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Microbiological, physicochemical properties and biogenic amine contents of the strained yoghurts from Turkish local markets

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Thirty three strained yoghurt samples were collected from local open markets in different provinces of Turkey (Afyon [AF], Aydın [AY], Burdur [B], Isparta [I] and Muğla [M]). Physicochemical and microbiological properties, as well as biogenic amine contents, were examined in each of the samples. The dry matter (17.90 to 26.44%), fat (5.51 to 10.44%), ash (0.65 to 1.48%), lactic acid (1.69 to 2.05 g l⁻¹), salt (0.61 to 1.68%), pH (3.52 to 3.94), protein (5.73 to 8.57%) and syneresis (1.67 to 9.22%) of samples differed as indicated. The mean of the lactobacilli counts ranged from 6.63 to 8.35 Log₁₀ CFU g⁻¹, lactococci ranged from 7.16 to 8.02 Log₁₀ CFU g⁻¹, while streptococci and propionic acid bacteria ranged from 6.71 to 7.27 and 5.60 to 8.38 Log₁₀ CFU g⁻¹, respectively. All the yoghurts were contaminated with fungi and coliforms at levels above the acceptable limits, indicating insufficient process hygiene and also raising concerns of consumer safety. Tryptamine is the only biogenic amine determined in all yoghurt samples. Cadaverine, putrescine, tyramine and b-phenylethylamine were not detected in any of the samples.

Key words: Strained yoghurt, physicochemical properties, biogenic amine, tryptamine.

INTRODUCTION

Strained yoghurt (sometimes known by other names, such as torba, kese, sūzme, filtered or concentrated yoghurt) is a concentrated fermented product which has been made in the Anatolia region for centuries (Kabak and Dobson, 2011). Strained yoghurt has higher nutritional value, preserves better and, has better taste and texture than normal yoghurt; so it has more acceptability among consumers (Yeganehzad et al., 2007).

Strained yoghurt contains 70.0 to 82.0% moisture, 4.46 to 9.22% protein, 6.0 to 10.4% fat, 4.46 to 9.22% lactose, and 0.56 to 0.82% minerals (Kırdar and Gün, 2001). In the Middle East, strained yoghurt is popularly known as “labneh,” and various labneh-related products are manufactured in other countries, such as labne zeer (in Egypt), Besa (in Bulgaria), skyr (in Iceland), and chakka and shrikhand (in India). Strained yoghurt is consumed as a main dish at breakfast in many Middle Eastern countries, such as Iraq, Iran and the Lebanon. It can also be served as a dip with garlic, dried herbs (usually mint and parsley) and red peppers, or with cucumber and olive oil (Ozer and Robinson, 1999; Kabak and Dobson, 2011).

Strained yoghurt is made from either cow, goat, or sheep milk. Traditionally, yoghurt is strained in a special cloth bag; drainage is achieved by holding yoghurt (16% w/v solids) in bags of double layer cheese cloth at 4°C for 18 to 20 h to remove the whey, and is then packed
and stored at a cool temperature (4°C). Additionally, salt can be added to strained yoghurt to enhance the shelf life of the product (Özer et al., 1997; Yaygin, 1999). However, the traditional method of producing strained yoghurt has drawbacks: lower yield, labor intensiveness, long production time, and unhygienic conditions during the straining stage. Therefore, efforts have been made to develop better techniques (Tamime, 1978; Abu-Jdayil et al., 2002; Tekinşen et al., 2008; Şenel et al., 2009). The following methods have been shown to produce a more modern version of concentrated yoghurt, with a broadly similar composition as the traditional product: direct recombination of full-cream milk powder; ultra-filtration of normal yoghurt; fermentation of milk concentrated to 230 g kg⁻¹ by ultra-filtration; reverse osmosis treatment of normal yoghurt; fermentation of milk concentrated to 230 g kg⁻¹ by reverse osmosis, and centrifugal separation of the whey from normal yoghurt (Robinson and Tamime, 1993).

