HOW STARTER CULTURES AFFECT THE SENSORIAL CHARACTERISTICS AND THE MICROBIOTA EVOLUTION OF DRY SAUSAGES

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Received: 21/12/2006
Accepted after revision: 16/03/2007

Abstract: The use of the culture starter in the processing of the meat products is of a crucial importance in order to improve sensorial and preservation characteristics of the final product. The aim of this study was to establish the influence of different starter cultures made of *Staphylococcus carnosus* and a mix between *Pediococcus acidilactici* and *Lactobacillus plantarum,* on the sensorial characteristics and microbiota evolution of the home-made dry sausages, comparing with a control batch. Our results showed an improvement of safety and quality of the fermented meat products with started cultures and confirm other similar experimental studies.

Keywords: *raw-dry sausages, starter cultures, nitrite, texture, safety.*

INTRODUCTION

The microbiota involved in the fermented meat product biotechnology is greatly varied; it acts in various stages of the manufacturing process and contributes to the specific characteristics of the final product. Today, the use of starter cultures from selected microorganisms is a common practice and it is a key necessity for the manufacturing of high quality short fermentation products.
Consumption-safe products of equal quality are obtained by controlling with starter cultures the fermentation process of the dry fermented sausages and salami and of the dry and ripened salty ham. By adding lactic acid bacteria, *Micrococcus*, *Pediococcus*, *Streptococcus*, and rarely yeast starter cultures to the composition of the dry fermented sausages and salami we can ensure: (i) color formation and stabilization by nitrite- and nitrate-reductase synthesis; (ii) protection of the fat against oxidation by catalase synthesis which generates hydrogen peroxide (metabolic product of some lactobacillus strains); (iii) development of the flavor by generating specific metabolites; (iv) increase of the acidity level of the products and thus contributing to: their safety by inhibiting the development of pathogenic and altering bacteria and by reducing cancer-inducing and toxic substance level (biogenic amines and nitrosamines); giving texture to the products [1, 2] and determining its slicing properties.
There are an increasing number of specialized firms which produce starter cultures for meats, guarantee their quality and sell them in small containers, thus enabling buyers to use them on a small scale.
The present study was aimed by: artisan-making of a few batches of dry sausages with and without added starter cultures, supervising the physical and microbial parameters during the entire manufacturing process and performing a sensory characterization of the product which is deemed "final".

PREPARATION OF THE EXPERIMENTAL BATCHES

We have artisan-made several experimental batches of dry sausages, using one or a mix of starter cultures. The dry sausage composition (mass percent) consisted of pork meat which was well separated from any fat and conjunctive tissue (65.7%); hard lard (28.117%); salt (2.35%); powdered skimmed milk (2.44%); sodium nitrite (0.008%), sodium nitrate (0.011%); sugar (0.75%); dextrose (0.47%); ascorbic acid (0.028%) and spices (granulated garlic 0.01%; powdered black pepper 0.066%). The following starter cultures were used: the Baktoferment 61 single starter culture (lot 2600068 Nubassa-Gewürzwerk GmbH - 25 g package) made up of *Staphylococcus carnosus* strains (experimental lot A) and the BioBak Sal Plus mixed culture (lot No 70059 WIBERG GmbH A-5020 Salzburg - 50 g package), made up of *Pediococcus acidilactici* and *Lactobacillus plantarum* (experimental lot B). The starter cultures were added in a proportion of 0.05 kg/100 kg of sausage composition. The letter M was assigned to the control lot which was made without any starter culture. We made three batches of each experimental sample.
Approximately 36 hours after being harvested, the refrigerated meat was bought from specialized shops of Galați; all spices and ingredients used were up to the standards required by dry fermented sausage production.

The sausage manufacturing process consisted in the following operations: separation of the meat from the fat and the connective tissue; chopping the meat and the hard lard into cubes of approximately 2 cm; mincing the hardened raw material with the laboratory electrical mincing machine through a sieve with holes of 3 mm; measuring the ingredients, adding them to the pork meat and lard mixture and manually homogenizing the composition. The resulting mix was stuffed as compact as possible into natural pork membranes and the sausages were formed into sticks of 25 cm length by twisting. After being tied in pairs, the sausage sticks were dried and ripened under artisan conditions. The temperature of the ripening room was checked daily with a thermo-humid meter, these physical parameters being regularly modified by normal ventilation. During the drying-ripening process, the temperature varied between 24 – 15 ^{o}C. The ripening process lasted 32 days, until the samples made with starter cultures reached a 30% moisture level. The sausage surface was periodically wiped with an acetic acid solution, to prevent the formation of the mould and samples were taken for analysis in order to evaluate the microflora during the drying- ripening process.

