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Microbiological Characteristics of Turkish Semi-Dry Fermented Sausage During Processing Stages and Storage

[1]Umit Gurbuz, [2]Mustafa Ardic and [3]Hilal Duygu Calim
[1]Department of Food Hygiene and Technology,
Faculty of Veterinary Medicine, Selcuk University, 42071 Konya, Turkey
[2]Department of Food Hygiene and Technology,
Faculty of Veterinary Medicine, Harran University, 63300 Sanliurfa, Turkey
[3]Ministry of Agriculture and Rural Affairs,
General Directorate of Agricultural Research, 06171 Ankara, Turkey

Abstract: This study was aimed to determine microbiological changes in various periods (raw material, after mixing, after stuffing, after heat application and in 10 days of storage) of Turkish semi-dry fermented sausage produced in a meat products plant located in Konya, Turkey. Coliforms, *Enterobacteriaceae*, yeasts-moulds counts of sausages stuffed into both natural and collagen casing decreased ($p<0.001$) after heat application and *Staphylococcus-Micrococcus* at the end of storage ($p<0.01$, $p<0.05$). On the other hand, significant increase ($p<0.01$, $p<0.05$) in lactic acid bacteria counts at the end of storage were found. In order to prolong the shelf life and to improve the microbiological quality of semi-dry fermented sausage, lower initial microbial levels of meat and other ingredients, effective heat treatment during heat application, careful handling of sausage and maintenance of appropriate chill temperature during storage are necessary.

Key words: Sausage, semi-dry fermented sausage, processing, microbiological characteristics

INTRODUCTION

Sausage is very popular and highly relished meat product all over the world. Recently, consumer's awareness has increased for microbiological quality of sausages. Thus, an understanding of microbial characteristics of sausage is vital. Microbial ecology of sausages is closely related to meat and other ingredients as well as environment, equipment, handling practices, processing, packaging and storage temperature (Sachindra *et al*., 2005). Microflora of sausage is composed of technological microorganisms (Lactic acid bacteria and Gram-positive cocci) that are useful for fermentation and flavour, spoilage microorganisms that can cause negative changes in appearance, odour, flavour and consistency of the final product due to their metabolic activity and may also include some pathogenic microorganisms (Lebert *et al*., 2007). In recent years, food operators have been urged to develop food hygiene procedures based on the principles of HACCP and good manufacturing practices, requiring that the safety of final products be demonstrated prior to marketing (Barbuti and Parolari, 2002).

Fermented sausages are divided into 2 subgroups. That is, they are named semidry or quickly fermented and dry or slowly fermented sausages (Savic, 1985). Both semidry and dry fermented sausages are produced in Turkey. Turkish dry fermented sausage has traditionally been produced without the addition of starter cultures. Both ripening and drying are carried out under natural climatic conditions during September and December (Soyer *et al*., 2005). Due to the long processing time and dependence on natural climatic conditions, dry fermented sausage production has been replaced by rapid ripening methods which mainly rely on the use of controlled drying chambers and starter cultures and heat application process to guarantee the safety and quality of the final product (Sanz *et al*., 1998).

During the last decade, modern plants in Turkey started to produce semi-dry fermented sausages with the addition of starter cultures and heat application. However, the characteristics of these sausages produced with the addition of starter cultures and heat application are quite different from the naturally fermented ones with respect to taste and flavour. Currently, there is a much higher market share for the sausages produced with starter cultures and heat application than the naturally fermented sausages (Soyer *et al*., 2005).

Corresponding Author: Mustafa Ardic, Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, Harran University, 63300 Sanliurfa, Turkey

Microbiological control of sausage at either production or storage stages for preparation of high quality product is the major concern. The safety of sausage is generally achieved by controlling or preventing growth of pathogen and spoilage microorganism during the process and reducing contamination to the lowest possible level. There are a great number of studies (Bozkurt and Erkmen, 2002; Aksu and Kaya, 2004; Kaban and Kaya, 2006; Colak *et al*., 2007) on dry fermented sausages in Turkey. On the other hand, information in the literature related to semi-dry fermented sausage very limited (Vural, 2003; Siriken *et al*., 2006a, b) and these studies have been generally carried out in final product and during storage. Thus, the aim of this study, was to investigate microbiological changes in some periods (raw material, after mixing, after stuffing, after heat application and in ten days of storage) of Turkish semi-dry fermented sausage produced in a meat products plant located in Konya, Turkey.

