

Physicochemical properties, antioxidant activity, sugar profile, volatile compounds, and sensory evaluation of water kefir fermented from pineapple juice and pomace at varying fermentation durations

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Abstract

Water kefir is a fermented, non-dairy beverage known for its probiotic content and associated health benefits. Recently, there has been growing interest in utilizing fruit by-products such as pomace in fermentation to enhance nutritional value and promote sustainability. This study aimed to investigate the effects of different fermentation substrates which is pineapple juice and pineapple pomace and fermentation durations (0, 24, and 72 hours at 4°C) on the physicochemical characteristics, antioxidant properties, sugar composition, volatile compound profiles, and sensory attributes of water kefir. Two water kefir formulations were prepared: one using 300 mL of diluted pineapple juice (F1), and the other using 300 mL of boiled and filtered pineapple pomace (F2). Both were supplemented with 20 g of sugar and inoculated with water kefir grains. Samples were analysed at specified fermentation intervals to assess sugar content, total phenolic content (TPC), total flavonoid content (TFC), antioxidant activity (DPPH and FRAP), pH, viscosity, Brix, volatile compounds, and sensory characteristics. Substrate type significantly influenced fermentation dynamics and kefir quality. In F1, enzymatic breakdown of complex sugars led to increased levels of glucose and fructose, promoting the formation of esters and alcohols and enhancing fruity aromas. In contrast, F2, with lower initial sugar content and higher fiber, fermented more slowly, producing more acids and esters, resulting in a tangier and more complex flavour profile. Antioxidant properties improved significantly in F2, especially after 72 hours of fermentation, with notable increases in TPC, TFC, and radical scavenging activity. F2 also showed a greater pH reduction and higher viscosity, while F1 maintained a relatively stable pH and moderate viscosity. Sensory evaluation indicated that F2 retained favourable qualities in taste, colour, and overall acceptability over time, while F1's sensory appeal declined during fermentation. Pineapple pomace serves as a valuable substrate in water kefir production, enhancing both its functional and sensory qualities while contributing to the sustainable reuse of fruit waste. Substrate selection and fermentation time are critical factors in optimizing the quality and nutritional value of functional fermented beverages.

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1. Introduction

With the rising global focus on health and wellness, the consumption of nutritious and functional foods has gained significant traction. Among such foods, fermented beverages have become increasingly popular due to their probiotic benefits. One such beverage is water kefir, a non-dairy, effervescent drink produced by

fermenting sugar water with kefir grains, a symbiotic culture of bacteria and yeast (Campbell-Falck *et al.*, 2000). Unlike traditional milk kefir, water kefir is vegan-friendly, low in alcohol (usually <1%), and suitable for individuals with lactose intolerance (Cufaoglu and Erdinc, 2023). Also known as tibicos or Japanese water crystals, water kefir is appreciated for its pleasant taste,

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health-promoting attributes, and ease of consumption (Adebamowo *et al.*, 2015). Studies have shown that water kefir consumption may enhance immune function, improve digestion, and contribute to cancer prevention (Heaney, 2004). Additionally, it contains important minerals like magnesium, calcium, and potassium that may support cardiovascular health (Heaney, 2004).

Pineapple (*Ananas comosus*), a tropical fruit from the Bromeliad family, is a popular substrate for water kefir fermentation due to its high sugar content, refreshing acidity, and rich composition of vitamins, antioxidants, and enzymes such as bromelain, which aid digestion and exhibit anti-inflammatory effects (Rathnavelu *et al.*, 2016). Pineapple juice provides an ideal medium for fermentation, nourishing the kefir grains while contributing to flavor complexity (Lee *et al.*, 2015; Mohd Ali *et al.*, 2020). Pineapple pomace, the fibrous residue from juice extraction, is rich in dietary fiber (about 76% of which is insoluble), vitamins, and minerals (Kumari *et al.*, 2020). Besides improving texture and viscosity, pomace contributes functional properties such as water absorption, oil holding, and swelling capacities (Kumari *et al.*, 2020). Its incorporation in food products promotes sustainability by reducing waste and enhancing nutritional value (Rocha-Gomes *et al.*, 2018; Santos *et al.*, 2021).

