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# Effect of different ingredients on the fermentation activity in tarhana

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**Abstract**

Tarhana is a fermented wheat flour–yoghurt mixture which is widely consumed in Turkey. Baker's yeast is also involved in the fermentation. In this study, fermentation activity of tarhana was investigated by monitoring the lactic acid bacteria and yeast population when the level of salt and amount of yoghurt used were varied. Fermentation activity was high during the first day of fermentation. Microbial counts dropped below the initial counts at the end of a 4-day fermentation. Fermentation activity of tarhana prepared without salt was found to be higher than the tarhana samples prepared with salt. 

## 1. Introduction

Fermented milk–cereal mixtures form an important part of the diets of many people in the Middle East. Tarhana, a popular traditional fermented yoghurt-wheat flour mixture food product in Turkey (Siyamoglu, 1961), is prepared by mixing yoghurt, wheat flour, yeast and a variety of cooked vegetables and spices (tomatoes, onions, salt, mint, paprika) followed by fermentation for one to seven days (Campbell-Platt, 1987). Lactic acid bacteria in yoghurt, and the yeast are responsible for acid formation during fermentation and the leavening effect. After fermentation, the mixture is sun dried. Tarhana has an acidic and sour taste with a strong yeasty flavour and is used for soup making. It is a good source of protein and vitamins and therefore is used largely for feeding children and elderly people (Ibanoğlu et al., 1995a). The low pH (3.8-4.4) and low moisture content (6-9%) make the 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 sign of deterioration (Salama et al., 1992).

The amount and type of ingredients may change from place to place. Wheat flour is sometimes replaced by bulgur (parboiled, cracked wheat kernel) and sour dough is used instead of wheat flour. The ratio of yoghurt to wheat flour is subject to the availability of milk in that particular season and in that particular region. There are some other products similar to tarhana such as kishk (sour milk–wheat mixture with boiled chicken stock) in Syria, Jordan and Egypt (Youssef, 1990) and kushuk (milk–sour dough mixture with turnips) in Iraq (Alnouri and Duitschaever, 1974).

The effects of the presence of salt and amount of yoghurt incorporated into tarhana during fermentation on the nutritional status (Siyamoglu, 1961) and rheological properties (Ibanoğlu et al., 1995b) of tarhana have been studied. However, no information is published regarding the microbial development during Turkish tarhana fermentation with and without salt and with different amounts of yoghurt. The purpose of this work was, therefore, to monitor the microbial changes during a typical 4-day tarhana fermentation when the level of salt and amount of yoghurt used were varied. Investigating the microbial changes during fermentation of tarhana with different ingredients would lead to a clearer understanding of the chemical changes in tarhana during fermentation.

## 2. Materials and methods

### 2.1. Materials

The ingredients used in tarhana preparation were purchased from local markets in Manchester, UK. The

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wheat flour used was regular finely ground commercial white wheat flour with a crude protein content of 12.3% (wet basis). The yoghurt used was full-fat set yoghurt (pH 3.9) made of cow's milk and had a solids and fat content of 14.0 and 3.6%, respectively. Tomato puree was double concentrated (30% dry solids). Yeast was baker's yeast in active dry form. The spices used were in powder form.

### 2.2. Preparation of tarhana

Three different tarhana formulations were prepared: (1) tarhana with a yoghurt to wheat flour ratio of 0.5 with 80.0 g salt $kg^{-1}$ wheat flour used (standard tarhana, S1); (2) tarhana with a yoghurt to wheat flour ratio of 1.0 with 80.0 g salt $kg^{-1}$ wheat flour used (S2) and; (3) tarhana with a yoghurt to wheat flour ratio of 0.5 without salt (S3). The amounts of ingredients used in the preparation of tarhana samples are given in Table 1.

