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A Research on Microbiological Properties of Torba Yoghurts Sold in İzmir Province

İzmir Pazarında Satışa Sunulan Torba Yoğurtlarının Mikrobiyolojik Özellikleri Üzerine Bir Araştırma

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ABSTRACT

In this study, microbiological quality of Torba yoghurt sold at local markets in İzmir was determined. In Torba yoghurt samples, numbers of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* were $2.1 \times 10^6 - 4.1 \times 10^9$ cfu/g and $1.0 \times 10^5 - 7.8 \times 10^8$ cfu/g respectively. The total numbers of total mesophilic aerobic, proteolytic and lipolytic bacteria, yeast-mould, coliform group bacteria, *Staphylococcus aureus* and *Enterococcus* ssp. which found in yoghurt as contaminant were $1.5 \times 10^6 - 7.5 \times 10^9$ cfu/g, $1.1 \times 10^1 - 5.4 \times 10^4$ cfu/g, $1.1 \times 10^1 - 2.1 \times 10^4$ cfu/g, $1.4 \times 10^2 - 8.1 \times 10^5$ cfu/g, $< 10 - 7.1 \times 10^2$ cfu/g, $< 10 - 1.6 \times 10^3$ cfu/g and $< 10 - 3.6 \times 10^4$ cfu/g respectively. The number of coliforms and *Staphylococcus aureus* in 13 samples and the counts of *Enterococcus* ssp. in 8 samples were found less than 10 cfu/g. It can be concluded that microbiological quality of Torba yoghurt samples were not at desired level. According to the high numbers of indicator bacteria counts, some Torba yoghurt samples could be potential risk factor for public health. The production conditions of torba yoghurt must be hygienically for marketing.

Anahtar Sözcükler:

Mikrobiyolojik kalite, torba yoğurdu, fermente süt ürünleri, süzme yoğurt

ÖZET

Bu çalışmada İzmir bölgesinin yerel pazarlarında satılmakta olan Torba Yoğurtlarının mikrobiyolojik kalitesi değerlendirilmiştir. Toplamda 30 adet torba yoğurdu örneği analiz edilmiştir. Torba yoğurdu örneklerinde *Lactobacillus delbrueckii* subsp. *bulgaricus* ve *Streptococcus thermophilus* sayıları sırasıyla $2.1 \times 10^6 - 4.1 \times 10^9$ cfu/g ve $1.0 \times 10^5 - 7.8 \times 10^8$ cfu/g bulunmuştur. Toplam mezofilik aerobik bakteri sayısı, proteolitik ve lipolitik bakteri sayıları, maya-küf sayısı, koliform bakteri sayısı, *Staphylococcus aureus* ve *Enterococcus* ssp. sayıları sırasıyla $1.5 \times 10^6 - 7.5 \times 10^9$ cfu/g, $1.1 \times 10^1 - 5.4 \times 10^4$ cfu/g, $1.1 \times 10^1 - 2.1 \times 10^4$ cfu/g, $1.4 \times 10^2 - 8.1 \times 10^5$ cfu/g, $< 10 - 7.1 \times 10^2$ cfu/g, $< 10 - 1.6 \times 10^3$ cfu/g ve $< 10 - 3.6 \times 10^4$ cfu/g olarak bulunmuştur. 13 örnekte Koliformlar ve *Staphylococcus* ssp. sayısı ve 8 örnekte *Enterococcus* ssp. sayısı 10 cfu/g' dan daha düşük düzeyde bulunmuştur. Bu çalışma sonucu torba yoğurdu örneklerinin mikrobiyolojik kalitesinin arzu edilebilir seviyede olmadığı göstermiştir. Yüksek indikatör bakteri sayısına sahip olduğu göz önüne alındığında bazı torba yoğurtlarının halk sağlığı açısından önemli bir risk oluşturabileceği görülmüş, bu nedenle torba yoğurdu üretiminde hijyenik şartlara dikkat edilmelidir.

INTRODUCTION

Yogurt is a fermented milk product that contains the characteristic bacterial cultures *Lactobacillus* subsp. *bulgaricus* and *Streptococcus thermophilus*. The Code of Federal Regulations

defines it as the food produced by culturing cream, milk, partially skimmed milk, or skim milk used alone or in combination with a characterizing bacterial culture that contains the lactic acid-producing bacteria, *Lactobacillus delbrueckii* subsp. *bulgaricus*

and *Streptococcus thermophilus* (Robinson et al 2006; Ozer 2006; Anonymous 2009).

