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Research on the Characteristic Properties of *Viburnum opulus* Nectar Fermented with Water Kefir Grains[§]

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Abstract: Water kefir is produced by fermenting a plant-based carbon source with water kefir grains under favorable conditions. The resulting drink had unique sensorial properties, high antioxidant capacity, and probiotic microorganism content. In this study, water kefir grains were used for fermentation of nectar obtained from *Viburnum opulus* for 24 hours at 25°C. The study investigated the fermented beverage's organoleptic, physico-chemical, antioxidant capacity, and microbiological content. The results showed that the sensory evaluation of the water kefir beverage obtained by fermenting *V. opulus* was generally low, but the product's color and fermented odor were highly appreciated. *Lactobacilli* spp., *Lactococci* spp., and yeast counts were approximately 6 log cfu/mL after fermentation. Even though the total soluble solids amount value was as low as 1.0, the microbial development in the plant nectar was favorable. Following the fermentation, there was a significant increase in the concentrations of malonic acid, lactic acid, citric acid, and propionic acid ($P<0.05$). Our sample color had a slightly red-yellow mixture based on instrumental color analysis. The total antioxidant capacity was determined as 937 mg GAE/L based on the results of the antioxidant capacity analysis. The conclusion was that *V. opulus* is a suitable substrate for fermentation with water kefir grains despite the low overall sensorial evaluation of the product.

Keywords: Antioxidant capacity; fermentation; *Viburnum opulus*; water kefir.

1. Introduction

The fast pace of daily life and the effects of fast eating habits on consumer health have become the subject of scientific studies. Unbalanced nutrition, which occurs due to the consumption of food products high in fat, salt, and sugar, can cause not only stomach and intestinal health problems but also food allergies, cardiovascular diseases, and damage to the immune system. Moreover, according to recent studies, it has been stated that it can also cause neurological diseases such as Parkinson's and

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Alzheimer's. The relationship between nutrition and human health was first put forward by Hippocrates about 2500 years ago with the expression, "Let your medicine be your food, and your food be your medicine" in Kos Island. Today, due to the increase in heart, circulatory system, and obesity-related disorders, consumers' trend towards functional foods and food supplements has increased. Functional foods encompass a wide variety of foods containing unique bioactive compounds. Among these, probiotic foods have particular importance due to the increasing consumer demand due to their health effects [1].

Water kefir grains have beneficial probiotic properties. They are symbiotic in a polysaccharide matrix formed by lactic acid bacteria and yeasts [2]. Grey-white, translucent water kefir grains are left to ferment with sugary water (sucrose, molasses) and fresh or dried fruits (like figs, apricots, and raisins) for 2-4 days. Separated water kefir grains are used in the subsequent fermentation, while the fermented beverage can be consumed directly or after being kept in the refrigerator [3]. Water kefir is a product that can be consumed primarily by individuals who have adopted a vegan diet, have lactose intolerance problems, or have an allergic condition. In addition, it has significant potential for producing new products with different sensory and functional properties using diverse agricultural products.

Recently, various substrates such as pear juice, passion fruit, mandarin, pomegranate juice, quinoa, demineralized whey, and persimmon have been used for fermentation with water kefir grains [4]. However, to our knowledge, no study has been carried out with *V. opulus* nectar. It has been reported that *V. opulus* fruit has anticancerogenic, antimicrobial, and antioxidant properties due to the acids in its content. The presence of amino acids such as aspartic acid, glycine, glutamic acid, proline, arginine, alanine, histidine, phenylalanine, phenylalanine, isoleucine, tyrosine, threonine, serine, leucine, lysine, and valine was found in the kernel content of *V. opulus* fruit. It was also reported that the seeds of *V. opulus* fruit were rich in fatty acid and free fat content [5].

The study aimed to investigate the fermentation characteristics of a water kefir drink obtained by fermenting *V. opulus* nectar.

