GIDA
THE JOURNAL OF FOOD
E-ISSN 1309-6273, ISSN 1300-3070

Research/Araştırma
GIDA (2023) 48 (4) 715-727
doi: 10.15237/gida.GD23039

PHYSICOCHEMICAL, MICROBIOLOGICAL AND SENSORY ANALYSES OF FUNCTIONAL DETOX JUICES FERMENTED WITH WATER KEFIR GRAINS

Ayca Gülhan*
Department of Food Technology, Vocational School of Technical Sciences, Aksaray University, Aksaray, Türkiye

Received /Geliş: 25.03.2023; *Accepted* / Kabul: 14.06.2023; *Published online* / Online baskı: 21.06.2023

Gülhan, A. (2023). Physicochemical, microbiological and sensory analyses of functional detox juices fermented with water kefir grains. GIDA (2023) 48 (4) 715-727 doi: 10.15237/ gida.GD23039

Gülhan, A. (2023). Su kefir taneleri ile fermente edilmiş fonksiyonel detoks sularının fizikokimyasal, mikrobiyolojik ve duyusal analizleri. GIDA (2023) 48 (4) 715-727 doi: 10.15237/ gida.GD23039

ABSTRACT
In this study, detox juice prepared from green fruit and vegetable juices was fermented with water kefir grains at 25 ℃ for 48 hours and stored at 4 ℃ for 6 days. The samples had pH values of 3.41-3.97, titratable acidity of 0.196-0.495 g/100 mL, and brix values of 8%-10.4%. The 0th day samples had less phenolic substance than the control (861.26±0.24 mg GAE/L) (P<0.05). DPPH (82.2±0.19%) and CUPRAC (1.18±0.05 mmol Trolox/g) were the highest on the 6th day of storage. At the end of fermentation, an increase in color values for L* and b* and a decrease in a* value were determined (P<0.05). The microorganism loads rose from the 2nd to the 6th day of storage. Green fruit and vegetable juices may be utilized to make water kefir, an innovative functional beverage for vegetarians and vegans who can not consume probiotic dairy products.
Keywords: Water kefir, detox juice, fermentation, microbiology, sensory analyses

SU KEFİR TANELERİ İLE FERMENTE EDİLMİŞ FONKSİYONEL DETOKS SULARININ FİZİKOKİMYASAL, MİKROBİYOLOJİK VE DUYUSAL ANALİZLERİ

ÖZ
Bu çalışmada, yeşil meyve ve sebze sularından hazırlanan detoks suyu, su kefiri taneleri ile 25 ℃ 'de 48 saat fermente edilmiş ve 4 ℃ 'de 6 gün depolanmıştır. Örneklerin pH değerleri 3.41-3.97, titre edilebilir asitlik değerleri 0.196-0.495 g/100 mL, brix değerleri %8-%10.4 arasında bulunmuştur. Toplam fenolik madde miktarı kontrol (861.26±0.24 mg GAE/L) ile karşılaştırıldığında 0. gün (798.41±0.32 mg GAE/L) numunelerinde istatistiksel olarak azalmıştır (P<0.05). DPPH (%82.2±0.19) ve CUPRAC (1.18±0.05 mmol Trolox/g) depolamanın 6. gününde en yüksekti. Fermantasyon sonunda L* ve b* için renk değerlerinde artış, a* değerinde azalma belirlenmiştir (P<0.05). Depolama süresinin 2. gününden 6. gününe kadar belirlenen mikroorganizma yükleri log KOB/mL olarak artmıştır. Probiyotik süt ürünlerini tüketemeyen vejetaryenler ve veganlar için yenilikçi fonksiyonel bir içecek olan su kefiri yapımında yeşil meyve ve sebze suları kullanılabilir.
Anahtar kelimeler: Su kefiri, detoks suyu, fermantasyon, mikrobiyoloji, duyusal analizler

* *Corresponding author* / Yazışmalardan sorumlu yazar
✉: aycakucukcuban@aksaray.edu.tr ☎: +90 (554) 309 9237
Ayca Gülhan; Orcid ID: 0000-0002-3435-7767

INTRODUCTION

Foods containing probiotics are among the most popular functional foods preferred by consumers due to their benefits to human health (Lillo-Perez et al., 2021). Most probiotic foods produced consist of fermented milk products such as yogurt. However, factors such as lactose intolerance increased allergies to milk proteins, high cholesterol content, and an increase in consumers adopting vegetarian or vegan diets are limiting the consumption of probiotic foods obtained from milk and its products (Rezaei and Koohsari, 2021; Chielle et al., 2022). For these reasons, the worldwide importance of non-dairy probiotic products is increasing daily (Corona et al., 2016). Fruits and vegetables are foods rich in carbohydrates, minerals, vitamins, dietary fibers, carotenoids, and phenolic compounds with antioxidant activity (Panghal et al., 2017; Silva et al., 2020). They also contain various prebiotics, such as inulin, fructooligosaccharides, or galactooligosaccharides, that support probiotic growth (Do and Fan, 2019). Because of these properties, fruits and vegetables can be used as suitable substrates for non-dairy probiotics (Rezaei and Koohsari, 2021). Mixed fruit and vegetable-based beverages, marketed as "detox", are part of popular detoxification diets favored by consumers looking for healthy food (Fraga et al., 2020; Silva et al., 2020). Phytochemicals found in detox beverages and probiotics have protective effects on cardiovascular risk factors (Chielle et al., 2022). There are studies on adding probiotics to mixtures produced using different fruits and vegetables (Di Cagno et al., 2011; Sharma and Mishra, 2013, Simsek et al., 2014, Panghal et al., 2017, Cui et al., 2019, Yang et al., 2020). Within non-dairy fermented beverages, water kefir is a carbonated, slightly acidic beverage produced by fermenting a solution of sucrose to which fresh or dried fruit has been added with water kefir grains (Corona et al., 2016, Lynch et al., 2021). Kefir grains contain predominantly lactic acid bacteria (LAB) and yeasts in a polysaccharide matrix named kefiran. Water kefir has beneficial effects on health, such as the ability to change the gut microbiota composition and activity (Ayed et al., 2020). The starter culture consists of LAB, the genera of *Leuconostoc*, *Lactobacillus*, *Streptococcus*, and *Lactococcus,* and yeasts the genera of *Saccharomyces*, *Zygosaccharomyces spp*., *Dekkera*, *Candida, Kluyveromyces*, and *Pichia*. In addition, acetic acid bacteria were also isolated due to oxygen (Ayed et al., 2020, Lynch et al., 2021). There are studies on some fruit and vegetable juices fermented with water kefir (Corona et al., 2016, Randazzo et al., 2016, Bueno et al., 2021, Ozcelik et al., 2021). However, no study has been found in the literature on the fermentation of fruit and vegetable juice mixtures with water kefir, which can show detox properties. Consumption of fruit and vegetable-based probiotic beverages commercially produced abroad is quite common. In recent years, the production of beverages that can also show detox properties by using different fruits and vegetables has increased in our country. It is important to investigate the activities of water kefir microorganisms as a fermentation medium in fruit and vegetable juice mixtures with detox effect. This study aimed to add probiotic food properties as a result of fermentation by adding water kefir microorganisms to the prepared detox juices. In addition, physicochemical and microbiological changes in the samples were determined at the end of fermentation and during storage, and the sensory acceptability of the products was evaluated.

