The effect of starter cultures on the qualitative indicators of dry fermented sausages made from poultry meat

O. Zinina^{1,*}, S. Merenkova¹, A. Soloveva², T. Savostina³, E. Sayfulmulyukov³, I. Lykasova³ and A. Mizhevikina³

¹South Ural State University (national research university), Department of Food and Biotechnology, Lenin Avenue 76, RU454080 Chelyabinsk, Russia ²Meat processing complex Sitno, Laznik street 30, RU455000 Magnitogorsk, Russia ³South Ural State Agrarian University, Gagarin street 13, RU457100 Troitsk, Chelyabinsk Region, Russia

*Correspondence: zininaov@susu.ru

Abstract. Changes in physicochemical, rheological and microbiological properties occurring throughout the ripening (on days 0, 7, 14, 21, and 28) of dry fermented sausages made from poultry meat were studied. The effect of starter bacteria on the microstructure and sensory attributes of dry fermented sausages has also been determined. The results of physicochemical analysis of dry fermented sausage shows no significant difference (P < 0.05) between the test (inoculated) and the control sausages in the protein, fat, moisture, salt, ash and nitrite content. However, the significant difference (P > 0.05) between the control and inoculated batches in lowering the pH level, changing the critical shear stress, growth of viable microorganisms, accumulation of amine nitrogen during ripening was established. The results show, that inculcation of starter cultures accelerates biochemical processes during fermentation and thereby provides the necessary functional and technological properties of minced meat. Sensory profiling showed a more significant (P < 0.05) acidic and spicy flavour and intensity of acidic and smoked meat aroma; and increased firmness and cohesiveness in inoculated sausage. The results of microstructural analysis showed that the dry fermented sausages that ripened with the starter bacteria (Lactobacillus curvatus, Staphylococcus carnosus, Pediococcus pentosaceus), differ from the control sample compacted as a thin surface layer which is formed during the drying, smoking and maturation, and that indicates more uniform moisture removal.

Key words: poultry meat, dry fermented sausage, starter bacteria, ripening.

INTRODUCTION

Traditional technologies recommend the use of beef and pork as the basic raw materials in the production of fermented sausages (Marco et al., 2008). In several studies, the compositions of fermented sausages from different meat types, such as goat and sheep (Stajic et al., 2013), horse (Kovačević et al., 2016), camel (Mejri et al., 2017b), and mutton (Zaho et al., 2011) have been considered. Given that currently the poultry is one of the most commonly consumed type of meat in the world (Tsirulnichenko et al., 2017), it is important to expand the assortment of products from poultry meat.

Traditional production of dry fermented sausages includes the grinding of meat, formulation, stuffing into a casing, fermentation, ripening and drying. During the extended drying process, the product becomes dehydrated, so the dry fermented sausages can be characterized by low moisture content, a significant amount of fat and protein, and consequently being of high energy.

The importance of fermented sausages from the point of view of healthy nutrition should be noted. Numerous studies have shown that fermented products that contain lactic acid bacteria can have a positive influence on the digestion of nutrients, so their consumption has a beneficial effect on the gastrointestinal tract and prevents intoxication in the human body (de Vuyst et al., 2008; Turhan et al., 2017). Different probiotic lactic acid bacteria might have different functional properties. Kim (2014) demonstrated that fermented sausages with probiotic starter cultures have similar physicochemical and functional properties to those with commercial starter cultures. Gallego et al. (2018) obtained results confirming the potential of dry fermented sausages as natural sources of bioactive peptides that can exert certain bioactivities such as antioxidant and ACE (angiotensin converting enzyme) inhibitory activities.

However, despite all the advantages of the product, there is a significant disadvantage – production of fermented sausages is one of the most complex technologies in the meat processing industry, characterized by its duration and laboriousness (Marco et al., 2008). Therefore, the problem of intensification of fermented sausages production, and reduction of the technological cycle, is quite topical. The solution to the problem includes issues related to the acceleration of structural changes, intensifying flavour, taste and colour formation through the application of starter cultures, protein supplements, glucono-delta-lactone, fermented sugars and other components (Sawitzki et al., 2008).

To ensure safety for the consumers and the typical characteristics of a fermented sausage as a colour and a flavour, it is very important to use starter cultures. Lactic acid bacteria (LAB) suppress the growth of pathogenic and spoilage bacteria through the antimicrobial properties of their metabolites such as organic acids, hydrogen peroxide and bacteriocins (Ammor & Mayo, 2007). Antimicrobial compounds synthesized by LAB are considered to be natural preservatives (Tabanelli et al., 2012; El Adab et al., 2015).

During the maturation of fermented sausages, biochemical, and microbiological processes take place depending on the activity of dominant microorganisms in the meat. LAB and coagulase negative cocci are the most active indigenous microorganisms, first in the acidification process, and second in denitrification, lipolysis and proteolysis (Hammes et al., 1998; Lucke, 2000).

