Fate of some pathogenic bacteria and molds in Turkish Tarhana during fermentation and storage period

Fulya Turantaş · Kemal Kemahlioğlu

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Abstract The survival of the molds and some pathogenic bacteria such as *E. coli* O157:H7, *S. aureus*, *S. typhimurium* and *B. cereus* during fermentation and storage period of *tarhana* (traditional Turkish fermented food) was investigated. *Tarhana* batches were produced with two different yogurt/flour ratio 0.5 (T_1) and 0.75 (T_2). The pH and E_h values of samples were around 4.0–4.5 and 130–160 mV during fermentation and storage period in both batches, respectively. Moisture and a_w values in T_1 and T_2 samples significantly decreased ($p < 0.05$) between the 3rd–7th days of production at 11.2–11.6% and 0.52–0.54, respectively. The numbers of *S. aureus* and *S. typhimurium* significantly decreased ($p < 0.05$) during the 1st and 2nd days of fermentation period, respectively and this decline gradually proceeded in both batches. No significant differences ($p > 0.05$) were observed in both *tarhana* batches among the molds, *E. coli* O157:H7 counts during the 1st, 2nd days of fermentation, and *B. cereus* counts during the all days of fermentation and storage periods, except the 16th day of storage.

Keywords *Tarhana* · Pathogen survival · Fermented foods · Acid-resistant pathogens

Introduction

Traditional fermented foods appear to be a cheap, practical, convenient and nutritious alternative for the modern world

where famine is a great threat. Among the Turkish cultural and traditional foods, *tarhana* has an important place in daily diet especially for the people who live in rural parts of the Turkey. It is produced with flour, yogurt, salt, onion, tomato, pepper and spices (mint, red pepper). In winter time, Turkish people prefer traditionally prepared foods more in their diets. Furthermore, the dried *tarhana* slices are consumed in different ways such as nugget or snack in local regions of Anatolia. Baker's yeast is generally added to the *tarhana* dough, leading to the lactic acid and alcohol fermentation (İbanoğlu and İbanoğlu 1999; Çopur et al. 2001; Erbaş et al. 2006). Therefore, *tarhana* has an acidic taste and yeast aroma.

The important nutrients such as organic acids, ascorbic acid, niacin, folic acid, pantothenic acid, minerals and different amino acids are produced during *tarhana* fermentation (Nout 1993; Svanberg and Lorri 1997; Nout and Motarjemi 1997; Steinkraus 2002). Because of its high nutritional value and bioavailability, *tarhana* is generally used for feeding children and elderly people (İbanoğlu and İbanoğlu 1999; Dağlıoğlu 2000; Ekinci 2005; Ekinci and Kadakal 2005; Özdemir et al. 2007; Bozkurt and Gürbüz 2008; Dalgıç and Belibağlı 2008; Bilgiçli 2009).

The effects of different ingredients, fermentation and storage conditions on the chemical, nutritional, rheological and functional properties of *tarhana* have been studied (Temiz and Pirkul 1990; Temiz and Pirkul 1991; İbanoğlu et al. 1995; İbanoğlu et al. 1999; Dağlıoğlu 2000; Köse and Çağındı 2002; Erbaş et al. 2005; Erbaş et al. 2006; Bilgiçli et al. 2006a; Bozkurt and Gürbüz 2008; Bilgiçli 2009). There are limited microbiological data about on the survival of some pathogenic bacteria during production of *tarhana* (Aytaç 1996; Dağlıoğlu 2000; Sağdıç et al. 2005). Lactic antagonism is an important example of the microbial interference. Among antimicro-

F. Turantaş (✉) · K. Kemahlioğlu
Ege Vocational School Food Technology Department,
Ege University,
35100 Bornova,
İzmir, Turkey
e-mail: fturantas@yahoo.com

bial factors, pH depression is generally known as the primary factor for inhibition of microorganisms. However, in recent years several studies have been carried out on the acid tolerance and survival characteristics of some pathogenic bacteria in different acidic and/or acidic-fermented foods (Skovgaard 2007; Stopforth et al. 2007; Alvarez-Ordóñez et al. 2009; Montet et al. 2009). From this perspective, it is important to determine the surviving conditions of some pathogenic bacteria and molds in acidic-fermented foods such as *tarhana*. Some of the researches have focused on *Lactococcus*, *Lactobacillus*, *Streptococcus*, and other species which are the dominant organisms in starter cultures used for different fermented foods which contain milk and yogurt (Smit et al. 2005). Yogurt added to *tarhana* dough contains mixed cultures of *Streptococcus salivarius* subsp. *thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* in a 1:1 ratio. Lactic acid bacteria (LAB) produce lactic acid and a small amount of other organic acids, which have an antimicrobial and antagonistic effect on pathogenic and spoilage microorganisms in foods (Bozkurt and Gürbüz 2008; Buyong et al. 1998; Dağlıoğlu 2000; El-Ziney et al. 1999; Jay et al. 2005; Kang and Fung 2000). However, no information is published regarding to the lactic antagonism on the survival of some pathogenic bacteria and molds during *tarhana* production.

