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ORIGINAL ARTICLE

Microbiological Quality of Sausage during Slicing at Food Retail Stores in Shiraz, Iran

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ABSTRACT

Background: Sausage can become contaminated via the environment, handlers, and equipment particularly slicing machines and cutting utensils during processing. We aimed to study the effect of slicing machines on microbiological quality of sausages at retail points in Shiraz, southern Iran.

Methods: Totally, 120 samples of sausage from different retail points were collected and analyzed for different microbiological indicators. The total viable microorganisms were enumerated using plate count agar (PCA). For coliforms and coagulase positive coagulase Staphylococci, violet red bile lactose agar (VRBL) and Baird-Parker agar were used, respectively. Enumeration of molds and yeasts was carried out using yeast extract chloramphenicol agar. For isolation of salmonella and *E. coli* national standard numbers 1810 and 2946 were used, respectively.

Results: The number of samples containing the total viable microorganism, coliform, positive coagulase Staphylococcus, yeast, and mold increased by about 140%, 151%, 123%, and 40%, respectively, during slicing but salmonella and *E. coli* were not detected in any of the samples. Moreover, differences in total viable microorganisms, coliform, and positive coagulase Staphylococcus counts were not significant in quadratic areas of Shiraz, whereas significant differences were detected in the counts of yeast and mold and the counts were higher in east Shiraz.

Conclusion: Generally, a relatively high microbial load of sausage samples indicated poor hygienic status of slicing machines, insufficient hand washing, improper handling and cleaning of equipment. Prevention of cross contamination and careful handling of the products and effective cleaning and sanitation programs play an important role in providing safety and quality of ready-to-eat meat products.

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Introduction

There is an increasing rise in the demand of meat products because of their pleasant taste and texture, fast cooking and preparation, and lower costs than red meat (1). Despite the enhancing popularity of these products, there is a concern about the microbial status of meat products (2). Sausage is one of the most famous meat products around the worlds (3). Generally, the main ingredients which are used for the production of sausage are meat, oil, and water. The nutritional composition of meat

products provides an appropriate environment for the growth and proliferation of food borne pathogens (4). Therefore, pathogen bacteria may contaminate sausage during processing which has contributed to food borne disease globally (5). Moreover, these products are known as a high risk food group (6).

It is known that microorganisms can contaminate sausage from meat, spices and other ingredients as well as the environment, handlers and equipment, particularly slicing machines and cutting utensils during processing that can have an important effect on the microbiological properties of the products (2). Based on epidemiological and microbiological studies, cross-contamination during processing and subsequent bacterial growth is the major reason of meat product contamination and disease (7). It is therefore essential that effective protection from post-cooking contamination and the inactivation of pathogenic bacteria are applied to maintain the safety and quality of meat products (8). Cross-contamination from food contact surfaces could contaminate food (9). Absent sanitizing and washing and improper cleaning procedures of slicer blades would lead to contamination of slicing machines that increase food borne disease in consumers (10). Therefore, training and supervision are necessary to ensure appropriate hand washing, proper cleaning and sanitizing in order to reduce cross-contamination (9). On the basis of the mentioned premises, we aimed to study the effect of slicing machines on microbiological quality of sausages at retail points in Shiraz, southern Iran.

Materials and Methods

All materials used were purchased from Merck, Germany. In this descriptive analytical study, 120 sausage samples (60 samples before slicing and 60 samples after slicing) from different retail points were collected randomly and transported in an ice box to the microbiological laboratory. Then, 10 gr of each sample was inserted into a stomacher bag containing 90 ml of 1% buffered peptone water and homogenized in a stomacher for 1 min. Then, decimal serial dilution (10^{-1}–10^{-5}) was prepared and used for microbiological analysis. This study was approved by the Local Ethics Committee.

The total viable microorganisms were enumerated by using plate count agar (PCA) and plates were incubated at 30°C for 72 hr (11). For Coliforms, violet red bile lactose agar (VRBL) was used followed by incubation at 37°C for 24 hr (12). Coagulase positive Staphylococci were counted in Baird-Parker agar and the plates were incubated at 37°C for 24-48 h (13). Enumeration of molds and yeasts was carried out using yeast extract chloramphenicol agar and the plates were incubated at 25°C for 3-5 days (14). Finally, results were expressed as log CFU/g.

The sausage samples were homogenized in buffered peptone water and incubated at 37°C for 20 hrs for pre-enrichment. In the enrichment stage, 1 ml of pre-enrichment culture was added into separate tubes containing selenite broth and tetrathionate broth. Selenite broth was incubated at 37°C for 24 hr and tetrathionate broth was incubated at 41.5°C for 24-48 hr. Following enrichment, culture from each enrichment broth was separately inoculated on plates of Salmonella-Shigella (SS) agar and xylose lysine deoxycholate (XLD) media by streaking. The inoculated plates were incubated at 37°C for 18-24 hr. Characteristic colonies from each media were confirmed biochemically on lysine iron agar and tested for Indole, Methyl red, Voges-Proskauer, Citrate (IMVIC) reaction (15).