Yogurt is more nutritious than many other fermented milk products because it contains a high level of milk solids in addition to nutrients developed during the fermentation process. Today, many types of concentrated fermented milks are produced in different countries to extend the keeping quality and, although these products have different local names, in practice they are similar. "Torba" yoghurt is one of the most consumed concentrated fermented milk product in Turkey. It is also known as "Süzme" or "Kese" yoghurt. Several types of concentrated yoghurt have been produced traditionally in Turkey. They are known as Kurut, Torba yoghurt, Tulum and Peskuten (Tamime & Robinson 1978; Ozdemir et al 1995). Among these, Torba yoghurt is the most consumed (Nergiz & Seckin 1997).

Similar strained products are manufactured by different methods in Balkan and Eastern Mediterranean countries, in Turkmenistan and the Indian subcontinent (Yazici et al 2004; Tamime & Robinson 2007). The traditional method of making Torba yoghurt is achieved by straining cold plain yoghurt using a cloth bag (Nergiz & Seckin 1997; Yazici et al 2004; Kesenkas 2010). Torba yoghurt has increased in popularity during recent years in the world. Its increasing economic importance has been achieved as a result of its perceived nutritional benefits and storage characteristics. Traditional and new methods have been used in removing yoghurt whey for the manufacture of Torba yoghurt. In the traditional method, yoghurt is strained in a special cloth bag. The basic principle of using the traditional cloth bag method is to remove whey from plain yoghurt until the desired level of total solids has been reached (Tamime et al 1991; Ozer 2006; Kesenkas 2010). New methods, centrifugation, ultrafiltration and reverse osmosis, have recently been employed to produce strained yoghurt (Tamime et al 1991, Abu-Jdayil et al 2002; Somer & Basyigit-Kilinc 2012). Studies on torba yoghurts are limited; however, some research has been focused on this variety in the last decade. This research aims to determine that microbiological safety and quality of Torba yoghurts sold in several İzmir markets.

MATERIALS and METHODS

A total of 30 different torba yoghurts collected from several market of Alsancak, Bornova, Menderes, Tire and Ödemiş in İzmir. All the samples were transferred to the laboratory under refrigeration and were stored at 4°C until their analysis. A total of 30

torba yoghurts (10g) were diluted in 90 mL Ringer's solution (Merck, Darmstadt, Germany), homogenized by stomacker and serially diluted with Ringer's solution. Serial 10^{-8} fold dilutions of samples were plated out in duplicate on Man Ragosa Sharpe Agar (MRS, Merck, Darmstadt, Germany) was used for enumeration *L. bulgaricus*, M17 Agar (Merck, Darmstadt, Germany) was used for enumeration *S. thermophilus*. MRS Agar plates were incubated at 42°C for 72 h anaerobically conditions using Anaerocult A (Merck, Darmstadt, Germany) for *L. bulgaricus*, M-17 Agar was incubated at 37°C for 72 h aerobically conditions for *S. thermophilus* (Terzaghi & Sandine 1975; Bracquart 1981). Plate Count Agar (Merck, Germany) to determine total mesophilic aerob bacteria (TMAB) and incubated at 30°C for 48 h, *Enterococci* was determined Kanamycin-Esculin Azide Agar (Merck, Germany) at 37°C for 24 - 48h. Coliform group bacteria was determined on Violet Red Bile Agar (Oxoid, UK, CM 107) and incubated at 37°C for 18 - 24 h (Anonymous 2005). Yeast and mould was cultivated on Yeast Glucose Chloramphenicol (YGC) Agar (Merck, Germany) at 25°C for 5 d (Kesenkaş 2010). *Staphylococcus aureus* was determined modification Baird Parker Agar BPA (Merck, Germany) with Egg Yolk Tellurite Emulsion (Oxoid SR 47) and at 37°C for 24 48 h. Proteolytic bacteria was determined Calcium Caseinate Agar (Merck, Germany) at 25°C for 24-48h. Lypolytic bacteria was determined Tributyrin Agar with Tyributrin (Merck, Germany) at $30 \pm 1^\circ\text{C}$ for 72 ± 2 h (Anonymous 2005).

