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A Survey on Microbiological and Chemical Quality of Vacuum-Packaged Frankfurters

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ABSTRACT : The objective of this research was to determine chemical and microbiological properties of frankfurter samples obtained from retail market in Erzurum. Aerobic plate, lactic acid bacteria, *Enterococcus* and *Bacillus* counts varied from <3.30 to 8.00 log CFU/g, <2.00 to 8.15 log CFU/g, <2.00 to 5.04 log CFU/g, <2.00 to 4.46 log CFU/g, respectively. Yeast - mould in 96.7 % of samples were <3.3 log CFU/g. *Enterobacteriaceae* and coliform group bacteria counts were also <1.00 log CFU/g in 93.3 % of the samples. None of the samples had *L. monocytogenes* and *Salmonella*. *E. coli*, *Pseudomonas* and *C. perfringens* counts were under detectable level in all samples. In addition, *S. aureus* numbers were below 3 MPN/g in 90.0 % of the samples. The moisture/protein ratio of samples ranged from 4.09 to 6.45 and pH values ranged from 4.26 to 6.61. The residual nitrite was detected in all samples with amounts lower than 30ppm. The hidroxyproline contents of samples were determined to be between 0.101 and 0.218 %.

Keywords: Frankfurter, pathogen flora, chemical properties

Vakum Uygulanarak Ambalajlanmış Sosislerin Mikrobiyolojik ve Kimyasal Özellikleri

ÖZET : Araştırmanın amacı, Erzurum piyasasından temin edilen sosis örneklerinin kimyasal ve mikrobiyolojik özelliklerinin belirlenmesidir. Örneklerin aerobik bakteri, laktik asit bakteri, *Enterococcus* ve *Bacillus* sayıları sırasıyla <3.30 to 8.00 log CFU/g, <2.00 to 8.15 log CFU/g, <2.00 to 5.04 log CFU/g, <2.00 to 4.46 log CFU/g arasında değişmektedir. Örneklerin %96.7'sindeki maya-küf sayısı <3.3 log CFU/g'dır. Ayrıca örneklerin %93.3'ünde *Enterobacteriaceae* ve koliform grubu bakteri sayıları <1.0 log CFU/g'dır. Örneklerden *L. monocytogenes* ve *Salmonella* izole edilememiştir. Bütün örneklerdeki *E. coli*, *Pseudomonas* ve *C. perfringens* sayıları ise saptanabilir seviyenin altındadır. *S. aureus* sayısı ise örneklerin%90'ında 3 MPN/g'ın altındadır. Örneklerin nem/protein oranı 4.09-6.45 arasında, pH değeri ise 4.26-6.61 arasında değişmektedir. Bütün örneklerde kalıntı nitrate miktarı miktarı 30 ppm'den daha azdır. Örneklerin hidroksiprolin içeriğinin ise %0.101 ve %0.218 arasında olduğu belirlenmiştir.

Anahtar Kelimeler: Sosis, Patojen Flora, Kimyasal Özellikler

INTRODUCTION

Emulsion-type sausages like wiener, bologna-type sausage and frankfurter may spoil more quickly due to high pH and a_w. Many vegetative cells can be inactivated with cooking process (Tändler 1986; Price and Schweigert 1987). Sausage may be contaminated after heat processing and during other processes such as slicing, packaging, peeling (Cygnarowicz-Provost et al. 1994; Mckellar et al. 1994). Many studies have determined the presence of food-borne pathogens in these products, such as *Listeria monocytogenes*, *Staphylococcus aureus*, *Clostridium perfringens*, and *Salmonella* spp. (Guang-Hua and Xiao-Ling 1994; Wang and Muraina 1994). 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 (Schmidt and Kaya 1990; Krämer and Baumgart 1992; Wang and Muraina 1994). However, there is a lack of information on chemical and microbiological properties of emulsion-type sausage in Turkey (Ertaş and Kolsarici 1983; Vural et al., 1996). Ertas and Kolsarici (1983) have reported that moisture, protein, fat, salt and hidroxyproline contents of samples randomly selected from the retail market in Ankara were 52.54-63.34%, 9.00-15.52%, 8.94 -

19.33%, 1.60-2.68%, 0.131-0.318 g/100g, respectively. Vural et al. (1996) demonstrated that minimum and maximum values for aerobic plate, yeasts - moulds, and coliform group bacteria were obtained as; $2.60x10^3$-$3.07x10^7$, $4.40x10^2$-$7.63x10^6$, <10-$1.51x10^5$ CFU/g, respectively. It was also determined that moisture, salt, fat ratios, pH value and residual nitrite content were 52.49-65.98%, 0.56-2, 33%, 10.70-24.52, 5.53-6.93, and 3.93 244.05 ppm, respectively (Vural et al. 1996). There has not been a detailed research on general composition and microbiological properties of emulsion type sausage on retail market in Turkey.