MATERIALS AND METHODS

Samples collection: Samples were taken four times in different periods of sausage production from a private meat products factory in Konya/Turkey. A flow diagram of Turkish semi-dry fermented sausage production is given in detail in Fig. 1. Samples were collected from raw material (meat), after grinding of meat, after mixing of meat with other ingredients, after stuffing and after heat application during production stages and in 10 days of storage. All samples were stored at 4°C and processed within 12 h of collection.

Microbiological analysis: Except Ringer's tablet, which was purchased from Merck (Darmstadt, Germany), all other microbiological media were obtained from Oxoid (Basingstoke, UK). For microbiological analysis, 25 g of samples were diluted in 225 mL of 1/4-strength Ringer's solution and homogenised in a Colworth Stomacher Lab-Blender 400 (Seward Medical, London, UK) for at least 2 min. The homogenate was decimally diluted in the

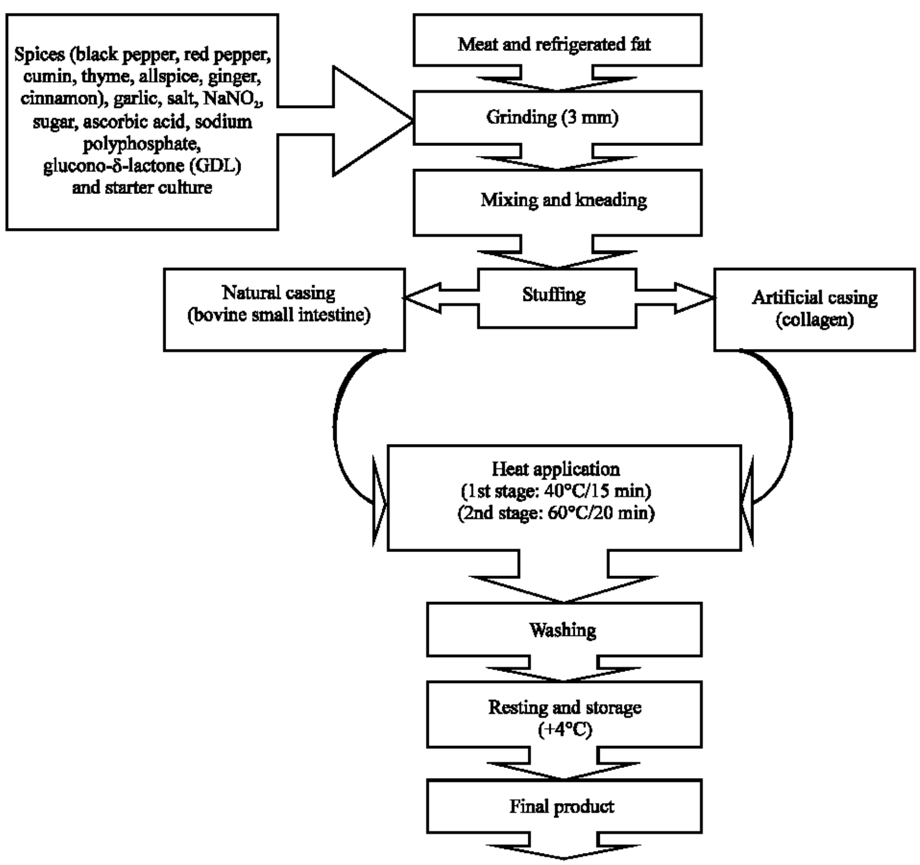

Fig. 1: Flow diagram of Turkish semi-dry fermented sausage production

same solution and each dilution was plated in duplicate on the specific media required for the different microbial groups to be examined (Messer *et al.*, 1992). The pour plate technique (1 mL) was used to determine Total Aerobic Mesophilic Bacteria (TAMB), yeasts and moulds and *Staphylococcus-Micrococcus* or the pour plate and overlay technique was utilized for determining coliforms, *Enterobacteriaceae* and Lactic Acid Bacteria (LAB) (Swanson *et al.*, 1992). Total aerobic mesophilic bacteria were enumerated on standard plate count agar after incubation at 30°C for 72 h. Yeasts and moulds were counted on potato dextrose agar acidified with 10% tartaric acid and incubated at 25°C for 5 days. *Staphylococcus-Micrococcus* were enumerated on mannitol salt agar at 37°C for 48 h. Coliforms were determined on violet red bile lactose agar after incubation at 37°C for 24 h. *Enterobacteriaceae* was enumerated on violet red bile glucose agar after incubation at 37°C for 24 h. LAB were determined on De Man, Rogosa, Sharpe agar after incubation at 30°C for 72 h. Plates with 30-300 colonies were counted and the results were expressed as logarithm of colony forming units (cfu) per gram ($\log_{10}$ cfu g^{-1}).