At the end of ripening, LAB are the dominant microorganisms due to their excellent adaptation to the meat environment and their faster growth rates during fermentation and sausage ripening. LAB play an important role in meat preservation and fermentation processes because they affect both the technological properties and the microbial stability of the final product (Lorenzo, 2012; Daba & Saidi, 2015; Cardinali et al., 2018).

Obtaining fermented sausages from poultry meat is much more complicated, which is due to the morphological and physicochemical composition of raw materials. Poultry meat is characterized by its significant water content, and such meat dehydrates much more slowly than necessary. Therefore, it is very difficult to process poultry meat, especially chicken broilers meat, in fermented sausages using traditional technologies. Due to the accelerated and considerable loss of water the smoked sausages ripen more slowly, which can lead to a crumbly consistency and considerable deformation of the product.

Mechanically deboned poultry meat was used in fermented sausages by Dhillon & Maurer (1975) and Mc Mahon & Dawson (1976). They observed that in order to obtain an acceptable texture and appearance of the product, ground beef or hand-deboned ground poultry had to be included in the formula.

In connection with the foregoing, the purpose of this research is to study the influence of starter bacterial cultures on the quality and characteristics of the model dry fermented sausages as made from poultry meat.

MATERIALS AND METHODS

Sausage production and sampling procedures

Sausage formulation includes boneless chicken breast and thigh meat (red and white poultry meat), and frozen pork back fat. The bacterial mixture 'Start Star' was used as a starter culture (Germany, HOLKOF GmbH), which consists of lactic acid bacteria of the *Lactobacillus curvatus, Staphylococcus carnosus, Pediococcus pentosaceus* strains.

The raw meat and fat were frozen before grinding at temperature of -4 $^{\circ}$ C. The sausage filling was cooked in a vacuum cutter (CFS/GEA Cutmaster). At first, pieces of frozen chicken thigh were added (45 kg), then chicken breast (40 kg), starter bacterial concentrate (10, 15 and 20 g per 100 kg of minced meat), salt (3 kg), sodium nitrite (0.01 kg), sugar (0.2 kg), ground black pepper (0.2 kg), complex food supplement 'Gypsy Plus' (0.9), and glucono-delta-lactone (0.5). For 1–2 minutes before the end of grinding, minced pork back fat (15 kg) was added. The mince for the control sample was prepared in a similar way, though without introducing the starter cultures.

The sausage batters were stuffed into edible collagen casing with a diameter of 45 mm and a length of 60 cm. Next was the pressing stage, in order to form linked sausages that were rectangular shape, after which the sausages were hung on the frames.

The linked sausage was cured for 24 h at 4 °C (85–90% RH) and the speed of air movement was held at 0.1 m s⁻¹.

After curing, the fermented sausages were smoked in chamber at 20 °C for 2 days by successive cycles in the 'smoke-air mixture and air mixture supply' mode, the total time within the smoke-air mixture supply being 7.4% of the total smoking time. The speed of the smoke-air mixture in the chamber was set to 5 m s⁻¹, ensuring a speed of passage through the product of up to 8 m s⁻¹.

After smoking, the fermented sausages were transferred into a climate chamber where they were dried in two stages. The first stage was the 5 days at 15 °C, with a relative humidity of 82% and air velocity of 0.1 m s⁻¹. The second stage lasted 23 days at a temperature of 12 °C and a relative humidity of 75%.

The pH, water-binding capacity, rheological properties, total amount of viable bacteria, amount of amino-ammonia nitrogen and volatile fatty acids were examined to assess the influence of starter cultures on microbiological, physicochemical and biochemical changes during ripening. For sampling, three sausages of each batch at 0 day (mix before stuffing) and after 7, 14, 21 and 28 days of ripening were taken for microbiological and physicochemical analyzes.

Microstructural analysis was carried out to study the effect of starter cultures on changes in the structure of poultry meat. At the end of the ripening three sausages from each batch were taken for sensory and physicochemical analysis.

Microbiological analysis

Microbial tests on Standard Plate Count Agar (PCA) were used to determine the total number of viable bacteria. Homogenized samples (10 g) were mixed with 90 mL of sterile double deionized water (dd water), and serial dilutions were prepared, then dilution (0.1 mL) was spread in PCA agar. They were incubated at 37 °C for 48 h, counted and expressed as log CFU g⁻¹.

Physicochemical analysis

Physicochemical analyses during the ripening period included the pH measurements, water-binding capacity, the amount of amino-ammonia nitrogen and volatile fatty acids.

The pH values were measured in homogenates prepared by blending 10 g of sausage with 40 mL of distilled water for 2 min. Measurements were taken with a digital pH meter (model 710 A+) equipped with a penetration probe. The water-binding capacity of the fermented sausages was determined as the difference between the moisture mass fraction in the samples and the quantity of moisture that were separated in the process of the heat treatment.

The amount of amino-ammonia nitrogen was determined according to a method based on the binding of amino groups and ammonia with formaldehyde in a neutral solution, followed by titration of carboxyl groups with sodium hydroxide, the amount of which is equivalent to the number of free amino groups.

