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STUDY ON FACTORS (pH, WATER ACTIVITY, SALT CONTENT) AFFECTING THE GROWTH OF LISTERIA MONOCYTOGENES IN RAW DRIED CURED SAUSAGES

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ABSTRACT

Commission Regulation (EC) 2073:2005 considers the factors which can support or inhibit the growth of *Listeria monocytogenes* in ready-to-eat foods. The aim of the experiments was to examine the values of pH, water activity (a_w), salt content and level of contamination with *Listeria monocytogenes* of some popular in Bulgaria raw dried cured vacuum packed sausages, produced from June 2006 till May 2008. 81 vacuum-packed samples were taken from 5 different meat producing plants during the period of study. Average water activity level of the tested sausages was 0,87 ± 0,035; pH level - 5,61 ± 0,59 and salt content - 4,12 ± 1,11%. Four specimens contained *Listeria spp.* (two samples *L.monocytogenes*, one *L.welshimeri* and one *L.innocua*). All contaminated raw dried cured sausages had a_w bellow ≤ 0,92 and pH ≥ 4,4 or pH ≥ 5. After 3 months of storage of the same contaminated samples at 4^0C, in three of them *Listeria spp.* (two *L.monocitogenes* and *L.welshimeri*) survived and was detected. Salt content of the samples varied from 2,46 to 6,28% and was not able to affect the growth of *L.monocytogenes*.
Data showed that the detected levels of a_w could support the growth of *L.monocytogenes* in only 6 (7,4%) of the tested samples. pH values lower than 5 were presented in three samples and only the combination with low a_w was able to inhibit the growth of *L.monocytogenes*. The detected levels of salt content did not affect the presence and growth of *L.monocytogenes*. Microbiological criterion' set in Commission Regulation (EC) No 2073/2005 for ready-to-eat foods unable to support the growth of *L. monocytogenes* can be applied to 75 (92,6%) of the tested sausages.

Key words: L.monocytogenes, raw dried cured sausage, water activity, pH, salt

INTRODUCTION

Commission Regulation (EC) 2073:2005 (1) considers the factors which can support or inhibit the growth of *L.monocytogenes* in ready-to-eat foods, such as water activity (a_w) and pH. According to ICMSF (8) raw cured shelf-stable meats are some low-acid dry sausages and high-acid fermented sausages in which low a_w or a combination of low pH and reduced a_w provides microbial stability. EFSA opinion (6) reported that all products, except hard cheeses and fermented sausages, were able to support the growth of the pathogen. However, depending on their physicochemical characteristics (pH, a_w, presence of antimicrobials etc.), many of these products may not support the growth of *L. monocytogenes*. An effective evaluation of the compliance to the safety criteria requires a comparison of data concerning the prevalence and concentration of the bacterium with the results from physicochemical examinations of the products (mainly pH, water activity (a_w), as well as the remaining shelf life of the products after the time of analysis. The need for such information was taken into account in the *L. monocytogenes* baseline survey (BS) in certain ready-to-eat foods for 2010/2011. Wijtes et al. (12) noted that temperature, pH and water activity were important factors controlling the microbiological safety of foods and they described the growth rate

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of *Listeria monocytogenes* in relation to these factors. Two equations were developed, both equations were based upon the Ratkowsky equation for temperature and growth rate. McMeekin et al. (10) considered factors such as low pH tolerance and low water activity tolerance when describing quantitative microbiology and predictable modeling in food microbiology.

The aim of the experiments was to examine the values of pH, water activity (a_w), salt content and level of contamination with *Listeria monocytogenes* of some popular in Bulgaria raw dried cured vacuum packed sausages, produced from June 2006 till May 2008.

MATERIALS AND METHODS

Sampling

81 different kinds of raw cured dried meat sausages were studied. All of them were vacuum-packed in meat factories and prepared for sale without any other treatments. All samples were taken from 5 different food business operators during all seasons included in the period of study (June 2006 – May 2008). Tested specimens were kept at storage temperature from 0 to 4^0C. The samples were transported and received at the laboratory up to 72 hours after processing.