Measuring of pH: The pH values of samples were measured by pH meter (InoLab pH 720 model; WTW GmbH, Hamburg, Germany) equipped with a combined electrode.

Statistical analysis: A one way analysis of variance was performed on data obtained at different stages of production after a log transformation for bacterial counts, using the SPSS/PC version 10.00 (SPSS Inc, Chicago, IL, USA). Differences between the groups and the processing stages were identified using Duncan's multiple range test.

RESULTS AND DISCUSSION

Microorganisms gain access into sausage from meat, spices and other ingredients, intestine, environment, equipment and handlers during processing stages and this affect microbiological quality of final product. Lower initial microbial load of sausage mix and maintenance of adequate temperature during storage would improve the microbiological quality and enhance the shelf life of sausage (Sachindra *et al.*, 2005; Siriken *et al.*, 2006b).

The changes in the counts of microbial groups enumerated during processing stages and storage of Turkish semi-dry fermented sausages stuffed into natural and collagen casing are presented in Table 1 and 2. Processing stages and storage significantly affected coliforms, *Enterobacteriaceae*, *Staphylococcus-Micrococcus* and yeasts-moulds counts of sausages, which had lower counts in sausages stuffed into both natural and collagen casing after heat application for coliforms ($p<0.001$), *Enterobacteriaceae* ($p<0.001$) and yeasts-moulds ($p<0.001$) and at the end of storage for *Staphylococcus-Micrococcus* ($p<0.01$, $p<0.05$). On the other hand, LAB were significantly increased in sausages stuffed into both natural and collagen casing at the end of storage ($p<0.01$, $p<0.05$).

Table 1: Changes in microbiological profile during processing stages of Turkish semi-dry fermented sausage stuffed into natural casing ($\log_{10}$ cfu g^{-1})[1]

	Processing stage					
Microorganism	Raw material	After grinding	After mixing	After stuffing	After heat application	Storage
Total aerobic mesophilic bacteria	6.26±0.45	6.92±0.32	6.51±0.43	6.93±0.36	5.68±0.86	7.16±1.41
Coliforms	4.54±0.31^{a}	5.14±0.38^{a}	4.71±0.25^{a}	4.88±0.29^{a}	1.26±0.73^{b}	nd^{c}
Enterobacteriaceae	4.45±0.48^{a}	5.19±0.71^{a}	4.92±0.26^{a}	5.03±0.24^{a}	1.28±0.74^{b}	nd^{b}
Lactic acid bacteria	4.88±0.27^{a}	5.78±0.25^{a}	5.89±0.28^{a}	5.85±0.27^{a}	5.50±0.42^{a}	7.88±0.74^{b}
Staphylococcus-Micrococcus	5.20±0.20^{a}	5.93±0.23^{b}	5.65±0.28^{ab}	5.91±0.18^{b}	5.28±0.14^{ab}	4.51±0.28^{c}
Yeasts and moulds	3.76±1.45^{a}	4.41±0.57^{a}	4.59±0.76^{a}	5.24±0.50^{a}	nd^{b}	nd^{b}

Table 2: Changes in microbiological profile during processing stages of Turkish semi-dry fermented sausage stuffed into collagen casing ($\log_{10}$ cfu g^{-1})[1]

	Processing stage					
Microorganism	Raw material	After grinding	After mixing	After stuffing	After heat application	Storage
Total aerobic mesophilic bacteria	6.26±0.45	6.92±0.32	6.51±0.43	6.60±0.36	5.78±0.83	7.20±1.05
Coliforms	4.54±0.32^{a}	5.14±0.37^{a}	4.71±0.25^{a}	4.92±0.23^{a}	0.74±0.74^{b}	0.75±0.75^{b}
Enterobacteriaceae	4.45±0.48^{a}	5.19±0.71^{a}	4.92±0.26^{a}	5.14±0.27^{a}	0.76±0.76^{b}	0.68±0.68^{b}
Lactic acid bacteria	4. 88±0.27^{a}	5.78±0.25^{a}	5.89±0.28^{a}	5.15±0.72^{a}	6.00±0.41^{a}	7.70±0.80^{b}
Staphylococcus-Micrococcus	5.20±0.20^{abc}	5.93±0.23^{a}	5.65±0.28^{ab}	5.69±0.20^{ab}	5.03±0.05^{bc}	4.84±0.35^{c}
Yeasts and moulds	3.76±1.45^{a}	4.41±0.57^{a}	4.59±0.76^{a}	4.37±0.66^{a}	nd^{b}	nd^{b}