RESULTS and DISCUSSION

The microbiological properties of Torba yoghurts are presented in Table 1. It was found that the lowest and the highest amounts of *L. bulgaricus* and *S. thermophilus*, the natural lactic acid bacteria of strained yoghurt, vary between 1.0×10^6 - 4.1×10^9 cfu/g, 1.0×10^5 - 7.8×10^8 cfu/g, respectively. In this context, it is determined that the number of *L. bulgaricus* in strained yoghurt is higher than *S. thermophilus*. Among all strained yoghurt samples, while number of *L. bulgaricus* is always higher than 10^6 cfu/g however number of *S. thermophilus* in some of the strained yoghurt samples are lower than 10^6 cfu/g. According to Turkish Food Codex, The Notification of Fermented Milk, the number of the specific microorganisms in yoghurts should be at least 10^7 . According to this, it is observed that among 30 samples, one sample in terms of *L. bulgaricus* and 11 sample in terms of *S. thermophilus* do

not provide these rates. Caglar et al (1997) determined that lactic acid bacteria numbers of strained yoghurt vary between 2.2×10^8 and 2.69×10^9 cfu/g. Kesenkas (2010) determined the number of *L. bulgaricus* ve *S. thermophilus* content from their produced strained yoghurt respectively as $1.17 \times$

$10^8 - 5.8 \times 10^8$ cfu/g, $1.8 \times 10^8 - 3.3 \times 10^8$ cfu/g. Ersoz et al (2011) determined the number of *L. bulgaricus* as $2.1 \times 10^6 - 4.8 \times 10^7$ cfu/g and *S. thermophilus* as $6.6 \times 10^6 - 10.3 \times 10^7$ cfu/g from strained yoghurt produced from sheep's milk by using phenolic compounds.

Table 1. Microbiological properties of Torba Yoghurts sold in İzmir Providence (cfu/g)

