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Physical, chemical, and sensory properties of water kefir produced from *Aronia melanocarpa* juice and pomace

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ABSTRACT

Water kefir is widely consumed all over the world due to its potential health benefits. The aim of this current study was to compare non-fermented juice and fermented beverage of water kefir produced from *Aronia melanocarpa* juice and pomace in terms of chemical, physical and sensory quality as well as valorisation of pomace in the production of water kefir. When compared to water kefir made with aronia juice, less reduction in total phenolic content (TPC), total flavonoid content (TFC) and total anthocyanin content (TAC) was observed in samples made with aronia pomace during the fermentation process. Similarly, greater antioxidant activity was demonstrated in water kefir made with aronia pomace than juice. Based on sensory evaluation, no difference was found in overall acceptability, taste, aroma/odor, and turbidity of water kefir made with aronia pomace before and after fermentation. Results indicated that aronia pomace has potential in water kefir production.

Introduction

Recently, the attention in the intake of healthy and functional foods has boosted together with the household making of fermented products. Among these products, water kefir (also recognized as aqua kefir or sugary kefir) has gained special attention and currently available all over the world. Water kefir is conventionally made from different unique probiotic containing gelatinous grains, called as water kefir grains. These gelatinous grains are a symbiotic culture of yeast and bacteria implanted in a polysaccharide matrix. Yeast, lactic acid and acetic acid bacteria are the main microbial community of the sugary kefir grain. Water kefir is generated by fermentation of sugary water containing dried raisins or figs in the presence of the microbial community in the water kefir grains (Guzel-Seydim, Gökırmaklı & Greene, 2021). Therefore, water kefir is a fruity, slightly alcoholic, sour, and carbonated beverage. Water kefir has several health benefits, including antimicrobial, anti-inflammatory, antioxidant activity (Alsayadi, Jawfi, Belarbi & Sabri, 2013), hepatoprotective action (Aspiras, Flores, & Pareja, 2015), and wound-healing effects, lowering cholesterol and LDL levels (Rocha-Gomes, Escobar, Soares, Silva, Dessimoni-Pinto, & Riul, 2018), and exerting gastroprotective effects (Brasil, Andrade Moraes, Prucoli Falsoni, Resende, Andrade & Lima, 2019). Since probiotics stimulate/promote intestine health and overall immune system, their role in fighting viral COVID-19 infections has been emphasized, especially in elderly people (Sundararaman, Ray, Ravindra & Halami, 2020).

The fermented product, namely, water kefir, includes viable microorganisms, residues of sugar and fruits and some metabolites such as lactic and acetic acids, ethanol, CO_2, vitamins (mainly B-complex), mannitol and some amino acids such as arginine, polysaccharides (glucans and levans) (Laureys & De Vuyst, 2014; Laureys & De Vuyst, 2017).

Water kefir grains consist of a dextran matrix and its structure is

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consisting of α-D-(1 → 6)-linked glucopyranosyl residues with (1 → 3)-connected side chains (Moinas, Horisberger & Bauer, 1980). The microbial composition of the grain has been described by many researchers. Davidović, Miljković, Antonović, Rajilić-Stojanović and Dimitrijević-Branković (2015) reported that *Lactobacillus hordei*, *Lactobacillus casei, Leuconostoc mesenteroides, Lactobacillus hilgardii* and *Lactobacillus nagelii* generate the dextran structure in water kefir grains. Prado et al. (2015) reported that the microbiological composition of water kefir grains varies with its origin, culture and growth methods. Some researchers indicated that typical microorganisms in water kefir grains include *Lactobacillus paracasei, Lb. nagelii, Lb. hilgardii,* and *S. cerevisiae* for the fermentation of water kefir (Laureys & De Vuyst, 2017). Other researchers showed that water kefir grains contained *Lactobacillus* sp. (70%), *Acetobacter* sp. (10%), *Leuconostoc* sp. (10%), *Bifidobacterium* sp. (5%), and other bacteria (5%) (Fiorda et al., 2017). Furthermore, researchers reported that water kefir grains consisted of roughly 39% *Lactobacillus* sp., 31% *Lactococcus* sp. and 30% yeasts (Gökırmaklı and Güzel-Seydim, 2022). All these studies reflect the fact that water kefir is usually made in an artisanal manner and the fermentation environment is generally variable. Therefore, the microbial composition of the grains and the fermented beverage depend on the fermentation conditions such as time and temperature and especially with different substrates such as source of sugars, fruits, and vegetables (Laureys, Aerts, Vandamme & Vuyst, 2018). Thus, the microorganisms and their metabolites in the beverage vary and accordingly the health properties of water kefir. This fermented beverage has distinctive physical, chemical and microbiological attributes. Thus, water kefir is considered as a potential prebiotic, probiotic, and antioxidant source for vegans and individuals who are intolerant/allergic to dairy products. Randazzo et al. (2016) developed non-dairy beverages from different fruits such as apple, quince, kiwifruit, grape, pomegranate and prickly pear with the usage of water kefir microorganisms. Corona et al. (2016) also produced kefir-like beverages by using vegetable juices (carrot, melon, fennel, tomato, onion, and strawberry) as fermentable substrates with water kefir microorganisms. These studies indicated the potential in developing value-added and functional fruit or vegetable based kefir like beverages. Recently, Darvishzadeh, Orsat and Martinez (2021) formulated a water kefir beverage with Russian olive as a non-dairy product with high antioxidant and probiotic properties. Ozcelik, Akan and Kinik (2021) also used fruit juices of Cornelian cherry, red plum, hawthorn, pomegranate and rosehip in the production of water kefir beverages while Hampton, Tang, Jayasree Subhash and Serventi (2021) and Bueno et al. (2021) prepared water kefir with pear juice or its puree and pitaya or apple pulp, respectively.

Aronia melanocarpa, called black chokeberry, belongs to the Rosaceae family and is cultivated as an ornamental shrub and utilised to produce juices, wines, jams, as well as natural food colorants. Since Aronia fruit contains high polyphenol content, it is regarded as a novel and good source of dietary antioxidants. Aronia fruit pomace is a by-product from juice processing and mainly discarded as waste. These residues are inadequately re-utilised due to a lack of innovative procedures for their valorization. Considerable research has put emphasis on the significant quantities of phenolic compounds (especially wall bound) that are retained in by-products of plants. These compounds indeed can be recovered via innovative methods for extraction and fractionation (Brazdauskas, Montero, Venskutonis, Ibañez & Herrero, 2016; Kitrytė, Kraujalienė, Šulniūtė, Pukalskas & Venskutonis, 2017). Therefore, this study involves valorization of *A. melanocarpa* pomace to determine its feasibility as a high antioxidant astringent juice and by-product to produce water kefir, which is valuable source for human consumption. The main objective of this study was to evaluate the effect of the fermentation process on chemical, physical and sensory quality of water kefir produced from *Aronia melanocarpa* juice and pomace.

Materials and methods

Chemicals and reagents

D-(+)-glucose (99.5 % GC), D-(-)-fructose (>99%), D-(+)-saccharose (HPLC, 99.5 %), (±)-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox; 97%), gallic acid (97.5-102.5%), (+)-catechin hydrate (≥98%), rutin hydrate (≥94%) were purchased from Sigma Aldrich (Steinheim, Germany).

Ethanol (99% denatured with 1% methyl ethyl ketone) was obtained from Walter CMP (Kiel, Germany), acetonitrile (UHPLC supergradient, >99.9%; PanReac AppliChem, Darmstadt, Germany), L-(+)-ascorbic acid (p.a., ≥99%) and sodium carbonate (≥99.5%) from Carl Roth (Karlsruhe, Germany), 2,2-diphenyl-1-picrylhydrazyl (abcr, Karlsruhe, Germany), copper (II)-chloride (p.a., 98%), sodium acetate anhydrous (p.a., ≥99.0%) and ammonium acetate (p.a.) from Chemsolute (Renningen, Germany), neocuproine (98.5%, J&K Chemicals, San Jose, USA), Folin-Ciocalteu phenol reagent (2 M, Merck, Darmstadt, Germany), aluminum chloride hexahydrate (p.a., ≥99.0%, Honeywell, Seelze, Germany).