The objective of this research was to determine the survival of molds and some pathogenic bacteria as indicators such as *Escherichia coli* (*E. coli*) O157:H7, *Staphylococcus aureus* (*S. aureus*), *Salmonella typhimurium* (*S. typhimurium*) and *Bacillus cereus* (*B. cereus*) which contaminated from different habitats in home-style domestic production during fermentation and storage period of *tarhana*.

Materials and methods

Materials Wheat flour (moisture 14%, protein 12%, ash 0.5% on dry basis), full fat yogurt (3.2% wet basis), tomato paste (double concentrated, brix 28–30%) and paprika paste (brix 18–19%) onion, mint, red pepper, salt and baker's yeast (*Saccharomyces cerevisiae*, press form) were purchased from local markets in Izmir, Turkey. Before processing, all the ingredients were appropriately handled and onion was peeled and chopped.

Test cultures The cultures of *E. coli* O157:H7 ATCC 43895, *S. aureus* ATCC 4012, *S. typhimurium* ATCC 7823, and *B. cereus* FCI 11778 obtained from Safe Spice and Food Control Institute of Izmir were used in the experiments. These microorganisms were maintained on slopes of nutrient agar (Oxoid).

Production of tarhana All ingredients of *tarhana* (wheat flour 1000 g, yogurt 500 g or 750 g, tomato paste 150 g, paprika paste 100 g, onion 150 g, salt 25 g, baker's yeast 25 g, red pepper 10 g, mint 10 g and water 100 g) were mixed. Two different *tarhana* doughs (T_1 and T_2) were prepared using the same ingredients and quantities with two different yogurt/flour ratio (0.5- T_1 and 0.75- T_2). Tomato and paprika paste, onion and salt were blended by using Waring blender. All other ingredients were added to the *tarhana* dough and kneaded in a Hobart mixer (A120-10, Hobart MFG, London) for 5 min at the highest speed. After kneading *tarhana*, herb (*Echinobhora sibthorpiana*) was added in dough to provide special aroma, and then the *tarhana* doughs (T_1 and T_2) were divided into two parts for microbiological and chemical analysis and placed in two sealed plastic containers (27×27×5 cm) to a depth of 2 cm. First part was inoculated with 5 ml of suitable dilutions of 24 h nutrient broth cultures. The initial numbers of cultures in T_1 and T_2 *tarhana* doughs were 3.6–3.5/5.3–5.8/6.1–6.8 and 4.1–4.8 log cfu/g for *E. coli* O157:H7, *S. aureus*, *S. typhimurium* and *B. cereus*, respectively. Following inoculations *tarhana* doughs were manually kneaded by hand using disposable gloves (5 ml culture/500 g *tarhana* dough). Second part of the *tarhana* doughs was used for chemical analysis. *Tarhana* samples were fermented at 28 °C for 4 days in an incubator. It was manually mixed every 12 h by a spoon to prevent surface drying and dough rising.

The numbers of *E. coli* O157:H7, *S. aureus*, *S. typhimurium*, *B. cereus*, molds, LAB and pH, E_h (oxidation-reduction potential), water activity (a_w) and moisture were determined at 0, 1st, 2nd, 3rd and 4th days of fermentation and 7th, 9th and 16th days of storage.

At the end of the fermentation period, *tarhana* doughs were divided into small pieces and dried at ambient temperature for a few days (between 4th–7th days) similar to home-style natural drying conditions (on the bench in the laboratory) and finally ground in a blender. The dried samples were kept in the screw-capped glass jar at ambient temperature until analysed. All analyses were carried out in triplicate.

