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ORIGINAL PAPER

Chemical, microbial, and volatile changes of water kefir during fermentation with economic substrates

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Abstract

Water kefir is a plant-based type of kefir and has significant differences from well-known milk kefir. Recently, various complicated substrates were fermented with water kefir grains, but an economical substrate is essential for the industrial aspect. This study aims to understand changes in fermentation products of water kefir grains during fermentation with economic substrates and investigates how fermentation progresses under almost ideal fermentation parameters. The fig-based medium provided high contents of *Lactobacillus* spp. and *Lactococcus* spp. Also, fig-based medium with high fructose content contained a higher amount of *Bifidobacterium* sp. Moreover, the fig-based medium resulted in more organic acid content, forming as much as ten times higher than the sugar-based medium for lactic acid after the same fermentation time ($p < 0.05$). On the other hand, volatile compounds such as acetaldehyde, ethyl acetate, ethanol, acetic acid, 2-ethyl-1-hexanol, oxime-, methoxy-phenyl, and phenylethyl alcohol were detected for both water kefir samples. Antioxidant capacity (TEAC and ORAC) was higher for the fig-based medium than for the sugar-based medium. This is the first study that comprehensively proved that only sugar solution was not a sufficient medium for water kefir fermentation, but fig containing solution had abundant nutrients to obtain preferable and acceptable water kefir beverage.

Keywords Water kefir grains · Sugary kefir · Tibicos · Fermentation · Volatiles

Abbreviations

AAB	Acetic acid bacteria
ABTS +	2,2'-Azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) diammonium salt
BS	Water kefir with white sugar
INP	Water kefir with dried figs
LAB	Lactic acid bacteria
ORAC	Oxygen radical absorbance capacity
TEAC	Trolox equivalent antioxidant capacity

Introduction

There are two types of kefir in nature: one of them is milk kefir and the other one water kefir [1]. Both fermented products are produced using unique grains with some similarities, but they have quite differences, such as microbial composition, chemical structure, and the final product [2, 3]. Traditionally, water kefir grains or tibicos are utilized to obtain water kefir beverages. The characteristics of the product may change depending on the preferred fermentation medium, but generally it is slightly sour and carbonated with a refreshing taste. It is a plant-based fermented food that does not contain any animal-originated compounds. For this reason, it is suitable for vegans and individuals who are sensitive to consuming animal-originated products for allergic or ethical reasons. Moreover, recently, anticarcinogenic, hepatoprotective, gastroprotective, cholesterol-lowering, blood sugar reducing, antimicrobial, anti-inflammatory, antioxidant, improved wound healing effects, and ACE-inhibitory activity were studied [2, 4, 5]. In previous studies, milk kefir had a more standardized production in terms of fermentation time, inoculation ratio, and fermentation medium. However, WK production has some uncertainties on the production factors [2]. For example, various inoculation ratios were presented in previous studies such as 5% (w/v), 7.5% (w/v), and 10% (w/v) [6–8]. Moreover, various WK fermentation lengths were suggested, 48 h and 72 h [9, 10]. Furthermore, various substrates, e.g., for fermentation media such

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as fruit juices [11], vegetable juices [12], rice [13], banana peel [14], soy whey [6, 15, 16], and *Spirulina* [17] have been experimentally utilized to produce water kefir.

According to available knowledge, a standard and economical production method is not yet available for water kefir. In this aspect, the study aims to determine the changes in the characteristics of water kefir with different economic fermentation substrates and investigate how fermentation progresses under almost ideal fermentation parameters.

Materials and methods

Water kefir grains

Water kefir grains were kindly provided by Danem Inc. (Isparta, Turkey, https://www.kefirdanem.com). The microbiota of water kefir grains were analyzed and published in our previous study [3]. According to the results of this study, our water kefir grains to obtain water kefir beverages contained *Bifidobacterium* spp., *Lactobacillus* spp. *Lactococcus* spp., *Streptococcus* spp. *Acetobacter* spp., and *Gluconobacter* spp.

Preparation of water kefirs

Firstly, trials were applied for both substrates with water kefir grains. In these preliminary trials, 2.5, 5.0, and 7.5% water kefir grains were fermented with 2.5, 5.0, and 7.5% sugar or fig, based on the fermentation medium. According to the results of these preliminary trials, 5.0% water kefir grain inoculation with 5% medium, e.g., sugar or fig, was selected according to the fermentatitive activity and microbial and sensory results.

Water kefir with white sugar (BS): 5% (w/v) white sugar was added into 1 L potable water (https://www.hayatsu.com.tr/) and autoclaved at 121 °C for 15 min. After cooling the sugar solutions to 25 °C, 5% water kefir grains (w/v) were added under a sterile cabinet. Two jars were fermented for 24 h and or 48 h at 25 °C. Samples were taken at the beginning (0), 24 h, and 48 h, and coded as BS0, BS24, and BS48.

Water kefir with dried figs (INP): Organic dried figs (Metro Chef Bio, Aydın Fig, 500 g) were utilized. For better sugar and bioactive extraction, each fig was cut into four equal parts, then wrapped in 50 × 50 sterile cheesecloth (to be able to well separate water kefir grains at the end of fermentation) and put into a glass jar. Then, 1 L potable water (https://www.hayatsu.com.tr/) was added, boiled for 2 min, and allowed to cool to room temperature. Then, 5% water kefir grains (w/v) were inoculated under a sterile cabinet. Two jars were fermented for 24 h and 48 h at 25 °C. Samples were taken at the beginning (0), 24 h, and 48 h, and coded as INP0, INP24, and INP48.

Physicochemical analyses

Water-soluble solids (Bellingham Stanley Limit 60/70 Refractometer, UK), pH (Schott instruments, pH-meter lab 850, USA), and total titratable acidity (in terms of lactic acid%) analyses were performed in accordance with AOAC (1992). Dry matter content was determined by Shimadzu MOC63U (Shimadzu) Moisture Analysis device.