Fermentation time plays a vital role in shaping the biochemical and sensory profile of water kefir. It influences microbial activity, sugar utilization, metabolite production, and ultimately, the quality of the final product. However, limited research has addressed how fermentation duration and substrate type (juice vs. pomace) affect water kefir's sugar content, antioxidant activity, and volatile compound development. Therefore, this study aims to evaluate the effects of fermentation time on the physicochemical, functional, and sensory characteristics of water kefir made from pineapple juice and pomace, while promoting sustainable food innovation.

2. Materials and methods

2.1 Materials

The pineapple used in this study was of the 'Yankee' variety, harvested at maturity stage 6. The pineapple was collected from a local farm in Meru, Klang, Malaysia.

2.2 Preparation of samples

2.2.1 Preparation of pineapple juice and pineapple pomace

The fresh pineapple was peeled, cored and cut into pieces. The pineapple then was grinded using a mechanical blender. The resulting mixture was filtered using a muslin cloth to separate pineapple juice from the pomace.

2.2.2 Preparation of water kefir from a pineapple juice (F1)

To produce water kefir with juice, 300 mL of pineapple juice was boiled together with 300 mL of tap water and 20 g of sugar. A total of 400 mL of the prepared substrate was transferred into a fermentation flask and allowed to cool to 4°C. Once cooled, water kefir grains were added. The flask was sealed with a fermentation tube. The 0-hour sample was stored immediately in a chiller, while the 24-hour and 72-hour samples were fermented at 4°C in the chiller. After the respective fermentation periods, the mixtures were filtered using a sieve to remove the water kefir grains.

2.2.3 Preparation of water kefir from a pineapple pomace (F2)

Fresh pineapple fruit was sorted, washed, and blended. The resulting mixture was filtered to separate the juice and pomace. The pomace was packed in airtight containers and stored at room temperature until required for fermentation. For the preparation of the pomace substrate, 300 g of pineapple pomace was boiled with 3 L of water and then simmered for 10 minutes. The mixture was subsequently filtered using a straining cloth and allowed to cool to 35°C.

To produce water kefir with pomace, 600 mL of the prepared pomace substrate was boiled with 20 g of sugar. Additionally, 51.1 g of sugar was incorporated into the water kefir base containing pomace. Then, 30 g of drained water kefir grains (Fairment, Berlin, Germany) was added to the substrate in a fermentation flask. The flask was sealed with a fermentation tube. The 0-hour sample was stored immediately in a chiller, while the 24-hour and 72-hour samples were fermented at 4°C in a chiller (Bakery Proofer L 834.1B, MEC, Rimini, Italy). After fermentation, the mixture was filtered through a sieve to remove the water kefir grains.

The fermented water kefir bases, prepared from either pomace or juice, were transferred into sterilized screw-cap jars and subsequently pasteurized in a convection oven (Rational iCombi Classic 6-1/1 Elektro, Rational Kombidampfer, Neuruppin, Germany) at 85°C for 30 minutes (Esatbeyoglu *et al.*, 2023).

2.3 Physicochemical analysis of water kefir from pineapple juice and pomace

2.3.1 Brix

Total soluble solids were measured as °Brix at 20°C using a handheld refractometer (Master-20M, ATAGO Co., Ltd., Tokyo, Japan).

2.3.2 pH

For pH, three readings were taken from different kefir samples. The pH of the sample liquid was measured using a calibrated bench-top meter (Sper Scientific Ltd., Scottsdale, AZ, USA) at room temperature (23–25°C).

2.3.3 Viscosity

Viscosity measurements were carried out using advanced equipment, LFRA Texture Analyzer, made by Brookfield Engineering. The experiments were carried out in the controlled stress mode to measure the viscosity of the samples. About 150 to 250 ml of samples were put into the stationary rheometer cup. The viscosities of the products were measured at temperatures between 25 to 26°C (± 1).

2.3.4 Colour

Visual color of sample solution was measured using a Minolta colorimeter (CR-400, Minolta Camera Co., Ltd., Osaka, Japan) with the Hunter Lab color system. The color values were expressed as L^* (whiteness or brightness/darkness), a^* (redness/greenness) and b^* (yellowness/blueness).

2.3.5 Total phenolic content

Total phenolic content was determined colorimetrically using the Folin–Ciocalteu reagent. Catechin hydrate was used as the positive control. A volume of 20 μ L from each sample, including the positive control, calibration standards (prepared from a 1 mg/mL gallic acid stock solution), and blank (distilled water), was dispensed into a 96-well microtiter plate in technical triplicates. Subsequently, 100 μ L of Folin–Ciocalteu reagent (0.2 mol/L) was added to each well. After a 5-minute incubation, 100 μ L of saturated sodium carbonate solution was added, and the mixture was shaken for 12 seconds. The plate was then incubated for 60 minutes, and absorbance was measured at 765 nm using a TECAN Infinite M200 plate reader (Männedorf, Switzerland) (Esatbeyoglu *et al.*, 2023).

