Determination of Microbial Dynamics and Some Metabolite Formation of Semi-Dried Tarhana Produced from Home-Made Yoghurt

Yekta GEZGİNÇ*

*Kahramanmaraş SütçüİmamUniversity, Faculty of Engineering and Architecture,
Department of Food Engineering, TR-46060, Kahramanmaraş,Turkey

ABSTRACT: In this study, home-made yoghurt samples was used for making tarhana and microbial and metabolic features of different semi-dried tarhana samples were analyzed. Lactic acid bacteria and yeasts were isolated from home-made yoghurt and semi-dried tarhana. Molecular characterization of isolates was performed with PCR approach using species-specific primers. Each sample was analyzed in terms of metabolite profile by using HPLC. Lactic acid contents of yoghurt were varied between 27.87±1.61 and 165.32±0.30µg/g, while this values was ranked between 1.70±0.35 and 22.03±1.05 µg/g in semi-dried tarhana. Acetaldehyde was found in the range of 15.32±2.56 and 179.37±3.99µg/g in yoghurt and of 1.89±0.25 and 61.08±1.35 µg/g in semi-dried tarhana.

Keywords:*Yoghurt, tarhana, yoghurt starter cultures, lactic acid and acetaldehyde metabolite*

Ev Tipi Yoğurtlardan Üretilen Yarı-Kurutulmuş Tarhananın Mikrobiyal Dinamiği ve Bazı Metabolitlerinin Belirlenmesi

ÖZET: Bu çalışmada, tarhana yapımı için kullanılan ev yoğurtları ve yarı-kurutulmuş tarhana örneklerinin mikrobiyal ve metabolit analizi yapılmıştır. Laktik asit bakterileri ve mayalar ev yapımı yoğurtlardan ve yarı kurutulmuş tarhanadan izole edilmiştir. Bu izolatların moleküler karakterizasyonu türe özel spesifik pirimerlerin kullanımı ile PCR'da gerçekleştirilmiştir. Her bir örneğin metabolit analizi HPLC ile yapılmıştır. Yoğurt örneklerinde laktik asit değişimi 27.87±1.61 ile 165.32±0.30 µg/g arasında ve yarı kurutulmuş tarhana örneklerinde 1.70±0.35 ile 22.03±1.05 µg/g arasında tespit edilmiştir. Asetaldehit içeriği, yoğurtta 15.32±2.56 ile 179.37±3.99µg/g arasında, yarı-kurutulmuş tarhanada 1.89±0.25 ve 61.08±1.35 µg/g aralığında bulunmuştur.

Anahtar Kelimeler: *Tarhana, yoğurt, yoğurt starter kültürü, laktik asit ve asetaldehit*

1. INTRODUCTION

Tarhana is a traditional fermented food made form of yoghurt-cereal mixture and it is consumed dried or semi-dried forms. The main microbial content of tarhana is lactic acid bacteria and yeast. Tarhana has been considered as one of the oldest probiotic foods [1-3]. It is consumed in nearly all regions of Turkey. It is produced both industrial and home-made types. Tarhana-like fermented products are also commonly consumed around the world; Kishk (Kushk) in Middle East Countries, Trahanas in Greece, Thanu in Hungary, Talkuna in Finland and Atole in Scotland [4-5]. Tarhana is mostly produced through a four main step process; i-tarhana dough mixing, ii- fermentation, iii- drying, and iv-grinding, depending on the country and the region, different heat processes and ingredients(yoghurt and cracked cereal, raw or cooked vegetables, spices and salt) may be used in its making [6-7].

The thyme and black cumin is used besides its main ingredients (cracked wheat: wheat derived and yoghurt). At first, cracked wheat is cooked in boiling water, kneaded, added to home-made yoghurt and then blended. The mixture is generally fermented overnight at outside temperature (±25°C). Following the fermentation process, the dough is laid on a thin layer bulrush cane and dried approximately for two days. At the end of first day semi-dried tarhana (SDT) is obtained and this stage of tarhana extensively consumed in Turkey [4-5]. SDT is an acidic fermented food[4] and contains high amount of living probiotic microorganisms, *Lactobacillus bulgaricus*(*L. bulgaricus*) and *Streptococcus thermophilus* (*S. thermophilus*) and yeasts (*Saccharomyces cerevisiae*). These microorganisms produce lactic acid, acetaldehyde, ethanol, carbon dioxide and other typical aromatic compounds in tarhana[9].