Microbiological analyses

Microbiological analyses were performed according to the method specified by Spencer and Spencer [18]. Briefly, 1 mL of sample was taken from each water kefir and placed in 9 mL of sterile peptone water. Serial dilutions of up to seven were then prepared. MRS agar (Merck) was utilized for *Lactobacillus* sp., M17 agar (Merck) for *Lactococcus* sp., PDA agar (Merck) for yeasts, MRS-NNLP agar for *Bifidobacterium* sp., and MRS-salicin agar for *L. acidophilus*. Incubation temperature and times were selected and applied according to the target microorganism's needs.

Organic acid content analysis

Analyses were performed on HPLC (Shimadzu SCL-10A, Scientific Instruments, Inc., Tokyo, Japan) instrument equipped with DAD detector (LC 20ADvp), pump (LC 10ADvp), gas separator (DGU 20A), and column oven (CTO 10Avp). Inertsil ODS 3 V (4.8 × 250 mm 5 µm) column was used for organic acid content. The column pressure was 8.8 Mpa. The flow rate was set as 1 mL/min. The absorbance value was measured at 210 nm. Oven temperature was set to 30 °C. The mobile phase was obtained by dissolving 5 mM H_2SO_4 in 1 L of distilled water. Samples were filtered through a 0.45 µm filter before each injection.

Sugar content analysis

Sugar analyses were performed on HPLC (Shimadzu SCL-10A, Scientific Instruments, Inc., Tokyo, Japan) instrument equipped with 4.6 mm i.d. × 250 mm Cosmosil Sugar-D packed column (Nacalai Tesque Inc., Japan), DAD detector (LC 20ADvp), pump (LC 10ADvp), gas separator (DGU 20A), and column oven (CTO 10Avp). Acetonitrile/water ratio was set as 75/25 as the mobile phase. The flow rate was set at 1.0 mL/min. The column temperature was set to 30 °C. The column pressure was kept constant at 4.2 MPa. Before each injection, samples were diluted and filtered through 0.45 µm syringe filters.

The samples were injected into the device by diluting them ten times with the mobile phase.

Antioxidant capacity analyses

$ABTS^+$ analysis

To determine the total antioxidant activity of water kefir samples, 7 mM 2,2′-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) diammonium salt ($ABTS^+$) stock solution and 2.45 mM potassium persulfate solution were reacted in the dark for 12–16 h, and $ABTS^+$ radical cation was obtained. $ABTS^+$ radical solution was diluted with phosphate buffer until the absorbance value was 0.700 ± 0.02 at a wavelength of 734 nm. 100 µL of sample or Trolox (prepared in different concentration ranges) was added to 2 mL of $ABTS^+$ radical, and the reaction was allowed to complete for 6 min at 30 °C. The reaction of antioxidants with the radical was measured by lowering the absorbance of the radical at 734 nm. Standard curve and % inhibition equations were established for different Trolox concentrations. Results were expressed as Trolox equivalent antioxidant capacity (TEAC) mM [19].

Oxygen radical absorbance capacity (ORAC) analysis

Water kefir samples and blank samples were placed into microplates, and the standard ORAC procedure was applied according to Dávalos et al. [20] and Singh and Singh [21]. To perform this assay, Synergy™ HT Multi-Detection Microplate Reader (Winooski, Vermont, USA) was utilized for kinetic measurement at an excitation–emission wavelength of 485 to 520 nm.

Volatile compound analysis

Headspace solid-phase microextraction method was used for isolation of volatile compounds from the sample matrix. For this purpose, the fiber (SPME Fiber Assembly 50/30 um DVB/CAR/PDMS. Stableflex (2 cm) 24 Ga. Manual Holder. 3pk (Gray-Notched)) was firstly conditioned at 270 °C for 0.5 h as specified in the user manual. HP 6890 GC and 7895 C mass selective detector (Agilent Technologies, Wilmington, DE, USA) and polar capillary column (HP-INNOwax 60 m length, 0.25 mm i.d., 0.25 µm df; J&W Scientific, Folsom, CA) were used. The pressure was set to 131.2 kPa, and the flow rate was adjusted to 1.2 mL/min. The total program duration was set to 62.5 min. 5 mL of each sample was taken in a 40 mL amber vial, and 1 g of salt and 10 µL of internal standard (4-methyl-2-pentanol and methyl-nonanoate mixture) were added. Then, the vial was closed, mixed for 15 s, and kept in a 40 °C water bath for 0.5 h. Then, at the end of that time, the lid was properly punctured, the fiber was inserted, and the vial was kept at 40 °C for 0.5 h with fiber. Then, the fiber was removed from the vial and placed in the injection port of the GC device, and the analysis program was started in such a way that the oven was first set from 40 °C (2 min) to 150 °C at 4 °C/min, from 150 °C (20 min) to 200 °C at 5 °C/min.

Descriptive sensory analysis

Sensory analysis was carried out according to the method explained by Tu et al. [16] and Ozcelik et al. [10] with slight modifications. Briefly, each sample and its parallel were coded with random numbers and kept in the refrigerator until testing. Drinkable water and pretzels were given to the panelists to use during the analysis. Before the test, panelists were informed. For each test, eight panelists, ages between 24 and 50 years (4 male and 4 female) participated. Panelists were informed and the required instructions were explained. Panelists scored between 1 and 10 points descriptive scale ratings of the characteristics of the products for foaminess, color, turbidity, consistency, fermented odor, sourness, sweetness, fruity flavor, alcohol content, freshness, and astringency. These characteristic properties were determined after preliminary trials.

Statistical analysis

The statistical results were analyzed by one-way analysis of variance (ANOVA) test using IBM SPSS v. 22.0 (SPSS Inc., Chicago, USA). Duncan's test was used for assessing the significantly different results ($p < 0.05$) for all analyses except volatile analysis. All analyses were carried out with two replications and four parallels, except sensory analysis test. Sensory analysis was obtained by 8 replications and 16 parallels.

