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ABSTRACT

Hardaliye is a traditional beverage produced by fermenting red grapes with mustard seeds and sour cherry leaves in the Thrace region of Turkey. There are few studies that have determined the microorganisms responsible for hardaliye fermentation, and these are limited to lactic acid bacteria (LAB) using culture-dependent techniques. The aim of this study was to determine the bacterial dynamics of hardaliye fermentation using a culture-independent approach, high-throughput sequencing of 16S rRNA amplicons. Hardaliye was produced using the traditional method, and samples were taken and analyzed on days 0, 2, 4, 6, and 10 of fermentation. During the fermentation period, pH decreased from 3.65 to 3.23. Amplicon sequencing showed that bacterial diversity was highest at 2 d, and lowest at 10 d, the final day. Although

Enterobacteriaceae was the most dominant family at 0 and 2 d, *Acetobacteriaceae*, specifically *Gluconobacter frateurii*, became dominant with ~50% relative abundance at 4 d, and increased its abundance to >98% at 6 and 10 d. Although a slight increase in the relative abundance of ~1% (0 d) to ~5% (4 d) was observed in LAB, their presence was limited. This study showed that acetic acid bacteria should not be overlooked in hardaliye fermentation.

Keywords: 16S rRNA targeted metagenomics; acetic acid bacteria; amplicon sequencing; fermented foods; *Gluconobacter frateurii*

1. Introduction

Hardaliye is a traditional grape-based beverage from the Thracian region of Turkey (Arici & Coskun 2001). It is produced by fermenting aromatic red grapes together with crushed raw mustard seeds, sour cherry leaves, sorbic and/or benzoic acid as a preservative for 7 to 10 d (Aydogdu et al. 2014; Coşkun et al. 2012). Yeast growth and ethanol fermentation in hardaliye are limited due to the protective effect of active compounds in mustard seeds together with the action of the preservative (Coşkun et al. 2012). After production, hardaliye is filtered and stored at 4 °C (Arici & Coskun 2001; Coskun 2017). Even if it is stored in the cold, there may be changes in its properties after one year of storage (Coşkun et al. 2012). Fruits and fruit products are important components of a healthy diet because of their bioactive compounds, poor fat content, and low sodium and potassium (Rodríguez et al. 2021). Fermentation is an alternative preservation technique to extend the short shelf life of fruits; moreover, it improves the functional properties, including nutritional value and sensory attributes (Prado et al. 2008). Because the fermentation takes place with the seeds and skins of grapes, hardaliye is rich in antioxidant polyphenols, such as resveratrol, gallic acid, and flavonoids, such as quercetin and anthocyanidins (Amoutzopoulos et al. 2013). Hardaliye consumption significantly decreased oxidative stress markers compared to the control group suggesting an antioxidative effect (Amoutzopoulos et al. 2013). Hardaliye also reduced the

formation of the lipid oxidation product, malondialdehyde, in meat products during in vitro-digestion, indicating a potential health effect when consumed together with meat (Aksoy et al. 2022). Because it is non-alcoholic and plant-based, hardaliye is a beverage suitable for the consumption of a wide variety of consumer groups, including children and vegetarians (Prado et al. 2008).

Hardaliye has been described as a lactic acid fermented product (Arici et al. 2017; Arici & Coskun 2001; Bayram et al. 2015; Coskun 2017, Pehlivanoglu et al. 2015). Studies on the microbiology of hardaliye have mainly focused on culture-dependent methods using plate counts, including total mesophilic aerobic bacteria, lactic acid bacteria (LAB), coliforms, and yeasts (Arici & Coskun 2001; Aydogdu et al. 2014; Bayram et al. 2015; Coşkun et al. 2012). Two studies have described the isolation of LAB species from hardaliye (Arici et al. 2017; Arici & Coskun 2001).

Culture-independent microbial profiling techniques, which involve extracting DNA directly from samples, allow analyzing microorganisms without isolating and culturing them. Owing to the decrease in sequencing costs and the increasing availability of bioinformatics analysis tools, high-throughput sequencing (HTS) techniques have been ubiquitously used in fermented food community analysis for the past two decades (Chen et al. 2017; Ferrocino & Cocolin, 2017; Rizo et al. 2020). In HTS, shotgun sequencing of whole genomes can be used for a comprehensive analysis involving identifying microbial communities and their functional potential (Rizo et al. 2020). Meanwhile, amplicon-based “targeted” approaches, which involve sequencing only an informative region of the genome, are more accessible and cheaper when the aim is to identify the community members and their relative abundance (Chen et al. 2017; De Filippis et al. 2016; Golebiewski & Tretyn, 2020). For the identification of bacteria, the most widely used taxonomically informative region is the 16S rRNA gene (Ferrocino & Cocolin, 2017; Golebiewski & Tretyn, 2020). Amplicon sequencing is performed after amplification of 16S rRNA with universal primers. The resulting operational taxonomic unit (OTU) abundance is proportional to the number of reads, allowing the method to be quantitative (De Filippis et al. 2016).