Microbiological analysis

The samples were analyzed according to the USDA method for meat foods, described by Ryser and Donelly (11). Five of all typical on PALCAM agar (Merck) colonies were taken and reinoculated on TSAYE agar (Merck, Darmstadt). Further examination comprised Gram staining, motility at 20-25^{0}C, growth at 35^{0}C, catalase activity (Hydrogen peroxide, Merck, Darmstadt), oxidase reaction (Oxidase reagent, bioMerieux) and β-hemolysis on blood agar (Merck, Darmstadt). Additionally, biochemical identification with API Listeria ID strip (bioMerieux, Inc., Hazelwood, Mo.) was done to all *Listeria spp.* isolates.

Physicochemical testing

Water activity (a_w) was estimated by HygroLab 3 rotronic AG instrument. Sample mass was cut to small pieces (3-5 mm) and put into a sample cup. The cup was filled to the upper edge. The probe was immediately put into the sample cup. The result was read as soon as the humidity and temperature values became stable.

pH was measured by Professional pH Meter Sartorius PP-15, according to interlaboratory procedure for testing, based on Bulgarian State Standard 1323 (2).

Salt content was determined according to AOAC Official method 935.47 Salt in Meat (Volumetric Method) (3).

RESULTS

Results showing the pH values of the tested raw cured dried meat products are presented in Table 1.

Table 1. Data of pH values of raw cured dried sausages, tested for presence of *Listeria spp.*

Number of samples	Limits of variation of pH values	Number of samples in fixed range of variation	*1.3 Ready-to-eat foods unable to support the growth of *L. monocytogenes* (8)	pH values of samples, contaminated with *Listeria spp.*
n = 81	4,0 to ≤ 4,4	0	≤ 4,4	-
	>4,4 to ≤ 5,0	8 (9,8%)	≤ 5,0	-
	> 5,0	73 (90,2%)	> 5,0	5,8; 7,2; 6,4; 5,8
	4,42 – 7,2	81	-	5,8; 7,2; 6,4; 5,8
X ± Sx = 5,61 ± 0,59				

**COMMISSION REGULATION (EC) No 2073/2005, Chapter 1. Food safety criteria, (8) Products with pH ≤ 4,4 or a_w. ≤ 0,92, products with pH ≤ 5,0 and a_w. ≤ 0,94, products with a shelf-life of less than five days shall be automatically considered to belong to this category. Other categories of products can also belong to this category, subject to scientific justification.*

Most of the specimens (90,2 %) had pH higher than 5 and only in 9,8% pH varied from 4,4 to 5 and could influence the growth of *L.monocytogenes*. Four samples were contaminated with *Listeria spp.* and all of them showed high pH values (>5).

Data for water activity of the tested specimens are reported in Table 2.

Great number of samples showed variation of a_w below 0,92 (92,6%). All contaminated with *Listeria* spp. samples belonged to the group of raw cured dried products with low a_w. Only 6 samples (7,4%) showed variation of a_w from 0,92 up to 0,94. None of the specimens had a_w higher than 0,94.

Table 2. Data of water activity (a_w) values of raw cured dried sausages, tested for presence of *Listeria spp*.

Number of samples	**Limits of variation of water activity (a_w) values**	**Number of samples in fixed range of variation**	***1.3 Ready-to-eat foods unable to support the growth of *L. monocytogenes*, (8)**	**a_w values of samples, contaminated with *Listeria spp.***
n = 81	0,70 to ≤ 0,92	75 (92,6 %)	≤ 0,92	0,906; 0,88; 0,88; 0,84
	>0,92 to ≤ 0,94	6 (7,4 %)	≤ 0,94	-
	> 0,94	0	> 0,94	-
	0,74 – 0,94	81	-	0,906; 0,88; 0,88; 0,84
X ± Sx = 0,87 ± 0,035				

**COMMISSION REGULATION (EC) No 2073/2005, Chapter 1. Food safety criteria, (8) Products with pH ≤ 4,4 or a_w. ≤ 0,92, products with pH ≤ 5,0 and a_w. ≤ 0,94, products with a shelf-life of less than five days shall be automatically considered to belong to this category. Other categories of products can also belong to this category, subject to scientific justification.*

Results presenting the sodium chloride values of the tested samples are shown in Table 3. Most of the samples (67,9%) had salt content between 3 and 4,5%. Two of them were contaminated with *Listeria spp.* Other 20 samples (24,7%) showed higher salt content values (≥ 4,5%) and also two of them contained *Listeria spp.*

