

The effect of thermal treatment by cooking and storage on viability of *Listeria monocytogenes* in frankfurters

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Scientific paper

Summary

Listeria monocytogenes differs in several aspects from other pathogens transmissible through food. Considering the fact that it is widespread and significantly resistant to different unfavorable conditions of growth and development, such as low pH values and high NaCl concentrations, its microaerophilic and psychrotrophic traits, contamination of food with this pathogen has become one of the leading problems of public health and food industry. Except for that, the danger of contamination of food production plants is also based on its ability to survive for a long period in an external environment, as at very low temperatures (from 0°C to 7°C), even at temperatures of deep-freezing (>-20°C), so at high temperatures (from 60°C to 90°C). Knowing the basic characteristics of *L. monocytogenes* and with the intent of contributing to the improvement of health safety of foodstuffs and protection of consumer health, the goals of research were testing the effect of different temperature regimes of thermal treatment of frankfurters on the viability of experimentally inoculated *L. monocytogenes*. There were also tested the effect of different initial number of experimentally inoculated *L. monocytogenes* and technological process of production and storage on dynamics of its viability and growth in the researched meat products. The obtained results of our research pointed to the possibility of viability of *L. monocytogenes* in frankfurters with the effect of different temperature regimes of thermal treatment and storage. Considering our results, we recommend an obligatory control of foodstuffs to the presence of *L. monocytogenes*, together with the necessary education of food producers, farmers and general public on basic measures of protection against the contamination of food by a pathogen, as well as measures of prevention of alimentary listeriosis.

Key words: microbiological control, *Listeria monocytogenes*, frankfurters

Introduction

Bacteria *Listeria monocytogenes* differs in several aspects from other pathogens transmissible through food. As it is widespread, it is resistant to different environmental conditions, including low pH values and high concentrations of NaCl. It is a microaerophilic and psychrotrophic bacteria. It has become one of the leading problems in food industry because of its ability to contaminate food production plants in different ways, to survive for a long period in an external environment (Fenlon, 1999), (Warriner et al., 2009), as well as its ability to grow at very low

temperatures (from 0°C to 7°C), and to survive in or on food for a longer period of time in bad conditions at temperatures of deep-freezing (Fraber et al., 1994).

Severe symptoms and mortality rate caused by *L. monocytogenes* bacteria demand suitable prevention measures which are conditioned by the mentioned characteristics of pathogen. Therefore it is not realistic to expect all foodstuffs of animal and plant origin to be free of listeria. Specificity and characteristics of listeriosis have caused a very lively scientific discussion and encouraged researches in different areas, such

as the conventional and methods of fast detection of listeria in food, its behavior in food, virulence genetics and molecular typing, as well as epidemiologic researches of this disease, all in the goal of suppression of listeriosis.

The data obtained during the last ten years related to the question of outbreaks of mass diseases caused by poisoning after consuming meat and meat products, indicate that some meat products become more prone to microbiological contamination due to the complexity of the procedures of production and processing. Knowing numerous ways of microbiological contamination

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of meat and meat products during production, processing and storage, the major determinant of microflora in meat products is easily noticed. Meat products which carry risk from the aspect of *L. monocytogenes* contamination are ready and semi-prepared meals which are refrigerated for a longer period of time, as well as the meals which are contaminated by microorganisms in the number of >100 CFU/g or ml (Walker et al., 1999). Microorganisms which get into meat from facilities for slaughter, processing and storage can cause a change in sensory traits and spoilage of meat and meat products. Some of them can even cause alimentary poisonings with severe or easy consequences. Meat products which were used in the experiment – frankfurters, also belong to the group of risky products.

Material and Methods

The research of the effect of technological process of production and storage on viability of *L. monocytogenes* in meat products – frankfurters, was conducted in the period of 2008 to 2009.

There were experimentally produced 69 frankfurter a' samples of 100 g. The preparation of raw materials was made in manufacturing facilities of the meat company "Bećarević" Sarajevo. Pathogen inoculation and completion of the technological process of production of meat products were performed in laboratories of the Department of Food Hygiene and Technology, Veterinary faculty, University of Sarajevo, where microbiological and physical-chemical analyses of experimentally produced meat products' samples were conducted.