Microbiological analysis Ten g of *tarhana* samples were aseptically homogenised for 2 min in a stomacher model 400 (Seward Medical, London, UK) with 90 ml of 0.1% peptone (Oxoid) water and serial decimal dilutions were made using the same diluent. *Salmonella*–*Shigella* agar, Baird Parker agar and MacConkey agar (Oxoid) were used for enumeration of *S. typhimurium*, *S. aureus* and *E. coli* O157:H7 by using spread plate method, respectively. The numbers were determined after incubation at 32 °C for 24–48 h (Tadesse et al. 2005). For *B. cereus* count, the serial dilutions were plated on Phenol-Red Egg Yolk Polymyxin Agar (Oxoid) incubated at 35–37 °C for 24 h (Rhodehamel and

Harmon 2001). The mold count was determined by the pour plate method using Oxytetracycline Glucose Yeast Extract Agar (Oxoid) and incubated at 20–25 °C for 3–5 days (Ünlütürk and Turantaş 2002). LAB were enumerated on Man-Rogosa and Sharp agar (MRS, Oxoid) with an overlay of the same agar and incubated for three days at 30 °C (Dağlıoğlu et al. 2002; Ünlütürk and Turantaş 2002). All microbiological analyses were performed in triplicate samples.

Chemical analysis A 10 g of *tarhana* sample was dissolved in 100 ml of distilled water. The pH and E_h of the samples was measured using a portable pH/ E_h meter (Sartorius AG, PT-10, Germany) during fermentation (0, 1st, 2nd, 3rd and 4th days) and 7th, 9th and 16th days of storage. At the end of the 16th day of storage, the total titratable acidity of the samples were expressed as percent lactic acid on dry base (İbanoğlu et al. 1999). a_w values of *tarhana* samples was measured using a water activity measurement device (Testo 400, Lenzkirch, Germany) with an accuracy of ± 0.001 at 25 °C during fermentation and storage period (0, 1, 2, 3, 4, 7, 9 and 16th days). Moisture content was measured by drying samples to a constant weight (AOAC, 1990), and calculating the percentage of weight loss during fermentation and storage period (0, 1, 2, 3, 4, 7, 9 and 16th days). All assays were performed in triplicate samples and results were given as means.

Statistical analysis All statistical calculations were performed by SAS Statistical Software (release 7.00 for

windows, SAS Institute Inc., Cary, NC, USA). Significance were evaluated with the analyses of variance ($p < 0.05$).

Results and discussion

The pH and E_h values of *tarhana* samples during the fermentation were shown in Fig. 1. The *tarhana* doughs used in these experiments had the pH of 4.43–4.45 and the E_h values of 131.6–132.8 mV immediately after kneading in 0.50 (T_1) and 0.75 (T_2) yogurt/flour ratio *tarhana* samples, respectively. The pH values of the T_1 and T_2 *tarhana* samples varied from 4.43 to 3.97 and 4.45 to 3.88, E_h values of T_1 and T_2 samples varied from 131.6 to 160.7 mV and 132.8 to 161.3 mV, respectively during the fermentation period. The pH values of T_1 and T_2 significantly decreased ($p < 0.05$) at the 4th day of fermentation from 4.43 to 3.97 and from 4.45 to 3.88, respectively. The pH values gradually decreased ($p < 0.05$) and the E_h values increased after the 4th day of fermentation and the final pH and E_h values of T_1 and T_2 samples after the 16th day of storage reached to 4.54–4.53 and 131.9–130.8 mV, respectively. Moisture and a_w values in T_1 and T_2 samples significantly decreased ($p < 0.05$) in drying stage (between 4th and 7th days), from 33.2–35.5% to 11.2–11.6% and from 0.63–0.66 to 0.52–0.54, respectively (Fig. 1).

Similar to our findings, Sağdıç et al. (2005) and Erbaş et al. (2005) reported that the pH values of *tarhana* doughs

Fig. 1 pH, E_h , moisture and a_w values of *tarhana* samples during the fermentation* and storage** period. Yogurt/flour ratio 0.5 • T_1 and 0.75 Δ T_2 , the values are the average of triplicate measurements ($n=3$). (* at 28 °C, ** at ambient temperature)

were around 4.0–4.5 during 3 days of fermentation. At the beginning of the fermentation, relatively low pH value (around 4.5) of fresh *tarhana* samples (T_1 and T_2) is attributed to different organic acids present and/or produced in dough by means of LAB and yeasts in our research. However, Erbaş et al. (2006) determined that lactic, acetic and propionic acids were found in fresh *tarhana*.