Average water activity level of the tested sausages was 0,87 ± 0,035; pH level - 5,61 ± 0,59 and salt content - 4,12 ± 1,11%. Four specimens contained *Listeria spp.* (two samples *L.monocytogenes*, one *L.welshimeri* and one *L.innocua*). All contaminated raw dried cured sausages had a_w bellow ≤ 0,92 and pH ≥ 4,4 or pH ≥ 5. After 3 months of storage of the same contaminated samples at 4^0C, in three of them *Listeria spp.* (two *L.monocitogenes* and *L.welshimeri*) survived and was detected. Salt content of the samples varied from 2,46% to 6,28% and was not able to affect the growth of *L.monocytogenes*.

Table 3. Data of sodium chloride (NaCl) values in % of raw cured dried sausages, tested for presence of *Listeria spp.*

Number of samples	**Limits of variation sodium chloride (NaCl) values in %**	**Number of samples in fixed range of variation**	**Sodium chloride (NaCl) values in % in samples, contaminated with *Listeria spp.***
n = 81	2,0 to ≤ 3,0	6 (7,4%)	-
	>3,0 to ≤ 4,5	55 (67,9%)	3,94; 3,40
	> 4,5	20 (24,7%)	5,04; 4,94
	2,46 – 6,28	81	3,94; 3,40; 5,04; 4,94
X ± Sx = 4,12 ± 1,11			

DISCUSSION

Our data illustrated the status of some popular in Bulgaria raw cured dried sausages, typical for the taste of Balkan region countries. Factors as pH, a_w and salt content, affecting the growth of *Listeria spp.*, especially *L.monocytogenes* showed values, influencing in different ways the survival and growth of *Listeria* spp. and *L.monocytogenes*. On average, salt content in the group of tested products (raw cured dried meat foods) was low. The main reason for this is the consumer demand in Bulgaria. The evaluated pH level of the examined samples was up to 5, despite the adding of starter culture in the process of preparation of raw cured dried products. Consumers in Bulgaria do not prefer a too acidulous taste of these kinds of products. Drying and related to it decrease in water activity level is the hurdle that stops the growth of *Listeria spp.* In our case all positive for *Listeria* spp. samples belonged to the category of ready-to-eat raw cured dried meat foods unable to support the growth of *L.monocytogenes*, according to Regulation (EC) 2073:2005(1). After 3 month of storage in refrigerator ($\leq 4^0$C) one of the samples contaminated with *Listeria* spp. was found to be free from the pathogen. EFSA (7) reported that there was influence of pH and water activity on the prevalence of *L.monocytogenes* in ready-to-eat foods (fish products) and concluded that data showed very slow growth in the period of storage. Coelho (4) concluded that fermented under variable temperatures (mostly between 25-30°C) salami (also raw cured dried sausages) which were not heat treated, had final pH between 4.8 and 5.2, and water activity around 0.85-0.90. In our raw cured dried sausages fermentation process, if carried out, did not decrease pH to such low levels. Lahti et al. (9) studied sausages, which were manufactured (fermented and dried) in a smoke chamber at 17–23°C for 15 days and further stored at 15–17°C for 34 days. *L. monocytogenes* counts decreased more rapidly in the high-inoculum sausages produced with starter A ($P<0.0001$) but no significant difference was detected between the starters in the medium-inoculum sausages. *L. monocytogenes* was eliminated from the medium-inoculum sausages after 49 days. Daskalov et al. (5) proved in experiment the fate of *L.monocytogenes* during process of drying and storing of already dried product.

CONCLUSIONS

Data showed that the detected levels of a_w could support the growth of *L.monocytogenes* in only 6 (7,4%) of the tested samples. pH values lower than 5 were presented in three samples and only the combination with low a_w was able to inhibit the growth of *L.monocytogenes*. The detected levels of salt content did not affect the presence and growth of *L.monocytogenes*. 'Microbiological criterion' set in Commission Regulation (EC) No 2073/2005 for ready-to-eat foods unable to support the growth of *L. monocytogenes* can be applied to 75 (92,6%) of the tested sausages.

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