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Microbiological Study of Cocktail Sausage During Shelf Life

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Abstract: Cocktail sausage is one of most famous and most adherent Sausage. Shelf life of meat products treated by heat depends on some factors. The most important factor is the microbiological quality of raw materials. This study was made on cocktail sausage produced indoor without vacuum-packaging. A total of 10 samples with the same production date taken randomly from different batches were collected. These samples were studied from production time to 5th week. The total bacterial, mold and yeast, coliforms, psychrophilic microorganisms` and coagulase positive *Staphylococcus aureus* counts were performed on basis of No. 2303, 1194,437,997 and 356 protocols of the Iranian Standard Institute. The results were analyzed by paired T-test and means of microbial counts were compared in the different weeks. The results showed a significant increase ($p<0.05$) in microbial counts in the different weeks. No coliforms were detected in the 5 week study period.

Key words: Microbial Properties · Cocktail Sausage · Shelf Life.

INTRODUCTION

Foodborne disease and microbial spoilage of food result from the failure or inability to control microorganisms at one or more stages of the food chain from raw material production to consumption of the final product. The implications of situations that result in food poisoning outbreaks or food spoilage can be severe for food producers, retailers, consumers and regulatory authorities. Traditionally, control of microorganisms in a food has been demonstrated by microbiological testing of samples at various stages of production and of the final product. The potential for growth and/or toxin production of residual microbial populations in finished products depends on the types of organisms present and their ability to grow to a level of concern under the storage conditions applied during the product shelf life [1]. Microbiological criteria should take an account of any organisms likely to be present. The levels of tolerance applied at the point of manufacture should be such that allowing for predictable growth of these organisms, the product will remain safe and whole some to the end of shelf life provided it is stored under the correct conditions. It is the industry practice to build in a safety margin for chilled products which allows for mild temperature abuse of product during storage. Meat products may be contaminated with microorganisms from meat handlers, who carry pathogenic microorganism during the processes of manufacturing, packing and marketing. Improper cooking, refrigeration or storage may lead to meat borne illness [2]. Foodborne pathogens are the leading causes of illness and death in developing countries costing billions of dollars in medical care, medical and social costs. Meat products are products that insist of at least one half of it meat. These products are one of the most consuming food products. Sausage is a popular and highly relished meat product world over. Dry Sausage is produced as a result of finding of new spices. These spices can help to improve flavor and increasing of shelf lif of meat. Many kinds of Sausage are produced depending on raw material in area and different climates [1-4]. Microbial growth during storage is one of the main factors affecting the quality of meat products, leading to spoilage and hence economic losses. Emulsion-type sausages like wiener, bologna-type sausage and hot smoked sausage may spoil more quickly due to high pH and aw (activity water). Many vegetative cells can be inactivated with cooking process [3, 4]. Sausage may be contaminated after heat processing and during other processes such as slicing, packaging and peeling [5, 6]. Many studies have determined the presence of foodborne pathogens in these products, such as *Listeria*

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monocytogenes, *Staphylococcus aureus*, *Clostridium perfringens* and *Salmonella spp*. [7]. As for *L. monocytogenes*, several studies showed that emulsion-type sausage is risky, because the bacteria have high tolerance to physical conditions compared to other pathogens. Moreover, this food-borne pathogen is able to grow at refrigeration temperatures [8, 9]. The safety of food of animal origin for human consumption has become an essential part of the public health debate. Thus, microbial ecology of meat products will mainly depend on the environment, kind of meat and raw materials, equipment, handling practices, processing, packaging and storage temperature. Information on microbiology of cocktail sausage is very limited; therefore objective of the study was to understand the microbial properties of cocktail sausage during shelf life.