As shown in Fig. 2, during the first 3 days of fermentation period, the numbers of *E. coli* O157:H7 slightly inclined from 3.6 to 3.3 log cfu/g in T_1 and T_2 *tarhana* samples. Although there was no consistent and appreciable difference between the numbers of *E. coli* O157:H7 at 1st, 2nd and 3rd days of fermentation in the T_1 and T_2 samples, these differences were found to be not significant either between days in the same *tarhana* sample or between two different *tarhana* samples for the same days ($p>0.05$). In contrast, the viable numbers of *E. coli* O157:H7 in T_1 and T_2 samples showed more than 2.5 log unit reduction within 1 day (from third to fourth day) at pH 3.97

and 3.88, at 160.7 and 161.3 mV of E_h values, respectively. The numbers of *E. coli* O157:H7 decreased below detectable level (<100 cfu/g) at the 4th day of storage in both *tarhana* batches (Fig. 2).

Sağdıç et al. (2005) determined that the growth of *E. coli* O157:H7 was inhibited at the end of the 2nd day of fermentation of seven different *tarhana* samples (control and added different spices). Aytaç (1996) reported that *E. coli* O157:H7 was able to survive on the 8th day of fermentation in *tarhana* at pH 3.10. In our research, *E. coli* O157:H7 was inhibited between the 4th and 7th days of storage period of T_1 and T_2 *tarhana* samples at approx. pH 4 (3.9/4.0/3.8). Similar to our findings, Dağlıoğlu et al. (2002) determined that *E. coli* O157:H7 survived until the 3rd day of fermentation in the inoculated samples; it was inhibited after the 5th day. This phenomena can be explained by the inexistence of a standardized production process for *tarhana* production. Application of different ingredients, the bacterial strains used and the fermentation

Fig. 2 Microbiological changes during tarhana fermentation* and storage** period. Yogurt/flour ratio 0.5 ● T_1 and 0.75 △ T_2 , the values are the average of triplicate measurements ($n=3$). (* at 28° C, ** at ambient temperature)

conditions might lead to varying results and survivability of microorganisms. From the stand point of the inhibition pathogenic and spoilage bacteria, it is clear that low pH, moisture, a_w and high E_h values were very important inhibitory factors in dry fermented foods.

It is determined that the conditions of fermentation are attributed to failure of some pathogenic bacteria such as *Salmonella*, *S. aureus*, *Listeria monocytogenes* and *E. coli* O157:H7. A wide variety of food-borne pathogens are either inhibited or killed and many spoilage microorganisms are affected similarly in fermented foods. Lactic antagonism has been attributed to the production of organic acids, hydrogen peroxide, bacteriocins, diacetyl and antibiotics by means of LAB. Organic acids reduce the pH value below 4.0–4.5, making it difficult for some spoilage and pathogenic microorganisms contaminated from different sources to survive. Many researchers observed a correlation between the decline in pH and antagonistic effect of LAB (Dağlıoğlu 2000; Blandino et al. 2003; Jay et al. 2005). However, Huang et al. (2001) demonstrated that the fermentation by-products decreased the viability of both *E. coli* O157:H7 and *S. aureus* depending on the concentrations and the lactic antagonism has received extensive attention and several reviews have been published, the most contemporary researches are listed as Fang et al. (1996), Nazeef et al. (2008) and Singh and Ramesh (2008).

The number of *S. aureus* decreased from 5.32 to 3.78 and from 5.85 to 3.92 during the first 24 h of fermentation period of T₁ and T₂ *tarhana* samples. Significant ($p<0.05$) decline in the number of *S. aureus* of T₁ and T₂ *tarhana* samples continued until the 3rd day of fermentation and the number of *S. aureus* decreased under the detectable level (<100 cfu/g, Fig. 2). This decline differed from the other test organisms which survived until the 4th, 7th, 9th and 16th days.

In the both *tarhana* batches (T₁ and T₂), the number of microorganisms decreased to undetectable level at the 3rd, 4th, 4th, 4th, and 16th days of fermentation and storage period of *tarhana* for *S. aureus*, molds, *E. coli* O157:H7, *S. typhimurium* and *B. cereus*, respectively (Fig. 2). It could be concluded that the numbers of *S. aureus* and *S. typhimurium* had shown gradual and significant decline ($p<0.05$) during the fermentation and storage period of *tarhana* batches (T₁ and T₂) differed from *E. coli* O157:H7 and *B. cereus*. In spite of, several researches have reported that *S. aureus* can grow and survive in environments with relatively low pH and high salt concentration (Koenig and Marth 1982; Halpin-Dohnalek and Marth 1989; Brunner and Wong 1992; Dağlıoğlu et al. 2002), in our research, the number of *S. aureus* significantly decreased ($p<0.05$) during the first day of fermentation and then gradual decline had continued until the 3rd day of fermentation (undetectable level) for both the *tarhana*

