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APPLICATION OF LACTIC ACID BACTERIA AS STARTER CULTURE FOR TARHANA FERMENTATION

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ABSTRACT

The suitability of lactic acid bacteria starter cultures *Lactobacillus sanfrancisco* CCM 7699 and *Lactobacillus plantarum* CCM 7039 for tarhana production was studied. Removed 2nd sentence Reducing saccharides served as an available source of energy for fermenting microbiota and their concentration dropped from 2.06 - 2.12 to 0.25 – 0.31 g.100g^{-1} at the end of the process. The most of lactic acid (0.81 g.100g^{-1} after 144 h) was produced in tarhana inoculated with *Lb. plantarum* CCM 7039. In all tarhana samples production of acetic and succinic acid were also observed. The citric acid concentration declined from 132.19 - 134.75 mg.100g^{-1} in unfermented dough to 6.81 – 55.65 mg.100g^{-1} in fermented tarhana. Fermentation of tarhana led to significant phytic acid reduction, while the highest phytic acid loss (88.75 %) was observed in sample with culture *Lb. sanfrancisco* CCM 7699. Fermentation process of tarhana samples caused also increase in mineral content (especially Ca and Mg). Enumeration of microorganisms showed, that lactic acid bacteria and yeasts proliferated in initial phases of fermentation (48 – 72 h), while in later phases their counts remained stable or decreased. Sensory evaluation of tarhana showed, that application of starter cultures significantly affected odour, taste and overall acceptance of samples due to different amounts of produced metabolites, especially organic acids. No significant differences were found in color and consistency of control sample and samples with starter cultures.

Keywords: Tarhana, fermentation, lactic acid bacteria, yeasts, organic acids, phytic acid

INTRODUCTION

Fermented cereals play a significant role in human nutrition in all parts of the world where cereals grow. Among all food fermentations (e.g. milk, meat, fish, soy or wine) cereal fermentations reach the highest volume **(Brandt, 2014)**. Fermentation is carried out to enhance taste, aroma, shelf-life, texture, nutritional value and other favourable properties of foods **(Georgala, 2013)**.

Tarhana is a traditional cereal based fermented food product consumed in Turkey with a potential for industrial processing and is prepared by mixing wheat flour, yoghurt, yeast and a variety of cooked vegetables (tomatoes, onions, green pepper etc.), salt and spices (mint, paprika) followed by fermentation for one to seven days. The temperature of fermentation is in range of 30 – 40 °C depending on the procedure applied **(Daglioğlu, 2000; Maskan and Ibanoğlu, 2002)**. Throughout fermentation lactic acid bacteria (LAB) (*Streptococcus thermophilus*, *Lactococcus lactis*, *Lactococcus diacetylatis*, *Lactobacillus bulgaricus*, *Lactobacillus acidophilus*, *Leuconostoc cremoris*, *Lactobacillus casei*) and yeasts *Saccharomyces cerevisiae* give the characteristic taste and flavour of tarhana by producing lactic acid, ethanol, carbon dioxide and some other organic compounds **(Daglioğlu *et al*., 2002)**. Tarhana is usually reconstituted with water and served as a hot soup generally consumed at lunch and dinner. Tarhana soup can be prepared from wet or dry tarhana **(Erbaş *et al*., 2005; Koca *et al*., 2002; Ozdemir *et al*., 2007)**. Tarhana has an acidic and sour taste with a strong yeasty flavor **(Kaya *et al*., 1999)**.

Tarhana is very nutritive food because of nutritional deficiency in wheat mostly eliminated by yoghurt. Its nutritional value is increased and digestion is facilitated by fermentation **(Dalgiç and Belibağh, 2008)**. The protein, carbohydrate and lipid components of tarhana mix are subjected to partial digestion and hydrolysis by lactic acid bacteria and yeasts during fermentation, resulting in a product with improved digestive properties **(Tamer *et al*., 2007)**. Tarhana is a good source of calcium, iron and zinc as well as some other minerals **(Daglioğlu, 2000)**. Fermentation of tarhana also results in significant increases of riboflavin, niacin, panthothenic acid, ascorbic acid and folic acid contents **(Bilgiçli, 2009)**.

The products similar to Turkish tarhana are known as trahana in Greece, kishk in Egypt, Syria and Jordan, kushuk in Iraq, and tahonya/talkuna in Hungary and Finland **(Isik and Yapar, 2012)**. The low moisture content (3-9 %) and low pH value (4.0-4.5) of the final product provide bacteriostatic effect against pathogenic and spoilage microorganisms and increase the shelf life of the product **(Sengun and Karapinar, 2012)**.