2. Materials and Methods

2.1. Preparation of Water Kefir

Bir Sey Gida Imalat Ltd. Sti kindly donated water kefir grains (www.birseygida.com.tr, Antalya, Turkey). Organic nectar of *V. opulus* (Arifoglu Baharat ve Gıda San. Ltd. Sti, Istanbul, Turkey) was purchased from a local market. The nutritional values of *V. opulus* nectar provided by the product manufacturer are as follows: 33.50 mg/100mL of vitamin C, 19.00 mg/100 mL of sodium, 13.54 mg/100 mL of iron, and 8.11 mg/100 mL of zinc. The water source used was natural spring water (www.hayatsu.com.tr). To produce water kefir, 500 mL of natural spring water was mixed with 500 mL of pasteurized *V. opulus* nectar in a sterilized glass jar. Then, water kefir grains (3% w/v) were added to the water-nectar mixture and fermented for 24 hours at 25°C. After 24 hours, the grains were sieved under sterile conditions, and the resulting beverage, i.e., water kefir, was refrigerated (+4°C) until analysis.

2.2. Physicochemical Analysis

Dry matter content was analyzed with an automatic device (Shimadzu MOC63U Moisture Analysis, Kyoto, Japan). For each measurement, 3.0 grams sample was used. Titration acidity was carried out based on a standard procedure [6] (for juice citric acid %, for water kefir lactic acid %). The total soluble solids amount (ºBrix) was measured with a digital refractometer (Atago Pal-1®, Tokyo, Japan), and pH (Mettler Toledo, Greifensee, Switzerland) was measured directly from the samples. The viscosity was determined using a spindle DV-2 at 100 rpm (Brookfield Viscosemeter, DV2T, USA). Instrumental color measurement was carried out as identified by a previous study [7]. HPLC conditions for organic acid analysis were carried out according to a previous study [8]. For organic acid analysis, briefly, a 2 mL sample was mixed with 10 mL of distilled water and shaken for 4-6 hours. The diluted samples were then filtered through a 0.45 m filter and placed in a vial for analysis. The mobile phase was prepared using 10 millimolar ammonium dihydrogen phosphate, and the pH was adjusted to 2.6 with orthophosphoric acid.

2.3. Antioxidant Capacity Analysis

The total phenolic content of the samples was measured using the Folin-Ciocalteu method, as previously described by the literature [9]. TEAC (ABTS+) assay was carried out based on the method described by a previous study [10]. The DPPH (2,2,-diphenyl-2-picryl-hydrazyl) method was applied according to the procedure outlined by a previous research [11].

2.4. Microbial Enumeration

Lactobacilli spp., *Lactococci* spp., and yeast contents of samples were analyzed with the same protocol by related literature [12]. Briefly, a 1 mL water kefir sample was mixed with 9 mL of sterile peptone water. The resulting mixture was then serially diluted. A 1 mL aliquot of the serially diluted mixture was then plated onto MRS Agar, M17 Agar, Plate Count Agar and Potato Dextrose Agar for the detection of *Lactobacilli* spp., *Lactococci* spp., total aerobic bacteria and yeast, respectively. The petri dishes were incubated for 48 hours at 37°C under anaerobic conditions containing 6% CO_2 to enumerate lactic acid bacteria and aerobic bacteria. For yeast enumeration, the dishes were incubated at 25°C for 5 days under normal atmospheric conditions.

2.5. Sensory Analysis

A descriptive sensory analysis was carried out as described by a previously described procedure by a previous study [13]. Before conducting the sensory test, participants were provided with all necessary information and directions. Two main criteria were applied to select suitable panelists: non-smoking and good health, in order to prevent any inability to smell or loss of smell/taste. The sensory analysis panel consisted of ten educated panelists (5 male and 5 female) between the ages of 20 and 50. Foaminess, color, turbidity, consistency, fermented odor, sourness, sweetness, fruity flavor, alcohol taste, refreshing taste, astringency and general acceptance were scored on a scale of 1 to 10.

2.6. Statistical Analysis

A paired samples *t*-test was used to detect significant differences between samples using IBM SPSS v. 22.0 (SPSS Inc., Chicago, USA). Except for the sensory analysis test, all results were obtained with two replications and four parallels. Sensory analysis was performed with eight replications and 16 parallels. All results are expressed as mean ± standard deviation.