batches. Similarly, surviving numbers of *S. typhimurium* showed a tendency to decline ($p<0.05$) starting from the second day of fermentation and decreased below the detectable level (<100 cfu/g) at the 4th day of storage while the *E. coli* O157:H7 and *B. cereus* counts did not significantly change ($p>0.05$) during the 3rd and 9th days of fermentation and storage period, respectively. It could be explained that *B. cereus* is a spore forming bacterium and can resist and survive for long periods under unfavorable conditions and *E. coli* O157:H7 used in this research can be relatively a low pH resistant strain. However, it is reported that the some food-borne pathogens like *S. typhimurium*, *E. coli* O157:H7 are able to survive under extremely low pH (pH 2–3) conditions (de Jonge et al. 2003).

A large number of investigators have shown that the normal saprophytic flora in foods offers protection against staphylococcal growth through antagonism; in addition, competition for nutrients and the modification of environmental conditions are less favourable for *S. aureus* and *Salmonella*. In addition to, the antagonistic effect of other bacteria such as *Acinetobacter*, *Aeromonas*, *Bacillus*, *Pseudomonas*, *S. epidermidis*, the Enterobacteriaceae, the Lactobacillaceae, enterococci and others create unfavourable conditions for *S. aureus* and *Salmonella* (Caplice and Fitzgerald 1999; Jay et al. 2005). Finally, it can be said that *S. aureus* and *Salmonella* are not competitive enough in *tarhana* and in many food systems.

The results of this research showed that the important food-borne pathogens in *tarhana* ingredients survived after the fermentation period. Especially, competitive and resistant pathogens such as *B. cereus* can survive under unfavourable conditions in wet and dry-fresh *tarhana* during storage period.

The gradual decline of the LAB counts continued until the 16th day of storage and the numbers of LAB decreased from 7.6 to 5.1 log cfu/g in all final T₁-T₂ *tarhana* samples (Fig. 2). Different researches had determined that the number of the LAB was nearly around 6.5–7.0 log cfu/g at the beginning of the fermentation period of *tarhana* containing 0.35 and 0.50 ratio of yogurt (Sağdıç et al. 2005; Erbaş et al. 2005). The surviving numbers of molds showed a tendency to decline ($p<0.05$) starting from the second day of fermentation and decreased below the detectable level (<10 cfu/g) at the 4th day of fermentation due to the restricting factors such as E_h , a_w , acid content. Similar results were founded in the previous work (İbanoğlu et al. 1999).

Similarly, the total titratable acidity (as lactic acid) of the dried and ground *tarhana* samples was nearly around at 1.8–2.5% were reported (İbanoğlu et al. 1999; Erbaş et al. 2005; Bilgiçli et al. 2006b). In our research, total acidity was 2.3 and 2.5% for dried samples on the 16th storage day of T₁ and T₂ samples, respectively. Similar to our findings,

Bozkurt and Gürbüz (2008) reported that the total acidity was generally (17%) higher when yogurt ratio was increased from 0.5 to 0.75 in *tarhana* dough. It can be concluded from the results of this study that increasing ratio of yogurt in the *tarhana* dough did not significantly affect the surviving of pathogens at the end of the 16th storage day. According to Bozkurt and Gürbüz (2008), high percentage yogurt (75%) containing *tarhana* samples involve higher amounts of lactic acid; but increased moisture and oil content would create a shelf-life risk in the long-term storage. Although researches did not contain any microbiological data, they can give an idea about some factors (such as acidity, moisture) affected by growth and survival of microorganisms. According to our findings, there was not any important pH, a_w , moisture content and total acidity differences ($p>0.05$) between two batches of *tarhana* containing different ratio of yogurt (0.5 and 0.75) at the end of the 16th day of storage period.

It should be kept in mind that there is no standardized production process for *tarhana* which is traditionally produced at different regions of Turkey, and different food-borne pathogens generally contaminate during the home-style domestic production period from the human, ingredients and environments. It should be noted that the numbers for a particular contamination source reported by different researchers do not always reflect the microbiological status of the product correctly under ideal production, handling and storage conditions.

Conclusion

Microbial flora of fermented foods is primarily created by the natural flora of raw foods. In addition to these microorganisms, especially in home-style domestic production, the seconder flora can be contaminated from the different environmental sources. From the stand point of the human health protection and the food spoilage prevention, some foods gain importance after the determination of acid-resistant pathogenic bacteria in the acid-fermented foods. Sometimes *tarhana* is consumed before drying. As a consequence, consumption of wet fresh *tarhana* is not recommended in the first 7 days of production the period of which microorganisms inactivate.

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