Tarhana was made in conditions that are not controlled and also yoghurt was made using home-made using home-made non-starters. Not only *L. bulgaricus* and *S. thermophilus* species but also environmental yeasts and other microorganisms take role in during fermentation of tarhana. Additionally, drying stage of tarhana occurs in an uncontrolled environment. Therefore, in order to determine the quality of the final product and to select the best starter culture for its industrial production, it is important to identify and enumerate microorganisms present in home-made yoghurt and tarhana.

Lactic acid fermentation helps for the formation of taste and aroma, shelf-life, nutritional value and other favorable properties of foods [9]. Lactic acid bacteria (LAB) and yeasts are responsible for the acid and ethanol formation during the fermentation of product. LAB and yeast fermentation proceeds through the Embden-Meyerhof Pathway (EMP) in which glucose is transformed into ethanol (via pyruvate and acetaldehyde), carbon dioxide and traces of other acids and carbonyl compounds [10-11]. As the inspection of the safety and desirability of the final product, its chemical composition should be detected by the measurement of pH and some metabolites such as lactic acid, acetaldehyde, and ethanol.

The main objective of this study, quantification and identification of lactic acid bacteria and yeasts from tarhana's yoghurt, SDT and determination of occurring lactic acid, acetaldehyde and ethanol amounts in yoghurt and SDT by using HPLC.

2. MATERIALS AND METHODS

2.1. Sample Collection

Yoghurt and SDT samples were collected from local market in Kahramanmaras, province of Turkey. Each sample was transported to the laboratory in sterile jars or plastic bags at ±5˚C.

2.2. Isolation and Enumeration of LAB and Yeast

The pH of collected samples were analyzed and each measurements carried out in triplicate by using digital pH meter (Mettler-Toledo AG,Schweiz) when microbial study were started.25 g of each SDT and yoghurt (a component used in SDT making) was taken aseptically, transferred to a sterile plastic bag, and homogenized with a blender in 225 mL of sterile physiological solution for 2 min. After decimal dilutions were prepared in sterile 0.85% (w/v) saline solution, 0.1 mL of each dilution was plated by using double layer technique on de Man–Rogosa and Sharpe Agar (MRS, Merck) for the isolation of Lactobacillus spp., or on M17 Agar (Merck) for the isolation of Streptococcus spp. The plates were incubated anaerobically at 37°C and 42°C for 48 h., respectively. Total yeast count was determined on potato dextrose agar (P DA), incubated aerobically at 25°C for 48 h. Colony counts were repeated three times per sample and results were expressed as logcfu/g.

All isolates were tested for cell morphology (colonies of various shapes of yeasts and bacteria by an optical microscope), gram staining ability and catalase activity (determined by transferring fresh colonies from a petri dish to a glass slide and adding H_2O_2 5%v/v on them). Gram-positive and catalase-negative colonies with rod or cocci shapes were considered as LAB and were selected for further examination. The pure cultures of these strains were stored in corresponding broth supplemented with 30% glycerol at −80°C. When required, the cultures were activated by two consecutive transfers to broth and they were incubated as described above.

2.3. Molecular Identification of LAB

Genotypic identification of isolated LAB strains was carried according to the method described by Tabasco et al., [12]. Genomic DNA was extracted using the Genomic DNA Extraction Kit (Fermentas) according to the manufacturer's protocol. The amount and the quality of DNA were determined by Nano-Drop and confirmed by agarose gel electrophoresis. Molecular identification of the isolates was conducted using specific primers within variable regions in the 16S rRNA encoding genes of *S. thermophilus* and *L. bulgaricus*(StF:5'ACGCTGAAGAGAGGAGCTTG3'-StR: 5'GCAATTGCCCCTTTCAAATA 3', and LbF: 5' TCAAAGATTCCTTCGGGATG 3' - LbR: 5' TACGCATCATTGCCTTGGTA 3'). These primers were obtained from Iontek(Istanbul, Turkey). The PCR amplification reaction was performed in a 40 µL mixture containing 1 µL of each primer (20 pmol), 4 µLof 10X reaction buffer, 1 µL of each dNTPs (250 µM each), 0.5 µlofTaq DNA polymerase and 1 µL of the isolated DNA. The PCR products were generated using an initial denaturation step of 4 min at 94°C, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 60°C for 1 min, elongation at 72°C for 1 min and final at 72°C for 5 min. PCR reactions were performed in an EppendorfMastercycler Personal system. Following the amplification, all PCR products were run through 1% (m/v)agarose gels at 80V for one hour. The separated fragments were stained with 0.5µgmL^{-1}ethidium bromide [13] and visualized under UV light.