2.3.6 Total flavonoid content

The total flavonoid content (TFC) assay was performed following the method described by Esatbeyoglu *et al.* (2023), with slight modifications. Rutin hydrate was used as the positive control. Fifty microlitres of each sample, including the positive control (rutin hydrate), calibration standards (prepared from a 0.5 mg/mL quercetin stock solution), and blank (ethanol), were dispensed into a 96-well microtiter plate in technical triplicates. Subsequently, 130 μ L of ethanol was added to each well. This was followed by the addition of 20 μ L of a

1:1 (v/v) mixture consisting of 10% aqueous aluminum chloride solution and 1 mol/L sodium acetate solution, and the plate was shaken for 12 seconds. After 40 minutes of incubation, the absorbance was measured at 415 nm using a TECAN Infinite M200 plate reader.

2.3.7 DPPH assay (2,2-Diphenyl-1-picrylhydrazyl)

A colorimetric method based on DPPH (2,2-diphenyl-1-picrylhydrazyl) (Sigma Aldrich, San Louis, MO, USA) was used to assess the antioxidant capacity of the kefir fractions. DPPH is a nitrogenous organic free radical, susceptible to react with antioxidant compounds, when those are capable of donate an electron. The reaction was carried out in ethanolic media and monitored spectrophotometrically at 520 nm, switching colour from violet to yellow when the reaction takes place (Esatbeyoglu *et al.*, 2023).

2.3.8 Ferric reducing antioxidant power (FRAP assay)

The method described by Benzie and Strain (1996) was followed. 2.3mL of the FRAP reagent was mixed with 0.7 mL of the aqueous extracts at different concentrations (0.5-5.0 mg/mL). The mixture was then incubated at 37°C for 30 min in the dark. The absorbance was measured at 593 nm against a blank having all the reagents excluding the sample using spectrophotometer (Systronics Visiscan 167). Increased absorbance of the reaction mixture indicates an increase of reduction capability. Samples were measured in triplicates. Ascorbic acid (Merck, India) was used as the standard.

2.4 Quantitative analysis of sugar content in water kefir from a pineapple juice and pomace by using high performance liquid chromatography (HPLC)

Analysis of the sugar's sucrose, glucose and fructose were 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 NH₂ (CS Chromatographie Service GmbH, Langerwehe, Germany; 250 \times 4.6 mm, 5 μ m) column with a NH₂ pre-column was used as a stationary phase. As mobile phase ultrapure water/acetonitrile (25/75, v/v) was used (isocratic elution). The flow rate was set at 1.0 mL/min. The injection volume was 20 μ L. The column oven and RI-detector temperature was set at 35°C. Samples were diluted using ultrapure water at a ratio of 1/5 and 1/10 (v/v) and centrifuged at 14.500 rpm for 10 min (Pico 21, Thermo Scientific, Darmstadt, Germany). All the standards such as 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

Table 1. °Brix, pH, viscosity, and colour (L*, a*, b*) properties of water kefir from pineapple juice (F1) and pomace (F2).

Analysis	Formulation 1 (F1)			Formulation 2 (F2)		
	Fermentation time (hour)			Fermentation time (hour)		
	0	24	72	0	24	72
Brix	10.10 ± 0.001 ^{bA}	10.90 ± 0.02 ^{aA}	9.16 ± 0.055 ^{cA}	6.08 ± 0.11 ^{aB}	3.92 ± 0.084 ^{bB}	3.00 ± 0.001 ^{cB}
pH	3.35 ± 0.01 ^{aB}	3.21 ± 0.017 ^{cB}	3.29 ± 0.019 ^{bB}	3.90 ± 0.05 ^{aA}	3.67 ± 0.046 ^{bA}	3.45 ± 0.019 ^{cA}
Viscosity	18.33 ± 3.357 ^{bB}	31.67 ± 2.357 ^{aB}	8.33 ± 2.187 ^{cB}	28.33 ± 2.813 ^{bA}	48.33 ± 2.314 ^{aA}	28.33 ± 2.042 ^{bB}
Lightness (L*)	38.34 ± 3.312 ^{aB}	37.50 ± 0.072 ^{bB}	36.24 ± 0.72 ^{cB}	43.66 ± 0.259 ^{aA}	40.48 ± 0.985 ^{bA}	38.61 ± 0.624 ^{cA}
Redness or greenness (a*)	-1.14 ± 0.025 ^{bB}	-0.70 ± 0.256 ^{aB}	-1.18 ± 0.085 ^{cB}	-0.15 ± 0.035 ^{bA}	-0.17 ± 0.072 ^{cA}	-0.12 ± 0.212 ^{aA}
Yellowness or blueness (b*)	9.35 ± 0.4 ^{aA}	8.42 ± 0.64 ^{bA}	8.32 ± 1.114 ^{cA}	2.09 ± 0.11 ^{cB}	2.17 ± 0.209 ^{bB}	2.53 ± 0.055 ^{cB}

