JMBFS

Journal of Microbiology, Biotechnology and Food Sciences

International peer-reviewed scientific online journal

Published by Faculty of Biotechnology and Food Sciences

BACTERIAL MICROBIOTA AND CHEMICAL PROPERTIES OF TURKISH TARHANA

Esra Ersoy Omeroglu[1], Özge Can[2], Şevval Nur Temiz[3], Rabia Al[4], Osman Altunbas[5], Ali Soyuçok[6], Mediha Nur Zafer Yurt[7], Veli Cengiz Ozalp[8] and Mert Sudagidan[7]*

Address(es):
[1] Biology Department, Basic and Industrial Microbiology Section, Faculty of Science, Ege University, 35040 Izmir, Türkiye.
[2] Department of Bioengineering, İzmir Institute of Technology, 35430 İzmir, Türkiye.
[3] Department of Molecular Biology and Genetics, Muğla Sıtkı Koçman University, 48000 Muğla, Türkiye.
[4] Department of Basic and Industrial Microbiology, Eskişehir Osmangazi University, 26040 Eskişehir, Türkiye.
[5] SARGEM R&D Center, Konya Food and Agriculture University, 42080 Konya, Türkiye.
[6] Department of Food Processing, Food Agriculture and Livestock Vocational School , Burdur Mehmet Akif Ersoy University, 15030 Burdur, Türkiye.
[7] KIT-ARGEM R&D Center, Konya Food and Agriculture University, 42080 Konya, Türkiye.
[8] Medical School, Department of Medical Biology, Atilim University, 06830 Ankara, Türkiye.

*Corresponding author: msudagidan@gmail.com

https://doi.org/10.55251/jmbfs.9143

ARTICLE INFO

Received 28. 6. 2022
Revised 14. 12. 2022
Accepted 21. 12. 2022
Published 1. 2. 2023

Regular article

OPEN ACCESS

ABSTRACT

Tarhana is one of the traditional Turkish fermented food and it is served as a soup. In this study, bacterial microbiota and chemical properties (acidity, salt, and moisture content) of tarhana samples (n=96) were examined. The metagenomic analysis revealed that Firmicutes were the dominant phylum and Bacillaceae, Enterococcaceae, Paenibacillaceae, Enterobacteriaceae, and Clostridiaceae were the dominant bacterial families. In the samples, *Bacillus*, *Enterococcus*, and *Paenibacillus* were mostly identified at the genus level. Alpha diversity and evenness showed that sample 30 had the highest diversity collected from İzmir. Principal Coordinate Analysis was used to identify relationships of samples at different taxonomic levels and it was found that most of the samples were closely related at the phylum level. Chemical analysis indicated that the acidity of tarhana samples varied between 5.00% and 42.5%, moisture contents were 4.39–18.66% and salt values were from 0.32% to 6.64%. The results of this study extensively demonstrated the chemical properties and the dominant bacterial communities present in tarhana samples collected from different parts of Türkiye.

Keywords: Tarhana, Microbiota, Metagenomics, Next Generation Sequencing

INTRODUCTION

Tarhana is one of the traditional Turkish fermented healthy food containing essential minerals, nutrients, organic acids, vitamins, and amino acids **(Goncu & Çelik, 2020; Kivanc & Funda, 2017; O'Callaghan *et al*., 2019)**. It is produced by both traditional and industrial methods using different kinds of flour (wheat, or chickpea flour), homemade dairy products like yogurt and cream, local raw vegetables (tomato, onion, paprika, and pepper), and various spices (mint and pickling herb) **(Coşkun, 2014; Gok, 2021)**. Lactic acid bacteria (LAB) and yeasts from yogurt and baker form the acidic characteristic of tarhana **(Temiz & Tarakçı, 2017)**. In addition, exopolysaccharide (EPS) production by LAB improves food quality during fermentation process **(Şentürk *et al*., 2020)**. Bacterial species *Enterococcus durans*, *Lactobacillus acidophilus*, *Companilactobacillus alimentarius*, *Levilactobacillus brevis*, *Lacticaseibacillus casei*, *L. paracasei*, *Lactiplantibacillus plantarum*, *Lactococcus lactis*, *Leuconostoc mesenteroides*, and *Pediococcus* spp. were dominant in the microbiota of tarhana **(Settanni *et al*., 2011; Şimşek, Özel, & Çon, 2017)**. Spontaneous or natural fermentation takes place in tarhana production **(Tamang, 2010)**. Any starter cultures are not used in the traditional fermentation process. For this reason, the type and amounts of used ingredients shape the microbiota of tarhana during the fermentation process **(Soyuçok *et al*., 2021)**. The bacterial communities present in tarhana fermentation lead to the production of special flavor and aroma compounds by bacterial enzymatic like proteolytic, lipolytic or amylolytic activities to improve its sensory quality **(De Vuyst & Leroy, 2007)**. Moreover, a recent study was identified the starter potential of *L. plantarum*, *L. alimentarius*, and *L. brevis* species for industrial tarhana production **(Özel *et al*., 2020)**. *L. alimentarius* co-cultured with *Pichia kudriavzevii* can produce desired flavor compounds and they also showed starter culture potential **(Özdemir *et al*., 2018)**.
The diversity of used materials in its production affects the vitamin and mineral content of tarhana and ingredients such as yogurt and wheat improve the nutritional properties of tarhana **(Başlar, Özçelik, & Çalışkan, 2022)**. Besides yogurt plays an important role in strengthening the immune system and bones, it is an important nutritional source for human health with its high-quality amino acid and fatty acid profile. Wheat contributes to human health through it is consist of high amounts of dietary fiber, A and B group vitamins, and amino acids **(Altundağ, Kenger, & Ulu, 2020; Yörükoğlu & Dayısoylu, 2016)**. Tarhana have positive effects on human health. Especially hypolipidemic effect, increasing protein digestibility and bioavailability of minerals **(Gok, 2021)**. In addition, tarhana serves important source of quercetin, a dietary flavonoids, having potential in the prevention of chronic diseases including cardiovascular and neurodegenerative diseases and cancer **(D'Andrea 2015; Lesjak *et al*., 2018)**. A recent study by **Tarakci, Erdem, & Dumen (2022)** indicated that tarhana combined with kefir led to weight loss in obese Wistar albino rats with improving intestinal microbiota by increasing LAB. This study was aimed to identify the microbiota of tarhana samples collected from different parts of Türkiye by Next Generation Sequencing (NGS) and metagenomic analysis. In addition, the chemical properties of tarhana samples based on acidity, moisture, and salt content were analyzed.

MATERIALS AND METHODS

Samples

Dried tarhana samples (n = 96) were collected from different locations in Türkiye **(Table 1)** in original packages and they were stored at +4 °C for a week until molecular and chemical analysis. Regional differences were seen in tarhana samples due to production techniques and used raw materials **(Figure 1)**.

DNA extraction from tarhana samples

Total DNA extraction from pre-enriched tarhana samples was performed by the phenol/chloroform/isoamyl alcohol method **(Liu *et al*., 2004)**. For this purpose, 1 g tarhana was added to 9 mL buffered peptone water (Oxoid, Basingstoke, UK) and incubated at 35 °C for 24 h with shaking at 200 rpm in a shaker incubator (IKA KS 4000ic, Germany). Then, 1 mL pre-enriched culture was centrifuged at 10,000 rpm for 5 min. The obtained pellet was dissolved in 0.5 ml 1xTE buffer (10 mM Tris-HCl and 1 mM EDTA, pH 8.0) including 4 mg/mL lysozyme (A3711,

Applichem, Germany) and 10 µg/mL lysostaphin (L7386, Sigma Aldrich Co., St. Louis, MO, USA). After that, the tubes were incubated at 37 °C overnight. For each sample, 20 µl proteinase K (P2308, Sigma Aldrich Co., St. Louis, MO, USA) and 250 µL sodium dodecyl sulfate (10%, w/v) (Sigma Aldrich Co., St. Louis, MO, USA) were added and the tubes were incubated at 56 °C for 2 h in a water bath. At the end of incubation period, 1 volume of the phenol/chloroform/isoamyl alcohol (25:24:1, VWR Amresco, USA) (~800 µl) was added and centrifuged at 14,000 rpm for 15 min at room temperature. Upper phase was collected in a new tube and 150 µL 5M NaCl (Sigma Aldrich Co., St. Louis, MO, USA) and 1 volume of 2-propanol were added. After mixing by a pipette, the tubes were centrifuged at 14,000 rpm for 10 min. The obtained DNA pellet was washed two-times with 70% (v/v) ethanol and then dried in a dry block. Finally, DNA was dissolved in 100 µL sterile ultrapure water. Total extracted DNA samples were quantitated in Take3 plate of a microplate reader (Epoch-2, BioTek, USA) at 260/280 nm. The DNA samples were stored at -20 °C until amplicon PCR.