CHEMICAL AND MICRO-BIOLOGICAL ANALYSIS

The moisture, fat and protein contents were determined according to the AOAC (1995) standard methods [3]. The total nitrite content determined according to the Romanian standard STAS 11581-83.
The pH measurements were realized according to [4]. An amount of 10 g of sample were homogenized for 2 minutes in a blender, with 90 mL of distilled water. The pH of the solution obtained through the filtration of the homogenate was determined with a digital Hanna pH-meter.
Bacterial analysis: the determination of total number of mesophile aerobic living microorganisms was realized according to standard procedures.
Sensorial analysis: the sensory characteristics (color, texture, smell and flavor) were rated by a panelists group of 10 members, based on the 5 points Hedonic scale (1 = extremely objectionable, 2 = slightly objectionable, 3 = neither objectionable nor acceptable, 4 = slightly acceptable, 5 = extremely acceptable). The texture rates were given by the panelists during the cutting and tasting of the product. The overall sensory quality was evaluated according to Bruna's formula [5]:

$$\text{Overall sensorial quality} = 0.1 \times \text{Color} + 0.15 \times \text{Smell} + 0.25 \times \text{Texture} + 0.5 \times \text{Flavor}$$

The texture coefficients were determined according to A. Ionescu's guidelines [6].

STATISTICAL ANALYSIS

Three experimental batches were realized for each kind of treatment. Statistical analysis, which consist in evaluating the mean values, standard error and standard deviation with the framing into the confidence interval of 95%, was performed using Sigma Plot 2001/Statistics Date software.

EVALUATING THE SENSORIAL CHARACTERISTICS

The evaluation of the sensorial characteristics of the sausages obtained by adding starter cultures of *Staphylococcus carnosus* and *Lactobacillus plantarum* combined with *Pediococcus acidilactici* was based on Hedonic scale method, and the average scores were compared with the one of the control samples. The statistic data for each sensory characteristic is shown below, in table 1.

Table 1. *The average score of the analyzed batches*

Experimental lot	Color	Texture (Consistency)	Smell	Flavor	Overall sensorial quality
Control sausage	4.02	4.21	4.14	4.22	4.19
Sausage A	4.81	4.71	4.71	4.72	4.73
Sausage B	4.73	4.73	4.53	4.64	4.65

The color was developed mainly during the early stages of manufacturing and fermentation, when the environment was favorable for chemical and biochemical reactions involved in turning the meat pigment into salting pigment. The coloring process was faster and more intense for the sausages made with added starter cultures than it was in the case of the other products. The lactic acid synthesized by lactic acid bacteria as final metabolite helped the conversion of the nitrite in nitrogen oxide, which is an extremely reactive compound involved in forming nitrosomyoglobin, a relatively stable pink-red salting pigment.

In the case of the sausage lot with added *Staphylococcus carnosus*, the panelists noticed a more intense coloration and consequently awarded the highest (4.81 points). Neubauer [7] mentions that the reduction of the nitrate to nitrite is the main task of the *Staphylococcus carnosus* starter culture during the salting process; this bacterium is important for the development of color and flavor. The same author noted that the *Staphylococcus carnosus* synthesizes nitrate reductases attached to the membrane and nitrite reductases as cytosolic enzymes. In the case of the batches with added *Lactobacillus plantarum* combined with *Pediococcus acidilactici*, the possible conversion of the most of the nitrite content into nitrogen oxide can be due to a strong acidification and a rapid decrease of the pH level below 5.2. Part of the nitrogen oxide can volatilize from the complex matrix of the product turning the sausage color into a faded red.

At a pH level lower than 5.2, the nitrogen oxide synthesis is practically stopped [8] and this process explains the higher content of residual nitrite in the case of sample B with respect to the sample A. The development of the salting pigment of all experimental batches was correlated in a positive way with the lactic acid deposits from the sausage and salami matrix, with the pH drop, with the increased number of bacteria and with the elimination of the nitrite from the system.