Preparation of water kefir

Two types of water kefir were prepared by using the Nero variety of *Aronia melanocarpa.* Pomace was obtained from Aronia ORIGINAL Naturprodukte (Dresden, Germany) and the juice from a supermarket (dm-drogerie markt, Karlsruhe, Germany). Water kefir from aronia pomace and juice were prepared according to Malchow et al. (2019).

Aronia pomace (300 g) was boiled with 3 L of water and then simmered for 10 min. Then, the mixture was filtered through a straining cloth and cooled down to 35 °C. For the production of water kefir with pomace, 600 mL of the prepared aronia pomace substrate was boiled with 10 g of raisins and 20 g of sugar. The raisins were filtered and 400 mL of the substrate was transferred to a fermentation flask. The substrate was finally cooled down at 35 °C. The Brix (PCE-DRW 2 digital refractometer, PCE Instruments, Meschede, Germany) and pH values (PH-Serie pH 5, Dostmann electronic, Wertheim-Reicholzheim, Germany) were measured for verification. The final product from pomace had a low sugar content with a 6.0 °Bx compared to that of juice with 11.1 °Bx. Therefore, 51.1 g of sugar was added to the water kefir base including pomace and the Brix value was measured again. Thirty grams of drained water kefir grains (Fairment, Berlin, Germany) was added to the substrate in the fermentation flask. The flask was covered with a fermentation tube and the mixture was then fermented for 72 h at 35 °C in a fermenter (Bakery Proofer L 834.1B, MEC, Rimini, Italy).

For production of water kefir with juice, 300 mL of aronia juice was boiled with 300 mL of tap water, 10 g of raisins and 20 g of sugar. The raisins were filtered and 400 mL of the substrate was transferred to a fermentation flask. The substrate in the flask was left to cool down to 35 °C and afterwards the water kefir crystals were added. The flask was sealed with a fermentation tube and the mixture was then fermented at 35 °C for 72 h. After fermentation, the mixture was poured through a sieve and the water kefir grains were removed.

The fermented water kefir bases obtained from pomace or juice were transferred into pasteurized screw jar, which were pasteurized in a convection oven (Rational iCombi Classic 6-1/1 Elektro, Rational Kombidaempfer, Neuruppin, Germany) at 85 °C for 30 min.

Physico-chemical analyses

The Brix value provides information about the sucrose content in water. The measurement works by comparing the different densities of pure water and a sucrose solution. The digital refractometer used calculates a value in degrees Brix based on the refractive index. 1 °Brix equals the same density as a solution of 1 g sucrose in 100 g water (1% solution). The Brix value was determined at 20 °C using a portable

refractometer (PCE-DRW 2 digital refractometer, PCE Instruments, Meschede, Germany). The pH value was determined with a digital pH meter (PH-Serie pH 5, Dostmann electronic GmbH, Wertheim-Reicholzheim, Germany).

Total phenolic content (TPC) assay

Total phenolics were colorimetrically measured using Folin–Ciocalteu reagent according to the methods of Khan & Kumar (2019) and Zhou, Yang, Zhu, Lin, Hao, & Xu, (2020) with modifications. Catechin hydrate was used as a positive control. Twenty microliters of sample, positive control, calibration standards (1 mg/mL gallic acid stock solution) and blank samples (bidistilled water) were added to a 96 well microtiter plate in a technical triplicate followed by addition of 100 µL Folin reagent (0.2 mol/L). After 5 min, 100 µL of a saturated sodium carbonate solution was added and shaken for 12 *sec*. After 60 min the absorbance was measured at 765 nm using the plate reader TECAN infinite M200 (Männedorf, Switzerland).

Total flavonoids content (TFC) assay

TFC of samples were analyzed according to the method of Nurcholis, Putri, Husnawati, Aisyah & Priosoeryanto (2021) with slight modifications. Fifty microliters of sample, positive control (rutin hydrate), calibration standard (0.5 mg/mL quercetin stock solution) and blank sample (ethanol) were added to a 96-well microtiter plate in the technical triplicate and then 130 µL of ethanol was added. Twenty microliters of a 1:1 (*v/v*) mixture of 10% aqueous aluminium chloride solution and 1 mol/L sodium acetate solution was added and shaken for 12 *sec*. After 40 min the absorbance at 415 nm was measured using the TECAN infinite M200.

Total anthocyanin content (TAC) assay

The total monomeric anthocyanin content (TAC) was carried out by the pH differential technique (Giusti and Wrolstad, 2001) using the TECAN infinite M200 spectrophotometer. The absorbance of the samples diluted in pH 1.0 and 4.5 buffers were measured at 520 and 700 nm. The monomeric anthocyanin pigment concentration of samples was calculated by using the Equation (1):

$$\text{Total monomeric anthocyanin content}\left(\frac{\text{mg}}{\text{L}}\right) = \frac{AxMWxDFx1000}{\varepsilon \text{ x l}} \quad (1)$$

where A = (A520nm − A700nm) $_{pH1.0}$ − (A520nm − A700nm) $_{pH4.5}$, MW is the molecular weight of cyanidin-3-*O*-glucoside (449.2 g/moL), DF is the dilution factor, 1000 is the conversion factor from g to mg, ε is the molar extinction coefficient of cyanidin-3-*O*-glucoside (26900 L/(mol.cm)), and l is the path length.

2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay for antioxidant activity

For the DPPH assay the method by Mishra, Ojha & Chaudhury (2012) with modifications were used. The assay was conducted by using the DPPH-radical reagent. Ascorbic acid was used as a positive control. Hundred microliters of the sample, the calibration standard (2.5 mmol/L Trolox stock solution), the positive control and blank (ethanol) were pipetted in technical triplicate in a microtiter plate. Then, 100 µL of 30 µmoL DPPH solution was added and shaken for 12 *sec* automatically with the TECAN infinite M200. The absorbance was measured after 30 min at 515 nm using the TECAN infinite M200.

Cupric ion reducing antioxidant capacity (CUPRAC) assay for antioxidant activity

CUPRAC was determined according to the method of Apak, Güçlü, Ozyürek & Karademir (2004) with small modifications. Briefly, 5 µL of sample, positive control (ascorbic acid), calibration standards (10 mmol/L Trolox stock solution) and blank sample (ethanol) were added to a 96-well microtiter plate in the technical triplicate and then 200 µL of a mixture of copper(II)-chloride solution (10 mmol/L), neocuproine solution (7.5 mmol/L), ammonium acetate solution with pH = 7.0 and bidistilled water were added in a 1:1:1:1 (*v/v/v/v*) ratio and was shaken for 12 *sec*. After 30 min, absorbance was measured at 450 nm with the TECAN infinite M200.

Colour analysis

Colour of water kefir beverages obtained from aronia pomace and juice was determined before (0 h) and after (72 h) fermentation using a CM-5 spectrophotometer (Konica Minolta Business Solutions Deutschland, Langenhagen, Germany) with the spectator set to 2° and the type of light being D65. The different variants were each examined as a biological duplicate and each analysed with a triple measurement (technical triplicate). The calibration was performed using the zero calibration box CM-A124, the calibration glass for petri dishes CM-A212 and the internal white calibration standard of the CM-5. After 10 mL of sample were poured into the petri dish CM-A128, darkened with the retaining ring CM-A519 and covered with the white calibration plate CM-A210. For the colour comparison of the samples, the CIE L^*, a^* and b^* values were measured by using CM-5 spectrophotometer and thus C* (chroma), h° (hue) and total colour differences (ΔE*) values were calculated by use of L^*, a^* and b^* measurements. The lightness (L^*) is evaluated from 0 (black) to 100 (white). The a^* coordinate takes positive values for reddish colours and negative values for greenish colours, and b^* takes positive values for yellowish colours and negative values for bluish colours. C*, h° and ΔE* were calculated by using Equations (2), (3), and (4).

$$C^* = \sqrt{a^{*2} + b^{*2}} \quad (2)$$

$$h^o = \tan^{-1}\left(\frac{b^*}{a^*}\right) \quad (3)$$

$$\Delta E^* = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}} \quad (4)$$

The measurement was recorded using SpectraMagic™NX software (version CM-S100w 3.20.0002).