Sample No	<i>Lactobacillus bulgaricus</i>	<i>Streptococcus thermophilus</i>	Total Aerobic Mesophilic Bacteria	<i>Enterococcus</i> sp.	Coliform Group Bacteria	Yeast and Moulds	<i>Staphylococcus aureus</i>	Proteolytic Bacteria	Lipolitic Bacteria
1	3.6×10^8	7.1×10^8	1.1×10^9	7.3×10^3	< 10	8.1×10^4	< 10	6.3×10^3	1.2×10^4
2	2.6×10^8	3.4×10^7	1.2×10^7	< 10	2.5×10^2	8.9×10^4	< 10	1.3×10^3	6.9×10^2
3	3.6×10^7	3.7×10^8	4.0×10^7	1.0×10^2	< 10	5.0×10^4	< 10	1.5×10^2	5.0×10^2
4	4.2×10^8	2.8×10^7	1.2×10^8	2.5×10^2	8×10^1	3.5×10^4	3×10^2	2.0×10^3	3.9×10^3
5	2.2×10^7	7.8×10^8	9.0×10^8	2.5×10^2	4.9×10^2	2.9×10^4	< 10	3.2×10^3	2.4×10^3
6	1.4×10^7	7.5×10^6	1.5×10^6	1.5×10^2	< 10	5.8×10^5	2×10^2	5.0×10^2	6.0×10^2
7	3.4×10^7	1.6×10^9	4.4×10^8	2.1×10^3	2.0×10^2	1.8×10^4	3×10^2	5.4×10^4	1.4×10^3
8	1.3×10^8	2.5×10^8	5.9×10^8	< 10	9×10^1	4.9×10^5	2×10^2	1.9×10^3	1.0×10^3
9	1.2×10^7	1.1×10^7	3.1×10^7	< 10	< 10	4.7×10^5	6.0×10^1	2.5×10^3	3.8×10^3
10	1.2×10^8	1.0×10^6	1.7×10^8	< 10	1.0×10^1	3.6×10^3	5.5×10^1	2.1×10^3	2.2×10^2
11	2.1×10^8	7.5×10^8	5.3×10^8	3.6×10^4	1.5×10^1	8.1×10^5	6.7×10^2	2.2×10^3	6.0×10^1
12	1.9×10^9	2.6×10^8	2.2×10^9	5.0×10^2	< 10	2.5×10^4	1.3×10^2	2.6×10^4	1.0×10^4
13	1.6×10^8	2.6×10^7	2.6×10^9	< 10	< 10	1.8×10^3	< 10	2.6×10^3	3.5×10^3
14	2.6×10^7	6.2×10^6	1.2×10^8	< 10	1.0×10^1	1.6×10^4	< 10	2.1×10^3	2.5×10^3
15	5.2×10^7	4.3×10^6	9.8×10^7	3.6×10^3	1.0×10^1	4.6×10^5	4.7×10^2	2.2×10^2	2.1×10^3
16	4.1×10^9	5.1×10^8	1.6×10^9	5.4×10^3	1.5×10^1	3.6×10^5	1.6×10^3	6.1×10^3	2.5×10^3
17	5.8×10^7	3.1×10^7	6.2×10^8	1.1×10^2	< 10	2.8×10^4	1×10^1	2.1×10^2	2.5×10^3
18	2.1×10^6	7.1×10^5	2.3×10^7	3.4×10^4	3.6×10^2	6.3×10^3	1.3×10^2	3.6×10^3	4.9×10^3
19	3.6×10^8	8.2×10^7	2.3×10^8	1.5×10^3	7.1×10^2	1.2×10^4	1.2×10^2	1.1×10^3	2.1×10^4
20	2.1×10^7	6.5×10^6	8.7×10^8	< 10	< 10	1.4×10^2	< 10	3.1×10^2	7.0×10^2
21	1.7×10^7	8.2×10^6	6.4×10^8	7.0×10^2	< 10	2.6×10^2	1.2×10^1	6.7×10^1	1.8×10^3
22	3.1×10^8	1.2×10^7	3.4×10^9	1.2×10^1	1.1×10^1	2.2×10^3	< 10	2.3×10^2	2.1×10^2
23	2.1×10^7	6.2×10^6	5.6×10^8	1.0×10^1	1.2×10^1	7.2×10^4	< 10	1.2×10^1	1.3×10^2
24	1.1×10^8	3.2×10^7	4.6×10^9	< 10	< 10	2.1×10^3	< 10	1.1×10^2	1.4×10^3
25	7.1×10^7	6.2×10^6	2.6×10^8	1.1×10^1	< 10	3.2×10^2	< 10	2.1×10^1	1.1×10^2
26	3.2×10^8	5.5×10^7	9.1×10^9	1.2×10^2	2.2×10^2	6.7×10^3	3.8×10^1	1.0×10^2	5.2×10^2
27	2.1×10^7	6.1×10^6	5.3×10^8	2.6×10^1	1.3×10^1	6.8×10^3	< 10	2.1×10^2	2.3×10^2
28	4.5×10^7	3.0×10^6	6.1×10^8	3.1×10^1	1.1×10^2	5.7×10^4	2.6×10^1	2.2×10^2	1.6×10^2
29	4.3×10^8	1.3×10^8	1.1×10^9	2.9×10^1	< 10	2.2×10^3	1.1×10^1	2.6×10^1	1.3×10^1
30	8.2×10^8	1.9×10^8	7.5×10^9	3.3×10^1	< 10	4.2×10^4	< 10	1.1×10^2	1.1×10^1
Minimum	2.1×10^6	1.0×10^5	1.5×10^6	< 10	< 10	1.4×10^2	< 10	1.1×10^1	1.1×10^1
Maximum	4.1×10^9	7.8×10^8	7.5×10^9	3.6×10^4	7.1×10^2	8.1×10^5	1.6×10^3	5.4×10^4	2.1×10^4

It may think that quality of raw materials of food, packaging materials, production and storage conditions is important in quality of product during the shelf-life. It is possible to obtain data about raw materials of food, excipients, packaging materials, operating conditions in general, storage after manipulation and shipping conditions by using total aerobic mezophilic bacteria counting, and to determine if they are compliant with the minimum standards. According to this, it may be determined the initiation of the spoilage of food and shelf-life. According to European Community, the only way of controlling microbiologic quality of raw milk is to count total aerobic mezophilic bacteria (Dogan & Tukul 2005). In this context, minimum and maximum total aerobic mezophilic bacteria numbers counted in strained yoghurt samples are 1.5×10^6 and 7.5×10^9 cfu/g, respectively. The number of total bacteria in 13 strained yoghurt samples of Erzurum country was found to be between 2.7×10^8 and 2.8×10^9 cfu/g from by Çağlar et al (1997). The number of total bacteria in strained yoghurt samples was found to vary between 1.2×10^3 – 1×10^8 cfu/g by Kirdar & Gun (2001). The results of our study are in compliance with mentioned studies.

