THE EFFECT OF STARTER CULTURES ON THE PHYSICAL AND BIOCHEMICAL CHARACTERISTICS OF DRIED SAUSAGES

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Abstract: We have monitored the physical and biochemical modifications which have taken place during the ripening of the home-made sausages with and without starter cultures. Baktoferment 61 (*Staphylococcus carnosus*) and Biobak Sal Plus (*Lactobacillus plantarum* and *Pediococcus acidilactici*) starter cultures have intensified the conversion of glucides to lactic acid, have reduced the value of the pH and increased the acidity and have sped up drying and forming of colours and textures processes. Also, the utilized starter cultures have led to a more intense proteolytic activity, the increase of the free amino acid and non-protein nitrogen content being higher than those of the sample with normal ripening. The rate of producing free amino acids was lower than that of the non-protein nitrogen but higher than that of forming the ammonia by oxidative deamination of some free amino acids. The presence of meat bacterial starter cultures selected, guarantees lower values of pH and the water's activity (a_w), and furthermore safety products.

Keywords: *dry sausage, culture starter, lactic acid bacteria, ripening, proteolysis, lipolyse, drying, water activity, safety.*

INTRODUCTION

Conventional technologies for manufacturing fermented dry and semi-dry sausages consist in: mixing the chopped meat and lard with different spices, aromatics, salt, nitrites and/or nitrates, glucides and different additives. The resulting mixes are filled into membranes and kept for fermentation a certain amount of time at different temperatures. After fermentation, the product is dried and ripened under controlled temperatures and humidity. In this case, the fermentation process during the sausages ripening is induced by the natural contamination microflora of the sausages mix. The desired microflora is composed of halophytic nonpathogen Gram-negative bacillus, *Micrococacceae,* yeasts, halophytic nonpathogen Gram-positive bacillus [1]. The development of the desired microflora is absolutely unintended and the ripening process is a very long one [2]. Within the spontaneous meat microflora, the lactic acid bacteria are responsible for the main processes of the fermented sausage ripening. The lactic acid bacteria convert glucides to lactic acid and smaller quantities of other metabolites, thus reducing the pH, improving the texture of the products, ensuring the prolonged stability against the proliferation of food pathogens and of altering bacteria [3] and producing flavour compounds [4 – 6]. The *Lactobacillus* is the main component of the meat and meat products microflora [7].

The modern technologies carefully control the ripening process by introducing selected microorganism strains and by utilizing the acclimated drying chambers [8].

Currently, there are bacterial starter cultures which belong to the *Lactobacillus*, *Pediococcus, Micrococcus* and *Staphylococcus* genus and which are commercialized for meats as singular cultures or as mixtures of 2 or 3 strands [9 – 11]. The bacterial starter cultures are available frozen, freeze-dried or as liquids stabilized at low temperatures. Through their physiological activity, the starter cultures ensure the rapid decrease of the pH, guarantee the safety of the product and the uniformity of its flavour, colour and texture and a shortening of the production cycles [12, 13]. Moreover, Erkila et al. [14] have reported the benefits of utilizing the starter cultures of *Lactobacillus rhamnosus* strains GG, LC-705, E-97800, *Pediococcus pentosaceus* E-90390 and *Lactobacillus plantarum* E-98098 as probiotics in fermented dried salami, when is respected the number of living lactic bacteria cells, the accepted level of biogenic amine and the flavour profile of the salami, compared to other commercial starter cultures.

The home-made sausages production in Romania is not very developed, the production means and methods used being very different thus the final products are very different from a microbial and sensorial point of view. Also, there is little information concerning the dynamics of the physical, biochemical and microbiological specific processes which play a part in the development, improvement and balancing of the sensorial characteristics and in the conservation of these products.

The goals of this study were to make new batches of dried home-made sausages with or without starter cultures, and to monitor physical and chemical parameters which are important for the ripening process during the entire manufacturing process.