The quality of tarhana, as with other fermented food products depends highly on microbial yield and type. The use of lactic starter culture is essential to provide a more controlled and standardized fermentation process (Herken & Çon, 2012). Therefore in this study the effect of starter cultures *Lactobacillus* (*Lb.*) *sanfrancisco* CCM 7699 and *Lactobacillus plantarum* CCM 7039 application on the fermentation process and sensory properties of tarhana were investigated. *Lb. sanfrancisco* was used due to its heterolactic metabolism, short lag phase during dough acidification, volatile compounds production and symbiotic relationship with *Saccharomyces cerevisiae* as was reported by **Zapparoli and Torriani (1997)**. *Lb. plantarum* was inoculated to tarhana due to its previous application as starter culture in tarhana preparation by **Herken and Çon (2012)** and also to other cereal fermented products **(Angelov *et al*., 2006; Coda *et al*., 2011; Coda *et al*., 2012)**. During tarhana fermentation, analysis of chemical parameters (pH, content of reducing saccharides, lactic, acetic, citric, succinic acid concentration, concentration of phytic acid and amount of Na, Ca, Mg) and determination of lactic acid bacteria and yeasts counts were performed. Sensory parameters (color, odour, taste, consistency, overall acceptability) of tarhana soups were also evaluated.

MATERIAL AND METHODS

Raw materials

The ingredients used in tarhana preparation (Table 1) were purchased from local markets in Bratislava, Slovakia. The wheat flour used was regular finely ground commercial white wheat flour with a moisture content of 13.16 %, crude protein content of 11.07 % and ash content of 0.43 % (w/w, dry basis). The yoghurt was laboratory prepared from bovine UHT milk (3.5 % fat) by using commercial yoghurt culture Lactoflora® (Milcom, Czech Republic) and incubated in thermostat for about 6 h at 39 °C. Bacterial cultures *Lb. sanfrancisco* CCM 7699 and *Lb. plantarum* CCM 7039 were purchased from the Czech collection of microorganisms (Masaryk University, Czech Republic) as lyophilised cultures and propagated in de Man, Rogosa and Sharpe (MRS) broth (Merck, Darmstadt, Germany) for 48 h. Cells were centrifuged at 5000 rpm for 20 min after incubation. Then cells were washed with sterile saline physiological solution and

resuspensed in sterile physiological solution before being added to the tarhana mix.

Table 1 Recipe of tarhana ingredients

Ingredient	Quantity (g)
Wheat flour	500
Yoghurt	250
Tomato puree	60
Onion	60
Salt	40
Baker's yeast	10
Sweet paprika	10
Dill	1
Mint	1
Water	150

Preparation of tarhana samples

Tarhana samples (control – without starter culture, tarhana with culture *Lb. sanfrancisco* CCM 7699 and tarhana with *Lb. plantarum* CCM 7039) were prepared as wet tarhana according to the recipe and method described by **Ibanoğlu and Maskan (2002)**. To prepare tarhana samples, onions were chopped, sliced to small pieces and blended for 30 s with 50 cm^3 of tap water. The tomato puree, salt, sweet paprika powder, dill powder and mint powder were added, blended for 30 s, brought to the boil and blended for 10 min. The mixture was left to cool to room temperature and then wheat flour, yoghurt, baker's yeast were added. Inoculum of cultures *Lb. sanfrancisco* CCM 7699 and *Lb. plantarum* CCM 7039 was then added to the relevant sample in amount of 0.5 % (w/v) with cell density 10^9 CFU.ml^{-1}. The mixture was kneaded after the addition of a further 100 cm^3 of tap water. The resulting mixture was taken into covered sterile containers and incubated in thermostat at 30 ± 1 °C for 144 h. During fermentation, samples were withdrawn in pre-specified time intervals for chemical analyses and microbiological determinations. Tarhana soups were prepared by mixing 40 g of tarhana sample with 500 cm^3 tap water and simmering for 10 min with constant stirring **(Erkan *et al*., 2006)**.

Chemical determinations

Reducing saccharides was determined according to Schoorl. The nonreacted Cu^{2+} was determined after formation of Cu_2O. The KI was oxidized by $CuSO_4$ to I_2 that was determined by titration with $Na_2S_2O_3$. Reducing saccharides were expressed as grams of glucose per 100 g of sample **(Kohajdová and Karovičová, 2005)**. Lactic, acetic, citric and succinic acids were determined by using of isotachophoretic method described by **Kohajdová *et al*. (2006)**. Phytic acid expressed as myo-inositol hexakisphosphate was measured by modified isotachophoretic method of **Dušková *et al*. (2000)**. Modified isotachophoretic method of **Zelenský *et al*. (1997)** was used for determination of minerals (Ca, Na, Mg). Isotachophoretic analyser ZKI 01 (Villa Labeco, Slovakia) with conductivity detector and two-line recorder TZ 4200 (Laboratórní přístroje, Czech Republic) was applied for analysis of organic acids. Isotachophoretic analyser EA 102 (Villa Labeco, Slovakia) equipped with software ITP Pro 32 version 1.0.4.26 (KasComp, Slovakia) was used for analysis of phytic acid and minerals. The composition of electrolytic systems used for identification and determination of organic acids, phytic acid and minerals as well as driving current applied during analysis are listed in Table 2.