2.4. Metabolic End Products Analysis by HPLC

The amounts of lactic acid, acetaldehyde and ethanol formed in each sample were analyzed using

HPLC as described by Ozogul et al., [14]. Briefly, 1mL of the extracted sample was centrifuged at 14.000 g for 5 min. The supernatant was filtered and placed in a fresh eppendorf tube. The sample was diluted 1 fold in 0.5% meta-phosphoric acid, and 20 µL of it was injected into the HPLC. Separation was performed on a Capcell Pak 5 µM C18 MG column (150×4.6 mm). Acetaldehyde concentration was calculated by using the absorbance at 277 nm, lactic acid by the absorbance at 210 nm and ethanol at 190 nm.

2.5. Statistical Analysis

In this study, yoghurt and SDT samples were analyzed in triplicates. Microbiological results were analyzed after using logarithmic transform. All statistical calculations were performed by one-way ANOVA using SPSS software (SPSS Inc., Chicago, IL, USA) SAS Statistical Software (release 7.00 for windows. SAS Institute Inc. Cary. NC.USA). Results are presented as Mean ± Standard Deviation. Significances were evaluated with the analysis of variance, followed by Duncan's Multiple Range Test, where p value lower than 0.05 ($p < 0.05$) was considered significant.

3. RESULTS AND DISCUSSION

Microbial enumeration and metabolite formation findings of the present study appear to be substantially different than previous studies [15-17]. These inequalities could be explained by the types and characteristics of tarhana samples evaluated between the studies. Tarhana may be produced with different ingredients, and the samples may be taken during the various steps of processing. In our study, SDT samples were taken at a different fermentation time (12 h) than they were taken in other studies. These variables may affect the results and may be considered as one of the reasons of the discrepancy between the values presented in preceding studies and ours.

The pH of each SDT and yoghurt (Y) sample, and the number of the colonies formed by the strains isolated from them were determined. SDT samples were characterized with lower pH values than yoghurt samples. As a result of the evaluation of pH values for tarhana's yoghurt, it was revealed that Y2 had the lowest pH value (3.69±0.05) while the highest value was presented by Y12 (5.04±0.46) (Table 1). In a previous study conducted by Birollo et al. [18] it was reported that pH in yoghurt samples ranged between 3.8 and 4.0. Similarly, Biberoglu and Ceylan [19] reported that the pH values in yoghurt samples were varied between 3.43 and 4.19 when produced traditionally. The observation of having slightly higher pH compared to what is presented in the literature could be resulted by the difference in storage duration and conditions that have been shown to affect the acidity of the product [20]. Additionally, the sample named SDT17 had the highest pH values (4.26±0.09), whereas the lowest pH value (3.34±0.23) was measured in the sample SDT5 in SDT. In a previous study reported by Sengun et al. [4], it was stated that pH values of tarhana varied from 4.0 to 5.0 at the start of fermentation and typically dropped at a degree of between 0.1 and 0.2 through the three day of fermentation. Considering that SDT is a half-product of tarhana, the pH values that we measured appear to be somewhat lower than the study mentioned above. This slight decrease might depend on the contents used in tarhana making, such as yoghurt and cracked wheat. Furthermore, when the pH values obtained for yoghurt and SDT samples evaluated in this study were compared, no significant difference ($p < 0.05$) was detected, even though the tendency of SDT samples to have lower pH values to some extent can still be observed through the data. pH is a reliable parameter of tarhana quality since the low pH makes tarhana unattractive to pathogenic and spoilage microorganisms while it produces an attractive sour taste desirable for consumers [21-22,19]. In the literature, a combined culture of yeast and Lactobacilli is demonstrated to cause more significant declines in pH of the fermented products than it is caused by the use of a single culture [23-24]. SDT making includes yoghurt processing, therefore takes a longer time which increases the chance of yeast content.

