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ORIGINAL ARTICLE

Microbiological quality of Tarhana, Turkish cereal based fermented food

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fermentation; safety; tarhana; wheat; yogurt.

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Abstract

Objectives Tarhana is a traditional fermented product produced from a mixture of yogurt and wheat flour in Turkey. The aim of the present study was to investigate the microbiological quality of tarhana and its raw material. *Methods* Samples were collected from eight different regions of Turkey during fermentation period and after drying process. Aerobic plate count, *Staphylococcus aureus*, *Salmonella* spp., *Clostridium perfringens*, coliform, *Escherichia coli* and *Bacillus cereus* analysis were applied for detecting the microbiological quality of tarhana samples. *Results* *E. coli* and *S. aureus* were not isolated from all the production steps used. Some of the tarhana samples were found containing coliform, *Salmonella*, *C. perfringens* and *B. cereus* at the beginning of the fermentation process; however, they were not isolated from other stages, except one dried tarhana sample, which were containing *C. perfringens* in low levels. It is found that yogurt and wheat flour samples met the microbiological criteria given for these products. Changes of pH and acidic values during tarhana fermentations were also detected in the study. *Conclusion* The results indicated that microbiological and chemical properties of tarhana change depending on the raw materials, fermentation time and techniques used in its production.

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Introduction

Tarhana is a cereal-based fermented traditional Turkish food made by mixing wheat flour, yogurt, variety of cooked vegetables (tomato, onion, pepper, etc.), yeast (some regions), salt, tarhana herb (*Echinophora sibthorpiana*) and other spices (mint, paprika etc.) (Figure 1). Different types of ingredients and production techniques can also be used in various regions (Table 1). Sometimes broken wheat is used instead of wheat flour in the formulation and this type of tarhana is called Göce tarhana. After mixing all ingredients, tarhana dough is obtained and it is followed with the lactic acid bacteria (LAB) and yeast fermentations for 1 to 7 days. LAB that take place in tarhana fermentations were studied in the other part of this study and it was found that the different production sites investigated all had individual LAB profiles but with *Pediococcus acidilactici* and *Streptococcus thermophilus* being isolated from the majority of samples as dominant LAB, while other minor group were identified as *Lactobacillus fermentum*, *Enterococcus faecium*, *Pediococcus pentosaceus*, *Leuconostocs pseudomesenteroides, Weissella cibaria*, *Lactobacillus plantarum, Lactobacillus delbrueckii* subsp. *bulgaricus*, *Leuconostoc citrenum*, *Lactobacillus paraplantarum* and *Lactobacillus casei* (Sengun *et al.*, 2009). This study, which defined the LAB flora of different tarhana samples, is the first microbiological investigation of tarhana using molecular biology-based methods. Although yeasts also take an important role in