In the case of the control batch, the development of the color is due to the participation of the meat's enzymatic equipment together with the one secreted by the spontaneous contamination microflora of the raw dry sausages, make up of lactic acid bacteria, *Micrococcus*, *Streptococcus*, *Pediococcus*, yeasts, *Enterococcus* and other microorganisms.

The evolution of the nitrate and nitrite content of the dry sausages, both with and without added starter cultures, was assessed (SAU monitored) experimentally during the manufacturing process. Analyzing the experimental data concerning the overall nitrite content evolution of the examined batches, it can be seen a constant decrease of the nitrite during the entire process (figure 1). The observed conversion rate of the nitrite depends on the type of sausage and on the period of the ripening process. The drastic decrease of the nitrite content during the first days of manufacturing corresponds with the formation of nitroso pigments through the conversion of the meat's pigments. Generally, only 50 – 70% of the initial nitrite can be analytically measured within the product immediately after being formed.

Sausage sticks consistency

One of the most important physical-chemical modifications which took place during the ripening of the dry fermented sausage was the transformation of the viscous-plastic raw composition into a strongly joined structure with an elastic consistency, specific to the final product. The texture characteristics of the sausages have been mostly depended on the presence of a starter culture in the matrix and on the nature of this culture. *Lactobacillus plantarum* and *Pediococcus acidilactici*, which are bacteria with high acidification ability, favored the texture improvement, reason for which the products with a mixed BioBak starter culture have obtained the highest score (4.73 points). The control sample had a lower consistency comparing with those benefiting from a starter culture (4.21 points). The sausage texture development was positively correlated with the growth of lactic acid bacteria, the acidification of the batter, the increase in salt content of the environment, the drop in moisture and the increase of the protein and lipid content.

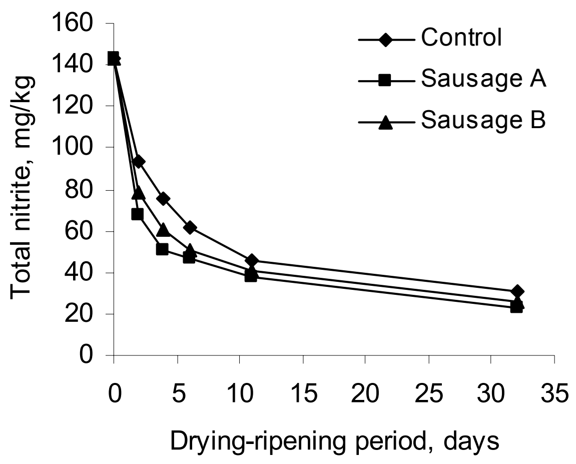

Figure 1. *Evolution of the total nitrite content during dry fermented sausage ripening*

The points given for the sensorial appreciation of consistency matched those obtained for texture by computing the texture coefficients I and II:

$$I = \frac{\%\ \text{protein}}{\%\ \text{water} + \%\ \text{fat}}; \quad II = \frac{\%\ \text{protein}}{\%\ \text{water}}$$

These coefficients had a positive evolution during drying-ripening but with differences depending on the starter culture used (figures 2 and 3). The increase of the hardness of the sausage and the improvement of their slicing properties was done constantly during the entire manufacturing process.