To prepare tarhana samples, onions were chopped and blended for 30 s with 50 ml of tap water by means of a coffee blender (Model A516, Kenwood Limited, New Lane, Havant). The tomato puree, salt, paprika, dill and mint were added, blended for 30 s, brought to the boil and simmered for 10 min. The mixture was left to cool to room temperature and then yoghurt, wheat flour and yeast were added and the mixture was kneaded (Chef 5 K7, Kenwood Limited, New Lane, Havant) at 40 rpm after the addition of a further 100 ml of tap water. The resulting dough was spread over a stainless steel tray ($30\times12\times3$ cm) to a depth of approximately 10 mm and incubated at $30\pm1$°C for 4 days to ferment, as typical of normal practice. Triplicate samples (1 g) were taken every 24 h during fermentation for microbial analyses. The mixture was manually blended prior to sampling to ensure homogeneity of the samples taken. After fermentation, the mixture was dried in an air oven (Status, Sainsbury Way, Hessle) at $50\pm1$°C for 48 h and finely ground (Lionhill Mill 14920, Copenhagen, Denmark) to a particle size of $<400$ μm for further analyses.

Table 1
Recipe of ingredients for tarhana samples

| Ingredients | Wheat flour used (g $kg^{-1}$) |
|---|---|
| Yoghurt | 500.0[a] |
| Water | 300.0 |
| Fresh onions | 120.0 |
| Tomato puree | 120.0 |
| Salt | 40.0[b] |
| Baker's yeast | 20.0 |
| Paprika powder | 20.0 |
| Dill powder | 2.0 |
| Mint powder | 2.0 |

[a] :1000 g in tarhana with increased yoghurt (S2).
[b]:Nil in tarhana without salt (S3).

### 2.3. Microbial analyses

Microbial analyses were carried out aseptically by homogenising 1 g samples in 20 ml sterile phosphate buffer (pH 7.0) and making serial dilutions from $10^{-1}$ to $10^{-8}$ levels. Pour plate technique was used for lactic acid bacteria (using deMan Rogosa and Sharpe agar) and total bacterial count (using plate count agar). Surface spread technique was used for yeasts and moulds (using malt extract agar). The plates were incubated at $25\pm1$°C for 5 days. The plates having less than 300 cfu (colony forming unit) were counted. The results were expressed as cfu/g dry weight. Both pour and spread plates were carried out in triplicate for each dilution.

### 2.4. Analytical methods

#### 2.4.1. Determination of pH

A 5-g dried and ground sample was blended using a laboratory blender with 100 ml of distilled water for 3 min, and the solution was filtered through Whatman 30 filter paper. The pH of the solution was then measured using a digital pH meter.

#### 2.4.2. Determination of acidity

Acid concentration of dried and ground samples (1 g) was determined by titration using 0.1 M NaOH and expressed as percent lactic acid (Kirk and Sawyer, 1991).

#### 2.4.3. Determination of protein content

Crude protein content of dried and ground samples (0.5 g) was determined by the Kjeldahl method (AOAC, 1984) using a Buchi apparatus (Models 430 and 320, Buchi Laboratoriums-Technic AG, Flawil, Switzerland).

#### 2.4.4. Fat determination

The total crude fat content of dried and ground samples (2 g) was determined by the Soxhlet method using a Soxtec HT system (Tecator, Sweden), with petroleum ether being the solvent.

#### 2.4.5. Moisture determination

The moisture content of dried and ground samples (0.5 g) was determined by drying the samples at $130\pm5$°C for 1 h in an air oven.

#### 2.4.6. Ash determination

Dried and ground samples (1 g) were ashed at $550\pm5$°C in a muffle furnace until a constant weight was obtained.

#### 2.4.7. Salt determination

Salt content of dried and ground tarhana samples (0.5 g) was determined by the Mohr method (Kirk and Sawyer, 1991).

