**RESEARCH ARTICLE**

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# Technological properties of some lactic acid bacteria and interactions with *Saccharomyces cerevisiae* PFC121 in tarhana dough during fermentation

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**ABSTRACT**

In this study, the interactions between *Saccharomyces cerevisiae* PFC121 and lactic acid bacteria (LAB; *Pediococcus acidilactici* PFC69, *Lactobacillus namurensis* PFC70, *Lactococcus lactis* PFC77 isolated from tarhana sourdoughs) in tarhana dough during fermentation period of 7 days were investigated. The technological properties of LAB were also determined. Presence of *Saccharomyces cerevisiae* PFC121 had significantly effect on the lactic acid bacteria counts and chemical properties. *L. namurensis* PFC70 and *Lc. lactis* PFC77 counts were decreased during fermentation period when used mix culture with yeast. The counts of *P. acidilactici* PFC69 with yeast decreased comparing the counts of *P. acidilactici* PFC69 alone. *S. cerevisiae* PFC121 counts in tarhana dough samples were not affected by the presence of lactic acid bacteria. Co-culturing *S. cerevisiae* with lactic acid bacteria was caused the decrease of lactic acid level and a significant affected other organic acid amounts of tarhana dough. *S. cerevisiae* PFC121 may be used with lactic acid bacteria because of being not too much interaction between them and the effect of yeast on the aroma formation.

**Key words:** Lactic acid bacteria, yeast, interactions, tarhana sourdough, organic acid

## Introduction

Tarhana, a traditional Turkish fermented cereal food, is prepared by mixing cereal flours, yoghurt, a variety of vegetables and spices and after that, fermented (lactic and alcoholic fermentation) for 1-7 days at room temperatures (Bozkurt & Gürbüz 2008; Erbaş et al. 2006; Settanni et al. 2011). After the fermentation, wet tarhana is sundried or dried with a drier and then granulated (Settanni et al. 2011). Tarhana is a good source of vitamins, minerals, organic acids and free amino acids (Bozkurt & Gürbüz 2008). It is commonly consumed as soup at lunch and dinner, and it is especially used for feeding children, elderly and medical patients (Karagözlü et al. 2008). Additionally, it is also locally consumed as a snack after the drying (Erbaş et al. 2006). Some products are produced in other countries similar to tarhana as "kishk" in Egypt, Syria and Jordan, "talkuna" in Finland, "kushuk" in Iraq, "tahonya" in Hungary, "trahana" in Greek and "atole" in Scotland (Tamer et al. 2007).

Tarhana fermentation is usually carried out by using yoghurt bacteria such as *Lactobacillus delbrueckii ssp. bulgaricus*, *Streptococcus thermophilus* and baker's yeast (*Saccharomyces cerevisiae*) (Bozkurt & Gürbüz 2008). Therefore, tarhana has a sour and acidic taste with characteristic yeasty flavor. Lactic acid bacteria (LAB) are responsible for the formation of acids during fermentation and yeast are responsible for the formation of flavor components like $CO_2$, alcohol, organic acids, aldehydes, ketones (Tarakci et al. 2004). At the same time, using yeast in tarhana fermentation can reduce the fermentation period and improve the taste and flavor properties of tarhana (Temiz & Pirkul 1990). LAB has also a positive effect on the hygienic quality and shelf life of tarhana because of their antimicrobial effect against spoilage and pathogenic microorganism (Devi & Halami 2011). For all that, yeasts and LAB fermentations occur simultaneously during tarhana production and the interaction between yeast and LAB have great importance for sensory, physical and textural properties of tarhana (Herken & Çon 2014). Although various microorganisms present in the dough at the beginning of the fermentation period, as LAB and yeasts can tolerate acid existing in the tarhana dough, they are the dominant microorganisms at the later stages of fermentation. Therefore, the interaction between the yeast and LAB selected as a starter has great importance. According to Ravyts & De Vuyst (2011), the use of well-selected LAB and yeast strains as starter cultures can result in positive differences in volatile profiles of the sourdough production.

Recently, several studies focus on the impact of single or mixed starter cultures of LAB and yeasts on sourdough

fermentation (Paramithiotis et al. 2006; Ravyts & De Vuyst 2011; Sieuwerts et al. 2018). Meignen et al. (2001) stated that using of single or mixed starters changed dough fermentation and end products. Paramithiotis et al. (2006) found that the presence of LAB had no effect on final yeast cell yield but ethanol production was affected negatively at initial because of maximum specific growth decreasing. Moreover, there are many competitive species like *L. plantarum*, *L. fermentum*, *S. cerevisiae*, and *L. brevis* on sourdough fermentation (Herken & Çon 2014; Vogelmann et al. 2009). In the literature, the microbiota in tarhana fermentation by using single or mixed different LAB and *S. cerevisiae* as starter culture has not been investigated. The aim of the study was to assess whether the yeast improved the tarhana properties produced with *Pediococcus acidilactici* PFC69, *Lactobacillus namurensis* PFC70 and *Lactococcus lactis* PFC77 isolated from different Turkish tarhana or not. In addition, possible interactions between the yeast and LAB were also investigated by studying final populations in tarhana sourdough and by comparing their lactic acid production ability.

## Materials and Methods

### *Microorganisms and growth conditions*

*Pediococcus acidilactici* PFC69, *Lactobacillus namurensis* PFC70 and *Lactococcus lactis* PFC77 cultures, previously isolated from tarhana sourdoughs (Şimşek et al. 2017), were obtained from Pamukkale University, Food Engineering Culture Collection (PUFECC). They were cultivated in Man Rogosa Sharpe (MRS) broth, MRS-5 broth (10 g tripton, 5 g meat extract, 5 g yeast extract, 10 g maltose, 5 g fructose, 5 g glucose, 5 g $C_2H_3NaO_2$, 3 g NHCL3, 2.6 g $K_2HPO_4$, 4 g $KH_2PO_4$, 0.1 g $MgSO_4$, 0.05 g $MnSO_4$, 0.5 g Cystein-HCI, 1 ml Tween 80, pH 5.8), and M17 broth (Merck, Darmstadt, Germany), respectively. *Saccharomyces cerevisiae* PFC121, previously isolated from tarhana sourdoughs (Özel et al. 2015), was propagated in Nutrient Broth (Merck, Darmstadt, Germany) including 1% glucose.