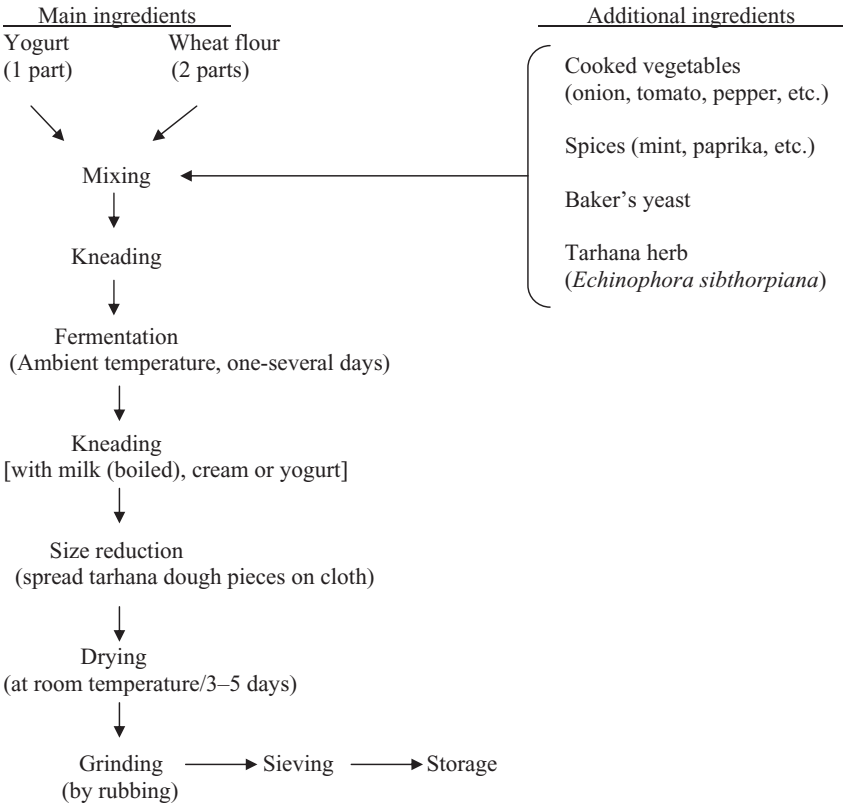

Figure 1 Flow diagram for traditional tarhana production.

Table 1 Sampling sites and ingredients used in the production of tarhana

Sample	Sampling region/site	Ingredients	Fermentation time (day)
A	Aydın/İncirliova	Flour (4 kg), homemade strained yogurt (2.5 kg), semolina (1 kg), tarhana herb, onion (2 kg), tomato (4 kg), red pepper (2 kg), salt	2
B	Milas/Bodrum-Gündoğan village	Broken wheat (1 kg), homemade strained yogurt (1 kg), salt	4
C	İzmir/Hatay	Flour (9 kg), homemade strained yogurt (5 kg), semolina (2.5 kg), tarhana herb, onion (5 kg), tomato (5 kg), red pepper (6 kg), salt	6
D	Manisa/Gölmarmara-Tiyenli village	Flour (added until reaching the right consistency), homemade strained yogurt (3 kg), onion (1 kg), cooked chickpea (1/2 kg), red pepper (4 kg), salt	15
E	İzmir/Urla-Gülbahçe village	Flour (6 kg), commercial set type yogurt (4 kg), tarhana herb, onion (3 kg), tomato (2 kg), red pepper (2 kg), salt	1
F	İzmir/Çeşme-Barbaros village	Flour (added until reaching the right consistency), homemade strained yogurt (2.5 kg), tarhana herb, onion, tomato, red pepper, salt	1
G	Uşak/City Center	Flour (5 kg), homemade strained yogurt (10 kg), red pepper (4 kg), green pepper (4 kg), onion (5 kg), yeast, mint, milk or milk cream, salt	21
H	Isparta/Şarkikaraağaç	Flour (added until reaching the right consistency), homemade strained yogurt (2 glass), semolina (1 glass), tomato (2 unit), red pepper (2 unit), onion (2 unit), egg (1), parsley, mint, dill, milk or milk cream (1 spoon), cooked chickpea, yeast (1 spoon), salt	3

tarhana fermentations, there is no study on the identification of this group.

Tarhana dough is sun dried, ground into fine powder and used for making soup by adding tarhana powder to boiling water. Different preparation techniques in distinct places lead to the production of various types of tarhana. Moreover, the preparation of tarhana is a traditional family art. There are also some fermentation products similar to tarhana known as kishk in Syria, Palestine, Jordan, Lebanon and Egypt; talkuna in Finland; kushuk in Iraq and Iran; thanu in Hungary; and trahanas in Greece (Siyamoglu, 1961; Ibanoglu & Ibanoglu, 1999; Blandino *et al*., 2003).

The low moisture content (3–9%) and low pH value (4.0–4.5) of the final product provide a bacteriostatic effect against pathogenic and spoilage microorganisms and increase the shelf life of the product (Ibanoglu & Ibanoglu, 1999). Powdered form of tarhana can be stored up to 1–2 years under dry and cooled conditions (Siyamoglu, 1961).

Tarhana is a good source of protein, vitamins and minerals, and it is thought to be a nutritious food for babies, children and persons who suffer from digestion problems (Pirkul, 1988). It has been reported that water-soluble vitamin contents of tarhana increased through the fermentation process, while it decreased during the drying stage (Ibanoglu *et al*., 1995; Ekinci, 2005; Erbas *et al*., 2005).

Although there are many studies related with the effects of different production techniques on tarhana characteristics (Ibanoglu *et al*., 1999; Tarakcı *et al*., 2004), only a few studies have been reported on the microbiological properties of tarhana (Siyamoglu, 1961; Temiz & Pirkul, 1990; Erbas *et al*., 2005).