Table 2 Systems for isotachophoretic analyses

Substance	Leading electolyte	Terminating electolyte	Driving current
Lactic, acetic, citric and succinic acids	10 mmol.dm^{-3} HCl, counter-ion 6-aminocapronic acid, additive 0.1 % MHEC, pH 4.3	5 mmol.dm^{-3} capronic acid	250 μA in the preseparation column
Phytic acid	10 mmol.dm^{-3} HCl, counter-ion 5.5 mmol.dm^{-3} BTP, additive 0.1 % HEC, pH 6.2	5 mmol.dm^{-3} MES	250 μA in the pre-separation and 40 μA in analytical column
Minerals (Ca, Na, Mg)	7.5 mmol.dm^{-3} H_2SO_4, counter- ion 7 mmol.dm^{-3} 18-Crown-6, additive 0.1 % HEC	10 mmol.dm^{-3} BTP, 20 mmol.dm^{-3} acetic acid	200 μA in the pre-separation and 30 μA in analytical column

MHEC – methylhydroxyethylcellulose, BTP - bis-tris-propan, HEC – hydroxyethylcellulose, MES – morpholinoethanesulfonic acid

Kinetic of acidification

The kinetic of acidification during fermentation was determined by using an pH-meter (Inolab WTW, Weilheim, Germany). Acidification data were fitted according to the Gompertz equation as modified by **Zwietering *et al*. (1990)**:

$$y = K + A\ exp\ \{ - exp[(V_{max}\, e / A)(\lambda - t) + 1]\}$$

where y is dpH/dt (units of pH/h); K is the initial level of the dependent variable to be fitted; A (ΔpH) is the difference in pH (units) between the initial value (pH_0) and the value reached in the stationary phase of lactic acid fermentation (pH_i); V_{max} is the maximum acidification rate as dpH/h; λ is the length of the latency phase of acidification expressed in hod; and t is the incubation time **(Servili *et al*., 2011)**. The experimental data were fitted through the non-linear regresion procedure of the statistic package Origin Version 5.0 (Microcal Software Inc., Northampton, USA).

Enumeration of microorganisms

Samples of tarhana (10 g) taken aseptically were dispersed in 90 cm^3 of sterile physiological saline solution (0.85 %). 1 cm^3 of primary 1/10 suspension was then withdrawn and further decimal serial dilutions were prepared from this homogenate in the same sterile diluents. The appropriate dilutions were subsequently used for enumeration of microorganisms in the samples, at each of the pre-determined time intervals during fermentation. De Man, Rogosa and Sharpe (MRS) agar (Merck, Darmstadt, Germany) was used to determine lactic acid bacteria (LAB) count. The LAB were analyzed for growth using total viable cell counts method **(STN ISO 15214, 2002)**. Aliquots of 1 cm^3 of dilution were plated, in duplicate, in plates containing MRS agar (spread plate method). The plates were anaerobically incubated at 37 ºC for 72 h. Plates containing 30 – 300 colonies were measured and recorded as log colony forming units (log CFU) per gram. Norm STN **ISO 7954 (1997)** was used for yeasts count determination. Briefly aliquots of 0.2 cm^3 of selected serial dilutions were plated, in duplicate, on the surface of yeast extract glucose chloramphenicol (YGC) agar (Merck, Darmstadt, Germany). Plates were incubated at 25 ºC for 48 h. Afterwards colonies were calculated and results were recorded as log colony forming units (log CFU) per gram of tarhana sample.

Sensory evaluation

Sensory parameters of tarhana soups prepared from wet tarhana fermented for 144 h was determined by method **Isik and Yapar (2012)**. The cooked samples were served to the volunteers at 80 ºC in random order **(Erkan *et al*., 2006)**. The 7 instructed assessors evaluated color, aroma, taste, consistency and overall acceptance on hedonic scale from 1 (dislike extremely) to 7 (like extremely).

Statistical analysis

All analyses were carried out using three independent determinations and expressed as mean ± standard deviation. One-way analysis of variance (ANOVA) and Fisher's least significant difference (LSD) multiple range test (minerals, counts of LAB and yeasts) or Duncan's multiple range test (sensory evaluation) were applied to data for establish significance of differences between samples at level p = 0.05. Stagraphic Plus version 3.1 (Statsoft, Tulsa, USA) was used as statistical analysis software.

RESULTS AND DISCUSSION

Kinetic of acidification and chemical determinations

The pH of tarhana is important for keeping its quality because low pH values make it unattractive to pathogenic and spoilage microorganisms **(Herken and Çon, 2012)**. Before fermentation process, the value of pH of the three tarhana samples was in the range 4.82 – 4.87. Control and sample with *Lb. sanfrancisco* CCM 7699 reached the value of pH 4.35 after 96 h of incubation. Tarhana with culture *Lb. plantarum* CCM 7039 needed approximately 48 h to get the same value of pH. The kinetic of acidification (Figure 1) of control sample and tarhana inoculated with culture *Lb. sanfrancisco* CCM 7699 showed similar values of A (ΔpH) 0.49 units of pH in 96 h, whereas tarhana with *Lb. plantarum* CCM 7039 had A value of 0.79 units of pH in 120 h. Sample with *Lb. plantarum* CCM 7039 reached the highest maximum acidification rate V_{max} (13.5×10^{-3} dpH/h), while control tarhana and sample with *Lb. sanfrancisco* CCM 7699 reached lower values of V_{max} (11.8×10^{-3} and 12.7×10^{-3} dpH/h). According to **Çelik *et al.* (2010)** production of acids by lactic acid bacteria during fermentation was the main reason of pH reduction in tarhana samples.