[1]Mean values and standard error of 4 samples; $^{a-c}$Means within the same line with different superscript letters are different ($p<0.05$) according to Duncan's multiple range test; nd: not detected

TAMB count of raw material (meat), which was detected 6.26 $\log_{10}$ cfu g^{-1}, was within the microbiological standards of raw red meat according to the Turkish Food Codex (2006). This initial TAMB count increased up to 7.16 $\log_{10}$ cfu g^{-1} in the sausage stuffed into natural casing and 7.20 $\log_{10}$ cfu g^{-1} in the sausage stuffed into collagen casing at the end of storage. However, the TAMB counts in both groups did not change significantly ($p>0.05$) during production stages and storage. TAMB counts of final product were lower than the findings of Siriken *et al.* (2006b), who found 73% (73/100) of the samples contained TAMB between the levels of 10^7-10^{10} cfu g^{-1}.

The initial count of coliforms in raw material was 4.54 $\log_{10}$ cfu g^{-1} and count increased at level 4.88 $\log_{10}$ cfu g^{-1} in sausage stuffed into natural casing and reached at level 4.92 $\log_{10}$ cfu g^{-1} in sausage stuffed into collagen casing after stuffing stage. But, the increase of coliforms count did not show any significance ($p>0.05$) during mixing and stuffing stages. After heat application, significant decrease was observed ($p<0.001$). This result demonstrates that heat application process was effective in reducing the coliforms counts. While coliforms were not detected in s a usage stuffed into natural casing at the end of storage ($p<0.001$), its count decreased to 0.75 $\log_{10}$ cfu g^{-1} in sausage stuffed into collagen casing ($p<0.001$). This observation confirms the strong competitive effect of LAB on the rest of the endogenous flora as is observed in sausage (Drosinos *et al*., 2005) and in other fermentations (Spyropoulou *et al*., 2001). Result obtained in this study was in agreement with those of Lücke (2000) and Chevallier *et al.* (2006), who stated that coliforms count declined very quickly and was totally inhibited within 7 days in more acidified sausages. On the other hand, Siriken *et al.* (2006b) have reported that contamination of semi-dry fermented sausages with coliforms and *Enterobacteriaceae* in high levels. This may be due to recontamination during handling of sausage.

The initial count of *Enterobacteriaceae* in raw material was 4.45 $\log_{10}$ cfu g^{-1}. Similar changes like coliforms were observed in the counts of *Enterobacteriaceae* in sausages stuffed into both natural casing and collagen casing ($p<0.001$).

The LAB may have an important contribution to the final flavour of the products due to their fermentation of carbohydrates (Lizaso *et al*., 1999). The initial count of LAB was low and other microorganisms such as *Staphylococcus-Micrococcus* had higher initial counts. LAB counts increased during processing stages and reached levels up to 7.88 and 7.77 $\log_{10}$ cfu g^{-1} of sausages stuffed into both natural and collagen casing at the end of storage, respectively ($p<0.01$, $p<0.05$). The count of LAB exceeded 7 $\log_{10}$ cfu g^{-1} and constituted the major microflora at the end of storage in sausages stuffed into both natural and collagen casing. Because of the good adaptation of LAB to the meat environment, the presence of NaCl and nitrite and their faster growth rates, which were displayed during fermentation and ripening of sausages (Drosinos *et al*., 2005; Chevallier *et al*., 2006), they became the dominant microflora at the end of storage.

Micrococcus and *Staphylococcus* are beneficial for their ability to reduce nitrates, hydrolyze lipids and destroy peroxides (Samelis *et al*., 1994; Fista *et al*., 2004). The initial count of *Staphylococcus-Micrococcus* (5.20 $\log_{10}$ cfu g^{-1}) was increased at level 5.91 $\log_{10}$ cfu g^{-1} in sausage stuffed into natural casing and reached at level 5.69 $\log_{10}$ cfu g^{-1} in sausage stuffed into collagen casing after stuffing stage. Thereafter, the counts decreased after heat application and storage stages in sausages stuffed into both natural and collagen casing. *Staphylococcus-Micrococcus* counts at the end of storage significantly lower than the other stages ($p<0.01$, $p<0.05$). Several authors have reported that the acidification and anaerobic condition inhibited the growth of *Staphylococcus-Micrococcus* during ripening of fermented sausage (Aksu and Kaya, 2004; Hu *et al*., 2008). Fista *et al.* (2004) stated that low temperatures of storage combined with high numbers of LAB and pH values below 5 cause growth of *Micrococcus and Staphylococcus* to cease. Our results were lower than those determined by Siriken *et al.* (2006b), who stated that 72% of the samples contained *Staphylococcus-Micrococcus* at the level $\geq 10^4$ cfu g^{-1}.