The purpose of this study was to determine chemical and microbiological qualities of frankfurters, sold in retail market in Erzurum.

MATERIALS AND METHODS

Samples

The study was carried out by using a total of 30 vacuum-packed frankfurters, stored at refrigerated shelves purchased randomly from retail markets in Erzurum, Turkey. The samples were transported to laboratory in an ice box and then microbiological and chemical analyses of samples were examined immediately.

Bacteriological Analysis

Each sample (25 g) was homogenized in 225 ml of physiological saline solution containing 0.85 % (w/v) NaCl using a stomacher (Lab Blender 400 BA 7021 SewardMedical). Serial dilutions were prepared using the homogenized solution for microbiological examination. All microbiological examinations were plating duplicate.

Aerobic plate counts (APC) was enumerated on Plate Count Agar (Fluka) incubated at 30 °C for 3 days. To count lactic acid bacteria, de Man Rogosa Sharpe Agar (Fluka) was incubated at 30°C for 3 days in anaerobic jars, and, catalase (-) colonies were examined. Citrate Azide Tween Carbonate Agar (Merck) was used for *Enterococcus* and incubated at 37°C for 48 h. Red or rose pink colonies were confirmed and counted using methods demonstrated by Baumgart et al. (1986) Potato Dextrose Agar (Merck) plates were incubated at 25°C for 5 days, and then examined for yeasts and moulds. To count *Enterobacteriaceae*, Violet Red Bile Dextrose Agar (Oxoid), incubated at 30°C for 48 h in anaerobic jars, was used. Violet Red Bile Agar (Oxoid), which was incubated at 37°C for 24 h, used for coliform counts. Dark red colonies were counted and confirmed using the methods stated by Gökalp et al. (1995). The colonies defined as coliform were also confirmed as *E. coli* by using IMVIC test and API 20 (Biomerieux, France). *Bacillus* spores enumerated according to the method described by Baumgart et.al (1986). Sulfite Polymyxin Sulfadiazin Agar (Fluka) was incubated at 37°C for 24 h in anaerobic jar used for *Clostridium perfringens*. *Staphylococcus aureus* was determined by using Most Probable Number (MPN) methods. For *Pseudomonas* counts, Cetrimid - Fusidin Cephaloridin (CFC) Agar (Oxoid) was used and incubated at 25°C for 48 h. Oxidase (+) colonies were counted (Baumgart et.al.1986).

For *Salmonella* detection, each sample (25 g) was homogenized in 225 ml of buffered peptone water (Fluka) and incubated at 37°C for 18 h. After this, 1 ml of the homogenized mixture was transferred into 10 ml of Rappaport Vassiliadis (RV) Broth (Merck) and Selenite Cystine Broth (SSB) (Fluka), and incubated at 43°C for 48 h in RV- Broth and at 37°C for 48 h in SSB. The enriched cultures were streaked onto plate of Brillant Green Agar (Fluka), Bismuth Sulfite Agar (Oxoid) and *Salmonella* - *Shigella* Agar (Merck) and incubated at 37°C for 24 h. Typical colonies were streaked onto Vio1et Red Bile Agar (Oxoid) and incubated at 37°C for 24 h (Baumgart et.al 1986). The colorless colonies were further confirmed by Triple Sugar Iron Agar (Oxoid), Lysine Iron Agar (Fluka) and Urea Broth (Merck) at 37°C for 24 to 48 h and finally API-20 E (Biomerieux, France).

For *Listeria* detection, each sample (25g) was mixed with 225 ml UVM broth (Merck) and incubated at 30°C for 24 h. Then, 0.1 ml was transferred into 10 ml of FRASER Listeria Selective Enrichment Broth (Merck) and incubated at 30°C for 24 h. Tubes containing black precipitates were streaked onto Listeria Se1ective Agar (Merck) and Palcam Agar (Merck). The plates were incubated at 37°C for 24 to 48 h. Typical colonies on Palcam Agar and suspected *Listeria* colonies selected by Henry's Oblique Light Technique on Listeria Selective Agar were streaked onto Tryticase Soy Agar (Oxoid) and incubated at 37°C for 24 h. Typical grayish blue colonies were selected by Henry's Oblique Light Technique and confirmed by biochemical and morphological methods, including Gram straining, motility (at 22°C), cata1ase, D-glucose, D-salicin, urease, oxidase, esculin, Voges - Proskauer . Confirmed isolates were identified by carbon hydrate fermentation (L-rhamnose, α-metil-D-mannosid, D- xylose), β hemolysis and a CAMP test (Schmidt 1989).