Ozer et al. (1997, 1998, 1999) have investigated the dynamic rheology of labneh produced by ultrafiltration and reverse osmosis membrane technique. Abu-Jdayil et al. (2002) also examined the effect of the manufacturing processes on the rheological behavior of labneh and the influence of temperature on the viscosity of labneh. Other investigators have studied how to enhance the quality and lengthen the shelf life of the yoghurts. Yeganehzad et al. (2007) showed the importance of total solid concentration of milk on the survival of probiotic strains, as well as the physiochemical and sensory properties of yoghurt. Seckin and Ozkilinc (2011) determined the physical, chemical, textural and sensory properties of concentrated yoghurts made from milks including some prebiotics (inulin and oligofructose) added in different ratios. Al Otaibi and El Demerdash (2008) investigated the quality and shelf life of concentrated yoghurt (labneh) by the addition of some essential oils. Şenel et al. (2009) also determined some compounds affecting aroma and flavour of strained yoghurt produced from goat milk.

Biogenic amines are generally recognized as a serious health hazard for humans, when present in food to significant levels (Pintado et al., 2008). Common chemical indicators of hygienic quality of food include such biogenic amines as histamine, tyramine, cadaverine, putrescine, tryptamine and 2-phenylethylamine. These compounds have been detected in many kinds of ripened cheeses, whenever a high protein content - coupled with an extensive proteolytic activity brought about by rennet or the microbiota - provide the precursors needed for decarboxylase activity (Vale and Gloria, 1998). Since the microorganisms involved in biogenic amine production may be the starter cultures used or may be introduced by contamination before, during, or after manufacturing and storage, the bacteriological quality of milk could be critical to controlling formation of biogenic amines (Novella-Rodriguez et al., 2000).

In recent years, the production of the strained yoghurt in modern dairies has increased, but strained yoghurt produced by small family manufacturers are still sold in the markets. Since no standardized production technique is applied in the manufacture of such strained yoghurts, there are important differences in quality attributes of the strained yoghurts sold in the markets (Tekinşen and Bayar, 2008). Food market studies have the essential function of providing insight into consumer purchasing patterns and provide directions to researchers and regulatory agencies for controlling safe and nutritious food production. The objectives of this study were to determine the microbiological, some physiochemical characteristics, and the biogenic amine contents of strained yoghurts sold in public markets and evaluate the relationships among the tested parameters.

**MATERIALS AND METHODS**

The research materials consisted of 33 strained yoghurt samples. The origins of the samples were different provinces of Turkey (Afyon [AF] (n = 5), Aydın [AY] (n = 3), Burdur [B] (n = 11), Isparta [I] (n = 9) and Muğla [M] (n = 5)). The strained yoghurts, made from cow’s milk and produced using traditional methods by farmers or small-scale producers, were collected from public markets.

**Microbiological analysis**

The viable counts of total mesophilic aerobic bacteria (TMAB), lactic acid bacteria (LAB), lactococci, streptococci, propionic acid bacteria (PAB), enterobacteria, yeasts and moulds were determined. For this purpose, 10 g of yoghurt were emulsified with 90 ml of sterile ¼ ringer solution. Decimal dilutions in ringer solution were prepared and plated in duplicate. TMAB were determined on Plate Count Agar (PCA) (Merck KGaA, Darmstadt, Germany) at 30°C for 2 days; LAB and lactococci were grown anaerobically (Anaero Gen Compact, OXOID, Basingstoke, Hants, UK) on de Man, Rogosa and Sharpe (MRS) agar (Merck KGaA, Darmstadt, Germany) and M17 agar (Merck KGaA, Darmstadt, Germany) at 30°C for 2 and 5 days, respectively. Streptococci were determined on M17 agar at 42°C for 48 h, and PAB were grown on Na-lactate agar (NLA) (Lab M, Lancashire, UK) at 32°C for 5 days under anaerobic conditions. Coliforms were enumerated on Eosin Methylene-blue Lactose Sucrose Agar (EMB) (Merck KGaA, Darmstadt, Germany) after incubation at 37°C for 24 h. Yeasts and moulds were grown on Potato Dextrose Agar (PDA) (Merck KGaA, Darmstadt, Germany), acidified with 14 ml of 10% (v/v) lactic acid (Merck KGaA, Darmstadt, Germany) at 25°C for 5 days. All analyses were performed in duplicate.