Results and discussion

Physicochemical analyses

A fermented product's acceptance is closely related to its acidity level, e.g., pH, since it is related to a wide range of quality parameters such as texture and flavor [7]. A significant change was observed in pH because of the fermentation ($p < 0.05$); at the beginning, the pH value of the sugar solution was 7.33. After 24-h fermentation, it was 4.99 ($p < 0.05$). However, there was no significant difference between the 24-h and 48-h fermentation times ($p > 0.05$). On the other hand, the pH value of the fig-based medium was 5.03, due to the presence of organic acids in the fig-based medium and this was significantly lower than the pH of the sugar-based medium at the beginning ($p < 0.05$). The pH value of fig-based water kefir significantly changed from

5.03 to 3.95 after 24-h fermentation ($p < 0.05$); then, there was no significant change between the 24-h and 48-h fermentation times. Previous studies reported various pH values from 3.33 to 5.00 for water kefirs [12, 22, 23]. The decrease in the pH of our water kefir samples was related to the formation of metabolites, mainly organic acids because various microorganisms' microbial activities naturally occur in water kefir [24, 25]. Also, water kefirs' pH value decreased gradually during the 24-h to 48-h fermentation. The buffer effect may occur during the 24th–36th of fermentation [26]. Fermentation parameters such as substrate, length, temperature, and activity of grain microbiota impacted the product's final pH [10]. Brix was adjusted to 4.5 in the fermentation medium at the beginning and significantly decreased in both samples at the end of the fermentation ($p < 0.05$), similar to other studies [7, 10–12, 26]. The sugars present in the medium were used by lactic acid bacteria and yeasts and converted into various metabolites during the fermentation. Decrease in pH and Brix and fermentation show efficient use of sugar as a carbon source by water kefir grain microbiota (Table 1). Brix values of water kefir beverages vary depending on the mainly used substrate during fermentation [10]. For instance, Brix values of various water kefir beverages were between 5.87 and 9.97 [11]. On the other hand, a reduction in pH value and an increase in titratable acidity value were observed during the fermentation for both water kefir samples. These results were compatible with our organic acid results (Table 3). A decrease in pH and increase in acidity values following an increase in lactic acid, acetic acid, and malic acid were observed at the end of fermentation. A similar trend was reported by Maldonado et al. [26].

Table 1 Physicochemical properties water kefir samples

	pH	Brix°	Dry matter (%)	Titratable acidity*
BS 0	7.33 ± 0.57^{a}	4.83 ± 0.52^{a}	5.87 ± 0.08^{a}	0.01 ± 0.00^{a}
BS 24	4.99 ± 0.41^{b}	3.87 ± 0.57^{b}	4.58 ± 0.06^{a}	0.02 ± 0.00^{a}
BS 48	4.71 ± 0.31^{b}	4.40 ± 0.00^{b}	4.90 ± 1.16^{a}	0.04 ± 0.00^{a}
INP0	5.03 ± 0.00^{b}	4.32 ± 0.30^{b}	5.32 ± 0.80^{a}	0.05 ± 0.00^{a}
INP24	3.95 ± 0.01^{c}	2.95 ± 0.06^{c}	2.76 ± 0.37^{b}	0.29 ± 0.11^{b}
INP48	3.59 ± 0.02^{c}	2.42 ± 0.05^{c}	1.56 ± 0.24^{b}	0.97 ± 0.01^{c}

*The different superscripts in the same column show a significant difference among the samples ($p < 0.05$). Results were expressed as mean ± standard deviation

Table 3 The sugar content of water kefir samples

Sample	Fructose (g/L)	Glucose (g/L)	Sucrose (g/L)
BS0	0.13 ± 0.06^{a}	*dnq	52.52 ± 5.70^{a}
BS24	2.44 ± 0.08^{b}	2.29 ± 0.07^{a}	36.59 ± 3.92^{b}
BS48	2.95 ± 0.68^{b}	2.22 ± 0.69^{a}	38.37 ± 3.52^{b}
INP0	22.80 ± 1.14^{c}	31.47 ± 1.93^{b}	3.43 ± 1.07^{c}
INP24	8.64 ± 1.66^{d}	3.93 ± 1.20^{a}	0.82 ± 0.23^{c}
INP48	5.42 ± 0.67^{e}	4.11 ± 2.39^{a}	0.17 ± 0.08^{c}

*The different superscripts in the same column significantly differ among the samples ($p < 0.05$). Results were expressed as mean ± standard deviation

*dnq: detected but not quantified

Microbiological analysis

Naturally, water kefir grains have unique, plenty, and diversified microbial consortia mainly composed of lactic acid bacteria (LAB), acetic acid bacteria (AAB), and yeasts [27]. Firstly, microbiological analysis of fermentation media was carried out before inoculation of water kefir grains to ensure that microbial changes of our samples resulted from microbial activity in water kefir grains. According to microbiological results, substrates did not contain any microorganisms before inoculating kefir grains.

The number of *Lactobacillus* sp. in the sugar-based medium after inoculation with water kefir grains was 4.46 log CFU/mL at the beginning. Typically, an increase in the number of *Lactobacillus* sp. was expected depending on fermentation. However, there was a slight decrease to 4.41 and 4.16 log CFU/mL after 24 h and 48 h of fermentation, respectively ($p > 0.05$). On the other hand, *Lactobacillus* sp. was significantly increased in a fig-based medium during fermentation ($p < 0.05$). *Lactobacillus* sp. was 5.18 log CFU/mL at the beginning, reaching 7.51 log CFU/mL and

Table 2 Microbiological contents of water kefir samples (Log CFU/mL)

Sample	*Lactobacillus* sp.	*L. acidophilus*	*Lactococcus* sp.	*Bifidobacterium* sp.	Yeast
BS 0	4.46 ± 0.12^{a}	4.66 ± 0.07^{a}	3.83 ± 0.21^{a}	3.24 ± 0.17^{a}	4.45 ± 0.52^{a}
BS 24	4.41 ± 0.14^{a}	4.98 ± 0.49^{a}	5.00 ± 0.63^{b}	2.26 ± 0.07^{b}	5.89 ± 0.52^{b}
BS 48	4.16 ± 0.32^{a}	5.43 ± 0.60^{b}	5.53 ± 0.05^{c}	2.13 ± 0.08^{b}	5.25 ± 0.42^{b}
INP 0	5.18 ± 0.11^{b}	5.15 ± 0.10^{a}	4.46 ± 0.11^{b}	3.10 ± 0.07^{a}	4.34 ± 0.10^{a}
INP 24	7.51 ± 0.11^{c}	7.43 ± 0.04^{c}	7.31 ± 0.05^{d}	6.01 ± 0.06^{c}	6.16 ± 0.14^{c}
INP 48	7.71 ± 0.16^{c}	7.67 ± 0.15^{c}	7.77 ± 0.07^{d}	6.09 ± 0.10^{c}	5.78 ± 0.06^{b}