MATERIALS AND METHODS

Preparation of the experimental batches

In home-making conditions, we have prepared several experimental batches of dried sausages with different addition starter culture (3 batches for each experiment). The sausage composition was prepared with well chosen pork meat (< 10% fat), hard lard, skinned powdered milk, salt, sodium nitrite and nitrate, sugar, dextrose, antioxidants and spices.

Table 1. *The formulations for dry sausage*

Ingredients	Quantity, kg/100 kg mix		
	Sausage A	**Sausage B**	**Control M**
Well chosen red pork meat	65.7	65.7	65.7
Fat	28.117	28.117	28.117
Skimmed powder milk	2.44	2.44	2.44
NaCl	2.35	2.35	2.35
Sugar	0.75	0.75	0.75
Dextrose	0.47	0.47	0.47
Sodium nitrate	0.008	0.008	0.008
Sodium nitrite	0.011	0.011	0.011
Ascorbic acid	0.028	0.028	0.028
Granulated garlic	0.01	0.01	0.01
Black pepper	0.066	0.066	0.066
Baktoferment 61 (*Staphylococcus carnosus*)	0.05	-	-
BioBak Sal Plus (*Lactobacillus plantarum* and *Pediococcus acidilactici)*	-	0.05	-

We have selected starter culture to be used, either singular culture, either microorganism mixes, on the basis of their characteristic properties which are important for the meat industry, especially for manufacturing dried sausages.
For the batch A we used the Baktoferment 61, lot 2600068 Nubassa-Gewürzwerk GmbH (a package of 25 g) starter culture, made up of only *Staphylococcus carnosus* strain, coagulase-negative bacteria, non-pathogen, chemoorganotroph with respiratory or fermentative metabolism, aerobic or optionally anaerobic.
The mix culture BioBak Sal Plus, lot no. 70059 WIBERG GmbH A-5020 Salzburg (a 50 g package), made up of *Pediococcus acidilactici* and *Lactobacillus plantarum*, was added to experimental batch B. *Lactobacillus plantarum* is a very common bacteria in fermented meat products, being linked to the spontaneous and traditional fermentation of raw sausage and responsible for producing lactic acid, for the slightly sour flavour of the sausage and for forming small quantities of acetic acid, ethylic alcohol, acetone, CO_2 and pyruvic acid during the fermentation process, depending on the sugars utilized as a carbon source and on the protein sources of the meat. *Lactobacillus plantarum* has an optimum development temperature of 30 – 35 ˚C. *Pediococcus acidilactici* is known as a bacterium which produces bacteriocines which block the *Listeria monocytogenes* in the fresh meat [15]. Batch M represents the witness sample, with no added starter culture.

Preparing the composition

The meat was bought from the stores from Galați and the used ingredients were those commonly used for the production of fermented sausage.
The manufacturing technology required: choosing of the connective tissue and fat meat, cutting the meat and the hard lard into 3 cm cubes; hardening the meat and the lard by cooling them in the freezer to a temperature of –3 ˚C; chopping the hardened materials with an electric mincing machine with a sieve which has 3 mm holes; measuring the ingredients; adding them to the meat and lard mix and homogenizing the composition. The meat paste was stuffed into pork intestines and the sausages were shaped by twisting them at the required length (25 cm long). The sausages were tied in pairs and subjected to the drying-ripening process. The temperature (24 – 15 ^{o}C) and the relative humidity (78 – 65%) into the ripening chamber were verified daily with a thermo-humid meter, the conditions into the chamber being regularly modified by normal ventilation. The ripening process lasted for 32 days until the desired sensorial, chemical and microbial characteristics of the products were achieved. Periodically, the surface of the membrane was wiped with an acetic acid solution in order to avoid the forming of mould on the surface of the sausages; also samples were taken for analysis in order to evaluate the ripening-drying process.