Figure 1 The shape and color of tarhana samples collected from different cities of Türkiye

NGS and metagenomic analysis

16S rRNA V3–V4 gene region amplicon sequencing was carried out as described previously **(Sudagidan *et al.*, 2021)**. DNA sequencing was performed in the iSeq100 system (Illumina, San Diego, California, USA) using i1 reagent cartridge V1 with a pair-end read of 151 bp. An Operational Taxonomic Unit (OTU) was used to determine the sequence identity of clustered sequences. The obtained raw sequencing data was analyzed using 16S Metagenomics, Version: 1.1.0 software (Illumina) with RefSeq Ribosomal Database Project (RDP) 16S v3 May 2018 DADA2 32 bp taxonomical interference and RDP Classifier in order to identify bacterial communities present in the microbiota of tarhana samples **(Wang *et al.*, 2007)**. Taxonomic distributions at different levels were determined based on the sequence reads. Alpha diversity (Shannon species diversity index) and evenness with the number of identified species were also specified. Furthermore, Principal Coordinate Analysis (PCoA) were carried out using 16S Metagenomics (Version: 1.1.0) software.

Table 1 The collection places of powder tarhana samples from different parts of Türkiye

Sample ID	Collection place
2	Trabzon-Akçaabat
3	Trabzon
4	Samsun
5	Konya
6	Konya
7	Konya-Beyşehir
8	Konya-Obruk
9	Kahramanmaraş
10	Balıkesir
11	Istanbul
14	İzmir
15	Konya
16	Beypazarı
17	Malatya
18	Silifke
19	Hatay
20	Hatay
21	Hatay
22	Uşak
23	Uşak
25	Uşak
29	İzmir-Karşıyaka
30	İzmir-Karşıyaka
31	İzmir-Karşıyaka
32	İzmir-Foça
34	İzmir-Foça
36	Manisa-Demirci
37	İzmir-Selçuk-Şirince
38	İzmir-Selçuk-Şirince
39	İzmir-Selçuk-Şirince
40	İzmir-Selçuk-Şirince
41	İzmir-Selçuk
42	İzmir-Selçuk
43	İzmir-Selçuk
44	İzmir-Selçuk
45	İzmir-Selçuk
46	Manisa-Kula
47	Manisa-Kula
48	Kütahya-Gediz
49	Uşak-Eşme
50	Isparta-İslamköy
51	Isparta-İslamköy
54	Isparta
55	Denizli
56	Denizli
57	Denizli
58	Denizli
59	Denizli
60	Denizli
61	Kahramanmaraş
62	Antalya-Korkuteli
63	Konya
64	Amasya
65	Aydın
66	Konya-Kadınhanı
67	Karaman-Ermenek
68	Erzincan
69	Konya
71	Sivas
72	Karaman-Ermenek
73	Kahramanmaraş
74	Beypazarı
76	Konya-Başarakavak
78	Uşak
79	Adapazarı
80	Konya-Seydişehir
81	Konya-Derbent
82	Artvin
83	Kayseri
84	Kayseri
85	Kayseri
86	Ankara
87	Kayseri
88	Kayseri
89	Kayseri
90	Kayseri
91	Kayseri
92	Kayseri
95	Mersin-Bozyazı
96	Çanakkale
98	Burdur
100	Adapazarı

101	Burdur
102	Çankırı
103	Elazığ
104	Mersin
105	Isparta
106	Bingöl
107	Kahramanmaraş
108	Adana
110	Nevşehir-Ürgüp
111	Manisa
112	Manisa
113	İzmir-Karşıyaka
114	İzmir
115	Manisa-Kula

Chemical analysis

Acidity

The acidity of tarhana samples (10 g) was measured after vigorously mixing with 50 ml of 67% (v/v) neutralized ethanol and it was filtered via Whatman® qualitative filter paper (Grade 4). The mixture (10 mL) was titrated with 0.1 M NaOH and the consumption of NaOH was multiplied by the dilution factor of 5. The obtained result was expressed as lactic acid equivalent of acidity (S°) **(Anonymous, 2004)**.

Salt content

NaCl salt content of tarhana samples (5 g) was analyzed after homogenization of the samples with hot water and to total volume was completed to 250 mL with cold water. Then, 20 mL from the upper clear part was titrated with 0.1 M $AgNO_3$ (Sigma Aldrich). The results were measured as g salt per 100 g of dry matter **(Anonymous, 2010)**.

Moisture content (dry matter)

In dry matter measurement, tarhana sample (2 g) in a glass petri dish was stored at 105 °C until constant weighing **(AOAC, 1990)**. Dry matter was expressed as g dry matter per 100 g tarhana sample.

Statistical analysis

Acidity, moisture, and salt content measurements were carried out in triplicates. All results were statistically assessment using Minitab® version 19.1.1 (Coventry, UK) software with the ANOVA test. Statistically differences ($p<0.05$) between samples were determined using Tukey multiple comparison test. The results were given as mean ± standard deviation. Furthermore, the result of mean value, standard error, minimum value, maximum value, and p-value of each parameter was given in **Table 2** and **Table 3**.

Table 2 Statistical parameters of chemically examined tarhana samples

	n	Mean	SE Mean	Minimum	Maximum	p-value
Acidity	96	19,930	0,545	5,00	42,5	0,007
Moisture	96	10,561	0,142	4,39	18,66	0,035
NaCl (%)	96	1,8872	0,066	0,32	6,64	0,028

Table 3 Chemical properties of tarhana samples based on acidity, moisture, and salt (NaCl) contents