### *Technological properties of microorganisms*

All lactic acid bacteria were tested for technological properties such as growth at different temperatures, pH and salt tolerance, ethanol tolerance, production of carbon dioxide, amylolytic and lipolytic activity, freezing and lyophilisation tolerance, antimicrobial activity and total acid production ability. Overnight bacterial culture was used for all tests. For growth at different temperatures, obtained cultures were transferred into suitable agar and incubated at 10°C, 15°C and 45°C accompanied maximum-minimum thermometer for 48 h (Tassou et al. 2002). Growth at different pH (3.0, 4.0 and 9.0), salt tolerance (3.5, 6.5 and 9%, w/v) and ethanol tolerance (final ethanol concentration of 7, 12 and 15%) were tested after 10 days of incubation. During the incubation period, tubes were controlled every day and growth of lactic acid bacteria was evaluated as positive (G-Alegria et al. 2004).

Total acid production ability of microorganisms was determined with the method of Karasu et al. (2010) with minor modifications. Suitable mediums for microorganisms were prepared as 100 mL in a flask and inoculated from 18-24 hours culture. Incubation was performed with orbital shaking incubator (IKA, KS 4000i control, Germany) at 30°C for seven days. Every day during incubation time, 5 mL of culture from samples was taken aseptically and pH was measured by pH meter (JENCO, model 6173 pH, USA) and titrations were made until pH 6.2 with 0.1 M NaOH. Percent acidity was calculated as follows:

$$Titratable\ acidity\ (\%) = VxNxEx100/m$$

where V is the volume of spent 0.1 N NaOH, N is the normality of alkaline spent for titration, E is the equivalent lactic acid, and m is the sample titrated.

The carbon dioxide production from glucose was carried out with the method reported by Schillinger & Lücke (1987) with some modifications. Durham tubes were reverse put into test tubes containing suitable broth prepared without citrate and then sterilized with an autoclave (JSR, Neoclave JSAX60, South Korea). After the inoculation, tubes were incubated at 30°C for 10 days. Production of gas into Durham tubes was controlled every day during the incubation period and culture producing gas was stated as positive. In regard, the amylolytic activity, the carbohydrate fermentation pattern of cultures was carried out using a biochemical test kit (API 50 CH BioMerieux, Inc., Durham, NC) and API 50 CH system was used for determination of lactic acid production from starch. Cultures were inoculated to CHL medium containing 1% of starch and incubated at 30°C for 10 days. After the incubation period, when the purple color was changed as yellow, result was indicated as positive (Şimşek et al. 2006). For lipolytic activity, overnight bacterial culture was point inoculated on Tributyrin agar (Merck, Darmstad, Germany) and incubated for at 30°C for 48 h. After the incubation period, the appearance of transparent zone around the colonies was regarded as an indicator for lipolysis (Thapa et al. 2006).

Freezing and lyophilisation tolerance of microorganisms were assayed with the method of (G-Alegria et al. 2004) with some modifications. Twenty-five µL of fresh cultures of lactic acid bacteria were added to 3 mL of suitable broth and cells were grown at 30° C for 24 h. Cells were collected by centrifugation at 2000 rpm (Hettich Zentrifugen, Universal 320R, Germany) for 15 min and obtained pellets were re-suspended in 0.5 mL of sterile skimmed milk medium and stored at -40°C. For each microorganism, while 2 vial containing cultures were stored at -40°C, 4 vial containing cultures were lyophilized at -80°C for 16 h under vacuum (Virtis BenchTop, SP Scientific Series, USA). The number of viable cells before and after freezing and lyophilisation was

determined with suitable agar (MRS agar, M17 agar, MRS-5C) using the spread plate method. The number of colonies was counted after 48 h at 30°C under anaerobic conditions (10% of CO2) and survival rate was calculated as follow:

$$Survival\ rate\ (\%) = \left(\frac{N}{N_0}\right) x100$$

where N0 is the number of microorganism before freezing and lyophilisation and N is the number of microorganism after freezing and lyophilisation.

Antagonistic activity of microorganisms on the growth of each other was determined with the agar spot method reported by Schillinger and Lücke (1987). For the agar spot test, 10 µL of an overnight culture was spotted on the surface of MRS-0.2, MRS-5C-0.2 supplemented vitamin B and M17-0.2 agar and incubated at 24°C for 24 h under anaerobic condition. Seven mL of suitable semi-solid agar were inoculated in the rate of 3% with LAB and S. cerevisiae (grown to OD600=0.2-0.3) and poured as a second layer on the agar plates which LAB grew on them before. The plates were incubated at 30°C for 24-30 h and then inhibition zones were measured. A clear zone of more than 1 mm around a spot was considered as positive.

***Preparation of tarhana***

To prepare tarhana at laboratory conditions, wheat flour (1000 g), yoghurt (700 g), paprika (35 g), tomato (85 g), onion (78 g), salt (12 g) dry mint (4.5 g) were used. The wheat flour was a commercial variety, Type 550 (Selva wheat flour, Konya, Turkey) and yoghurt was full-fat commercial brand (Ulker Icim, Istanbul, Turkey) made from cow's milk. The other ingredients, tomato, fresh onion bulbs (peeled, washed and chopped), paprika, dry mint and salt were obtained from local markets in Samsun, Turkey.