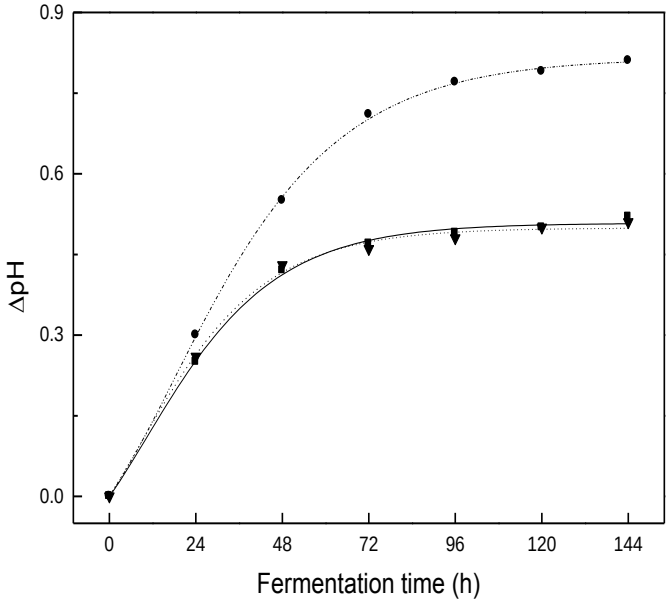

Figure 1 Kinetic of acidification of control (■), sample with *Lb. sanfrancisco* CCM 7699 (▼) and with culture *Lb. plantarum* CCM 7039 (●)

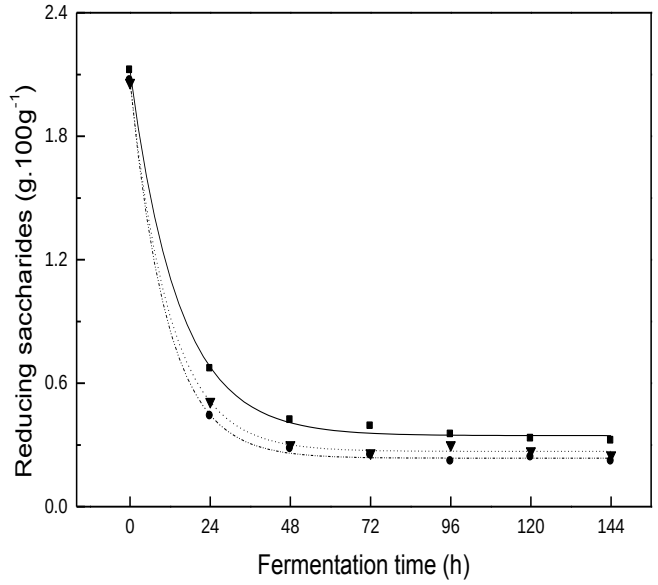

Figure 2 Reducing sacharides concentration in g.100g^{-1} of control sample (■), sample with *Lb. sanfrancisco* CCM 7699 (▼) and *Lb. plantarum* CCM 7039 (●)

Reducing saccharides are fermentable saccharides and their concentration decreases with increasing period of fermentation **(Singh *et al.*, 2013)**. The content of reducing saccharides expressed as glucose (Figure 2) varied from 2.12 to 2.06 g.100g^{-1} at the beginning of the fermentation. Over a period of first 48 hours the content of saccharides rapidly declined. In further fermentation process was observed moderate decrease of reducing saccharides concentration and after 144 h was determined reducing saccharides in range 0.25 – 0.31 g.100g^{-1}. **Tamer *et al.* (2007)** reported, that the content of reducing saccharides in various tarhana samples varied between 0.22 – 1.85 g.100g^{-1} at the end of fermentation.

The major organic acid in fermented tarhana dough is lactic acid and is produce by the fermentable carbohydrates found in a cereal flour and yoghurt mixture **(Değirmencioğlu *et al.*, 2005)**. In addition to lactic acid, other organic acids such as acetic, propionic and pyruvic acids are formed **(Bozkurt and Gürbüz, 2008)**. The concentration of lactic acid (Figure 3) increased during fermentation from 0.18 to 0.81 g.100g^{-1}. The most of lactic acid was produced in tarhana sample inoculated with *Lb. plantarum* CCM 7039 (0.81 g.100g^{-1} after 144 h). In this sample the highest utilization of reducing saccharides by fermenting microbiota was also observed (reduction from 2.06 to 0.31 g.100g^{-1}). Acetic acid in fermented products contributes to the aroma and prevents mould spoilage **(Leroy and De Vuyst, 2004)**. In our experiments, the fermented tarhana samples contained at the end of fermentation between 0.19 g.100g^{-1} (*Lb. plantarum* CCM 7039) and 0.26 g.100g^{-1} (control and tarhana with *Lb. sanfrancisco* CCM 7699) of acetic acid. Production of acetic acid during tarhana samples fermentation is shown in Figure 4. According to **Sroka and Tuszyński (2007)** the acetate quantity depends mainly on the concentration of carbohydrates and the source of nitrogen as well as the pH. Similar production of lactate and acetate during tarhana fermentation was reported in previous studies by **Bozkurt and Gürbüz (2008)** and **Erbaş *et al.* (2006)**.