Sample ID	Acidity %	Moisture %	Salt, NaCl %
2	8,00±1,68^{rs}	9,64±1,06^{g-s}	0,32±0,04^{aj}
3	17,00±3,57^{f-s}	4,39±0,48^{t}	3,5±0,42^{de}
4	19,00±3,99^{e-s}	7,46±0,82^{rst}	0,88±0,11^{ab-aj}
5	5,00±1,05^{s}	9,45±1,04^{i-s}	1,56±0,19^{n-ag}
6	17,00±3,57^{f-s}	9,83±1,08^{f-s}	1,42±0,17^{q-ai}
7	11,00±2,31^{p-s}	8,32±0,92^{t}	1,5±0,18^{o-ah}
8	20,50±4,31^{d-s}	9,64±1,06^{n-s}	1,15±0,14^{u-aj}
9	19,50±4,10^{e-s}	9,47±1,04^{g-s}	1,5±0,18^{o-ah}
10	10,50±2,21^{p-s}	10,5±1,16^{f-s}	1,2±0,14^{t-aj}
11	24,50±5,15^{b-q}	7,64±0,84^{rst}	6,64±0,80^{a}
14	27,50±5,78^{a-o}	7,25±0,80st	4,66±0,56^{bc}
15	12,00±2,52^{n-s}	9,48±1,04^{h-s}	3,88±0,47^{cd}
16	25,00±5,25^{b-q}	13,83±1,52^{b-g}	1,68±0,20^{m-ae}
17	28,00±5,88^{a-n}	8,09±0,89^{p-t}	0,74±0,09^{af-aj}
18	29,00±6,09^{a-m}	10,32±1,14^{f-s}	2,42±0,29^{g-o}
19	30,50±6,41^{a-j}	8,51±0,94^{m-t}	1,68±0,20^{m-ae}
20	16,50±3,47^{g-s}	9,05±1,00^{k-s}	1,39±0,17^{r-ai}
21	21,00±4,41^{d-s}	10,56±1,16^{e-s}	1,3±0,16^{s-ai}
22	36,00±7,56^{a-d}	9,25±1,02^{i-s}	2,28±0,27^{h-r}
23	30,00±6,30^{a-k}	10,48±1,15^{f-s}	3,44±0,41^{def}
25	30,00±6,30^{a-k}	10,7±1,18^{d-s}	3,01±0,36^{d-h}
29	11,00±2,31^{p-s}	11,33±1,25^{d-s}	5,28±0,63^{b}
30	10,50±2,21^{p-s}	8,01±0,88^{p-t}	2,51±0,30^{f-m}
31	11,50±2,42^{o-s}	7,19±0,79st	2,45±0,29^{g-n}
32	17,00±3,57^{f-s}	11,31±1,24^{d-s}	1,26±0,15^{t-ai}
34	15,50±3,26^{i-s}	13,36±1,47^{b-i}	2,03±0,24^{i-w}
36	12,00±2,52^{n-s}	11,41±1,26^{d-s}	5,06±0,61^{b}
37	13,00±2,72^{m-s}	12,06±1,33^{c-q}	0,75±0,09^{ae-aj}
38	21,50±4,51^{c-r}	12,79±1,41^{b-l}	0,79±0,09^{ac-aj}
39	14,00±2,94^{k-s}	12,36±1,36^{b-o}	0,64±0,08^{ag-aj}
40	16,50±3,47^{g-s}	11,37±1,25^{d-s}	1,14±0,14^{u-aj}
41	21,00±4,41^{d-s}	14,84±1,63^{a-d}	1,84±0,22^{k-aa}
42	20,50±4,31^{d-s}	9,28±1,02^{i-s}	3,59±0,43^{de}
43	17,00±3,57^{f-s}	12,01±1,32^{c-q}	1,92±0,23^{j-y}
44	20,00±4,2^{d-s}	11,29±1,24^{d-s}	1,71±0,21^{m-ac}
45	17,50±3,68^{f-s}	11,52±1,27^{d-r}	1,33±0,16^{s-ai}
46	20,50±4,31^{d-s}	18,66±2,05^{a}	2,33±0,28^{h-q}
47	29,50±6,19^{a-l}	10,84±1,19^{d-s}	1,2±0,14^{t-aj}
48	20,25±4,25^{d-s}	14,77±1,62^{a-e}	2,69±0,32^{e-l}
49	25,00±5,25^{b-q}	10,45±1,15^{f-s}	1,97±0,24^{i-y}
50	25,00±5,25^{b-q}	11,06±1,22^{d-s}	1,51±0,18^{o-ag}
51	12,25±2,57^{n-s}	11±1,21^{d-s}	1,8±0,22^{l-ab}
54	10,50±2,21^{p-s}	9,2±1,01^{i-s}	1,13±0,14^{u-aj}
55	34,50±7,25^{a-e}	11,2±1,23^{d-s}	1,12±0,13^{v-aj}
56	36,00±7,56^{a-d}	10,97±1,21^{d-s}	0,98±0,12^{z-aj}
57	26,50±5,57^{a-p}	16,16±1,78^{abc}	2,79±0,33^{e-j}
58	32,50±6,83^{a-g}	13,96±1,54^{b-f}	0,97±0,12^{z-aj}
59	39,00±8,19^{ab}	16,34±1,80^{ab}	2,75±0,33^{e-k}
60	31,50±6,62^{a-i}	10,93±1,20^{d-s}	1,71±0,21^{m-ac}
61	20,50±4,31^{d-s}	8,04±0,88^{p-t}	1,43±0,17^{p-ai}
62	21,00±4,41^{d-s}	13,33±1,47^{b-j}	2,8±0,34^{e-j}
63	25,00±5,25^{b-q}	10,03±1,10^{f-s}	2,05±0,25^{i-v}
64	14,50±3,05^{j-s}	9,4±1,03^{i-s}	1,05±0,13^{y-aj}
65	18,00±3,78^{f-s}	10,28±1,13^{f-s}	0,55±0,07^{aiaj}
66	28,50±5,99^{a-p}	10,01±1,10^{f-s}	1,84±0,22^{k-aa}
67	15,50±3,26^{i-s}	8,87±0,98^{k-s}	1,7±0,20^{m-ad}
68	22,00±4,62^{c-r}	12,69±1,40^{b-m}	1,58±0,19^{m-af}
69	19,50±4,1^{e-s}	11,07±1,22^{d-s}	1,58±0,19^{m-af}
71	25,00±5,25^{b-q}	9,99±1,10^{f-s}	2,77±0,33^{e-k}
72	19,50±4,10^{e-s}	9,41±1,04^{i-s}	1,2±0,14^{t-aj}
73	17,50±3,68^{f-s}	8,88±0,98^{k-s}	2,22±0,27^{h-s}
74	25,00±5,25^{b-q}	12,93±1,42^{b-k}	3,02±0,36^{d-h}
76	33,00±6,93^{a-f}	12,57±1,38^{b-m}	2,76±0,33^{e-k}
78	13,00±2,73^{m-s}	9,34±1,03^{i-s}	1,14±0,14^{u-aj}
79	10,50±2,21^{p-s}	9,21±1,01^{i-s}	1,17±0,14^{u-aj}
80	20,50±4,31^{d-s}	11,23±1,24^{d-s}	1,24±0,15^{t-aj}
81	9,50±2^{qrs}	8,64±0,95^{l-s}	2,02±0,24^{i-x}
82	25,00±5,25^{b-q}	12,57±1,38^{b-m}	1,23±0,15^{t-aj}
83	42,50±8,93^{a}	7,92±0,87^{q-t}	1,49±0,18^{o-ah}
84	7,50±1,58^{rs}	9,21±1,01^{i-s}	1,15±0,14^{u-aj}
85	14,50±3,05^{j-s}	8,1±0,89^{p-t}	1,32±0,16^{s-ai}
86	25,00±5,25^{b-q}	8,87±0,98^{k-s}	0,97±0,12^{z-aj}
87	18,00±3,78^{f-s}	9,64±1,06^{g-s}	1,19±0,14^{u-aj}
88	5,00±1,05^{s}	8,88±0,98^{k-s}	1,15±0,14^{u-aj}
89	10,75±2,26^{p-s}	8,94±0,98^{k-s}	0,69±0,08^{af-aj}
90	20,50±4,31^{d-s}	9,45±1,04^{i-s}	0,57±0,07^{ah-aj}
91	6,50±1,37^{rs}	9,14±1,01^{i-s}	1,09±0,13^{x-aj}
92	7,00±1,47^{rs}	9,15±1,01^{i-s}	0,94±0,11^{aa-aj}
95	14,00±2,94^{k-s}	10,53±1,16^{f-s}	0,77±0,09^{ad-aj}
96	21,00±4,41^{d-s}	12,61±1,39^{b-m}	1,85±0,22^{k-aa}
98	25,00±5,25^{b-q}	9,51±1,05^{h-s}	2,71±0,33^{e-l}
100	16,00±3,36^{h-s}	13,71±1,51^{b-h}	0,52±0,06^{aiaj}
101	32,00±6,72^{a-h}	12,47±1,37^{b-n}	2,36±0,28^{h-p}
102	16,00±3,36^{h-s}	9,1±10^{j-s}	3,82±0,46^{cd}
103	10,50±2,21^{p-s}	12,2±1,34^{b-p}	2,06±0,25^{i-u}
104	36,00±7,56^{a-d}	12,79±1,41^{b-l}	1,11±0,13^{w-aj}
105	37,50±7,88^{abc}	13,04±1,43^{b-k}	2,38±0,29^{h-o}
106	7,00±1,47^{rs}	12,45±1,37^{b-n}	0,98±0,12^{z-aj}
107	25,00±5,25^{b-q}	8,31±0,91^{n-t}	1,88±0,23^{j-z}
108	7,50±1,58^{rs}	10,55±1,16^{e-s}	2,87±0,34^{e-i}
110	19,00±3,99^{e-s}	8,5±0,94^{m-t}	2,23±0,27^{h-s}
111	15,50±3,26^{i-s}	8,19±0,90^{o-t}	1,58±0,19^{m-af}
112	17,00±3,57^{f-s}	12,24±1,35^{b-p}	0,98±0,12^{z-aj}
113	12,50±2,63^{n-s}	8,3±0,91^{n-t}	2,13±0,26^{h-t}
114	13,50±2,84^{l-s}	11,06±1,22^{d-s}	2,28±0,27^{h-r}
115	31,00±6,51^{a-i}	11,01±1,21^{d-s}	3,35±0,40^{d-g}

a-aj (↓): The difference between the means shown with different letters within a column is statistically significant ($p<0.05$).

