

Influence of Tarhana Herb (*Echinophora sibthorpiana*) on Fermentation of Tarhana, Turkish Traditional Fermented Food

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Summary

Tarhana herb (*Echinophora sibthorpiana*) (TH) is used as a spice in tarhana. It has a pleasant flavour and stimulates some microorganisms. In this study, the fermentation activity of tarhana was investigated by monitoring the lactic acid bacteria (LAB) and yeast populations when TH was used as additive. It can be said that tarhana herb (*Echinophora sibthorpiana*) prevented the decrease in the counts of LAB and yeast below the initial number during tarhana fermentation.

Key words: tarhana, tarhana herb, lactic acid bacteria, fermentation, yeast

Introduction

Almost any food can be produced by fermentation of cereals (*e.g.* wheat, barley), leguminous plants (*e.g.* soya, beans), vegetables (*e.g.* cabbage, cucumbers), fruits (*e.g.* grapes, apples), milk, meat or fish. The two main reasons for fermenting food are (i) to improve its keeping properties against the attack of unacceptable microorganisms, and (ii) to create foods with better flavour, aroma, and/or texture through acceptable production of acids, alcohols, aromatic compounds, and other by the use of microorganisms (1).

Tarhana is a fermented cereal food and one of the oldest traditional Turkish soups. Scottish porridge, atole, kishk, kushuk, tahonya and tarhana are a class of hot gruel foods and are widely consumed in many countries. The gruel made from tarhana is an important part of diet in the Middle East, Asia, Africa and Europe as a good source of proteins, minerals and vitamins, so its products are widely consumed by people of all ages

(2–4). Tarhana is produced by mixing cereal flour, yogurt, baker's yeast (*Saccharomyces cerevisiae*), vegetables (tomatoes, onions, green peppers and red peppers), salt and spices (mint, thyme, dill, tarhana herb, *etc.*), followed by fermentation for one to seven days (5). At the end of this period, the fermented dough is usually sun dried at a domestic level or oven dried at an industrial level and ground to fine particle dimensions (<1 mm) (4,6). The low moisture content (6–9 %) and pH (3.8–4.4) make tarhana a poor medium for pathogens and spoilage organisms; tarhana is not hygroscopic and it can be stored for 2 to 3 years without any signs of deterioration (5).

Baker's yeast in combination with lactic acid bacteria is often used in the production of beverages and fermented foods (7). Yeast and bacteria are the most important fermentative microorganisms for cereal products (1). Fermentation causes functional and chemical changes in tarhana. For this reason tarhana has sour and

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acidic taste with yeast flavour (5,8). The nature and the concentration of microbial species present in the fermentation media markedly affect the nature of the final products (e.g. aroma). In cereal fermentations, heterofermentative lactic acid bacteria form lactic acid, acetic acid, ethyl esters of these acids, carbon dioxide, and several aromatic compounds. Homofermentative lactic acid bacteria form mainly lactic acid. Yeast fermentation proceeds through the Embden-Meyerhof pathway, in which glucose is transformed into ethanol (via pyruvate and acetaldehyde), carbon dioxide, and traces of other acids and carbonyl compounds. Lactic acid bacteria and yeasts readily ferment sugar sources present as fermentable sugars or derived from starchy materials by the action of cereal, fungal, or bacterial amylases (1). In essence, lactic acid bacteria produce several acids that lower the pH, while yeasts mainly form ethanol and carbon dioxide gas (1).

The amounts and types of ingredients and fermentation conditions may vary from place to place in Turkey, affecting chemical compositions, nutritional content and sensory attributes of tarhana. The ratio of yogurt to wheat flour is usually 1:1, in some regions the content of yogurt may be reduced or increased (4). Various spices are used as flavouring agents (mint, thyme, dill, tarhana herb) in different parts of Turkey.