Values were the mean ± standard deviation of three replicates analysis. The different small letter a-c is significantly different at ($p < 0.05$) between samples and the different capital letters A-B are significantly different between fermentation time.

were stated as mg fructose/glucose/sucrose per 100mL (Esatbeyoglu *et al.*, 2023).

2.5 Analysis of volatile compounds in water kefir from a pineapple juice and pomace by using SPME-GCMS

Three grams of sample were transferred into a 20 mL headspace vial containing 2 mL of 20% NaCl solution and incubated at 60°C for 15 minutes. Volatile compounds were extracted for 30 minutes using solid-phase microextraction (SPME) with a 50/30 µm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS, 2 cm) fiber (Sigma-Aldrich, Steinheim, Germany). The analysis of volatiles was performed using an Agilent 7890B gas chromatograph (Agilent, Waldbronn, Germany) equipped with a DB-Wax capillary column (60 m × 0.25 mm i.d × 0.25 µm df; J and W Scientific, Folsom, USA) coupled to an Agilent 7010B mass spectrometry system. The split-splitless injection port was heated to 250°C, with a split ratio of 1:10. After a 5-minute desorption, the oven temperature was initially held at 40°C for 4 minutes, increased at 3°C/min to 90°C, then at 4°C/min to 130°C and held for 4 minutes, followed by a final ramp of 5°C/min to 240°C, where it was maintained for an additional 8 minutes. Helium (He) was used as the carrier gas. The ionization energy was set at 70 eV, and mass spectra were recorded in the range of 30–600 m/z. Quantification of volatiles was carried out using 4-nonanol as the internal standard (Esatbeyoglu *et al.*, 2023).

2.6 Sensory evaluation of water kefir from a pineapple juice and pomace by using SPME-GCMS

The sensorial quality of the water kefir samples derived from water kefir from pineapple and pomace was evaluated by fifthly panellists aged between 20 and 24 years, all of whom were regular consumers of either commercial or homemade water kefir. The panellists assessed the beverages using a 9-point hedonic scale, ranging from 1 ("disliked extremely") to 9 ("liked extremely"), based on predefined sensory attributes including colour, aroma, taste, viscosity, and overall flavour acceptance.

Refrigerated samples (15 mL, approximately 8°C)

were served in transparent glasses, each labelled with randomly assigned three-digit codes. The evaluations were conducted under diffused lighting, and water was provided for palate cleansing between samples.

2.7 Statistical analysis

Statistical analyses were conducted using IBM SPSS Statistics Version 28 (San Diego, USA). To determine statistically significant differences among the samples, a one-way analysis of variance (ANOVA) was performed, followed by Duncan's multiple range test at a significance level of $p < 0.05$. Two independent experimental replicates of the water kefir samples were prepared, and all measurements were conducted in triplicate. Results were expressed as mean ± standard deviation

3. Results and discussion

3.1 Physicochemical analysis of water kefir from a pineapple juice (F1) and pomace (F2)

Determination on the physicochemical properties of water kefir from a pineapple juice and pomace which includes brix, pH, viscosity, and colour are shown in Table 1. All the properties were measured at three different fermentation intervals (0-hour, 24-hour and 72-hour). Degree Brix (°Brix) is the sugar content of an aqueous solution. The results showed an initial difference in Brix values where F1 is significantly ($p < 0.05$) higher than F2. At 24-hour fermentation, the Brix for both samples decreased significantly ($p < 0.05$) to 10.90 for F1 and 3.93 for F2. This indicates that sugars in both ingredients were actively used during fermentation. The decrement in Brix is indicative of microbial activity during fermentation, where yeasts and lactic acid bacteria convert sugars into by-products such as ethanol and carbon dioxide (Randazzo *et al.*, 2016). Therefore, the significant reduction in Brix in F2 can be attributed to the difference in the sugar composition of substrates, with the pomace having a lower starting sugar level, leading to reduction of Brix value.