MATERIALS AND METHODS

A total of 10 samples of cocktail sausage with similar production date from different batches were collected and transported in an ice box at 4°C to laboratory of food hygiene in Islamic Azad University Tabriz branch. Study on microbial properties during shelf life from first week until fifth week was done. Culture of samples and identification of the suspected colonies was done according to the standard methods [10-14]. For microbial counts, 10 grams of each cocktail sausage representative sample were removed aseptically and homogenized in 90 ml of 1.5% peptone water, using a stomacher and inoculated onto plate count agar (Merck) (incubated at 37°C for 48h), using the pour-plate method and violet neutral red bile lactose agar (Merck) (incubated at 37°C for 24 h), for coliform counts. Cooked meat salt medium (Merck) and Bird Parker agar (Merck) were used for coagulase positive *Staphylococcus aureus* counts (incubated at 37°C for 48h). For mold and yeast total counts, Sabaroud Dextrose agar (Merck) plates incubated at 25°C for 5 days were used. Psychrophilic microorganisms' counts were carried out by use of nutrient agar (Merck) plates incubated at 5-20°C for 24h [10-14]. Data were analyzed by using SPSS (version 12) software and Paired T- test.

RESULTS

Results of this Study Were Divided to 5 Parts: The microbiological analysis results of this study showed that, the count at first, second and fourth weeks was lesser than 10^5CFU/gm (Maximum permissible count for total bacteria is). By attention to results of this part during 5th weeks, total bacterial count mean comparing with those in weeks 5 showed significant difference ($p<0.05$) (Table 1).

Table 1: Mean, standard deviation and standard error mean of cocktail sausage total bacterial counts (One-Sample Statistics).

Week	Sample No.	Mean	Std.Deviation	Std. Error Mean
1	10	0.6200	0.69250	0.21899
2	10	3.3800	8.00747	2.53218
3	10	59.3000	67.51798	21.35106
4	10	13.2700	7.41681	2.34540
5	10	549.5000	825.87977	261.1661

Table 2: Mean, standard deviation and standard error mean of cocktail sausage coagulase positive *Staphylococcus aureus* counts (One-Sample Statistics).

Week	Sample No.	Mean	Std.Deviation	Std. Error Mean
1	10	0.3100	0.30350	0.9597
2	10	0.2300	0.25408	0.08035
3	10	2.6100	4.52780	1.43182
4	10	4.7700	5.95503	1.88314
5	10	9.6000	21.39159	6.76461

Table 3: Mean, standard deviation and standard mean of cocktail sausage total psychrophilic microorganism counts (One-Sample Statistics).

Week	Sample No.	Mean	Std.Deviation	Std. Error Mean
1	10	0.5300	0.55187	0.17452
2	10	1.0800	1.72292	0.54483
3	10	33.0600	98.74584	31.22618
4	10	51.2000	82.80806	26.18621
5	10	510.8470	934.37661	295.4758

Table 4: Mean, standard deviation and standard mean of cocktail sausage total mold and yeast counts (One-Sample Statistics).

Week	Sample No.	Mean	Std.Deviation	Std. Error Mean
1	10	1.8600	4.62390	1.46221
2	10	2.7800	4.54552	1.43742
3	10	4.0400	4.40585	1.39325
4	10	37.1000	62.95312	19.90753
5	10	537.5600	918.53513	290.4663

Permissible count for coagulase positive *Staphylococcus aureus* is 0 CFU/gm. In this study, the count in first week got through the permissible range. Results of coagulase positive *Staphylococcus aureus* count means in weeks 1, 2 and 4 showed significant difference ($p<0.05$) (Table 2).

The acceptable psychrophilic microorganisms` count is 10^2 CFU/gm, in this study, the count of psychrophilic microorganisms during 5 weeks has been increased and this increase was very significant in the 5^{th} week in comparison with other weeks (Table 3).

The permissible count of mold and yeast in Iranian standard institute is 10^2 CFU/gm and the samples in the 5th week showed abnormal range. For this group of microorganisms, between weeks a significant difference was not observed (Table 4).