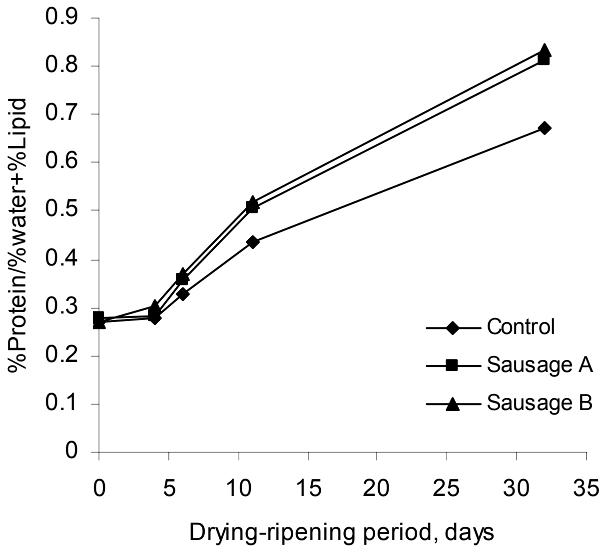

Figure 2. *Texture I index variation during ripening of the dry sausages*

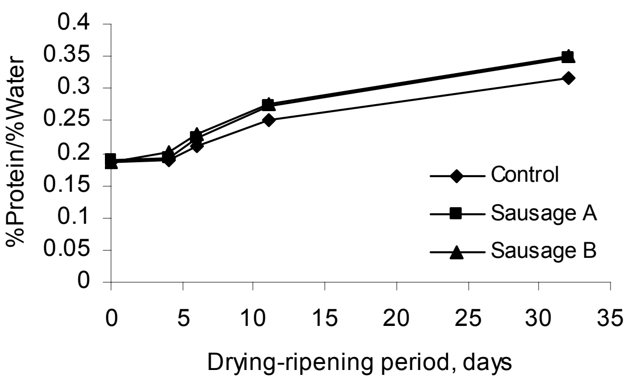

Figure 3. *Texture II index variation during ripening of the dry sausages*

Flavor and smell

The sausages with added starter cultures had relatively different flavor profiles comparing with the control sample. The scores attributed to these sensory indicators of the experimental samples with started cultures were higher than those of the control sample. The most appreciated batch in terms of flavor and smell was sausage batch A with *Staphylococcus carnosus* (4.71 points for smell and 4.72 points for flavor) which had a stronger ripening flavor. The *Lactobacillus plantarum* and *Pediococcus acidilactici* combination led to a predominantly acid flavor and smell which gained only 4.53, respectively 4.64 points, still a higher score than the control sample (4.14 and 4.22 points respectively). Our results match those of other researchers [9 – 11] who have noted that different starter cultures can generate different flavor components in the dry fermented sausages. The flavor of the dry fermented sausage that we have obtained, can be due to a complex combination of volatile (aldehydes, ketones, alcohols and esters) [12] and non-volatile (amino acids, amines, peptides with a small molecular mass) compounds. The majority of these substances are formed either by enzymatic reactions (glycolysis, proteolysis, oxidative deamination, transamination or decarboxylation) and by chemical processes (lipid autooxidation, Strecker degradation, Maillard reactions) which usually take place during the ripening of the salted and fermented meats [1, 2], or results from the meat, the used supplementary materials or from the spices.

We consider that the main chemical and biochemical modifications involved in smell and flavor profile development, that took place during the ripening of dry fermented sausages consist in both (i) acidification of the composition mixture as result of the glucides conversion to lactic acid through lactic acid bacteria and of the reduction of nitrites to nitrogen oxide, and (ii) change of the most chemical components profile (proteins, lipids) and of other ingredients.

The microorganism growth in the sausage together with endogenous enzyme activity led to an important number of flavor and sapidity compounds. The type and the concentration of these compounds are important factors in defining the overall flavor of the dry fermented sausages and salami. They are mainly the result of the microbial peptidases, which cause the oligopeptides hydrolysis thus increasing the quantity of non-protein nitrogen and free amino acids; the latter compounds are the starting point for several chemical reactions and for the growth of microorganisms, forming different flavour compounds. According to Montel [13], the proteolytic enzyme system of the common lactic acid bacteria present in the spontaneous microflora or in the starter cultures (such as *Lactobacillus sakei* or *Staphylococcus carnosus)* had have and important part in the process of degrading proteins. The *Lactobacillus sakei* microorganism is found in the indigenous flora of the dry fermented sausages and it synthesizes an aminopeptidase, a tripeptidase and a dipeptidase, which are responsible for the release of the amino acids in dry fermented sausages and salami. The meat's myoglobin is an endogenous inhibitor of the muscle proteinases, of the aminopeptidase's and of lipases. Meynier et al. [14], have identified 80 flavor compounds in the Milan sausage out of which 60% are compounds from spices, 19% are lipid oxidation product, 12% are the result of amino acid catabolism and only 5% are formed during fermentation. Mateo and Zumalacárregui [15] have identified 52 aromatic compounds in the dry fermented sausage batches made with added *Staphylococcus carnosus* and *Lactobacillus plantarum* starter culture. During the storage of the dry fermented sausages, the fractions of aldehydes and of terpenes develop [16]. The lipolyse plays an essential role in developing the flavor of the ripened dry sausage. Several research projects have studied the triglycerides conversion into free fatty acids, diglycerides and monoglycerides during ripening. Lipids autooxidation is also an important source of flavor and smell substances although it is not known exactly which of those are important for sausage ripening. There are probably the carbonyl compounds that are responsible for determining the flavor because they generally have a low perception threshold from mg/kg to µg/kg level [2]. The salt added to the original composition greatly contributes to the flavor of the dry fermented sausages and salami. The level of the salt content increases gradually as the water evaporates, especially in the final drying-ripening stage.

