

The Effect of Starter Cultures on the Physico-chemical, Microbiological and Sensory Characteristics of Semi-dried Sausages

Shadi Al-ahmad

Engineer-Department of Food Science- Faculty of Agriculture.
P. O. box. 30 621 University of Damascus-Syria

Abstract: among batches with mixed starters. The sausage inoculated with the combination of *Lactococcus lactis*, *Lactobacillus sakei*, *Staphylococcus carnosus* and *Staphylococcus xylosus* (S-S3) The aim of this research was to study the effect of starter cultures on the physico-chemical, microbiological and sensory characteristics of semi-dried sausages comparing with spontaneously fermented sausage. Three types of commercial combinations of starter cultures were used in this study (*Staphylococcus carnosus* and *Staphylococcus vitulinus*), (*Lactococcus lactis* and *Lactobacillus sakei*), and (*Lactococcus lactis*, *Lactobacillus sakei*, *Staphylococcus carnosus* and *Staphylococcus xylosus*). The use of starter cultures resulted in a rapid decrease in pH values, inhibition in the growth of Enterobacteriaceae, yeasts and molds.

Sausages inoculated with mixed starter cultures resulted in a rapid pH decrease, during the 48 h of fermentation at 30°C. The changes in non-protein nitrogen (NPN) and free amino acid indicated severe hydrolysis of protein occurred during fermentation. No significant differences for texture and appearance were found) gained the highest scores for flavor and taste. There was an apparent positive correlation ($r = 0.87$) between the NPN and appearance, taste and flavor in sausages, else a positive correlation ($r = 0.663$ $P < 0.01$) showed between flavor and *Staphylococcus* count in products .whereas pH value showed a significantly negative correlation ($r = -0.832$ $P < 0.01$) with taste.

Keywords: Fermented sausages, Starter cultures, Quality parameters.

Introduction

Dry and semi-dry fermented sausages are typical Mediterranean meat-products. The acceptability of these products by the consumer is strongly influenced by their final flavor. The flavor of fermented sausages depends on many factors such as raw meat quality and breed, processing conditions (traditional or industrial), additives, and spices. In fermented sausage preparation, fermentation of added carbohydrate is accomplished by starter culture processes involving added bacterial cultures or by traditional processes involving fermentation by bacteria within the natural microflora of the meats¹. After fermentation, the pH of the sausage will be lowered to 4.8-5.3, which helps prevention of the growth of other microorganisms². Flavor of the sausage is derived from microbial metabolism of carbohydrates, proteins and lipids, as well as the addition of spices³. However, after the commercial introduction of pure bacterial starter cultures in the 1950s, traditional sausage production shifted to starter culture processes in order to provide a controlled fermentation for especially large scale production requirements. Safe final products of high quality standards were obtained⁴. Today, processors around the world use cultures to produce Genova salami, Thuringer, Cervelat, etc. Using a culture helps improve batch

consistency and reduce the guesswork associated with so-called “wild fermentation”. Starter culture use has actually increased over the past decade with the demand for reducing/eliminating *Escherichia coli* 0157:H7 in dry-fermented sausages⁵. Safe final products of high quality standards were obtained^{4,6}.

The use of starter cultures could be a guarantee to get products with repeatable hygienic and organoleptic properties in a shorter ripening time. However, commercial starter cultures not always are able to compete with house flora, resulting in losses of desirable sensory properties. Therefore, appropriate starter cultures should be selected from indigenous microorganisms, which are well adapted to meat environment and more competitive because of their specific metabolic capabilities⁷.

Lactic acid bacteria (LAB) represent a significant part of the naturally fermented sausage microflora. The presence of *Lactobacillus plantarum*, *Lactobacillus curvatus*, *Lactobacillus sakei* as well as *Pediococcus* spp. and *Leuconostoc* spp. in fermented sausages has often been reported^{8,9}. In European fermented sausages, strains of *Lactobacillus sakei*, *Lactobacillus curvatus*, *Lactobacillus plantarum* and *Lactobacillus pentosus* are widely used as starter organisms⁶. In sausage fermentations performed at 18–23 °C, the indigenous microflora is usually dominated by strains of *L. sakei* and *L. curvatus*^{10,11}. Lactic Acid bacteria are responsible of flavor development and preservation of fermented meat products by producing antimicrobial compounds such as lactic acid and acetic acid, hydrogen peroxide, diacetyl, and bacteriocins^{12,13}.

Two of the most important cultures added in starter preparations commonly used in longer fermentation periods and at lower temperatures are *Staphylococcus carnosus* and *S. xylosus* due to their ability to delay rancidity through peroxide decomposition, to reduce nitrate for color formation and to improve flavor as a result of proteolysis^{14,15,16}.