The objectives of this study were to investigate the microbiological quality of tarhana produced traditionally at different locations, to investigate microbiological quality of yogurt and wheat flour/broken wheat used in tarhana receipts, to investigate the effects of fermentation and drying steps on the microbiological profile of tarhana, and to determine the changes of pH and acidic value of tarhana samples during fermentation period.

Materials and methods

Sample collection

Tarhana samples, which are produced traditionally by using homemade products with different formulations in eight different cities of the Aegean region in Turkey (Table 1), were collected at different times of fermentation (tarhana dough) and after drying period (dried tarhana). Main tarhana ingredients used in the preparation of tarhana, namely yogurt and wheat flour/broken wheat, were also collected separately. One of the samples (sample B) was Göce tarhana, which contains broken wheat instead of wheat flour in the formulation (Table 1). Except sample E (set type, Pınar brand), all other yogurt samples are homemade strained yogurt. This type of yogurt is produced traditionally by adding old yogurt as a starter culture to mild temperature milk. After fermentation is completed (yogurt container is covered with cloths to keep the mild temperature for 5–7 h during fermentation), it is transferred to fabric bags to separate the excess water and finally strained yogurt is obtained. All samples were carried to the laboratory in sterile jars under cooled conditions and analyzed immediately. Time elapsed between sample collection and analysis did not exceed 8 h.

Microbiological analysis

Microbiological quality of yogurt samples were detected by applying coliform, *Escherichia coli*, mould and yeast analysis as expressed in Turkish Standards (Anon, 2001a), while aerobic plate count (APC), coliform, *E. coli, Bacillus cereus, Clostridium perfringens, Salmonella* spp., rope spore and mould analysis were applied for wheat flour/broken wheat samples (Anon, 2001b). To determine microbiological quality of dried and powdered form of tarhana, APC, *Staphylococcus aureus*, *Salmonella* spp., *C. perfringens*, coliform, *E. coli* and *B. cereus* analysis, which are given in Turkish Standards for vegetable soups used after cooking, were applied to tarhana dough and dried tarhana samples (Anon, 2001b). Sample (10 g) was transferred to 90 mL 0.1% peptone water (pH 6.3 $\pm$ 0.2, Oxoid-L37, Basingstoke, Hampshire, England) and homogenized with Stomacher Lab-Blender 400 (Seward Medical, London, UK). Appropriate 10-fold dilutions of the samples were prepared in peptone water and plated in duplicate on/in growth media.

APC was determined by using pour plate method on plate count agar (pH 7.1 $\pm$ 0.2, Oxoid-CM325) and incubating the plates at a temperature of 30 °C for 24–48 h [Bacteriological Analytical Manual (BAM), 2001a]. Mould and yeast count was determined in acidified potato dextrose agar (pH 3.5, Oxoid-CM139) with 10% of tartaric acid (Merck, Art-802) by using pour plate method and plates were incubated at 25 °C for 3–5 days (BAM, 2001b). *S. aureus* was determined by surface plating on baird–parker agar (pH 6.8 $\pm$ 0.2, Oxoid-CM275) and incubating plates at 37 °C for 30–48 h. Coagulase test was applied for typical *S. aureus* colonies (BAM, 2001c). *Salmonella* was detected after preen-

Table 2 Microbiological results of wheat flour/broken wheat samples used in tarhana production

	CFU g^{-1}					MPN g^{-1}		
Sample[1]	*C. perfringens*	*Salmonella* spp.	*Bacillus cereus*	Aerobic plate count	Mould-yeast	Rope spore	Coliform	*Escherichia coli*
A	<10	Negative	$<10^2$	1.3×10^3	1.4×10^3	<3	<3	<3
B	<10	Negative	$<10^2$	9.5×10^4	1.1×10^2	1.1×10^3	2.5×10^2	4.5×10^1
C	<10	Negative	$<10^2$	7.0×10^2	1.0×10^3	4	<3	<3
D	<10	Negative	$<10^2$	8.0×10^2	4.5×10^1	4	<3	<3
E	<10	Negative	$<10^2$	3.0×10^2	2.8×10^2	<3	<3	<3
F	<10	Negative	$<10^2$	2.5×10^3	1.0×10^3	<3	2.5×10^1	9
G	<10	Negative	1.0×10^2	5.5×10^4	2.0×10^2	1.4×10^3	<3	<3
H	<10	Negative	$<10^2$	1.2×10^2	1.6×10^2	4	<3	<3

MPN, Most Probable Number.
[1]For explanations, see Table 1.