Preparation of *L. monocytogenes* inoculation and inoculation

A commercial reference *L. monocytogenes* strain ATCC 13932 was



Photo 1. Thermal treatment of frankfurters at 65°C and 72°C. (Technological laboratory of the Department of Food Hygiene and Technology)

used for inoculation. Colonies of *L. monocytogenes* bacteria were transferred from the preserved condition (semi-viscous agar) by swabs to Brain-Heart Infusion Broth (BHI., Ref M 210 Himedia, India), nutritious broth. After a 24-hour incubation at 37°C, the inoculum was over-inoculated at Listeria agar (Fluka Rb 16233, Sigma-Aldrich, India), which was incubated for 48 hours at 37°C. Characteristic grown colonies from agar were inoculated by swabs (1-2 colonies) in 10 ml Fraser secondary enrichment broth base (Fluka Sigma-Aldrich, PW 10043RB, USA). 1 ml of inoculated broth was taken after 24 hours of thermostating at 37°C, which was used to make decimal dilutions from -1 to -10. Out of each dilution there was sowed per 0.1 ml at Listeria agar in order to determine the exact count of bacteria/ml which will be inoculated to meat products.

Previously prepared different concentrations of *L. monocytogenes* in Fraser Broth were inoculated into stuffing and raw materials for meat products by previously sterilized spray can. Homogenization of the raw material and added inoculate

was performed after that. There followed further stages of technological production process after the inoculation and homogenization.

Experimental production of frankfurters

Beef and baby beef of first and second class were used for the production of frankfurters, and the mixture of spices and additives which are added in a suitable quantity according to the producer's specification and valid normative documents, Rules of Regulations on meat products quality (Sl. List No. 2/92, 13/94 and 14/94). The total quantity of prepared stuffing for inoculation was 10 kg. Out of that, 2.5 kg of raw material was used to prepare control samples of frankfurters, and 7.5 kg for the preparation of frankfurters used for inoculation with bacteria *L. monocytogenes*.

Cooled meat of the first and second class was grinded in a chopping machine ("wolf"), then cut up in small pieces in a cutter ("Bizerba") where it was added a mixture of prepared spices according to the producers' specification. After homoge-



Photo 2. Colonies of *L. monocytogenes* on a selective base Aloa agar (Collection of the Department of Food Hygiene and Technology)

nization and shaping of the stuffing, the mixture was inoculated with *L. monocytogenes* of the initial number 10^3 - 10^4 /g. In the next stage, the inoculated stuffing was filled into a casing of 1.8 cm diameter (sheep casing) by a manual filling machine (TEFAL „Le Hachoir 1500“) of 1.8 cm diameter. As the final stage (Photo 1) there followed warm smoking, cooking and showering in an atmosphere chamber of laboratory kind (SPAKO MCTT 6, Holland). Temperatures of cooking inside of products were 65°C and 72°C in the period of 30 minutes. Frankfurters prepared in such way were cooled (cold showering), packed in a vacuum packaging (Krupps F380 – China) and refrigerated at 0.5°C before sampling.

Sampling

Marking of the samples was performed based on the kind of products and the period of storage and thermal treatment. All samples of the experimentally produced products in all analyses were done in a triplicate.

After the described experimental production and inoculation, frankfurter samples were divided into groups according to temperatures of thermal treatment and stored at 0.5°C. Storing period of 45 days and the mentioned temperature were chosen based on the producer's specification. The schedule of frankfurter sampling for both thermal

Table 1. Microbiological status of raw material for the production of frankfurters before the experimental inoculation

<i>Salmonella</i> spp. / 25 g	<i>E. coli</i> / 0.01 g	Coagulase-positive staphylococci/ 0.1 g	Sulphite reducing clostridia /0.1 g	<i>Proteus</i> spp. /0.01 g	<i>L. monocytogenes</i> /0.1 g
Negative	Negative	Negative	Negative	Negative	Negative