Coagulase-negative staphylococcus (CNS) and lactic acid bacteria (LAB) are the most important microorganisms used as starter cultures in meat fermentations. The organoleptic properties of fermented sausages, in fact, are due to the metabolic activities of these microorganisms together to the activity of endogenous meat enzymes¹⁷. Several studies suggested that *Staphylococcus* species, rather than LAB, play an important role in the development of sensory properties (flavor, texture, color) of fermented sausages by reduction of nitrates, proteolytic and lipolytic activities^{18,19}. Additionally, the ability of CNS to produce antimicrobial compounds may improve safety and shelf-life of sausages^{17,20}.

However, little is known about the microbiological and biochemical changes that occur during fermentation of sausages with a co-culture of LAB and staphylococcus. The purpose of this study was to compare the physico-chemical, microbiological and sensory characteristics of fermented sausage produced by different combinations of LAB and staphylococcus.

Materials and methods

Starter cultures:

Starter 1: {S1} *Staphylococcus carnosus*, *Staphylococcus vitulinus*; Starter 2: {S2} *Lactococcus lactis*, *Lactobacillus sakei* and Starter 3: {S3} *Lactococcus lactis*, *Lactobacillus sakei*, *Staphylococcus carnosus*, *Staphylococcus xylosus* were purchased from Danisco A/S Brabrand, Denmark. Starter cultures mixed with batches as powder until the cell concentration was adjusted to 7–9 log c.f.u. g⁻¹.

Preparation of fermented sausage:

Frozen beef (5 kg) was thawed in running tap water and then gutted, and minced with (1.5 kg) from cow fat through a deboner (Model 694, Baader North America, New Bedford, MA, USA) with a drum having 6 mm-diameter perforations. The processed samples were then mixed with 3% NaCl, 2% dextrose and 30 g black pepper. Starter cultures were inoculated with a final level of 6–7 log c.f.u. g⁻¹. Four separated batches were prepared with different mixed starter cultures: S-S1 (*Staphylococcus carnosus*, *Staphylococcus vitulinus*); S-S2 (*Lactococcus lactis*, *Lactobacillus sakei*), S-S3 (*Lactococcus lactis*, *Lactobacillus sakei*, *Staphylococcus carnosus*, *Staphylococcus xylosus*) and a batch without any starter (S-Non) as control were prepared. Each sausage batter was stuffed into collagen casings (ø 40 mm, RL2, Naturin, Weinheim, Germany) with a filling machine (SZ-200, Guangzhou, China) and fermented at 30C, RH 85% for 48 h.

Microbiological analysis:

Twenty-five gram samples and 225 ml 0.1% peptone water were weighed aseptically into sterile stomacher bags, sealed, and homogenized at 25°C for 2 min. appropriate decimal dilutions of the samples were prepared using the same diluent and 0.1 ml of each dilution was plated in triplicate on different growth media. Results were expressed as colony-forming units per gram ($\log \text{c.f.u.g}^{-1}$). The following media and incubation conditions were used: (1) Plate count agar (PCA) incubated at 37°C for 2 days for total viable count (TVC), (2) MRS agar incubated at 30°C for 1–2 days for LAB count, (3) Mannitol Salt Agar (MSA) incubated at 30°C for 2–3 days for *Staphylococcus* count, (4) Violet red bile glucose agar (VRBG) incubated at 37°C for 24 h for Enterobacteriaceae, and (5) Rose Bengal Dichloran Agar (DRBC) incubated at 25°C for 3–4 days for yeast and mold counts.

Chemical analysis

Protein, fat, ash and moisture content were determined according to the AOAC official procedures²¹. pH measurement was carried out according to the procedure of²². Ten gram samples were homogenized with 90 ml deionized water and the pH was measured with a digital pH meter (Mettler Toledo 320-s). Non-protein nitrogen (NPN) was determined by the method of²³.

Free amino acids were determined by reversed phase high-performance liquid chromatography (RPHPLC) (Agilent 1100 Series, Palo Alto, CA, USA). The preparation of amino acid extracts was made according to the method described by²⁴. Precolumn amino acid derivatization was done using o-phthalaldehyde (OPA)²⁵. Peak identification and quantification were accomplished by determining retention time and recoveries of free amino acid standards (Sigma Chemical, St Louis, MO, USA).

Sensory evaluation

At the end of the fermentation, fermented sausage samples were sliced into 0.5 cm thick slices. Three semicircle of the sausage samples were coded, randomized and served uncooked in white paper plate at room temperature to 15 people. Samples were assessed for appearance, flavor, color, taste and texture on a scale of 1–9; with 1 being dislike extremely, 5 is neutral, and 9 is like extremely²⁶.