Göçmen *et al.* (9) determined 41 aroma active compounds in different tarhana samples. The aroma active compounds identified from different types of tarhana were mainly aldehydes, esters, ketones, alcohols, terpenes, furan, phenols, sulfur compounds, acids, and other compounds. Aldehydes were the largest single class of aroma compounds in tarhana. Other differentiating aroma compounds included alcohols, terpenes and phenols such as geraniol, terpinolene and 4-vinylguaiacol, among others. Flavour compounds in dough fermentation are mainly formed as a result of enzymatic reactions by microflora.

Cossignani *et al.* (10) showed that the highest production of volatiles with a marked reduction of ethylacetate (volatile produced by lactic acid bacteria) characterized the fermentation by baker's yeast. While dough with the highest ratio (10 000:1) between lactic acid bacteria and yeast had ethylacetate as the main volatile compound, the use of mixed fresh-cell starters (lactic acid bacteria and yeast) with *S. exiguous* M14 (maltose negative) gave the fermented dough with the most balanced profile of volatile compounds.

Tarhana herb belongs to the *Apiaceae* family and originates from West Asia. It is a perennial shrub that grows from 20 to 50 cm and has strong, dense branches without spines. Its leaves are hairy and bipinnate. The upper surface of the leaves is dark green. Tarhana herb grows naturally in Turkey, the Balkans, Azerbaijan, Afghanistan, Italy, Sicily, Turkistan and temperate regions of the Mediterranean. The leaves of tarhana herb are used as a spice in tarhana, pickles and meatballs in various regions of Turkey (11,12).

There are many studies about tarhana. However, no information is published regarding the microbial development during Turkish tarhana fermentation with TH. Tarhana herb (*Echinophora sibthorpiana*) (TH) is used as a

spice in tarhana. It has a pleasant flavour and stimulates some microorganisms. The aim of this study was to determine the microbial, chemical, and pH changes when the three different ratios of TH (0.5, 1 and 1.5 %) were used. Sample codes are shown in Table 1.

Table 1. Sample codes

Code	Sample
S1	Standard tarhana sample without tarhana herb
S2	Tarhana sample with 0.5 % tarhana herb
S3	Tarhana sample with 1.0 % tarhana herb
S4	Tarhana sample with 1.5 % tarhana herb

Materials and Methods

Ingredients

Wheat flour (Toru Wheat Flour Cooperation, Bandırma/Turkey) with a moisture content of 13 %, ash content of 0.55 %, crude protein content of 10 %, on dry basis, was used. The yogurt (Sütaş A.Ş, Bursa, Turkey) used was full fat (4 %, wet basis). Tomato paste and red pepper paste (Penguen A.Ş, Bursa, Turkey) were concentrated with a solid content of 30 and 20 %, respectively. Pressed baker's yeast (*S. cerevisiae*) (Pakmaya A.Ş, İzmit, Turkey), table salt, onion and green pepper were purchased from the local markets in Bursa, Turkey. Tarhana herb was purchased in Kütahya, Turkey.

Production of tarhana

Onion and green pepper were blended for 30 s in a Moulinex Masterchef 70 (France) electronic blender and then flour (approx. 1000 g), yogurt 50 %, blended onion 10 %, blended green pepper 5 %, tomato paste 2.5 %, red pepper paste 7.5 %, salt 7.5 %, baker's yeast 1 % and dry-ground tarhana herb (0.5, 1.0 and 1.5 % level, based on flour weight) were mixed together and kneaded in a commercial percussion kneader (55 rpm) for 5 minutes. The tarhana dough was fermented at 25 °C for 4 days. This method was a modified procedure of Unal (13).

Triplicate samples (1 g) were taken every 48 h during fermentation for microbial analyses. The mixture was manually blended prior to sampling to ensure homogeneity of the samples taken. After the fermentation the dough was separated into small pieces of about 5–6 g and dried in air-oven at (50±1) °C until the moisture content of about 10 % was reached. Dried samples were milled in a hammer mill and sifted through a 1-mm screen sieve. The resulting samples were stored in a glass jar and refrigerated until tested.

Analytical methods

Moisture, ash, crude protein and salt levels were determined by Tarhana Standard Methods (TS 2282) (14). Titratable acidity and pH values of the samples were determined as described by Ibanoglu *et al.* (15).