In the study, strains were used as single and mixed combinations for tarhana productions and seven different tarhana formulations were prepared (Table 1). Production of tarhana dough was implemented with modifying of the technique used by Herken & Çon (2014). To prepare tarhana samples, onions, paprika and tomatoes were chopped, sliced to small pieces and then all these ingredients blended by using a blender (Sinbo, SHB 3024, Turkey). Obtained vegetable mix was pasteurized at 65°C for 30 min and left to cool to room temperature. Also, yoghurt was pasteurized (batch method) at 65°C for 30 min. Pasteurized yoghurt, wheat flour, salt, and dry mint were added into the vegetable mix. All ingredients were continued mixing until a homogenous mixture was obtained. The LAB (106–107 CFU/g) and yeast (103–104 CFU/g) culture suspensions were added to the samples after growing 24 hours in a suitable medium. For this purpose, fresh cultures were centrifuged at 5000 g for 10 min (4°C) (Hettich Zentrifugen, Universal 320R, Germany) and re-suspended in sterile distilled water (Paramithiotis et al. 2006). Prepared tarhana dough samples were fermented for 7 days at 30°C in the fermentation chamber (Yucebas Machine, Turkey). All dough samples were mixed aseptically with sterilized spoons twice every day during fermentation time to prevent growing and drying on the surface and provide homogenous fermentation.

**Table 1.** *Microorganisms used in the tarhana production*

| Dough code | Microorganisms |
|---|---|
| S1 | *P. acidilactici* PFC69 |
| S2 | *L. namurensis* PFC77 |
| S3 | *Lc. lactis* PFC77 |
| S4 | *S. cerevisiae* PFC121 |
| S5 | *P. acidilactici* PFC69 + *S. cerevisiae* PFC121 |
| S6 | *L. namurensis* PFC70 + *S. cerevisiae* PFC121 |
| S7 | *Lc. lactis* PFC77 + *S. cerevisiae* PFC121 |

***Microbiological analysis of tarhana samples***

Wet tarhana samples were analysed for LAB and *S. cerevisiae* on 0, 1, 3, 5 and 7 days of fermentation. For this purpose, 10 g of dough sample was taken aseptically with sterilized spoons homogenized for 1.5 min in 90 mL of sterile physiological solution with a stomacher (BagMixer 400, Interscience Co., Chemin du Bois Saint Nom, France) and after that serially diluted. Diluted samples were plated for enumeration into specific media by using the spread plate method. LAB was counted on MRS, MRS-5C agar (MRS-5 broth with 1.4% agar) and M17 agar with 0.01% Cycloheximide after incubation at 30°C for 48 hours under 10% $CO_2$ (Memmert GmbH, Schwabach, Germany) (Şimşek et al. 2006). Yeast was counted on Dichloran Rose Bengal Chloramphenicol (DRBC) agar after incubation at 28-30°C for 48 h and results were expressed as log CFU/g (Özel et al. 2015).

***Determination of pH, total acidity, acidity number and water activity***

For analysis pH and total acidity of dough samples, 5 g of sample and 25 mL of distilled water were mixed homogenically with stomacher (WiseStir, HS-50A, DAIHAN, Korea), then completed to 50 mL with distilled water. At this stage pH measurement of the samples were performed by pH meter (JENCO, model 6173 pH, USA). For total acidity analyses, samples were titrated with 0.1 N NaOH until pH 8.1 after pH measurement, and results given as equivalent lactic acid.

Acidity number of the samples was determined according to Turkish Tarhana Standard (Institution 1981) and defined as the quantity of 0.1 M NaOH used to neutralize the acidity of 10 g tarhana sample dissolved in ethyl alcohol (67%). Ten grams of tarhana sample was put in a flask and neutral ethyl alcohol was added on it. The obtained mixture was shaken for

5 min and then filtrated from filter paper. Ten mL of filtrate was mixed a little distilled water for bleaching and titrated with 0.1 N NaOH.

Water activity values of tarhana samples were measured with a Novasina Labmaster aw apparatus (Novasina, Lachen, Switzerland).

***Determination of organic acids***

Organic acid extraction was performed with the method of Zannini et al. (2009) with some modification. For this purpose, 10 g of sample were homogenized with 90 mL of distilled water using a stomacher apparatus for 180 s. Ten mL of homogenate was mixed with 5 mL of 0.1 mM $HClO_4$ solution. The mixtures were centrifuged for 15 min at 4 000 g at 4°C and the obtained supernatants were neutralized to pH 7.0 using 2M NaOH, and then volume was adjusted to 25 mL with distilled water. After that, 5 mL of sample were passed from cationic solid phase extraction column (GracePureTM SPE Cation-X (W. R. Grace & Co.-Conn., Maryland, USA). The eluates of samples were filtered through 0.45 µm cellulose filters (Millipore) and then analyzed by HPLC.

HPLC analyses were performed with a Shimadzu HPLC system (Japan). The analyses were carried out at a flow rate of 0.7 mL/min with a pump LC-20AT using 10 mM $HClO_4$ as the mobile phase. The injection volume was adjusted as 20 µL with using an autosampler SIL-10A. Chromatographic separation was performed using Inertsil® ODS-3 $C_{18}$ column (250 x 4.6 mm 5 µm, Japan). The column temperature was maintained at 65oC with a Column oven CTO-10AS VP. The detection was carried out at the wavelengths of maximum absorption of organic acids at 210 nm using a Photodiode Array Detector (PDA) SPD-M20A. Organic acids (lactic, acetic, citric, propionic, oxalic, malic, tartaric and formic acid) were defined using external standards (Sigma Aldrich) and the quantification was performed depending on standard curves obtained with five different concentration standard solutions in the range of 10 to 500 mL/L.

***Statistical analysis***

MINITAB16 software was used to perform statistical analyses. For comparison of samples, a one-way analysis of variance (ANOVA) and Tukey was used for determination of differences at $P \leq 0.05$.