Statistical analysis

Duncan's Multiple Range Test was employed to determine statistical difference within treatments, the test for significance was conducted at $P < 0.05$. All statistical analyses were performed using SPSS 16 statistic program²⁷.

Result:

Microbiological analyses

The initial total aerobic plate counts (APC) in the sausage batter were in the range of 3.5–4.5 $\log \text{c.f.u.g}^{-1}$. However, the batches with added cultures significantly increased to the level of high $\log \text{c.f.u.g}^{-1}$ after 36 h fermentation and then started to stabilize or decline during the later stages (Fig. 1a). It seemed likely that the higher acidity and bacteriocins produced by the starters could have suppressive action against the growth of APC in the final product²⁸. LAB counts increased during fermentation for both the control and starters added samples (Fig. 2b). Initial LAB counts in the samples with starters S2 and S3 added were significantly higher than in the control samples and samples with starters S1 due to the inoculation of starter strains. During 36 h fermentation, LAB numbers increased and reached levels up to 9 $\log \text{c.f.u.g}^{-1}$ in batches of sausage inoculated with starters S2 and S3, which indicated that meat is suitable for the growth of LAB. A significant negative correlation ($r = -0.988$) was obtained between LAB and pH decline during fermentation.²⁹ have reported similar correlation between pH and LAB counts. In contrast, the control samples and samples with starters S1 LAB increased more slowly, LAB counts increased to 6.5–7 $\log \text{c.f.u.g}^{-1}$ during 36 h.