Content of sugars (sucrose, fructose and glucose) determined by HPLC-RI

Analysis of the sugars sucrose, glucose and fructose was done on an Agilent HPLC 1200 series equipped with a G7162A RI-detector (Agilent Infinity II 1260 series), G1312A binary pump and G1329A autosampler. Lichrospher NH_2 (CS Chromatographie Service GmbH, Langerwehe, Germany; 250 × 4.6 mm, 5 µm) column with a NH_2 pre-column was used as stationary phase. As mobile phase ultrapure water/acetonitril (25/75, *v/v*) was used (isocratic elution). The flow rate was set at 1.0 mL/min. The injection volume was 20 µL. The column oven and RI-detector temperature were set at 35 °C. Samples were diluted using ultrapure water at a ratio of 1/5 und 1/10 (*v/v*) and centrifuged at 14.500 rpm for 10 min (Pico 21, Thermo Scientific, Darmstadt, Germany). All standards (glucose, fructose and sucrose) were prepared in ultrapure water. The concentration of the stock solutions was 10 mg/mL. Calibration standards were set in the range of 2.0 to 9.0 mg/mL. All results were stated as g fructose/glucose/sucrose per 100 mL.

SPME-GC/MS analysis of volatile components

Threegrams of sample were transferred in a 20 mL headspace vial with 2 mL NaCl (20%) and incubated at 60 °C for 15 min. The volatiles were then extracted for 30 min by solid phase micro extraction (SPME) using a 50/30 µm d_f Divinylbenzene/Carboxene/Polydimethylsiloxane (DVB/CAR/PDMS, 2 cm) Sigma-Aldrich (Steinheim, Germany). Analysis of volatiles was performed using an Agilent 7890B (Agilent, Waldbronn, Germany) gas chromatograph equipped with a DB-Wax (60 m × 0.25 mm i.d × 0.25 µm d_f; J&W Scientific-Folsom, USA) capillary column coupled to a Agilent 7010B mass spectrometry system. The split–splitless-injection port was heated to 250 °C, and the split ratio was set to 1:10. After desorption for 5 min, the column temperature was held at

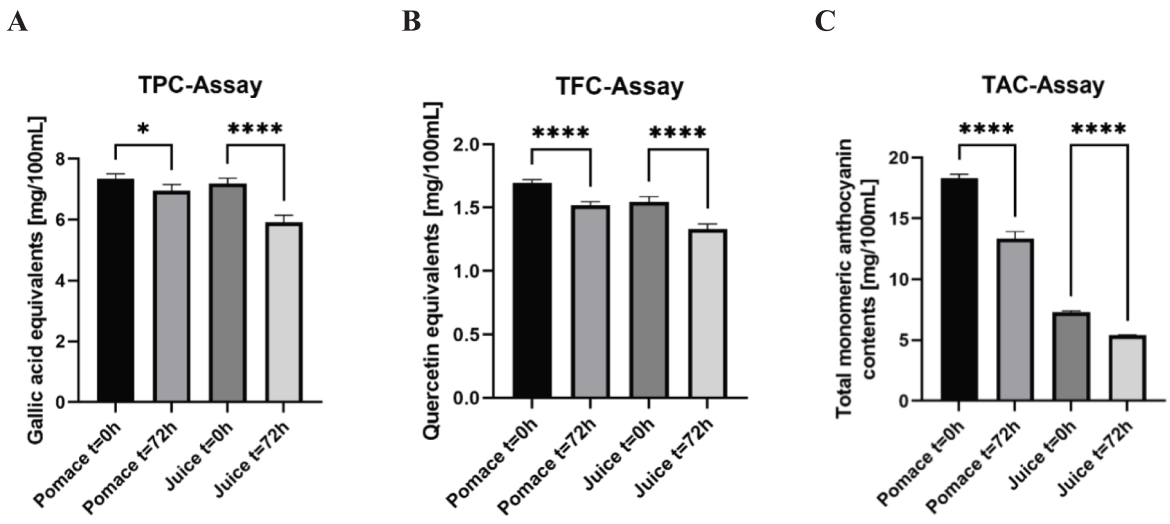

Fig. 1. Effects of fermentation (*A. melanocarpa* pomace and juice water kefir at 0 h and 72 h) on the (A) total phenolic content (TPC) shown as gallic acid equivalents, (B) total flavonoid content (TFC) shown as quercetin equivalents and (C) total monomeric anthocyanin content (TAC) shown as cyanidin-3-*O*-glucoside. The data presented in this figure consist of average values ± standard deviation of a biological duplicate and a technical triplicate. One-way ANOVA followed by Tukey's multiple comparison test * $p < 0.05$, **** $p < 0.0001$: Statistical differences between at 0 h and at 72 h.

40 °C for 4 min, and then increased at 3 °C/min to 90 °C, and then increased at 4 °C/min to 130 °C where it was held for 4 min. After which, the temperature was adjusted to 240 °C by rising 5 °C per minute and kept at this temperature for further 8 min. Helium (He) was used as carrier gas. The ionisation energy was 70 eV and the mass range 30–600 *m/z*. The volatiles were then quantified utilizing the internal standard with 4-nonanol.

Sensory evaluation

Sensorial quality of the water kefir samples obtained from aronia pomace or juice were evaluated by eleven panellists (aged 25–50) who were regular consumers of commercial and/or homemade milk kefir. Panellists rated water kefir beverages using a 10-point hedonic scale varying from 1 (disliked extremely) to 10 (liked extremely) based on predefined attributes including colour, turbidity, attractiveness (optical), attractiveness (taste), aroma/odour, acidity, sweetness, sparkling (CO_2) and favor (overall acceptance). Ten millilitres of refrigerated samples (~8 °C) were served in transparent glasses. Samples were randomly coded with three-digit numbers and served with water under diffused lighting. Based on the scores obtained by panellists, each attribute was indicated as mean ± standard deviation.

Statistical analysis

Statistical analysis was done with Graphpad Prism version 9.3.1 (San Diego, USA). To determine the statistically significant difference a one-way ANOVA (Analysis of Variance) was carried out followed by a Tukey's multiple comparison test. Two experimental replicates of water kefirs was prepared and measurements were repeated three times. The values are mean ± standard deviation.

Results and discussion

Brix and pH values

Before fermentation (0 h), the Brix values of the pomace- and juice-based fermentation substrate were 10.4 ± 0.1 and 11.8 ± 0.5, respectively. After fermentation (72 h), there was a significant reduction ($p < 0.05$) in the Brix values to 6.10 ± 0.28 for pomace and 7.65 ± 0.92 for juice. During the fermentation process, sugars were converted to CO_2 and ethanol and thereby the sugar content of water kefir beverages made with aronia pomace and juice decreased to 42% and 31%, respectively. Similar results were reported in previous studies for water kefir like beverages (Randazzo et al., 2016).

The pH values of water kefir made from pomace and juice (0 h) were 3.72 ± 0.05 and 3.63 ± 0.07, respectively, whereas pH decreased in the kefir sample made from pomace (3.39 ± 0.01) but slightly increased in kefir produced from juice (3.59 ± 0.01). Corona et al. (2016) found that the pH value of the water kefir-like beverages from fruits changed between 3.43 and 4.11. Moreover, Ozcelik et al. (2021) reported that the pH values of all water kefir beverages produced from hawthorn, cornelian cherry, roseship, red plum, and pomegranate juices varied from 3.45 to 3.97 at the end of fermentation. Similar results (pH < 4) were also obtained by Randazzo et al. (2016) for water kefir produced from Mediterranean fruit juices. Furthermore, Hampton et al. (2021) indicated that pH values of water kefir made from pear juice and puree appeared to be slightly different and this was attributed the different levels of ash content and also to lactic acid production from different strains of lactic acid bacteria during the fermentation process.