*The different superscripts in the same column show a significant difference among the samples ($p < 0.05$). Results were expressed as mean ± standard deviation

Table 4 Organic acid contents of water kefir samples

Sample	Tartaric acid (g/L)	Malic acid (g/L)	Lactic acid (g/L)	Acetic acid (g/L)	Citric acid (g/L)
BS 0	–	0.145 ± 0.009^{a}	0.169 ± 0.010^{a}	0.539 ± 0.013^{a}	–
BS 24	–	0.170 ± 0.025^{a}	0.232 ± 0.031^{a}	0.612 ± 0.051^{a}	–
BS 48	–	0.168 ± 0.031^{a}	0.252 ± 0.039^{a}	0.653 ± 0.044^{a}	–
INP 0	0.09 ± 0.026	0.787 ± 0.173^{b}	0.254 ± 0.011^{a}	0.896 ± 0.179^{a}	0.506 ± 0.012^{a}
INP24	–	1.482 ± 0.490^{c}	1.514 ± 0.404^{b}	1.672 ± 0.181^{b}	0.392 ± 0.046^{a}
INP48	–	1.226 ± 0.470^{c}	2.693 ± 0.938^{c}	3.531 ± 0.237^{c}	0.062 ± 0.013^{b}

*The different superscripts in the same column show a significant difference among the samples ($p < 0.05$). Results were expressed as mean ± standard deviation

7.71 log CFU/mL after 24-h and 48-h fermentation time, respectively (Table 2). According to our results, the type of substrate resulted in a significant difference in the count of *Lactobacillus* sp. ($p < 0.05$). In other studies, the use of various substrates such as soy, whey, fruit juices, vegetable juices, sugared water, raw sugar solution, and *Spirulina* resulted in 5.0–9.5 log CFU/mL *Lactobacillus* sp. [8, 9, 24, 26, 28, 29]. Azi et al. [15] reported about 8 log CFU/mL lactic acid bacteria after 48-h fermentation. According to our results (Table 2), only white sugar as a substrate was not enough for *Lactobacillus* sp. Growth, since it did not contain essential nutrients (carbon and nitrogen sources) to support their growth [30]; however, the present *Lactobacillus* sp. were able to use carbon source for their survival, lower the pH, and produce organic acids Various *Lactobacillus* sp. were identified from water kefir grains such as *Lactobacillus acidophilus, L. brevis, L. hordei, L. nagelii,* and *L. satsumensis* [3].

L. acidophilus content of sugar-based medium did not change significantly for the first 24 h of fermentation. However, it significantly increased from 4.98 to 5.43 log CFU/mL between 24 and 48 h of fermentation. On the other hand, *L. acidophilus* content of fig-based medium significantly increased from 5.15 to 7.43 log CFU/mL within 24-h fermentation; then, there was no significant change at the end of the 48-h fermentation. The microbial content of the fig-based fermentation medium was higher than that of sugar solution due to the rich nutritional content of dried figs in carbohydrates, vitamins, and minerals [31].

Lactococcus sp. count of the sugar-based medium was increased significantly from 3.83 to 5.00 and 5.53 log CFU/mL after 24-h and 48-h fermentation, respectively ($p < 0.05$). On the other hand, *Lactococcus* sp. of the fig-based medium was 4.46 log CFU/mL at the beginning. Then, it significantly increased and reached 7.31 log CFU/mL after 24-h fermentation ($p < 0.05$). Then, there was no significant change between 24-h and 48-h fermentation, probably due to decreased nutrients in the medium via metabolic activities of microorganisms. In addition, according to our results, different types of substrates caused a significant change in *Lactococcus* sp. number for BS0 and INP0. Our *Lactococcus* sp. results were compatible with the previous results, which also reported a significant increase in the number of *Lactococcus* sp. due to water kefir fermentation (Magalhaes et al. [29]; Randazzo et al. [11]). Also, based on the present study's results, white sugar and fig were preferable substrates for the growth of *Lactococcus* sp. present in water kefir grains. *Lactococcus lactis* and *Lc. taiwanensis* were identified from water kefir grains as *Lactococcus* sp. [3].

The *Bifidobacterium* sp. content of sugar-based medium decreased significantly from 3.26 to 2.26 log CFU/mL after the first 24 h of fermentation. Then, its number was not changed significantly at the end of fermentation. On the contrary, its number was nearly doubled for the fig-based medium, as much as 6.01 log CFU/mL after 24 h of fermentation ($p < 0.05$), and then did not change significantly until the end of fermentation (Table 2). The fig-based medium contained a high amount of fructose at the beginning of the fermentation. Fructose is one of the most favorable carbon sources for lactobacilli and bifidobacteria [32].On the other hand, even though fructose content increased during the fermentation of the sugar-based medium, its amount was significantly lower (as much as 170 times) than in the fig-based fermentation medium (Table 4). For this reason, it was concluded that the sugar-based medium was not preferable for *Bifidobacterium* sp. growth in water kefir. *Bifidobacterium adolescentis*, *B. aquikefiri*, *B. crudilactis,* and *B. subtile* were identified in water kefir grains [3].

The yeast content of sugar-based medium significantly increased from 4.45 to 5.89 log CFU/mL after the first 24 h of fermentation ($p < 0.05$). Then, its number decreased to 5.25 log CFU/mL between 24-h and 48-h fermentation. For the fig-based medium, the yeast population significantly increased from 4.34 to 6.16 in the first 24 h of fermentation ($p < 0.05$); then, similar to the sugar-based medium, yeast content decreased maybe due to the death phase (Table 2). According to the results of the present study, 24 h of fermentation was suitable enough to reach a maximum number of yeasts. Moreover, the fermentation medium was not a significant parameter for yeast growth, unlike *Lactococcus* sp. and *Bifidobacteria* sp. In other studies, yeast contents were reported between 3.3 and 8.0 CFU/mL [11, 12, 17, 29,

33]. There is a symbiotic relation between LAB and yeast. An acidified environment was formed during the fermentation process due to bacterial metabolic activities, resulting in favorable conditions for growing yeasts. On the other hand, some essential nutritional factors such as vitamins and soluble nitrogen compounds necessary for the growth of bacteria were provided in the medium as the results of yeast activities [29]. Similar to the study of Laureys and De Vuyst [34], yeast number was lower than lactic acid bacteria in kefir samples.