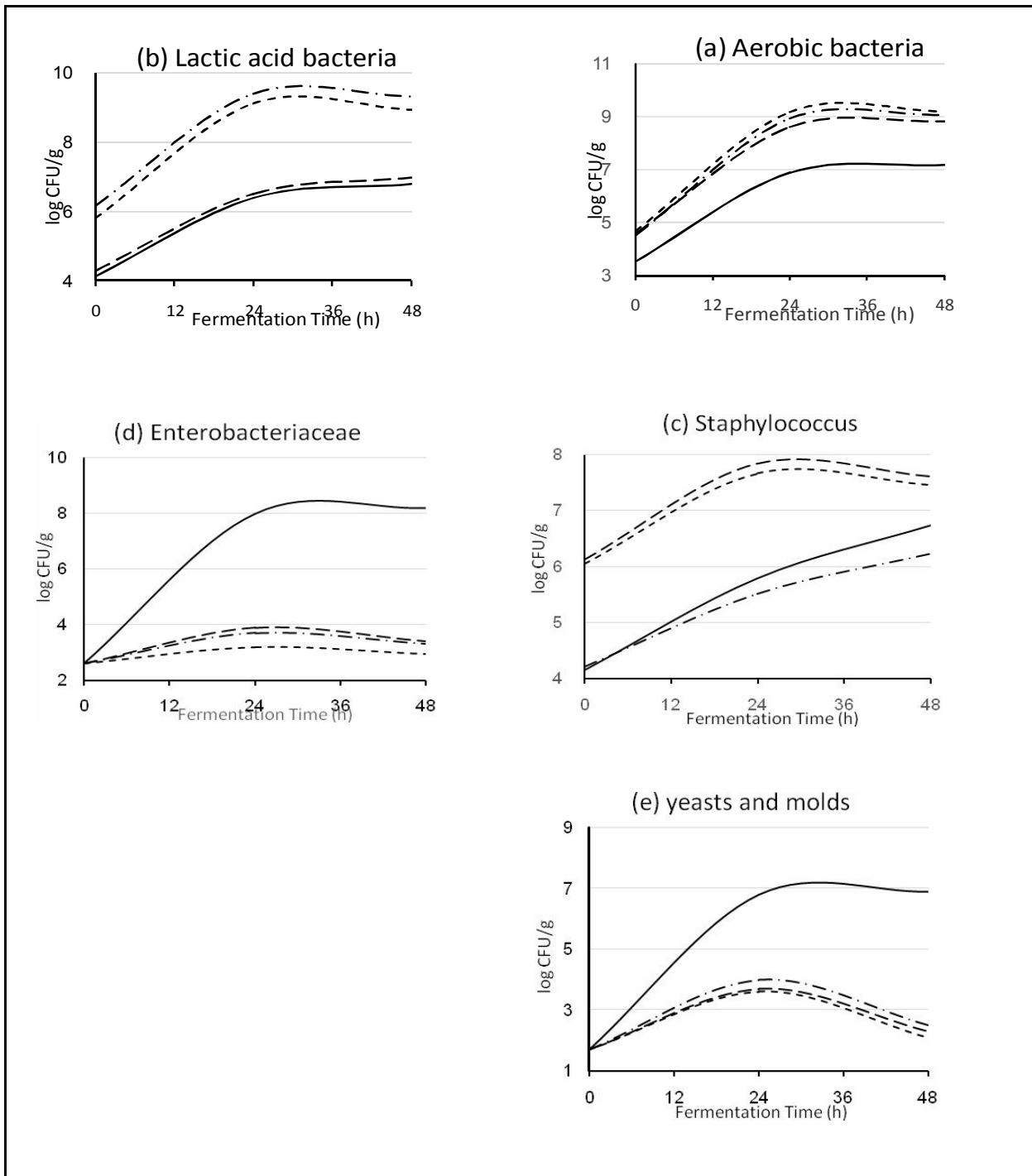


Fig. 1 Microbiological changes in fermented sausage

S-NON - - - - 1S-S - · - · 2S-S · · · · 3S-S

S-NON Sample no starter added, **S-S1:** Sample with *Staphylococcus carnosus* and *Staphylococcus vitulinus*; **S-S2:** Sample with *Lactococcus lactis* and *Lactobacillus sakei*; **S-S3:** Sample with *Lactococcus lactis*, *Lactobacillus sakei*, *Staphylococcus carnosus* and *Staphylococcus xylosum*.

The prime counts of *Staphylococcus* in samples were in the range of 4–6 log c.f.u. g⁻¹ depending upon the starter, where counts were obviously higher for sausages added with starter S1 and S3. The counts of *Staphylococcus* were significantly increased in the batches inoculated with starters S1 and S3 to a level of 8 log c.f.u. g⁻¹ at 24 h, thereafter, the counts decreased significantly in batches with starters S1 and S3 except the control and samples with starters S2 (Fig. 3c). Several authors have reported that the acidification and anaerobic conditions inhibited the growth of *Staphylococcus* during ripening of fermented sausages^{30,31}.

Enterobacteriaceae counts of sausages with or without starters were between 2.3 and 2.6 log c.f.u. g⁻¹ at the beginning of fermentation; no differences between batches were observed. After 48 h of fermentation, the sausages inoculated with starter cultures significantly inhibited the growth of Enterobacteriaceae (Fig. 1d). This result was in agreement with the result of ³² who reported that Enterobacteriaceae counts were significantly decreased with starter cultures added to Turkish Soudjoucks (a fermented meat product). The rapid decline in pH value as well as probably production of bacteriocin by lactic acid bacteria and antagonistic characteristics of *Staphylococcus* might be responsible factors for suppression of such spoilage bacteria like Enterobacteriaceae ^{33,34}. A highly significant correlation ($r = 0.753$, $P < 0.01$) between pH and Enterobacteriaceae reflected that the pH was the main hurdle for the growth of those bacteria. The yeast and mold counts increased in the batches inoculated with mixed starters to a level of 3.7–4.1 log c.f.u. g⁻¹ at 24 h, thereafter, the counts decreased significantly with processing time to 1–2 log c.f.u. g⁻¹. While the control showed higher counts of yeasts and molds in final products (Fig. 1e).

Chemical analysis

No differences ($p > 0.05$) in moisture, protein contents were found in the analysis of variance of main effects of starter culture combination and control after fermentation. An increase in protein and fat ($p < 0.05$) content was found between the before fermented and fermented sausage mixes which was attributed to the decrease ($p < 0.05$) in moisture content between the mixes (Table 1). Loss of moisture from the sausage mixes was likely the result of evaporative effects associated with temperature differences fermentation (48 h ending at 30 °C). The shift in protein, and possibly fat contents (not significant in Table 1), due to moisture loss agrees with previously reported results for other types of fermented sausages ^{35,36}.

NPN is an indicator for the degree of proteolysis in fermented sausages. Fermentation resulted in substantial increases in NPN content. All NPN values for fermented mixes were significantly different ($p < 0.05$) from the respective before fermented mixes. The increase in NPN was highest for S-S1 and S-S3 inoculated sausages and lowest for the control. This result agreed with the result of ³⁷ who have been reported that the mixed culture of *S. xylosus* and LAB possessed higher amount of NPN values in dry fermented sausage. Similarly, strong proteolytic activity of starter *S. xylosus*–12 in dry fermented sausage has been noticed, which caused an increase in the NPN values in the final products ³⁸. Mixes with *S. carnosus* and *S. xylosus* or *S. vitulinus* showed a faster rate of initiating NPN constituents during 48 h of holding as compared to mixes containing only LAB. The increase found during fermentation is consistent with the findings of ³⁵ for chorizo and ³⁹ for summer sausage ^{15,40,41} have also associated the NPN increase with proteolysis effects occurring in the fermentation phase of dry sausage production.