Table 2
Microbial changes during tarhana fermentation (cfu $g^{-1}$ dry wt ± SD, $n = 3$)*

| Days | 0 | 1 | 2 | 3 | 4 |
|---|---|---|---|---|---|
| S1 | | | | | |
| TB | $5.3\times10^7\pm1\times10^5$(a) | $2.7\times10^8\pm2\times10^5$(b) | $6.9\times10^7\pm9\times10^4$(c) | $6.6\times10^7\pm1\times10^5$(d) | $4.5\times10^7\pm1\times10^4$(e) |
| LAB | $6.6\times10^7\pm4\times10^5$(a) | $9.6\times10^7\pm3\times10^5$(b) | $2.7\times10^7\pm1\times10^5$(c) | $4.1\times10^6\pm3\times10^4$(d) | $4.0\times10^6\pm2\times10^4$(d) |
| Y | $8.4\times10^6\pm3\times10^4$(a) | $2.8\times10^7\pm3\times10^4$(b) | $9.1\times10^6\pm3\times10^4$(c) | $2.3\times10^6\pm1\times10^4$(d) | $2.2\times10^6\pm1\times10^4$(e) |
| S2 | | | | | |
| TB | $5.1\times10^8\pm2\times10^5$(a) | $1.3\times10^9\pm3\times10^5$(b) | $1.2\times10^7\pm1\times10^5$(c) | $6.4\times10^6\pm2\times10^4$(d) | $6.4\times10^6\pm1\times10^4$(d) |
| LAB | $2.0\times10^8\pm2\times10^5$(a) | $1.5\times10^8\pm3\times10^5$(b) | $3.7\times10^7\pm1\times10^5$(c) | $1.6\times10^7\pm1\times10^5$(d) | $1.5\times10^7\pm1\times10^5$(e) |
| Y | $8.6\times10^6\pm1\times10^5$(a) | $2.9\times10^7\pm4\times10^5$(b) | $9.2\times10^6\pm1\times10^5$(c) | $2.2\times10^6\pm1\times10^4$(d) | $2.2\times10^6\pm1\times10^4$(e) |
| S3 | | | | | |
| TB | $5.5\times10^7\pm1\times10^5$(a) | $1.4\times10^8\pm1\times10^5$(b) | $8.3\times10^7\pm1\times10^5$(c) | $2.3\times10^7\pm4\times10^4$(d) | $1.6\times10^7\pm1\times10^5$(e) |
| LAB | $9.2\times10^7\pm3\times10^5$(a) | $4.4\times10^8\pm3\times10^5$(b) | $6.8\times10^8\pm5\times10^5$(c) | $6.9\times10^7\pm2\times10^4$(d) | $3.8\times10^7\pm2\times10^5$(e) |
| Y | $8.5\times10^6\pm4\times10^5$(a) | $2.8\times10^7\pm1\times10^5$(b) | $9.3\times10^6\pm1\times10^5$(c) | $2.4\times10^6\pm8\times10^4$(d) | $2.3\times10^6\pm1\times10^5$(e) |

S1: Standard tarhana sample; S2: tarhana sample with increased yoghurt; S3: tarhana sample without salt.
TB: Total bacterial count; LAB: lactic acid bacteria, Y: yeast.
*Mean values bearing different letters in parenthesis in a row are significantly different at $p = 0.05$ (Duncan's multiple comparison test).

## 3. Results and discussion

The fermentation of tarhana is a result of the action of a mixed population of microbes. Although many micro-organisms may be present, principal organisms are lactic acid bacteria from yoghurt and *Saccharomyces cerevisae* from baker's yeast (Robinson and Cadena, 1978). These organisms are responsible for the production of carbon dioxide, alcohol, acids, aldehydes, ketones and other fermentation products, thus giving tarhana its characteristic flavour (Campbell-Platt, 1987).

The results of microbial analyses during tarhana fermentation are shown in Table 2. Microbial populations of all tarhana samples increased significantly ($p < 0.05$) during the first day of fermentation and then decreased gradually below the initial number at the end of the fermentation. This could be expected since no additional substrate (yoghurt and/or wheat flour) was added through the fermentation. The increase in yeast number indicated that yeast fermentation was also involved in the tarhana fermentation. During the first day of fermentation, the production of carbon dioxide and therefore leavening of the batter was observed, as the yeast readily fermented the free sugars available in the mixture.