**Chemical analysis**

The samples of strained yoghurts were analyzed in duplicate for their total solid, fat, protein, titratable acidity, NaCl, ash contents, pH, syneresis and biogenic amine content. Total solid content was determined by gravimetric method by drying in a laboratory oven Binder GmbH (Tuttlingen, Germany) at 105°C (TSI, 1999). Fat and salt for strained yoghurt were determined by the Gerber method and the potentiometric titration method as described in Turkish standards TS 1018 (TSI, 1981) and TS 4708 (TSI, 2001), respectively. The titratable acidity was also determined according to the TS 1330 (TSI, 1999). Total nitrogen was measured by micro-
Kjeldahl method (IDF, 1962). Ash content was quantified by dry ashing the samples in a muffle furnace at 550°C for 24 h; before samples were placed in the muffle furnace, they were dried in an oven at 105°C. The pH of the samples was measured with a pH-meter (Hanna pH 211, Michigan, USA). The susceptibility of yoghurt to syneresis was determined using the method of Keogh and O’Kennedy (1998).

Biogenic amine analysis

Biogenic amine contents of yoghurt samples were determined according to a modified method of Anh et al. (2004). A 2.5 g sample of yoghurt was extracted with 25 ml of 0.4 M perchloric acid in a blender and cooled to 3°C for crystallizing most of the fat. Then the sample was centrifuged at 10,000 rpm for 10 min at 3°C. 400 µl sample, 400 µl Na2CO3 (2 g/100 ml H2O) and 400 µl dansyl chloride were incubated in a water bath for 30 min. Then, 200 µl Na-L-glutamate monohydrate (200 mg/4 ml H2O) was added and incubated 1 more hour in water bath. After the incubation, 1 ml acetonitrile was added and centrifuged at 2,500 rpm for 10 min. The supernatant was injected into the high-performance liquid chromatography (HPLC) system (Shimadzu, Tokyo, Japan) equipped with a Prodigy 5 µ ODS(2) column (250 × 4.6 mm). The mobile phase consisted of acetonitrile/water (18:100 v/v) and the flow rate was 0.5 ml/min. Quantification was carried out based on a mixture of biogenic amine standards: cadaverine, putrescine, tyramine, tryptamine and phenylethylamine. All determinations were performed in duplicate. The amounts of cadaverine, histamine, phenylethylamine, putrescine, tryptamine and tyramine were tested in all yoghurt samples.

Statistical analysis

All analyses were replicated. Data collected for microbiological, physicochemical properties and biogenic amine contents were analyzed using the MINITABV.14.1 Statistical Analysis System (MINITAB Inc. USA, 2003). The generated data were subjected to analysis of variance (ANOVA). Differences among mean values were established using Tukey’s test and were considered significant when p < 0.05.

RESULTS AND DISCUSSION

Microbiological analysis

The means of the microbial counts of the strained yoghurts collected from different provinces are shown in Table 1. The mean of the total mesophilic aerobic bacteria (TMAB) was determined to be between 6.25 to 8.13 Log10 CFU g−1 for the yoghurt samples. All provinces have significant variation in total viable count (p < 0.01), but the highest count was found in yoghurt samples collected from I province. Çağlar et al. (1997) determined the mean of the TMAB of 13 strained yoghurt as approximately 9.4 Log10 CFU g−1. Kirdar and Gün (2000) reported the TMAB between 2.30 to 6.61 Log10 CFU g−1 in strained yoghurts obtained from Burdur local markets in the summer season.