3.1.Enumeration and identification of *Laccobacillus bulgaricus*, *Streptococcus thermophilus* and yeasts

Bacteria and yeasts used in the culture during the production of tarhana have great importance in the nutritional and commercial value of the final product. In yoghurt and tarhana industry, available strains of microorganisms are *Streptococcus thermophilus* (*S. thermophilus*), *Laccobacillus bulgaricus*(*L. bulgaricus*) and *Saccharomyces cerevisia* [25]. In our study, all bacteria isolates that were catalase-negative, gram positive and with rod or cocci shapes(phenotypic methods) were selected for further analysis. On the basis of these tests, the bacteria isolated from tarhana's yoghurt and SDT were identified as *S. thermophilus* and *L. bulgaricus*.18 of the isolates produced a 157 bp long amplification on the gel and were consequently determined as *S.thermophilus* whereas another 18 were identified as *L. bulgaricus* species for generating a visible 232 bp PCR product on the gel (data not shown). The yeast isolates(*S. cerevisae*) were identified by using Biolog Gen III Micro plate YT MicroPlate diagnostic kits (for yeasts) assisted by computer software and were found to be *S. cerevisiae*(95%). LAB from tarhana's yoghurt and SDT were enumerated on two different media and at two different temperatures. $Log_{10}cfumL^{-1}$ of LAB and yeast determined on MRS, M17 and PDA media incubated at 37°C, 42°C and 25°C for 2 days, 3 days and 3 days, respectively (Table 1).

Table 1. Yoghurt(Y), Semi-Dried Tarhana(SDT) pH and (logcfu/mL) of (LAB) and yeast determined on MRS, M17 and PDA media incubated at 37°C, 42°C and 25 °C.