The amount of volatile fatty acids was determined by a method based on the separation of volatile fatty acids accumulated in poultry meat during the hydrolysis and oxidation of lipids using steam distillation and the determination their amount by titration with a solution of potassium hydroxide. The amount of volatile fatty acids is expressed in milligrams of potassium hydroxide used for titrating volatile fatty acids isolated from 100 g of sample.

Moisture, fat, protein and ash content in sausages samples were determined according to standards recommended by the International Organization for Standardization. The percentage of moisture was calculated by the weight lost by the experimental sample (5 g) maintained in an oven (Memmert, UL 60) at 105 °C, until constant weight was achieved. Fat and protein content were analysed using Soxhlet extraction and protein determination by the Kjeldahl method, respectively.

The sodium chloride and sodium nitrite contents were determined according to the AOAC methodology (2002).

Investigations of rheological properties (critical shear stress) of fermented sausages during maturation were carried out at room temperature (20 ± 1 °C), using a texture analyser (Brookfield R/S) and a rotary viscometer.

Microstructural analysis

Changes taking place in tissues under the influence of biotechnological processing were determined via a histological study of the samples. After the sampling, the samples were fixed in a 10% water solution of neutral formalin, which were then washed with

cold water and compacted in gelatin. The blocks were cut and compacted in 20% formalin solution over 12 hours after cooling the gelatin solution with the samples. Then the blocks were washed, pieces were cut of $15 \times 15 \times 4$ mm in dimension, and the sections were created on a freezing microtome. A section was placed on a glass slide treated with an ovalbumin and glycerol. The sections were painted with haematoxylin-eosin. Histological preparations were examined under a light microscope (Leika DM 1000).

Sensory analysis

To conduct a sensory analysis, sausages were subjected to microbiological research and their microbiological safety was proved (Solovyova, 2015).

A sensory evaluation was performed on the control and inoculated sausages at the end of the ripening process. A total of 15 experienced panelists, including staff members of Department of Food and Biotechnology and experts from food enterprises who had experience in assessing fermented meat products were chosen to perform a sensory characterization of the batches (ISO 8586–2:1996). The panellists were trained for 2 weeks according to the attributes and scale recommended by the International Organization for Standardization (2012).

The preparation of the samples for the sensory evaluation consisted of removing the casings and slicing cold sausages into equally sized pieces, each 0.5 cm thick. Single slices were placed into odourless, plastic white dishes and served at room temperature. Each sample was evaluated three times. Water and unsalted crackers were provided to cleanse the palate between samples.

The sensory quality was characterized on the basis of 16 sensory traits: five odour attributes (smoked meat aroma, rancid aroma, spicy aroma, acid aroma, mould aroma); three appearance attributes (fat distribution, colour intensity and colour homogeneity); three texture attributes (firmness, cohesiveness, fattiness); five attributes of flavour (smoked meat flavour, spicy flavour, acid flavour, rancid flavour and saltiness).

Each parameter in the sensorial analysis was evaluated by means of a scale from 1 to 9 with anchors of 1 meaning no intensity or extremely low intensity, 5 meaning regular intensity of dry-fermented sausage and 9 meaning extremely high intensity.

Statistical Analysis

The values are presented as the mean \pm SEM. Probability values ≤ 0.05 were taken to indicate statistical significance. The data were analysed by One-way ANOVA using free web-based software offered by Assaad et al. (2014).

RESULTS AND DISCUSSION

Microstructural analysis

Microstructural studies can identify changes occurring in individual structural components and differentiate the characteristics of tissue and cellular structures. Proteolysis of proteins occurs during the ripening of fermented sausages under the influence of enzymes present in meat tissues or by enzymes of microbial origin from added starter cultures (Mejri et al., 2017a), which leads to certain changes in the structure of animal tissues. These changes cause the formation of a specific texture of raw sausages (Katsaras & Budras, 1992), and their intensity can be detected using microstructural studies.

In a microstructural study, it was found that the mince of the control (Fig. 1) and the test (Fig. 2) samples of the sausages was homogeneous, dense-friable, and slightly vacuolized.

The basis of the mince is a fine-grained protein mass with fat cell components uniformly distributed throughout the sample. There are isolated groups of unchanged striated muscle bundles which have retained their shape and size and which fit tightly to each other (Fig. 1, a).

Fig. 1, b shows that the muscle fibres are swollen bundles that have retained their integrity, and the boundaries between them are difficult to distinguish. There is practically no transverse and longitudinal striation of muscle fibres. Bunches of collagen fibres and smooth muscle tissue are weakly expressed in a state of colloidal mucoid swelling.



Figure 1. Microstructure of the control sample of the dry fermented sausage, magnified ×200.

Individual fragments and conglomerates of fibres and bundles of transverse striated and smooth muscle with a pronounced degree of treatment are noticeable in the prototype (Fig. 2, a). This is due to the formation of a spatial framework, accompanied by the destruction of the cellular structure of the tissues, as caused by the activity of microorganisms, the development of the autolysis processes and the morphological features of muscle tissue.



Figure 2. Microstructure of the test sample of the dry fermented sausage, magnified ×200.