LAB counts in this study ranged from 6.63 to 8.35 Log10 CFU g−1. The number of the LAB from region AF and M were significantly higher than the other provinces (p < 0.01). Lactococci ranged from 7.16 to 8.02 Log10 CFU g−1. The number of the streptococci and PAB were between 6.71 to 7.27 Log10 CFU g−1 and 5.60 to 8.38 Log10 CFU g−1, respectively. There were no significant differences in the number of lactococci and streptococci among all five provinces (p > 0.01). The highest LAB and PAB were found in M province. Limited data is available on the microbiology of strained yoghurts. Çağlar et al. (1997) and Hocalar et al. (2009) have reported the LAB count in the range of 9.43 to 9.44 and 5.04 to 6.92 Log10 CFU g−1, respectively.

The means of the yeasts and moulds in strained yoghurts from different regions were measured at levels of 6.15 to 7.12 Log10 CFU g−1. These values are quite a bit higher than the limits of the Turkish Food Codex, Fermented Milk Notification (Anonymous, 2001). Fungal contamination from the environment in milk may carry over due to insufficient heat treatment of the yoghurt base (El-Diasty and El-Kaseh, 2007). Other authors have also reported the presence of excessive levels of fungi in strained yoghurt in Turkey. Kirdar and Gün (2000) tested samples from local Burdur markets and reported that the number of yeasts and moulds in strained yoghurt samples was between 2.17 to 7.04 Log10 CFU g−1. These values were close to those reported by Gökçe et al. (2000). Tekingen et al. (2008) investigated 45 strained yoghurt samples produced under nine different trademarks in Konya province in Turkey. They determined the average number for yeast and mould to be about 4.55 Log10 CFU g−1. Similar to our study, a study conducted in Egypt on laban zeer (a labneh-related product) indicated that the total counts of aerobic bacteria averaged 8.37

Table 1. Microbial counts of strained yogurts (log CFU/mL).

<table>
<thead>
<tr>
<th>Province</th>
<th>LAB1</th>
<th>Lactococci</th>
<th>Streptococci</th>
<th>PAB2</th>
<th>TMAB3</th>
<th>Yeast and mould</th>
<th>Coliform</th>
</tr>
</thead>
<tbody>
<tr>
<td>A(5)</td>
<td>7.19±0.75ab</td>
<td>7.39±1.06a</td>
<td>7.27±0.98a</td>
<td>5.60±0.26d</td>
<td>6.25±0.66c</td>
<td>6.52±0.45a</td>
<td>6.04±0.37a</td>
</tr>
<tr>
<td>A(3)</td>
<td>8.35±0.11a</td>
<td>7.51±0.49a</td>
<td>6.86±0.55a</td>
<td>8.38±0.17a</td>
<td>6.96±0.47abc</td>
<td>6.90±0.41a</td>
<td>6.63±0.52a</td>
</tr>
<tr>
<td>I(9)</td>
<td>6.77±1.12b</td>
<td>7.58±0.54a</td>
<td>7.26±0.68a</td>
<td>7.37±0.43b</td>
<td>8.13±0.66a</td>
<td>6.81±1.22a</td>
<td>6.29±0.84a</td>
</tr>
<tr>
<td>B(11)</td>
<td>6.71±1.39d</td>
<td>7.16±0.88a</td>
<td>6.71±1.09a</td>
<td>6.17±1.18c</td>
<td>6.77±1.56bc</td>
<td>6.15±1.30a</td>
<td>5.96±1.16d</td>
</tr>
<tr>
<td>M(5)</td>
<td>6.63±0.41c</td>
<td>8.02±0.37a</td>
<td>6.82±0.54a</td>
<td>6.69±0.36ab</td>
<td>7.91±0.17abc</td>
<td>7.12±0.22a</td>
<td>6.01±0.53c</td>
</tr>
</tbody>
</table>

Means sharing the same letter within a column are not significantly different at 5% significance level. 1Lactic acid bacteria, 2propionic acid bacteria, 3total mesophilic aerobic bacteria 4Afyon province, 5Aydın province, 6Isparta province 7Burdur province 8Muğla province.

```python
AF
A
I
B
M
```
Table 2. Chemical composition of strained yoghurts.