Table 2. Physical and chemical values of experimentally produced frankfurters before thermal treatment

Water %	Fat %	NaCl %	a_w	pH
65.89	17.1	1.98	0.975	6.00

Table 3. Physical and chemical values of frankfurters stored up to 45 days at 0.5°C

Sample	After thermal treatment					After 45 days of storage				
	Water %	Fat%	NaCl%	a_w	pH	Water%	Fat%	NaCl%	a_w	pH
Frankfurters 65° C	64.02	18.98	2.34	0.970	5.50	58.82	22.68	4.54	0.940	5.0
Frankfurters 72° C	59.58	21.05	2.77	0.964	5.50	54.08	24.25	5.17	0.930	4.8

treatments (65°C and 72°C) in the goal of analyses was made: one, five, ten, 15, 20, 25, 30, 35, 40 and 45 days after the day of inoculation and thermal treatment. Except for the detection and counting of the bacteria *L. monocytogenes*, there were performed physical and chemical researches and water activity (a_w) was determined.

The principle of isolation and counting of *L. monocytogenes*

Isolation and counting of the bacteria *L. monocytogenes* in the experimentally inoculated frankfurters were performed by an official NMLK method, Nordic Committee no Food Analysis No 136 rd ed. 2004, Lončarević et al. (2008).

Preparing procedure of samples for analyses

There was taken aseptically 50 g of the sample approximately and it was homogenized in a stomacher (Lab-Blender 3500) for 30 to 60 seconds. After the homogenization, 20 g were taken for further analysis for primary enrichment. The rest of the homogenized sample was stored at 0.5°C until the end of the analysis.

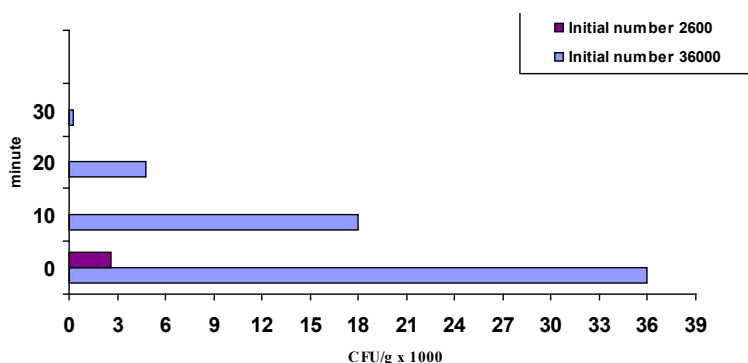
Procedures of enrichment, isolation and reading

Procedures of isolation and counting can be conducted simultaneously or counting can start only after typical or suspicious colonies are isolated on a selective medium, after the primary or secondary enrichment. Equal initial dilutions of the sample can be used for counting, as well as the procedures of enrichment. Alternatively, these procedures can be performed from separate dilutions too, but in this case it is important to start from the same homogenized sample. The procedure of two-stage enrichment was used in the method of isolation.

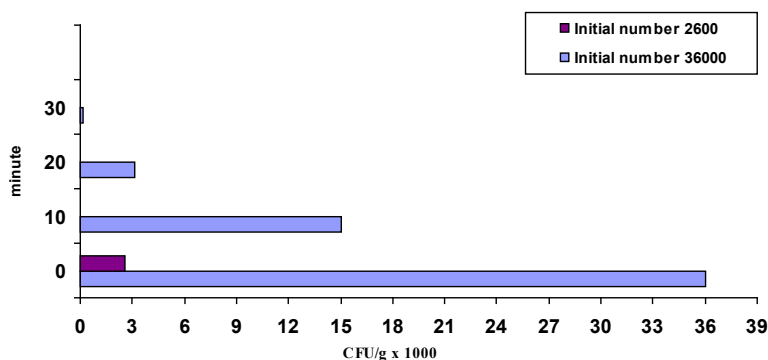
For primary enrichment, 20 g of the homogenized sample were taken aseptically and there was added 225 ml of primary enrichment broth (Half Fraiser Broth), previously warmed at 30°C - 37°C. Homogenization in the length of 30 seconds followed.