There are single islets from unchanged muscle bundles that have retained their shape and dimensions and fit tightly to each other (Fig. 2, b). However, unlike the control sample, bundles of smooth muscle and connective tissue fibres have a more restructured and unfolded structure.

The results of microstructural studies show that the addition of the starter cultures caused significant changes in the structure of the muscular and connective tissues, namely the loosening of the bundles into separate fibrils and their fragments. This is due to accumulation of lactic acid during the starter bacteria reproduction and their proteolytic activity. This contributes to the softening of raw materials. Aktas & Kaya (2001) determined the destructive effect of lactic acid on animal tissues. Katsaras & Budras (1992) noted a change in the structure of native muscle proteins results from different technological processes (chopping, salting, and fermentation). The salting leads to changes in the original protein structure through swelling and partial dissolution of myofibrils. The formation of a matrix of fermented sausage and its specific structure occurs during the maturation of sausages under the influence of lactic acid and due to the gradual loss of water (Katsaras & Budras, 1992).

Physicochemical analysis

Such physicochemical parameters as pH, salt and moisture content can be attributed to 'typical' characteristics for dry fermented sausage (Montanari et al., 2018).

The pH values underwent a rapid reduction in the control and inoculated sausages (Fig. 3), but in the samples with 0.02% starter cultures, the pH decreased more quickly and reached a value of 4.95 (P < 0.05) for by the 14th day. When 0.015% starter cultures were added, the pH reached 4.9 (P < 0.05) after 21 days of ripening, whereas in the control sample over the same period, the pH reached 5.32 (P < 0.05). Sawitzki et al. (2008) determined a similar dependence where during the first 7 days of fermentation the pH values decreased from 5.60 to 4.97 in the inoculated salami, while in the control, pH values decreased from 5.68 to 5.34.



Figure 3. Dynamics of pH change during ripening.

During the ripening of fermented meat products, LAB fermented glucose to lactic acid, which was responsible for the decrease in pH (Parente et al., 2001; Drosinos et al., 2007).

The results show (Fig. 3) that by day 28 of the ripening/fermentation, the rate of decrease in pH was slowing down. The same disposition was defined in the study of fermented sausages conducted by Zdolec et al. (2008). These gradual changes in pH are explained mainly by the reduction in the number of LAB due to the exhaustion of the sugar and, secondly, to the proteolytic activity generated by microorganisms (Katsaras & Budras, 1992; Rai et al., 2010).

Water-binding capacity (WBC) affects the formation of the sausage structure. The results of the experimental studies (Fig. 4) show that large decrease in WBC was observed in samples using starter cultures, which correlates with the dynamics of pH change. Similar results were obtained by Nesterenko (2014), who found that in samples with added starter cultures, the water-binding capacity is lower than in the control sample by 2%.



Figure 4. Dynamics of water-binding capacity change during ripening.

This effect is due to rapid glycolysis and the accumulation of acidic metabolic products of bacteria that reduce the pH value and, accordingly, affect the properties of muscle proteins (Mejri et al., 2017b). The decrease in pH causes a reduction in the waterbinding capacity of the meat, accelerating the drying process aging (Työppönen et al., 2003). Mauriello et al. (2004) reported that water-binding capacity decrease is related to pH decrease. When the pH rates are close to the isoelectric point of protein, the functional properties of meat proteins are reduced and dehydration occurs.

One of the characteristics that makes sausage such an appreciated product is its texture, which is related to the mechanical properties of the product (Daros et al., 2005). Consistency of minced and finished products is best characterized by the value of the

critical shear stress (Figs 5, 6). This indicator was used for technological assessment of minced meat during the process of ripening.



Figure 5. Dynamics of critical shear stress change during ripening.



Figure 6. Example of a diagram obtained on the texture analyser (Brookfield R/S) when examining the critical shear stress of the dry fermented sausage samples after 28 days of ripening.

The data presented in Fig. 5 indicates that the compression of minced meat in a test sample with the addition of starter cultures is faster than the control. After 7 days of ripening, the critical shear stress in the test samples (1655, 1685 and 1695 Pa, respectively, (P < 0.05)) significantly exceeds this value in the control sample (1510 Pa (P < 0.05)). Structure formation for the control sample was complete by 21 days. Reducing the pH level to the value of the isoelectric point of muscle proteins leads to a decrease in the hydration properties of the meat system. Reducing the moisture content correlates with the formation of a dense structure of sausages, therefore, the shear stress increases. These results are in agreement with those found by Mejri et al. (2017b), who determined that the hardness of the control and inoculated sausages increased by 28 days of ripening. The authors attributes this to a decrease in the moisture content (drying of the sausage) and a corresponding increase in the level of fat. In addition, this was perhaps due to the accumulation of nonprotein products, namely exopolysaccharides, as a result of the lactic acid bacteria activity (Ruas-Madiedo et al., 2002), and this directly effects the structural formation of minced meat during maturation.

Thus, the use of starter cultures contributes to the formation of a dense surface layer and the monolithic structure of minced meat.