## Results and Discussion

***Technological properties of microorganisms***

The results of growth in different temperature, pH and salt tolerance, ethanol tolerance, production of carbon dioxide, amylolytic and lipolytic activity, freezing and lyophilisation tolerance, antim10icrobial activity and total acid production ability for all lactic acid bacteria are shown in Table 2 and 3. All lactic acid bacteria used in this study grew at 15°C but not at 10 and 40°C. All strains grew at pH 4.0 and 9.0, no growth was detected at pH 3.0. While all of them grew at 3.5% (w/v) salt concentration, only *P. acidilactici* PFC69 was detected at higher values (6.5 and 9% w/v) of salt concentrations. When considering these results, it might be said that all strains were technologically sufficient for tarhana production due to the lower salt content of tarhana. With regard to ethanol tolerance, all strains grew in the presence of 7% ethanol in the medium. But none of them grew at increasing ethanol content up to 12 and 15%. As shown in Table 2, it was determined that all microorganisms did not produce gas from glucose. All strains did not show amylolytic and lipolytic activity.

Total acid production and pH changes of strains in suitable mediums are shown in Figure 1. It was determined that all strains used in the study caused a little pH decline and a little acidity rise except *L. namurensis* PFC70 significantly decreasing the pH and increasing acidity at the day of 1. Although these two results support each other, changes have been lower than expected. According to the results, it was seen that *L. namurensis* PFC70 was not the fastest acidifying strain, but the strain with the highest acidification extent.

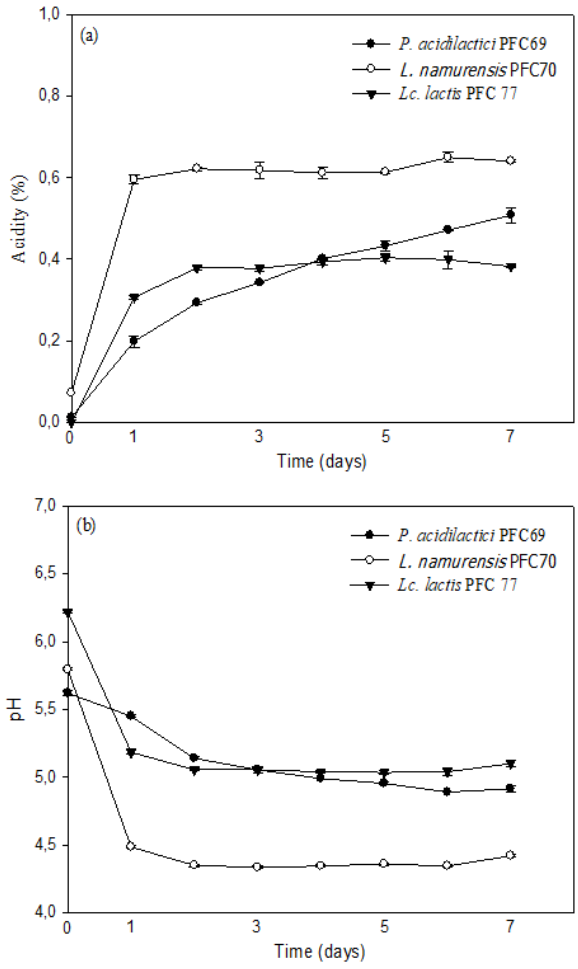


**Figure 1.** *Total acid production ability (a) and pH values (b) of LAB during incubation.*

**Table 2.** *Technological properties of microorganisms used in the tarhana production*

| Microorganism | Temperature (°C) | | | pH | | | Salt concentration (%) | | | Ethanol concentration (%) | | | Survival of microorganism (%) | | Gas production from glucose |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 10 | 15 | 40 | 3.0 | 4.0 | 9.0 | 3.5 | 6.5 | 9 | 7 | 12 | 15 | Freezing | Lyophilisation | |
| *P. acidilactici* PFC69 | - | + | - | - | + | + | + | + | + | + | - | - | 100.69 | 101.40 | - |
| *L. namurensis* PFC70 | - | + | - | - | + | + | + | - | - | + | - | - | 96.64 | 91.99 | - |
| *Lc. lactis* PFC77 | - | + | - | - | + | + | + | - | - | + | - | - | 94.96 | 83.63 | - |

**Table 3.** *Antagonistic activity of microorganisms on growth of each other.*

| Microorganism | Indicator microorganism (inhibition zone, mm) | | | |
|---|---|---|---|---|
| | PFC69 | PFC70 | PFC77 | PFC121 |
| *P. acidilactici* PFC69 | | 4.5 | 0.5 | - |
| *L. namurensis* PFC70 | 13.5 | | 1.5 | - |
| *Lc. lactis* PFC77 | - | 1 | | - |

*P. acidilactici* PFC69, *L. namurensis* PFC70 and *Lc. lactis* PFC77 were subjected to freezing and lyophilisation and according to the results, all strains successfully survived after the both of process (Table 3). *P. acidilactici* PFC69 was a more resistant strain to both freezing and lyophilisation than *L. namurensis* PFC70 and *Lc. lactis* PFC77. Today, freezing and lyophilisation are still the most widely used culture preservation techniques. Therefore, the resistance to freezing and lyophilisation of starter cultures or probiotics is a very important criterion. The results of our study indicated that all strains can be easily stored using these techniques.

The antagonistic activity of used strains against to each other was determined using agar spot test. Results of the agar spot test showed that *L. namurensis* PFC70 and *P. acidilactici* PFC69 exhibited antagonistic activity towards the growth of each other (Table 3). There was no determined activity among the other strains. Moreover, the growth of S. cerevisiae was not negatively effected in the presence of LAB. These results indicated that the use of *L. namurensis* PFC70 and *P. acidilactici* PFC69 together in mixed culture might be a disadvantage.