Chemical analysis

The dried sausages samples were harvested at different time intervals. After removing the membrane and cutting the sample into pieces, the samples were finely chopped and homogenized with a Braun mixer. The resulting homogenized mixture was introduced in special containers, hermetically sealed and kept at +4 °C until the analysis was carried out. The samples were analyzed the same day they were harvested. For each type of analysis double samples were taken.
Humidity, fat and proteins were determined according to the AOAC standard procedure [16]. The non-protein nitrogen content was determined using the Kjeldahl method, after previously precipitating the proteins from the samples with trichloroacetic acid 10%, according to Ionescu et al. [17]; the free amino acid content was determined through the method indicated by Vâță et al. [18].
The ammonia was determined according to the Romanian Standard STAS 9065-5/73.
The titrable acidity was determined as lactic acid by titration the watery extract with 0.1N NaOH, using phenolphthalein as an indicator [19].
The NaCl content was determined trough the Mohr method according to the Romanian Standard STAS 9065-5/73.
The TBA number was determined trough the colorimetric method described by Ionescu [20].
The pH measurements were realized according to [21]. (10 g of sample which were homogenized for 2 minutes, in a blender, with 90 mL of distilled water). The pH was determined with a Hanna digital pH-meter on the solution obtained after the filtration of the homogenate.

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. Experimental data were fitted using Table Curve 2D software and the regression equations were established based on statistical criteria (R2, Fit Standard Error or F Statistic).

RESULTS AND DISCUSSIONS

Evolution of the physical and chemical parameters

The analytical data concerning the global chemical composition are shown in table 2. From economically and technologically viewpoints, a special attention is paid to the evolution of the global chemical composition of the dried sausages which usually depends on the composition of the mix, the diameter of the roll, the type and level of additives, the ripening temperature, the relative humidity and the air speed, the presence or absence of lactic acid bacteria and their nature. To emphasize these modifications, we have measured the water, total proteins and fats contents. Analyzing numerical data presented in table 2, it can be noted a sustained decrease of the moisture content, higher at the beginning of the drying-ripening process in the case of the samples with added lactic bacterial starter cultures comparing to the sample without added starter cultures, and lower in the final stage (figure 1). At the same time with the decrease in the humidity level, a concentration of the main components of the dried sausages (proteins, lipids, NaCl) have had place. With respect to 100 g dried mass of sample, we could notice that these chemical components appear practically unaffected throughout the entire manufacturing process.

The process of water elimination was different for each batch due to the different acidity of each composition mix caused by the started culture. The *Staphylococcus carnosus* starter culture led to a lower acidity level compared to the *Lactobacillus plantarum* and *Pediococcus acidilactici* starter cultures. In the case of A and B samples, the pH values reached more rapidly the isoelectric point of the myofibrillar proteins which have lost their water-retaining ability. This process has facilitated the outside transfer of water from the inner of the sample and its later elimination through evaporation.

After 32 days of ripening, the humidity of the samples with added starter cultures decreased by 30% in the case of sample A and by 29.58% in the case of sample B compared to the control sample which has reached a value of 34%, showing the importance of the lactic bacteria and *Micrococcus* in speeding up the water evaporation process and shortening the ripening time for the dried sausages.

Another consequence of water content modification is the continuum decrease of the raw sausage weight during the entire period of ripening-drying period for all samples.

Table 2. *The evolution of global chemical composition of sausages during drying-ripening period*

Drying-ripening period, days	Sample	Moisture, w%	Dried mass, w%	Proteins		Fat		NaCl	
				w%	w % d.s.	w%	w % d.s.	w%	w % d.s.
0	Control M	56.49	43.51	15.18	34.89	25.08	57.66	2.83	6.50
	Sausage A	55.79	44.21	15.42	34.88	25.65	58.02	2.88	6.51
	Sausage B	56.59	43.41	15.15	34.90	25.12	57.86	2.82	6.45
4	Control M	55.56	44.44	15.45	34.77	25.88	58.23	3.03	6.82
	Sausage A	55.03	44.97	15.66	34,82	26.07	57.98	3.01	6.69
	Sausage B	53.71	46.29	16.18	34.95	26.87	57.86	2.92	6.30
6	Control M	51.64	48.36	16.82	34.78	28.06	58.02	3.29	6.80
	Sausage A	49.43	50.57	17.64	34.88	29.15	57.65	3.29	6.66
	Sausage B	48.65	51.35	17.98	34.83	30.12	58.65	3.24	6.31
11	Control M	44.48	55.52	19.29	34.74	32.07	57.77	3.81	6.86
	Sausage A	40.99	59.01	20.64	34.98	34.27	58.02	3.70	6.27
	Sausage B	40.07	59.93	20.72	34.57	34.84	58.13	3.78	6.31
32	Control M	34.08	65.92	22.88	34.71	38.52	58.03	4.60	6.98
	Sausage A	30.03	69.97	24.36	34.81	40.08	57.28	4.67	6.77
	Sausage B	29.58	71.42	24.69	34.57	40.68	56.96	4.85	6.79