To evaluate the effect of starter culture on lipolysis and proteolysis intensity, volatile fatty acids and amine nitrogen content were determined through ripening period. Organic acids, in particular volatile fatty acids (VFA) and amino acids that accumulate in the minced meat, are associated with the formation of a specific aroma and taste of the sausage. The results of the studies (Figs 7, 8) show that during the ripening of minced meat in experimental samples, intensive accumulation of VFA and amine nitrogen can be observed.



Figure 7. Dynamics of accumulation of amine nitrogen during ripening.

Thus, after 7 days of ripening, the maximum amine nitrogen content and VFA in the test samples was 4.05 and 0.34 mg in 100 g (P < 0.05), respectively, and in the control sample such values were achieved by 12 days in terms of the VFA content, and only by 28 days in terms of the amine nitrogen content. The dynamics of amine nitrogen and VFA accumulation indicate an acceleration of the ripening in inoculated sausages in comparison with the controls.

The hydrolysis of meat proteins generates polypeptides that can be further degraded to smaller peptides and free amino acids, where such degradation can be caused by endogenous and microbial enzymes, as reported by a number of different authors (Hughes et al., 2002; AroAro et al., 2010). El. Adab et al. (2015) demonstrated that in sausages inoculated with *S. xylosus* and *L. plantarum*, the free-acid content increased from 2,059.82 mg kg⁻¹ to 3,461.07 mg kg⁻¹ as a result of proteolysis. This difference in total free amino acid accumulation was related to the proteolytic activities of microbial enzymes. These amino acids play an important role in development of characteristic taste and flavor of the final product (Casaburi et al., 2008; Lorenzo & Franco, 2012).



Figure 8. Dynamics of accumulation of volatile fatty acids during ripening.

The results of physicochemical analysis of dry fermented sausage (Table 1) shows that there are no significant differences (P > 0.05) between the test (inoculated) and the control sausages in terms of the protein, fat, moisture, salt, ash and nitrite contents. The moisture content in the sausages was between 29.8 and 31.4 (P < 0.05), which is typical for dry fermented sausages. Sawitzki et al. (2008) and Mejri et al. (2017b) obtained similar results, where these authors observed no significant difference (P > 0.05) between the inoculated and the control sausages. However, the determined that the moisture content was somewhat higher than the value reported in dry fermented Chinese-style sausage by Rai et al. (2010).

Indicator	Value of indicator for samples of dry fermented sausages, %			
	Control	Test 1	Test 2	Test 3
Protein	28.7 ± 0.64^{b}	29.6 ± 0.52^{ab}	$31.1\pm0.42^{\rm a}$	$31.2\pm0.58^{\rm a}$
Fat	34.2 ± 0.86	33.2 ± 0.94	32.9 ± 0.74	33.5 ± 0.62
Moisture	$31.4\pm0.20^{\rm a}$	30.8 ± 0.36^{ab}	$30.2\pm0.22^{\text{ab}}$	$29.8\pm0.41^{\text{b}}$
Ash	4.52 ± 0.16	4.46 ± 0.16	4.44 ± 0.12	4.4 ± 0.14
Salt	3.76 ± 0.12	3.74 ± 0.08	3.8 ± 0.06	3.82 ± 0.08
Nitrite	0.003 ± 0.001	0.003 ± 0.001	0.003 ± 0.001	0.003 ± 0.001

Table 1. The results of the physicochemical analysis of dry fermented sausages

Designation: test 1 – inoculated sausage 0.010%; test 2 – inoculated sausage 0.015%; test 3 – inoculated sausage 0.020%. Values are means \pm SEM, n = 5 per treatment group. Means in a row without a common superscript letter differ (P < 0.05) as analyzed by one-way ANOVA and the TUKEY test.

Microbiological analysis

It is known that the maturation process of fermented products is based on the vital activity of lactic acid bacteria which gradually become dominant, suppressing the development of pathogenic bacteria (Drosinos et al., 2007; El Adab et al., 2015).

However, it is not always possible to set the ripening process on the right track for fermented products, and as a result bacterial deterioration can take place (Sawitzki et al., 2008). According to Drosinos et al. (2007), Sawitzki et al. (2008) and Mejri et al. (2017b), the microbial population of lactic acid bacteria in fermented sausages is equal to or lower than 4.5 log CFU g^{-1} at the beginning of fermentation without introduction of starting cultures. We obtained similar results at the start of fermentation where the amount of viable bacteria was 3.4 log CFU g⁻¹ in the control sample and from 3.8 to $4.1 \log CFU g^{-1}$ in the test samples (Fig. 9). The amount of viable bacteria by day 21 was 6.0 log CFU g⁻¹ in control sample and up to 9.2 log CFU g⁻¹ in the test samples, and which thereafter remained at the same level until the end of ripening.



Figure 9. Growth of microbial population during ripening.

The results of the studies indicate a high survival rate of the microorganisms included in the starter cultures, the metabolism of which ensures the microbiological safety of dry fermented sausages. Gao et al. (2014) demonstrated that lactic acid bacteria improve the safety, stability and shelf life of meat products.