MATERIALS AND METHODS

Materials

The fruits and vegetables used in the preparation of green fruit and vegetable juices and sucrose were obtained from a local market in Aksaray/Turkey. The water kefir grains were obtained from Danem Dairy Products (Isparta, Turkey).

Preparation of green detox juice

For the production of beverages, fruits and vegetables were effectively washed after being sorted. Green detox juices were obtained separately using a juicer (Vestel NS3100). The formulation of green fruit and vegetable (detox) juice was chosen as a result of the researches made by examining the components of detox juices of different brands produced in the market and considering the nutritional value in the literature. In the preparation of the detox juice, 30% mint

juice, 20% cucumber juice, 20% spinach juice, 15% lettuce juice and 15% apple juice were used. For the growth of microorganisms in water kefir grain and the formation of fermentation products, sucrose was added at a rate of 3% as a carbon source (Lynch et al., 2021). Prepared fruit and vegetable juices were pasteurized at 80 °C for 5 min (Rezaei and Koohsari, 2021) in a water bath (Thermomac WB15). Then pasteurized juices wereimmediately cooled to 25 °C for microbial inoculation. 3 g of water kefir grains were added to the prepared 500 mL detox juice and mixed. Then the prepared mixture was incubated at 25 °C for 48 hours under anaerobic conditions (Corona et al., 2016). Fermentation was not applied to the control (C) samples that did not contain water kefir grains. The shelf life of fruit and vegetable juice mixtures (detox juices) that do not contain any additives or preservatives sold commercially is between 3 and 5 days. For this reason, the products obtained were stored at 4 °C for 6 days. Samples were taken on at the end of the fermentation, 2nd, 4th and 6th days and examined in terms of physical, chemical, microbiological and sensory properties.

pH analysis

The pH values of the prepared beverages were measured at 20 °C using a pH meter (Seven Easy GMBH 8603, Switzerland) (AOAC, 2007).

Titratable acidity (TA) analysis

The TA of the samples was determined by the titration method followed by a pH meter. For this purpose, samples were titrated with 0.1 N NaOH up to pH 8.1. TA was calculated from the amount of spent NaOH and expressed as "g citric acid/100 mL detox juice" (AOAC, 2007).

Total soluble solid (°Brix) analysis

The total soluble solid (°Brix) of the samples were measured using a refractometer (Atago, PAL-3, Japan) (AOAC, 2007).

Color analysis

Color measurements of the samples were made with a Konica Minolta brand (CR-400, MINOLTA Co., Osaka, Japan) color measuring device. Lightness (*L**), redness (*a**), yellowness (*b**) and the index of total color difference (ΔE) were detected (Do and Fan, 2019).

Serum separation analysis

The colloidal stability of the samples was determined by a phase separation analysis during the storage period (0, 2, 4 and 6 days) at 4 °C. For this purpose, 50 mL detox juice samples were placed in 50 mL measuring cylinder and the volumes of the separated phases were read out (Bernat et al., 2014).

Determination of carbon dioxide amount

Carbon dioxide was calculated by indirect method based on weight loss before and after fermentation (Ozcelik et al., 2021).

DPPH radical scavenging activity

The ability of samples to scavenge free radicals was assessed using a modified version of the DPPH technique reported by Brand-Williams et al. (1995). 20 µL of the diluted sample (0.5 mg/mL) was added to 180 µL of a 0.2 mM methanolic DPPH solution in a 96-well microplate (Micro Well, Thermo Fisher Scientific, France), and the mixture was left to stand. Thermo Fisher Scientific's Multiskan Go, F1-01620 microplate reader was used to measure the absorbance at 517 nm, which is the wavelength where the most DPPH is absorbed. After 25 min of incubation at room temperature (15-20 °C), the Asample and Ablank without extract were measured. DPPH inhibition was calculated as:

Equation 1: % inhibition=100 x (Ablank - Asample) / Ablank

CUPRAC assay

500 µL of $CuCl_2$ solution and 500 µL of $C_2H_7NO_2$ (1 M pH: 7.0) solution were placed in test tubes for this investigation. Each tube received 500 µL of neocuproin ($C_{14}H_{12}N_2$) (7.5×10^{-3} M) solution. On it was poured 100 µL of lyophilisate solution at a concentration of 1 mg/mL and 550 µL of distilled water was added. The extract was replaced with distilled water in the blank samples. It was incubated for 30 min at room temperature and in a 50 °C water bath. The absorbance at 450 nm was measured in comparison to a blank sample, and ascorbic acid served as a reference (Apak et al., 2006).

Determination of total phenolic compounds
Spectrophotometric techniques were used to evaluate the samples' total phenolic compund (TPC) levels. In order to test the detox juices samples at 760 nm, distilled water (7.9 mL), Folin-Ciocalteu reagent (0.5 mL), and 20% Na_2CO_3 (1.5 mL) were combined. The mixture was then maintained at 25 ℃ 2 hours Three measurements were obtained concurrently using gallic acid as the standard and expressed as mg GAE/L (Singleton and Rossi, 1965).