Changes in sugar profile and organic acid contents

Enzymes of microorganisms in water kefir grains break down sucrose to its monomers due to their microbial activities. Monomers of sucrose are the primary substrate for water kefir microorganisms.

Changes in sugar contents during water kefir fermentations are given in Table 3. The fructose content of the sugar-based medium increased significantly after 24-h fermentation ($p < 0.05$), but no significant change was observed between the 24-h and 48-h fermentation period. On the contrary, the fructose content of the fig-based medium significantly decreased from 22.80 g/L to 8.64 g/L and 5.42 g/L after 24-h and 48-h fermentation periods, respectively ($p < 0.05$). The substrate type significantly impacted the fructose content of water kefir ($p < 0.05$).

The glucose content of the sugar-based medium changed significantly, reached 2.29 g/L concentration after 24 h of fermentation ($p < 0.05$), and was nearly stable between 24-h and 48-h fermentation. On the other hand, the glucose content of the fig-based medium decreased significantly, e.g., about tenfold, after the first 24-h fermentation period ($p < 0.05$). Then, an insignificant increase occurred until the end of fermentation. The glucose content of samples was significantly affected by the type of substrate at the beginning ($p < 0.05$).

Sucrose altered significantly from 52.52 to 36.59 g/L after 24-h fermentation of sugar-based medium ($p < 0.05$). However, no significant change in its concentration was observed between 24-h and 48-h fermentation. On the other hand, the fig-based medium had a significantly lower amount of sucrose than the sugar-based medium ($p < 0.05$). Also, the sucrose amount of fig-based medium decreased insignificantly from 3.43 to 0.82 and 0.17 g/L following the 24- and 48-h fermentation periods. The type of utilized substrate significantly affected the sucrose content of samples ($p < 0.05$). Our results were compatible with other studies [22, 23, 25, 34–38].

Glucose and fructose concentrations increased significantly at the end of fermentation for sugar-based water kefir samples ($p < 0.05$). It was because the enzymes of microorganisms metabolized sucrose and turned it into glucose and fructose. The increased amount of monosaccharides was consumed and degraded by lactic acid bacteria and *Gluconobacter* sp. until depleted [23, 25, 34, 35]. On the other hand, in this study, sucrose significantly decreased, and the monosaccharides formed were probably used during 24-h fermentation; some remaining monosaccharides were also present. The subcomponents of sucrose, e.g., glucose and fructose, were essential carbon sources for LAB and *Bifidobacteria* sp. This functional symbiotic relationship attributed unique properties to water kefir [15]. Sucrose was the primary carbohydrate source for the BS sample, while ready-to-use fructose and glucose were the main carbohydrate sources for the INP sample. Thus, glucose and fructose concentrations in the INP sample were higher at the beginning due to fig content [39].

Organic acids in the products may contribute to their palatability by increasing organoleptic characteristics and functional properties [16]. The changes in organic acid contents during water kefir fermentations are given in Table 4. Tartaric and citric acids were not detected in the sugar-based medium, but found in the fig-based medium at the beginning. Their concentrations decreased during the fermentation ($p < 0.05$). On the other hand, the malic acid content of water kefirs reached their maximum concentration at 24 h of fermentation, similar to Tu et al. [16] (Table 4). The lactic acid and acetic acid contents of our samples increased during the fermentation. Primarily, the fig-based medium's lactic acid content increased significantly during the 24-h and 48-h fermentation period ($p < 0.05$). The water extract of fig provided preferable nutrients for LAB growth (Table 2). However, the concentration of acetic acid was higher than lactic acid. This may be related to the consumption of lactic acid by AAB during fermentation. AAB's presence during the fermentation was confirmed by also detecting high ethyl acetate concentration (Table 5). Our samples' lactic acid and acetic acid contents were compatible with those in previous studies [2, 8, 11, 22, 28, 34]. However, our fig-based water kefir had a higher acetic acid concentration at 48 h ($p < 0.05$)

Table 5 Antioxidant capacity analyses results of the samples

Sample	TEAC (mmol TE/L)	ORAC (μmol TE/mL)
BS 0	0.01 ± 0.00^{a}	0.00 ± 0.00^{a}
BS 24	0.01 ± 0.00^{a}	0.02 ± 0.01^{a}
BS 48	0.01 ± 0.00^{a}	0.05 ± 0.00^{a}
INP 0	0.58 ± 0.28^{b}	1.65 ± 0.02^{b}
INP 24	0.85 ± 0.02^{c}	2.04 ± 0.02^{c}
INP 48	1.00 ± 0.07^{c}	1.96 ± 0.05^{d}

*The different superscripts in the same column show a significant difference among the samples ($p < 0.05$). Results were expressed as mean ± standard deviation

than other vegetables (carrot, fennel, melon, onion, tomato) and fruit (apple, strawberry, quince, grape, kiwifruit, prickly pear, and pomegranate) juices-based kefirs in other studies [11, 12]. The existence of acetic acid in addition to lactic acid implies that homofermentative and heterofermentative LAB are present together in our samples. Also, acetic acid contributes to the sensorial quality and acceptability of the product and naturally protects the product from undesirable microorganisms [12].

Acetic acid is one of the major end products for water kefir. Its amount was related to boosting AAB found in grain because of yeast-oriented ethanol production and the amount of sucrose converted into fermentation metabolites [8, 22]. Our samples' organic acid concentrations increased except for tartaric and citric acid, depending on the fermentation time. In general, the total soluble solid content decreased due to microorganisms' fermentation and metabolic activity. Thus, the formation of organic acids and an increase in their concentration were expected results [26]. Moreover, malic acid and acetic acid were two dominant organic acids at the beginning of the fig-based fermentation medium, as reported by Pande and Akoh [40] and Slatnar et al. [39]. According to our results, the substrate type significantly impacted the formation of organic acids during the fermentation ($p < 0.05$).