Sensory analysis

The four types of sausages were subjected to a sensory evaluation of 16 characteristics as carried out by 15 experienced panellists. Fig. 10 shows the results of the descriptive sensory analysis of the sausages at the end of the ripening period.

The complex studies concerning the importance of sensory attributes and their perception by consumers have shown that flavour is always the most important attribute of food products, followed by texture and appearance (Jaworsk & Hoffmann, 2008).

Among the 16 descriptors considered in the test, significant differences were obtained in the traits characterizing the flavour, texture and appearance of the sausages.

Regarding the flavour, the addition of commercial starter bacteria significantly affected the acidic and spicy flavours and saltiness. The highest values of acidic and spicy tastes were obtained from the batch containing 0.02% bacterial concentrate. The lowest value corresponded to the control sausages. On the other hand, we observed slightly higher scores for rancid flavour and smoked meat flavour intensity in the control batch, but the differences were not felt to be significant. Furthermore, the saltiness of the sausages was significantly reduced by the addition of starter bacteria.



Figure 10. Sensory evaluation of control and test (inoculated) sausages.

The highest acid taste score in test batches agreed with those reported by Lorenzo et al. (2016), who also observed that non-inoculated sausages gave the lowest acid taste. Our results are consistent with the data obtained for pH values, where, as commented above, test sausages gave significantly lower pH values than the control sausages.

The extent of the rancid flavour in the control samples agreed with the results reported by Cenci-Goga et al. (2012), who found a more pronounced rancid taste in

salami made without the addition of starter cultures. The results of the sensory analyses confirm the antioxidant effect of the starter cultures in controlling lipid oxidation, which positively influence the sensory properties of sausages.

During the analysis of the aroma of inoculated sausages, a positive correlation between the amount of starter bacteria and the acid, spicy and smoked meat aroma intensities was established.

Starter bacteria noticeably influenced the texture of the sausages compared with the control sample. Inoculated sausages presented greater firmness and cohesiveness; they had a better texture, which is in agreement with the instrumental rheological analysis. No significant difference was found between any of the samples in terms of fattiness.

The test sausages presented higher colour intensity and colour homogeneity than the control sample. The most acceptable appearance was observed in sausages containing 0.02% starter bacteria. These findings were in agreement with the results obtained by other authors, who found a more intense red colour in inoculated than in control sausages (Andrade et al., 2010; Essid & Hassouna, 2013). The colour formation is related with the nitrate reductase activity of the starters used in this study, which contained *L. curvatus, S. carnosus, P. pentosaceus* with its high nitrate reductase activity.

CONCLUSIONS

This study demonstrated that the introduction of starter cultures accelerates biochemical processes during fermentation/ripening and thereby provides the necessary functional and technological properties of minced meat in the production of dry fermented sausages from poultry meat. This was confirmed by the results of physical and chemical studies, which showed a greater decrease in pH, WBC, accumulation of amine nitrogen, and increased critical shear stress in the test (inoculated) samples compared to the control sample. Microstructural and microbiological studies also confirm the effectiveness of using the starter bacteria in the production of dry fermented sausages from poultry meat. The results of intensive proteolysis and lipolysis, which develop due to the enzymatic activity of microorganisms, is responsible for the formation of the specific taste and aroma of the sausages, as determined during the sensory analysis.

ACKNOWLEDGEMENTS. The work was supported by Act 211 Government of the Russian Federation, contract №02.A03.21.0011. The authors thank the managers and employees of the meat processing complex Sitno for their assistance in the production of sausages and their research, as well as the reviewers for valuable comments in preparing this article.

REFERENCES

- Aktas, N. & Kaya, M. 2001. The influence of marinating with weak organic acids and salts on the intramuscular connective tissue and sensory properties of beef. *European Food Research and Technology* 213, 88–94.
- Ammor, M.S. & Mayo, B. 2007. Selection criteria for lactic acid bacteria to be used as functional starter cultures in dry sausage production. *Meat Science* **76**, 138–146.
- Andrade, M.A., Cyrdoba, J.J., Casado, E.M., Cyrdoba, M.G. & Rodriguez, M. 2010. Effect of selected strains of Debaryomyces hansenii on the volatile compound production of dry fermented sausage 'salchichyn'. *Meat Science* 85, 256–264.