Microbiological analyses
Dilutions between 10^{-1} and 10^{-7} were prepared from the samples, and analyzes were carried out by sowing by pour-plate method into empty petri dishes in which 1 mL was planted. Plate count agar (PCA) was used for the aerobic mesophilic count (TMC) and incubated aerobically at 30 ℃ for 72 h. Total LAB analysis was performed using MRS (De Man Rogosa Sharp Agar) and M17 Agar. Incubation conditions are anaerobically at 30 ℃ for 48 h. To perform the yeast analysis, DRBC (Dichloran Rose Bengal Chloramphenicol) agar was used and incubated aerobically at 25 ℃ for 48 h. Count plates were performed in duplicate. The microbial population was expressed as log CFU/mL (Randazzo et al., 2016).

Sensory quality
Green fruit and vegetable juices fermented with water kefir microorganisms and unfermented C samples were served to the panelists for sensory evaluation. Before the sensory evaluation, the panelists were informed about fruit and vegetable-based beverages and water kefir. Sensory analysis of the samples was carried out by 8 panelists using a 9-point hedonic scale, ranging from 1 to 9 (9 = like extremely and 1 = dislike extremely). Samples were evaluated for appearance, aroma, consistency, taste, mouth feel, and overall acceptability (Panghal et al., 2017).

Statistical Analysis
Minitab version 21.3 software was used for statistical analysis of the obtained data. All results are given as means (SEM) obtained from three independent experiments ($n \geq 3$). At least three replications were made for each sample in the study. Variability between means of results was determined by ANOVA and Tukey's multiplex analysis of variance. Signifcance levels were considered statistically signifcant for $P \leq 0.05$.

RESULTS AND DISCUSSION

Properties of green detox juice
pH, one of the most important parameters affecting food fermentation, is directly related to the structural changes of microbiota and phytochemicals during fermentation. The stability of various phenolic components in detox juice may vary depending on pH. They are very unstable in alkaline solutions but stable in acidic solutions. Storage time and temperatures may affect the metabolic activities of some bacteria and yeast species in a water kefir medium. It may result in lowering of pH and Brix and an increase in TA (Bueno et al., 2021). The changes in pH, TA, °Brix, and color properties of the prepared green detox juice are given in Table 1. The pH value of the prepared detox juice before fermentation was 3.97, the titratable acidity value was 0.196 g citric acid/100 mL, and the brix value was 10.4%. The L^*, a^*, b^*, and ΔE values of the detox juice were 28.58±0.02,-4.30±0.03, 6.55±0.06 and 53.52±0.02, respectively. Fraga et al. (2020) calculated the pH value of Green (Detox) juice prepared from orange juice, cabbage, mint, ginger, and cucumber as 4.27, the total titratable acidity as 163.57 mg citric acid/100 mL, and the brix value as 4.9 g/100 g. Di Cagno et al. (2011) stated that brix value was 10.8 green smoothies prepared using kiwifruits (40%, w/w), fennels (7%, w/w), spinach (8%, w/w) and papaya (15%, w/w). The pH values of commercially available detox beverages were found to be between 3.02 and 4.63, soluble solids (brix) between 4.23 and 11.76, and acidity (%) between 0.10 and 0.53 (Silva et al., 2020). The pH value of green fruit and vegetable juices decreased to 3.41 after 48 h of fermentation with water kefir microorganisms. The pH values of the samples taken on the 2nd, 4th and 6th days of storage were measured as 3.42, 3.43 and 3.45, respectively (Table 1). No significant change was observed in pH values during storage (P>0.05). Corona et al. (2016) stated that the pH of the water kefir-like

beverages from carrot, melon, onion, tomato, strawberry, and fennel juices ranged from 3.6 to 5.0. It was stated that the pH values of water kefir beverages produced from different fruit juices (cornelian cherry, hawthorn, roseship, pomegranate, red plum) varied between 3.11-3.65 during 28 days of storage (Ozcelik et al., 2021). TA value of green fruit and vegetable juices increased to 0.455 g citric acid/100 mL at the end of fermentation. On the second day of storage, this value increased to 0.495 g citric acid/100mL (Table 1). In the data revealed by some research results, regular increases in titratable acidity values were detected after the fermentation of fruit and vegetable juices with water kefir microorganisms and during storage (Corona et al., 2016; Randazzo et al., 2016; Ozcelik et al., 2021). It can be said that factors such as the components of the raw materials used in the production of water kefir, the microbial population in the water kefir, the storage time change the pH and titratable acidity values of the beverages. After the fermentation of water kefir, there was a decrease in the brix values. While the brix value of fresh detox juices was 10.4, this value decreased to 8.6 at the end of fermentation. Brix value showed no statistically significant (P>0.05) change during storage (2^{nd}-6^{th} days). During the fermentation, fermentable sugars are converted to ethyl alcohol and CO_2. Therefore, the sugar contents of beverages are decreased after fermentation. It can be said that there is a decrease in brix values due to the decrease in sugar (Corona et al., 2016; Ozcelik et al., 2021). Randazzo et al. (2016) stated that the brix value before fermentation was 12.03% in apples, 14.93% in grapes, 11.73% in kiwi, 15.73% in pomegranate, 14.07 in prickly pear, 11.67 in quince, and after fermentation %8.70, 8.47%, 9.97%, 9.37%, 9.67% and 5.87, respectively. Ozcelik et al. (2021) determined that brix values of cornelian cherry, hawthorn, roseship, pomegranate, and red plum juices were 10.92, 10.85, 11.42, 12.80 and 9.85, respectively. At the end of 48 h of fermentation with water kefir microorganisms, these values decreased to 8.94, 9.16, 7.36, 7.34, and 7.38. Do and Fan (2019) stated that the pH value of the vegetable juice mixture (jicama, winter melon, and carrot) before fermentation with *Lactobacillus plantarum* was 5.97, the brix value was 6.4, and the titratable acidity (% lactic acid) was 0.09. After 48 h of fermentation, these values changed to 3.40, 5.0 and 0.95. In this study, it is seen that the changes in pH, titratable acidity and brix values at the end of fermentation and during storage are consistent with other research results. In the scientific researches, it is thought that differences in factors such as used fruits or vegetables, microbial load of water kefir grains, fermentation and storage conditions caused different results in each fermented product Randazzo et al., 2016; Corona et al., 2016, Ozcelik et al., 2021). Changes in color values of fermented detox juices are shown in Table 1.