***Microbiological results of tarhana samples***

LAB and yeast counts of tarhana samples during fermentation are shown in Figure 2 and 3 as comparatively. Initial LAB and yeast number of samples were found between 5.77-6.35 log CFU/g and 3.89-4.26 log CFU/g, respectively and at the end of the fermentation period LAB and yeast counts were determined, ranging from 6.18 to 7.38 log CFU/g and 5.82 to 6.14 log CFU/g, respectively. For all fermentation days, the highest LAB numbers for P. acidilactici, L. namurensis PFC70 and Lc. lactis PFC77 were found as 9.32, 8.26 and 8.64 log CFU/g, respectively. And highest yeast number (*S. cerevisiae* PFC121) was determined as 6.71 log CFU/g. In all samples, generally, LAB counts significantly increased until day 3 of the fermentation period ($P<0.05$) and after that decreased except S3 and S7 samples. LAB counts of these samples were decreased after the fifth day of fermentation. Similar results are obtained by İbanoğlu et al. (1999) and Özel et al. (2015) who reported that LAB numbers of tarhana samples increased during the first days and then decreased.

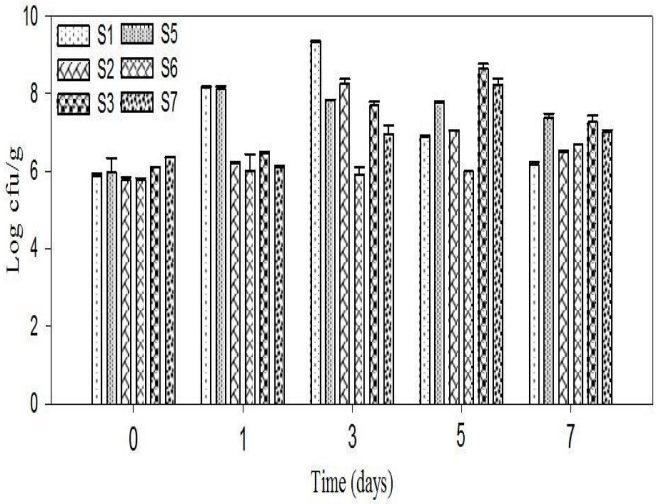


**Figure 2.** *LAB counts of tarhana dough samples during fermentation period*
*S1: P. acidilactici PFC69, S2: L. namurensis PFC70, S3: Lc. lactis PFC 77, S5: P. acidilactici PFC69 + S. cerevisiae PFC121, S6: L. namurensis PFC70 + S. cerevisiae PFC121, S7: Lc. lactis PFC 77 + S. cerevisiae PFC121*

When *S. cerevisiae* PFC121 used with *P. acidilactici* PFC69 as culture in S5 sample, *P. acidilactici* PFC69 counts were significantly decreased at the day of 3 compared S1 samples ($P<0.05$). Towards the end of fermentation period, *P. acidilactici* PFC69 counts were stable in S5 sample and at the end of fermentation, *P. acidilactici* PFC69 counts in S5 were found higher than S1 ($P<0.05$). It probably depended on pH values of tarhana samples as pH value of S1 was lower than S5 at the end of fermentation. The counts of *L. namurensis* PFC70 was affected negatively by the presence of yeast due to the negative correlation between them during five days of the fermentation period. At the end of the fermentation, *L. namurensis* PFC70 counts in S6 was found higher than the dough S2, but there was no significantly difference ($P>0.05$).

Similarly, *S. cerevisiae* PFC121 affected growing of *Lc. lactis* PFC77 negatively and *Lc. lactis* PFC77 counts in S7 sample were found lower compared to the sample S3 for all fermentation periods. The results of the study on the interaction between LAB and yeast in tarhana sourdough showed that bacterial growth decreased except *P. acidilactici* PFC69 when used mixed cultures. This is the result of *S. cerevisiae* having faster consumption of maltose and especially glucose by more effective high-affinity transport systems without depending on external systems (De Vuyst & Neysens 2005; Gurbuz et al. 2010). Paramithiotis et al. (2006) found that maltose and glucose increased during the fermentation period of 24 h when LAB was used as monocultures. This situation probably occurs hydrolytic activity of amylases already present in the flour. LAB used for fermentation of tarhana dough in our study was not shown amylolytic character. However, when yeast was grown either as a monoculture or with a lactic acid bacteria, all carbon sources were depleted after 12 h of fermentation period and this is probably the result of an imbalance between starch hydrolysis and carbohydrate consumption (Paramithiotis et al. 2006). On the other hand, it was reported that the positive effect of yeast presence on the growing of LAB was observed only in flours including different soluble carbohydrate (Corsetti & Settanni 2007; Gobbetti et al. 1994).

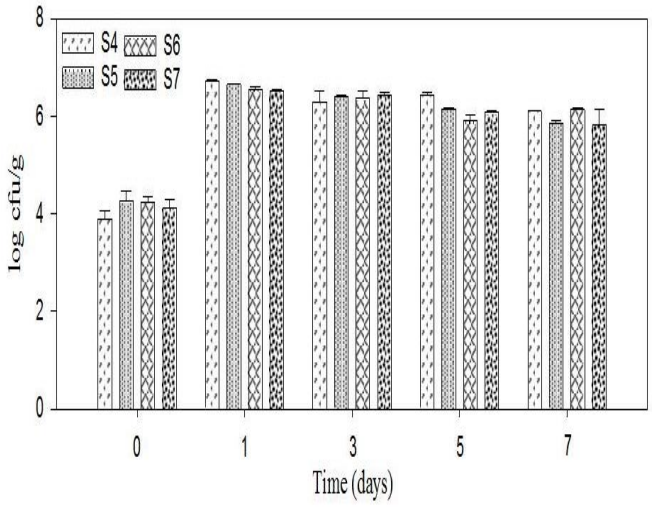


**Figure 3.** *S. cerevisiae counts of tarhana dough samples during fermentation period*

*S4: S. cerevisiae PFC121, S5: P. acidilactici PFC69 + S. cerevisiae PFC121, S6: L. namurensis PFC70 + S. cerevisiae PFC121, S7: Lc. lactis PFC 77 + S. cerevisiae PFC12*