Yogurt Sample	**pH**	**Number of colony (cfu/g)**		
		L. bulgaricus	***S. thermophilus***	***S. cerevisiae***
Y1	4.16±0.08 ^{de}	7.75±0.12 ^{bc}	8.14±0.93 ^{ab}	ND
Y2	3.69±0.05 ^{f}	6.93±0.01 ^{ef}	8.65±2.13 ^{a}	ND
Y3	4.36±0.15 ^{abcd}	7.83±0.07 ^{bc}	7.85±1.81 ^{ab}	7.14±0.02 ^{b}
Y4	4.30±0.13 ^{bcd}	7.14±0.05 ^{de}	7.47±2.37 ^{bcd}	3.69±0.23 ^{fg}
Y5	4.82±0.12 ^{ab}	6.44±0.12 ^{g}	8.61±2.45 ^{a}	3.54±0.02 ^{g}
Y6	4.76±0.07 ^{abc}	7.16±0.05 ^{de}	7.37±2.55 ^{bcde}	6.26±0.02 ^{d}
Y7	4.83±0.12 ^{ab}	7.74±0.05 ^{cd}	7.87±2.57 ^{ab}	1.84±0.02 ^{j}
Y8	4.38±0.12 ^{abcd}	7.47±0.10 ^{cd}	7.65±2.56 ^{bc}	3.94±0.02 ^{f}
Y9	4.78±0.13 ^{abc}	8.48±0.14 ^{a}	6.54±2.58 ^{defg}	4.46±0.02 ^{e}
Y10	4.34±0.09 ^{abcd}	6.56±0.09 ^{fg}	6.84±2.57 ^{cdef}	1.88±0.01 ^{j}
Y11	4.36±0.09 ^{abcd}	6.47±0.13 ^{g}	6.54±2.54 ^{efg}	ND
Y12	5.04±0.46 ^{a}	6.76±0.12 ^{efg}	5.74±0.55 ^{g}	2.64±0.02 ^{ı}
Y13	4.94±0.13 ^{ab}	5.87±0.12 ^{h}	6.46±2.17 ^{efg}	7.84±0.06 ^{a}
Y14	4.12±0.10 ^{de}	7.94±0.04 ^{b}	8.07±1.85 ^{ab}	7.69±0.12 ^{a}
Y15	4.34±0.12 ^{abcd}	5.53±0.13 ^{h}	6.39±1.68 ^{fg}	6.71±0.12 ^{c}
Y16	4.24±0.12 ^{cde}	6.38±0.13 ^{g}	6.85±1.36 ^{cdef}	3.68±0.14 ^{fg}
Y17	4.74±0.12 ^{abc}	5.03±0.35 ^{ı}	6.24±1.02 ^{fg}	2.40±0.14 ^{ı}
Y18	5.02±0.45 ^{a}	5.88±0.09 ^{h}	8.25±2.26 ^{ab}	3.13±0.05 ^{h}
SDT Sample	**pH**	***L. bulgaricus***	***S. thermophilus***	***S. cerevisiae***
SDT1	4.03±0.12 ^{abc}	3.6±1.37 ^{cd}	4.15±0.09 ^{efg}	1.68±0.14 ^{m}
SDT2	3.82±0.12 ^{bcdef}	1.76±0.94 ^{g}	3.52±0.13 ^{fg}	2.94±0.08 ^{l}
SDT3	3.72±0.12 ^{cdefg}	3.47±1.13 ^{cde}	5.28±0.14 ^{bc}	7.89±0.18 ^{d}
SDT4	3.68±0.12 ^{cdefg}	4.24±0.86 ^{a}	5.36±0.91 ^{b}	11.83±0.05 ^{b}
SDT5	3.34±0.23 ^{gh}	3.58±1.30 ^{cde}	6.59±0.07 ^{a}	4.53±0.07 ^{ı}
SDT6	4.14±0.05 ^{ab}	2.50±1.37 ^{f}	5.15±0.08 ^{bcd}	5.26±0.07 ^{h}
SDT7	4.24±0.12 ^{a}	2.59±1.42 ^{f}	3.55±0.13 ^{fg}	5.94±0.01 ^{g}
SDT8	3.42±0.22 ^{fgh}	4.28±1.23 ^{a}	4.16±0.25 ^{efg}	3.53±0.03 ^{k}
SDT9	3.66±0.12 ^{defg}	2.17±0.76 ^{fg}	4.27±0.13 ^{ef}	3.26±0.03 ^{k}
SDT10	4.14±0.06 ^{ab}	4.16±1.31 ^{ab}	5.06±0.17 ^{bcd}	3.94±0.01 ^{j}
SDT11	4.24±0.15 ^{a}	3.77±1.26 ^{bc}	3.46±0.17 ^{g}	1.33±0.01 ^{n}
SDT12	3.44±0.02 ^{gh}	4.27±1.13 ^{a}	4.42±0.18 ^{de}	7.26±0.01 ^{e}
SDT13	3.84±0.01 ^{bcdef}	3.17±1.09 ^{e}	5.29±0.06 ^{bc}	6.93±0.00 ^{f}
SDT14	3.67±0.03 ^{defg}	4.36±1.38 ^{a}	5.57±0.06 ^{b}	5.42±0.05 ^{h}
SDT15	3.86±0.09 ^{bcde}	3.67±0.65 ^{cd}	4.60±0.10 ^{cde}	8.24±0.01 ^{c}
SDT16	3.52±0.13 ^{efgh}	2.46±0.60 ^{f}	5.35±0.02 ^{b}	6.65±0.05 ^{f}
SDT17	4.26±0.09 ^{a}	3.20±0.27 ^{de}	4.35±0.13 ^{e}	12.51±0.01 ^{a}
SDT18	4.02±0.01 ^{abcd}	4.17±0.33 ^{ab}	5.16±0.20 ^{bcd}	4.28±0.03 ^{ı}

ND: Not determined

When the numbers of the microorganisms found in yoghurt and SDT were compared, all three of them were detected at significantly higher levels in yoghurt than they were in SDT ($p < 0.05$).