Total phenolic content (TPC)

Changes in TPC of water kefir made with aronia pomace and juice are given in Fig. 1A. Before fermentation, TPC of water kefir of pomace had 7.33 gallic acid equivalents (GAE) mg/100 mL TPC content, whereas it was 7.19 GAE mg/100 mL in the water kefir from juice. Aronia pomace and juice had higher contents of TPC before fermentation (0 h) than those after fermentation (72 h). Significant differences were found between the TPC of water kefir made from pomace and juice before and after fermentation ($p < 0.05$), TPC content of water kefir samples declined considerably compared to the initial of fermentation ($p < 0.05$). The highest decrease was observed in water kefir made from juice. Corona et al. (2016) found a decrease in TPC of water kefir produced from fruits, especially in fennel kefir like beverage (49%) and a slight increase in TPC of carrot and tomato kefir like beverages. A decrease in TPC was also reported in water kefir made from vegetables (Randazzo et al. 2016) and hawthorn, Cornelian cherry, roseship, red plum, and pomegranate juices (Ozcelik et al., 2021). However, Hampton et al. (2021) reported that a decrease in free phenolic and an increase in bound phenolic of water kefir obtained from pear juice and puree. Du & Myracle (2018) prepared aronia kefir made from cow's milk with aronia

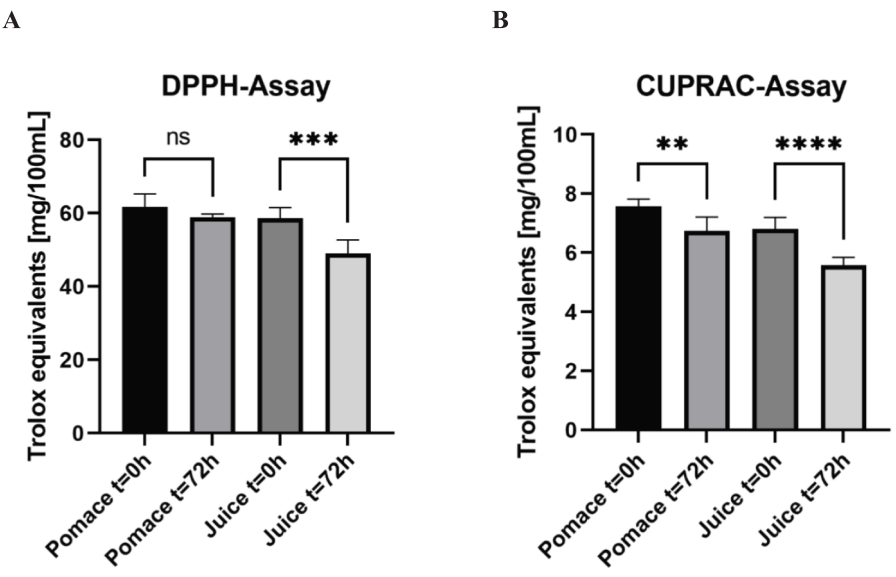

Fig. 2. Changes in total antioxidant capacity (A: DPPH, B: CUPRAC) of the non-fermented (t = 0 h) and fermented (t = 72 h) *A. melanocarpa* pomace and juice water kefir samples. The data presented in this figure consist of average values + standard deviation of two independent batches and three technical replicates. One-way ANOVA followed by Tukey's multiple comparison test, ns = not significant ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$: Statistical differences between at 0 h and at 72 h. CUPRAC, cupric ion reducing antioxidant capacity; DPPH, 2,2–diphenyl-1-picrylhydrazyl.

juice. They found that aronia kefir has less phenolic compound than the non-fermented control. Septembre-Malaterre, Remize & Poucheret (2018) attributed these decreases to the metabolic degradation of phenolic compounds by different strains of lactic acid bacteria involved in the fermentation.

Total flavonoids content (TFC)

The results showed that initial TFC of the water kefir made with pomace and juice were 1.69 and 1.55 quercetin equivalent (QE) mg/100 mL, respectively. As in TPC results, water kefir-like beverages made from aronia pomace and juice had higher TFC at 0 h of fermentation than those after 72 h of fermentation, respectively (Fig. 1B). The TFC of water kefir-like beverages aronia pomace and juice decreased after fermentation (72 h) from 1.69 to 1.52 and from 1.55 to 1.33 QE (mg/100 mL), respectively. The highest TFC was found in water kefir produced from aronia pomace at the beginning of fermentation (0 h). Fermentation (72 h) caused a decrease ($p < 0.0001$) in TFC for both kefir-like beverages. Similar results were reported in previous studies for milk kefir (Yirmibeşoğlu & Öztürk, 2020). Łopusiewicz et al. (2019) developed a non-dairy kefir-like fermented beverage based on flaxseed oil cake and they reported fluctuations in TPC and TFC during cold storage. This decrease was attributed to the fact that phenolic compounds containing flavonoids are utilized as a nutrient by lactic acid bacteria for bacterial growth (Irkin, Dogan, Degirmencioglu, Diken & Guldas, 2015). It was suggested that these are due to some factors affecting microbiota strains present in kefir grains, including the origin of the kefir grains, fermentation condition, the type of the substrate, and culture methods (Prado et al., 2015).

Total anthocyanin content (TAC)

Anthocyanin is known as a colour pigment (mostly red, blue or purple) present in vegetables and fruits. This pigment is a member of the flavonoid group with health benefits, including a scavenger of free-radicals, antiviral, antimicrobial, and anticarcinogenic properties (Kabakci et al., 2020). Total anthocyanin content of water kefir made of aronia pomace and juice before and after fermentation are shown in Fig. 1C. Products with aronia pomace had higher TAC (17.8 and 13.3 mg/100 mL kefir for 0 h and 72 h, respectively) compared to those with aronia juice (7.30 and 5.34 mg/100 mL kefir at 0 h and 72 h, respectively). Based on the 240 mL of serving size, water kefir made of aronia pomace provides 43 mg and 31 mg cyanidin-3-*O*-glucoside (cy-3-glc)/100 mL before (0 h) and after (72 h) fermentation, respectively. Similarly, water kefir made of juice contained 17.5 mg cy-3-glc/100 mL before fermentation and 12.8 mg cy-3-glc/100 mL after fermentation. Thus, these products provide more anthocyanin than the daily recommended intake which is 12.5 mg/day/person in the U.S. (Wu et al., 2006). Additionally, high anthocyanin intake results in improved insulin resistance, reduced inflammation levels, lowered LDL and total cholesterol, and decreased risk of cardiovascular disease (Bakuradze et al., 2019).

Antioxidant activity

The DPPH- and CUPRAC assays were assessed for the determination of the antioxidant activity of water kefir samples. Regarding the DPPH assay, DPPH radical scavenging activities of water kefir obtained from aronia pomace and its juice are shown in Fig. 2A. The DPPH of non-fermented samples (0 h) was 61.6 mg Trolox equivalents (TE)/100 mL for water kefir based on aronia pomace and 58.7 mg TE/100 mL for water kefir from aronia juice. After fermentation (72 h), DPPH values decreased not significant in aronia pomace based water kefir (58.9 mg TE/100 mL) and significantly decreased ($p < 0.001$) in aronia juice based water kefir (49.0 mg TE/100 mL). Aronia pomace kefir demonstrated a greater DPPH radical-scavenging activity than aronia juice kefir. Corona et al. (2016) developed water kefir-like beverages made from vegetable juices and they observed a decrease in DPPH values apart from melon and tomato. Randazzo et al. (2016) also produced non-dairy beverages produced from Mediterranean fruit juices and found decreases in DPPH values except quince with slight increase after fermentation. Ozcelik et al. (2021) used of hawthorn, Cornelian cherry, roseship, red plum, and pomegranate juices in the production of water kefir beverages. They found that during storage, DPPH values tended to decline apart from pomegranate beverage. On the other hand, high antioxidant activity of fermented plant beverages was reported for soy whey by Tu, Azi, Huang, Xu, Xing & Dong (2019), for pomegranate juice and whey by Sabokbar & Khodaiyan (2016), peanut by Bensmira and Jiang (2015) for Russian olive fruit by Darvishzadeh et al. (2021), flaxseed oil cake by Łopusiewicz et al. (2019). One of main reasons of the high antioxidant activity of water kefir is due to the lactic acid bacteria in the kefir grain as well as bioactive compounds in exopolysaccharide structure formed during fermentation (Alsayadi et al., 2013; Ozcelik et al., 2021). In the current study, antioxidant capacity of the