Yeasts and moulds count level of raw material was within the microbiological standards of raw red meat according to the Turkish Food Codex (2006). The initial count of yeasts and moulds in raw material was 3.76 $\log_{10}$ cfu g^{-1} and this count reached at level 4.59 $\log_{10}$ cfu g^{-1} after mixing of meat with other ingredients. Yeasts and moulds count reached 5.24 in sausage stuffed into natural casing and decreased 4.37 $\log_{10}$ cfu g^{-1} in sausage stuffed into collagen casing after stuffing stage. After heat application, yeasts and moulds were not detected in the samples ($p<0.001$). Similar results, were observed by Sachindra *et al.* (2005). They stated that cooking process was effective in reducing the yeasts and moulds counts substantially in sausage. Conversely, the counts of yeasts and moulds recorded in this study at the end of storage are not in agreement with the finding of Siriken *et al.* (2006b). They reported that 17% of the semi-dry fermented sausage samples were found highly contaminated with yeasts and moulds at

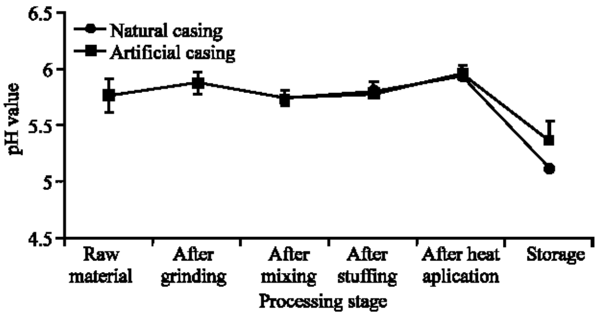

Fig. 2: Changes in pH values during production stages of Turkish semi-dry fermented sausage stuffed into natural and collagen casing

the level of 10^4 cfu g^{-1} and 34% of the contaminated samples were considered as non-consumable products with relation to Turkish Food Codex (2000).

According to Turkish Food Codex (2000), maximum pH value of sausage applied heat treatment should be 5.8. The changes in the pH values during processing stages and storage of Turkish semi-dry fermented sausage stuffed into natural and collagen casing are presented in Fig. 2. The initial pH value of raw material was 5.76. This value increased in grinding stage and decreased after mixing stage. After stuffing and heat application stages, pH values of sausages stuffed into both natural and collagen casing were again increased. During storage, pH values significantly decreased to 5.11 and 5.36 in both groups, respectively ($p<0.001$, $p<0.05$). The drop of pH because of the production of lactic acid by the increasing population of LAB at the end of storage, antagonism by other metabolic products produced by LAB and depletion of nutrients may have prevented the increase. In this respect, pH values was correlated to LAB counts at the end of storage and significant correlation ($r = -627$, $p<0.01$ for sausage stuffed into natural casing; $r = -598$, $p<0.01$ for sausage stuffed into collagen casing) was found between LAB and pH. Many researchers (Muguerza *et al*., 2002; Kayaardi and Gok, 2003; Vural, 2003) stated organic acids, mainly lactic acid are formed in fermented sausages as a result of carbohydrate breakdown during fermentation giving rise to the reduction in pH. The pH values obtained in this study at the end of storage were lower than those reported in semi-dry fermented sausages by Siriken *et al*. (2006a, b).

CONCLUSION

Hygienic quality of raw material has an important effect on final microbial load of sausage. Heat application is also the main stage for the elimination of non-desired microorganisms during the production of semi-dry fermented sausage. In order to prolong the shelf life and to improve the microbiological quality of semi-dry fermented sausage, lower initial microbial levels of meat and other ingredients, effective heat treatment during heat application, careful handling of sausage and maintenance of appropriate chill temperature during storage are necessary. In addition, the use of HACCP based control programs improves the quality and safety of the sausage during processing stages because hygienic status of the processing environment and equipment plays an essential role in the microbial stability and safety of the final products.

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