In the case of our experiment, lipids oxidation, which is responsible for both the developing of the desired flavor of the fermented sausage [9] and the deterioration in color, texture and nutritional value by forming toxic compounds when in an advanced stage [17], was relatively reduced.

Staphylococcus carnosus presents a lipolytic activity (it synthesizes a lipase with high substrate specificity, stable at a pH level of 5.0) but inhibits the oxidation of free fatty non-saturated acids [18] and has a catalase activity related to the cell. It also has an anti-oxidation activity by synthesizing the superoxid dismutase which is a metalloenzyme that detoxify the super oxides radicals. Catalases play a role in conversion of the hydrogen peroxide which is formed by some Lactobacillus strains in water and oxygen. The two anti-oxidative enzymes have a high activity at the manufacturing conditions of the dry fermented sausages [19].

According to our results, the sausages made with added starter cultures have had similar rates of acceptability (4.73 points for sample A and 4.65 points for sample B) but noticeably superior to those of the control sample (4.19 points).

The conclusions reached after the sensory analysis confirms the results of the physical, chemical and microbiological analyses conducted during each manufacturing stage of the dry fermented sausages.

THE EVOLUTION OF THE BACTERIOLOGICAL PARAMETERS

The drying and ripening processes generally lead to safe and stable meat products, with a high resistance to storage. The stability of these products is due mainly to the low final pH level which is the result of an important accumulation of lactic acid, especially in its dissociated state. The lactic acid is synthesized by the lactic acid bacteria which are naturally occurring in the meat or are added as starter cultures. Bio-protection or bio-conservation is reached by inhibiting the altering and pathogen bacteria by forcing them to compete with the lactic acid bacteria for their nutrients, by assuring low pH levels and by using the antimicrobial compounds (bacteriocines, H_2O_2). In normal manufacturing conditions, the safety and stability of the dried-ripened meat products closely depends on the water activity (a_w), on the time/temperature/ relative moisture relationship, on the salt content, on the residual nitrite and on the types of present microorganisms. We have investigated how the presence ant the type of a starter culture can affect the evolution of the overall microbial load.

In table 2 are presented all the data concerning the evolution of the overall microbial load in the raw sausages with or without added starter cultures.

According to these data, the initial overall microbial load of the pates was slightly different for the control and the experimental batches, being situated between $8.22 \cdot 10^3$ – $1.399 \cdot 10^5$ CFU/g after the sausages were stuffed and had time to settle. During the first

6 days of drying-ripening, at a temperature of 24 – 15 °C, the number of mesophile aerobic microorganisms increased differently, depending on the presence and type of starter culture. Thus, the number of microorganisms in samples A (with added *Staphylococcus carnosus)* and B (with added *Lactobacillus plantarum* and *Pediococcus acidilactici)* increased from $1.40 \cdot 10^4$ CFU/g to $1.17 \cdot 10^6$ CFU/g, respectively from $1.61 \cdot 10^4$ CFU/g to $5.49 \cdot 10^6$ CFU/g; these numbers are higher than in the case of the control sample where the number grew from $8.23 \cdot 10^3$ CFU/g up to $8.16 \cdot 10^4$ CFU/g. During this stage of the drying-ripening process, a series of glycolytic and microbial processes were initiated and they have mainly involved the lactic bacteria from the spontaneous microflora of the meat and from the starter cultures and, in a lesser measure, bacteria of genus *Micrococcus* and *Staphylococcus.*

Table 2. *The overall microbial load evolution in the dry sausages during different technological stages (ufc/g)*

Duration of the drying/ripening process in days	Overall number of microorganisms, CFU/g		
	Control M	Sausages – sample A	Sausages – sample B
0	$8.23 \cdot 10^3$	$1.40 \cdot 10^4$	$1.61 \cdot 10^4$
4	$5.98 \cdot 10^4$	$4.89 \cdot 10^5$	$4.27 \cdot 10^5$
6	$8.16 \cdot 10^4$	$1.17 \cdot 10^6$	$5.49 \cdot 10^6$
11	$1.01 \cdot 10^5$	$7.82 \cdot 10^5$	$7.12 \cdot 10^5$
32	$5.06 \cdot 10^5$	$8.23 \cdot 10^5$	$9.01 \cdot 10^5$

The microbial load shows the same increasing level throughout the entire drying ripening cycle, with a slight drop at the end of the manufacturing process depending on the nature of the sample.