RESULTS AND DISCUSSION

Metagenomic analysis of tarhana microbiota

NGS is a culture-independent method and it is successfully applied to identify the microbiota of fermented foods **(Demirci *et al*., 2022; Ucak *et al*., 2022; Soyuçok *et al*., 2021; Yegin *et al*., 2022)**. In this study, a total of 459,430 high-quality reads were obtained by NGS. Metagenomic analysis was performed from the reads to identify bacterial communities present in tarhana microbiota from the kingdom to the species level. All samples were belong to Bacteria kingdom. Firmicutes were determined as the dominant phylum **(Figure 2)**. In the samples 7, 15, 84, 88, 103, and 112, the Proteobacteria population was higher than Firmicutes. Moreover, in the samples 14, 91, 108, and 113, Proteobacteria was identified with high read numbers **(Figure 2)**.

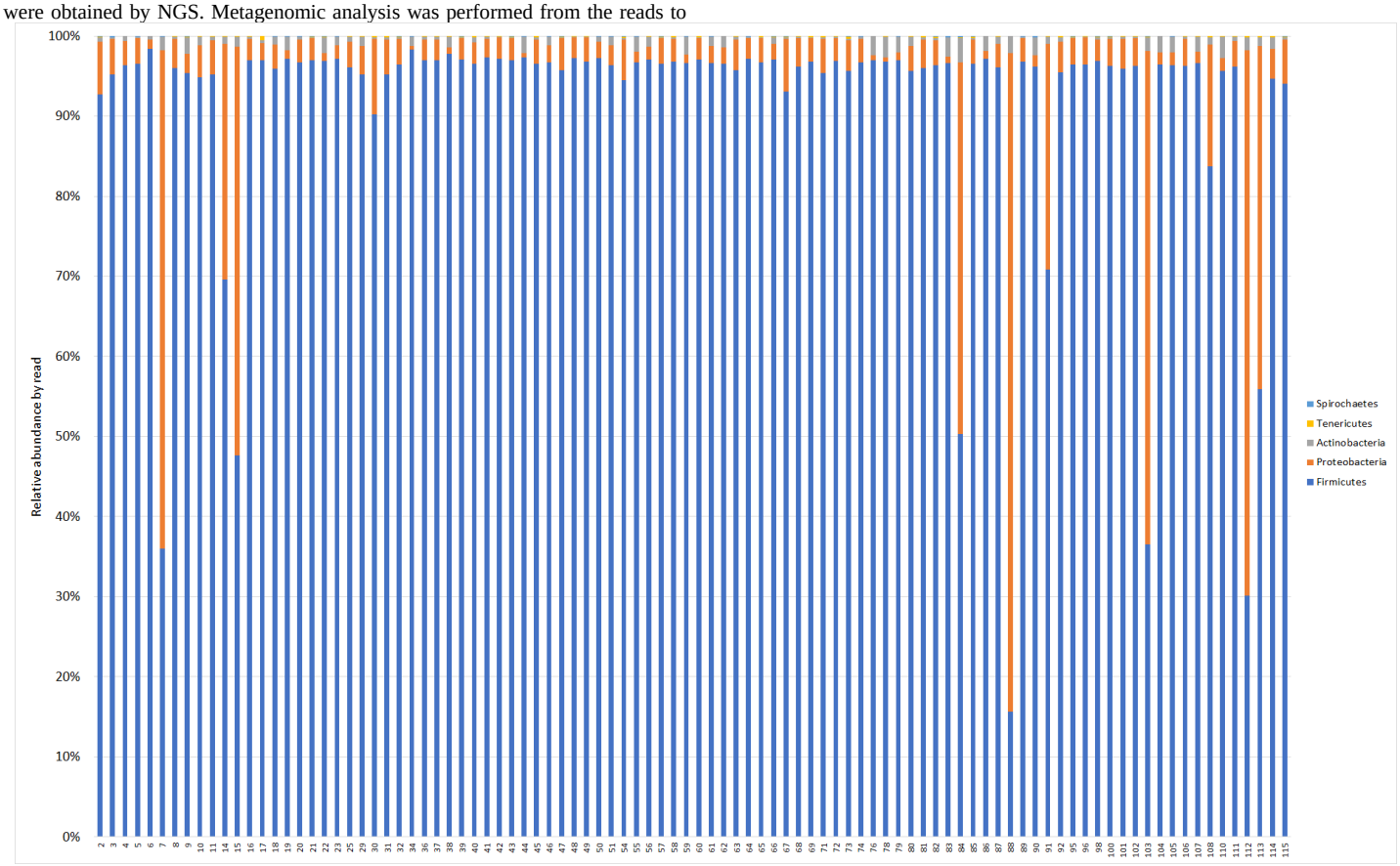

Figure 2 Bacterial community structure of tarhana microbiota at the phylum level

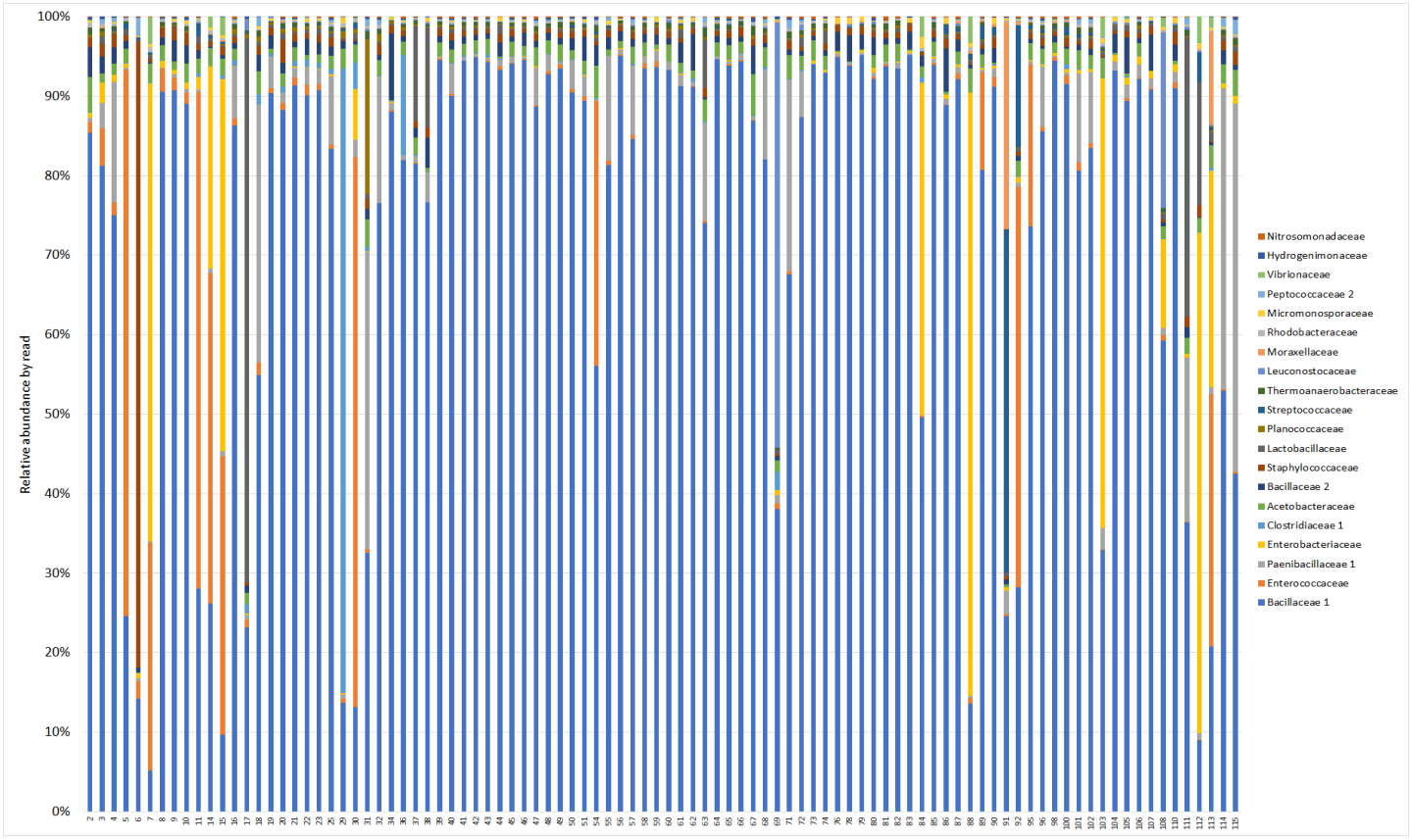

Figure 3 Identified the top 20 bacterial families in tarhana samples by metagenomic analysis