Chemical Analysis

Total protein, fat, moisture and pH analyses were performed using procedures outlined by Gökalp et al**.** (1995). Hidroxyproline, residual nitrite and salt were determined according to the methods outlined by Tauchmann (1987).

RESULTS AND DISCUSSION

The microbiological analysis results showed that none of the samples had *Listeria monocytogenes*. However, *Listeria innocua* was isolated and identified from only one sample. Heat treatment in frankfurter processing is sufficient to inactivate *Listeria* (Zaika et al. 1990). A probable explanation for the presence of *L. innocua* is that it may result from post processing contamination (Schmidt and Kaya 1990). *Salmonella* spp. was not determined in any sample, although two different selective enrichments were done. Both *E. coli* and *C. perfringens* counts were found to be <1.00 log CFU/g and *Pseudomonas* was also <2.00 log CFU/g in all samples. In addition, *S. aureus* numbers were below 3 MPN/g in 90.0 % of the samples (Table 1). The samples were generally thought as safe from pathogen flora. However, aerobic plate counts of the frankfurters were found to be high. Aerobic plate counts were between <3.30 and 8.00 log CFU/g (Table 1). This APC range is similar to the range reported by Vural et al**.** (1996). Lactic acid bacteria were usually predominant in the samples. Lactic acid bacteria counts were between <2.00 and 8.15 log CFU/g (Table 1). Lactic acid bacteria are considered a major component of the microbial population found on various types of vacuum-packaged emulsion-type sausages (Schmidt and Kaya 1990). The frankfurters may be recontaminated with lactic acid bacteria mainly during processing stages after cooking (Korkeala and Björkroth 1997). Also, some heterofermentative lactic acid bacteria may survive heat processing, and can multiply at low temperatures (Schmidt and Kaya 1990; Makela and Korkeala 1992). Both *Enterobacteriaceae* and coliform group bacteria counts were <1.0 log CFU/g in 93.3 % of the samples (Table 1). Coliform group bacteria counts in this study were lower than those determined by Vural et al**.** (1996). *Enterococcus* counts were <2.00-5.04 log CFU/g (Table 1). *Enterococcus* is known as indicator bacteria in food processing. *Entereococcus* counts are 4- 5 log cfu/g in only 3 samples. It can be said that cooking process is sufficient in all samples in terms of *Enterococcus*, except these 3 samples. *Bacillus* and *Clostridium* spores can be found in this kind of products, because they could not be inactivated by cooking process (Price and Schweigert 1987).*Bacillus* spore count were <2.00-4.46 log CFU/g (Table 1). Yeast - mould count in 96.7 % of samples were <3.3 log CFU/g (Table 1) with only one sample having high yeast and mould counts. Vacuum packaging is generally used for cooked products, and these products have scarcely high yeast and mould counts (Jay 1992). High yeast and mould counts may have originated from insufficient vacuum and/or refracted vacuum.

The mean, maximum and minimum values of moisture, protein, moisture/protein, fat, salt, residue nitrite, hidroxyproline, pH of the samples were shown in Table 2.

The difference of moisture content may be a result of from using different raw materials and different processing methods. Moisture ratios in 33.3 % of the samples were higher than 65 % moisture ratio limited by Turkish Sausage Standard (TSE 1984) The data in this study have been partially similar to the data of Ertaş and Kolsarici (1983) and Vural et al. (1996). Fat content were also found to be similar to that indicated by Vural et al. (1996), but it was found to be different from the study conducted by Ertaş and Kolsarici (1983). Protein content in 96.6% of the samples was lower than 15% that has been stated as the lowest protein content by Turkish Sausage Standard (TSE 1984). Price and Schweigert (1987) demonstrated that the range protein content has been 11.2-11.5% in the frankfurter made from beef.

Salt content of the samples were lower than 3%, except one sample. This data was similar to the data reported by Ertas and Kolsarici (1983). Residual nitrite contents in all samples were below 100 ppm limit as indicated by Wirt (1984). The nitrite contents of the samples were found to be similar to the results of frankfurter and wiener study conducted by Müller (1991) and different from another study by Vural et al. (1983). It is recorded that pH of emulsion-type meat product is between 6.0 and 6.4 Tändler (1986). The pH content of samples was generally low, similar to pH value determined by Vural et al. (1983). pH was under 6.0 in 63.4% of the samples. These results show that temperature was not controlled appropriately at the markets during 2-3 months of cold storage and therefore the acidity of the products had increased. Maximum pH was set as 6.3 by Turkish Sausage Standard (TSE 1984), but no minimum level was stated. Hidroxyproline content was varied between 0.101 and 0.218 g/100g.