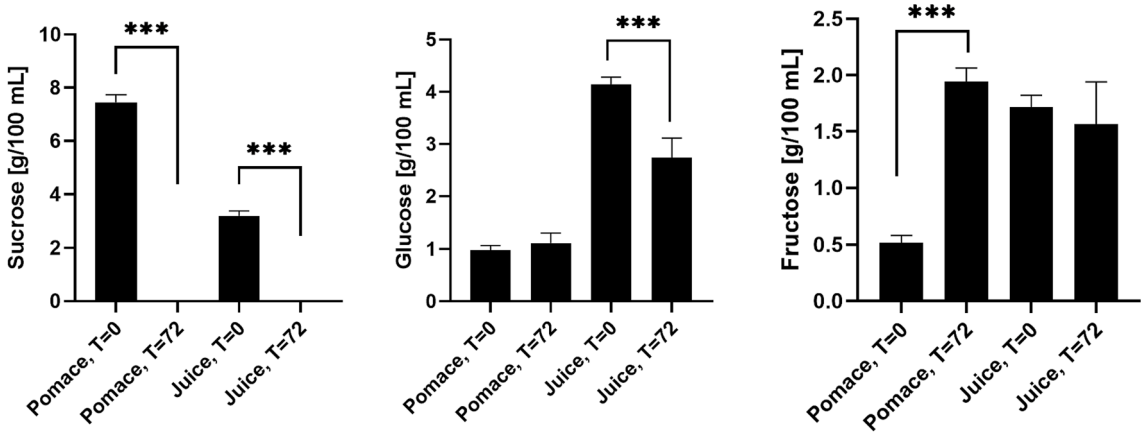

Fig. 3. Changes in sugars (sucrose, fructose and glucose) of the non-fermented (t = 0 h) and fermented (t = 72 h) *A. melanocarpa* pomace and juice water kefir samples determined by HPLC-RI. The data presented in this figure consist of average values + standard deviation of two independent batches and three technical replicates. One-way ANOVA followed by Tukey's multiple comparison test, *** $p < 0.001$: Statistical differences between at 0 h and at 72 h. Limit of detection (LOD): 0.1 mg/mL, limit of quantification (LOQ): 1.0 mg/mL.

water kefirs based on juice decreased after fermentation (72 h) and this could be due to the structure of phenolic compounds which can be affected by activity of microbial enzymes that convert them into other molecules, thus affecting the antioxidant activity of the beverage. In addition, the stability of some natural phenols and antioxidant compounds depends on pH which influence antioxidant activity and total phenolic content of the product during fermentation process (Hur, Lee, Kim, Choi & Kim, 2014).

The antioxidant capacity by the CUPRAC-assay values from the water kefir based on aronia pomace and juice decreased significantly after the 72 h fermentation period (Fig. 2B). Hence, CUPRAC declined from 7.35 to 6.68 TE mg/100 mL ($p < 0.01$) in aronia pomace based water kefir and in aronia juice based water kefir the CUPRAC values decreased from 6.81 to 5.57 TE mg/100 mL ($p < 0.0001$). Similar to TPC, the greatest antioxidant activity was observed in both aronia pomace and juice based kefir before fermentation (0 h) and the lowest antioxidant activity was found in aronia juice beverage after fermentation (72 h). There was a positive correlation of TPC to antioxidant activity for all samples before and after fermentation. As found in this study, it was shown that TPC and antioxidant activity exhibit similar pattern before and after fermentation in fruit and vegetable juices (Ozcelik et al., 2021; Corona et al., 2016).

Sugars (sucrose, fructose and glucose)

Fig. 3 shows the sugar contents of water kefir with aronia pomace and juice. Sucrose is a disaccharide composed of the monosaccharides glucose and fructose. Sucrose content was the highest in water kefir made from pomace (7.75 g/100 mL) followed by juice (3.19 g/100 mL) at initial (0 h) of fermentation. This could be due the fact that during preparation of kefir with aronia pomace, total soluble solid was too low (6.00 °Brix), and thus more sugar was added (10.4 °Brix). Glucose content was not detectable after 72 h of fermentation in water kefir produced from both pomace and juice. Laureys & Vuyst (2014) reported that sucrose level of 50 g/L was completely consumed after 24 h of fermentation in water kefir. They also indicated that during the initial 24 h of fermentation, sucrose content decreased (approximately 98%) in a water kefir (Martínez-Torres, Gutiérrez-Ambrocio, Heredia-del-Orbe, Villa-Tanaca, & Hernández-Rodríguez, 2017) whereas the levels of ethanol raised, followed by lactic acid, glycerol, acetate and mannitol. Sucrose consumption during the first 24 h of fermentation is positively associated with ethanol formation by yeasts with invertase that hydrolyses sucrose resulting in a rise in fructose and glucose, which are then utilised by lactic acid and acetic acid bacteria (Pendón, Bengoa, Iraporda, Medrano, Garrote & Abraham, 2022).

Table 1
Changes of colour values of water kefir beverages made with aronia juice and pomace before (t = 0 h) and after (t = 72 h) fermentation.

		L^*	a^*	b^*	C*	h°	ΔE*
Pomace	t = 0 h	4.46 ± 0.15^x	8.15 ± 0.06^x	3.25 ± 0.02^x	8.78 ± 0.04^x	21.75 ± 0.26^x	2.00 ± 0.10
	t = 72 h	3.80 ± 0.33^x	9.95 ± 0.23^y	3.14 ± 0.03^x	10.44 ± 0.21^y	17.55 ± 0.02^x	
Juice	t = 0 h	1.56 ± 0.06^x	5.21 ± 0.18^x	1.73 ± 0.02^x	5.51 ± 0.19^x	18.37 ± 0.53^x	10.62 ± 3.46
	t = 72 h	5.91 ± 1.43^y	14.85 ± 1.31^y	2.60 ± 0.22^y	15.09 ± 1.33^y	10.14 ± 0.04^y	

The values are mean ± standard deviation. Experiments were replicated two times and measurements were repeated three times. Significant differences ($p < 0.05$) between at 0 h and at 72 h within the groups are indicated by different superscript letters (x, y) in the column.

The reducing sugars showed increase in aronia pomace based water kefir, but a reduction in aronia juice based water kefir. The fructose content was 0.52 g/100 mL at the initial time (0 h) of fermentation and increased to 1.94 g/100 mL (increase of 3.7 fold) after 72 h of fermentation for water kefir made from aronia pomace whereas its content was 1.72 g/100 mL at 0 h and decreased to 1.56 g/100 mL at 72 h for water kefir with aronia juice. The glucose content of water kefir based on aronia pomace was 0.98 g/100 mL at 0 h and its level increased slightly to 1.11 g/100 mL whereas its content for aronia juice based kefir was 4.15 g/100 mL at the beginning of fermentation (0 h) and decreased to 2.74 g/100 mL after fermentation (72 h). Microbial growth depends on sugar catabolism, indicating the higher consumption of available sugars the higher microbial activity (Baú, Garcia & Ida, 2015). During fermentation sucrose breakage take places, and results in formation of glucose and fructose. In this work, at a 72 h fermentation time, an increase in fructose was observed for kefir from aronia pomace but a decrease was observed for kefir from aronia juice. Similar patterns were also found for glucose content. However, Destro, Prates, Watanabe, Garcia, Biz & Spinosa (2019) reported that reducing sugars showed increasing levels in water kefir including jaboticaba pulp and the fructose level ranged from 1.5 g/L at the initial time to 5.5 g/L and glucose, from 1.2 to 3.9 g/L at the final 24 h fermentation time (see Fig. 3).

Table 2
Changes in volatile compounds of the non-fermented (t = 0 h) and fermented (t = 72 h) *A. melanocarpa* pomace and juice water kefir samples determined by GC–MS.