<table>
<thead>
<tr>
<th>Provinces</th>
<th>pH</th>
<th>Lactic acid (g/L)</th>
<th>Fat (%)</th>
<th>Salt (%)</th>
<th>Dry matter (%)</th>
<th>Ash (%)</th>
<th>Syneresis (%)</th>
<th>Protein (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AF^1 (n=5)</td>
<td>3.78±0.22a</td>
<td>1.72±0.45b</td>
<td>10.44±2.69a</td>
<td>1.68±1.56b</td>
<td>26.44±4.65a</td>
<td>1.48±1.29a</td>
<td>1.67±1.94b</td>
<td>8.30±2.96a</td>
</tr>
<tr>
<td>AY^2 (n=3)</td>
<td>3.52±0.12c</td>
<td>2.04±0.25a</td>
<td>5.51±1.97c</td>
<td>0.61±0.11c</td>
<td>17.90±2.11d</td>
<td>0.65±0.03b</td>
<td>6.72±4.49a</td>
<td>6.21±0.49bc</td>
</tr>
<tr>
<td>I^3 (n=9)</td>
<td>3.94±0.31a</td>
<td>1.69±0.68a</td>
<td>11.61±2.18a</td>
<td>1.01±0.61bc</td>
<td>22.85±2.56ab</td>
<td>0.65±0.08b</td>
<td>6.05±5.90a</td>
<td>5.73±3.02bc</td>
</tr>
<tr>
<td>B^4 (n=11)</td>
<td>3.79±0.19b</td>
<td>1.76±0.41b</td>
<td>8.48±3.30abc</td>
<td>0.84±0.31bc</td>
<td>22.23±4.10bc</td>
<td>0.73±0.14b</td>
<td>9.22±3.41a</td>
<td>6.48±2.37bc</td>
</tr>
<tr>
<td>M^5 (n=5)</td>
<td>3.77±0.37b</td>
<td>2.05±0.32b</td>
<td>6.50±2.40bc</td>
<td>0.69±0.15bc</td>
<td>21.35±1.71bc</td>
<td>0.69±0.07b</td>
<td>7.03±4.85a</td>
<td>8.57±2.12bc</td>
</tr>
</tbody>
</table>

Means sharing the same letter within a column are not significantly different at 5% significance level.  
1) Afyon province, 2) Aydın province, 3) Isparta province, 4) Burdur province, 5) Muğla province.

Log_{10} CFU g^{-1}, and the average count of yeasts and fungi was 7.57 Log_{10} CFU g^{-1} (Ibrahim et al., 1999).

Packaged strained yoghurt, as a concentrated product with a high concentration of lactic acid and limited access to air during refrigerated storage, is thought to provide ideal conditions for the growth of yeasts (Nsabimana et al., 2005). Şahan et al. (2004) determined that the addition of 10 mg/kg natamycin had a significant inhibitory effect on yeast and mould growth. It has been suggested that when the traditional production method is used, especially when good manufacturing practices are not fully followed, contamination of labneh by yeasts cannot be prevented (Nsabimana et al., 2005). The transfer of yoghurt from filter bags used for straining to packaging materials may contribute to fungal and coliform contamination. In addition, the hygienic conditions of the packaging material, storage, and market places may also cause microbial contamination (Hocalar et al., 2009). Although the low pH value of yoghurt limits growth of pathogenic microorganisms, yeast and mould are still able to grow in yoghurt.

Our study found high numbers of coliforms (ranging from 5.96 to 6.29 Log_{10} CFU g^{-1}) in strained yoghurt samples from markets in Turkey. These results are quite a bit higher than those reported by Kirdar and Gün (2000) and Gökcé et al. (2000). Coliforms are indicators of poor hygiene and possible contamination with microorganisms of fecal origin. The presence of these organisms reported in this study could also be an indication of fecal contamination of the water used for processing; this might have far reaching effects on the health of the consumers and the general public health. Strained yoghurts are often manufactured using pasteurized milk (Ozer et al., 1999; Nsabimana et al., 2005), but in this study the samples were collected from local bazaars; the yoghurts were produced mostly in small-scale factories, thus the milk might not be pasteurized properly. Since all yoghurt samples tested in this study were contaminated with coliforms, this may reflect very poor hygienic conditions and improper sanitation during manufacture of the yoghurts.