Antioxidant capacities of water kefirs

Some LAB such as *Lactobacillus*, *Lactococcus*, *Leuconostoc,* and *Streptococcus* sp. can scavenge reactive oxygen species, thus increasing the antioxidant capacity of the foods [15]. Natural phenols and antioxidant substances' stability may change depending on pH. For this reason, the content and structure of phenolic substances are affected by alterations of pH along the fermentation process, which impacts the antioxidant capacity of the foodstuffs [41]. During fermentation, the pH values of the samples change, and these changes possibly contribute to the antioxidant activity of the samples. Moreover, the microbial activity stimulated by water kefir fermentation causes hydrolysis of microbial enzymes, breaking the substrate cell wall structure and releasing diverse bioactive compounds. Furthermore, microorganisms metabolize these bioactive compounds to convert more simplified compounds to increase their bioactivities [41]. It was postulated that water kefir antioxidant activity is related to the bioactive compounds found in its grain exopolysaccharide structure, specifically produced throughout fermentation.

According to TEAC analysis results, no changes were observed for sugar-based water kefir samples (0.01 mmol TE/L), but a significant increase was observed for fig-based water kefir samples during the fermentation period (from 0.58 to 1.00 mmol TE/L) ($p < 0.05$) (Table 5). On the other hand, the antioxidant capacity increased at the end of fermentation for both samples, according to ORAC results. It was changed from 0.00 to 0.05 µmol TE/mL for sugar-based water kefir samples and 1.65 to 1.96 µmol TE/mL for fig-based water kefir samples. In general, our results were compatible with previous studies [28, 42]. This is because the antioxidant activity of foods is changeable depending on the type of LAB, the type of fermentable sugar in the environment, and the radical scavenging capacity of cellular or intracellular extracts [10].

Volatile compounds

Changes in pH values significantly impacted the product's volatile compounds [10]. Also, sugars were metabolized and new substances were formed such as exopolysaccharides, ethanol, carbon dioxide, lactic acid, glycerol, acetic acid, mannitol, and various aromatic compounds by water kefir grain microbiota as a result of the metabolic activity of microorganisms [38].

The volatile compounds formed in water kefirs are given in Table 6. The primary volatiles of sugar-based water kefir samples were acetaldehyde, ethyl acetate, ethanol, benzene, 1,2,3-trimethyl-, acetic acid, 2-ethyl-1-hexanol, oxime-, methoxy-phenyl- and phenylethyl alcohol. On the other hand, primary volatiles of fig-based water kefir samples were acetaldehyde, ethyl acetate, ethanol, isobutylalcohol, isoamyl alcohol, hexanoic acid, ethyl ester, 1-hexanol, 3-buten-1-ol, 3-methyl-, acetoin, ethyl lactate, acetic acid, 1-octanol, furfuryl alcohol, 2-ethyl-1-hexanol, 2,3-butanediol, 2-furanmethanol, benzyl alcohol, and phenylethyl alcohol. The ethanol concentration of sugar-based water kefir reached its maximum after 48-h fermentation from 105.04 mg/L to 858,76 mg/L. A similar trend was observed for the fig-based water kefir sample. INP 48 sample had the highest ethanol concentration (15 g/L). It was reported that the ethanol content of water kefir was as high as 38 g/L after 7–10 days of fermentation. Moreover, its ethanol content was measured as 16 g/L even after 48-h fermentation (Rabl et al. [43]). Similarly, Laureys et al. [22] reported a 7.65–20.60 g/L ethanol concentration for various water kefir beverages. Ethanol is mainly produced via the activity of yeasts [16].

The acetaldehyde concentrations of sugar-based water kefir were 2.52 and 5.21 mg/L after 24- and 48-h fermentation periods (Table 6). In the same time frame, fig-based water kefir contained 14.49 and 3.11 mg/L acetaldehyde, respectively. Generally, *Lactobacillus* sp. can convert acetaldehyde to ethanol along with fermentation [11]. In our samples, this trend was observed, especially for fig-based water kefir samples. Ethyl acetate concentrations of sugar-based and fig-based water kefirs were 6.77 and 150.72 mg/L after 48-h fermentation. On the other hand, the acetic acid concentration of sugar-based and fig-based water kefir samples reached their maximum concentration after 48-h