The total and lactic acid bacteria counts showed variations before fermentation due to different formulations (Table 2). The total and lactic acid bacteria count of tarhana with increased yoghurt (S2) was higher than that of other tarhana samples. This was expected, since more yoghurt is incorporated into the sample.

The final lactic acid bacteria population of tarhana with increased yoghurt (S2) was higher than in standard tarhana (S1), which is indicated by a higher concentration of lactic acid produced when compared with the other samples (Table 3). Damir et al. (1992) also showed a positive relationship between lactic acid concentration and lactic acid bacteria count during kishk fermentation (a fermented wheat-milk product).

The tarhana sample without salt (S3) had a higher lactic acid bacteria population than the other two samples at the end of fermentation (Table 2). Therefore, if salt is added before fermentation, the fermentation activity and lactic acid concentration are lower when compared with the same composition of tarhana without salt (Table 3). This could be explained by the higher water activity of the fermenting medium in which less salt is present to bind some of the free water available. This could favour the growth of lactic acid bacteria.

The ash content of tarhana without salt was the lowest (Table 3). The reason could be the absence of salt in the sample, reducing the ash content. The results of microbial analysis done on the dried and ground tarhana samples are given in Table 4. The microbial population of the tarhana sample without salt was higher than that of the other samples.

Table 3
Proximate composition and pH of dried and ground tarhana samples on dry basis (mean ± SD, $n = 3$)*

| | S1 | S2 | S3 |
|---|---|---|---|
| Moisture (%) | 7.7 ± 0.1(a) | 9.5 ± 0.1(b) | 6.9 ± 0.3(c) |
| Lactic acid (%) | 1.8 ± 0.1(a) | 2.0 ± 0.1(a,b) | 2.5 ± 0.1(c) |
| Crude protein (%) (N×6.25) | 16.2 ± 0.1(a) | 16.7 ± 0.1(b) | 16.0 ± 0.1(a) |
| Crude fat (%) | 3.8 ± 0.1(a) | 4.5 ± 0.2(b) | 3.5 ± 0.2(a) |
| Salt (%) | 5.9 ± 0.3(a) | 5.7 ± 0.3(a) | 2.2 ± 0.3(b) |
| Ash (%) | 7.4 ± 0.2(a) | 7.7 ± 0.1(a) | 1.8 ± 0.2(b) |
| pH | 4.80 ± 0.01 | 4.42 ± 0.01 | 4.21 ± 0.01 |

S1: Standard tarhana; S2: tarhana with increased yoghurt; S3: tarhana without salt.
*Figures in the same row sharing a common letter in parenthesis are not significantly different at 0.05 level (Duncan's multiple comparison test).

Table 4
Microbial populations of dried and ground tarhana samples (cfu $g^{-1}$ dry wt ± SD, $n = 3$)*

| | S1 | S2 | S3 |
|---|---|---|---|
| TB | 1200 ± 18(a) | 1900 ± 17(b) | 2200 ± 17(c) |
| LAB | 720 ± 20(a) | 1100 ± 50(b) | 1500 ± 20(c) |
| Y | 650 ± 17(a) | 590 ± 20(b) | 780 ± 13(c) |

S1: Standard tarhana; S2: tarhana with increased yoghurt; S3: tarhana without salt.

TB: Total bacterial count; LAB: lactic acid bacteria; Y: yeast.

*Figures in the same row bearing a different letter in parenthesis are significantly different at 0.05 level (Duncan's multiple comparison test).

No mould growth was seen in any of tarhana samples under investigation. The presence of moulds in tarhana fermentation should be avoided since some toxic products may be produced by them.

## 4. Conclusions

The results of this study showed that the fermentation activity decreased after the first day of tarhana fermentation. It can be concluded that tarhana which is fermented for more than one day requires addition of extra yoghurt and/or wheat flour to keep the fermentation activity high when a more acidic and sour taste is desired. Excluding the salt from the formulation and adding some extra yoghurt and/or wheat flour after the first day of fermentation would be useful for this purpose.

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