Table 1. Changes in physicochemical properties of fermented detox juice during 6 days of storage

Paramaters	Control	0^{th}	2^{nd}	4^{th}	6^{th}
pH	3.97±0.12^{a}	3.41±0.07^{b}	3.42±0.08^{b}	3.43±0.06^{b}	3.45±0.07^{b}
TA	0.196±0.17^{b}	0.455±0.31^{a}	0.495±0.34^{a}	0.493±0.32^{a}	0.494±0.33^{a}
°Brix	10.4±0.27^{a}	8.6±0.14^{b}	8.0±0.19^{b}	8.1±0.17^{b}	8.0±0.13^{b}
Colors					
*L**	28.58±0.02^{b}	32.57±0.01^{a}	32.49±0.02^{a}	32.79±0.01^{a}	32.87±0.01^{a}
*a**	-4.30±0.03^{b}	-4.66±0.02^{a}	-4.60±0.04^{a}	-4.75±0.01^{a}	-4.70±0.02^{a}
*b**	6.55±0.06^{b}	7.00±0.04^{a}	6.91±0.07^{a}	7.10±0.05^{a}	6.95±0.06^{a}
ΔE	53.52±0.02	52.59±0.01	52.70±0.01	52.37±0.01	52.37±0.01

^{a,b} Different superscripts within the same row demonstrate significant differences (P<0.05) (n=3±SD),$^{-}$ No significant difference(P>0.05), TA: g citric acid/100 mL, °Brix: %

As a result of the fermentation of prepared detox juices with water kefir microorganisms, an increase in lightness (L^*) and b^* values and a decrease in a* and the total color differences (ΔE) values were determined. The increase in L^* values indicates that the samples fade in color tone. The negative measure of a^* value is compatible with the green color of the beverages. As the storage time (from 2nd to 6th day) increased, the change in a^* value was found to be statistically insignificant ($P<0.05$). L^* generally increased after fruit juices (apple, grape, kiwifruit, pomegranate, prickly pear and quince) were fermented with water kefir microorganisms (Randazzo et al., 2016). Corona et al. (2016) reported significant differences in color parameters after carrot, fennel, melon, onion, tomato and strawberry juices were fermented with water kefir microorganisms. The total color difference varies between 2.94 (carrot) and 11.55 (fennel). The change in L^*, a^* and b^* values as a result of fermentation depending on the number of LAB in the prepared vegetable juice mixtures showed parallelism with the research results of Do and Fan (2019).

The carbon dioxide content of the detox juices calculated after fermentation was 3.78 g/100 mL. Ethanol and carbon dioxide, found in small amounts in water kefir beverages, are produced by the yeasts in the water kefir grains during fermentation (Lynch et al., 2021). It can be concluded that the decrease in the brix value at the end of fermentation is related to the breakdown of sugars and the formation of CO_2. It was stated that the CO_2 contents in water kefir beverages prepared from cornelian cherry, hawthorn, red plum, roseship, and pomegranate juices ranged from 1.41 to 4.10 g/100 mL (Ozcelik et al., 2021). After fermentation by water kefir microorganisms, the CO_2 amounts of carrot, fennel, melon, onion, tomato, and strawberry juices were 1.51, 0.87, 3.39, 0.14, 1.29, and 1.71, respectively (Corona et al., 2016). In fruit and vegetable-based beverages, solid and liquid phases are separated during storage. This separation should be stated quantitatively. It was determined that serum separation values of green fruit and vegetable juices tended to decrease during storage. On days 0, 2, 4, and 6 of storage, 7.5%, 10%, 17.5%, and 20% serum separation were observed, respectively.

This decrease was found to be statistically significant ($P<0.05$). In a study, Baysal et al. (2013) stated that while the serum separation in carrot and pumpkin juices was 10.12% and 8.50%, depending on the increase in fermentation time, these values changed as 40, 50 and 35% in carrot and pumpkin juice powders produced by drum drying. In another study, it was reported that serum separation value in fermented hazelnut milk stored at 4 °C was 11, 28% after 1 day of storage and 25.1% at the end of storage (Bernat et al., 2014).

Measurement of total phenolic content and antioxidant activities

The dietary trends recommended for the prevention of chronic diseases that are widespread in the world are to increase the intake of fruits and vegetables. Fruits and vegetables are food groups rich in biomolecules, vitamins, minerals, polyphenols, and phytochemicals. Consumption of fruit and vegetable juices enriched or fermented with probiotic supplements has been a popular topic recently. Lactic acid bacteria (LAB) can metabolize different substrates and produce biochemical modifications in the molecules in their content. In addition, it is thought that foods' nutritional properties, taste and health-related aspects can be improved with LAB fermentation (Karovicova et al., 2011). TPC changes in the samples taken during 6 days of storage of detox juices fermented in a water kefir medium are given in Table 2. According to the results obtained, it was determined that there was a statistical decrease ($P<0.05$) in the 0th day (798.41±0.32 mg GAE/L) samples compared to C (861.26±0.24 mg GAE/L). In line with the results of the current study, Corona et al. (2016) determined decreases in the total phenolic component content at the start of fermentation in some vegetable juice substrates (carrot, fennel, melon, onion, tomato and strawberry) that they fermented with water kefir. They explained that the most significant decrease was in fennel (49%). This was interpreted as the cloudiness of fruit and

vegetable juices at the beginning of fermentation and the decrease in the phenolic content and antioxidant activity of detox juice, which is high in bioactive components. Sabokbar et al. (2015) showed that the TPC of different beverages increased significantly during fermentation, in contrast to our results. They said that the TPC increase during fermentation might be related to the metabolic activities of microorganisms in kefir grains, which can alter the levels of bioactive components such as different phenolic compounds. Other researchers have reported that fermentation by lactic acid bacteria or other microorganisms can increase the level of total phenolic content (Dordevic et al., 2010; Coda et al., 2012). In addition, since the optimum pH is known to affect the release of enzymes derived from microorganisms, the difference in pH of different fermentations may be another reason for these results.

Table 2. Changes in TPC, CUPRAC, and DPPH of fermented detox juice during 6 days of storage

Storage time (day)	Antioxidant parameters		
	TPC (mg GAE/L)	CUPRAC (mmol Trolox/g)	DPPH (% inhibition)
C	861.26±0.24^{b}	1.01±0.06^{b}	65.4±0.35^{c}
0	798.41±0.32^{c}	0.91±0.09^{c}	53.9±0.31^{d}
2	820.39±0.27^{b}	1.04±0.07^{b}	68.5±0.29^{c}
4	823.57±0.21^{b}	1.04±0.07^{b}	74.3±0.37^{b}
6	927.62±0.13^{a}	1.18±0.05^{a}	82.2±0.19^{a}

$^{a.b.c.d}$ Different superscripts within the same row demonstrate significant differences (P<0.05) (n=3±SD).$^{-}$ No significant difference(P>0.05).