Considering the scarcity of studies on hardaliye microbiology, our objective was to determine the bacterial community of hardaliye during the 10-d fermentation period using a culture-independent method, namely, 16S rRNA-targeted amplicon sequencing, and to correlate this with the pH changes.

2. Materials and Methods

2.1. Hardaliye production

Hardaliye was produced using traditional methods (Arici & Coskun 2001; Aydogdu et al. 2014). A traditional French red grape variety Alphonse Lavallée (*Vitis vinifera* L.) (Aubert & Chalot, 2018) cultivated in Tekirdag was used in production. Black mustard (*Brassica nigra* L.) seeds were obtained from local stores in Istanbul, and sour cherry (*Prunus cerasus* L.) leaves were picked from a tree at the Istanbul Sabahattin Zaim University campus the same day the fermentation was started. Two parallel samples (fermentation samples 1 and 2) were used for the fermentation process. Five-L plastic barrels with a tap at the bottom were filled in three layers with 5 kg crashed grapes, 2 g/kg crushed raw black mustard seeds, 2.5 g/kg sour cherry leaves, and 0.5 g/kg each sodium benzoate and potassium sorbate as preservatives. The contents were fermented at room temperature (~25°C) for 10 d and mixed with a sterile ladle every other day.

2.2. Chemical analyses

The pH of both samples on days 0, 2, 4, 6, 8, and 10 of fermentation was measured using an HI 2211 pH meter (Hanna Instruments Inc., Woonsocket, RI, USA).

2.3. DNA isolation

For bacterial diversity analysis, genomic DNA was extracted directly from hardaliye samples without microorganism cultivation using samples obtained at 0, 2, 4, 6, and 10 d of the fermentation of sample 1 and at 10 d (the final day) of the fermentation of sample 2. The Meta-G-Nome™ DNA isolation kit (Epicentre Biotechnologies, Madison, WI, USA) was used for extraction. To prepare samples for DNA isolation, 50 mL hardaliye sample was first filtered through four layers of Miracloth (Merck KGaA, Darmstadt, Germany) and then through a 1.2-µm filter supplied with the kit to remove impurities. The bacteria were then captured using a 0.45-µm filter and the kit protocol was followed. Isolated DNA in 50

μL TE buffer (10 mM Tris-HCl [pH 7.5] and 1 mM ethylenediaminetetraacetic acid [EDTA]) was stored at -20°C. The quality of the metagenomic DNA was analyzed using the BioSpec Nano spectrophotometer (Shimadzu, Kyoto, Japan).

2.4. 16S rRNA amplicon sequencing

Amplicons were sequenced using Macrogen (Seoul, Korea). The sequencing library was first prepared using the Illumina (San Diego, CA, USA) 16S metagenomic sequencing library preparation protocol. This protocol involves amplification of the ~460 bp hypervariable V3–V4 region of the 16S rRNA gene using Kapa HiFi HotStart Polymerase Chain Reaction (PCR) Kit (Kapa Biosystems, Cape Town, South Africa) with adapter-added primers (Klindworth et al. 2012). After purification of the PCR products using AMPure XP beads (Beckman Coulter Inc, Indianapolis, IN, USA), a second index PCR was conducted to add dual indices and sequencing adapters using the Nextera XT Index kit (Illumina). PCR cleanup was conducted, and the library size and quantity were determined using the 2100 DNA 1000 reagent kit and 2100 Bioanalyzer (Agilent Technologies, Inc., Waldbronn, Germany). Library sequencing was conducted using the Miseq system (Illumina) with its own software, MiSeq Control v 2.2. Base-calling was conducted using Real Time Analysis v 1.18 (Illumina), and FASTQ files were generated using the package bcl2fastq v 1.8.4 (Illumina). The adapter sequences were removed using Scythe v 0.991 Beta (https://github.com/vsbuffalo/scythe) and Sickle (https://github.com/najoshi/sickle). FLASH was used for assembly (Magoč & Salzberg 2011). Denoising, OTU clustering (97% cutoff), and taxonomic and diversity analyses were conducted using CD-HIT-OTU, rDnaTools, and QIIME, respectively (Caporaso et al. 2010; Li et al. 2012; Schloss et al. 2009). Sequences were submitted to GenBank (accession numbers: OK217199-OK217226).