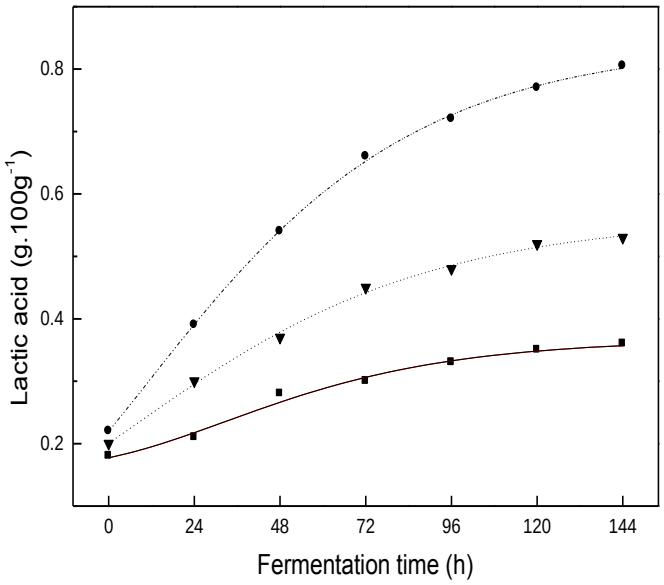

Figure 3 Change in lactic acid concentration in g.100g^{-1} during the tarhana fermentation: control (■), sample with *Lb. sanfrancisco* CCM 7699 (▼) and *Lb. plantarum* CCM 7039 (●)

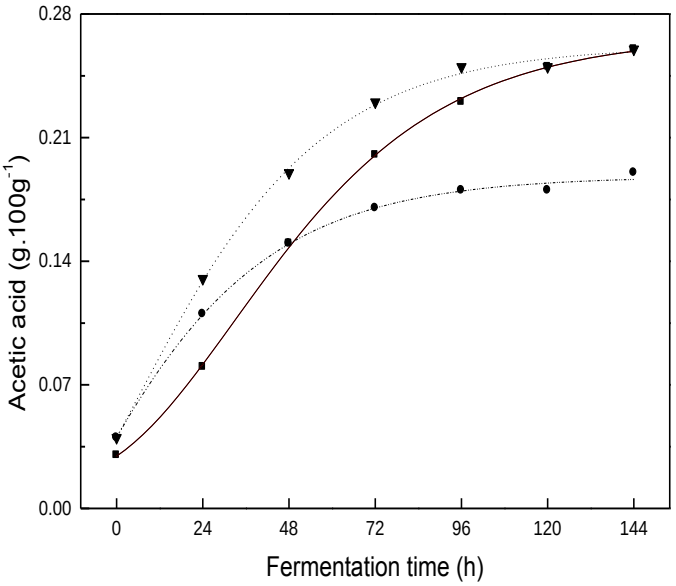

Figure 4 Amount of acetic acid in g.100g^{-1} produced in control (■), tarhana with *Lb. sanfrancisco* CCM 7699 (▼) and tarhana with *Lb. plantarum* CCM 7039 (●)

Concentration of citric acid (Figure 5) gradually decerased from 134.75 - 132.19 mg.100g^{-1} in unfermented dough to 55.65 – 6.81 mg.100g^{-1} after 144 h fermentation process. The decrease in citric acid content during fermentation was attributed to its usage as a substrate in secondary reactions during fermentation **(Caplice and Fitzgerald, 1999)**. **Helland *et al.* (2004)** described that the end products of citrate metabolism are CO_2, acetate, diacetyl, acetoin and 2,3-butanediol. The rate of citric acid degradation was comparable in control sample and tarhana with *Lb. sanfrancisco* CCM 7699, whereas sample inoculated with *Lb. plantarum* CCM 7039 reached highest degradation rate of citric acid. In previous study reported **Magala *et al.* (2013)** similar decrease in citric acid concentration during tarhana fermentation. Determination of succinic acid (Figure 6) showed, that its concentration markedly increased in first 48 h and further fermentation led to only slight increase or remained constant (after 144 h was concentration of succinic acid in range 128.66 - 138.44 mg.100g^{-1}).