richment and enrichment steps. For preenrichment, 25-g sample was blended with 225 mL of lactose broth (LB; pH 6.9 ± 0.2, Oxoid-CM137) and incubated at 35 °C for 24 h. For enrichment, 0.1 mL inoculum was transferred from LB to 10 mL of rappaport–vassiliadis medium (pH 5.2 ± 0.2, Oxoid-CM669) and another 1 mL mixture to 10 mL Tetrathionate Broth (Oxoid-CM671) and incubated at 42 °C and 35 °C for 24 h, respectively. *Salmonella* isolation was performed by using streak plate method on xylose lysine desoxycholate agar (pH 7.4 ± 0.2, Oxoid CM 469) and brillant green agar (pH 6.9 ± 0.2, Oxoid CM 263) and incubating the plates at 37 °C for 24 h. Presumptive *Salmonella* colonies were confirmed using biochemical tests [triple sugar iron agar (pH 7.4 ± 0.2, Oxoid) and lysine iron agar (pH 6.7 ± 0.2, Oxoid) reactions] and serological tests (BAM, 2007). The presence of *C. perfringens* was tested by using pour plate method on sulphite polimyxin sulphadiazin agar (pH 7.0 ± 0.2, E. Merck, Darmstadt, Germany), and plates were incubated at 37 °C for 24 h in anaerobic jar. Typical colonies formed after incubation period were inoculated to cooked meat medium (pH 7.2 ± 0.2, Oxoid-CM81) covering the surface with 2% agar (Agar Bacteriological, Agar No:1, Oxoid-LP0011) and incubated at 37 °C for 24 h (Karapinar, 1995). Enumeration of coliform bacteria was performed by most probable number technique using lauryl sulphite tryptose broth (LSTB; pH 6.8 ± 0.2, Oxoid-CM451). Tubes were incubated at 37 °C for 24–48 h, and after incubation period the tubes that produced gas were confirmed with brilliant green lactose bile broth (pH 7.4 ± 0.2, Oxoid-CM31) incubating the tubes at the same conditions. To enumerate *E. coli*, LSTB-positive tubes were inoculated to *E. coli* broths (pH 6.9 ± 0.2, Difco Laboratories, Detroit, MI, USA) and incubated at 44.5 °C for 24–48 h. After incubation period, gas-produced tubes were inoculated to tryptone water (Oxoid, CM87) to confirm indole production (BAM, 2002). *B. cereus* count was determined by using surface plating on mannitol egg yolk polimixin agar (pH 7.2 ± 0.2, Oxoid-CM0929) and plates were incubated at 30 °C for 24 h (BAM, 2001d). Laboratory-pasteurized samples (in boiling water bath for 30 min) were enumerated for rope spore-forming bacteria by the most probable number technique using glucose tryptone broth (pH 6.9 ± 0.2, Oxoid-CM73) after incubation at 30 °C for 48 h (Anon, 1992).

Chemical analysis

The pH value of yogurt, tarhana dough and dried tarhana samples were determined by using pH meter (Nel Mod 821 brand). Acid content (v/v) of the samples was determined by titrimetric method and the results were expressed as the acidic value that passed from 67% ethyl alcohol for tarhana samples (Anon, 1981) and percentage lactic acid for yogurt samples (Anon, 2001a).

Results and discussion

Yogurt and flour samples used in tarhana receipts were analyzed in order to explore the relationship between the microbiological quality of raw material and tarhana. It is found that mould and yeast counts of yogurt samples were ranged between 6.4×10^4 CFU g^{-1} and 2.5×10^7 CFU g^{-1} and no samples contained coliform and *E. coli*. When the results are compared with the Turkish Standards developed for yogurt (Anon, 2001a), it is observed that samples met the microbiological criteria given in the standard. Table 2 shows the microbiological results of wheat flour/broken wheat samples. It is found that no wheat samples contained *C. perfringens* and *Salmonella* spp., while one wheat flour sample (sample G) includes *B. cereus* in low levels. These results showed that all wheat flour samples met the microbiological criteria given in the flour standard in Turkey. On the other

Table 3 Microbiological results of eight tarhana dough (at the beginning of the fermentation) samples from different cities in Turkey