3. Results and Discussion

3.1. Change of pH during hardaliye fermentation

The pH of hardaliye decreased from 3.65 (±0.10) to 3.23 (±0.04) (average values of two samples) during fermentation (Figure 1). A study examining the hardaliye samples (n=26) obtained from the market has indicated that pH varies between 3.21 and 3.97 (Arici & Coskun 2001). The final pH of the hardaliye

produced in the present study was close to the lower end of the pH range in the study by Arici and Coskun (2001). The differences in the final pH values might result from a variety of factors, such as the raw materials, microorganism load on the raw materials, fermentation temperature, fermentation duration, and storage period.

Laboratory-produced hardaliye samples in most of the previous studies showed a similar decrease in pH during the fermentation period (Arici & Coskun 2001; Aydogdu et al. 2014; Coskun & Arici 2006), while some studies indicated an increase in pH but not pH > 4 (Çoşkun & Arici 2011) or a relatively constant value remaining close to pH 4.0 (Bayram et al. 2015). Coskun and Arici (2011) have shown that different grape varieties result in different pH trends and that the pH trend of the same grape variety is similar even though different mustard seeds are used. This indicated that grape variety was more effective than the type of mustard seeds in determining pH trend. The Alphonse Lavallée grape variety was used in the present study, which might have been responsible for our relatively lower pH values compared to that of other studies. In a previous study using the same grape variety for hardaliye production, pH decreased from 3.86 to 3.39 in 7 days of fermentation (Arici & Coskun 2001) with low pH values similar to that of our study. Aydoğdu et al. (2014) also observed a pH decrease from 4.24 to 3.82 with the Alphonse Lavallée grape variety; however, those pH values were higher than that in the current study.

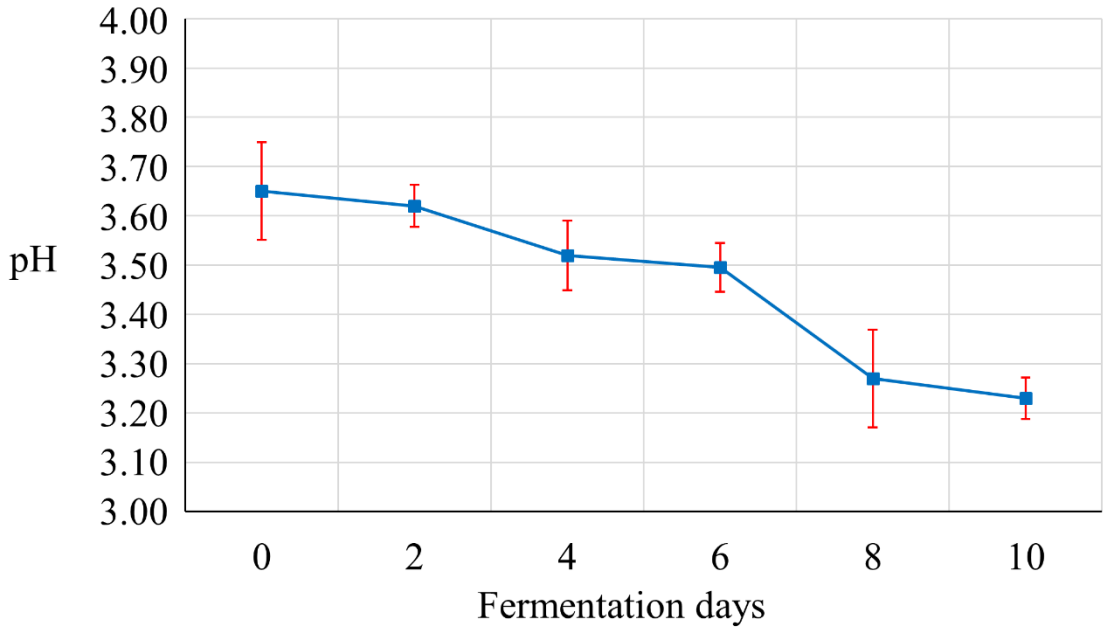

Figure 1-Change in pH during hardaliye fermentation

3.2. Bacterial dynamics of hardaliye

Amplicon sequencing was conducted at 0, 2, 4, 6, and 10 d of fermentation for sample 1 and at 10 d of fermentation for sample 2. The number of reads obtained by sequencing the six samples were between 1,327,260 and 1,548,056, which were reduced between 459,864 and 614,148 after adapter removal and assembly (Table S1). Quality scores, Q20 and Q30, for the assembled data were between 97.19 and 97.47 and between 90.10 and 90.97, respectively (Table S1). As a result of the analysis, 140 OTUs were obtained. After cleaning chloroplast and mitochondrial sequences, 135 OTUs were determined (Table S2).

