. **In**: *Yeast Biotechnology: Diversity and Applications*, T. Satyanarayana, G. Kunze (eds). Netherlands: Springer, p. 65–84.
2. Al-Zeyara S.A., Jarvis B., 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*, Vol. 145, p. 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, p. 1–25.
4. Bērziņš A., Hörman A., Lundén J., Korkeala H. (2007) Factors associated with *Listeria monocytogenes* contamination of cold-smoked pork products produced in Latvia and Lithuania. *International Journal of Food Microbiology*, Vol. 115, p. 173–179.
5. Bredholt S., Nesbakken T., Holck A. (2001) Industrial application of an antilisterial strain of *Lactobacillus sakei* as a protective culture and its effect on the sensory acceptability of cooked, sliced, vacuum-packaged meats. *International Journal of Food Microbiology*, Vol. 66, p. 191–196.
6. Comi U., Iacumin K.R., Cattaneo C.C., Cocolin L. (2005) Characterisation of naturally fermented sausages produced in the North East of Italy. *Meat Science*, Vol. 69, Iss. 3, p. 381–392.
7. Drosinos M.M., Xiraphi G.M., Gaitis J.M. (2005) Characterization of the microbial flora from a traditional Greek fermented sausage. *Meat Science*, Vol. 69, Iss. 2, p. 307–317.
8. Farber J.M. Peterkin P.I. (1991) *Listeria monocytogenes*, a food-borne pathogen. *Microbiology and Molecular Biology Reviews*, Vol. 55, Iss. 4, p. 476-511.
9. Glass K.A. Doyle M.P. (1989) Fate of *Listeria monocytogenes* in processed meat products during refrigerated storage. *Applied and Environmental Microbiology*, Vol.55, Iss 6, p. 1565–1569.
10. Korkeala H., Alanko T., Tiusanen T. (1992) Effect of sodium nitrite and sodium chloride on growth of lactic acid bacteria. *Acta Veterinaria Scandinavica*, Vol. 33, Iss. 1, p.27–32.
11. Lahti E., Johansson T., Honkanen-Buzalski T., Hill P., Nurmi E. (2001) Survival and detection of *Escherichia coli* O157:H7 and *Listeria monocytogenes* during the manufacture of dry sausage using two different starter cultures. *Food Microbiology*, Vol.18, Iss. 1, p. 75–85.
12. Leroy F., de Vuyst L. (1999) The Presence of Salt and a Curing Agent Reduces Bacteriocin Production by *Lactobacillus sakei* CTC 494, a Potential Starter Culture for Sausage Fermentation. *Applied and Environmental Microbiology*, Vol. 65, Iss. 12, p. 5350–5356.
13. Liu D., Lawrence M.L., Ainsworth A.J., Austin F.W. (2005) Comparative assessment of acid, alkali and salt tolerance in *Listeria monocytogenes* virulent and avirulent strains. *FEMS Microbiology Letters*, Vol. 243, Iss. 2, p. 373–378.
14. Lunden J., Autio T., Korkeala H. (2003) Persistent and nonpersistent *Listeria monocytogenes* contamination in meat and poultry processing plants. *Journal of Food Protection*, Vol. 66, No. 11, p. 2062–2069.
15. McKellar R.C. (1994) Use of the CAMP Test for identification of Listeria monocytogenes. *Applied and Environmental Microbiology*, Vol. 60, No. 12, p. 4219–4225.
16. Miller A.J. (1992) Combined water activity and solute effects on growth and survival of *Listeria monocytogenes* Scott A. *Journal of Food Protection*, Vol. 55, Iss 3, p. 414–418.

17. Paleari M.A., Moretti V.M., Beretta G., Mentasti T., Bersani C. (2003) Cured products from different animal species. *Meat Science*, Vol. 63(4), p. 485-489.
18. Petran R.L., Zottola E.A. (1989) A study of factors affecting growth and recovery of *Listeria monocytogenes* Scott A. *Journal of Food Science*, Vol. 54(2), p. 458–460.
19. Samapundo S., Ampofo-Asiama J., Anthierens T., Xhaferi R., Van Bree I., Szczepaniak S., Goemaere O., Steen L., Dhooge M., Paelinck H., Dewettinck K., Devlieghere F. (2010) Influence of NaCl reduction and replacement on the growth of *Lactobacillus sakei* in broth, cooked ham and white sauce. *International Journal of Food Microbiology*, Vol. 143(1), p. 9–16.
20. Shank F.R., Eliot E.L., Wachsmuth I.K., Losikoff M.E. (1996) US position on *Listeria monocytogenes* in foods. *Food Control*, Vol. 7(4-5), p. 229–234.
21. Schillinger U., Kaya M., Lücke F.K. (1991) Behavior of *Listeria monocytogenes* in meat and its control by a bacteriocin-producing strain of *Lactobacillus sake*. *Journal of Applied Bacteriology*, Vol. 70(6), p. 473–478.
22. Terra N.N., Sossela de Freitas R.J., Cichoski A.J. (2007) Water activity, pH, moisture and growth of *Staphylococcus xylosus* during processing and storage of cured, matured and fermented pork shoulder. *Ciência e Tecnologia de Alimentos*, Vol. 27, Iss. 4, p. 2026–2031.
23. Thevenot D., Delignette-Muller M.L., Christieans S., Ver-nozy-Rozand C. (2005) Fate of *Listeria monocytogenes* in experimentally contaminated French sausages. *International Journal of Food Microbioogy*, Vol. 101. Iss. 2, p. 189–200.
24. Tolvanen R., Hellström S., Elsser D., Morgenstern H., Björkroth J., Korkeala H. (2008) Survival of *Listeria monocytogenes* strains in a dry sausage model. *Journal of Food Protection*, Vol. 71, Iss. 8, p. 1550–1555.
25. Työppönen S., Markkula A., Petaja E., Suihko M., Mattila-Sandholm T. (2003) Survival of *Listeria monocytogenes* in North European type dry sausages fermented by bioprotective meat starter cultures *Food Control*, Vol. 14, p. 181–185.
26. Vermeiren D., Debevere A. (2004) Evaluation of meat born lactic acid bacteria as protective cultures for the biopreservation of cooked meat products. *International Journal of Food Microbiology*, Vol. 96, Iss. 2, p. 149–164.
27. Vermeulen A., Gysemans K.P., Bernaerts K., Geeraerd A.H., Van Impe J.F., Debevere J., Devlieghere F. (2007) Influence of pH, water activity and acetic acid concentration on *Listeria monocytogenes* at 7 degrees C: data collection for the development of a growth ⁄ no growth model. *International Journal of Food Microbiology*, Vol. 114, Iss. 3, p. 332–341.
28. Visessanguan S.B., Panya C.K. Assavanig C. (2005) Influence of minced pork and rind ratios on physico-chemical and sensory quality of Nham-a Thai fermented pork sausage. *Meat Science*, Vol. 69, Iss. 2, p. 355–362.