	CFU g^{-1}					MPN g^{-1}	
Sample[1]	*Staphylococcus aureus*	*C. perfringens*	*Salmonella* spp.	*Bacillus cereus*	Aerobic plate count	Coliform	*Escherichia coli*
A	<10	1.0×10^1	negative	<10	4.1×10^6	<3	<3
B	<10	7.0×10^1	negative	5.0×10^2	7.0×10^6	<3	<3
C	<10	0.5×10^1	negative	1.0×10^2	4.0×10^7	4	<3
D	<10	<10	negative	<10	3.5×10^7	15	<3
E	<10	<10	positive	<10	2.3×10^6	<3	<3
F	<10	<10	positive	<10	1.6×10^5	<3	<3
G	<10	<10	positive	2.5×10^2	8.0×10^6	45	<3
H	<10	<10	negative	<10	1.6×10^6	<3	<3

MPN, Most Probable Number.
[1]For explanations, see Table 1.

hand, it is found that sample B, which is the one sample used in tarhana formulation as a broken wheat, contained *E. coli* above the limit given in the standard and was not suitable microbiologically (Anon, 2001b).

The microbial counts of tarhana samples, at the beginning of fermentation period, are represented in Table 3. As it can be seen from the table, none of the samples contained *S. aureus* and *E. coli.* Three out of eight samples were found to contain *C. perfringens* (samples A, B and C) in low numbers, which were not sufficient levels to produce toxin. Additionally, samples B, C and G were found to contain *B. cereus* approximately 10^2 CFU g^{-1} level, whereas samples E, F and G were found positive for *Salmonella*. On the other hand, after 24 h fermentation period, no *Salmonella, C. perfringens, S. aureus, B. cereus,* coliform and *E. coli* were detected from the samples. It can be thought that microbiological quality of ingredients used in the production of tarhana, but not analyzed in this study, such as spices, pepper, etc, which are known as potential sources of *Salmonella, C. perfringens* and *B. cereus* [International Commission on Microbiological Specifications for Foods (ICMSF), 1998], possibly affected the quality of tarhana. Vegetables used in tarhana formulations can be thought as safe foods, because they were used after cooking process. On the other hand, spices used in this study as ingredients of tarhana were commonly prepared by drying under the sun. During sun drying of spices, contamination risk increases and controlling conditions, especially for protecting the product from rain, overnight dew and vermin, become harder (ICMSF, 1998). It is clearly seen that only APC numbers, which mainly consisted of LAB and yeast, were enumerated at the end of the fermentation process while other microorganisms were not detected for all of the samples. Different researchers also studied the effects of tarhana fermentations on pathogenic microorganisms and similar results were reported. Daglioglu *et al*. (2002) inoculated tarhana dough with *E. coli* O157:H7 and *S. aureus* and observed that *E. coli* O157:H7 survived until the third day of fermentation while *S. aureus* was enumerated at the end of tarhana fermentation. In the other study, *Yersinia enterocolitica* and *E. coli* O157:H7 inoculated in tarhana dough and it is found that *Y. enterocolitica* survived until the second day of fermentation while *E. coli* O157:H7 could be isolated from the end of fermentation but not from the drying step (Aytac, 1996). On the contrary, Sagdıc *et al.* (2005) reported that he could not isolate *E. coli* O157:H7 from the second day of tarhana fermentation. These studies showed that the pathogen growth could be reduced during the production of tarhana, depending on the type of hazard being considered, the nature of the raw material and the precise details of the process used. Fermented foods are prime examples of the hurdle or multiple barrier approach to food preservation, where the overall antimicrobial effect seen is the aggregate effect of a number of different factors (Adams & Mitchell, 2002). A number of antimicrobial factors produced by LAB have been identified and their role has been reviewed periodically (Lindgren & Dobrogosz, 1990; Ouwehand, 1998; Adams, 2001). In recent years, considerable efforts have been devoted to the isolation and study of LAB antimicrobials such as bacteriocins, and this has tended to obscure the fact that the principal inhibitory contribution of LAB during lactic fermentations is the production of organic acid at levels up to and exceeding 100 mM and the consequent decrease in pH. For any inhibition to occur, LAB requires a large numerical superiority over any pathogens present (Adams & Mitchell, 2002).