Hashemi et al. (2017) fermented it with *Lactobacillus plantarum* LS5 to produce a probiotic juice to preparesweet lemon juice. They stated that the samples showed decreases in their phenolic component capacity immediately after fermentation. They also found that phenolics and antioxidants decreased in fermented samples during storage. They attributed this to the fact that the bioactive substances in fruit juices contain highly unstable compounds at the beginning of fermentation and can degrade rapidly under storage conditions. Similarly, Othman et al. (2009) reported that the fermentation process caused a significant reduction (32-58%) in the total phenolics of olives. Parallel to these results, Mousavi et al. (2013) found that the concentration of phenolic compounds decreased during the fermentation of pomegranate juice while the antioxidant activity of fermented pomegranate juice increased. In the current study, it was determined that there was an increase in TPC values after day 0th and the highest TPC (927.62±0.13 mg GAE/L) was on day 6th (P<0.05). Fraga et al. (2020) determined the total phenolic content of the detox mixture they prepared with green juices and vegetables as 2833.60 mg GAE/g. Compared to the current study, the high phenolic content may be because it is not a fermented product or the difference in the products that make up the prepared green detox juice. In another study, Dani et al. (2007) reported a positive correlation between total phenol content and antioxidant activity for all samples before and after fermentation in their vegetables and juices. High antioxidant activity was noted, especially for the fermented beverage prepared from strawberries. One of the most essential mechanisms of antioxidant capacity tests is proton-radical capture. The results of DPPH and CUPRAC methods and antioxidant capacity tests in detox juice fermented with water kefir are given in Table 2. Zero-day results of the DPPH and CUPRAC tests were statistically decreased compared to C. These values were determined as 53.9±0.31% for DPPH and 0.91±0.09 mmol Trolox/g for CUPRAC at zero-day. The results showed statistical reductions at zero-day for DPPH (65.4±0.35%) and (53.9±0.31%), CUPRAC (1.01±0.06 mmol Trolox/g) and (0.91±0.09 mmol Trolox/g), compared with C

(P<0.05). It was observed that the antioxidant capacity results determined by both methods (DPPH: 82.2%±0.19 and CUPRAC: 1.18±0.05 mmol Trolox/g) reached the highest levels on the 6th day of storage. These results showed that the total content of polyphenols was positively associated with antioxidant activity before and after fermentation. Many studies have reported that water kefir has high antioxidant activity (Alsayadi et al., 2013). One of the most critical factors of water kefir's strong antioxidant capacity is its rich microbiota. There are numerous studies on the potent antioxidant potential of LAB (Amaretti et al., 2013; Alsayadi et al., 2013; Aligita et al., 2020). In addition, it has been stated that the antioxidant activity of water kefir is due to the bioactive components in the exopolysaccharide structure produced, especially during fermentation. It strengthens the immune system and prevents oxidative stress, reduces oxidative damage and reduces the production of active oxygen components (Barboza et al., 2018). On the other hand, antioxidant activity varies according to the type of lactic acid bacteria, the radical scavenging capacity of cellular or intracellular extracts, and the type of fermentable sugar in the medium (Wu et al., 2010). Alsayadi et al. (2013) found that DPPH radical scavenging activity was over 70% in water kefir samples. This shows that the antioxidant activity of water kefir is higher than soy-whey kefir, cow and goat milk kefir, rice milk kefir, and whey kefir. These results revealed that water kefir has much higher antioxidant capacity than other milk-based kefir products (Monajjeni et al., 2012). Similarly, Luang-In et al. (2018) showed high DPPH scavenging culture activity from water kefir, especially from *Acetobacter pasteurianus*. Mechmeche et al. (2019) detected high radical scavenging activity of bioactive peptides obtained from tomato seed substrate fermented in a water kefir medium. Another reason for the increase in TPC and antioxidant capacity during fermentation may be that the metabolic activities of microorganisms in kefir grains increase their levels by contributing to the modification of bioactive components.

Microbiological assesments

Water kefir grains contain LAB and yeasts. LAB convert carbohydrates to lactic acid by homofermentative pathway. It converts lactic acid, acetic acid, ethyl alcohol, and carbon dioxide in a heterofermentative way. Yeast converts these organic substances formed by heterofermentative LAB into alcohol (Koh et al., 2019). Microbiological analysis results of fermented detox juices are given in Figure 1.

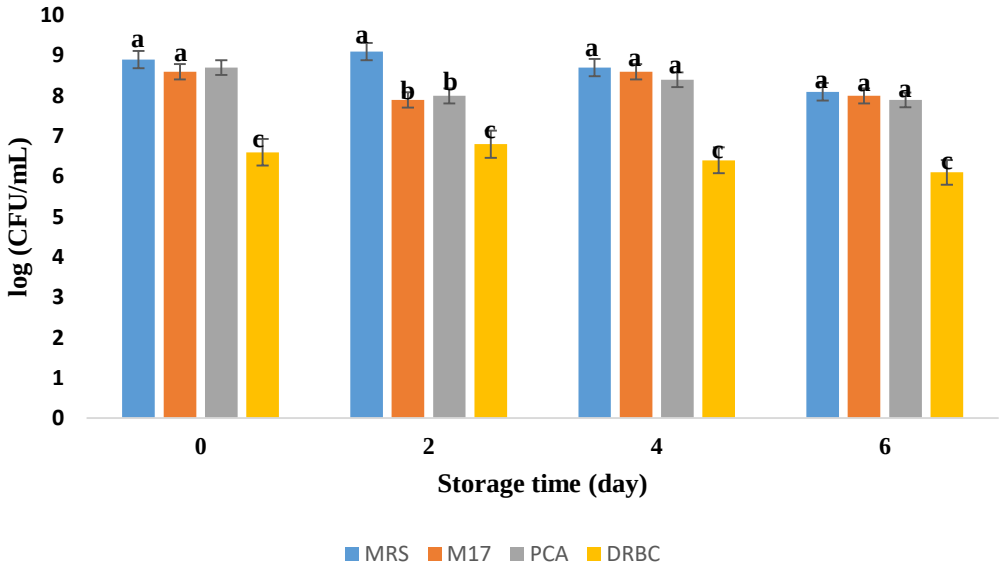

Figure 1. Changes in microbiological of fermented detox juice during 6 days of storage