The homogenized sample with the primary enrichment broth was incubated at the temperature of $30.0 \pm 1.0^\circ\text{C}$ through 24 ± 3 h. After 24 hours of incubation, there followed

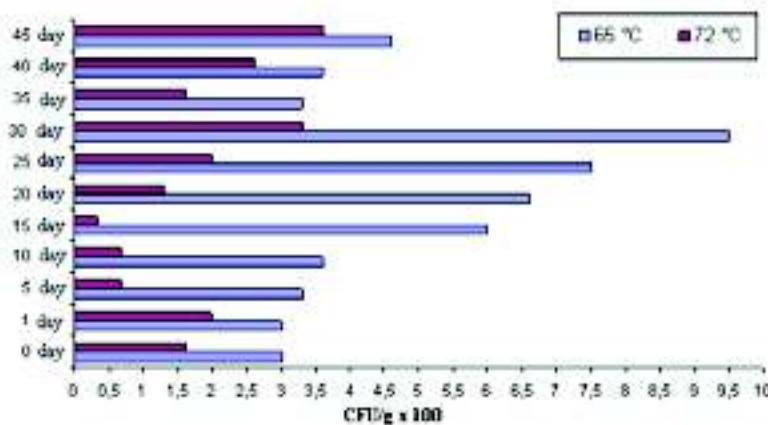
Graph 1. Viability of *L. monocytogenes* of different initial number in conditions of thermal treatment of frankfurters by cooking at the temperature of 65°C inside the product in the period of 30 minutes



Graph 2. Viability of *L. monocytogenes* of different initial number in conditions of thermal treatment of frankfurters by cooking at the temperature of 72°C inside the product in the period of 30 minutes



Graph 3. Viability of *L. monocytogenes* in previously thermally treated (65°C and 72°C) frankfurters while storing at 0.5°C in the period of 45 days



broth (full strength Fraser broth) and incubated at $37.0 \pm 1.0^\circ\text{C}$ in the length of 48 ± 4 h.

After the secondary enrichment, there was transferred by a micropipette from test tubes per 0.1 ml of sample to the surface of the previously prepared plate for detection and counting of *L. monocytogenes*-

ter which the plates were incubated at the temperature of $37.0 \pm 1.0^\circ\text{C}$ in the length of 24 ± 3 h. Reincubation of plates for the extra 24 ± 3 h and then reading was done in cases of negligible growth or in situations when there was no growth of colonies or if there were no noticed typical colonies after 24 ± 3 h of incubation. *L. monocytogenes* was con-

firmed based on appropriate tests (Gram staining, mobility, enzymatic reaction – catalase and β –hemolysis on blood agar). Characteristic green- blue colonies of *L. monocytogenes* surrounded by an opaque halo (Photo 2) were stained after that and their count was multiplied with the dilution in order to get the final count cfu/g.

Physical and chemical parameters of meat products (water, fat, NaCl, a_w and pH value)

Determining parameters of the content of water, fat and NaCl in experimentally produced frankfurters was performed in accordance with the regulations of the Ordinance on the Methods of Chemical Analyses and Super-analyses of Meat, Fat and Oil Products (Regulation SI.I. BiH 2/92, 13. and 14/94). Determining water activities in experimentally produced meat products was performed by a device for determining water activity "Testo 650" (Schwarzwalz) according to the producer's instructions. Determining pH values in frankfurters was performed by a potentiometric method, pH-meter "Hanna 210" (Italy), according to the producer's instructions.

Results

Analyses' results are represented for each experimentally produced product (frankfurters), both in forms of tables and graphs. Each result represents mean value of three measurements at all performed analyses.

The microbiological status made according to the Ordinance on conditions in microbiological safety which all foodstuffs have to comply with (SI.I. BiH No. 2/92, 13 and 14/94), the raw materials used for the production of the mentioned products and the examination of the presence of *L. monocytogenes* before inoculation are presented in Table 1.