Chemical compound (%)	Juice		Pomace	
	t = 0 h	t = 72 h	t = 0 h	t = 72 h
Alcohols				
3-Octanol	0.14 ± 0.00			
3-Heptanol	0.23 ± 0.01^{a}		0.13 ± 0.01^{b}	
1-Hexanol	0.36 ± 0.01^{xa}	0.76 ± 0.01^{yA}	8.84 ± 0.19 ^{xb}	0.16 ± 0.01^{yB}
1-Hexanol, 2-ethyl-	5.70 ± 0.10 ^{xa}	0.63 ± 0.00 ^{yA}	3.60 ± 0.04 ^{xb}	0.72 ± 0.01^{yB}
1-Octanol	0.26 ± 0.01 ^{xa}	0.09 ± 0.00 ^{yA}	0.12 ± 0.01 ^{xb}	0.16 ± 0.00^{yB}
3-Octen-2-ol	0.30 ± 0.01			
2,5-Dimethylcyclohexanol	0.67 ± 0.03			
Benzyl alcohol	0.61 ± 0.01 ^{xa}	1.66 ± 0.01 ^{yA}	4.96 ± 0.27 ^{xb}	0.51 ± 0.05^{yB}
Phenylethyl Alcohol	1.30 ± 0.10^{a}	5.24 ± 0.08 ^{yA}	0.37 ± 0.12 ^{xb}	18.7 ± 0.42^{yB}
E-11,13-Tetradecadien-1-ol	0.52 ± 0.01			0.20 ± 0.01
Ethanol		30.3 ± 0.51^{A}	6.23 ± 0.45 ^{x}	35.2 ± 0.64^{yB}
1-Butanol		9.21 ± 0.46^{A}		15.0 ± 0.54^{B}
2,3-Butanediol, [S-(R*,R*)]-		0.31 ± 0.01^{A}		0.47 ± 0.03^{B}
2,3-Butanediol				0.12 ± 0.04
4-Hexen-1-ol		0.28 ± 0.00		
4-Nonanol		0.24 ± 0.00^{A}		0.09 ± 0.00^{B}
Hexan-2-ol		0.14 ± 0.02		
3-Decyn-2-ol		0.07 ± 0.00		
1,6,10-Dodecatrien-3-ol		0.34 ± 0.00^{A}		0.32 ± 0.02^{A}
4-Penten-1-ol, 3-methyl-			0.25 ± 0.01	
3-Hexen-1-ol, (E)-			3.66 ± 0.05	
2-Hexen-1-ol, (Z)-			2.05 ± 0.13	
2,6-Octadien-1-ol, 2,7-dimethyl-		0.07 ± 0.00	0.08 ± 0.01	
1-Nonanol			0.15 ± 0.05 ^{x}	0.09 ± 0.01^{x}
1-Heptanol, 6-methyl-			0.31 ± 0.09	
1-Decanol			0.10 ± 0.00	
1-Octanol, 3,7-dimethyl-			0.31 ± 0.07	
Benzenemethanol,.alpha.-[(methylamino)methyl]-			0.06 ± 0.00	
Total	**10.1 ± 0.02** ^{xa}	**49.3 ± 0.90** ^{yA}	**31.2 ± 0.40** ^{xb}	**71.6 ± 0.41^{yB}**
Acids				
Acetic acid		0.57 ± 0.03^{A}		1.53 ± 0.04^{B}
Formic acid	0.17 ± 0.04 ^{xa}	1.06 ± 0.03 ^{yA}	0.16 ± 0.03 ^{xa}	0.10 ± 0.01 ^{xB}
Carbonic acid	0.27 ± 0.03^{a}		0.17 ± 0.01^{b}	
Pentanoic acid (valeric acid)	0.23 ± 0.00 ^{xa}	0.14 ± 0.01 ^{yA}	0.12 ± 0.01 ^{xb}	0.09 ± 0.00^{yB}
Hexanoic acid (caproic acid)	2.86 ± 0.10 ^{xa}	0.37 ± 0.03 ^{yA}	0.80 ± 0.00 ^{xb}	0.57 ± 0.04 ^{yB}

Table 2 (*continued*)

Chemical compound (%)	Juice		Pomace	
	t = 0 h	t = 72 h	t = 0 h	t = 72 h
Hexanoic acid, 2-ethyl-	0.09 ± 0.01 ^{xa}	0.28 ± 0.01 ^{yA}	0.06 ± 0.00 ^{xa}	0.43 ± 0.00^{yB}
Heptanoic acid	0.48 ± 0.39^{a}		0.50 ± 0.49^{a}	
Octanoic acid (caprylic acid)	3.86 ± 0.31 ^{xa}	2.93 ± 0.43 ^{xA}	1.87 ± 0.19 ^{xb}	3.35 ± 0.61 ^{xA}
Nonanoic acid	6.10 ± 1.35 ^{xa}	0.92 ± 0.50 ^{yA}	1.87 ± 0.15 ^{xb}	1.12 ± 0.02 ^{yA}
Propanoic acid (propionic acid)	0.08 ± 0.01 ^{x}	0.25 ± 0.00 ^{yA}		0.17 ± 0.00^{B}
Alpha-Hydroxyisocaproic acid		0.15 ± 0.04^{A}		0.11 ± 0.01^{A}
n-Decanoic acid		2.44 ± 1.14^{A}		1.86 ± 0.03^{A}
Pentadecanoic acid		0.11 ± 0.01		
Guanidineacetic acid	0.11 ± 0.00			
Total	**14.3 ± 0.79** ^{xa}	**8.69 ± 0.15** ^{yA}	**5.55 ± 0.32** ^{xb}	**7.80 ± 0.29^{yA}**
Esters				
Benzoic acid, ethyl ester		0.10 ± 0.01^{A}		0.10 ± 0.00^{A}
1-Butanol, 3-methyl-, acetate		0.47 ± 0.05^{A}		0.16 ± 0.02^{B}
Hexanoic acid, ethyl ester		0.14 ± 0.02^{A}		0.24 ± 0.02^{B}
Nonanoic acid, 5-methyl-, ethyl ester		1.74 ± 0.30	0.17 ± 0.08	
Decanoic acid, ethyl ester		3.79 ± 0.04^{A}		0.26 ± 0.03^{B}
Dodecanoic acid, ethyl ester (lauric acid)		7.72 ± 0.57^{A}		0.46 ± 0.06^{B}
E-11-Hexadecenoic acid, ethyl ester (palmitic acid)		0.90 ± 0.00		
Ethyl Acetate		0.68 ± 0.02^{A}	0.54 ± 0.02 ^{x}	0.59 ± 0.03 ^{xA}
Ethyl palmitate	0.22 ± 0.01			
Phenol, 2-methoxy-4-(2-propenyl)-, acetate		0.07 ± 0.01		
7-Octenethiol, acetate				0.19 ± 0.00
Phthalic acid	0.70 ± 0.20 ^{xa}	0.15 ± 0.04 ^{xA}	0.45 ± 0.06 ^{xa}	0.16 ± 0.02^{yA}
Total	**0.92 ± 0.36** ^{xa}	**15.7 ± 0.33** ^{yA}	**1.17 ± 0.16** ^{xa}	**2.17 ± 0.04^{yB}**
Aldehydes				
Acetaldehyde		0.07 ± 0.01^{A}		0.09 ± 0.02^{A}
Butanal, 3-hydroxy-	0.11 ± 0.05 ^{x}	0.10 ± 0.02 ^{x}		
Butanal, 3-methyl-	0.13 ± 0.00			
Hexanal	1.04 ± 0.32^{a}		0.21 ± 0.00^{a}	
Heptanal	0.13 ± 0.05^{a}		0.08 ± 0.03^{a}	
2-Hexenal, (E)-	0.11 ± 0.01			
Octanal	0.21 ± 0.01^{a}		0.11 ± 0.03^{a}	
Nonanal	0.54 ± 0.03 ^{xa}	0.17 ± 0.00 ^{yA}	0.22 ± 0.00 ^{xb}	0.13 ± 0.03^{yA}
2-Octenal, (E)-	0.11 ± 0.01			
Furfural	1.54 ± 0.35 ^{xa}	0.13 ± 0.00 ^{yA}	0.87 ± 0.03 ^{xa}	0.10 ± 0.01^{yB}
Benzaldehyde	35.39 ± 0.05 ^{xa}	4.42 ± 0.12 ^{yA}	10.86 ± 0.05 ^{xb}	2.39 ± 0.07^{yB}
Benzaldehyde, 2,4-dimethyl-	1.22 ± 0.01^{a}		0.83 ± 0.02^{b}	

(*continued on next page*)

Table 2 (*continued*)