Yoghurt samples were found to contain between 5.03±0.35logcfumL^{-1} and 8.48±0.14logcfumL^{-1}of *(L. bulgaricus)* on MRS. Saccaro et al. [26], found that the number of *L. bulgaricus* in yoghurt was in the range of 5.61-8.90logcfumL^{-1}. Albeit slightly lower, our findings are in accordance with these results. As for our SDT samples, the number of *L. bulgaricus* was detected as between1.76±0.94 and 4.36±1.38 logcfumL^{-1} (Table 1). The count of *L. bulgaricus* was seen to be high in yoghurt but low in SDT suggesting that naturally found *L. bulgaricus* in yoghurt are destroyed during the SDT-making process, as it was demonstrated in another study of which *Lactobacillus spp.* count in tarhana dough was dropped from6.41±0.01to5.44±0.03 logcfumL^{-1} till the third day of fermentation [27].

The yoghurt samples studied were determined to contain between 5.74±0.55 and 8.65±2.13logcfumL^{-1}of *S. thermophilus.* The highest *S.thermophilus* number for yoghurt sample were noted in the sample Y2 (8.65±2.13logcfumL^{-1}), and the lowest was found in Y12 (5.74±0.55logcfumL^{-1}).In the report published by Saccaro et al. [26], the *S. thermophilus* count remained at 5.97-9.15 logcfumL^{-1} and Canganella et al. [28] also found that the counts for lactic streptococci were around of 8-9 logcfu mL^{-1}. These results are in accordance withthe fact that our counts were so low might be due to that flavored yoghurts contain added sugar which acts as a fermentable growth substrate and increases the yeast proliferation whereas traditional Turkish yoghurts do not contain it.

As for in SDT, the sample with the highest yeast count was SDT17 (12.51±0.01 logcfumL^{-1}) while SDT11 was the sample with the lowest count (1.33±0.01 logcfumL^{-1}). Settani et al. [32], found the counts of yeasts in tarhana samples to be between 7.2±0.3 and 8.3±0.3 logcfumL^{-1}. Considering that our upper limit was nearly 50% higher than what was presented by the study, we did not find it surprising since comparable concentrations of LAB and yeasts are generally reported for this product [4]. Our findings, which indicate that the yoghurts used in our studies are comparable to the ones used in previous studies. Moreover, the number of S.*thermophilus* in SDT was detected in the range of3.46±0.17 to6.59±0.07 logcfu mL-1, which was significantly lower than yoghurt ($p<0.05$). The difference in the microorganism count between yoghurt and SDT sample suggest that *S. thermophilus* found naturally in yoghurt may also be destroyed during the SDT-making process due to heat process and salt concentration.

The results concerning the total yeast in yoghurts were varied between1.88±0.01 and7.84±0.06 logcfumL^{-1} throughout the samples. Of all yoghurt samples studied, no yeast was detected in Y1, Y2, and Y11; however, Y13 had the highest amount of grown yeasts (7.84±0.06logcfu mL^{-1}) while Y10 showed the lowest growth rate (1.88±0.01logcfu mL^{-1}). Although yoghurt environment is regarded as selective for yeast growth because of its high acidity [29], a considerable amount of yeast species is reported to grow in milk and fermented milk products such as yoghurt [30]. It has been suggested that some undesirable properties of milk and yoghurt, such as gas production, yeasty (or other off-) flavors, changes in color and texture may result from the over growth of yeasts in these products. In the literature, average yeasts counts were encountered as 10^4cfu/mL especially in the fruit-based and flavored yoghurts [31].

3.2.Metabolite Production by Tarhana's Yoghurt and SDT

The typical yoghurt flavour is caused by lactic acid, which imparts an acidic and refreshing taste, and a mixture of various carbonyl compounds like acetone, diacetyl, and acetaldehyde of which the latter is considered the major flavor component [33]. Lactic acid is the most important organic acid and produced by the fermentable carbohydrates found in cracked wheat and yoghurt mixture[7]. Metabolic characteristics of yoghurt and SDT samples were determined by HPLC. The difference of the lactic acid produced in these samples was determined as statistically significant ($P<0.05$). Analyses for tarhana's yoghurt (shown Table 2) revealed that the maximum amount of lactic acid was produced by Y2 (165.32±0.30 μg g^{-1}) while the minimum value was measured inY12 (27.87±1.61 μgg^{-1}). When lactic acid production was compared among the SDT samples; however, F5 was found to be the most productive sample (22.03±1.05 μg g^{-1}) whereas SDT11 was detected as the least productive (1.70±0.35μg g^{-1}). Although, to our knowledge, there has been no study to measure lactic acid in traditionally made Turkish SDT in the literature, a recent study conducted in our lab investigated lactic acid producing potentials of lactic acid bacteria isolated from traditional Turkish Yoghurts. In this study, we found that lactic acid produced by *S. thermophilus* and *L. bulgaricus* ranged from 0 to 77.9 mg/kg and from 0 to 103.5 mg/kg, respectively [34]. However, it has been well known that the mixture of these organisms can produce different amount of lactic acid than they produce by themselves. Therefore, the fact that the lactic acid amount we measured exceeded the upper limit of the values presented in our previous study is not only reasonable but also expected.