The growth of the microorganisms that caused the acidification was in close relation with the pH level of the composition. The increase of the lactic acid content during the composition preparation, stuffing and settling stage and during the first 6 days of manufacturing is directly correlated with the growth stage of the lactic acid bacteria, *Micrococcus* and *Streptococcus* which synthesize nitrite and nitrate reductase, and of the bacteria which are unaffected by the NaCl which slows down the growth of the Gram-negative germs.

The massive accumulation of lactic acid is directly linked to the growth of lactic bacteria until a pH level below 5.0 is reached, when it can be observed a drop in the numbers of viable microorganisms in all samples (figures 4 and 5); this can be also possible due to the decrease of the biologically available water content (drop of the a_w) or to the depletion of the nutritive substrate.

The strong competition of the lactic bacteria and the acid reaction of the substrates did more than inhibit the growth of unwanted microorganisms, but it also determined the gradual decrease of their numbers.

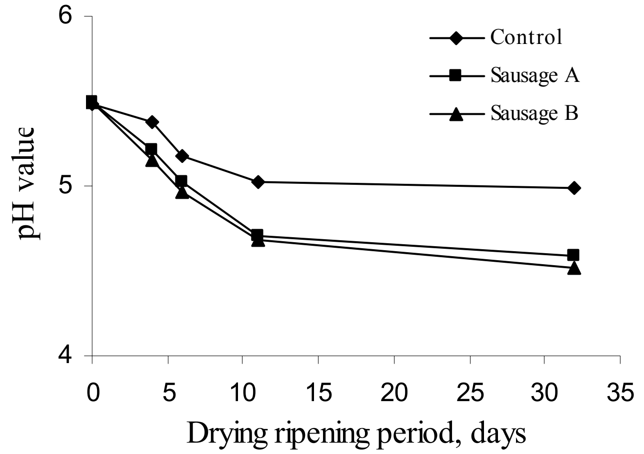

Figure 4. *The pH variation during drying-ripening*

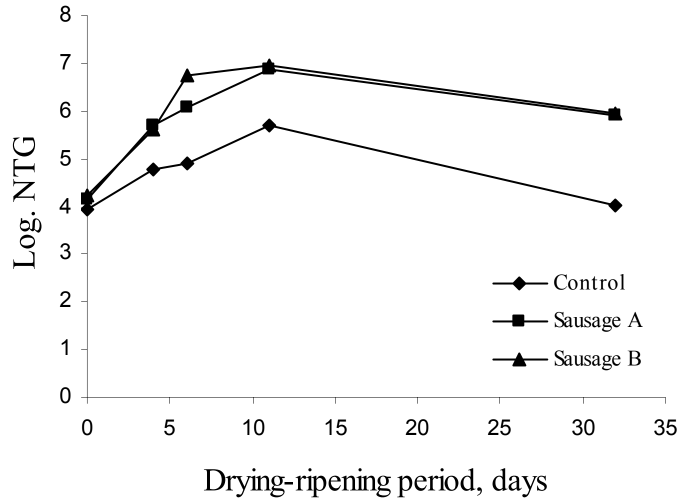

Figure 5. *The influence that the duration of the drying-ripening stage has on the log_{10} of the overall number of aerobe microorganisms*

CONCLUSIONS

The starter cultures we have used have had singular effects on the growth and metabolic rates, on the proteolytic activity and on the development of the flavour compounds.

The mixed *Lactobacillus plantarum* and *Pediococcus acidilactici* cultures we have used were more efficient; they provided a better control over the pathogen and altering bacteria growth and they contributed to the improvement of safety and quality of the fermented meat products.

Presently, the lactic bacteria starter cultures utilized in raw dry sausage manufacturing are considered to be probiotics since they survive in a highly acidic environment and they colonize the terminal ileum and the colon, thus being beneficial for the human health.