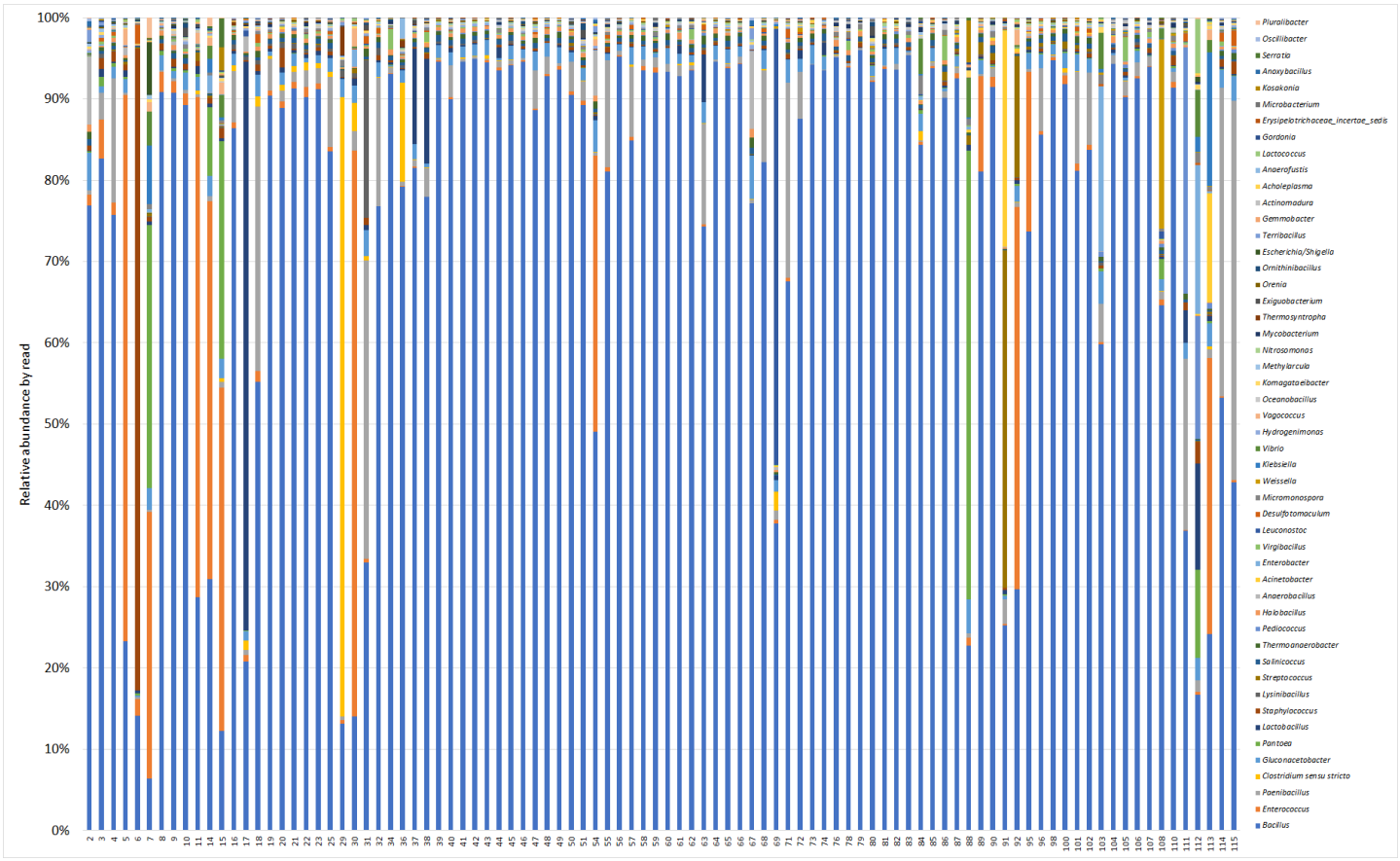

Figure 4 The top 50 identified bacterial genera in tarhana microbiota

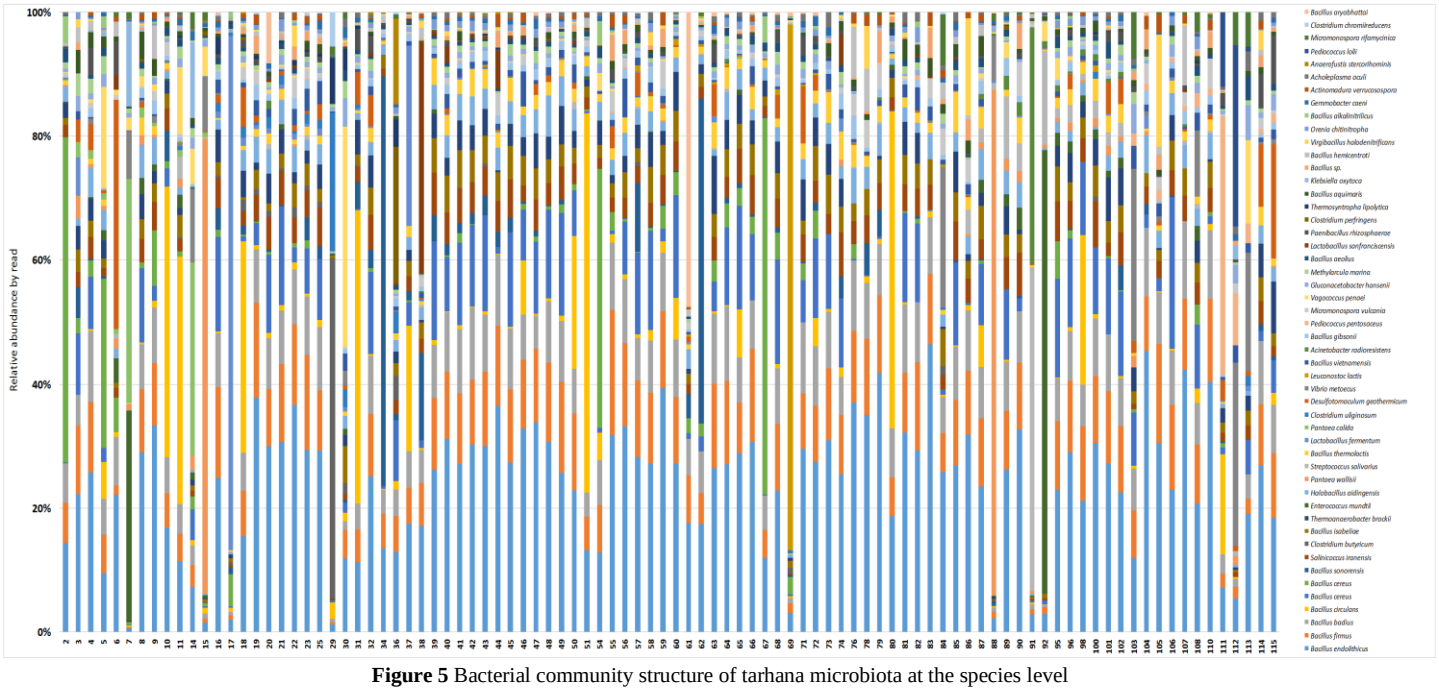

Figure 5 Bacterial community structure of tarhana microbiota at the species level

The presence of Actinobacteria, Tenericutes, and Spirochaetes bacterial phyla was less abundant. At the family level taxonomic analysis of tarhana samples demonstrated that Bacillaceae, Enterococcaceae, Paenibacillaceae, Enterobacteriaceae, and Clostridiaceae were the top five dominant bacteria **(Figure 3)**. Lactobacillaceae was dominant in the samples 17, 37, 38, 111, and 112 which are collected from Malatya, İzmir, İzmir, Manisa, and Manisa, respectively. Furthermore, Staphylococcaceae was found to be dominant in sample 6 collected from Konya. *Bacillus*, *Enterococcus*, and *Paenibacillus* were the top three bacterial communities at the genus level **(Figure 4)**. Moreover, *Clostridium sensu stricto* was found to be dominant in the samples 29 and 36 collected from İzmir and Manisa, respectively. **Yucel Sengun *et al.* (2009)** have been previously examined the presence of LAB in tarhana samples and their results showed that *Pediococcus acidilactici* (27%) were dominant in the samples. Moreover, *Streptococcus thermophilus*, *L. fermentum*, and *E. faecium* were also identified with 19-12% abundance.