Table 2. Amounts of Acetaldehyde, Lactic Acid and Ethanol (μg g^{-1}) produced by tarhana's yoghurt (Y) and Semi-Dried Tarhana (SDT).

No	Yoghurt		
	Lactic Acid	**Acetaldehyde**	**Ethanol**
Y1	132.01 ±2.43 cd	132.73 ±2.18 de	202.86 ±3.18 l
Y2	165.32 ±0.30 a	115.58 ±3.35 h	ND
Y3	144.65 ±1.78 b	135.14 ±0.30 d	143.15±3.09 k
Y4	124.63 ±4.54 f	121.67 ±3.55 f	297.04 ±1.19 h
Y5	143.95 ±3.87 b	127.58 ±4.84 ef	146.71±1.46 jk
Y6	125.74 ±4.73 ef	123.77 ±2.50 fg	562.18±4.25 bc
Y7	112.75 ±1.30 h	124.40 ±0.62 fg	504.35±6.45 de
Y8	143.43 ±3.05 b	156.24 ±3.85 b	482.29±3.64 e
Y9	117.72 ±1.70 g	184.37 ±3.99 a	365.25±3.80 f
Y10	131.45 ±0.43 cd	130.70 ±0.17 de	151.13±1.34 j
Y11	126.18 ±1.79 ef	94.91 ±1.16 i	104.44 ±8.38 kl
Y12	27.87 ±1.61 k	71.84 ±2.07 j	357.55±6.98 fg
Y13	127.33 ±3.68 cd	143.68 ±1.52 c	614.55±4.04 a
Y14	126.37 ±2.67 i	47.12 ±1.62 l	314.35±3.11 gh
Y15	135.11 ±2.97 c	56.58 ±2.70 k	200.46±7.06 l
Y16	122.61 ±2.19 f	122.73 ±1.77 fg	599.44±9.51 ab
Y17	130.12 ±2.17 de	14.32 ±2.56 m	529.63±8.64 cd
Y18	75.77 ±1.32 j	41.68 ±0.89 l	91.30±6.53 l
	SDT		
No	**Lactic Acid**	**Acetaldehyde**	**Ethanol**
SDT1	4.19 ±0.64 g	5.88 ±0.31 j	127.74±1.12 bc
SDT2	12.65 ±0.25 cd	37.22 ±1.24 cd	130.47±4.05 bc
SDT3	4.41±0.38 g	38.61±0.81 c	92.93±0.84 cd
SDT4	5.74±0.57 f	19.68 ±1.76 fg	118.28±1.01 bc
SDT5	22.03±1.05 a	28.94 ±0.62 e	37.44 ±1.67 de
SDT6	6.91±0.85 f	17.77 ±0.09 gh	27.91 ±5.39 de
SDT7	2.63 ±0.23 hı	21.35 ±0.71 f	3.46 ±0.14 e
SDT8	6.95 ±0.52 f	61.08 ±1.35 a	29.48 ±1.49 de
SDT9	2.32 ±0.04 l	1.89±0.25 k	1.25 ±0.53 e
SDT10	6.08 ±0.52 f	35.87 ±0.35 d	14.37 ±0.72 de
SDT11	1.70 ±0.35 i	7.89 ±1.21 j	12.94 ±1.43 e
SDT12	3.64 ±0.69 gh	2.16 ±1.49 k	55.09 ±1.04 cde
SDT13	13.78 ±0.39 bc	10.74 ±0.01 i	37.71 ±0.80 de
SDT14	12.21 ±0.09 d	5.94 ±0.79 j	39.09 ±1.65 de
SDT15	14.29 ±0.98 b	30.68 ±1.32 e	140.15 ±8.50 b
SDT16	12.20 ±0.09 d	6.04 ±0.65 j	14.60 ±3.69 de
SDT17	12.06 ±1.04 d	16.32 ±1.37 h	176.35 ±1.66 a
SDT18	9.23 ±0.63 e	57.53 ±1.54 b	58.77 ±0.67 cde

