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Comparison of bacteriological status during ripening of traditional fermented sausages filled into different diameter artificial casings

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Abstract

The ripening process of fermented sausages is affected by diameter and type of sausage casings, and depends on changes in the microflora, important from hygienic and technological aspects. The aim of this study was to compare the bacteriological status sausages which were stuffed in artificial collagen sausage casings of different diameters (35 mm and 60 mm) during ripening and drying. The sausage stuffing was the same, as was the uncontrolled ripening conditions. In bigger diameter sausages, significantly higher average total bacterial count, enterobacteria and lactic acid bacteria counts were found than in smaller diameter sausages.

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Keywords: fermented sausages; artificial collagen sausage casings; bacteriological status

1. Introduction

Fermented sausages filled in casings which can be natural or artificial, but they must be firm, elastic and must follow the contraction of the filling during drying, permeable to smoke, water vapor and gases. Artificial casings

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were created at the beginning of the twentieth century, when in some countries, the requirements of the meat industry, which was rapidly developing, overcame the supply of natural casings. Following the development of machines for filling, artificial casings were adapted to the requirements of these systems, especially when it comes to uniformity. For sausage casings that are used for the production of fermented sausages, it is extremely important that they contact the filling, and not only after filling, but also during the drying period, when the volume filling decreases[1,2]. Artificial casings have advantages from the hygienic point of view, because the microbial contamination is negligible, storage at low temperatures unnecessary, and there is no problem with the failure of the product during storage and transport [3]. Today, for the production of large diameter, artificial sausage casings are a better choice, while they are equivalent to natural casings when it comes to the production of sausages of small diameter[4]. In the production of raw fermented sausages, properly selected casings act as a microbial barrier, but also take an active part in stimulating the growth of useful microorganisms.

During the fermentation process, leading to decomposition of carbohydrate by microorganisms, mainly lactic acid is formed. The metabolic activity of microorganisms, present in the sausage mixture, are significantly impacted by the permeability of sausage casings, through the influence of the amount of oxygen, pH and a_w. As a result of the fermentation process, the pH is lowered from 4.9 to 5.2, which, in addition to low a_w value[5], is the priority factor for achieving the microbiological safety of traditional dry fermented sausage[15]. Also, casing diameter is a very important factor for prediction of required time for fermentation.

Making traditional dry sausages is exclusively based on the activity of natural microflora present in the raw materials and production space, where the process of fermentation and ripening takes place at low temperatures for a prolonged period. Lactic acid bacteria are essential in the production of dry sausages. The greatest risk to human health is growth of pathogenic microorganisms, which affects the stability and safety of the final product[6,7,8,9]. *Enterobacteriaceae* are common contaminants of meat and therefore can be found in the filling in amounts that depend on the initial number, type of sausages and the ripening phases. The combined effects of low pH, low temperature and low a_w mean these microorganisms tend to disappear during the process of fermentation and at the end of the ripening phase raw fermented sausages are considered shelf-stable even at higher temperatures[8,10]. The aim of this study was to compare the bacteriological status of fermented sausages filled into artificial casings of different diameters.

2. Materials and methods

The filling of fermented sausages was suitable pork meat of first and second categories (ham, shoulder and neck pork meat with removed binding and fatty tissue) and a proportion fatty tissue. The ratio of these components was not determined exactly, but is based on the empirical experience of the manufacturer, according to a recipe of Western Slavonia, without starter culture or glucono delta-lactone (GdL), finished in a private household.