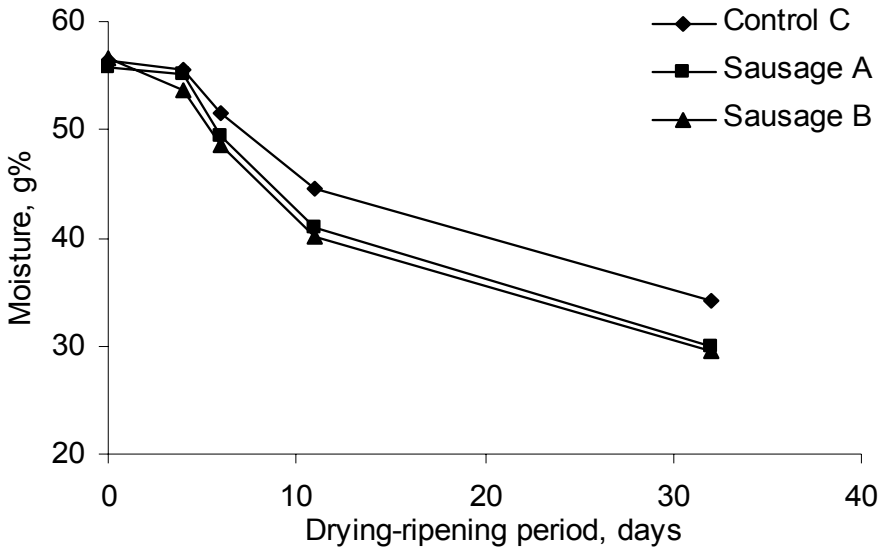

Figure 1. *The evolution of the moisture content of fermented sausages during drying-ripening period*

The evolution of the pH and of the total titrable acidity

During dried sausages' manufacturing, both the added and the existent sugars were converted to lactic acid. The fermentation of the sausage sugars was influenced by the lactic acid bacteria from the natural microflora of the meat and from the one added as a starter culture. The accumulated lactic acid led to the decrease of the pH level, this process being more rapid during the first 6 days and slower during the ripening-drying period (figure 2) depending on the sample type. The starter cultures have accelerated the

formation of lactic acid and have led to a drastic decrease of the pH level corresponding to the increased acidity of the sausages (figure 3).