Good's coverage index showing how well the data represented the larger set was near 1 for all samples (Table 1). The microbial diversity during the course of fermentation was estimated using the number of OTUs, Chao1 richness, and Shannon and Simpson diversity indices (Table 1). The number of OTUs first increased from 23 at 0 d to 95 at 2 d and then decreased to 15 at 10 d. Similarly, the second fermentation sample contained 14 OTUs at 10 d. All diversity estimates indicated that although the highest diversity was observed at 2 d, the lowest was observed at the end of the fermentation period at 10 d (Table 1). Starter culture is not used in hardaliye production; therefore, fermentation is spontaneous and driven by the microorganisms either in the raw materials or the environment in which fermentation takes place. Diversity analyses indicated that in the nutrient-rich fermentation medium, the bacterial diversity first increased up to day 2 of fermentation, after which it began to decrease and reached its lowest values by the end of the process, parallel to the decrease in pH. Towards the end of the fermentation period, dominance by the main microorganism eliminated the other species.

Table 1-Diveristy analyses of hardaliye samples: Community richness and alpha diversity indices

Fermentation Sample No	**Fermentation Day**	**Number of OTUs**	**Chao1 Richness**	**Shannon Diversity Index**	**Simpson Diversity Index**	**Good's Coverage Index**
1	0	23	63.5	1.9440	4.1383	0.9695
	2	95	95.5	3.0674	8.8777	0.9999
	4	32	32.1	1.6842	3.2071	0.9997
	6	38	38.2	0.0913	1.0237	0.9999
	10	15	17.0	0.0572	1.0191	0.9999

2	10	14	26.5	0.0582	1.0164	0.9999

Notes: OTU, operational taxonomic unit.

Taxonomical analyses showed that at 0 d, the bacteria in the fermentation medium was composed mainly of *Acetobacteriaceae* and *Enterobacteriaceae* (Figure 2A). At the species level, *Gluconobacter frateurii* and *Tatumella pytseos* had the greatest relative abundances (Figure 2B). At 2 d, the bacterial diversity increased and *Escherichia*/*Shigella* sp. within *Enterobacteriaceae* became dominant. At 4 d, when pH was slightly decreased, *Acetobacteriaceae*, specifically *G. frateurii* at the species level, became dominant with ~50% relative abundance (Figure 2, Table S2). Beginning at 6 d until the end of the fermentation period at 10 d, fermentation was nearly entirely (>98%) dominated by *G. frateurii* (Figure 2, Table S2). The presence of *G. frateurii* with a similar relative abundance of ~99% at the end of the fermentation period was confirmed using the second fermentation sample (Figure 2, Table S2). Although LAB species, such as *Levilactobacillus brevis*, *Companilactobacillus musae/farciminis* clade, *Streptococcus thermophilus*, *Lactococcus lactis*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Limosilactobacillus fermentum*, and *Weissella confusa/cibaria* clade were detected, their numbers were limited and greatly decreased after 4 d (Figure 2, Table S2).