Table (1) chemical composition of fermented sausages with different starters

Parameter	Sample				
	BF	S-NON	S-S1	S-S2	S-S3
Moisture (%)	62.89±0.23 ^b	43.28±0.21 ^a	43.16±0.22 ^a	42.96±0.29 ^a	43.06±0.31 ^a
Protein (%)	16.77±0.13 ^a	36.85±0.34 ^b	27.13±0.22 ^b	27.34±0.29 ^b	27.31±0.15 ^b
Fat (%)	16.53±0.08 ^a	24.45±0.18 ^b	25.11±0.23 ^c	25.45±0.13 ^c	25.39±0.17 ^c
Ash (%)	2.45±0.01 ^a	4.26±0.04 ^c	4.19±0.03 ^{bc}	4.17±0.06 ^b	4.13±0.04 ^b
Carbohydrate (%)	1.36±0.01 ^d	1.16±0.01 ^c	0.41±0.01 ^b	0.08±0.01 ^a	0.11±0.01 ^a
pH	5.74±0.03 ^d	5.36±0.03 ^c	5.24±0.02 ^b	4.84±0.03 ^a	4.86±0.02 ^a
NPN(mg per 100 g)	84±6 ^a	1052±142 ^b	1773±102 ^c	1234±115 ^b	1698±122 ^c

BF before fermentation, S-NON Sample no starter added, S-S1: Sample with *Staphylococcus carnosus* and *Staphylococcus vitulinus*; S-S2: Sample with *Lactococcus lactis* and *Lactobacillus sakei*; S-S3: Sample with *Lactococcus lactis*, *Lactobacillus sakei*, *Staphylococcus carnosus* and *Staphylococcus xylosus*.

^{a-d} Values with different superscript letters in the same row are significantly different ($P < 0.05$)

Free amino acids:

Free amino acid concentrations (dry matter basis) present in the NPN fractions of sausages inoculated with different starter culture combinations are presented in Table 2. Glutamine, taurine, alanine and glutamic

acid were the predominant amino acids in non-fermented sausage, which is in agreement with the data obtained by ^{40,42,43}.

Table (2) Changes in free amino acids of sausages before and after 48 hfermentation with/without starters

FAA	Sample				
	BF	S-non	S-S1	S-S2	S-S3
Glu-acid	112.4	131.1	171.6	142.2	204.8
His	19.9	21.5	33.8	23.4	28.9
Lys	30.6	64.8	107.3	96.9	103.6
Orn	39.9	89.5	120.5	121.1	132.9
Thr	29.1	29.6	38.2	37.2	30.6
Ser	26.8	14.9	24.1	19.8	26.3
Gly	29.36	40.5	75.6	62.8	72.1
Ala	185.2	203.2	256.6	249.5	358
Cys	11.2	23.9	75.7	62.8	72
Met	10.3	21	48.7	48.7	44.5
Val	27.1	65.3	86.5	69.7	98.7
Ile	47.8	66.3	67.8	59.9	60.4
Leu	66.4	163.5	168.7	172.2	164
Tyr	17.3	23.1	18.6	22	19.3
Phe	23.3	25.6	23.8	33.9	24.1
Glu	222.3	244.2	250.5	289.7	261.5
Tau	140.1	153.4	142.5	164.1	163.1
a-ABA	-	16.3	37.8	20.3	47.7
Total	939.1	1,397.7	1,748.3	1,696.2	1,912.5

After fermentation, leucine, ornithine, lysine and valine appeared to be the predominant amino acids overall in addition to glutamine, taurine, alanine and glutamic acid. In general, most amino acid concentrations increased after fermentation except for serine which showed decreases. Lysine, valine, a-amino-n-butyric acid and glutamic acid increased in samples sausages with starters S1 and S3, including *S. xylosus*, *S. carnosus* or *S. vitulinus*, while leucine, tyrosine, phenylalanine and glutamine increased in samples sausages with starters S2. In previous work, the free amino acids were highly correlated with the flavor development of fermented product ⁴⁴; their presence in fermented sausage seemed to be beneficial to develop the specific volatile components.