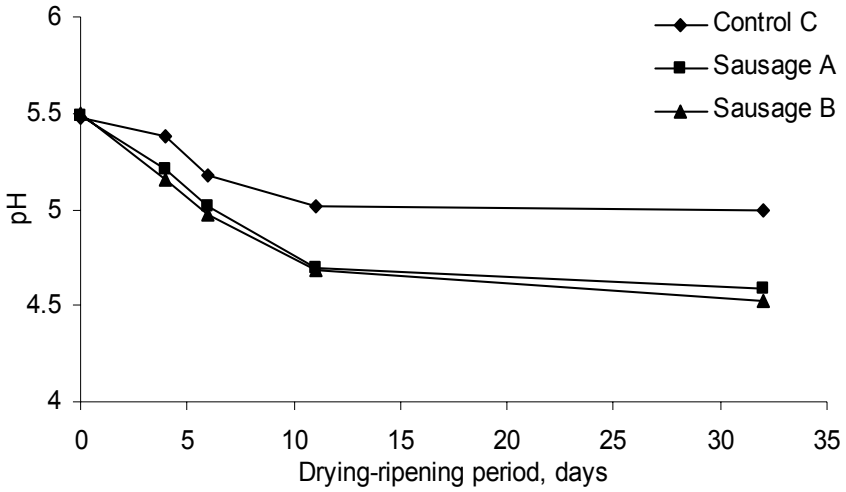

Figure 2. *Variation of the pH values during drying-ripening period*

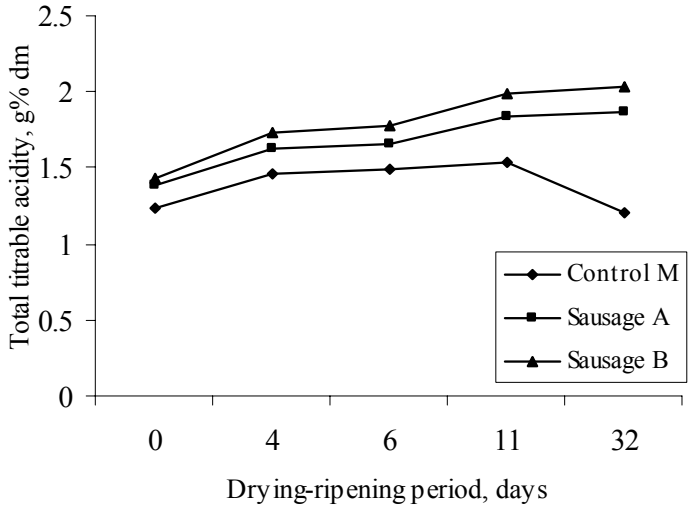

Figure 3. *Variation of the sausages' acidity during drying-ripening period*

Minimum and final values of the pH were lower in the case of the samples with added starter cultures when compared with to the control sample.

The gradual decrease of the pH level in the final stage of the ripening-drying process is due to the large quantity of added sugars and powdered milk, the source of nutrients for the lactic acid bacteria from starter culture and natural contamination of the meat. The presence of lactic acid causes a particular taste of the product and improves the consistency of its texture due to the acid denaturation of the meat proteins. Moreover,

the starter cultures prevent the growing of Gram-negative *Staphylococcus* and of *Escherichia coli* through the increased acidification of the sausage composition. *Lactobacillus plantarum* and *Staphylococcus carnosus* have a large inhibitive radius that covers the pathogen microorganisms (*Listeria monocytogenes, Shigella dysenteriae*) and the germination of the *Bacillus subtilis, Bacillus cereus* and the *Pseudomonas fluorescens* spores.

The proteolytic process dynamics

During the ripening-drying period of the sausages with or without added starter culture proteins' degradation processes took place. In order to obtain an indication about the freshness of the samples, the proteolytic activity of the spontaneous contamination microflora and from the starter cultures was estimated by determining the ammonia evolution throughout the manufacturing process of the products. Data regarding the ammonia accumulation are presented in figure 4.