Our results demonstrated that *Bacillus* was dominant in almost all of the tarhana samples (79/96) at the genus level. The dominant *Bacillus* species were *Bacillus endolithicus*, *B. firmus*, *B. badius*, *B. circulans*, *B. cereus*, and *B. sonorensis* **(Figure 5)** and all were endospore formers. They can survive in dried tarhana samples with low moisture values. Shelf life dried powder tarhana samples vary from 1 year to 2 years because it is not hygroscopic. Its composition do not change due to low moisture content **(Kaya, Ibanoglu, & Kaya, 1999)**. **Uçar & Çakıroğlu (2011)** examined microbiological quality of 20 tarhana samples collected from Ankara-Türkiye by culture-dependent methods and their results indicated that *B. cereus* (10^2–10^3 cfu/g) and *Staphylococcus* spp. (10^2–3.8×10^3 CFU/g) were present in the samples.

In this study, the tarhana samples were pre-enriched in buffered peptone water. Because in directly studied food samples especially containing low bacterial counts and dried foods, frequent amplification of bacterial 16S rRNA from extracted total DNA samples could not be achieved. This pre-enrichment process can lead to growing of fastidious bacteria, but in this issue, we compare the dominant bacterial communities present in tarhana samples collected from different parts of Türkiye. According to our experience, without pre-enrichment, amplification of bacterial DNA is sometimes difficult in dried fermented foods.

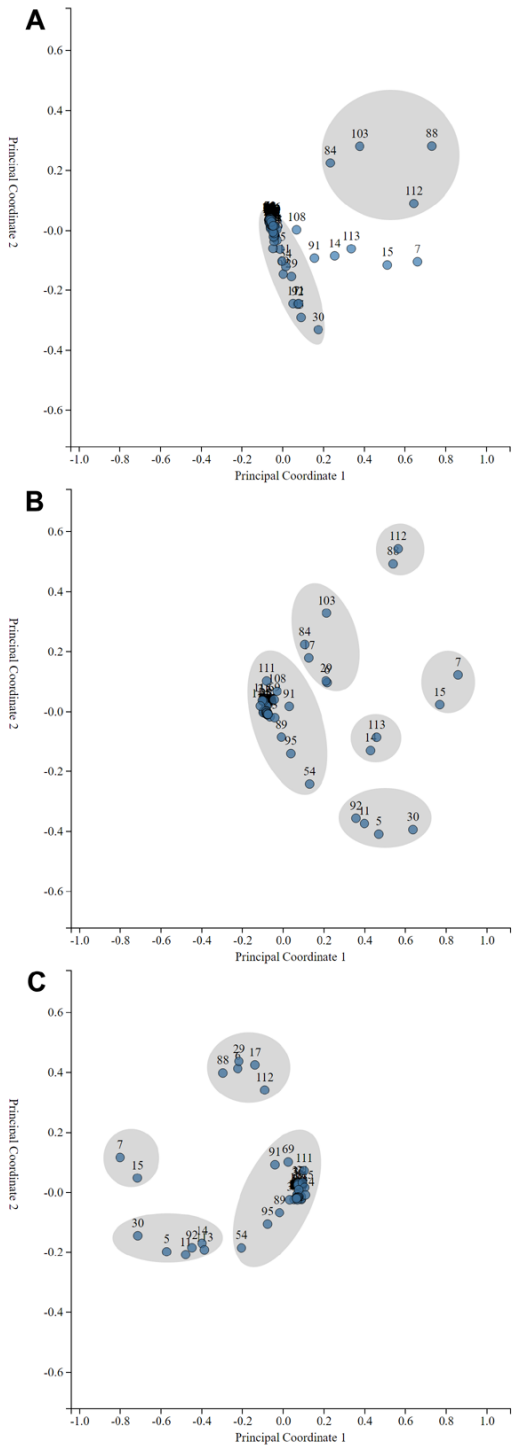

Figure 6 PCoA plot of tarhana samples at the phylum level (A), at the family level (B), and at the genus level (C)

Bacterial diversities

Alpha diversity (Shannon species diversity index) and evenness values were used to compare tarhana samples with respect to their bacterial richness in their microbiota. The lowest evenness value shows the highest diversity and among tarhana samples, the samples 30 collected from İzmir showed the highest diversity. The lowest diversity was recorded in sample 7 collected from Konya. The number of identified species also represents the richness of bacterial communities. The highest number of identified species was found in sample 31 collected from İzmir and the lowest one was the sample 73 from Kahramanmaraş **(Table 4)**.

Table 4 Shannon species diversity index, the number of identified species and evenness diversity values of tarhana samples after metagenomic analysis

Sample ID	Shannon Species Diversity Index Values	Number of Identified Species	Evenness
2	1.330	80	0.304
3	0.921	42	0.246
4	0.874	93	0.193
5	0.696	68	0.165
6	0.440	43	0.117
7	1.741	114	0.368
8	0.697	58	0.172
9	0.896	75	0.208
10	1.007	92	0.223
11	0.696	98	0.152
14	0.887	99	0.193
15	1.180	120	0.246
16	0.711	68	0.169
17	1.157	42	0.310
18	1.055	86	0.237
19	0.790	98	0.172
20	0.717	65	0.172
21	0.673	70	0.158
22	0.771	76	0.178
23	0.788	91	0.175
25	0.715	81	0.163
29	1.440	94	0.317
30	0.540	117	0.113
31	1.184	171	0.230
32	0.720	105	0.155
34	1.353	92	0.299
36	0.987	112	0.209
37	0.978	71	0.229
38	1.066	84	0.241
39	0.648	77	0.149
40	0.634	103	0.137
41	0.695	95	0.153
42	0.632	72	0.148
43	0.626	96	0.137
44	0.761	106	0.163
45	0.631	104	0.136
46	0.721	92	0.159
47	0.667	54	0.167
48	0.597	69	0.141
49	0.610	63	0.147
50	0.755	81	0.172
51	1.119	104	0.241
54	1.050	90	0.233
55	0.716	88	0.160
56	0.692	86	0.155
57	0.738	69	0.174
58	0.626	68	0.148
59	0.691	62	0.167
60	0.699	56	0.174
61	1.024	44	0.271
62	1.111	82	0.252
63	0.844	64	0.203
64	0.590	59	0.145
65	0.704	74	0.164
66	0.621	75	0.144
67	1.339	77	0.308
68	0.672	62	0.163
69	1.181	40	0.320
71	0.696	89	0.155
72	0.681	65	0.163
73	0.600	35	0.169
74	0.722	42	0.193
76	0.778	64	0.187
78	0.814	66	0.194
79	0.640	46	0.167
80	0.987	60	0.241
81	0.675	53	0.170
82	0.681	53	0.172
83	0.704	47	0.183
84	0.790	83	0.179
85	0.647	50	0.165
86	0.869	67	0.207