For the manufacture of sausages, white meat pig of Landrace breed, grown in Lijevče Polje, aged 12 months, weighing approximately 180 kg, which was fed a variety of foods was used. Preparation of raw meat for making the stuffing included cooling meat for 24 h, cutting the meat into small pieces with an electric meat grinder (number 42) and grinding, carried out to the desired degree of fragmentation, which, by traditional technology, passed holes of 8 mm on the grinding board. Ingredients, added in amounts which were not precisely defined and also based on empirical experience were: salt, extract of garlic and sweet and hot pepper. For smaller diameter sausages, artificial collagen sausage casings, diameter 35 mm were used, and for bigger sausages, artificial collagen sausage casings, 60 mm were used. Different diameter fermented sausages were ripened in identical conditions (smoking, drying). The manufacturing process for small diameter sausages lasted up to 31 days, and for large diameter sausages up to 61 days. Samples for bacteriological examination were taken on days 0, 7, 14, 21 and 31 day for both groups of sausages and for large diameter sausages on days 41, 51 and 61. Microbiological tests were conducted based on standard methods: determination of aerobic total viable mesophilic count - ISO 4833- 2003; determination of lactic acid bacteria count - Cook, 1991; determination of the total *Enterobacteriaceae* count - ISO 21528-1: 2004, ISO 21528-2: 2004 standard. Statistical analysis included the mean and variation measures. Statistically significant differences were determined using Student's t-test and analysis of variance. Data processing was done using Microsoft Excel, 2007. Value of $p < 0.05$, $p < 0.01$ and $p < 0.001$ were considered significant.

3. Results and discussion

The results of total viable count, total *Enterobacteriaceae* count and lactic acid bacteria count in sausages were consistent with the literature data about replacement of microflora. Increasing numbers of lactic acid and total mesophilic bacteria and acidification in sausages created environmental conditions that prevents the growth *Enterobacteriaceae*[3,6,7,8,9,10,11,12,13,14,16,17].

Table 1. Total viable count, total *Enterobacteriaceae* count and lactic acid bacteria count in samples of smaller and bigger diameter sausages ($\log_{10}$ cfu/g) (X±SD).

Day of ripening	Diameter of sausages	Group of examined bacteria		
		Total viable count	***Total Enterobacteriaceae count***	***Lactic acid bacteria count***
0	*Smaller diameter sausages*	3.19 ± 0.20	4.26 ± 0.19	$1.40^{x}\pm0.15$
	Bigger diameter sausages	3.27 ± 0.19	4.34 ± 0.28	$1.00^{x}\pm0.00$
7	*Smaller diameter sausages*	$3.36^{a}\pm0.23$	4.18 ± 0.18	$1.73^{x}\pm0.04$
	Bigger diameter sausages	$3.74^{a}\pm0.39$	4.19 ± 0.24	$2.34^{x}\pm0.21$
14	*Smaller diameter sausages*	$4.02^{x}\pm0.14$	3.76 ± 0.20	$4.63^{x}\pm0.26$
	Bigger diameter sausages	$4.59^{x}\pm0.27$	3.78 ± 0.27	$4.83^{x}\pm0.14$
21	*Smaller diameter sausages*	4.18 ± 0.17	$2.96^{x}\pm0.09$	$4.45^{A}\pm0.29$
	Bigger diameter sausages	4.17 ± 0.30	$4.14^{x}\pm0.19$	$4.82^{A}\pm0.12$
31	*Smaller diameter sausages*	$3.96^{a}\pm0.05$	-	$5.02^{A}\pm0.05$
	Bigger diameter sausages	$4.24^{a}\pm0.27$	3.69 ± 0.33	$4.91^{A}\pm0.15$
41	*Smaller diameter sausages*		-	-
	Bigger diameter sausages	4.43 ± 0.25	3.60 ± 0.21	4.99 ± 0.15
51	*Smaller diameter sausages*	-	-	-
	Bigger diameter sausages	3.98 ± 0.17	2.65 ± 0.12	5.02 ± 0.15
61	*Smaller diameter sausages*		-	
	Bigger diameter sausages	3.97 ± 0.07	-	4.95 ± 0.12

Legend*: Same letter shows statistically significant difference* ^{x} *$p < 0.001$;* ^{A} *$p < 0.01$;* ^{a} *$p < 0.05$.*

When comparing different diameter sausages, statistically significant differences in total viable count (TVC) were not seen on days 0 and 21 of ripening, while on days 7, 14 and 31, TVC was statistically significantly higher ($p < 0.05$; $p < 0.001$) in bigger diameter sausages. Total *Enterobacteriaceae* count on days 0, 7, and 14 of ripening was not significantly different in different diameter fermented sausages, while on day 21, the total number of enterobacteria was statistically significantly higher in bigger diameter fermented sausages ($p < 0.001$). At the end of the process of drying (ripening) in smaller diameter sausages (day 31) and bigger diameter sausages (day 61), there was no evidence of *Enterobacteriaceae*. Except on days 0 and 31, total lactic acid bacteria counts were statistically significantly higher in bigger diameter fermented sausages, but with different level of significance ($p < 0.01$; $p < 0.001$).