There is no consensus on *Enterococcus* species that are found in high rates in dairy products and other foods, if they could be seen as food borne pathogens despite the fact that they have some biotechnologic features like bacteriosin production, probiotic characters and utility of dairy industry. Furthermore, these bacteria are regarded as an indicator of fecal contamination of water (Giraffa 2003; Kinzelman et al 2003; Hussain et al 2007). *Enterococcus* ssp. rates found in strained yoghurt samples varied between < 10 - 3.6×10^4 cfu/g. However *Enterococcus* species were not found in 9 samples.

Counts of coliform group bacteria, changed between < 10 - 7.1×10^2 cfu/g. According to Turkish Food Codex, the yoghurt samples should not be contaminated with *E. coli*. While sixteen of 30 samples were contaminated with coliform group bacteria, in thirteen of them were determined coliform bacteria. Occurrence coliform microorganisms in foods shows improper sanitation conditions in food protection. With respect to this data, it is observed that hygienically rules are not applied during production,

storage and sales of strained yoghurt sold in public markets. These findings are supported by Atamer et al (1988), Gokce et al (2001), and Kirdar & Gun (2002) who studied the hygienic quality of strained yoghurt.

Table 1 indicates that the numbers of minimum and maximum yeast-mold count found in strained yoghurt vary between 1.4×10^2 – 8.1×10^5 cfu/g. The number of yeast and mould should not be below 10^2 – 10^3 cfu/g. Gokce et al (2001) 57 samples were collected from 10 distinct retail shops selling strained yoghurt in Denizli city center in summer and winter and some of the chemical and microbiological properties of samples were determined. General yeast-mold numbers of strained yoghurt samples were between $< 1.0 \times 10^1$ and 4.5×10^6 cfu/g and 85% of samples were determined as inappropriate in terms of yeast-mold. Tekinsen et al (2008) determined average yeast number as 3.5×10^4 cfu/g and average mold number as 1.4×10^3 cfu/g in a study on the microbiological properties of strained yoghurt produced in Konya. According to results obtained from this study, not all samples did comply with the Communication on Fermented Milks values. Similar results obtained by other researchers such as Uysal (2002) and Karabiyik (2006).

While the number of *Staph. aureus* that identified in the samples varies between < 10 - 1.6×10^3 , it was under the detectable level in 13 samples. *Staph. aureus* strains can cause food poisoning and is supposed not to be higher than 1.0×10^2 cfu/g (Anonymous 2010). *Staph. aureus* levels given in Table 1, indicates that some strained yoghurt samples may have significant level of risk.

The number of proteolytic bacteria detected in 30 strained yoghurt samples was between 1.1×10^1 – 5.4×10^4 cfu/g (Table 1). Proteolytic bacteria cause undesirable taste and odors by breaking proteins in milk and dairy products. This microorganisms that are undesirable in milk and dairy products cause notable quality loss at long term storage processes (Samaržija et al 2012; Machado et al 2013).

The lipolytic bacteria numbers in strained yoghurt examples vary between 1.1×10^1 - 2.1×10^4 cfu/g. Lipase-producing microorganisms in nature spread over a wide area. The bacterial species showing lipolytic activity is located in the genus of *Pseudomonas*, *Alcaligenes*, *Moraxella* and

Staphylococcus, the mold species is located in the genus of *Rhizopus*, *Geotrichum*, *Aspergillus*, *Mucor* and *Penicillium* and yeast species are located in the genus of *Candida*, *Rhodotorula* and *Hansenula*. For this reason the lipolytic bacteria numbers in dairy products matter (Dogan et al 2005).

CONCLUSIONS

In conclusion, according to the high numbers of indicator bacteria counts, some Torba yoghurt samples were potential risk factor for public health and microbiological quality of samples was not at

desired level. Also, it is seen that most of Torba yoghurts didn't cohere Turkish Food Codex Fermented Milks Bulletin. The results of this study shows the production, storage and marketing conditions of Torba yoghurts should be kept under control to improve the microbial quality.

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