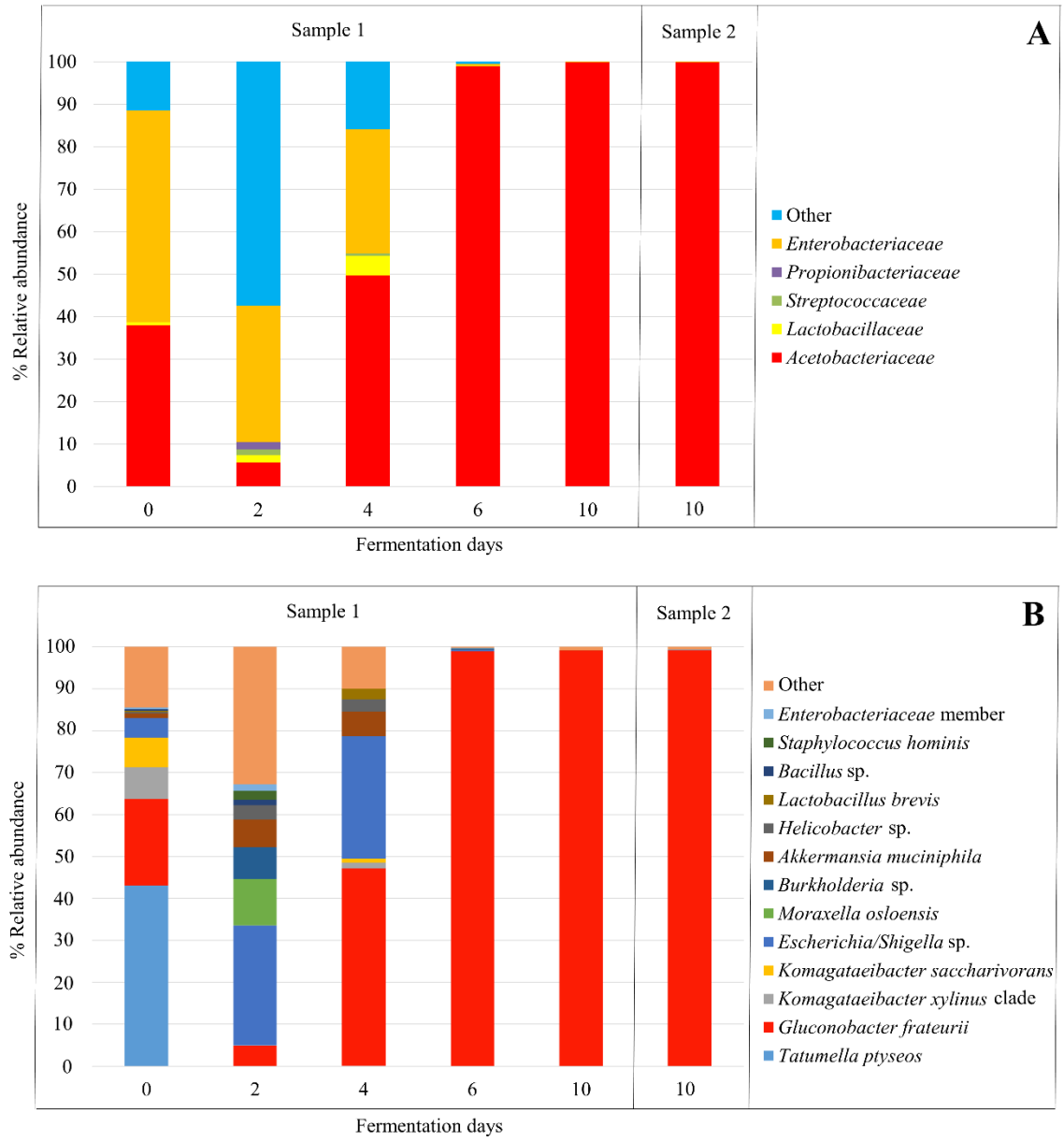

Figure 2-

Bacterial diversity of hardaliye during the fermentation period. A) Diversity at the family level. **B)** Diversity at the species level determined using the five most abundant species on each fermentation day. The remaining species are represented by "other".

Previous studies identifying bacteria species in hardaliye were focused on LAB. One such study identified *Lacticaseibacillus paracasei, Lacticaseibacillus casei, Limosilactobacillus pontis, L. brevis, Lactobacillus acetotolerans, Fructilactobacillus sanfranciscensis,* and *Paucilactobacillus vaccinostercus* (Arici & Coskun 2001). In another study, mostly *Lactiplantibacillus plantarum* and *Lactiplantibacillus pentosus* were isolated in addition to *L. brevis* and *Secundilactobacillus collinoides* (Arici et al. 2017). The common LAB species we identified in these two studies was *L. brevis*.

In the present study, we detected acetic acid bacteria (AAB) as the leading microorganism group in hardaliye fermentation. AAB have not been enumerated or isolated in previous hardaliye studies because, in general, AAB have not been studied as widely as other food-related bacteria (Pothakos et al. 2016), most likely because cultivation and isolation are difficult in spontaneously fermented food ecosystems harboring a variety of different bacteria and yeasts (De Roos & De Vuyst 2018). In addition, AAB are known to have a viable-but-not-culturable state, especially under low oxygen conditions, which causes an underestimation of the population (Bartowsky & Henschke 2008; Pothakos et al. 2016). The process of determining AAB in various fermented foods has increased, especially after the use of culture-independent high-throughput techniques (De Roos & De Vuyst 2018; Pothakos et al. 2016). For example, the unexpected presence of AAB, specifically *Gluconobacter* species, has been demonstrated in the spontaneous fermentation of the Grenache grape variety using HTS (Portillo & Mas 2016). Similar to that study, HTS has also shown that low sulfited wine fermentations involve AAB, specifically *Gluconobacter* species, more often than LAB (Bokulich et al. 2015). AAB has also been reported to represent an important group of microbiota in various fermented products, such as vinegar (Buyukduman et al. 2022; Lynch et al. 2019), Lambic beer and other sour beers (Bouchez & De Vuyst, 2022; De Roos J. et al. 2018), water kefir (Martínez-Torres et al. 2017), Kombucha (Villarreal-Soto et al. 2020), and cocoa (De Vuyst & Leroy 2020).