87	0.815	69	0.192
88	1.202	57	0.297
89	0.631	57	0.156
90	0.753	63	0.182
91	1.286	74	0.299
92	1.274	66	0.304
95	0.640	75	0.148
96	0.638	76	0.147
98	0.751	76	0.173
100	0.713	49	0.183
101	0.659	63	0.159
102	0.713	92	0.158
103	0.732	70	0.172
104	0.724	47	0.188
105	0.802	67	0.191
106	0.628	58	0.155
107	0.842	56	0.209
108	0.684	68	0.162
110	0.749	60	0.183
111	1.289	96	0.282
112	1.079	89	0.240
113	0.914	82	0.207
114	0.846	119	0.177
115	0.679	79	0.155

Beta diversity PCoA results demonstrated that the microbiota of most of the tarhana samples was closely related at the phylum level **(Figure 6A).** Only the samples 84, 88, 103 and 112 were separated from the other samples. At the family level analysis of PCoA showed that there was the main group and the other tarhana samples were separated into five sub-groups **(Figure 6B)**. Samples 88 and 112 were distinguished from the others at the family level. Similarly, most of the samples were closely related at the genus level PCoA and the other samples were separated into three sub-groups. The most distinguished samples were 7 and 15, and in another group 6, 17, 29, 88 and 112 **(Figure 6C)**. Technological properties of tarhana have been improved using especially EPS-producing *Lactiplantibacillus plantarum* strains by increasing 2-fold higher consistency index value than in the absence of EPS-producing strains **(Taşdelen & Şimşek, 2021)**. The increasing amount of yogurt in tarhana fermentation leads to increase in total LAB while influencing acidity of the final product **(Ozdemir, Gocmen, & Yildirim Kumral, 2007)**.

Chemical properties of tarhana samples

Spontaneous fermentation of tarhana leads to compositional changes in tarhana sourdough by the addition of fresh mixture of vegetable pulps containing both microorganisms and enzymes **(İçen, Karakaş-Budak, & Certel, 2021)**. The activity of either plant or animal based microorganisms and enzymes influences pH, acidity, and sensory properties of tarhana. Especially production of acids by LAB during fermentation process of tarhana decrease pH **(Çelik, Işık, & Yılmaz, 2010)**. In our study, chemical properties of tarhana samples were analyzed according to Turkish Standards **(Anonymous 2004; Anonymous 2010)** and the American Association of Cereal Chemists **(AOAC, 1990)**. The statistical parameters of tarhana samples were shown in **Table 3**. Differences in acidity, moisture, and salt content were significant ($p<0.05$). It was also determined that the acidity of tarhana samples varied between 5.00–42.5%, moisture content of 4.39–18.66%, and salt values between 0.32–6.64%. According to the TS 2282 tarhana standard, it is stated that acidity, moisture, and salt should be at most 40%, 10%, and 10%, respectively. In addition, **Erbaş, Certel, & Uslu (2005)** reported the chemical composition of tarhana: 38.95% dry matter, 16.79% protein, and 6.48% salt content. **Karagozlu, Ergonul, & Karagozlu (2008)** also showed that the water content of instant tarhana was 5.8±0.66% and pH value was 4.19±0.04. Our results demonstrated that the acidity values of all groups, except for sample 2, were appropriated to TS 2282 tarhana standard. Moisture results of 52 samples were higher than 10%, whereas the salt values determined in our study were within the limits specified in the tarhana standard. The mean acidity values of tarhana samples were found to be higher in Denizli among the provinces. It was determined that the humidity and salt averages were at similar levels in all provinces. The differences in the production techniques, fermentation time, used local raw materials and microbial communities present in the raw materials were explained to have an effect on the chemical properties of tarhana **(Kivanc & Funda, 2017; Koç & Özçıra, 2019; Tamang, 2010)**. High acidity and low moisture content of tarhana prevents growth of foodborne pathogens like *Escherichia coli*, coliforms and *Salmonella* spp. **(Uçar & Çakıroğlu, 2011)**. We discussed the situation as mentioned in our previous study and our study revealed that some pathogens were inhibited at the end of the 21st day via high acidity of the drying process **(Soyuçok *et al.*, 2021)**. In this study, a direct relationship between chemical properties and bacterial community structure was not found. For example, tarhana samples with high acidity values (83, 59, 56, and 22) showed similar bacterial microbiota with the others. The acidity and moisture content of tarhana occurred according to the regional taste and consumer, however, it has been found that the amount of salt is generally similar even in different regions, since the salt content affects product acceptability.

CONCLUSION

The results of this study indicated that the bacterial microbiota of tarhana samples from different regions of Türkiye contains mostly Firmicutes phylum, Bacillaceae family, and *Bacillus* genus. In the production and drying process of tarhana, it could be contaminated by environmental *Bacillus* spores. By performing a culture-independent NGS method and a metagenomic analysis of 16S rRNA amplicon reads, for the first time in Türkiye, a study was carried out using so many tarhana samples. The obtained results could be helpful for the food industry to select potential starter cultures for industrial tarhana production.

Acknowledgements: The bacterial 16S rRNA gene amplicon sequencing data generated in this study using NGS was submitted to NCBI Sequence Read Archive (SRA) database with the BioProject accession number PRJNA817420. Authors thank to Kit-ARGEM R&D Center Konya Food and Agriculture University for providing lab facilities.
Conflict of Interest: The authors declare no conflict of interest.
CRediT author statement: Esra Ersoy Omeroglu, Özge Can, Şevval Nur Temiz, Rabia Al, Osman Altunbas, Ali Soyuçok, and Mediha Nur Zafer Yurt: Methodology; **Veli Cengiz Ozalp**: Project administration, Writing—Review & Editing; **Mert Sudagidan**: Conceptualization, Methodology, Investigation, Writing—Original Draft.

REFERENCES

Altundağ, Ö. Ö., Kenger, E. B., & Ulu, E. K. (2020). Farklı tarhana türlerinin sağlık yönünden değerlendirilmesine yönelik bir çalışma. *Sağlık Akademisi Kastamonu*, *5*(2), 143-157.
Anonymous (2004). TS 2282. Tarhana Standard, Turkish Standards Institution, Ankara, Türkiye.
Anonymous (2010). TS 5000. Bread Standard, Turkish Standards Institution, Ankara, Türkiye.
AOAC (1990). American Association of Cereal Chemists. Approved Methods of the AACC, 8th Ed., The Association, St. Paul, MN.
Başlar, M., Özçelik, G. N., & Çalışkan, H. (2022). A Comprehensive Review on Beyşehir Tarhana, A Turkish Traditional Food. *International Journal of Gastronomy Research*, *1*(1), 35-43.
Coşkun, F. (2014). History of Tarhana and varieties of Tarhana in Turkey. *Electronic Journal of Food Technologies*, *9*(3), 69-79.
Çelik, I., Işık, F., & Yılmaz, Y. (2010). Chemical, rheological and sensory properties of tarhana with wheat bran as a functional constituent. *Akademik Gıda*, *8*(3), 11-17.
D'Andrea, G. (2015). Quercetin: A flavonol with multifaceted therapeutic applications? *Fitoterapia*, *106*, 256-271. https://dx.doi.org/10.1016/j.fitote.2015.09.018
De Vuyst, L., & Leroy, F. (2007). Bacteriocins from lactic acid bacteria: Production, purification, and food applications. *Journal of Molecular Microbiology and Biotechnology*, *13*, 194-199. https://dx.doi.org/10.1159/000104752
Demirci, T., Akın, N., Öztürk, H. I., & Oğul, A. (2022). A metagenomic approach to homemade back-slopped yogurts produced in mountainous villages of Turkey with the potential next-generation probiotics. *LWT-Food Science and Technology*, *154*, 112860. https://dx.doi.org/10.1016/j.lwt.2021.112860
Erbaş, M., Certel, M., & Uslu, M. K. (2005). Microbiological and chemical properties of Tarhana during fermentation and storage as wet-sensorial properties of Tarhana soup. *LWT-Food Science and Technology*, *38*, 409-416. https://dx.doi.org/10.1016/j.lwt.2004.06.009
Gok, I. (2021). Functional potential of several Turkish fermented traditional foods: Biotic properties, bioactive compounds, and health benefits. *Food Reviews International*. https://dx.doi.org/10.1080/87559129.2021.1962340
Goncu, A., & Çelik, İ. (2020). Investigation of some properties of gluten-free tarhanas produced by red, green and yellow lentil whole flour. *Food Science and Technology (Campinas)*, *40*(Suppl.2), 574-581. https://dx.doi.org/10.1590/fst.34919
İçen, S., Karakaş-Budak, B., & Certel, M. (2021). Effect of sourdough starter and fungal proteases on gluten content and sensory properties of tarhana. *International Journal of Food Science and Technology*, *56*, 2557-2564. https://dx.doi.org/10.1111/ijfs.14895
Karagozlu, N., Ergonul, B., & Karagozlu, C. (2008). Microbiological attributes of instant tarhana during fermentation and drying. *Bulgarian Journal of Agricultural Sciences*, *14*(6), 535-541.
Kaya, S., Ibanoglu, S., & Kaya, A. (1999). Moisture sorption characteristics of Tarhana, a fermented Turkish cereal food. *Journal of Food Quality*, *22*, 95-100. https://dx.doi.org/10.1111/j.1745-4557.1999.tb00930.x
Kivanc, M., & Funda, E. G. (2017). A functional food: a traditional Tarhana fermentation. *Food Science and Technology (Campinas)*, *37*, 269-274. https://dx.doi.org/10.1590/1678-457x.08815