REFERENCES

1. Montel, M.C., Masson, F., Talon, R.: Bacterial role in flavour development, *Meat Science*, **1998**, **49**, Supplement 1, S111-S123.
2. Ordonez, J.A., Hierro, E.M., Bruna J.M., de la Hoz L.: Changes in the components of dry-fermented sausages during ripening, *Crit. Rev. Food Sci. Nutr.*, **1999**, **39**(4), 329-367.
3. AOAC – Association of Official Analytical Chemists: *Official methods of analysis*, 16th ed. Washington D.C.: AOAC, **1995**.
4. AOAC – Association of Official Analytical Chemists: *Official method of analysis*, 14th ed. Washington, **1984**.
5. Bruna, J. M., Fernandez, M., Hierro, E. M., Ordonez, J. A., de la Hoz, L.: Improvement of the sensory properties of dry fermented sausages by the superficial inoculation and/or the addition of intracellular extracts of *Mucor racemosus, J. Food Sci.*, **2000**, **65**, 731–738.
6. Ionescu, A.: *Studies Concerning the Improvement of Technological Processes Regarding the Nitrosamine Content Reduction in Meat Products*, PhD Thesis, University "Dunărea de Jos" Galați, **1990**.
7. Neubauer, H., Götz,F.: Physiology and interaction of nitrate and nitrite reduction in *Staphylococcus carnosus*, *J. Bacteriol.*, **1996**, **178**, 2005-2009.
8. Banu, C., Alexe, P., Vizireanu, C.: *Procesarea industrială a cărnii*, Editura Tehnică, Bucureşti, **1997**.
9. Montel, M.C., Reitz, J., Talon, R., Berdagué, J.L., Rousset-Akrim, S.: Biochemical activities of *Micrococcaceae* and their effects on the aromatic profiles and odours of a dry sausage model, *Food Microbiology*, **1996**, **13**, 489-499.
10. Stahnke, L.: Aroma components from dried sausages fermented with *Staphylococcus xylosus*, *Meat Sci.*, **1994**, **38**, 39-53.
11. Berdagué, J., Monteil, P., Montel, M., Talon, R.: Effects of starter cultures on the formation of flavour compounds in dry sausage, *Meat Sci.*, **1993**, **35**, 275-287.
12. Johansson, G., Berdagué, J., Larsson, M., Tran, N., Borch, E.: Lipolysis, proteolysis and formation of volatile components during ripening of a fermented sausage with *Pediococcus pentosaceus* and *Staphylococcus xylosus* as starter cultures, *Meat Sci.*, **1994**, **38**, 203-218.
13. Montel, M.-C., Talon, R., Cantonnet, M., Berdagué, J.L.: *Activités métaboliques des bactéries lactiques des produits carnés*, in Novel, G., Lequerler J.-F. (ed.): *Les bactéries lactiques*, Ardia Normandie, Caen, France, **1992**.
14. Meynier, A., Genot, C., Gandemer, G.: Oxidation of muscle phospholipids inrelation to their fatty acid composition with emphasis on volatile compounds, *J. Sci. Food Agric.*, **1999**, **79**, 797-804.
15. Mateo, J., Zumalacárregui, J.: Volatile compounds in chorizo and their changes during ripening, *Meat Sci.*, **1996**, **44**, 255-273.
16. Misharina, A.T., Andreenkov, A.V., Vashchuk, A.E., Changes in the Composition of Volatile Compounds during Aging of Dry-cured Sausages, *Applied Biochemistry and Microbiology*, **2001**, **37**(4), 413-418.
17. Morrissey, P.A., Sheehy, P.J.A., Galvin, K., Kerry, J.P., Buckley, D.J.: Lipid stability in Meat and Meat Products, *Meat Sci.*, **1998**, **49**, 73-86.

18. Talon, R., Walter, D., Montel, M.C.: Growth and effect of staphylococci and lactic bacteria on unsatured free fatty acid, *Meat Sci*., **2000**, **54**, 41-47.
19. Barriere, C., Leroy-Setrin, S., Talon, R.: Characterization of Catalase and Superoxide Dismutase in *Staphylococcus carnosus* 833 strain, *Appl Microbiol.*, **2001**, **91**(3), 514-519.