3. Results and Discussion

3.1. Physicochemical Analysis Results

The pH value changed significantly at the end of fermentation ($P<0.05$). Similarly, significant changes were observed for the other parameters ($P<0.05$). A decrease in pH value was expected due to the microbial activity of water kefir fermentation in most cases [14]. Consistent with our results, A previous research [13] also observed a decrease in pH and an increase in titratable acidity. The viscosity of the water kefir may vary depending on how much sugar is added or which fruit component is utilized. In a study [8], it was measured the viscosity of water kefir made from demineralized whey with 2% and 5% lactose at 10.20-14.40 cP and 11.20-14.80 cP, respectively. The viscosity values of green Indian juice kefir with high fructose syrup were 3.52, 3.66, 2.56, 2.12, and 1.28 cP, respectively [15]. As can be observed, the materials utilized in water kefir manufacturing have a distinct influence on product viscosity.

The L value indicates the whiteness of the sample, while the a and b values indicate the yellowness and redness of the sample, respectively. According to our results, the color of our sample had a slightly red-yellow colour mixture (Table 1).

The organic acid contents of the samples are given in Table 2. According to the results, the tartaric acid content of the samples was not significantly changed ($P>0.05$). On the other hand, the lactic acid, citric acid, and propionic acid contents of *V. opulus* nectar were significantly increased, whereas malonic acid decreased during the fermentation ($P<0.05$). The lactic acid content of various fruit-based water kefirs was reported as 0.02-1.00 g/L [16]. The main factors related to changes in organic acid content during water kefir fermentation Zannini et al. [17] are the alteration of the dominant microbial community, their metabolic activities, and their relationship with the fermentation substrate.

Table 1. Physicochemical analysis results of the samples

Parameters	GN (T=0)	GNWK (T=24)
pH	3.05±0.00[a]	3.02±0.00[b]
Dry matter content (%)	1.08±0.02[a]	0.87±0.02[b]
Total soluble solids amount (°Brix)	1.00±0.00[a]	1.13±0.05[b]
Titratable acidity (%)	0.12±0.00[a]	0.17±0.04[b]
Viscosity (cP)	*NT*	14.05±0.38
L*	*NT*	23.78±0.32
a*	*NT*	9.64±0.39
b*	*NT*	4.73±0.56

NT: Not measured. The different superscripts in the same row show a significant difference among the samples ($p < 0.05$). Results were expressed as mean ± standard deviation. *GN*: Nectar of *V. opulus*. *GNWK*: Water kefir obtained by fermentation of *V. opulus* nectar.

Table 2. Organic acid content of *V. opulus* nectar and water kefir

Sample	Tartaric acid (ppm)	Malonic acid (ppm)	Lactic acid (ppm)	Citric acid (ppm)	Propionic acid (ppm)
GN	4335.69±59.42[a]	4261.50±58.40[a]	7566.13±103.69[a]	6602.98±90.49[a]	6076.06±83.27[a]
GNWK	4113.01±439.94[a]	2397.38±351.53[b]	13065.05±1272.82[b]	8150,32±1101,02[b]	6903,46±275,38[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. GN: Nectar of *V. opulus*. GNWK: Water kefir obtained by fermentation of *V. opulus* nectar.

3.2. Antioxidant Capacity Analysis

The antioxidant capacity of water kefir is presented in Table 3. Previously, the total phenolic content of water kefirs was reported as 22.34 mg GAE/L and 49.30 mg GAE/L [12]. Our result was higher than those reported results. Similarly, our DPPH result was higher than the previously reported result [12]. *V. opulus* is rich in phenolic compounds, vitamin C, carotenoids, and triterpenes and, thus, has high antioxidant activity [18]. Polyphenols are partially absorbed, and thus, most of them reach the colon, where they can stimulate several important bacterial species, including *Lactobacilli* and *Bifidobacteria* [19]. The compatibility of probiotics and polyphenols in the body will highlight foods containing them. The high antioxidant capacity is also related to the activity of lactic acid bacteria during fermentation and the formation of bioactive substances in the exopolysaccharide structure during the fermentation process [20].

Table 3. Antioxidant capacity of water kefir made using *V. opulus* nectar*

Total phenolic content (mg GAE/L)	937±142.29
DPPH (% Inhibition)	35.77±1.06
TEAC (mmol TE/L)	1.24±0.05

*Results were expressed as mean ± standard deviation.