As for acetaldehyde content of our yoghurt samples, the one that generated it the least was the sampleY17 (14.32±2.56μg g^{-1}) howbeit Y9 was the sample produced the highest amount of it(184.37 ±3.99 μg g^{-1}).However, in SDT samples, the lowest amount of acetaldehyde was generated by SDT9 (1.89±0.25μg g^{-1})while SDT8 (61.08±1.35 μg g^{-1}) was the sample that produced it the most. In general; however, yoghurt was the better manufacturer of acetaldehyde, and the amount generated by yoghurt was significantly higher than by SDT ($p<0.05$). In a previous study, *S. thermophilus*has been reported to produce acetaldehyde in the range of 1.0 to 13.5 μg g^{-1} much as *L. bulgaricus* has been shown to generate it between the values of 1.4 and 77.5 μg g^{-1}[35]. In our study, the amount of acetaldehyde produced in yoghurtsamples remarkably higher than study[36]. This could be explained by the possible contamination during traditional yoghurt processing and the synergistic effect of using combined starter cultures on

the flavor components of yoghurt.On the other hand, the fact that such a synergy has not been observed in SDT samples might be due to the difficulty of interaction between the species co-cultured in a solid phase media.

Of all the carbonyl compounds which comprise the main aromatic substances in yoghurt, acetaldehyde is the most important compound for its typical flavor and yoghurts are considered to be good flavored only when they contain proper levels (23–40 mg/kg and at least 8–10 mg/kg) of acetaldehyde in them, and acetaldehyde levels in most yoghurts with a mixed starter culture are reported to be between 2.0 and 41.0 mg/kg [37]. Both the lower and the upper limits of our measurement exceed the literature (14.32±2.56 μg g^{-1} 184.37 ±3.99 μg g^{-1}, respectively) and it may be resulted by the fact that the levels presented in the literature are of the yoghurt produced in controlled environments, where the risk for additional microorganisms was broadly eliminated. Our yoghurt samples; however, were highly susceptible to contamination since there was no control of it through the whole manufacturing process.

Ethanol, a common terminal end product in the breakdown of glucose and catabolism of amino acids [38], was found to be produced between the amounts of 91.30±6.53 and 614.55±4.04 μg g^{-1} in yoghurt by the samples Y18 and Y13, respectively, whereas the values varied from 1.25±0.53μg g^{-1} to 176.35±1.66 μg g^{-1} in SDT samples; the lowest generated by SDT9 and the highest by SDT17. Ethanol is the principal alcohol in yoghurt, and despite the common report of ethanol as a major volatile compound, its contribution to the overall aroma and flavor is not clear. Some studies indicate that the amount of ethanol produced during lactic acid fermentation is so low that it has no practical importance in the flavor [39], while others suggest that it probably provides a complementary flavor to yoghurt[40]. In a comprehensive review on volatile compounds of yoghurt by Cheng[36], ethanol content of cow's milk yoghurt was declared to be in the range 0.2 to 9.9 mg/kg and it was stated that the values were generally lower in the yoghurts made from goat and sheep milk. These high values, similar to the increase in acetaldehyde, may be the results of the traditional yoghurt processing that allows the contamination of various microorganisms, thereby altering the metabolite production.

4. CONCLUSION

The present study has provided supplementary information on Turkish traditional yoghurts and tarhana commonly consumed. Microbial content of Turkish traditional yoghurts have higher amounts of lactic acid bacteria and yeast. Limited relation was observed between yoghurt and tarhana metabolite production. In tarhana, metabolite formation could be resulted from yeast activity rather than bacteria due to wheat addition.

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