AAB are able to oxidize ethanol, carbohydrates, and sugar alcohols to their corresponding oxidation products (Lynch et al. 2019). For example, the conversion of ethanol to acetic acid is the key reaction taking place in vinegar production. Gluconic acid is another metabolite of AAB converted from glucose prominently by *Gluconobacter* species (García-García et al. 2017). While gluconic acid is a mild organic acid providing a refreshing sour taste, acetic acid provides an astringent and strong acidic flavor (Li et al. 2022; Sainz et al. 2016). Kombucha contains gluconic acid and acetic acid as the primary organic acids, and the presence of the former one in higher proportion was reported to be correlated with higher sensory scores (Li et al. 2022). Because ethanol production in hardaliye is limited, the main organic acid produced by AAB might be expected to be gluconic acid, which can be analyzed in future studies.

The finding that AAB rather than LAB is dominant in hardaliye fermentation might reorient the studies on starter culture for hardaliye that previously involved only LAB (Coşkun et al. 2012; Coskun & Arici 2006); however, we admit that the information obtained from this culture-independent study should be confirmed by culture-dependent analyses in addition to metabolomics, which will give a more complete view of hardaliye fermentation in terms of the microorganisms involved and the metabolites produced.

4. Conclusions

In the present study, the bacterial dynamics of hardaliye, a traditional grape-based beverage in the Thracian region of Turkey, was determined for the first time using a culture-independent method. The pH decreased from 3.65 to 3.23 during the 10-d fermentation period. Bacterial diversity increased at 2 d, then decreased until the end of the fermentation period along with pH decrease. HTS of 16S rRNA amplicons revealed the dominance of AAB, specifically *G. frateurii*, especially at 4 d. To our knowledge, this is the first report of AAB in hardaliye fermentation and has important implications for the development of starter culture. In future studies, comprehensive analyses involving both culture-dependent and culture-independent techniques for determining microbial dynamics in addition to a metabolomics approach will present a more complete picture of hardaliye fermentation.

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References

Aksoy AS, Arici M, & Yaman M (2022). The effect of hardaliye on reducing the formation of malondialdehyde during in vitro gastrointestinal digestion of meat products. *Food Bioscience* 47: 101747 https://doi.org/10.1016/j.fbio.2022.101747

Amoutzopoulos B, Löker GB, Samur G, Çevikkalp SA, Yaman M, Köse T & Pelvan E (2013). Effects of a traditional fermented grape-based drink 'hardaliye'on antioxidant status of healthy adults: a randomized controlled clinical trial. *Journal of the Science of Food and Agriculture* 93(14): 3604-3610 https://doi.org/10.1002/jsfa.6158

Arici M & Coskun F (2001). Hardaliye: Fermented grape juice as a traditional Turkish beverage. *Food Microbiology* 18(4): 417–421 https://doi.org/10.1006/fmic. 2001.0413