Table 6 Volatile compounds of the samples

Volatile compounds	RI*	BS0 mg/L	BS24	BS48	INP0	INP24	INP48
Acetaldehyde	711	n.d	2.52 ± 1.89	5.21 ± 4.15	3.51 ± 0.70	14.49 ± 5.50	3.11 ± 1.11
Ethyl acetate	856	n.d	7.54 ± 3.36	6.77 ± 0.07	2.53 ± 1.52	112.53 ± 41.69	150.72 ± 22.22
Ethanol	905	105.04 ± 55.27	674.27 ± 106.91	858.76 ± 482.91	136.42 ± 57.80	10,950.51 ± 2339.84	14,496.59 ± 1902.78
2,3-butandione	937	n.d	n.d	n.d	8.29 ± 2.75	n.d	4.13 ± 1.79
Butanoic acid, 2-methyl-, ethyl ester	1004	n.d	3.23 ± 2.21	n.d	n.d	2.14 ± 0.04	n.d
Isobutyl alcohol	1053	n.d	n.d	n.d	n.d	58.42 ± 9.11	99.31 ± 12.66
Isoamyl alcohol	1170	n.d	n.d	n.d	n.d	1340.09 ± 347.68	n.d
Ethyl valerate	1110	n.d	n.d	n.d	n.d	2.14 ± 0.49	n.d
Ethyl heptanoate	1301	n.d	n.d	n.d	n.d	3.01 ± 2.46	3.06 ± 1.20
1-butanol, 3-methyl-, acetate	1098	n.d	n.d	n.d	n.d	n.d	4.15 ± 0.83
Hexanoic acid, ethyl ester	1200	n.d	n.d	n.d	n.d	15.47 ± 2.48	6.63 ± 1.19
1-Hexanol	1318	n.d	n.d	n.d	14.93 ± 3.78	75.74 ± 9.81	n.d
Benzene, 1,2,3-trimethyl-	1209	1.17 ± 0.74	1.43 ± 0.90	1.61 ± 0.05	2.37 ± 0.48	n.d	2.03 ± 0.49
Hexadecanoic acid, 2-methyl-, methyl ester	1356	n.d	n.d	1.66 ± 0.88	n.d	n.d	n.d
Heptyl isobutyl ketone	1363	n.d	n.d	1.28 ± 0.19	n.d	n.d	n.d
3-Buten-1-ol, 3-methyl-	1214	n.d	n.d	n.d	n.d	1.46 ± 0.48	42.35 ± 31.26
Hexanal	1040	n.d	n.d	n.d	1.98 ± 0.64	n.d	n.d
Acetoin	1255	n.d	n.d	n.d	2.95 ± 0.09	9.31 ± 1.24	10.17 ± 2.62
Ethyl lactate	1310	n.d	n.d	n.d	n.d	3.00 ± 1.30	13.80 ± 1.47
Ethyl octanoate	1401	n.d	n.d	0.68 ± 0.13	n.d	7.12 ± 2.48	5.01 ± 1.20
Octanoic acid, 2-methyl ester	1349	2.99 ± 0.45	n.d	n.d	n.d	n.d	n.d
Acetic acid	1415	3.16 ± 0.29	3.06 ± 1.04	5.83 ± 2.05	5.35 ± 2.20	45.08 ± 5.24	191.81 ± 13.32
Furfural	1433	n.d	n.d	n.d	11.79 ± 4.14	n.d	n.d
1-Octanol	1514	n.d	n.d	n.d	8.95 ± 3.49	53.53 ± 21.46	47.98 ± 5.55
Furfuryl alcohol	1618	n.d	n.d	n.d	2.82 ± 1.60	54.08 ± 28.17	31.35 ± 2.86
Diethyl succinate	1641	n.d	n.d	n.d	n.d	3.68 ± 1.96	1.86 ± 1.23
Benzene, 1,2-dimethoxy-	1690	n.d	n.d	n.d	1.65 ± 0.80	1.86 ± 0.57	n.d
2-Ethyl-1-hexanol	1454	30.62 ± 14.48	22.68 ± 2.18	41.01 ± 27.26	20.97 ± 10.75	104.67 ± 12.84	184.85 ± 1.39
Benzaldehyde	1491	n.d	n.d	n.d	16.70 ± 6.91	n.d	n.d
Propanoic acid	1503	n.d	n.d	n.d	n.d	n.d	1.48 ± 0.17
2,3-Butanediol	1541	n.d	n.d	n.d	7.18 ± 1.91	n.d	49.02 ± 29.78
1-Ethyl-2-formyl pyrrole	1571	n.d	n.d	n.d	1.09 ± 0.69	n.d	n.d
1-Decene	1615	n.d	n.d	n.d	0.81 ± 0.64	n.d	n.d
Isobutyric acid	1531	n.d	n.d	n.d	n.d	n.d	1.41 ± 0.87
Ethyl decanoate	1594	n.d	n.d	n.d	n.d	n.d	1.43 ± 0.62
Isovaleric acid	1625	n.d	n.d	n.d	n.d	n.d	2.92 ± 0.23
Oxime-, methoxy-phenyl-	1721	3.64 ± 0.91	5.01 ± 1.94	3.42 ± 2.41	7.06 ± 2.59	3.55 ± 1.12	8.14 ± 3.62
Ethylphenyl acetate	1746	n.d	n.d	n.d	n.d	1.11 ± 0.48	n.d
Tetrasiloxane, decamethyl-	1769	n.d	n.d	n.d	2.93 ± 0.58	2.74 ± 0.66	3.75 ± 0.43
Damascenone	1784	n.d	n.d	n.d	n.d	2.38 ± 1.00	n.d

Table 6 (continued)

Volatile compounds	RI*	BS0 mg/L	BS24	BS48	INP0	INP24	INP48
Hexanoic acid	1809	n.d	n.d	n.d	n.d	5.49 ± 2.19	n.d
Benzyl Alcohol	1837	n.d	n.d	n.d	9.80 ± 2.55	58.32 ± 27.16	65.13 ± 13.13
Benzenepropanoic acid, ethyl ester	1841	n.d	n.d	n.d	0.88 ± 0.75	n.d	n.d
Benzaldehyde, 4-propyl-	1794	n.d	0.74 ± 0.21	n.d	n.d	n.d	n.d
Phenylethyl alcohol	1878	n.d	9.85 ± 3.85	16.11 ± 9.52	9.41 ± 2.44	239.45 ± 42.75	418.03 ± 242.96
Octanoic acid	2016	n.d	n.d	n.d	n.d	n.d	9.35 ± 0.84

Experiments were carried out with two replications and four parallels. Values were given as mean ± standard deviation

*Retention index (Kovat index) based on C20M column

**n.d: not detected

fermentation at 5.83 and 191.81 mg/L, respectively. Similar to our results, Laureys and De Vuyst (2014) reported that negligible amounts of ethyl acetate and acetic acid were formed besides ethanol during fermentation. Also, our samples' acetic acid and ethyl acetate concentrations increased at the end of fermentation. It was reported that an increase in acetic acid concentration resulted in a loss of fruity flavor and contributed to a harsh acidic taste and aroma [44]. However, acetic acid may produce a pleasant aroma and flavor [4]. Also, ethyl acetate caused a solvent-like aroma [44]. On the other hand, ethyl octanoate was detected at 7.12 mg/L and 5.01 mg/L for fig-based samples after 24-h and 48-h fermentation, respectively. Ethyl decanoate was 1.43 mg/L from the fig-based water kefir sample after 48 h of fermentation. Similar ethyl octanoate and ethyl decanoate concentrations for water kefir were reported by Laureys et al. [23]. While ethyl octanoate contributed to the fruity aroma of the samples, ethyl decanoate contributed to the floral aroma [34]. 2-Ethyl-1-hexanol was one of the critical aromatic compounds for our water kefir samples. Its amount changed from 30.62 to 41.01 mg/L and 20.97 to 184.85 mg/L after 48-h fermentation for sugar-based and fig-based water kefir samples, respectively. Moreover, 2-ethyl-1-hexanol contributed to a slightly floral-rosy odor [45]. Some samples contained 2,3-butanediol produced by LAB via the butanediol fermentation pathway [11]. On the other hand, the amount of phenyl ethyl alcohol increased throughout the fermentation period of water kefir samples (Table 6). It was probably produced via the metabolic activity of yeasts found in water kefir grains [46]. It contributes to a rose-like odor of the samples [47].