Each dilution was added to tubes containing lauryl sulfate tryptose (LST) broth and was incubated at 37°C. The tubes were examined for gas production after 24 hrs. From each LST tube containing gas, a loopful was transferred into EC broth. The EC tubes were incubated at 44°C and were examined for gas production after 24 hrs. The complete test for *E. coli* was performed by taking each gassing EC tube and streaking it for isolation on LEMB agar plate. Isolates were tested for IMVIC reaction (16).

Probability value of less than 5% defined statistically significant. Statistical analysis was performed by SPSS software, version16. Frequency, mean, and standard deviations were calculated and Kruskal-Wallis, Wilcoxon, and McNemar's tests were used as appropriated.

Results

In the present study, positive coagulase Staphylococci was isolated from 2 samples of the post-sliced sausage. None of the post-sliced sausages were found positive for the presence of *E. coli* and Salmonella. Also, the contamination

rate of sausage samples before and after slicing in quadratic regions of Shiraz with respect to microbial factors is shown in tables 1 and 2.

Discussion

In this study the effect of slicing on microbial quality of sausage in Shiraz has been investigated. The counts of total viable microorganism, coliform, positive coagulase Staphylococcus, mold and yeast exceeded the Iranian standards in 8.3%, 3.3%, 6.7% and 25% of the pre-sliced sausages, respectively. The unacceptable microbial loads could indicate poor hygienic practices during preparation or storage, insufficient heat processing, contaminated raw material, improper handling and post-process contamination. For example, poor hygienic quality of raw material such as meat and spices could have led to contamination of final products. High microbial loads in the raw meat and the emulsion have been found by other authors. Rahimi and colleagues reported that 68%, 62%, 50%, 53%, and 21% of sausage paste samples exceeded the legal limit of standard for *Staphylococcus aureus*, yeast, *E. coli*, S*almonella spp.* and mold, respectively, which could be associated with contaminated raw material and insufficient heat processing (18). Moreover, Gungor and Gokoglu also evaluated the microbial contamination sources at a Frankfurter sausage processing line. They concluded that high microbial levels of minced meat and spice mix affected contamination of the final products (2).

In our study, no salmonella and *E. coli* were detected in the pre-sliced sausages. It is reported that the detection of salmonella in ready-to-eat meats is a potential risk of food borne disease (19). In order to inactivate salmonella, it is necessary to heat the sausages at 70°C for a minimum of 2 min (20). So, the presence of *Salmonella spp.* in the sausage samples could indicate inadequate cooking process or post-process contamination (21). Luiz and co-workers found that the pathogen was present in 6 out of 185 samples of the raw meat and the emulsion whereas there was no incidence of salmonella in cooked Frankfurter sausage (22). This showed that effective heat processing during cooking would eliminate the pathogen from the end-product.

In the present study, the mean microbiological count value of total viable microorganism, coliform, positive coagulase staphylococcus, yeast, and mold exceeded the Iranian standard limit in 20%, 8.3%, 15% and 35% of the post-sliced sausage samples, respectively; but salmonella and *E. coli* were not detected in any of the sausage samples. Furthermore, the number of samples containing the total viable microorganism, coliform, positive coagulase

Table 1: Comparison between microbiological count value and standard limit in sausage samples during slicing.

Microbial groups	Samples	Log CFU/g (Mean±SD)	Standard limit* (Log CFU/g)	*p*-value
Total viable microorganisms	Pre-sliced sausage	3.23±1.23	5	0.001
	Post-sliced sausage	4.17±1.10	5	
Coliform	Pre-sliced sausage	0.11±0.60	1	0.29
	Post-sliced sausage	0.25±0.91	1	
Positive coagulase Staphylococcus	Pre-sliced sausage	0.19±0.73	<1	0.006
	Post-sliced sausage	0.47±1.10	<1	
Yeast and mold	Pre-sliced sausage	0.92±1.26	2	0.01
	Post-sliced sausage	1.47±1.47	2	

*Iranian national standard, Number: 2303 (17).

Table 2: Comparison between microbiological count values of post-sliced sausage samples in quadratic areas of Shiraz.