⁴⁵indicated that alanine, leucine and isoleucine were the amino acids primarily responsible for the increases in total amino acids over semi-dry fermented sausage ripening in addition to glutamine, arginine, lysine, phenylalanine, valine, threonine, tyrosine, histidine, serine and glycine. ⁴⁶ reported increases in concentrations of leucine, valine, alanine, isoleucine, praline and phenylalanine during ripening of traditional Italian fermented sausages. Some of the results of these authors for individual free amino acid generation after fermentation in sausages were different from those of the present study, likely due to differences in time, temperature and microflora of the sausage mixture during fermentation, which makes it difficult to compare the individual amino acids generated ^{45,46}.

Sensory evaluation

The addition of starters that have improved sensory characteristics of the sausage fermented in all mixtures compare to the control. The samples inoculated with mixed cultures were superior in appearance, taste, texture, flavor and color compare to the control (Fig. 4). The control was worst in quality due to less sour (taste), too fishy smell (flavor), and gray (color), wherever the samples treated with mixed cultures were acceptable for sensory quality. However, no significant differences for texture, and appearance were found among batches with mixed starters. The samples S-S1 and S-S3 had significantly higher scores for flavor than the S-S2, the sample S-S3 had significantly higher scores for taste than the S-S1 and S-S2. Therefore, S-S3 was the most preferred by panelists. The better flavor score of batch S-S3 could be linked to a higher concentration of free amino acids, such as alanine, valine, glutamine, taurine, ornithine and glutamic acid. Some authors

reported the addition of free amino acids or enhancing the release of amino acids could result in an improvement of the overall quality of fermented sausages^{19,47}. From correlation analysis, appearance, taste and flavor showed a positive correlation ($r = 0.749$, 0.720 and 0.942 , respectively) with NPN of the products, else a positive correlation ($r = 0.663$ $P < 0.01$) showed between flavor and *Staphylococcus* count in products. whereas pH value showed a significantly negative correlation ($r = -0.832$ $P < 0.01$) with taste.

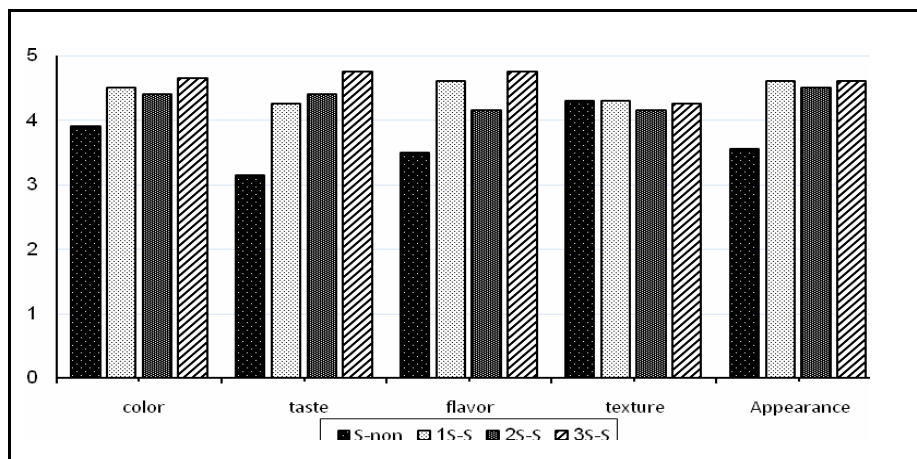


Fig. 2 Sensory evaluation of fermented sausage with/without starters

In conclusion, the mixed starter cultures decrease the pH quickly, inhibit the growth of contaminant microorganisms present in the raw materials and result in a better flavor and a finer texture of end-products.

Conclusions:

The addition of starters improved sensory characteristics in fermented sausage mixture and the S-S3 gave better sensory characteristics and approve the initiator of the third component of the bacterium *Lactococcus lactis*, *Lactobacillus sakei*, *Staphylococcus carnosus* and *Staphylococcus xylosus* in the manufacture of fermented sausage to give it the best organoleptic characteristics.

References:

1. Acton, J.C. 1977. The chemistry of dry sausages. In: Proceedings of 30th annual reciprocal meats conference. American Meat Science Association, Chicago, 49–62.
2. Savic, I.V. 1985. Small-scale sausage production, FAO animal production and health paper 52. Food and Agriculture Organisation of the United Nations, Rome.
3. Talon, R., Leroy-Setrin, S. and Fadda, S. 2002. Bacterial starter involved in the quality of fermented meat products. In: Research advanced in the quality of meat and meat products, Toldra, S. (Ed.). Research Signpost, India. pp. 175-191.
4. Incze, K. (1998). Dry fermented sausages. *Meat Science*,49:169–177.
5. Pond, T. J., Wood, D. S., Mumin, I. M., Barbut, S. and GriYths, M. W. 2001. Modeling the survival of *Escherichia coli* O157:H7 in uncooked semidry fermented sausage. *Journal of Food Protection*, 64: 759-766.
6. Jessen, B. 1995. Starter cultures for meat fermentation. In: Campbell- Platt, G. and Cook, P.E. Fermented meats. Chapman & Hall, New York. 130-159.
7. Leroy, F., Verluyten, J. and De Vuyst, L. 2006. Functional meat starter cultures for improved sausage fermentation. *International Journal of Food Microbiology*, 106: 270–285.
8. Schved, F.A., Lalazar, A., Henis, Y. and Juven, B.J. 1993. Purification, partial characterisation and plasmid-linkage of pediocin SJ-1 bacteriocins produced by *Pediococcus acidilactici*, *Journal of Applied Microbiology*,74: 67-77.
9. Parente, E., Greco, S. and 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.
10. Hugas, M., Garriga, M., Aymerich, T. and Monfort, J. M. 1993. Biochemical characterization of lactobacilli from dry fermented sausages. *International Journal of food Microbiology*, 18: 107-113.

11. Lücke, F. K. 1998. Fermented sausages. In: Wood, B.J.B. Microbiology of Fermented Foods. Blackie, London. 441-583.
12. Ray, B. 1992. Bacteriocins of starter culture bacteria as food biopreservatives: an overview. In: Ray, B. and M. Daeschel, (eds.). Food Biopreservatives of Microbial Origin. 177–205. Boca Raton, CRC Press, Florida.
13. Papamanoli, E., Tzanetakis, N., Litopoulou-Tzanetaki, E. and Kotzekidou, P. 2003. Characterization of lactic acid bacteria isolated from a Greek dry-fermented sausage in respect of their technological and probiotic properties. *Meat Science*, 65: 859-867.
14. Lücke, F. K. 1994. Fermented meat products. *Food Research International*, 27: 299- 307.
15. Verplaetse, A. 1994. Influence of raw meat properties and processing technology on aroma quality of raw fermented meat products. In: Proceedings of the 40th international congress on meat science and technology, The Hague, The Netherlands, 45–65.
16. Hammes, W.P. and Knauf, H.J. 1994. Starters in the processing of meat products. *Meat Science*,36:155–168.
17. Martin, A., Colin, B., Aranda, E., Benito, M. J. and Cordoba, M. G. 2007. Characterization of Micrococcaceae isolated from Iberian dry-cured sausages. *Meat Science*, 75: 696-708.
18. Mauriello, G., Casaburi, A., Blaiotta, G. and Villani, F. 2004. Isolation and technological properties of coagulase negative staphylococci from fermented sausages of Southern Italy. *Meat Science*, 67: 149-158.
19. Olesen, P. T., Meyer, A. S. and Stahnke, L. H. 2004. Generation of flavour compounds in fermented sausages - The influence of curing ingredients, Staphylococcus starter culture and ripening time. *Meat Science*, 66: 675-687.
20. Simonova, M., Strompfova, V., Marcinakova, M., Laukova, A., Vesterlund, S. and Moratalla, M. 2006. Characterization of Staphylococcus xylosus and Staphylococcus carnosus isolated from Slovak meat products. *Meat Science*, 73: 559-564.
21. AOAC. 2000. Meat and meat products. In: Cunnif P, editor. Official methods of analysis of AOAC international. DC., AOAC International, Washington. 16th Ed,1-23.
22. Wang, F. S. 2000. Effects of three preservative agents on the shelf life of vacuum packaged Chinese-style sausage stored at 20C. *Meat Science*,56:67–71.
23. Dierick, N., Vanderkerckove, V. and Demeyer, D. 1974. Changes in non-protein nitrogen compounds during dry sausage ripening. *Journal of Food Science*,39:301–304.
24. Fekkes, D. 1996. State-of-the-art of high-performance liquid chromatographic analysis of amino acids in physiological samples. *Journal of Chromatography B: Biomedical Sciences and Applications*,682:3–22.
25. Antoine, F., Wei, C., Littell, R., Quinn, B., Hogle, A. and Marshall, M. 2001. Free amino acids in dark- and white-muscle fish as determined by o-phthalaldehyde precolumn derivatization. *Journal of Food Science*, 66:72–74.
26. Lawless, H. T. and Heymann, H. 1999. *The Sensory evaluation of food principle and practice*. ANASDN publication, Gaithersburg-Maryland.
27. Sheridan, C. J. and Lyndall, S. G. 2001. *SPSS Analysis without Anguish*. Australia.
28. Yin, L. J., Pan, C. L. and Jiang, S. T. 2002. Effect of lactic acid bacterial fermentation on the characteristics of minced mackerel. *Journal of Food Science*, 67:786–792.
29. Bloukas, J. G., Paneras, E.D. and Fournitzis, G. C. 1997. Effect of replacing olive oil for pork backfat on processing and quality characteristics of fermented sausages. *Meat Science*,45:133–144.
30. Aksu, M. I. and Kaya, M. 2004. Effect of usage *Urtica dioica* L. on microbiological properties of sucuk, a Turkish dry-fermented sausage. *Food Control*,15:591–595.
31. Kaya, M. and Gokalp, H. Y. 2004. The behavior of *Listeria monocytogenes* in sucuks produced with different lactic starter cultures. *The Turkish Journal of Veterinary and Animal Sciences*, 28:1113–1120.
32. Aryanta, R. W., Fleet, G. H. and Buckle, K. A. 1991. The occurrence and growth of microorganisms during the fermentation of fish sausage. *International Journal of Food Microbiology*, 13:143–155.
33. Fadda, S., Oliver, G. and Vignolo, G. 2002. Protein degradation by *Lactobacillus plantarum* and *Lactobacillus casei* in a sausage model system. *Journal of Food Science*, 67:1179–1183.
34. Sakhare, P. Z. and Rao, N. 2003. Microbial profiles during lactic fermentation of meat by combined starter cultures at high temperatures. *Food Control*, 14:1–5.
35. Lois, A. L., Gutierrez, L. M., Zumalacarregui, J. M and Lopez, A. 1987. Changes in several constituents during the ripening of ‘Chorizo’— a Spanish dry sausage. *Meat Science*,19:169–177.