Microbial enumeration

Triplicate samples of dough or dry ground tarhana (10 g) were homogenized in 90 mL of sterile physiological saline solution (0.85 %). Dilutions were also made in this solution, as needed for plating. The enumeration of particular microbial groups was performed in the following media and culture conditions: de Man-Rogosa-Sharpe (MRS) agar (pH=6.2) (Merck 1.10660, Germany) for lactic acid bacteria (LAB) and incubated at 30 °C for 3 days; plate count agar (PCA) (Oxoid CM325, England) for total mesophilic bacteria and incubated at 30 °C for 2 days; potato dextrose agar (PDA) (Merck 1.10130, Germany) for yeasts and molds and incubated at 25 °C for 5 days. Microbial colonies from the plates containing 30–300 colony forming units (CFU) were counted and transformed to log₁₀CFU/g.

Statistical analyses

The standard deviation was calculated by analysis of variance (ANOVA) using Minitab statistical package (16). Furthermore, Duncan’s multiple range test was used to determine the differences between variances by using MSTAT statistical package (17).

Results and Discussion

The microbial changes during tarhana fermentation are given in Figs. 1–3. The basic microorganisms are LAB from yogurt and *S. cerevisiae* from the baker’s yeast in tarhana fermentation. These two microorganism groups are responsible for the tarhana fermentation.

In general, the imbalance between yeast consumption and starch hydrolysis by flour enzymes leads to the rapid depletion of fermentable carbohydrates during fermentation, which, in turn, decreases LAB acidification due to microbial competition. Most reports show that LAB multiply and produce lactic and acetic acids more slowly in the mixtures with yeasts than in pure culture (7).

It was, however, determined that the LAB counts of all samples of tarhana dough with TH increased during fermentation, but LAB counts of dough without TH decreased during the fermentation in this study. After 4 days of fermentation, the highest LAB count and titratable acidity level were determined in S2. These properties were not as affected by 1.0 and 1.5 % TH as by 0.5 % TH (Figs. 1 and 4).

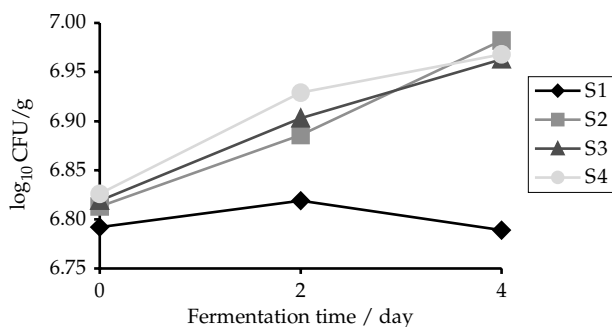


Fig. 1. Changes in viable LAB counts (log₁₀CFU/g) during tarhana fermentation (S1, standard sample without TH; S2, sample with 0.5 % of TH; S3, sample with 1.0 % of TH and S4, sample with 1.5 % of TH)

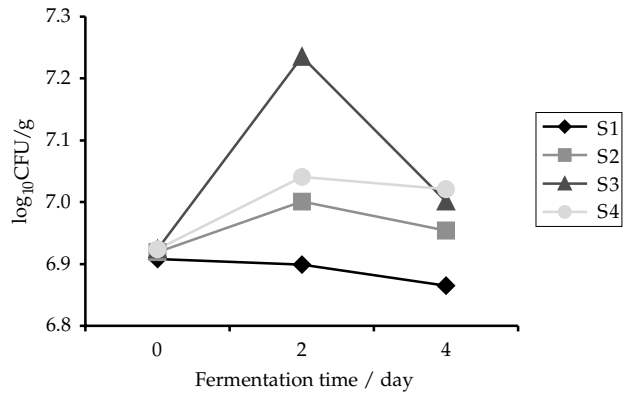


Fig. 2. Changes in viable yeast counts (log₁₀CFU/g) during tarhana fermentation (S1, standard sample without TH; S2, sample with 0.5 % of TH; S3, sample with 1.0 % of TH and S4, sample with 1.5 % of TH)