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References

1. Savic I and Z Savic. *Sausage Casings*, 1st ed. Vienna: Victus; 2002.
2. Urso R, Comi G, Cocolin L. Ecology of lactic acid bacteria in Italian fermented sausages: isolation, identification and molecular characterization. *Sys Appl Microbiol* 2006;**29**:671-80.
3. Coppola S, Mauriello G, Aponte M, Moschetti G, Villani F. Microbial succession during ripening of Naples-type salami, a southern Italian fermented sausage. *Meat Sci* 2000;**56**:321-29.
4. Hammes WP, Knauf HJ. Starters in the Processing of Meat Products. *Meat Sci* 1994;**36**:155-68.
5. Incze K. Dry Fermented Sausages. *Meat Sci* 1998;**49**:169-77.
6. Talon R, Leroy S, Lebert I. Microbial ecosystems of traditional fermented meat products: The importance of indigenous starters. *Meat Sci* 2007;**77**:55-62.
7. Papamanoli E, Tzanetakis N, Litopoulou-Tzanetaki E, Kotzekidou P. Characterization of lactic acid bacteria isolated from a Greek dry-fermented sausage in respect of their technological and probiotic properties. *Meat Sci* 2003;**65**:859-67.
8. Gonzalez B, V Diez. The effect of nitrite and starter culture on microbological quality of "chorizi"- a Spanish dry cured sausage. *Meat Sci* 2002;**60**:295-98.
9. Zdolec N, Hadziosmanovic M, Kozacinski L, Cvrtila Z, Filipovic I, Leskovar K, Vragovic N, Budimir D. Fermentirane kobasice proizvedene u domacinstvu – mikrobioloska kakvoca. *Meso* 2007;IX,**6**:318-24.
10. Rubio B, Martinez B, Sanchez MJ, Garsia-Cachan MD, Rovira J, Jaime I. Study of the shelf life of a dry fermented sausage "salchichon" made from raw material enriched in monounsaturated and polyunsaturated fatty acids and stored under modified atmospheres. *Meat Sci* 2007;**76**:128-37.
11. Drosinos EH, Mataragas M, Xiraphi N, Moschonas G, Gaitis F, Metaxopoulos J. Characterizaion of the microbial flora from a traditional Greed fermented sausage. *Meat Sci* 2005;**68**:307-17.
12. Lebert I, Leroy S, Giammarinaro P, Lebert A, Chacornac S, Bover-Cid S, Vidalcarou MC, Talon R. Diversity of microorganisms in the environment and dry fermented sausages of small traditional French processing units. *Meat Sci* 2007;**76**:112-22.
13. Fereira V, Barbosa J, Silva J, Vendeiroa S, Mota A, Silva F, Monteiroa MJ, Hogga T, Gibbs BP, Teixeira P. Chemical and microbiological characterisation of "Salpicao de Vinhas" and "Chourica de Vinhas": Traditional dry sausages produced in the North of Portugal. *Food Microbiol* 2007;**24**:618-23.
14. Danilovic B, Jokovic N, Petrovic Lj, Veljovic K, Tolinacki M, Savic D. The characterisation of lactic acid bacteria during the fermentation of an artisan Serbian sausage (Petrovska Klobasa). *Meat Sci* 2011;**88**:668-74.
15. Sawitzki MC, Fiorentini ÂM, Anildo Cunha Junior, Bertol, TM., Ernani Sebastião Sant'Anna. *Lactobacillus plantarum* AJ2 isolated from naturally fermented sausage and its effects on the technological properties of Milano-type salami. *Ciênc Tecnol Aliment* 2008;**28**(3):709–17.
16. Kozacinski L, Zdolec N, Hadziosmanovic M, Cvrtila Z, Filipovic I, Majic T. Microbial flora of the Croatian fermented sausage. *Arch Lebensmittelhyg* 2006;**57**:141–47.
17. Lucke FK. Utilization of microbes to process and preserve meat". *Meat Sci* 2000;**56** (2):105–15.