- AroAro, J.M., Nyam-Osor, P., Tsuji, K., Shimada, K.I., Fukushima, M. & Sekikawa, M. 2010. The effect of starter cultures on proteolytic changes and amino acid content in fermented sausages. *Food Chemistry* 119, 279–285.
- Assaad, H.I., Zhou, L., Carroll, R.J. & Wu, G. 2014. Rapid publication-ready MS-Word tables for one-way ANOVA. *Springer Plus* **3**, 474.
- Cardinali, F., Milanović, V., Osimani, A., Aquilanti, L., Taccari, M., Garofalo, C., Polverigiani, S., Clementi, F., Franciosi, E., Tuohy, K., Mercuri, L., Altissimi, M.S. & Haouet, M.N. 2018. Microbial dynamics of model Fabriano-like fermented sausages as affected by starter cultures, nitrates and nitrites. *International Journal of Food Microbiology* 278, 61–72.
- Casaburi, A., Di Monaco, R., Cavella, S., Toldra, F., Ercolini, D. & Villani, F. 2008. Proteolytic and lipolytic starter cultures and their effect on traditional fermented sausages ripening and sensory traits. *Food Microbiology* 25, 335–347.
- Cenci-Goga, B.T., Rossitto, P.V., Sechi, P., Parmegiani, S., Cambiotti, V. & Cullor, J.S. 2012. Effect of selected dairy starter cultures on microbiological, chemical and sensory characteristics of swine and venison (Dama dama) nitritefree dry-cured sausages. *Meat Science* 90, 599–606.
- Daba, H. & Saidi, S. 2015. Detection of bacteriocin-producing lactic acid bacteria from milk in various farms in north-east Algeria by a new procedure. *Agronomy Research* 13(4), 907–918.
- Daros, F.G., Amico, S.C. & Masson, M.L. 2005. A Methodology for the Evaluation of Mechanical Properties of Sausage Based on Tensile and Compression Tests. *International Journal of Food Engineering* 3(1), Article 2.
- De Vuyst, L., Falony, G. & Leroy, F. 2008. Probiotics in fermented sausages. *Meat Science* 80, 75–78.
- Dhillon, A.S. & Maurer, A.J. 1975. Utilization of mechanically deboned chicken meat in the formulation of summer sausages. *Poultry Science* **54**, 1164–1174.
- Drosinos, E.H., Paramithiostis, S., Kolovos, G., Tsikouras, I. & Metaxopoulos, I. 2007. Phenotypic and technological diversity of lactic acid bacteria and staphylococci isolated from traditionally fermented sausages in southern Greece. *Food Microbiology* 24, 260–270.
- El Adab, S., Essid, I. & Hassouna, M. 2015. Microbiological, biochemical and textural characteristics of a Tunisian dry fermented poultry meat sausage inoculated with selected starter cultures. *Journal of Food Safety* **35**, 75–85.
- Essid, I. & Hassouna, M. 2013. Effect of inoculation of selected Staphylococcus xylosus and Lactobacillus plantarum strains on biochemical, microbiological and textural characteristics of a Tunisian dry fermented sausage. *Food Control* **32**, 707–714.
- Gallego, M., Mora, L., Escudero, E. & Toldrá, F. 2018. Bioactive peptides and free amino acids profiles in different types of European dry-fermented sausages. *International Journal of Food Microbiology* 276, 71–78.
- Gao, Y., Li, D. & Liu, X. 2014. Bacteriocin-producing Lactobacillus sakei C2 as starter culture in fermented sausages. *Food Control* **35**, 1–6.
- Hammes, W.P. & Hertel, C. 1998. New developments in meat starter cultures. *Meat Science* **49**(1), 125–138.
- Hughes, M.C., Kerry, J.P., Arendt, E.K., Kenneally, P.M., McSweeney, P.L.H. & O'Neill, E.E. 2002. Characterization of proteolysis during the ripening of semi-dry fermented sausages. *Meat Science* 62, 205–216.
- Jaworska, D. & Hoffmann, M. 2008. Relative importance of texture properties in the sensory quality and acceptance of crispy products. *Journal of the Science of Food and Agriculture* 88, 1804–1812.
- Katsaras, K. & Budras, K.D. 1992. Microstructure of fermented sausage. *Meat Science* **31**(2), 121–134.