Sensorial properties of water kefirs

Water kefir samples were analyzed for their organoleptic properties. Similar sensorial properties were attributed to both sugar-based water kefir samples (Table 7). According to our results, the sugar-based medium was not a preferred for water kefir grains. As a result of this phenomenon, fermentation metabolites were detected in lower concentrations. Fortification with fig resulted in higher sensorial scores. For this reason, alcohol flavor, sourness, and astringency had low scores from sensorial analysis results ($p < 0.05$), which were in accordance with aroma and volatile profiles (Table 6). In addition, they had substantially lower ethanol content than fig-based water kefir samples ($p < 0.05$). Sugar-based water kefir may be classified as a soft drink with floral and mildly alcoholic notes. The overall acceptance for sugar-based water kefir samples was scored at about 5.5. This means that these kefir

Table 7 Sensory analysis of water kefir

Parameters	BS24	BS48	INP24	INP48
Foaminess	1.20 ± 0.45^{a}	0.75 ± 0.50^{a}	1.81 ± 0.95^{b}	2.00 ± 0.89^{b}
Color	8.93 ± 1.38^{a}	8.93 ± 1.44^{a}	7.33 ± 1.15^{b}	5.50 ± 2.14^{c}
Turbidity	3.80 ± 1.64^{a}	4.29 ± 0.49^{a}	4.92 ± 1.68^{b}	8.00 ± 1.91^{c}
Consistency	1.67 ± 0.79^{a}	1.80 ± 0.45^{a}	3.50 ± 2.59^{b}	6.20 ± 2.48^{b}
Fermented odor	2.71 ± 1.68^{a}	3.55 ± 2.16^{a}	7.17 ± 1.11^{b}	8.00 ± 2.24^{b}
Sourness	1.57 ± 0.79^{a}	2.33 ± 1.58^{a}	5.64 ± 2.34^{b}	8.67 ± 1.25^{c}
Sweetness	5.00 ± 2.80^{a}	3.93 ± 2.16^{a}	7.00 ± 1.70^{b}	8.50 ± 1.50^{b}
Fruity flavor	2.29 ± 1.25^{a}	3.88 ± 2.53^{a}	7.18 ± 2.40^{b}	6.17 ± 3.85^{b}
Alcohol	3.21 ± 2.46^{a}	3.07 ± 2.46^{a}	5.55 ± 1.81^{b}	7.33 ± 2.36^{b}
Refreshing	4.21 ± 2.39^{a}	4.29 ± 2.52^{a}	5.25 ± 2.22^{a}	6.00 ± 2.52^{a}
Astringency	1.25 ± 0.50^{a}	1.00 ± 0.00^{a}	5.25 ± 2.09^{b}	8.33 ± 1.89^{c}
Overall assessment	5.50 ± 2.10^{a}	5.29 ± 2.02^{a}	7.27 ± 1.79^{a}	7.33 ± 2.05^{a}

*The different superscripts in the same raw show a significant difference among the samples ($p < 0.05$). Results were expressed as mean ± standard deviation. 1: lowest point e.g., lowest acceptability, 10: highest point e.g., highest acceptability

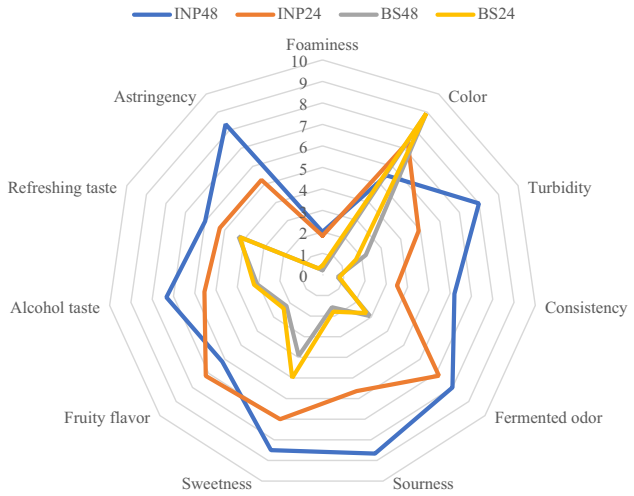

Fig. 1 Sensorial acceptance of samples

samples were preferable above average (Fig. 1). On the other hand, according to our results, the fig was a preferable medium agent for water kefir grains. Fermentation of water kefir grains within a fig-based medium was well continued. These samples had more fruity flavors and aromatic compounds than sugar-based water kefir samples (Table 6). For this reason, these water kefir samples had higher organoleptic scores. The acceptability of fig-based kefir samples was slightly higher than that of sugar-based samples without a significant difference ($p > 0.05$). It was probably due to the higher sweet-acidic taste of fig-based samples, which is a desirable property for most fermented products [35]. Generally, color was acceptable for all samples, except INP48, as a result of probably its high turbidity value ($p < 0.05$). In contrast to Tu et al. [16], 24-h fermented water kefir beverage (INP 24) was a preferable sample to a 48-h fermented water kefir beverage (INP 48). For this reason, 24-h fermentation is good enough to obtain fig-based water kefir beverage with characteristic sensorial properties.

Conclusion

Water kefir grains are a valuable source of bioactive compounds and fermentation metabolites depending on the preferred fermentation medium. In this study, sugar and fig as substrates in water were used to understand the nature of water kefir grains fermentation. According to our results, the fig-based medium was preferable for microbiota growth in water kefir grains. However, the sugar-based medium was not acceptable for fermentation of water kefir grains. Also, a 24-h fermentation time resulted in the optimum length for water kefir fermentation in microbiological counts, fermentation metabolites, and sensory analysis.

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Data availability The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Conflict of interest The authors declare that there is no conflict of interest.

Compliance with ethical requirements The authors declare this study complies with all ethical requirements.

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