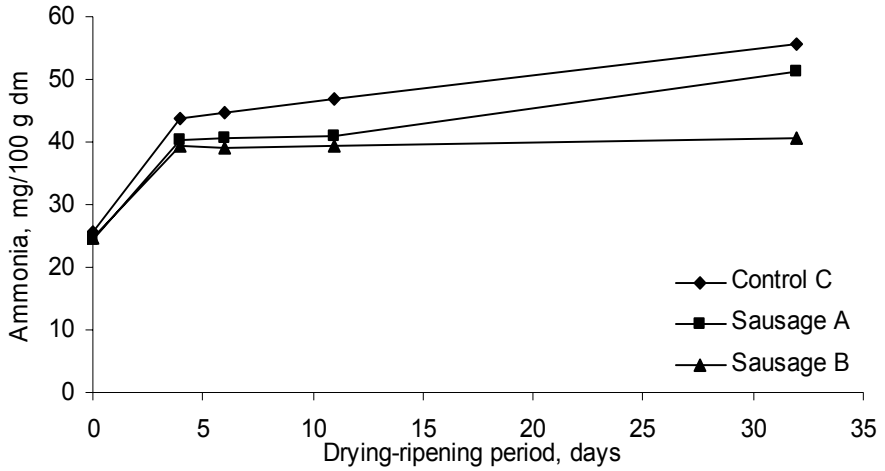

Figure 4. *The influence of starter cultures adding on ammonia levels*

Analyzing the fitting curves it can be noticed the constant slight raise of the ammonia level in all products during fabrication, with differences between the various product types. The ammonia accumulations have been slightly lower in the case of dried sausages with added starter cultures than in the case of spontaneous fermentation samples; within the experimental lots with added starter cultures only small differences were detected. The oxidative deamination of the free amino acids took place rapidly during the first 5 – 6 days of fabrication for both starter culture sausages and the control sample; after this period, the deamination speed starts to decrease for the sample with the Biobak starter culture. The ammonia accumulations from the first stages of the manufacturing process are correlated with a high water and glucides content, a low level of salt and a greater pH level; all these factors allow the growth of the microflora which secrets different enzymes with a role in protein hydrolysis and amino acid decarboxylation or deamination.

The proteolytic processes during the dry sausage processing lead to an increase of the non-protein nitrogen and free amino acids content (figures 5 and 6). The quality and the quantity of the non-protein nitrogen compounds contribute to the general flavour of salted meat products (raw-dry sausages, ripe salted bacon). Sanz et al., [22] believe that in raw sausages, the microbial proteinases play an important role in the hydrolysis of the oligopeptides released by the specific proteinases of the meat. As part of the spontaneous microflora and of the starter cultures (the *Lactobacillus sake* strain's case), the proteolytic system of the lactic bacteria can play a major role in the proteolytic phenomenon. *Lactobacillus sake* synthesizes proteolytic enzymes such as: aminopeptidase, tripeptidase and dipeptidase.

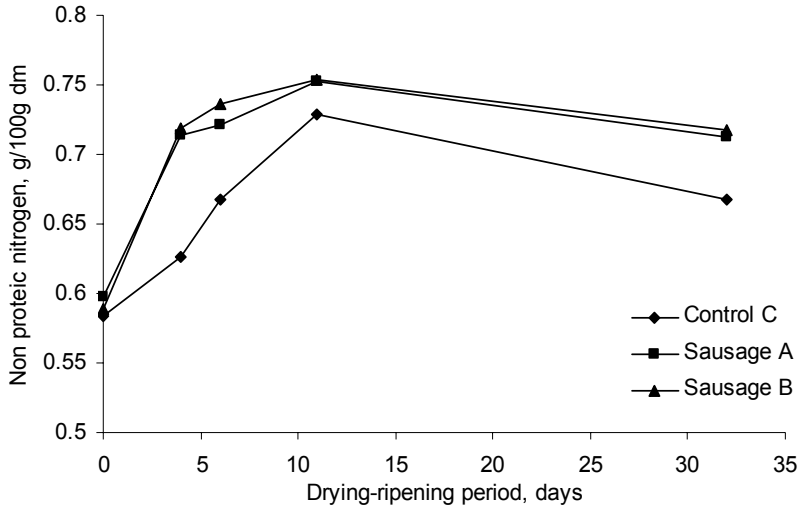

Figure 5. *The dynamic of the non protein nitrogen content of sausages during drying-ripening period*