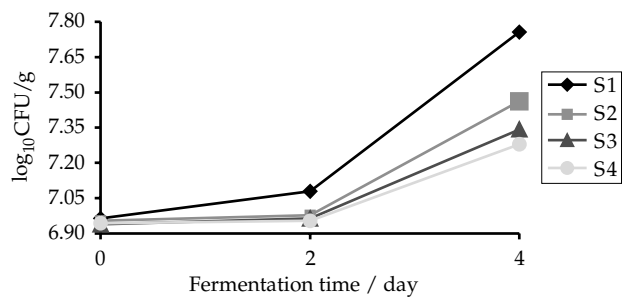


Fig. 3. Changes in viable total mesophilic bacteria counts (log₁₀CFU/g) during tarhana fermentation (S1, standard sample without TH; S2, sample with 0.5 % of TH; S3, sample with 1.0 % of TH and S4, sample with 1.5 % of TH)

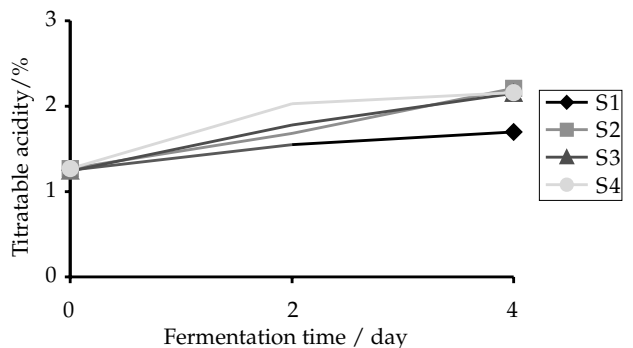


Fig. 4. Changes in titratable acidity levels during tarhana fermentation (S1, standard sample without TH; S2, sample with 0.5 % of TH; S3, sample with 1.0 % of TH and S4, sample with 1.5 % of TH)

Contrary to LAB counts, yeast populations of all samples of dough with TH increased during the first two days of fermentation and then decreased, but not below the initial number at the end of the fermentation. Contrary to this, yeast count decreased in dough without TH during the whole fermentation period (Fig. 2). Narvhus and Gadaga (18) reported that such interaction may be a stimulation or inhibition of growth of one, or both, of the co-cultured strains. The co-cultured organisms may compete for growth nutrients or they may produce metabolic products that inhibit each other’s growth. Yeasts may produce vitamins that enhance the growth of LAB.

Titrateable acidity levels of all samples of dough increased during fermentation (Fig. 4). The lowest titrateable acidity level was obtained in dough without TH and the highest value was obtained in S2 at the end of fermentation. pH values of all samples of dough decreased during the fermentation, but the highest pH value was determined in the dough without TH and the lowest pH was obtained in S2 at the end of fermentation (Fig. 5). According to Mugula *et al.* (19), a combined culture of yeasts and lactobacilli was also reported to bring about a more significant decrease in pH and a simultaneous increase in acidity in fermented millet than the use of single cultures.

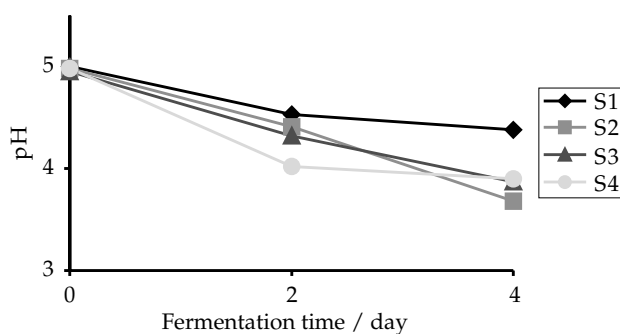


Fig. 5. Changes in pH during tarhana fermentation (S1, standard sample without TH; S2, sample with 0.5 % of TH; S3, sample with 1.0 % of TH and S4, sample with 1.5 % of TH)