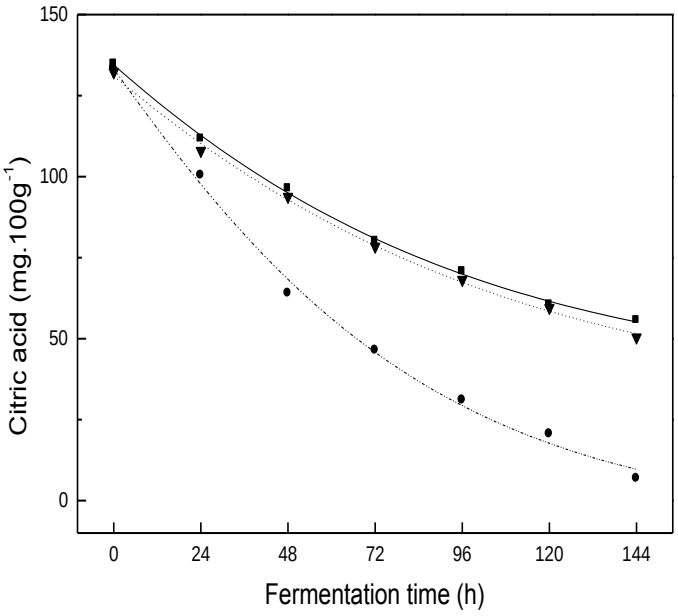

Figure 5 Dependence between citric acid concentration (mg.100g^{-1}) and time of fermentation: control (■), *Lb. sanfrancisco* CCM 7699 (▼) and *Lb. plantarum* CCM 7039 (●)

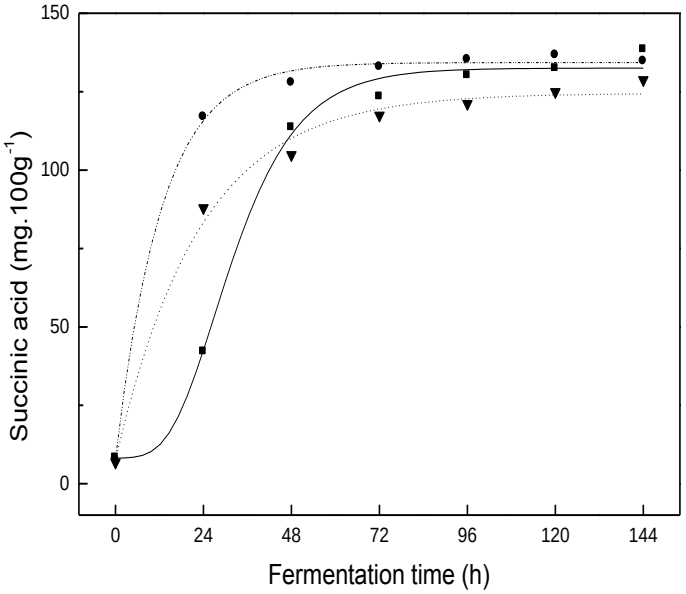

Figure 6 Effect of fermentation on succinicic acid concentration (mg.100g^{-1}) of control (■), sample with *Lb. sanfrancisco* CCM 7699 (▼) and *Lb. plantarum* CCM 7039 (●)

Phytic acid (PA), or *myo*-inositol hexakisphosphate, is a major component of most of plant seeds, constituting 1-3 % by weight of many cereals **(Wu *et al.*, 2010)**. The six phosphate groups of phytic acid have strong chelating capacity to essential divalent cations such as calcium, magnesium, iron, zinc and manganese, forming largely insoluble complex, and thereby decreasing their nutritional bioavailability, so phytic acid has long been considered to be a kind of antinutrient **(Wang *et al.*, 2013)**. Fermentation is one of the processes known to reduce PA. In general, lower pH, longer fermentation time and more yeast addition result in a more intensive degradation of PA **(Bilgiçli *et al.*, 2006)**. From the results of PA determination (Figure 7) concluded, that fermentation process of all tarhana samples led to PA reduction (from 318.6 – 324.2 mg.100g^{-1} at the beginning of the fermentation to 39.4 – 225.3 mg.100g^{-1} after 144 h). The highest PA reduction (88.75 %) was observed in sample with culture *Lb. sanfrancisco* CCM 7699. Similar degradation rate of PA reached control sample (81.44 %). This could be explained by the pH values suitable for phytase activity established during fermentation of control (pH 4.87 – 4.37) and sample inoculated with *Lb. sanfrancisco* CCM 7699 (pH 4.89 – 4.40). According to **Lopez *et al.* (2002)** optimum pH for PA hydrolysis is 4.5 - 5.0. Moreover some yeast, such as *Saccharomyces cerevisiae* have been shown to produce intracellular as well as extracellular phytate-degrading enzymes. Also some strains of LAB possess phytate-degrading activity, but its activity is quite low **(Konietzny and Greiner, 2002)**. In tarhana with culture *Lb. plantarum* CCM 7039 was recorded the lowest PA reduction (49.85 %). This could be due to low pH value for phytase activity (4.27 – 4.01 during 48 – 144 h). Previous studies also reported degradation of PA during tarhana fermentation **(Bilgiçli *et al.*, 2006; Bilgiçli and Ibanoğlu, 2007).**