Growing of *S. cerevisiae* PFC121 was not affected by using with different LAB culture (P>0.05). But, the *S. cerevisiae* PFC121 number in S4 was determined lower than other samples prepared with LAB on the first day of fermentation. Afterwards of fermentation periods, the yeast counts were found similarly for all tarhana samples. The results of *S. cerevisiae* PFC121 enumeration are in accordance with the results obtained by Değirmencioğlu et al. (2005) and Herken & Çon (2014). Similarly, Paramithiotis et al. (2006) stated that the final cell yield of *S. cerevisiae* in the sourdough was unaffected from the presence of lactic acid bacteria. But, the maximum specific grow rate was impressed negatively in all situations except when using with *L. sanfranciscensis* in the sourdough production.

***pH, total acidity, acidity number and water activity results of tarhana samples***

At the beginning of fermentation, pH values of dough samples including starter culture were found between 4.75-5.16 and 4.94 as mean (Figure 4a). The differences between initial pH values may be the result of not using starter cultures used but kneading all dough samples separately. At the end of fermentation, pH values were determined between 3.88 and 4.81 in all dough samples. The literature and our study results show that there are differences in tarhana samples depending on pH values. This may be the cause of the microorganism strains used in the production of tarhana dough.

To prevent the growing of undesirable microorganisms presented on the first days of fermentation, the rapid pH decrease has a big importance. It was found that pH values of tarhana samples slowly reduced when used yeast with LAB as starter culture except for S5. This reducing can be explained by the competition between yeast and LAB for carbohydrate sources (Çelik et al. 2005). At the end of fermentation, the lowest pH values were determined as 3.91 in the dough prepared with only *P. acidilactici* PFC69 (S1) and 4.07 in the sample produced with *P. acidilactici* PFC69 and *S. cerevisiae* PFC121 (S5). For the S2 sample, the lowest pH value was determined as 3.88 and S6 sample had the lowest pH value as 4.61. When *Lc. lactis* PFC77 was used alone (S3) and combined with yeast, the lowest pH values were established 4.00 and 4.45, respectively. As expected, the pH decrease of S4 occurred more slowly than the other dough samples except for S6. At the end of fermentation, the S6 sample had the highest pH value probably due to negative effect between *S. cerevisiae* PFC121 and *L. namurensis* PFC70. On the contrary to our results, Paramithiotis et al. (2006) found that the use of LAB with the yeast for fermentation of sourdough had no effect on the pH value.

The lowest pH value was determined in S2 sample at the end of fermentation. This is based on rapid acid production ability of *L. namurensis* PFC70. Because of *Lc. lactis* PFC77 having low acid production capacity, pH value of dough samples prepared with *Lc. lactis* PFC77 were a bit higher in comparison with other samples. Like our results, Gurbuz et al. (2010) determined lower pH values of tarhana samples not including yeast in comparison with samples produced with yeast and they stated that the cause of this result can be