Detection results and counting of *L. monocytogenes*

The results of microbiological analyses of the effect of the technological process of production and storage of frankfurters on growth and development of the *L. monocytogenes* bacteria are shown in Graphs 1, 2, and 3.

Results of researching physical and chemical parameters

Determining the content of water, fat, NaCl, a_w and pH value was performed with all experimentally produced frankfurters in the beginning and at the end of the technological process of processing and storage. The results are presented in Tables 2 and 3.

Discussion

Semi - prepared meat product-frankfurters, inoculated with two different initial numbers of *L. monocytogenes* were exposed to thermal treatment by cooking at temperatures of 65°C and 72°C in the period of 30 minutes, as it is required by the technological production process (Hadžibeganović, 1975), (Živković, 2001).

Results of microbiological analyses presented in Table 1 show that raw material for the product was negative for the presence of bacteria *L. monocytogenes* and other bacterial contaminants and pathogens. Except for that, the values of physical and chemical parameters (Table 2) complied with the regulations of the Ordinance on the methods of performing microbiological analysis and super-analysis of foodstuffs (Regulation Sl.I. BiH 2/92, 13. and 14/94) and technological requirements of the production (Hadžibeganović, 1975, 1983), (Živković, 1986).

The obtained results of the effect of different regimes of thermal treat-

ment on *L. monocytogenes* in frankfurters indicate similarity with the results of other authors (Čaklović, 2010), (Houben et al., 2006). Graph 1 presents the results of viability of *L. monocytogenes* in frankfurters experimentally inoculated in the initial concentration of 36 000 CFU/g, which was 300 CFU/g at the end of the thermal treatment by cooking in the period of 30 minutes at 65°C. During the activity of the same temperature in the same time interval and substrate with the initial number of 2600 CFU/g, as soon as in the 10th minute the count of *L. monocytogenes* was reduced completely.

The temperature of cooking of 72°C in frankfurters with the initial number of 36 000 CFU/g decreases the number during the period of 30 minutes to 200 CFU/g, whereas the initial number of *L. monocytogenes* of 2 600 CFU/g was again completely reduced in the first 10 minutes (Graph 2). Based on these results, we conclude that viability of *L. monocytogenes* in frankfurters on listed temperatures and time intervals depends directly on the quantity of the initial number of microorganisms. Similar results were obtained by other authors too (Bersot et al., 2001), (Fraber et al., 1990), (Jemmi et al., 2006), (Ryser et al., 2001) and (Shigenobu et al., 2007). This information is particularly important for producers of meat products of this type because it indicates to the importance of complying with standard operative procedures in production.

Monitoring the effect of temperature of storing of 0.5°C during the period of 45 days to the growth and viability of *L. monocytogenes* in the experimentally produced frankfurters was presented in Graph 3. The results indicate clearly not only to viability but to the evident growth of bacterial population of *L. monocytogenes*. They also justify the given hypothesis that longer storage can

have a favorable influence to its recovery after the thermal treatment at cooking temperatures, which was also explained by other authors in their papers (Gill et al., 1989), (McClure et al., 1997) and (Uyttendaele et al., 2009).

After cooking at 65°C, on the day 0 of storage frankfurters had 300 CFU/g of *L. monocytogenes*, whereas that number was three times higher on the 30th day, and at the end of the 45th day it was 460 CFU/g. After the thermal treatment of frankfurters at 72°C and their storage, the count of *L. monocytogenes* was 160 CFU/g on day 0; then it was double on the 30th day, and at the end of storage it is marked by the highest growth of 310 CFU/g. Different growth dynamics of *L. monocytogenes* with the mentioned thermal treatments in frankfurters during storage is also explained by physical and chemical parameters presented in Table 3. The results for frankfurters after the thermal treatment at 65°C are 64.02% for water, then 2.34% for NaCl and 0.970 for water activity, whereas with thermal treatment of 72°C lower values for the same parameters were obtained, except for pH mean which was 5.5 in both thermal treatments. Physical and chemical parameters of frankfurters after the 45th day of storage with the temperature regime of 65°C, water, NaCl, water activity and pH value were still somewhat higher than the values of the mentioned parameters with the thermal treatment of 72°C (Table 3). The obtained results show us that loss of water appears as the consequence of the increase of cooking temperature, which affects the values of share of water and water activity which decrease, and the count of *L. monocytogenes* is slightly higher at the end of storage with frankfurters that were cooked at 65°C with pH values which didn't change significantly.