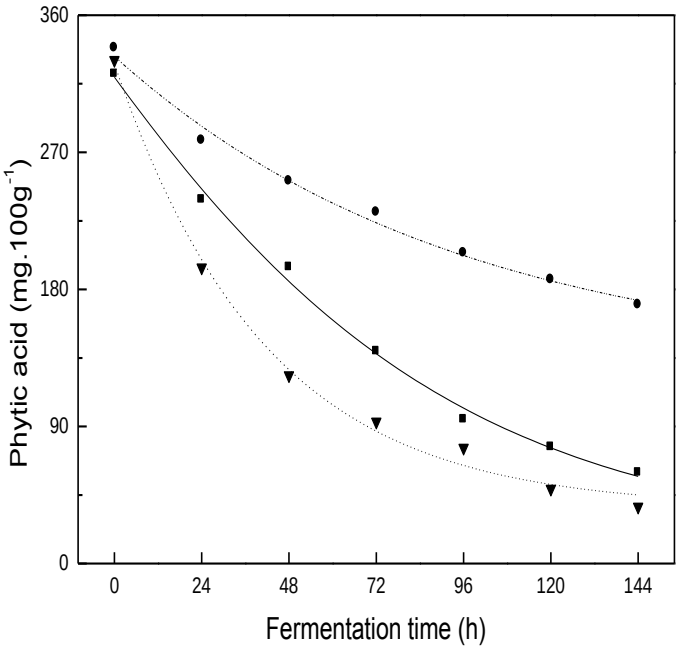

Figure 7 Degradation of phytic acid (mg.100g^{-1}) during tarhana fermentation: control (■), tarhana with *Lb. sanfrancisco* CCM 7699 (▼) and tarhana with *Lb. plantarum* CCM 7039 (●)

Concentration of minerals (Ca, Na, Mg) determined by isotachophoresis in 0 and 144 h of tarhana fermentation are showed in Table 3. Results of measured data showed, that significant increase ($p = 0.05$) in mineral concentration between unfermented dough (0 h) and fermented tarhana (144 h) was observed in samples inoculated with *Lb. sanfrancisco* CCM 7699 and *Lb. plantarum* CCM 7039 in case of Ca and in all samples in case of Mg. No significant differences in Na content of unfermented dough and fermented tarhana was indicated in all prepared samples. This increase in mineral content could be attributed, at least in part, to the decrease in phytic acid levels observed during the fermentation (Figure 7), which forms complexes with minerals **(Albarracín *et al.*, 2013; Toufeili *et al.*, 1999)**. **Bilgiçli *et al.* (2006)** reported similar differences in minerals content of unfermented dough and tarhana at the end of fermentation.

Table 3 Concentration of minerals in tarhana

Sample	Ca (mg.100g^{-1})		Na (mg.100g^{-1})		Mg (mg.100g^{-1})	
	0 h	144 h	0 h	144 h	0 h	144 h
Control	126.10±1.39^{a}	132.63±0.40^{a}	371.67±1.44^{a}	375.83±1.04^{a}	170.80±0.69^{a}	175.87±0.12^{b}
Lb. sanfrancisco CCM 7699	131.86±0.83^{a}	139.67±0.29^{b}	352.23±0.56^{a}	356.23±0.32^{a}	172.70±0.30^{a}	186.90±0.36^{b}
Lb. plantarum CCM 7039	125.73±0.21^{a}	133.29±0.47^{b}	353.11±0.67^{a}	354.57±0.51^{a}	176.33±0.47^{a}	184.73±0.21^{b}

Superscript with different letter denotes statistically significant difference between columns (0 and 144 h) at level $p = 0.05$. Fisher's LSD test.

Enumeration of microorganisms

The nature and the concentration of microbial species present in the fermentation media markedly affect the nature of the final products (e.g. aroma) **(Değirmencioğlu *et al*., 2005)**. Development of LAB during tarhana processing is shown in Table 4. In general, highest CFU.g^{-1} were obtained during the first 72 h of fermentation, when cell counts of LAB proliferated approximately about one logarithmical order. Later in the fermentation the LAB counts were either stable or showed a decreasing trend probably reflecting beginning lack of nutrients. Reasons of the decrease in the LAB count during fermentation could be the increase in acid content and formation of components such as carbon dioxide, hydrogen peroxide, diacetyl, ethanol and bacteriocins **(Erbaş *et al*., 2005)**. Similar increasing trend in LAB counts in first 72 h and subsequent decrease in later phases of the fermentation reported **Daglioğlu *et al*. (2002)** and **Settanni *et al*. (2011)**.

Table 4 Counts of LAB during tarhana processing

Time (h)	LAB (log CFU.g^{-1})		
	Control tarhana	Tarhana with culture *Lb. sanfrancisco* CCM 7699	Tarhana with culture *Lb. plantarum* CCM 7039
0	7.14 ± 0.03^{a}	8.16 ± 0.14^{a}	8.27 ± 0.03^{a}
24	7.98 ± 0.07^{b}	8.26 ± 0.09^{a}	8.47 ± 0.04^{b}
48	8.18 ± 0.12^{b}	8.99 ± 0.04^{b}	7.32 ± 0.02^{b}
72	8.25 ± 0.05^{b}	9.08 ± 0.12^{b}	7.30 ± 0.08^{b}
96	8.02 ± 0.08^{b}	8.10 ± 0.17^{a}	7.36 ± 0.05^{b}
120	7.99 ± 0.02^{b}	8.06 ± 0.08^{a}	7.41 ± 0.04^{b}
144	7.95 ± 0.06^{b}	7.88 ± 0.05^{b}	7.40 ± 0.02^{b}

Different superscript letter within each column denote statistical significance of fermentation time by Fisher's LSD test (p = 0.05).