During the storage of fermented detox juices, *Lactobacillus* spp. number was higher than *Lactococcus* spp. During the 6-day storage period, the number of *Lactobacillus* spp. varied between 8.1 and 9.1 log CFU/mL. The highest number of *Lactobacillus* spp. was determined on the 2nd day of storage. *Lactococcus* spp. count of the samples varied between 7.9 and 8.6 log CFU/mL during the storage period. The aerobic mesophilic bacteria counts of the fermented detox juices varied between 7.9 and 8.7 log CFU /mL during storage. *Lactococcus* spp. and aerobic mesophilic bacteria counts were highest after 48 h of fermentation. During storage, the yeast counts of fermented beverages varied between 6.1 and 6.8 log CFU/mL. On the 2nd day of storage, an increase in the yeast counts of the samples was detected. However, as the storage time increased, the yeast counts of the samples decreased. When all the microbiological analysis results were examined, it was determined that the microbial load tended to decrease with the increase in storage time, but the high cell count was preserved. During the storage, no statistically significant (P>0.05) change in cultivations for different microbiological media existed. Fruit and vegetable juices are suitable substrates for lactic acid fermentation (Garcia et al., 2020). Studies have shown that different strains of probiotics can grow and survive at constant levels during storage in fruit and vegetable juices (Di Cagno et al., 2011; Cui et al., 2019; Do ve Fan, 2019, Güney and Güngörmüşler, 2020). Corona et al. 2016 subjected some fruit and vegetable juices to fermentation with water kefir microorganisms at 25 °C for 48 h. Microbial loads (log CFU/mL) were found to be 8.5, 8.5, 8.4 and 6.7, respectively, after microbiological cultivation on MRS, M17, PCA, and DRBC agars in carrot juice. In tomato juice, these values are indicated as 8.9, 8.9, 9.0 and 7.1, respectively. In another study, total mesophilic counts ranged from 7.4 to 8.4 log CFU/mL, rod LAB 7.6 to 8.0 log CFU/mL, mesophilic coccus LAB 6.6 to 8.3 log CFU/mL, and yeasts 7.4 to 8.0 log CFU/mL in juices fermented with water kefir microorganisms (apple, grape, kiwifruit, pomegranate, prickly pear, and quince) (Randazzo et al., 2016). It has been stated that the numbers of LAB and yeast in water kefir vary depending on the fermentation time and temperature, sugar type, sugar concentration, and storage time (Ozcelik et al., 2021).

Sensory analyses

The sensory properties of the beverages were evaluated in terms of appearance, aroma, consistency, taste, mouth feel, and overall acceptability. The results are shown in Figure 2.

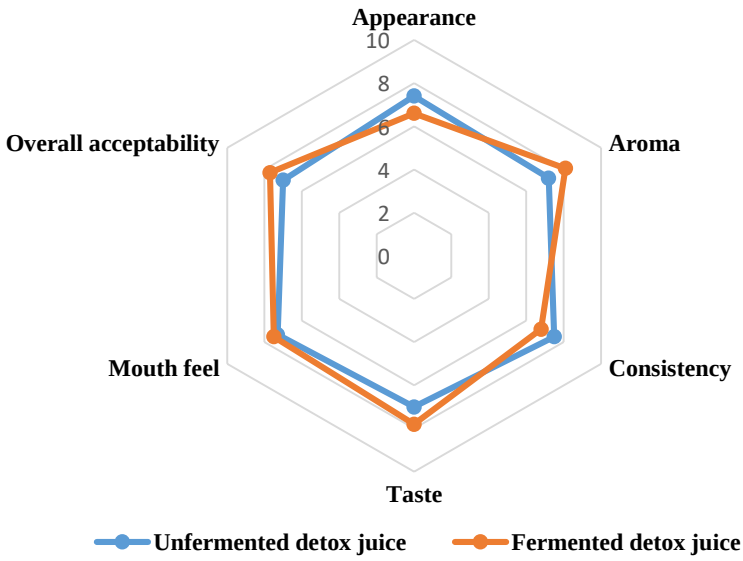

Figure 2. Changes in sensory analyses of fermented detox juice during 6 days of storage

Detox juice fermented with water kefir microorganisms was more accepted (7.7±0.05) than fresh juice (7.0±0.09). Fermented detox juices were preferred regarding aroma, taste, mouth feel, and overall acceptability. In studies where the sensory properties of beverages produced by water kefir fermentation are determined, the microorganism content of water kefir grains, fermentation conditions, storage temperature and time, fruits or vegetables used in production are the factors affecting the results (Randazzo et al., 2016, Corona et al., 2016, Ozcelik et al., 2021). The desirable flavor of many fruit and vegetable juices fermented with water kefir grains is due to volatile esters from the reaction of acids with alcohol (Ayed et al., 2020).

CONCLUSIONS

In recent years, consuming different fruit/vegetable juice mixtures with a detox effect has become widespread. Adding probiotics to fruits and vegetables rich in vitamins, minerals, antioxidants, and dietary fibers will improve the quality of these foodstuffs. In this study, the use of detox juices in the production of water kefir was investigated. It was determined that fermented detox juices'physicochemical characteristics, microbial composition, and sensory profiles were acceptable during storage. It has been determined that detox juice-based water kefir beverages have very high antioxidant activity. Prepared detox juice was a suitable substrate for water kefir microorganisms during 6 days of storage at 4 ℃. In addition to the benefits of detox juices for human health, a new and different flavor beverage was produced as a result of fermentation with water kefir grains, which may attract consumers' attention. It will also be an important alternative for people who are lactose intolerant and who cannot consume probiotic dairy products due to health problems such as milk protein allergy, as well as consumers who adopt a vegetarian/vegan diet. By preparing fruit or vegetable juices in different formulations, it should be studied which formulations will create suitable substrates for microorganisms in water kefir grains. More studies are needed to determine the positive effects of fruit/vegetable juices on the human body, which can display detox properties.

CONFLICT OF INTEREST

The author has declared no conflict of interest.