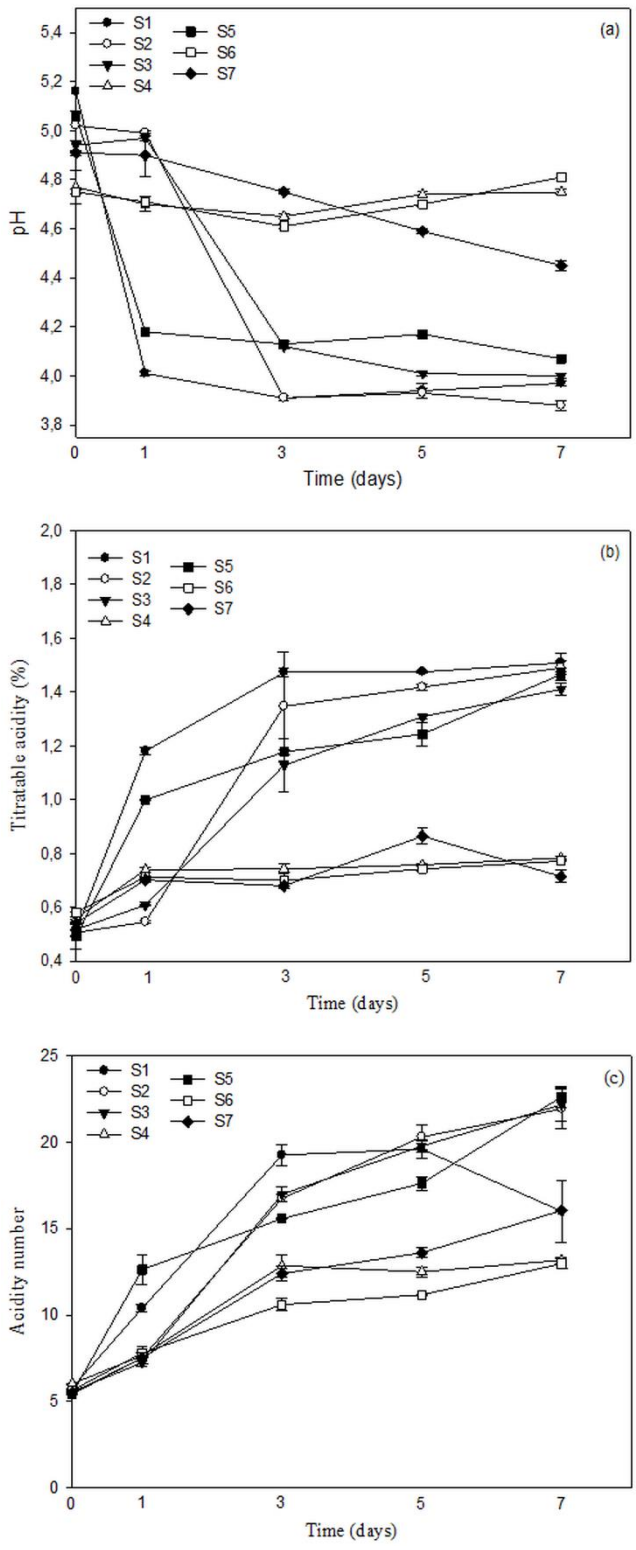


**Figure 4.** *pH value (a), total acidity (b) and acidity number (c) results of tarhana dough samples during fermentation period. S1: P. acidilactici PFC69, S2: L. namurensis PFC70, S3: Lc. lactis PFC 77, S4: S. cerevisiae PFC121, S5: P. acidilactici PFC69 + S. cerevisiae PFC121, S6: L. namurensis PFC70 + S. cerevisiae PFC121, S7: Lc. lactis PFC 77 + S. cerevisiae PFC121.*

explained with due to the competition between LAB and yeast. Similarly, Erbaş et al. (2005) determined pH values of wet tarhana dough samples between 4-5, and Bilgicli (2009) determined between 4.12 and 4.5. Nevertheless, Gabrial et al. (2010) found that pH values of tarhana samples fermented for three days by using probiotic cultures in different concentrations were found between 4.89-3.92.

Total acidity results of this study related to pH results (Figure 4b) and during all fermentation time, total acidity values of all dough samples were increased except S7. The highest total acidity value was determined on the S1 sample (1.59%) and total acidity value of S1 increased faster than the other samples. This result shows that *P. acidilactici* PFC69 is the fastest microorganism produces acid in tarhana dough. Whereas, the tarhana samples produced with LAB and yeast had a slower increase in total acidity except for S5. This situation was verified by pH results. Considering the results of many studies about the interaction of LAB and yeast in sourdough, bacterial growth and acid production decreased due to the faster consumption of carbohydrates especially glucose by yeast (Gurbuz et al. 2010). On the other hand, it was found that total acidity increased at a slower rate in S4. This result is an agreement with the study of Meignen et al. (2001) implicitly.

According to Turkish tarhana standard, acidity number of tarhana must be at least 10. When the results are examined (Figure 4c), it was seen that while S1 sample reached enough acidity number value on the first day of fermentation and the other samples reached after the first day. That was in accordance with the total acidity results. At the end of the fermentation period, the highest acidity number value was found in S5. It could be occur by positive interactions between *P. acidilactici* and *S. cerevisiae*. Acidity number of our Tarhana samples is similar to other researches (Herken & Çon 2014).

Values of water activity which is one of the most effective factors on microorganism growing were determined between 0.955-0.957 initially in tarhana dough samples ($P>0.05$). On the other fermentation days, the values decreased a little bit and were determined between 0.937-0.946 at the end of fermentation. Water activity results of the study are suitable with Erbaş et al. (2005) but higher than some studies reported by Colak et al. (2012) and Settanni et al. (2011). This situation can be explained due to the ingredients of tarhana dough and fermentation conditions such as fermentation time, temperature, humidity, cover (yes/no), etc.

### *Organic acid profiles of tarhana samples*

Changes in the organic acid composition of tarhana samples during the fermentation period are shown in Figure 5. The organic acids in tarhana come from its ingredients and are produced by LAB metabolism from fermentable

carbohydrates (Bozkurt & Gürbüz 2008; Erbaş et al. 2006; Kumral 2015). And also, it was reported that organic acids might also be produced by yeast (Erbaş et al. 2006). In tarhana, the major organic acid is lactic acid. As can be seen from the results, lactic acid concentration in all samples was increased and the highest lactic acid value was determined in S3 sample on the 7 days of the fermentation period. This result is in accordance with the other chemical results of this study. Lactic acid amount in the samples produced with mixed cultures was lower than the samples prepared with single cultures. In S4, lactic acid concentration was increased slowly in comparison with the other samples as expected. The bacteria found in the flour used for the study are responsible for the rise of lactic acid in S4. The lactic acid results are not suitable with Erbaş et al. (2006) and Magala et al. (2013). The differences between studies are the result of different tarhana ingredients and the amount of them and also microorganisms producing different organic acid.

Acetic acid having importance on the formatting of aroma and preventing fungi spoilage in fermented foods is the most important organic acid following lactic acid (Leroy & De Vuyst 2004; Magala et al. 2013). The highest acetic acid value (3995.75 mg/kg) in tarhana was observed in S5. It was determined that the production of acetic acid was faster and higher than the other samples (S5 and S1) prepared *with P. acidilactici* PFC69. This result is strange for *P. acidilactici* PFC69 as a homofermentative lactic acid bacterium. But when viewing citric acid results, it can be seen that there is a relation between acetic and citric acid for this bacterium. The lowest values of citric acid concentration were detected in sample S5 and S1. And this result shows the possibility that *P. acidilactici* PFC69 may convert citric acid to acetic acid. Citric acid amounts were increased in the other samples during the fermentation period. This result may be caused by yeast acid production.