Chemical compound (%)	Juice		Pomace	
	t = 0 h	t = 72 h	t = 0 h	t = 72 h
Pyrazole-4-carboxaldehyde, 1-methyl-	0.09 ± 0.00 ^{x}	0.07 ± 0.00^{y}		
Total	**40.6 ± 0.68** ^{xa}	**4.97 ± 0.19** ^{yA}	**13.2 ± 0.10** ^{xb}	**2.70 ± 0.12**^{yB}
Ketones				
2-Butanone		0.76 ± 0.02	0.21 ± 0.01	
3-Penten-2-one	3.82 ± 0.10^{a}		2.64 ± 0.43^{b}	
1-Propanone	0.08 ± 0.00			
3-Heptanone, 5-methyl-	0.10 ± 0.01^{a}		0.13 ± 0.01^{a}	
2-Heptanone, 4-methyl-	0.37 ± 0.00^{a}		0.26 ± 0.01^{b}	
Cyclohexanone	0.23 ± 0.01^{a}		0.09 ± 0.01^{b}	
1-Hepten-6-one, 2-methyl-			0.09 ± 0.01	
5-Hepten-2-one, 6-methyl-	0.11 ± 0.05			
Isophorone	0.09 ± 0.01			
2(3H)-Furanone, dihydro-5-pentyl-				0.20 ± 0.02
2,5-cyclohexadien-1-one		0.40 ± 0.01^{A}		0.29 ± 0.01^{B}
Dihydro-3-methylene-5-methyl-2-furanone	1.06 ± 0.04^{a}		2.40 ± 0.04^{b}	
2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (R)-	1.63 ± 0.04			0.41 ± 0.01
Total	**7.50 ± 0.01** ^{xa}	**1.16 ± 0.01** ^{yA}	**29.6 ± 0.37** ^{xb}	**0.90 ± 0.03**^{yB}
Alkane				
Heptane	0.30 ± 0.03^{a}		0.14 ± 0.00^{b}	
Heptane, 2,4-dimethyl-	0.56 ± 0.03^{a}		0.13 ± 0.01^{b}	
Propane, 2-(ethenyloxy)-			0.18 ± 0.00	
Octane, 4-methyl-	0.15 ± 0.00			
Octane, 2-methyl-	0.25 ± 0.00			
Isobutane		0.47 ± 0.02^{A}		1.24 ± 0.04^{B}
Total	**1.26 ± 0.18** ^{xa}	**0.47 ± 0.02** ^{yA}	**0.46 ± 0.01** ^{xb}	**1.24 ± 0.04**^{yB}
Hydrocarbons				
Toluene	0.25 ± 0.04^{a}		0.10 ± 0.01^{b}	
Benzene, 1,3-bis(1,1-dimethylethyl)-	3.35 ± 0.22 ^{xa}	2.80 ± 0.09 ^{yA}	2.22 ± 0.05 ^{xb}	3.05 ± 0.14^{yA}
Cyclohexane, [(1-methylpropyl) thio]-	0.71 ± 0.04^{a}		0.30 ± 0.03^{b}	
Cyclohexane, 1,4-dimethyl-2-octadecyl-			0.19 ± 0.03	
Cyclododecane		0.23 ± 0.02		
3-Trifluoroacetoxytridecane			0.08 ± 0.00	
Decane, 1-fluoro-			0.16 ± 0.06	
1,4,7,10,13,16-Hexaoxacyclooctadecane	0.09 ± 0.00			
Total	**4.41 ± 0.16** ^{xa}	**3.04 ± 0.11** ^{yA}	**3.06 ± 0.18** ^{xb}	**3.05 ± 0.14**^{xA}
Other compounds				
Linalool		0.41 ± 0.01^{A}	0.22 ± 0.02 ^{x}	0.43 ± 0.00^{yA}
L-α-Terpineol		0.19 ± 0.00^{A}	0.34 ± 0.06 ^{x}	0.11 ± 0.01 ^{yB}
Butylated Hydroxytoluene		9.71 ± 0.31^{A}		3.31 ± 0.16^{B}
2,4-Di-*tert*-butylphenol	14.02 ± 0.13 ^{xa}	2.92 ± 0.19 ^{yA}	12.56 ± 0.47 ^{xa}	3.73 ± 0.03 ^{yB}
CO_2	0.75 ± 0.07 ^{xa}	0.11 ± 0.01^{yA}	0.34 ± 0.04 ^{xb}	0.11 ± 0.02 ^{yA}
Total	**14.8 ± 0.05** ^{xa}	**13.4 ± 0.50** ^{xA}	**13.5 ± 0.51** ^{xa}	**7.69 ± 0.14** ^{yB}

Mean values ± standard deviation; results from two independent repetitions and three technical replicates. Significant differences ($p < 0.05$) between at 0 h and at 72 h within the same groups are indicated by different superscript letters (x, y) in the rows. Significant differences ($p < 0.05$) between juice and pomace at 0th h are indicated by different superscript letters (a, b) in the rows. Significant differences ($p < 0.05$) between juice and pomace at 72 h are indicated by different superscript letters (A, B) in the rows.

Colour properties

Colour values of water kefir beverages obtained from aronia pomace and juice measured before (0 h) and after fermentation (72 h) are shown in Table 1. L^*, a^* and b^* values of water kefir made with aronia juice increased significantly after fermentation ($p < 0.05$). Similarly, while a^* value of kefir made with aronia pomace increased after fermentation, no significant change was observed in L^* and b^* values after fermentation ($p > 0.05$). The increase in the chroma value of both samples after fermentation showed that the colour of kefir becomes more vivid with aronia juice and pomace, in other words, its saturation was higher. As observed in many fermented beverages, it was determined that the hue angle value (h°) decreased especially in water kefir prepared with aronia juice (Randazzo et al., 2016). The total colour difference (ΔE*) between fermentation time (0 h and 72 h) was calculated for water kefir made with aronia juice and pomace and found as 10.62 and 2.00, respectively. Mahy Van Eycken & Oosterlinck (1994) stated that a value greater than 2.3 indicates that the colour difference is visible. In line with this, slight differences were detected in the colour evaluations by the panellists in the sensory evaluation. Similarly, Corona et al. (2016) found the total colour difference in water kefir prepared with carrot and fennel to be 2.94 and 11.55, respectively, and Randazzo et al. (2016) reported the total colour difference as 3.41 and 14.91 for water kefir prepared with kiwifruit and prickly pear, respectively.

Analysis of volatile compounds

Volatile compounds of water kefir beverages made from aronia juice and pomace were extracted by the Solid Phase Micro Extraction (SPME) method and identified by GC–MS. A total of 99 volatile compounds were identified in water kefir before and after fermentation, including 14 acids, 12 esters, 28 alcohols, 13 aldehydes, 13 ketones, 6 alkanes, 8 hydrocarbons, 2 terpenes, 2 phenols and CO_2 (Table 2). Without fermentation, the main volatile components of water kefir made from aronia juice were benzaldehyde (35.4%) and 2,4-di-*tert*-butylphenol (14.0%). As for water kefir made from aronia pomace, the main volatile components were 3-penten-2-one (26.4%), benzaldehyde (10.9%) and 2,4-di-*tert*-butylphenol (12.6%) at the initial of fermentation. Benzaldehyde was the dominant aromatic aldehyde in both aronia juice and aronia pomace without fermentation. Hirvi & Honkanen (1985) also indicated benzaldehyde derivatives as the main components of aronia. Similarly, benzaldehyde was found as the basic volatile constituent in chokeberries by Butorova, Vitova & Polovka (2016) and Kraujalytė, Leitner, and Venskutonis (2013). On the other hand, Romani et al. (2016) reported that the most abundant compound in commercial chokeberry (*Aronia melanocarpa*) was 3-penthen-2-one (23.6%), as