Area	Log CFU/g (Mean±SD)			
	Total viable microorganisms	Coliform	Positive coagulase Staphylococcus	Yeast and mold
North	3.56±0.62	0±00	0.50±1.22	0.38±0.94
South	4.01±0.96	0.18±0.81	0.36±0.94	1.77±1.36
East	4.37±1.25	0.33±1.15	0.13±0.46	1.88±1.42
West	4.35±1.18	0.35±0.90	1.13±1.63	0.92±1.60
P value	0.29	0.714	0.176	0.048

staphylococcus, yeast and mold increased by about 140%, 151%, 123%, and 40%, respectively, during slicing. According to statistical analyses, significant differences were detected in the counts of total viable microorganism; positive coagulase Staphylococcus, yeast and mold before and after slicing but the counts were lower than Iranian standard limits. Although differences in coliform counts during the slicing were not significant, the counts of samples containing coliform increased by about 151%. Several causes could explain the relatively high counts of the bacterial groups during slicing procedures. For example, incorrect cleaning of slicing machines could lead to contamination of sausages during slicing. Our results are consistent with other studies indicating that slicing is a critical control point for contamination and transfer of pathogens to sliced products (23). High load of coliform, *E. coli* and *S. aureus* on meat slicer blades and in cooked sliced meats indicated that slicing machines could be a source of contamination and cross contamination. Another study also reported contamination during slicing as cause of high microbial levels in retail sliced dry sausages collected from different retail shops in Addis Ababa, Ethiopia. In fact, slicing can be a microbial hazard due to the potential for transfer of pathogens via the slicing blade (21). On the other hand, many microorganisms such as salmonella can be transferred from the slicer to meat products during slicing procedures (24). Similar finding have been reported by another study showing that *S. aureus* and *E. coli* O157:H7 can transfer from the blade of slicing machines to all sliced meat products (25).

Based on the statistical analysis in the present study, it can be stated that differences in total viable microorganisms, coliform and positive coagulase staphylococcus counts were not significant in quadratic areas of Shiraz, whereas significant differences were detected in the counts of yeast and mold and the counts were higher in the east of Shiraz. Differences observed in quadratic areas of Shiraz could be due to variable levels of cleaning and disinfection during slicing procedures. The cleaning and disinfection of equipment surfaces during processing and slicing could affect the safety and quality of the end-product (6). These finding were similar to another study showing that tested equipment such as knives, tables and mincing machines were highly (>4 log CFU/cm^2) contaminated by pathogens. Moreover, 11.7%, 26.4% and 11.7% of the food contact surfaces were contaminated by *Listeria monocytogenes*, *Salmonella spp.* and *S. aureus*, respectively (26). In another report, the researchers found that the design of equipment plays an important role in the cleaning and sanitizing program and microbial stability and quality of the final products (6). In fact, poor hygienic design could lead to decreased sanitizing and cleaning frequency via biofilm formation. Consequently, microorganisms could be transferred from contaminated surfaces to products that pass over them. In this sense, Keskinen and colleagues investigated impact bacterial stress and biofilm-forming ability on transfer of surface-dried *L. monocytogenes* during slicing of delicatessen meats. They indicated that the combination of time, product, injury and biofilm-forming ability affected *L. monocytogenes* transfer during slicing (27). Therefore, attention needs to be given to appropriate cleaning and sanitizing of food contact surfaces to inhibit the formation of resistant strains in niches during slicing.

One of the important risks of food contamination resulted from handling food with bare hands by food handlers and transfer of disease-causing microorganisms from food handlers to the products during handling. Moreover, poor hygienic status such as negligence to wash hands by workers could lead to contamination of the products (2). Hence, training the personnel about proper hand washing and hygienic handling of products has a positive effect on food safety. As it was shown by Gillespie and co-workers who reported that premises where the manager received advanced food hygiene training (14%) provided significantly less cold ready-to-eat sliced meats of unacceptable microbial quality compared with premises which the manager received intermediate (23%), basic (26%) or no (33%) food hygiene training. Moreover, significantly higher cold ready-to-eat sliced meat samples of unsatisfactory microbial quality were from the catering premises that used re-usable cloths (28%) compared with premises which used disposable cloths (24%) or both types of cloth (22%) (28).

Conclusion

Generally, a relatively high microbial load of sausage indicated poor hygienic practices and

lack of quality control during production or after processing of the products. Therefore, prevention of cross contamination and careful handling of the products and effective cleaning and sanitation program are essential to produce safer products. Since the knowledge and attitude of personnel is affected the food hygiene, training the staffs for appropriate hand washing and glove use are also advisable.

According to the results of the study, a well implemented hazard analysis and critical control points (HACCP) system and monitoring of procedures on good manufacturing practice is necessary to assure microbial quality of the products and consumers' health. Finally, application of vacuum packages and official supervision by inspectors plays an important role in providing safety of ready-to-eat meat products.

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Conflict of Interest

None declared.

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