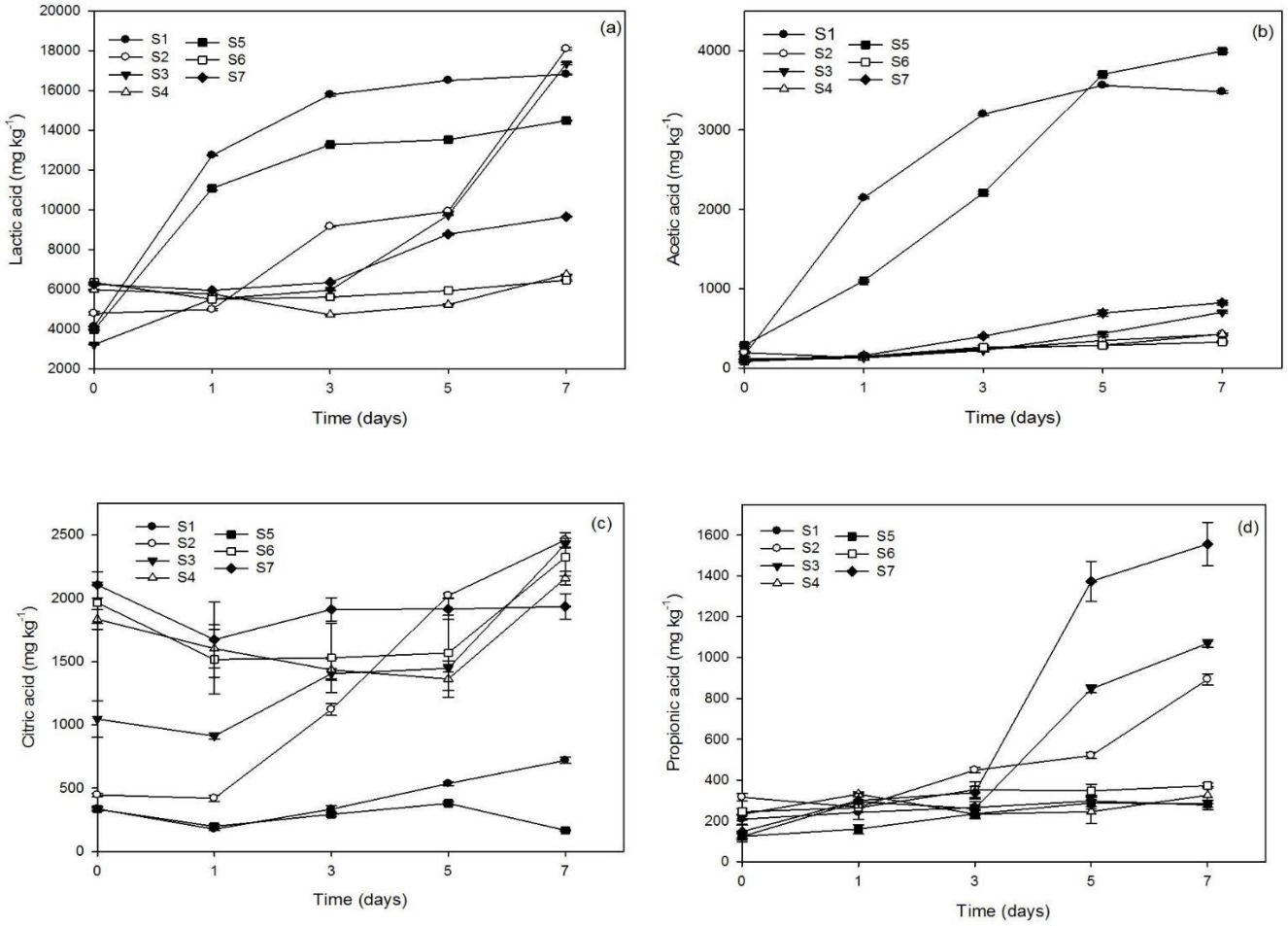


**Figure 5.** *Lactic acid concentrations (a), acetic acid concentrations (b), citric acid concentrations (c) and propionic acid concentrations (d) of tarhana dough samples during fermentation period.*
*S1: P. acidilactici PFC69, S2: L. namurensis PFC70, S3: Lc. lactis PFC 77, S4: S. cerevisiae PFC121, S5: P. acidilactici PFC69 + S. cerevisiae PFC121, S6: L. namurensis PFC70 + S. cerevisiae PFC121, S7: Lc. lactis PFC 77 + S. cerevisiae PFC121.*

Propionic acid is formed propionic acid bacteria coming from the flour used for tarhana production. As can be seen from the results propionic acid values are similar in samples except for S7. In this sample, yeast existing might stimulate propionic acid bacteria metabolism. In addition to lactic and acetic acid, other organic acids can be produced (Bozkurt & Gürbüz 2008; Lefebvre et al. 2002). But it was reported that the amount of them could be neglected for total acidity (Bilgicli 2009) and some of them come from vegetables used for tarhana production (Bozkurt & Gürbüz 2008).

## Conclusion

Overviewing the results obtained in this study, *P. acidilactici* PFC69, *L. namurensis* PFC70 and *Lc. lactis* PFC77 counts were significantly affected by the presence of *S. cerevisiae* PFC121. When *L. namurensis* PFC70 used with yeast, it was more stable than used alone. Moreover, a similar change of *Lc. lactis* PFC77 counts was observed when used with yeast during the fermentation period. But, *P. acidilactici* PFC69 was affected negatively when used yeast at the day 3 and at the end of the fermentation the counts of *P. acidilactici* PFC69 used with yeast were found higher than *P. acidilactici* PFC69 alone. Similar changes of pH, titratable acidity and acidity number were observed depending on LAB numbers. However, the counts of yeast were not changed in the presence of LAB. When considering that the major organic acid is lactic acid, determining the quality of tarhana in sense of both chemical and aroma, it is found that the existence of yeast had a negative effect on tarhana fermentation. The samples prepared without yeast had the highest values of lactic acid concentrations while fermentation time. In general, using yeast with LAB for tarhana production may be recommended because of being not too much interaction between them and the effect of yeast on the aroma formation. Further researches are needed to be done for trying LAB with yeast strain like *Candida*, *Kudrievzeii*, *Kazakistania* isolated sourdough and tarhana dough.

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