Koç, G. Ç., & Özçıra, N. (2019). Chemical composition, functional, powder, and sensory properties of tarhana enriched with wheat germ. *Journal of Food Science and Technology*, *56*, 5204-5213. https://dx.doi.org/10.1007/s13197-019-03989-y

Lesjak, M., Beara, I., Simin, N., Pintać, D., Majkić, T., Bekvalac, K., Orcic, D., & Mimica-Dukic, N. (2018). Antioxidant and anti-inflammatory activities of quercetin and its derivatives. *Journal of Functional Foods*, *40*, 68-75. https://dx.doi.org/10.1016/j. jff.2017.10.047

Liu, D., Ainsworth, A. J., Austin, F. W., & Lawrence, M. L. (2004). Use of PCR primers derived from a putative transcriptional regulator gene for species-specific determination of *Listeria monocytogenes*. *International Journal of Food Microbiology*, *91*, 297-304. https://dx.doi.org/10.1016/j.ijfoodmicro.2003.07.004

O'Callaghan, Y. C., Shevade, A. V., Guinee, T. P., O'Connor, T. P., & O'Brien, N. M. (2019). Comparison of the nutritional composition of experimental fermented milk:wheat bulgur blends and commercially available kishk and tarhana products. *Food Chemistry*, *278*, 110-118. https://dx.doi.org/10.1016/j.foodchem.2018.11.026

Ozdemir, S., Gocmen, D., & Yildirim Kumral, A. A. (2007). Traditional Turkish fermented cereal food: Tarhana. *Food Reviews International*, *23*(2), 107-121. https://dx.doi.org/10.1080/87559120701224923

Özdemir, N., Yazıcı, G., Şimşek, Ö., Özkal, S. G., & Çon, A. H. (2018). The effect of lactic acid bacteria and yeast usage on aroma development during tarhana fermentation. *Food Bioscience*, *26*, 30-37. https://dx.doi.org/10.1016/j.fbio.2018.09.004

Özel, B., Şimşek, Ö., Settanni, L., & Erten, H. (2020). The influence of backslopping on lactic acid bacteria diversity in tarhana fermentation. *International Journal of Food Microbiology*, *335*, 108886. https://dx.doi.org/10.1016/j.ijfoodmicro.2020.108886

Settanni, L., Tanguler, H., Moschetti, G., Reale, S., Gargano, V., & Erten, H. (2011). Evolution of fermenting microbiota in Tarhana produced under controlled technological conditions. *Food Microbiology*, *28*, 1367-1373. https://dx.doi.org/10.1016/j.fm.2011.06.008

Soyuçok, A., Yurt, M. N. Z., Altunbas, O., Ozalp, V. C., & Sudagidan, M. (2021). Metagenomic and chemical analysis of Tarhana during traditional fermentation process. *Food Bioscience*, *39*, 100824. https://dx.doi.org/10.1016/j.fbio.2020.100824

Sudagidan, M., Yurt, M. N. Z., Tasbasi, B. B., Acar, E. E., Ömeroğlu, E. E., Uçak, S., Ozalp, V. C., & Aydın, A. (2021). 16S bacterial metagenomic analysis of herby cheese (otlu peynir) microbiota. *Acta Veterinaria Eurasia*, *47*, 188-196. https://dx.doi.org/10.5152/actavet.2021.21037

Şentürk, D. Z., Dertli, E., Erten, H., & Şimşek, Ö. (2020). Structural and technological characterization of ropy exopolysaccharides produced by *Lactobacillus plantarum* strains isolated from Tarhana. *Food Science and Biotechnology*, *29*(1), 121-129. https://dx.doi.org/10.1007/s10068-019-00641-5

Şimşek, Ö., Özel, S., & Çon, A. H. (2017). Comparison of lactic acid bacteria diversity during the fermentation of Tarhana produced at home and on a commercial scale. *Food Science and Biotechnology*, *26*, 181-187. https://dx.doi.org/10.1007/s10068-017-0024-3

Tamang, J. P. (2010). Diversity of fermented foods. In JP Tamang and K Kailasapathy (Eds). Fermented foods and beverages of the World (pp. 41-84). London, UK: CRC Press.

Tarakci, N. G., Erdem, N. Z., & Dumen, E. (2022). Probiotic foods are effective on weight loss, biochemical parameters, and intestinal microbiota in Wistar Albino rats with obese microbiota. *International Journal of Clinical Practice*, *2022*, 4569100. https://dx.doi.org/10.1155/2022/4569100

Taşdelen, E., & Şimşek, Ö. (2021). The effects of ropy exopolysaccharide-producing *Lactiplantibacillus plantarum* strains on tarhana quality. *Food Bioscience*, *43*, 101282. https://dx.doi.org/10.1016/j.fbio.2021.101282

Temiz, H., & Tarakçı, Z. (2017). Composition of volatile aromatic compounds and minerals of tarhana enriched with cherry laurel (*Laurocerasus officinalis*). *Journal of Food Science and Technology*, *54*, 735-742. https://dx.doi.org/10.1007/s13197-017-2513-z

Ucak, S., Yurt, M. N. Z., Tasbasi, B. B., Acar, E. E., Altunbas, O., Soyucok, A., Aydin, A., Ozalp, V. C., & Sudagidan, M. (2022). Identification of bacterial communities of fermented cereal beverage Boza by metagenomic analysis. *LWT-Food Science and Technology*, *153*, 112465. https://dx.doi.org/10.1016/j.lwt.2021.112465

Uçar, A., & Çakıroğlu, F. P. (2011). Comparison of some chemical and microbiological quality of homemade tarhana in Ankara, Turkey. *Journal of Food Agriculture and Environment*, *9*(3&4), 34-37.

Wang, Q., Garrity, G. M., Tiedje, J. M., & Cole, J. R. (2007). Naïve bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Applied and Environmental Microbiology*, *73*(16), 5261-5267. https://dx.doi.org/10.1128/AEM.00062-07

Yegin, Z., Yurt, M. N. Z., Tasbasi, B. B., Acar, E. E., Altunbas, O., Ucak, S., Ozalp, V. C., & Sudagidan, M. (2022). Determination of bacterial community structure of Turkish kefir beverages via metagenomic approach. *International Dairy Journal*, *129*, 105337. https://dx.doi.org/10.1016/j.idairyj.2022.105337

Yucel Sengun, I., Nielsen, D. S., Karapinar, M., & Jakobsen, M. (2009). Identification of lactic acid bacteria isolated from Tarhana, a traditional Turkish fermented food. *International Journal of Food Microbiology*, *135*, 105-111. https://dx.doi.org/10.1016/j.ijfoodmicro.2009.07.033

Yörükoğlu, T. & Dayısoylu, K.S. (2016). Yöresel Maraş Tarhanasının Fonksiyonel ve Kimyasal Bazı Özellikleri. *Atatürk Üniversitesi Ziraat Fakültesi Dergisi*, *47*(1), 53-63.