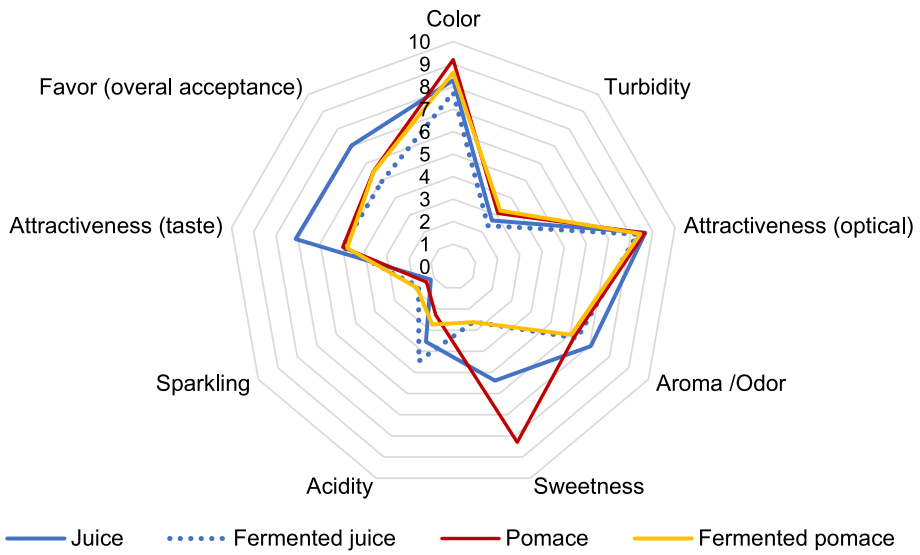

Fig. 4. Sensory analysis of before (t = 0 h) and after fermentation (t = 72 h) *A. melanocarpa* pomace and juice water kefir samples on a hedonic scale of 1–10. n = 11 panellists.

noted in aronia pomace before fermentation in this study. 2,4-Di-*tert*-butylphenol is a volatile organic acid compound, belongs to the phenol class, and displays antimicrobial and antioxidant activity (Varsha, Devendra, Shilpa, Priya, Pandey & Nampoothiri, 2015). Different natural sources and bioactivity of 2,4-di-*tert*-butylphenol, including its antioxidant capacity in terms of free radical scavenging, were reviewed by Zhao, Wang, Lucardi, Su & Li (2020). However, this compound is reported for the first time on aronia juice and pomace in this study. It was determined the main components of water kefir from aronia juice after fermentation were ethanol (30.3%), 1-butanol (9.21%) and butylated hydroxytoluene (9.71%). After fermentation, the main volatile components of water kefir made with aronia pomace were also ethanol (35.2%), phenylethyl alcohol (18.7%) and *n*-butanol (15.0%). As expected, it was observed that fermentation significantly increased alcohols in both groups ($p < 0.05$). Total alcohol contents of water kefir beverages made from aronia juice and pomace at the beginning of fermentation were 10.1% and 31.2%, respectively. After fermentation, the total alcohol in these kefirs was found as 49.3% and 71.6%, respectively. In this study, it was also determined that the total ester content in kefir made from aronia juice and pomace significantly increased from 0.92% to 15.7% and from 1.17% to 2.17%, respectively ($p < 0.05$). Similarly, Magalhães et al. (2011) stated that volatile higher alcohols and their corresponding esters increase during kefir fermentation.

Corona et al (2016) reported that the total acid content of vegetable-based kefir like beverages (carrot, fennel, melon, onion, strawberry and tomato) increased after fermentation. Similarly, Randazzo et al. (2016) stated that the acid content of kefir-like beverages made from grape, pomegranate and quince increased after fermentation. In parallel with these studies, a significant increase was observed in water kefir beverages made from aronia pomace after fermentation in this study ($p < 0.05$), but not in water kefir made from aronia juice ($p > 0.05$). However, in both kefir products, the decrease in hexanoic acid, heptanoic and nonanoic acid concentrations associated with unpleasant odour might contributed positively to their aroma and thus to their acceptability. In addition, antimicrobial acids such as acetic, propionic and α-hydroxysocaproic acid (also known as anticatabolic agent by Sumi, Sakuda, Munakata, Nakamura & Ashida, 2021) and decanoic acid with anticonvulsant activity (Chang et al., 2016) increased in both kefir groups after fermentation, having potential health benefits.

After fermentation, it was determined that the total aldehyde content significantly decreased from 40.6% to 4.97% in water kefir made with aronia juice and from 13.2% to 2.70% in water kefir made with aronia pomace ($p < 0.05$). Total ketone contents were also considerably reduced ($p < 0.05$). In particular, the absence of 3-penten-2-one, known as a lipid oxidation product, after fermentation in both groups shows a positive impact on kefirs with high quality. Laureys & De Vuyst (2016) also stated that aldehydes such as hexanal, furfural and benzaldehyde, which they detected before fermentation, were not found in water kefir after fermentation. Alike our results, Wang, Zhang & Lei (2021) determined that ketones and aldehydes reduced in all fermented pear juices. It can be said that the reduction of aldehydes and ketones results from their degradation or oxidation to alcohols or acids by LAB metabolism (Xu, Bao, Wu, Lao, Hu & Wu, 2019). It is known that the number of volatile compounds directly affects the flavour of the product, its organoleptic quality and thus its acceptability by the consumer, consequently it is important to identify these components. The production of water kefir with aronia juice and pomace with the kefir starter culture used in this study was successful, as it was evidenced by the detection of compositional changes in volatile compounds, similar to previously reported results.

Sensory evaluation

Water kefir is a non-dairy kefir, made from sucrose with or without fruit, by fermenting kefir grains. Unlike milk kefir, these products offer alternative healthy choice for a wider range of consumers including vegans and people with lactose intolerance. The unique flavour of water kefir is due to the fact that it is a self-carbonated product with ongoing lactic acid and alcoholic fermentations. In this study, the sensory properties of water kefir beverages made with aronia juice and pomace before and after fermentation were evaluated in terms of colour, turbidity, attractiveness (optical and taste), aroma, sweetness, acidity, sparkling (CO_2) and favour (overall acceptance) (Fig. 4). No difference was observed between the evaluations of colour, turbidity, optical attractiveness and sparkling in all tested groups. The lowest acidity values were observed in pomace with 2.29 before fermentation, and the highest in water kefir fermented with aronia juice with 4.46. In terms of sweetness, pomace and juice scored highest, while fermented forms scored the lowest, which can be attributed to the low sugar level at the end of fermentation. While aronia juice was the most acceptable group according to the aroma and attractiveness of taste scores, no noticeable difference was examined between the other groups. Similarly, when the groups were compared in terms of overall acceptability, it was

determined that the most preferred group was aronia juice, but its fermented forms were also within the acceptable limits. Kefir has a naturally sour, frothy and slightly acidic taste and is therefore difficult to compare with fruit juices. However, it could be also said that aronia juice and pomace (side stream from the juice industry) creates an important potential for the production of fruit-based kefir like beverages with health properties.

Conclusions

Aronia melanocarpa juice and its pomace were employed for the first time for the production of water kefir in order to valorise aronia pomace resulted from beverage industry. The resulting water kefir-like beverages were compared in terms of chemical, physical and sensory quality. As characteristics of kefir are influenced by raw materials, in this study, physico-chemical and sensory properties of water kefir made from aronia pomace were compared to those of water kefir produced from aronia juice. Although the total phenolic, flavonoid and anthocyanin contents decreased with the fermentation (72 h), the resulting water kefir still contained high phenol, flavonoid, anthocyanin contents and high antioxidant activities, providing health benefits. In conclusion, aronia pomace is regarded as a novel and a good substitute to produce water kefir with high polyphenol and antioxidant activity, especially for vegan and individual's intolerant/allergic to dairy products.

CRediT authorship contribution statement

Tuba Esatbeyoglu: Conceptualization, Methodology, Supervision, Funding acquisition, Project administration. **Annik Fischer:** Investigation, Data curation, Software. **Alessandra Legler:** Investigation, Data curation. **Manolya E. Oner:** Investigation, Data curation. **Henrik Wolken:** Investigation, Data curation. **Magdalena Köpsel:** Investigation, Data curation. **Yesim Ozogul:** Writing – original draft, Writing – review & editing. **Gülsün Özyurt:** Data curation, Writing – original draft. **Daniela De Biase:** Writing – review & editing, Funding acquisition. **Fatih Ozogul:** Writing – review & editing.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Fatih OZOGUL reports was provided by Leibniz University Hannover.

Data availability

Data will be made available on request.

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