In present study, no coliforms were isolated from the collected samples during the 5 weeks and this agrees with the Iranian standard institute acceptability.

DISCUSSION

Microbial contamination may be added or reduced at different stages of processing of hot smoked sausage. Friedhoff *et al*. [15] have described the use of simple microbiological criteria, including aerobic mesophilic colony counts, *Enterobacteriaceae* counts and in some instances, enumeration of yeast, performed on samples taken during processing in small businesses to verify good manufacturing practices. This verification through monitoring was found to be an attractive alternative to the examination of end products. Coliform bacteria are one of most important indicator organisms that are most commonly used to ensure food safety [16-18]. This verification through monitoring was found to be an attractive alternative to the examination of end products. Many investigators stated that coliform bacteria, fecal coliform bacteria, *E. coli*, total *Enterococcus* spp. and aerobic plate count (APC) the most important indicator organisms that are most commonly used to ensure food safety [19-21]. In present study, coliform bacteria were not isolated. Dowdell and Board [22] carried out a microbiological survey of British Fresh Sausage and reported the presence of coliform bacteria. Also in one study by Sachindra *et al*. [23] who isolated coliforms from raw and cooked sausage and in cooked form of sausage less than raw sausage and added that cooking process reduce the microbial counts substantially in the sausage. Staphylococcus aureus is one of the most common agents in bacterial food poisoning outbreaks. It is also a major causative pathogen of clinical or subclinical mastitis of dairy domestic ruminants. Poultry, meat and egg products as well as milk and milk products have been reported as common foods that may cause staphylococcal food poisoning [24]. Foods of animal origin, especially milk and dairy products, are associated with food borne disease [25]. Staphylococcus aureus is one of the commonest etiological agents of bacterial diseases worldwide due to its ability to produce a broad range of exotoxins and other virulence factors. Among them, the staphylococcal enterotoxins produced by some *S. aureus* strains are the main causal agents of one of the most widespread food borne intoxications. The staphylococcal food poisoning and, together with toxic shock syndrome toxin-1, are responsible for toxic shock syndrome and staphylococcal scarlet fever [26, 27]. Sachindra *et al*. [23] reported the absence of *Staphylococcus aureus* in raw and cooked sausage. The present study showed that *Staphylococcus aureus* was isolated from hot smoked sausage samples in the first week. In a study made by Apaidin *et al* [28] on the chemical quality of vacuum-packaged hot smoked sausage, *Staphylococcus aureus* was isolated from the studied samples. These results show the importance of *Staphylococcus aureus* in food industry because this agent causes dangerous diseases in human. Vacuum packaging is generally used for cooked products and these products have scarcely high yeast and mold counts [29, 30]. High yeast and mold counts may have originated from insufficient vacuum and/or refracted vacuum. In this study, yeast and mold counts ranged from $1.86\pm2.92\times10^3$ CFU/gm in first week to $5.37\pm5.8\times10^5$ CFU/gm in fifth week and these results agree with Apaidin *et al*. [28], but this range in comparison with a study by Sachindra *et al*. [23] is high. Psychrophilic organisms` counts in the first week of shelf life was non permissible and a significant different ($p>0.05$) between all weeks of shelf life were not observed.

Sachindra *et al*. [23] reported that these microorganisms show a low growth during time of the study [23]. In a study on vacuum packed cooked ring sausage during storage, a shelf life of 20-28 days [31] and 43 days [32] has been found. A shelf life of 49 days in hot smoked sausage packed in CO_2 was recorded [33]. Some studies showed that the augment of the additives, onion, garlic meal, pepper and E vitamin can decrease the microbial agents in cooked sausage products [34-37].In conclusion, the study revealed the pattern of microbial profile associated with the preparation of cocktail sausage. Lower initial microbial levels of sausage mix, effective heat treatment during cooking, careful handling of cooked sausage and maintenance of adequate chill temperature during storage would improve the microbiological quality and enhance the shelf life of cocktail sausage.

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