After the fermentation process is completed, drying step was applied for the samples. In this step, APC was decreased markedly, except Samples E and F, which have the shortest fermentation times. It is found that the counts of APC were ranged between 1.4×10^3 CFU g^{-1} and 4.0×10^6 CFU g^{-1} in dried tarhana samples. These values are seen to be higher than the counts reported for dried

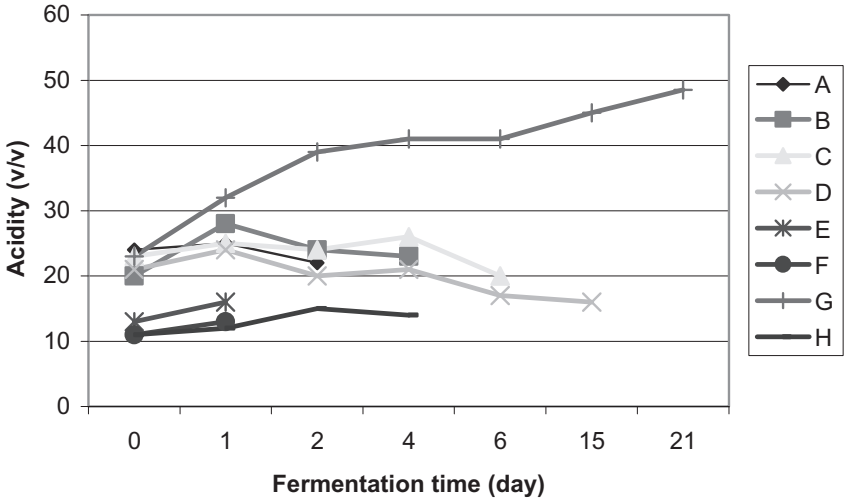

Figure 2 Changes in acidic values (passed from 67% ethyl alcohol) of tarhana samples during fermentation period.

tarhana which were found to average at 4.4×10^3 CFU g^{-1} (Coskun, 2002). It is observed that only one dried tarhana sample (E), which does not contain *C. perfringens* at the beginning of fermentation, contained *C. perfringens* in low levels (5.0×10^1 CFU g^{-1}). This finding means that although contaminated raw materials used in tarhana formulation affected the microbiological quality of the final product, some contamination can occur during the drying process. By the way, no *Salmonella, S. aureus, B. cereus,* coliform and *E. coli* were detected in any of the dried tarhana samples. Similarly, coliform bacteria could not be found in dried tarhana samples in other studies (Ozbilgin, 1983; Arıcı, 2000; Coskun, 2002). It is reported that drying reduces the moisture content of tarhana to 3–9% and it has a bacteriostatic effect on pathogenic microorganisms with low pH between 4.0 and 4.5 (Ibanoglu & Ibanoglu, 1999; Daglioglu, 2000; Bilgicli *et al.*, 2006). Various systems can be used for Tarhana drying and they are important for obtaining safe products. It is reported that microwave energy is more effective than conventional method to destroy the microorganisms in food preservation processes (Mudgett, 1989; Heddleson & Doores, 1994). Daglioglu *et al.* (2002) used two different drying methods for tarhana dough, inoculated with *E. coli* O157:H7 and *S. aureus*, and found that microwave drying completely destroyed the pathogen *S. aureus* and it was more efficient than the conventional method in reducing microbial population.

When we interpret the microbiological profile of yogurt, wheat and tarhana samples, it is observed that there is no exact correlation between the microbiological profile of raw material and tarhana. For example, as it can be seen from Tables 2 and 3, one broken wheat and one wheat flour sample (sample B and F) were found to contain coliform and *E. coli* while these microorganisms were not isolated from the related tarhana samples. It is thought that microbiological quality of other ingredients used in the production of tarhana such as spices, pepper, tomatoes, etc., could also affect the quality of tarhana. It is reported that while many pathogens can gain access to a product as a result of contamination during processing and storage, raw materials are often the principal source of hazards (Adams & Mitchell, 2002).

Figures 2 and 3 show acid content and pH value changes during tarhana fermentations. As it can be seen from Figure 2, the acid content of all samples rapidly increased at the end of the first day of fermentation while the pH value of tarhana samples commonly decreased sharply in the same period and then decreased gradually up to the fourth day (Figure 3). Samples E, F and H have the shortest fermentation times and the acidic values of these samples were found to be lower than the other samples, while sample G (tarhana produced by kneading the dough with fresh yogurt once a day during 21 days of fermentation) has the highest acidic value at the end of the fermentation process (Figure 2). It can be concluded that increasing the amount of yogurt and fermentation time had a significant effect on lactic acid formation (Figures 2 and 3). Bozkurt & Gürbüz (2008) also reported that the increasing fermentation time and the ratio of yogurt in tarhana dough increased the total acids of which lactic acid is the primary. As a result, the acidity produced in the product depends on the availability of fermentative substrates in tarhana samples and the fermentation time and temperature used in the production. Other researchers also reported that accelerated acidification occurred in the first 24 h fermentation period and resulted in a pH decrease from an initial