Arici M, Coşkun F, Çelikyurt G, Mirik M, Gülcü M & Tokatli N (2017). Some technological and functional properties of lactic acid bacteria isolated from hardaliye. *Journal of Agricultural Sciences* 23(4): 428–437 https://doi.org/ 10.15832/ankutbd.385870
Aubert C, & Chalot G (2018). Chemical composition, bioactive compounds, and volatiles of six table grape varieties (*Vitis vinifera* L.). *Food Chemistry*, 240: 524-533 https://doi.org/10.1016/j.foodchem.2017.07.152
Aydogdu H, Yildirim S, Halkman A K & Durgun T (2014). A study on production and quality criteria of hardaliye; a traditional drink from Thrace region of Turkey. *Gida (The Journal of Food)* 39(3): 193–145 http://doi.org/10.5505/gida.36844
Bartowsky E J & Henschke P A (2008). Acetic acid bacteria spoilage of bottled red wine—A review. *International Journal of Food Microbiology* 125(1): 60–70 https://doi.org/10.1016/j.ijfoodmicro.2007.10.016
Bayram M, Esin Y, Kaya C, İlhan M, Akin G & Etdöğer R (2015). Geleneksel yöntemle müşküle üzümünden üretilen hardaliyenin bazı özelliklerinin belirlenmesi. *Akademik Gıda (Academic Food Journal)* 13(2): 119–126 (Article in Turkish).
Bokulich N A, Swadener M, Sakamoto K, Mills D A & Bisson L F (2015). Sulfur dioxide treatment alters wine microbial diversity and fermentation progression in a dose-dependent fashion. *American Journal of Enology and Viticulture* 66(1): 73 https://doi.org/10.5344/ajev.2014.14096
Bouchez A & De Vuyst L (2022) Acetic acid bacteria in sour beer production: Friend or foe? *Frontiers in Microbiology* 13: 957167. https://doi.org/10.3389/fmicb.2022.957167.
Buyukduman E, Kirtil H E, & Metin B (2022) Molecular identification and technological properties of acetic acid bacteria isolated from Malatya apricot and home-made fruit vinegars. *Microbiology and Biotechnology Letters* 50(1):81-88 https://doi.org/10.48022/mbl.2109.09017
Caporaso J G, Kuczynski J, Stombaugh J, Bittinger K, Bushman F D, Costello E K, Fierer N, Peña A G, Goodrich, J K, Gordon, J I, Huttley G A, Kelley S T, Knights D, Koenig J E, Ley R E, Lozupone C A, McDonald D, Muegge B D, Pirrung M, … Knight R (2010). QIIME allows analysis of high-throughput community sequencing data. *Nature Methods* 7(5): 335–336 https://doi.org/10.1038/ nmeth.f.303
Chen G, Chen C, & Lei Z (2017). Meta-omics insights in the microbial community profiling and functional characterization of fermented foods. *Trends in Food Science & Technology* 65, 23-31 https://doi.org/10.1016/j.tifs.2017.05.002
Coskun F (2017). A traditional Turkish fermented non-alcoholic grape-based beverage, "hardaliye." *Beverages* 3(2) https://doi.org/10.3390/beverages3010002
Coskun F & Arici M (2006). The effects of using different mustard seeds and starter cultures on some properties of hardaliye. *Annals of Microbiology* 56(4): 335 https://doi.org/10.1007/BF03175027
Çoşkun F & Arici M (2011). Hardaliyenin bazi ozellikleri üzerine farkli hardal tohumlari ve üzüm cesitleri kullaniminin etkisi. *Akademik Gida (Academic Food Journal)* 9: 6–11 (Article in Turkish)
Coşkun F, Arıcı M, Çelikyurt G & Gülcü M (2012). Farklı yöntemler kullanılarak üretilen hardaliyelerin bazı özelliklerinde depolama sonunda meydana gelen değişmeler. *Tekirdağ Ziraat Fakültesi Dergisi* 9(3): 62–67 (Article in Turkish)
De Filippis F, Parente E, & Ercolini D (2017). Metagenomics insights into food fermentations. *Microbial Biotechnology*, 10(1): 91-102 https://doi.org/10.1111/1751-7915.12421
De Roos J & De Vuyst L (2018). Acetic acid bacteria in fermented foods and beverages. *Current Opinion in Biotechnology* 49: 115–119 https://doi.org/10.1016/j.cop bio.2017.08.007
De Roos J, Verce M, Aerts M, Vandamme P, De Vuyst L & Schaffner Donald W (2018). Temporal and spatial distribution of the acetic acid bacterium communities throughout the wooden casks used for the fermentation and maturation of Lambic beer underlines their functional role. *Applied and Environmental Microbiology* 84(7): e02846-17 https://doi.org/10.1128/AEM.02846-17
De Vuyst L & Leroy F (2020). Functional role of yeasts, lactic acid bacteria and acetic acid bacteria in cocoa fermentation processes. *FEMS Microbiology Reviews* 44(4): 432–453 https://doi.org/10.1093/femsre/fuaa014