REFERENCES

Aligita, A. W., Tarigan, P. N., Susilawati, E. (2020). Antiinflamatory and antioxidant activity of kefir water. *International Journal of Biology, Pharmacy and Allied Sciences*, *9(1),* 2454-2464. https://doi.org/10.31032/ijbpas/2020/9.1.4904.

Alsayadi, M., Al Jawfi, Y., Belarbi, M., Sabri, F.Z. (2013). Antioxidant potency of water kefir. *Journal of Microbiology, Biotechnology and Food Sciences*, *2(6),* 2444.

Amaretti, A., di Nunzio, M., Pompei, A., Raimondi, S., Rossi, M., Bordoni, A. (2013). Antioxidant properties of potentially probiotic bacteria: In vitro and in vivo activities. *Applied Microbiology and Biotechnology, 97(2),* 809–817. https://doi.org/10.1007/s00253-012-4241-7.

AOAC, 2007, Official Methods of Analysis of the association of official analytical chemists, 18th edition, AOAC International, USA, 326 p.

Apak, R., Güçlü, K., Özyürek, M., Karademir, S. E., Erça, E. (2006). The cupric ion reducing antioxidant capacity and polyphenolic content of some herbal teas. *International Journal of Food Sciences and Nutrition*, *57,* 292-304. https://doi.org/10.1080/09637480600798132.

Ayed, L., M'hir, S., Hamdi, M. (2020). Microbiological, biochemical, and functional aspects of fermented vegetable and fruit beverages. *Journal of Chemistry*, *12*, 5790432. https://doi.org/10.1155/2020/5790432.

Barboza, K. R. M., Coco, L. Z., Alves, G. M., Peters, B., Vasquez, E. C., Pereira, T. M. C., Campagnaro, B. P. (2018). Gastroprotective effect of oral kefir on indomethacin-induced acute gastric lesions in mice: Impact on oxidative stress. *Life Sciences, 209,* 370-376. https://doi.org/10.1016/j.lfs.2018.08.035.

Baysal, T., Ergün, A. R., Bozkır, H., Gedik, S. K., Özer, M. B., Demir, E. (2013). Quality characteristics of carrot and pumpkin juice

powders produced by drum drying. *Academic Food Journal*, *11(3-4)*, 27-32.

Bernat, N., Chafer, M., Chiralt, A., Gonzalez-Martınez, C. (2014). Hazelnut milk fermentation using probiotic *Lactobacillus rhamnosus* GG and inulin. *International Journal of Food Science and Technology*, *49*, 2553–2562. https://doi.org/10.1111/ijfs.12585.

Brand-Williams, W., Cuvelier, M. E., Berset, C. L. W. T. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT-Food Science and Technology*, *28*, 25-30.

Bueno, R. S., Ressutte, J. B., Hata, N. N. Y., Henrique-Bana, F. C., Guergoletto, K. B., de Oliveira, A. G., Spinosa, W. A. (2021). Quality and shelf life assessment of a new beverage produced from water kefir grains and red pitaya. *LWT-Food Science and Technology*, *140,* 110770.

Chielle, E. O., Vecchia, D. D., Rossi, E. M., Chielle, A. P. O., Bonadiman, B. S. R., Marafon, F., Bagatini, M. D. (2022). Supplementation with detox juice added with probiotic improves atherogenic parameters in healthy individuals. *Brazilian Journal of Pharmaceutical Sciences*, *58*, e20581. http://dx.doi.org/10.1590/s2175-97902022e20581.

Coda, R., Lanera, A., Trani, A., Gobbetti, M., Di Cagno, R. (2012). Yogurt-like beveragesmade of a mixture of cereals, soy and grape must: Microbiology, texture, nutritional and sensory properties. *International Journal of Food Microbiology, 155*, 120-127. https://doi.org/ 10.1016/ j.ijfoodmicro.2012.01.016.

Corona, O., Randazzo, W., Miceli, A., Guarcello, R., Francesca, N., Erten, H., Moschetti, G., Settanni, Luca. (2016). Characterization of kefir-like beverages produced from vegetable Juices. *LWT-Food Science and Technology*, *66*, 572-581. https://doi.org/10.1016/j.lwt.2015.11.014.

Cui, S., Zhao, N., Lu, W., Zhao, F., Zheng, S., Wang, W., Chen, W. (2019). Effect of different Lactobacillus species on volatile and nonvolatile flavor compounds in juices fermentation. *Food Science and Nutrition, 7*, 2214-2223. https://doi.org/10.1002/fsn3.1010.

Dani, C., Oliboni, L. S., Vanderlinde, R., Bonatto, D., Salvador, M., Henriques, J. A. P. (2007). Phenolic content and antioxidant activities of white and purple juices manufactured with organically-or conventionally-produced grapes. *Food and Chemical Toxicology, 45(12),* 2574-2580. https://doi.org/10.1016/j.fct.2007.06.022.

Di Cagno, R., Minervini, G., Rizzello, C. G., Angelis, M., Gobbetti, M. (2011). Effect of lactic acid fermentation on antioxidant, texture, color and sensory properties of red and green smoothies. *Food Microbiology, 28*, 1062-1071. https://doi.org/10.1016/j.fm.2011.02.011.

Do, T. V. T., Fan, L. (2019). Probiotic viability, qualitative characteristics, and sensory acceptability of vegetable juice mixture fermented with lactobacillus strains. *Food and Nutrition Sciences*, *10*, 412-427. https://doi.org/10.4236/ fns.2019.104031.

Dordevic, T.M., Siler-Marinkovic, S.S., Dimitrijevic-Brankovic, S.I. (2010). Effect of fermentation on antioxidant properties of some cereals and pseudo cereals. *Food Chemistry, 119*, 957-963. https://doi.org/10.1016/ j.foodchem.2009.07.049.

Fraga, J. L., Sant'Ana, G. C. F., Silva, K. A., Amaral P. F. F. (2020). Green (Detox) juice physicochemical properties and stabilization effect of naturals emulsifiers. *Ciência Rural, Santa Maria*, *50 (3),* e20190739. https://doi.org/ 10.1590/0103-8478cr20190739.

Garcia, C., Guerin, M., Souidi, K., Remize, F. (2020). Lactic fermented fruit or vegetable juices: past, present and future. *Beverages, 6 (8)*, 1-31. https://doi:10.3390/beverages6010008.