3.3. Microbial Analysis Results

The results of the microbial enumeration are given in Table 4. Favorable microbial activity was observed as a result of the fermentation process. A significant increase was observed for the lactic acid bacteria and yeasts ($P<0.05$). Similar to the trends observed in our results, previous studies also reported an increased number of microorganisms [14], [16]. Based on the results, *V. opulus* nectar was an ideal substrate for the fermentation of water kefir grains.

Table 4. Microbial enumeration results of the samples

Group of microorganisms	GN (T=0)	GNWK (T=24)
Lactobacilli spp. (Log CFU/mL)	4.35±0.30[a]	6.32±0.20[b]
Lactococci spp. (Log CFU/mL)	<3[a]	5.97±0.10[b]
Yeasts (Log CFU/mL)	<3[a]	6.72±0.13[b]
Total aerobic bacteria (Log CFU/mL)	4.20±0.10[a]	6.25±0.04[b]

The different superscripts in the same row show a significant difference among the samples ($p < 0.05$). Results were expressed as mean ± standard deviation. [GN]: Nectar of *V. opulus*. GNWK: Water kefir obtained by fermentation of *V. opulus* nectar.

3.4. Sensory Analysis Results

Water kefir obtained from *V. opulus* was tested for sensory acceptability. A descriptive sensory analysis was preferred for this purpose. According to the results, the water kefir drink had a low overall acceptance by the panelists. Its astringency, sweetness, and acidity were rated high, which is undesirable for water kefir beverages. On the other hand, its color and fermented odor were rated highly, which is favorable for a water kefir drink (Figure 1).

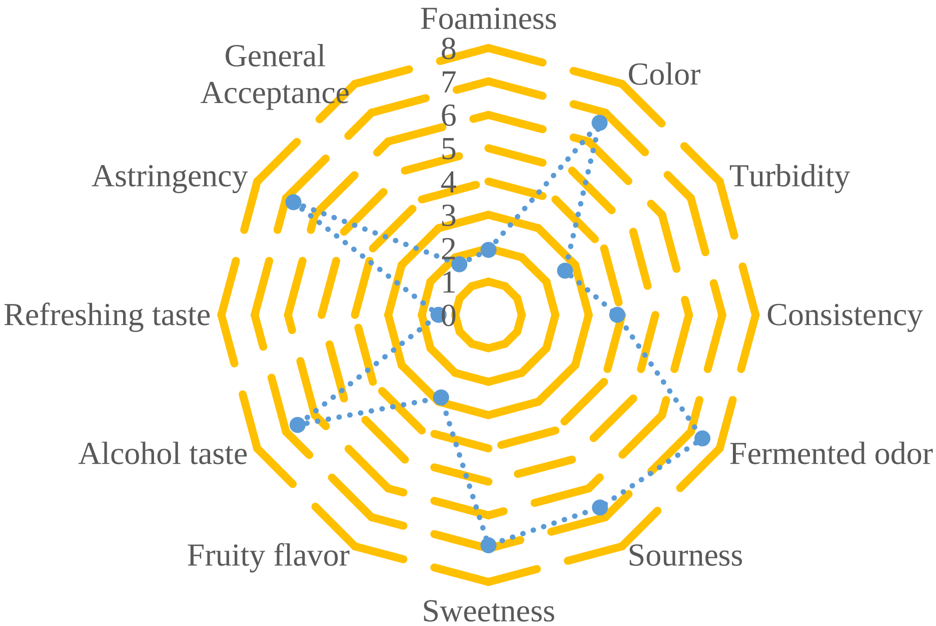

Figure 1. Sensory analysis results for water kefir beverage

4. Conclusion

Viburnum opulus is a valuable plant due to its nutritional content. It is important to note that this study is limited to the functionality of *V. opulus* in water kefir production and does not provide a comprehensive evaluation of its overall nutritional value. Its nectar was found to be a favourable medium for the fermentation of water kefir grains, resulting in a significant increase in organic acid content and microorganism numbers. The resulting water kefir beverage has a relatively high antioxidant capacity. However, the product's sensorial acceptance was low. With the addition of various natural substances, the sensorial properties could be improved.

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