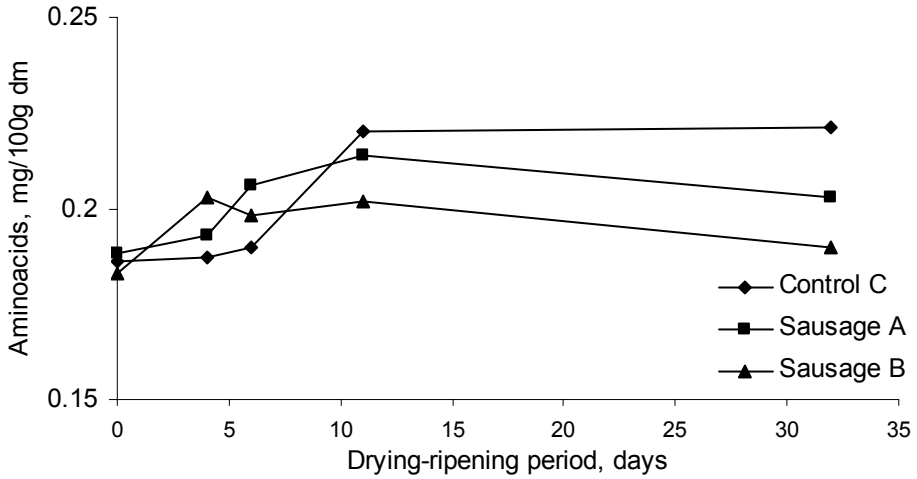

Figure 6. *The accumulation of amino acids during drying-ripening period*

The myoglobin of meat limits the production of free amino acids in the meat systems, by inhibiting the activity of aminopeptidase released by the *L. sake* and of those ones which are specific to the muscle [23].
The concentration of myoglobin depends on the metabolic type of the muscle and on the meat/lard ratio used for the sausage mix. By using the *Staphylococcus carnosus* starter culture, the accumulations of ammonia and biogenic amines, tyramine, cadaverine and putresceine, but not those of tryptamine and phenyl amine, can be drastically reduced.
The biogenic amines are known to be formed through the microbial decarboxylation of the free amino acids formed through the proteolytic processes which take place during the ripening of fermented sausages. The *Enterococcus* bacteria from the spontaneous microflora of the sausage composition and of dry fermented sausages, participate to the some biogenic amines formation. Shalaby [24], estimates that the main and most studied biogenic amine is the histamine found in raw dry sausages, due to its toxic effects. The decrease of the free amino acid content during the second stage of the ripening process is credited to the sausage microflora which consumes them and to their decarboxylation or deamination. During the last stage of ripening-drying we notice a reduction of the proteolytic activity at the same time with the drastic decrease of the water content (due to the drying process), which leads to a value of the water activity below 0.9, and of the microflora metabolic activity.

Evolution of the water activity

Water activity has a major importance for the raw-dry meat products' stability and quality, as a way of measuring how water influences the microbial and biochemical processes which take place in a food product. The data concerning water activity during different stages of manufacturing for all experimental lots with added starter cultures and for the control lot, have been estimated starting from the analytical data using the regression equation [25]:

$$y = 1.050 - 0.01144 \times S \tag{1}$$

where:

$$S = \frac{\%NaCl}{\%NaCl + \%H_2O} \times 100 \tag{2}$$

Analyzing the values of water activity (a_w) depicted in table 3, can be noticed a constant decrease during the drying-ripening process, the more accentuated drop-off being recorded during the final stage of the raw sausages drying. The regression equation indicates a linear variation in time of the water activity, the variation curbs following a similar path for all the studied cases. The best fitting was obtained for the sample B, the regression coefficient (r^2) being 0.987. The drying process was similar and uninfluenced by the nature of the sample.
The danger of *Clostridium*-type and other pathogen bacteria's development appears when water activity levels are above 0.950, level found in the control samples before the 11th day of fabrication. During the first stages of fabrication (raw paste, stuffed product and the last stage of drying-ripening), the pathogen bacteria development was limited by the low pH levels, by the simultaneous presence of high levels of residual nitrite and by the relatively low ripening temperature. In the case of the samples with added starter

cultures the final values of water activity were under 0.9, lower than those of the control sample; at this level of the water activity, it is no longer possible for the toxin to be released by *Clostridium botulinum*, bacteria which synthesizes the botulinum toxin at water activity levels higher than 0.940. By adding starter cultures to the raw sausages we can extend the storage period of these products compared to the samples without any starter cultures.