The possible function of yeasts in fermented foods are fermentation of carbohydrates, production of aroma compounds, stimulation of LAB providing essential metabolites, inhibition of mycotoxin-producing moulds, production of tissue-degrading enzymes such as cellulases and pectinases and probiotic properties **(Kohajdová and Karovičová, 2007)**. The evolution of yeast cells at the time of tarhana samples fermentation is shown in Table 5. During first 48 h yeast counts significantly (p=0.05) proliferated in all tarhana samples. In later phases of fermentation yeast counts remained stable or slightly decreased. Sample with starter *Lb. plantarum* CCM 7039 showed yeasts cells grow in 24 h and consequently rapidly decreased. **Paramithiotis *et al*. (2006)** reported that in mixed cultures, yeasts growth can be negatively affected by the rapid pH drop caused by the LAB and the metabolites produced, i.e. lactic and acetic acid, what explains decreasing counts of yeast in later phases. Similar counts of yeasts in tarhana during fermentation reported **Erbaş *et al*. (2005)**.

Table 5 Yeast cells concentration during tarhana fermentation

Time (h)	Yeasts (log CFU.g^{-1})		
	Control tarhana	Tarhana with culture *Lb. sanfrancisco* CCM 7699	Tarhana with culture *Lb. plantarum* CCM 7039
0	6.11 ± 0.06^{a}	6.17 ± 0.08^{a}	6.47 ± 0.05^{a}
24	6.18 ± 0.04^{a}	6.28 ± 0.02^{a}	6.50 ± 0.03^{a}
48	6.31 ± 0.10^{b}	6.34 ± 0.05^{b}	5.65 ± 0.02^{b}
72	6.19 ± 0.05^{a}	6.31 ± 0.07^{b}	5.52 ± 0.06^{b}
96	6.16 ± 0.07^{a}	6.30 ± 0.04^{a}	5.54 ± 0.03^{b}
120	6.10 ± 0.05^{a}	6.18 ± 0.03^{a}	4.70 ± 0.02^{b}
144	6.04 ± 0.04^{a}	6.00 ± 0.02^{b}	4.63 ± 0.04^{b}

Different superscript letter within each column denote statistical significance of fermentation time by Fisher's LSD test (p = 0.05).

Sensory evaluation

Fermentation process is an important stage for the development of sensory profile **(Erbaş *et al*., 2005)**. Sensory evaluation of tarhana (Table 6) showed, that no significant differences (p=0.05) were found in color and consistency of samples. It was also observed that samples with starter culture significantly differ from control sample in odour. This could be attributed to higher rate of aroma active compounds production. Sample inoculated with *Lb. plantarum* CCM 7039 had significantly lower value of taste due to excessively sour taste caused by highest amount of produced organic acids. Overall acceptance of control and sample with *Lb. sanfrancisco* CCM 7699 showed similar values, whereas tarhana with *Lb. plantarum* CCM 7039 reached significantly lower value of overall acceptance than control sample.

Table 6 Sensory parameters of tarhana

Sample	Color	Odour	Taste	Consitency	Overall acceptance
Control	6.57 ± 0.35^{a}	5.66 ± 0.40^{a}	6.50 ± 0.31^{a}	6.53 ± 0.33^{a}	6.76 ± 0.25^{a}
Lb. sanfrancisco	6.47 ± 0.26^{a}	6.30 ± 0.32^{b}	6.61 ± 0.34^{a}	6.79 ± 0.25^{a}	6.69 ± 0.18^{a}
Lb. plantarum	6.40 ± 0.21^{a}	6.09 ± 0.22^{b}	6.29 ± 0.31^{b}	6.64 ± 0.28^{a}	5.99 ± 0.37^{b}

Duncan′s multiple range test. Means (± standard error) with different superscript letter within column are significantly different (p = 0.05).

CONCLUSION

Fermented products play an important role in the daily diet not only for the attractiveness but also for improvement of shelf-life and nutritional properties that derive from fermentation **(Coda *et al*., 2014)**. During tarhana samples fermentation, pH value decreased due to production of organic acids such as lactic, acetic and succinic. The concentration of reducing saccharides decreased since they served as an available source of nutrients for microorganisms. It was also concluded that tarhana fermentation led to substantial reduction in phytic acid content. Enumeration of lactic acid bacteria and yeasts showed, that their counts increased in first phases of the process and remained stable or decreased in later phases. Sensory evaluation of tarhana soups showed, that starter culture addition positively affected odour, while taste and overall acceptance reached significantly lower value in tarhana sample with *Lb. plantarum* CCM 7039. From the results of present study it can be concluded, that application of lactic acid bacteria starter cultures *Lb. sanfrancisco* CCM 7699 and *Lb. plantarum* CCM 7039 is suitable for tarhana preparation.

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