Ferrocino I, & Cocolin L (2017). Current perspectives in food-based studies exploiting multi-omics approaches. *Current Opinion in Food Science*, 13, 10-15 https://doi.org/10.1016/j.cofs.2017.01.002
García-García I, Cañete-Rodríguez A M, Santos-Dueñas I M, Jiménez-Hornero J E, Ehrenreich A, Liebl W, García-Martínez T & Mauricio J C (2017). Biotechnologically relevant features of gluconic acid production by acetic acid bacteria. *Acetic Acid Bacteria*, 6(1): 6458 https://doi.org/10.4081/aab.2017.6458
Gołębiewski M, & Tretyn A (2020). Generating amplicon reads for microbial community assessment with next-generation sequencing. *Journal of Applied Microbiology* 128(2): 330-354 https://doi.org/10.1111/jam.14380
Klindworth A, Pruesse E, Schweer T, Peplies J, Quast C, Horn M & Glöckner F O (2012). Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Research* 41(1): e1 https://doi.org/10.1093/nar/gks808
Li R., Xu Y, Chen J, Wang F, Zou C, & Yin J (2022) Enhancing the proportion of gluconic acid with a microbial community reconstruction method to improve the taste quality of Kombucha. *LWT* 155: 112937 https://doi.org/10.1016/j.lwt.2021.112937
Li W, Fu L, Niu B, Wu S & Wooley J (2012). Ultrafast clustering algorithms for metagenomic sequence analysis. *Briefings in Bioinformatics* 13(6): 656–668 https://doi.org/10.1093/bib/bbs035
Lynch K M, Zannini E, Wilkinson S, Daenen L & Arendt E K (2019). Physiology of acetic acid bacteria and their role in vinegar and fermented beverages. *Comprehensive Reviews in Food Science and Food Safety* 18: 587-625 https://doi.org/10.1111/1541-4337.12440
Magoč T & Salzberg S L (2011). FLASH: fast length adjustment of short reads to improve genome assemblies. *Bioinformatics* 27(21): 2957–2963 https://doi.org/10.1093 /bioinformatics/btr507
Martínez-Torres A, Gutiérrez-Ambrocio S, Heredia-del-Orbe P, Villa-Tanaca L & Hernández-Rodríguez C (2017). Inferring the role of microorganisms in water kefir fermentations. *International Journal of Food Science & Technology* 52(2): 559–571 https://doi.org/10.1111/ijfs.13312
Prado FC, Parada JL, Pandey A, & Soccol CR (2008). Trends in non-dairy probiotic beverages. *Food Research International*, 41(2): 111–123 doi:10.1016/j.foodres.2007.10.010
Pehlivanoglu H, Gunduz H H, Ozulku G, Demirci M (2015) An investigation of antimicrobial activity of wheat grass juice,barley grass juice, hardaliye and boza. I*nternational Interdisciplinary Journal of Scientific Research* 2(1): 8-14
Portillo M del C & Mas A (2016). Analysis of microbial diversity and dynamics during wine fermentation of Grenache grape variety by high-throughput barcoding sequencing. *LWT - Food Science and Technology* 72: 317–321 https://doi.org/ 10.1016/j.lwt.2016.05.009
Pothakos V, Illeghems K, Laureys D, Spitaels F, Vandamme P & De Vuyst L (2016). Acetic acid bacteria in fermented food and beverage ecosystems. In K. Matsushita H Toyama, N Tonouchi & A Okamoto-Kainuma (Eds.), *Acetic Acid Bacteria: Ecology and Physiology* (pp. 73–99) Springer Japan. https://doi.org/10.1007/ 978-4-431-55933-7_3
Rizo J, Guillén D, Farrés A, Díaz-Ruiz G, Sánchez S, Wacher C & Rodríguez-Sanoja R (2020). Omics in traditional vegetable fermented foods and beverages. *Critical Reviews in Food Science and Nutrition* 60(5): 791-809 https://doi.org/10.1080/10408398.2018.1551189
Rodríguez LGR, Gasga VMZ, Pescuma M, Van Nieuwenhove C, Mozzi F, & Burgos J (2021). Fruits and fruit by-products as sources of bioactive compounds. Benefits and trends of lactic acid fermentation in the development of novel fruit-based functional beverages. *Food Research International*, 140: 109854 https://doi.org/10.1016/j.foodres.2020.109854

Sainz F, Navarro D, Mateo E, Torija M J & Mas A (2016). Comparison of D-gluconic acid production in selected strains of acetic acid bacteria. *International Journal of Food Microbiology* 222:40-47 https://doi.org/10.1016/j.ijfoodmicro.2016.01.015.
Schloss P D, Westcott S L, Ryabin T, Hall J R, Hartmann M, Hollister E B, Lesniewski R A, Oakley B B, Parks D H, Robinson C J, Sahl J W, Stres B, Thallinger G G, Van Horn D J & Weber C F (2009). Introducing mothur: Open-Source, platform-ındependent, community-supported software for describing and comparing microbial communities. *Applied and Environmental Microbiology* 75(23): 7537 https://doi.org/10.1128/AEM.01541-09
Villarreal-Soto S A, Bouajila J, Pace M, Leech J, Cotter P D, Souchard J P, Taillandier P & Beaufort S (2020). Metabolome-microbiome signatures in the fermented beverage, Kombucha. *International Journal of Food Microbiology* 333: 108778 https://doi.org/10.1016/j.ijfoodmicro.2020.108778