Table 1. The microbiological analysis results (log CFU/g)

Sample Number	APC[1]	LAB[2]	*Entero*[3]	CGB[4]	*Enterococcus*	*Bacillus* spore	Yeast - Mold	*S.aureus* (MPN/g)
1	5.49	5.73	<1.00	<1.00	<2.00	<2.00	<2.00	<3
2	5.20	4.32	<1.00	<1.00	<2.00	3.30	<2.00	<3
3	6.20	5.70	<1.00	<1.00	<2.00	<2.00	<2.00	<3
4	6.32	4.91	<1.00	<1.00	<2.00	<3.30	<2.00	<3
5	<3.30	<3.30	<1.00	<1.00	<2.00	<2.00	<2.00	<3
6	5.69	4.08	<1.00	<1.00	<2.00	<2.00	<2.00	<3
7	7.04	7.28	<1.00	<1.00	<2.00	<3.30	<2.00	20
8	5.97	5.43	<1.00	<1.00	<2.00	<2.00	<3.30	<3
9	<3.30	<2.00	<1.00	<1.00	<2.00	<3.30	<3.30	<3
10	6.36	5.89	<1.00	<1.00	<2.00	<3.30	<3.30	<3
11	4.08	3.98	<1.00	<1.00	<2.00	<2.00	<3.30	<3
12	5.34	5.65	<1.00	<1.00	<2.00	3.90	<2.00	<3
13	4.41	4.46	<1.00	<1.00	<2.00	<3.30	<3.30	<3
14	4.75	7.28	<1.00	<1.00	<2.00	<3.30	<3.30	<3
15	7.89	7.88	<2.30	<2.30	<2.00	<3.30	<2.00	<3
16	4.52	5.72	<1.00	<1.00	<2.00	3.62	<2.00	<3
17	4.48	<3.30	<1.00	<1.00	<2.00	<3.30	<3.30	<3
18	6.57	5.40	<1.00	<1.00	<2.00	<3.30	<3.30	<3
19	6.51	6.61	<1.00	<1.00	<2.00	<3.30	<3.30	<3
20	5.93	6.62	<1.00	<1.00	<2.00	4.45	<2.00	<3
21	6.11	6.65	<1.00	<1.00	4.81	4.46	<3.30	15
22	5.56	5.36	<1.00	<1.00	<2.00	<3.30	<2.00	<3
23	4.56	3.83	<1.00	<1.00	<2.00	3.86	<2.00	<3
24	8.00	7.95	<1.00	<1.00	<2.00	<3.30	<3.30	<3
25	7.91	8.15	<1.00	<1.00	<2.00	<3.30	<3.30	<3
26	6.00	5.73	4.91	<2.30	<2.00	<3.30	<2.00	<3
27	5.99	<3.30	<1.00	<1.00	<2.00	3.96	<3.30	<3
28	7.00	7.11	<1.00	<1.00	5.04	3.38	<3.30	<3
29	7.88	7.59	<1.00	<1.00	<2.00	3.62	4.76	<3
30	7.84	7.86	<1.00	<1.00	<3.30	4.08	<3.30	23
Min.	<3.30	<2.00	<1.00	<1.00	<2.00	<2.00	<2.00	<3
Max.	8.00	8.15	4.91	3.30	5.04	4.46	4.76	23

[1] APC: Aerobic Plate Count, [2]LAB: Lactic Acid Bacteria, [3]*Enterobacteriaceae* [4]CGB: Coliform Group Bacteria

Table 2. Chemical Analysis Results

Properties	Minimum	Maximum	Mean	Standard Deviation
pH	4.26	6.61	5.52	0.73
Moisture (%)	59.20	68.09	63.3l	2.80
Protein (%)	10.08	15.74	13 .19	1.24
Water/Protein	4.09	6.45	4.85	0.62
Fat (%)	11.98	21.42	16.52	2.67
Salt (%)	1.65	3.11	2.35	0.29
Nitrite (ppm)	2.42	28.02	8. 16	5.99
Hidroxyproline (g/100g)	0.101	0.218	0.162	0.027

CONCLUSIONS

The results of the research show that frankfurters, sold in retail market in Erzurum, were generally safe in terms of *Listeria monocytogenes*, *Salmonella* spp., *Staphylococcus aureus*, and *Clostridium perfringens*. But aerobic plate counts were high and, lactic acid bacteria were predominant, accordingly, pH was under 6.0 in a majority of the samples. In addition, moisture/protein ratios are high and protein levels are low, therefore the producers have to look out for Turkish Sausage Standard during manufacturing for providing the desired food quality.

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