The obtained values for content

of water, table salt, water activity and pH value with different thermal treatments of frankfurters still represent a favorable environment for growth and propagation of *L. monocytogenes* and that is the reason for the fact that its count at 0.5°C during storage is higher on the 45th day in comparison to its count on day 0.

The results have shown that the viability and development dynamics of *L. monocytogenes* in frankfurters after the thermal treatment by cooking at different temperatures with equal period of storage, along with the initial number, depends directly on the share of water in the product and water activity, which is necessary for the viability and development of all microorganisms and competitive microflora. Other authors, Jemmi et al., (2006), Kanabel et al., (1990) and Ryser et al., (2001) also mention the possibility of recovery of *L. monocytogenes* after the thermal treatment and the appearance of its development at longer storage of products in the environment with pH values below 5, which was confirmed by our results.

Both a disadvantage and an advantage of this paper is its concept which is significant for Bosnia and Herzegovina only, because it relates to meat products without determined control measures when it comes to *L. monocytogenes*. By pointing to the implicated food and a recommendation of the significance of control, as well as taking appropriate measures to prevent contamination with *L. monocytogenes* in plants for production and handling of food, a contamination chain can be stopped and large epidemics can be prevented.

By knowing that the experimentally researched product belongs to the group of meat products with an increased risk of viability of *Listeria* and a possible transmission to peo-

ple, and by adopting research results of the effect of different technological procedures of production, storage and viability and development of *Listeria* in a frankfurter, a justification of this experimental research is completed.

Conclusions

Based on the results of the research of the development and viability of *L. monocytogenes* during the technological process of production, processing and storage of experimentally produced meat products-frankfurters, the following can be concluded:

Storage temperature of 0.5°C doesn't stop the development of *L. monocytogenes* in frankfurters

It has been confirmed that *L. monocytogenes*, in comparison to most pathogenic causative agents, is extremely resistant to the effects of different temperatures of thermal treatment (65°C, 72°C) in contaminated products.

The obtained results indicate to the necessity of the introduction of the HACCP control system in the production of frankfurters and its strict application in the goal of the elimination of risk of contamination of these products by *L. monocytogenes*, then more effective protection of health of end consumers and prevention of the occurrence of alimentary listeriosis.

The results of our paper indicate to the necessity of convergence of legislation of Bosnia and Herzegovina to the European legislation in terms of control of *L. monocytogenes* bacteria in food of animal origin. Namely, the current Ordinance on conditions in microbiological safety which all foodstuffs have to comply with (Sl.I. BiH No. 2/92, 13 and 14/94) does not require control of food on the presence of *L. monocytogenes*,

whereas similar ordinances in the EU require not only a legally obligatory control of food on the presence of this pathogen, but also an application of a wider program of monitoring *L. monocytogenes* in food.

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Pravilnik o kvalitetu mesnih proizvoda (Sl. List BiH broj 2/92, 13/94 i 14/94)

Pravilnik o metodama obavljanja

mikrobioloških analiza i superanaliza živežnih namirnica (Sl. I. BiH br. 2/92, 13 i 14/94).

Pravilnik o uslovima u pogledu mikrobiološke ispravnosti kojima moraju udovoljavati živežne namirnice u prometu (Sl.I. BiH broj 2/92, 13 i 14/94).

Pravilnika o metodama vršenja hemijskih analiza i superanaliza proizvoda od mesa, masti i ulja (Uredba Sl.I. BiH 2/92 , 13. i 14./94).

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Received: 23 May 2011

Accepted: 22 April 2011 

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