Güney, D., Güngörmüşler, M. (2021). Development and comparative evaluation of a novel fermented juice mixture with probiotic strains of lactic acid bacteria and bifidobacteria. *Probiotics and Antimicrobial Proteins*, *13*, 495–505. https://doi.org/10.1007/s12602-020-09710-2.

Hashemi, S. M. B., Khaneghah, A. M., Barba, F. J., Nemati, Z., Shokofti, S. S., Alizadeh, F. (2017). Fermented sweet lemon juice (*Citrus limetta*) using *Lactobacillus plantarum* LS5: Chemical composition, antioxidant and antibacterial activities. *Journal of*

Functional Foods, *38*, 409-414. https://doi.org/10.1016/j.jff.2017.09.040.

Karovicova, J., Kohajdova, Z. (2011). Lactic acid fermented vegetable juices. *Horticultural. Sciences, 30,* 152-158. https://doi.org/10.17221/3878-hortsci.

Koh, W. Y., Uthumporn, U., Rosma, A., Effarizah, M. E., Wan Rosli, W. I. (2019). Assessment of yeast, acetic and lactic acid bacteria isolated from water kefir grains and their application as starter culture in the production of fermented pumpkin-based water kefir beverages in improving gastrointestinal tract digestive tolerance and inhibition against α-glucosidase. *International Food Research Journal*, *26(2),* 429-439.

Lillo-Perez, S., Guerra-Valle, M., Orellana-Palma, P., Petzold, G. (2021). Probiotics in fruit and vegetable matrices: Opportunities for nondairy consumers. *LWT-Food Science and Technology, 151*, 112106. https://doi.org/10.1016/j.lwt.2021.112106.

Lynch, K. M., Wilkinson, S., Daenen, L., Arendt, E. K. (2021). An update on water kefir: microbiology, composition and production. *International Journal of Food Microbiology, 345*, 109128. https://doi.org/10.1016/j.ijfoodmicro.2021.109128.

Mechmeche, M., Ksontini, H., Hamdi, M., Kachouri, F. (2019). Production of bioactive peptides in tomato seed protein isolate fermented by water kefir culture: optimization of the fermentation conditions. *International Journal of Peptide Research and Therapeutics, 25 (1)* 137-150. https://doi.org/10.1007/s10989-017-9655-8.

Monajjemi, M., Nurul, Aminin, L. A., Ilkhani, A., Mollaamin, F. (2012). Nano study of antioxidant activities of fermented soy whey prepared with lactic acid bacteria and kefir. *African Journal of Microbiology Research, 6(2),* 426–430. https://doi.org/10.5897/ajmr11.1249.

Mousavi, Z. E., Mousavi, S. M., Razavi, S. H., Hadinejad, M., Emam-Djomeh, Z., Mirzapour, M. (2013). Effect of fermentation of pomegranate juice by Lactobacillus plantarum and Lactobacillus acidophilus on the antioxidant activity and metabolism of sugars, organic acids and phenolic compounds. *Food Biotechnology, 27(1),* 1-13. https://doi.org/10.1080/08905436.2012.724037.

Othman, N. B., Roblain, D., Chammen, N., Thonart, P., Hamdi, M. (2009). Antioxidant phenolic compounds loss during the fermentation of Chétoui olives. *Food Chemistry*, 116(3), 662–669.

Ozcelik, F., Akan, E., Kinik, O. (2001). Use of Cornelian cherry, hawthorn, red plum, roseship and pomegranate juices in the production of water kefir beverages. *Food Bioscience, 42*, 101219. https://doi.org/10.1016/j.fbio.2021.101219.

Panghal, A., Virkar, K., Kumar, V., Dhull, S. B., Gat, Y., Chhikara, N. (2017). Development of probiotic beetroot drink. *Current Research in Nutrition and Food Science*, *5(3)*, 257-262. https://doi.org/10.12944/crnfsj.5.3.10.

Randazzo, W., Corona, O., Guarcello, R.,, Francesca, N., German, M. A., Erten, H., Moschetti, G., Settanni, L. (2016). Development of new non-dairy beverages from Mediterranean fruit juices fermented with water kefir microorganisms. *Food Microbiology, 54*, 40-51. https://doi.org/10.1016/j.fm.2015.10.018.

Rezaei, R., Koohsari, H. (2021). Using several fruit and vegetable juices as substrates for producing non-dairy probiotic beverages. *Iranian Food Science and Technology Research Journal,* 16(6), 111-121. https://doi.org/10.22067/ifstrj.v16i6.84472.

Sabokbar, N., Khodaiyan, F., Moosavi-Nasab, M. (2015). Optimization of processing conditions to improve antioxidant activities of apple juice and whey based novel beverage fermented by kefir grains. *Journal of Food Science and Technology, 52*(6), 3422-32. https://doi.org/10.1007/s13197-014-1397-4.

Sharma, V., Mishra, H.N. (2013). Fermentation of vegetable juice mixture by probiotic lactic acid bacteria. *Nutrafoods, 12*, 17–22. https://doi.org/10.1007/s13749-012-0050-y.

Silva E. B. M., Augusti, R., Melo, J. O. F., Takahashi, J. A., Araújo, R. L. B. (2020). Physicochemical characterization, antioxidant activity and fingerprints of industrialized "detox"

mixed beverages by paper spray mass spectrometry. *Química Nova*, *43(3),* 319-324. http://dx.doi.org/10.21577/0100-4042.20170490.

Simsek, S., Nehir El, S., Kilinc, A. K., Karakaya, S. (2014). Vegetable and fermented vegetable juices containing germinated seeds and sprouts of lentil and cowpea. *Food Chemistry*, *156*, 289-295. https://doi.org/10.1016/j.foodchem.2014.01.095.

Singleton, V. L., Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, *16*, 144-158.

Wu, S., Wang, F., Pan, C. (2010). The comparison of antioxidative properties of seaweed oligosaccharides fermented by two lactic acid bacteria. *Journal of Marine Science and Technology*, *18(4),* 537–545. https://doi.org/10.51400/2709-6998.1908

Yang, F., Wang, Y. P., Zhao, H. (2020). Quality enhancement of fermented vegetable juice by probiotic through fermented yam juice using *Saccharomyces cerevisiae*. *Food Science and Technology*, *40(1),* 26-35. https://doi.org/10.1590/fst.29918.