The results of the chemical analyses on the dried-ground tarhana samples are given in Table 2. Moisture contents of all samples were below 11 %. These levels were suitable for long-term storage without deterioration (4). As moisture content of tarhana is low, it can be

stored for 2 or 3 years (4,5). Crude protein contents of all samples were between 12.61 and 12.68 %. For this reason, tarhana is a good source of proteins for children and elderly people (6). The highest titrateable acidity level (2.3 %) and the lowest pH (3.71) were observed in S2. The significant differences ($p < 0.01$) in pH between the sample without TH and samples with TH were determined in this study. Organic acids, formed throughout lactic acid fermentation, lower the pH to 3.8–4.2 and thus tarhana becomes a poor growth medium for pathogens and spoilage microorganisms (4).

The microbial populations of dried ground tarhana samples are given in Table 3. Dried ground tarhana samples with TH had significantly ($p < 0.01$) higher microbial populations compared to tarhana without TH. No mould growth was seen in any of the tarhana samples.

Conclusions

The results of this study showed that LAB counts of all samples of tarhana dough with TH increased during fermentation, and not decreased. In contrast to these results, LAB population of dough without TH decreased during the fermentation. Yeast populations of all samples of dough with TH increased during the first two days of fermentation and then decreased, but not below the initial number. It can be said that tarhana herb (*Echinophora sibthorpiana*) prevented the decrease in the counts of LAB during the fermentation and in the populations of yeast during the first two days of fermentation. In contrast to an earlier study of Ibanoglu *et al.* (5), microbial counts did not drop below the initial counts in samples with TH at the end of the fermentation in this study.

Table 2. Chemical and pH changes of dried and ground tarhana samples

Sample code	pH	Titrateable acidity	Moisture	Ash	Crude protein (N×6.25)
	*	%	%	%	%
S1	4.38±0.09a	1.8±0.2a	10.2±0.4a	5.22±0.10a	12.61±0.03a
S2	3.71±0.11b	2.3±0.3a	10.0±0.4a	5.30±0.09a	12.63±0.04a
S3	3.91±0.09b	2.2±0.3a	10.6±0.7a	5.33±0.16a	12.68±0.03a
S4	3.94±0.05b	2.2±0.1a	10.4±0.4a	5.51±0.09a	12.64±0.03a

*Mean values bearing different letters in a row are significantly different at 0.01 level (Duncan’s multiple range test)
 ns: mean values bearing different letters in a row are not significantly different at 0.01 level (Duncan’s multiple range test)

Table 3. Microbial populations of dried and ground tarhana samples

Sample code	LAB	Y	TB
	(CFU/g)	(CFU/g)	(CFU/g)
S1	(1.27·10 ⁶)±0.04c	(4.05·10 ⁵)±0.001d	(7.10·10 ⁶)±0.04a
S2	(1.78·10 ⁶)±0.01a	(5.85·10 ⁶)±0.002a	(2.40·10 ⁶)±0.03b
S3	(1.22·10 ⁶)±0.04c	(1.13·10 ⁶)±0.025c	(2.37·10 ⁶)±0.03b
S4	(1.47·10 ⁶)±0.03b	(1.51·10 ⁶)±0.045b	(1.75·10 ⁶)±0.003c

LAB, lactic acid bacteria; Y, yeast; TB, total mesophilic bacteria
 Mean values bearing different letters in a row are significantly different at 0.01 level (Duncan’s multiple range test)

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Utjecaj začinske biljke *Echinophora sibthorpiana* na fermentaciju tarane, turske tradicionalne fermentirane hrane

Sažetak

Začinska biljka *Echinophora sibthorpiana* koristi se u pripremi tarane. Ima ugodnu aromu i stimulira rast nekih mikroorganizama. U ovom je radu ispitivana fermentacijska aktivnost tarane prateći rast bakterija mliječne kiseline (LAB) i kvasca u prisutnosti te začinske biljke. Utvrđeno je da ona sprečava snizivanje broja bakterija mliječne kiseline i kvasca ispod početne količine tijekom fermentacije tarane.