36. Johansson, G., Berdague, J. L., Larsson, M., Tran, N. and Borch, E. 1994. Lipolysis, proteolysis and formation of volatile components during ripening of a fermented sausage with *Pediococcus pentosaceus* and *Staphylococcus xylosus* as starter cultures. *Meat Science*,38:203–218.
37. Bover-Cid, M., Izquierdo-Pulido, M. and Vidal-Carou, M. C. 2000. Mixed starter cultures to control biogenic amine production in dry fermented sausages. *Journal of Food Protection*,63:1556–1562.
38. Bover-Cid S, Holzapfel WH (1999) Improved screening procedure for biogenic amine production by lactic acid bacteria. *International Journal of Food Microbiology*, 53:33–41.
39. Wardlaw, F. B., Skelley, G. C., Johnson, M. G. and Acton, J. C. 1973. Changes in meat components during fermentation, heat processing and drying of a summer sausage. *Journal of Food Science*, 38:1228–1231.
40. Garcia, I., Diez, V. and Zumalacarregui, J. M. 1998. Changes in nitrogen fraction and free amino acids during ripening of Spanish dried beef. *Journal of Muscle Foods*, 9:257–266.
41. Flores, J., Marcus, J. R., Nieto, P., Navarro, J. L. and Lorenzo, P. 1997. Effect of processing conditions on proteolysis and taste of dry-cured sausages. *Zeitschrift fuer Lebensmittel-Untersuchung und –Forschung*,204:168–172.
42. Hierro, E., de la Hoz, L. and Ordonez, J. A. 1999. Contribution of the microbial and endogenous enzymes to the free amino acid and amine contents of dry fermented sausages. *Journal of Agricultural and Food Chemistry*,47:1156–1161.
43. Demasi, T. W., Wardlaw, F. B., Dick, R. L. and Acton, J. C. 1990. Nonprotein nitrogen (NPN) and free amino acid contents of dry, fermented and nonfermented sausages. *Meat Science*,27:1–12.
44. Benito, M. J., Rodriguez, M., Martin, A., Aranda, E. and Cordoba, J. J. 2004. Effect of the fungal protease EPg222 on the sensory characteristics of dry fermented sausage "salchichon" ripened with commercial starter cultures. *Meat Science*,67:497–505.
45. Hughes, M. C., Kerry, J. P., Arendt, E. K., Kenneally, P. M., McSweeney, P. L. H. and O'Neill, E. E. 2002. Characterization of proteolysis during the ripening of semi-dry fermented sausages. *Meat Science*,62:205– 216.
46. Casaburi, A., Aristoy, M. C., Cavella, S., Di Monaco, R., Ercolini, D., Toldra, F. and Villani, F. 2007. Biochemical and sensory characteristics of traditional fermented sausages of Vallo di Diano (Southern Italy) as affected by the use of starter cultures. *Meat Science*,76:295–307.
47. Herranz, B., De la Hoz, L., Hierro, E., Fernandez, M. and Ordonez, J. A. 2005. Improvement of the sensory properties of dry-fermented sausages by the addition of free amino acids. *Food Chemistry*,91:673–682.