Table 3. *Evolution of the water activity during the ripening-drying period*

Ripening-drying period, days	Water activity		
	Control	**Sausage A**	**Sausage B**
0	0.995	0.994	0.996
4	0.991	0.991	0.991
6	0.981	0.979	0.980
11	0.960	0.955	0.957
32	0.913	0.896	0.899
Regression equation	Y = 0.996 – 0.0026X	Y = 0.997 – 0.0032X	Y = 0.998 – 0.0032X
Regression coefficient, r^2	0.981	0.984	0.987

Evaluation of the fat oxidation during dry sausage ripening

The level of the fat oxidation during the dry sausage ripening process was estimated by determining the content of substances which react with the 2-thiobarbituric acid (TBA). Higher values of the TBA number in sausage composition in the initial moment (time 0, figure 7) are due to the presence of ascorbic acid in the dry sausages, acid which is known to react with the 2-thiobarbituric acid and to artificially increase the TBA value. The significant increase of the TBA value is obvious in all samples after 11 days of ripening, increase which continues throughout the ripening-drying process. The oxidative processes were more prominent in the sample without a starter culture, where the TBA value reached 1.12 mg MDA/kg (MDA- malondialdehyde). The low TBA values show that there were no adequate conditions in the sausage composition for lipid oxidation processes which would have led to the apparition of secondary oxidation products (aldehydes, ketones) reacting with the 2-thiobarbituric acid. A TBA value > 1.0 mg MDA/kg of product matches very well the sensorial characteristics which define the rancid taste and smell [26]. Slightly higher TBA values for the *Staphylococcus carnosus* sample is due to this bacterium's ability to generate methyl ketones by incomplete β-oxidation of the fatty acids followed by decarboxylation [27].

CONCLUSIONS

In home-made manufacturing conditions, the Baktoferment 61 (*Staphylococcus carnosus*) and the BioBak Sal Plus (*Lactobacillus plantarum* and *Pediococcus acidilactici)* starter cultures have had a positive effect on the physical and biochemical processes during the ripening of dried sausages.

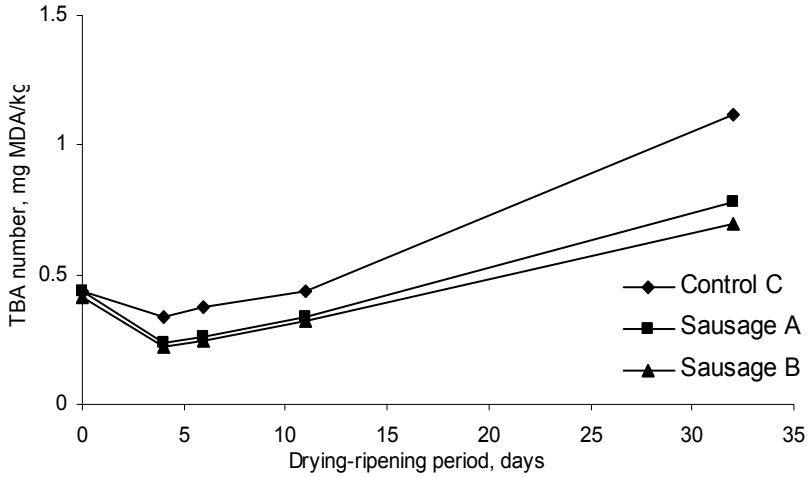

Figure 7. *Variation of TBA number during drying-ripening period*

Comparing with the control sample, the samples with added starter cultures have generated significantly larger quantities of lactic acid at the beginning of the ripening process. The lower final water activity and pH levels in the samples with added starter cultures guarantee stable and safe products for consumers.

Taking into consideration the decrease in oxidative deamination of the amino acids, the levels of non-protein nitrogen type compounds and of the free amino acids in particular, which are all responsible for the product flavour, have been higher in case of the samples with added starter cultures.

Unlike the in control sample, both used starter cultures have contributed to reducing the intensity of the β-oxidation of lipids which generates compounds which react with the 2-thiobarbituric acid.

The starter cultures used by us have significantly reduced the length of the manufacturing process of dry sausages.

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