In this study, the strained yoghurts were collected during the spring and summer seasons from open local markets of different provinces. The high number of TMAB, yeast and mold, and coliform bacteria in the tested yoghurt samples may also be explained by high seasonal temperatures.

Chemical analysis

The physicochemical properties of the strained yoghurts from different provinces are shown in Table 2. Significant differences (p < 0.05) were observed for all of the parameters. The total solids content ranged between 17.90 and 26.44%. While significant differences in total solids content were determined among all provinces, AF province was comparably higher than the other provinces. These values are in agreement with those reported in previous studies (Özer et al., 1998, 1999; Kirdar and Gün, 2002; Tekinşen et al., 2008; Şimşek et al., 2010). In contrast to our results, Çağlar et al. (1997) found the average of the 13 strained yoghurts’ total solid value as 32.36. The milk solids content (including the fat content) for yoghurt ranges from around 9% for skim milk yoghurt to more than 20% for certain types of concentrated yoghurt (Lee and Lucey, 2010).

It has been reported that the variations in total solids content of the strained yoghurts can be due to the variety of factors: chemical composition of the milk used in the production of yoghurts, especially fat content; production technique; a lack of dry matter standardization; and variations in the applied time, temperature and pressing method during the straining stage (Tekinşen and Bayar, 2008). There were variations among the fat contents of the tested yoghurts from different provinces. The fat content ranged from 5.51 to 11.61%. Similar to our findings, other researchers have reported values ranging between 6.0 to 10.40% (Gökcé et al., 2001; Kirdar and Gün, 2001; Tekinşen et al., 2008; Köse and Oçak, 2011) in strained yoghurts. Abu-Jdayil et al. (2002) also determined the fat content of the labneh as 6.35 to 10.00%. Results of our study indicated that solid content is associated with fat content; notably AY and M provinces had the lowest fat content and also the lowest total solids content. The variation in fat could be attributed to variations in the raw milk, treatments used to reduce fat, or practices such as adulteration of milk by adding water (Mukisa and Kyoshabire, 2010).

The highest protein content was found in the strained
Biogenic amine analysis

Cadaverine, putrescine, tyramine and b-phenylethylamine were not detected in any of the yoghurt samples analysed in this study. Tryptamine was the only biogenic amine determined in all yoghurt samples. Levels of the tryptamine was between 3.33 to 5.52 ppm. The biogenic amines such as histamine, putrescine, tyramine, tryptamine, b-phenylethylamine, and cadaverine may be formed during storage or during processing of these products (Balamatsia et al., 2006). The tryptamine content was the lowest in yoghurts from B province, and the highest in yoghurts from M province. On the other hand, yoghurts from AF, AY and I provinces exhibited similar levels of tryptamine. The most probable factor that influences low tryptamine production in strained yoghurts is their low pH (Pintado et al., 2008). These results are in accordance with the findings of Küçükçetin (2008), determined the biogenic amine content of strained yoghurts sold in local markets in Antalya province. No official limits for biogenic amine concentrations in dairy products have been set.

Conclusions

This study concluded that there are variations in the quality characteristics of strained yoghurts produced by small-scale manufacturers and sold in local open markets in five different provinces of Turkey. Some of the quality problems stem from microbial contamination. Fungi and coliform contamination in these yoghurts indicates insufficient process sanitation and also raises concerns of consumer safety. This study indicated that tryptamine was the only biogenic amine detected in all of the yoghurt samples.

The results of this study indicate that possible safety and quality problems are major concerns regarding strained yoghurts produced by small-scale manufacturers. Therefore, further research and a broad control system are needed to improve safety of these products and to retain product quality characteristics to satisfy consumer demands. Additionally, training and technical support services for hygienic product scale processors should be provided to small-scale processors.

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