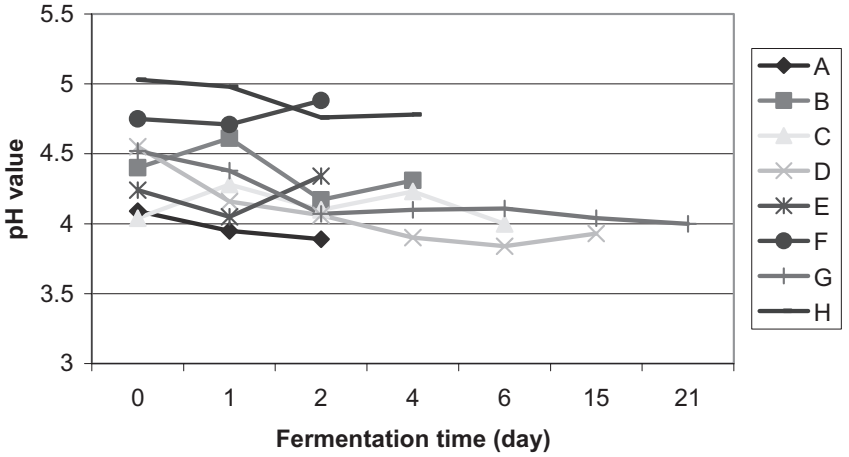

Figure 3 Changes in pH values of tarhana samples during fermentation period.

Table 4 pH value and acidity of dried tarhana and yogurt samples

Samples[1]		A	B	C	D	E	F	G	H
Dried tarhana	pH value	4.00	4.28	3.99	3.84	4.34	4.88	3.96	4.73
	Acidity[2]	18.00	20.00	20.00	18.00	16.00	16.00	48.50	14.80
Yogurt	pH value	3.35	3.71	3.61	3.76	3.57	3.85	3.96	3.82
	Acidity	1.37	0.88	0.82	0.88	1.23	0.83	1.00	1.16

[1]For explanations, see Table 1.
[2]Acidity (passed from 67 ml/100 ml ethyl alcohol).

value, followed by a phase of slow acidification that resulted in a further decrease in pH to around 4.00 (Ozbilgin, 1983; Ibanoglu *et al.*, 1995; Erbas *et al.*, 2005). Table 4 shows the pH and acidic values of dried tarhana and yogurt samples. As it can be seen from the table, these values are not so different from the values of samples that have just completed the fermentation periods (Figures 2 and 3). On the other hand, the pH and acidic values of yogurt samples used in tarhana fermentations were not directly affecting the acidic values of tarhana samples. However, LAB found in yogurt, which takes a role in the fermentation process by producing lactic acid and other compounds, affects the acidity of tarhana (Sengun *et al.*, 2009). The lactic acid content of tarhana produced with different dough treatments was determined by Bozkurt & Gürbüz (2008). They found that increasing fermentation time had a significant effect on lactic acid formation as well as on the total organic acid content of tarhana. On the other hand, when yogurt content was increased from 50% to 75%, total acidity in the first 48 h was 17.0% greater than the samples with 50% yogurt. Yogurt level had a significant effect on all the analytic parameters (Bozkurt & Gürbüz, 2008). Similarly, Temiz & Pirkul (1990) reported that the acid production of tarhana is related with the type and amount of yogurt used in the production.

It can be concluded from the results of this study that microbiological and chemical properties of tarhana change depending on the raw materials, fermentation time and techniques used in the production of tarhana. On the other hand, it remains to be investigated how other factors, such as additives like spices, the processing environment and the actual handling methods used, affect the microbiology of tarhana. Many pathogens can gain access to a product as a result of contamination from raw material or during processing and storage. Although tarhana is consumed as a soup after boiling process, microbiological safety is also important especially for toxin-producing microorganisms. The combined effect of organic acids produced in the fermentation period and the low moisture content may possibly exert bacteriostatic effect on spoilage organisms and pathogens that might be present.

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