- Kim, Y.J., Park, S.Y., Lee, H.Ch., Yoo, S.S., Se, J.O., Hyeong, S.K. & Chin, K. 2014. Evaluation of Fermented Sausages Manufactured with Reduced-fat and Functional Starter Cultures on Physicochemical, Functional and Flavor Characteristics. *Korean Journal for Food Science* of Animal Resources 34(3), 346–354.
- Kovačević, D., Mastanjević, K., Pleadin, J. & Frece, J. 2016. Physicochemical, microbiological and colour attributes of horse salami established during the ripening period. *Italian Journal of Food Science* **28**, 96–106.
- Lorenzo, J.M. & Franco, D. 2012. Fat effect on physio-chemical, microbial and textural changes through the manufactured of dry-cured foal sausage lipolysis, proteolysis and sensory properties. *Meat Science* 92, 704–714.
- Lorenzo, J.M., Gymez, M., Purricos, L. & Fonseca, S. 2016. Effect of commercial starter cultures on volatile compound profile and sensory characteristics of dry-cured foal sausage. *Journal* of the Science of Food and Agriculture 96(4), 1194–1201.
- Lu"cke, F.K. 2000. Utilization of microbes to process and preserve meat. *Meat Science* 56, 105–115.
- Marco, A., Navarro, J.L. & Flores, M. 2008. The sensory quality of dry fermented sausages as affected by fermentation stage and curing agents. *European Food Research and Technology* 226, 449–458.
- Mauriello, G., Casaburi, A. & Villani, F. 2004. Isolation and technological properties of coagulase negative Staphylococci from fermented sausages of Southern Italy. *Meat Science* 67(1), 149–158.
- McMahon, E.F. & Dawson, L.E. 1976. Influence of mechanically deboned meat and phosphate salts on functional and sensory attributes of fermented turkey sausage. *Poultry Science* 55, 103–112.
- Mejri, L., Vásquez-Villanueva, R., Hassouna, M., Marina, M.L. & García, M.C. 2017a. Identification of peptides with antioxidant and antihypertensive capacities by RP-HPLC-Q-TOF-MS in dry fermented camel sausages inoculated with different starter cultures and ripening times. *Food Research International* **100**, 708–716.
- Mejri, L., Ziadi, A., Adab, S.E., Boulares, M., Essid, I. & Hassouna, M. 2017b. Effect of commercial starter cultures on physicochemical, microbiological and textural characteristics of a traditional dry fermented sausage reformulated with camel meat and hump fat. *Food Measure* 11, 758–767.
- Montanari, C., Gatto, V., Torriani, S., Barbieri, F., Bargossi, E., Lanciotti, R., Grazia, L., Magnani, R., Tabanelli, G. & Gardini, F. 2018. Effects of the diameter on physicochemical, microbiological and volatile profile in dry fermented sausages produced with two different starter cultures. *Food Bioscience* **22**, 9–18.
- Nesterenko, A.A. 2014. The impact of starter cultures on functional and technological properties of model minced meat. *Austrian Journal of Technical and Natural Sciences* 4(7–8), 77–80.
- Parente, E., Grieco, S. & Crudele, M.A. 2001. Phenotypic diversity of lactic acid bacteria isolated from fermented sausages produced in Basilicata (Southern Italy). *Journal of Applied Microbiology* 90, 943–952.
- Rai, K.P., Zhang, C. & Shui, X.W. 2010. Effects of pure starter cultures on physico-chemical and sensory quality of dry fermented Chinese-style sausage. *Journal of Food Science and Technology* 47(2), 188–194.
- Ruas-Madiedo, P., Tuinier, R., Kanning, M. & Zoon, P. 2002. Role of exopolysaccharides produced by Lactococcus lactis subsp. cremoris on the viscosity of fermented milks. *International Dairy Journal* 12, 689–695.

- Sawitzki, M.C., Fiorentini, Â.M., Cunha, J.A., Bertol, T.M. & Sant'anna, E.S. 2008. Lactobacillus plantarum AJ2 isolated from naturally fermented sausage and its effects on the technological properties of Milano-type salami. *Ciencia e Tecnologia de Alimentos* (Campinas, Brazil) 28(3), 709–717.
- Sensory analysis-general guidelines for the selection, training and monitoring of selected assessors and expert sensory assessors: ISO 8586:2012. 2012. Available at: https://www.iso.org/obp/ui/#iso:std:iso:8586:ed-1:v2:en, 7 March 2018.
- Solovyova, A.A. 2015. Assessment of fermented sausage safety. *Food Processing: Techniques* and Technology **38**, 55–61 (in Russian).
- Stajic, S., Perunovic, M., Stanisic, N., Zujovic, M. & Zivkovic, D. 2013. Sucuk (Turkish-style dry-fermented sausage) quality as an influence of recipe formulation and inoculation of starter cultures. *Journal of Food Processing and Preservation* 37(5), 870–880.
- Tabanelli, G., Coloretti, F., Chiavari, C., Grazia, L., Lanciotti, R. & Gardini, F. 2012. Effects of starter cultures and fermentation climate on the properties of two types of typical Italian dry fermented sausages produced under industrial conditions. *Food Control* **26**, 416–426.
- Tsirulnichenko, L., Potoroko, I., Krasulya, O. & Gudina, I. 2017. Increasing the level of hydration of biopolymers in meat processing systems based on the use of acoustically activated brines. Agronomy Research 15, 1419–1425.
- Turhan, E.U., Erginkaya, Z., Polat, S. & Ozer, E.A. 2017. Design of probiotic dry fermented sausage (sucuk) production with microencapsulated and free cells of Lactobacillus rhamnosus. *Turkish Journal of Veterinary and Animal Sciences* 41, 598–603.
- Työppönen, E.S., Markkula, A., Petaja, E., Suihko, M.L. & Mattila-Sandholm, T. 2003. Survival of Listeria monocytogenes in North European type dry sausages fermented by bioprotective meat starter cultures. *Food Control* **14**, 18–85.
- Zaho, L., Jin, Y., Ma, C., Song, H., Li, H. & Wang, Z. 2011. Physico-chemical characteristics and free fatty acid composition of dry fermented mutton sausages as affected by the use of various combinations of starter cultures and spices. *Meat Science* 88(4), 761–766.
- Zdolec, N., Hadžiosmanović, M., Kozačinski, L., Čvrtila, Ž. & Filipović, I. 2008. Microbial and physicochemical succession in fermented sausages produced with bacteriocinogenic culture of Lactobacillus sakei and semi-purified bacteriocin mesenterocin Y. *Meat Science* 80(2), 480–487.