The pH values in F1 and F2 at different fermentation intervals (0, 24 and 72 hours) showed changes in acidity content. At 0 hours, the pH value of F2 (3.35) showed

Table 2. Total phenolic content (TPC), total flavonoid content (TFC), 2,2-Diphenyl-1-picrylhydrazyl (DPPH), and ferric reducing antioxidant power (FRAP) properties of water kefir from pineapple juice (F1) and pomace (F2).

Antioxidant Analysis	Formulation 1 (F1)			Formulation 2 (F2)		
	Fermentation time (hour)					
	0	24	72	0	24	72
TPC (mg GAE/g)	7.88 ± 0.024 ^{ba}	11.60 ± 0.045 ^{aA}	6.29 ± 0.028 ^{cA}	2.86 ± 0.031 ^{bb}	5.92 ± 0.067 ^{ab}	2.90 ± 0.008 ^{bb}
TFC (mg QE/g)	0.06 ± 0.009 ^{cA}	0.34 ± 0.034 ^{aA}	0.09 ± 0.030 ^{ba}	0.00 ± 0.000 ^{cb}	0.15 ± 0.046 ^{ab}	0.04 ± 0.017 ^{bb}
DPPH, IC ₅₀ (mg/ml)	435.09 ± 0.212 ^b	500.40 ± 2.425 ^{aB}	316.34 ± 0.361 ^{cB}	N/A	675.38 ± 18.625 ^{ba}	731.39 ± 34.726 ^{aA}
FRAP (mM Trolox/g)	3.34 ± 0.010 ^{aB}	3.21 ± 0.017 ^{cb}	3.29 ± 0.019 ^{bb}	3.91 ± 0.050 ^{aA}	3.67 ± 0.046 ^{ba}	3.45 ± 0.019 ^{ca}

Values were the mean ± standard deviation of three replicates analysis. The different small letter a-c is significantly different at (p<0.05) between samples and the different capital letters A-B are significantly different between fermentation time.

significantly (p<0.05) higher compared to F1 (3.90). This initial difference in pH could be due to the high sugar content in F1 as reported previously, which may facilitate more rapid fermentation and acid production. After 24 hours of fermentation, the pH for both formulations decreases significantly (p<0.05) and this might be due to microbial activity, including the production of organic acids by lactic acid bacteria (Hampton *et al.*, 2021). However, at 72 hours of fermentation, the pH value in F1 showed significant (p<0.05) increment to 3.29, while in F2, the pH continued to decrease to 3.49. The slight pH increment in F1 at 72 hours could be attributed to the dynamics of the microbial fermentation process where some bacterial strains may initially produce more lactic acid, but as fermentation slows down, the pH begins to stabilize (Ozcelik *et al.*, 2021).

The viscosity F1 and F2 varied significantly with fermentation time, influenced by substrate composition and microbial activity. Initially, F2 showed significant (p<0.05) higher viscosity (28.33 cP) than F1 (18.33 cP) due to its higher fiber and solid content, consistent with Aly *et al.* (2020), who noted increased viscosity in fiber-rich fermented juices. After 24 hours, viscosity peaked in both samples—F2 at 48.33 cP and F1 at 31.67 cP—attributed to exopolysaccharide production by lactic acid bacteria, as also observed in fermented pumpkin-coconut kefir (Aly *et al.*, 2020). By 72 hours, viscosity declined, especially in F1 (8.33 cP), likely due to polysaccharide breakdown, while F2 (28.33 cP) retained a thicker consistency due to its fibrous matrix. These results align with Randazzo *et al.* (2016) and Corona *et al.* (2016), highlighting how substrate type and fermentation duration influence the viscosity and sensory appeal of fruit-based kefir beverages.

The colour properties of F1 and F2 changed significantly during fermentation, particularly in lightness (L*) values as shown in Table 1, which decreased due to browning reactions likely caused by polyphenol oxidase activity under semi-aerobic conditions. F1 dropped significantly (p<0.05) from 38.94

to 3.29, while F2 also decreased significantly (p<0.05) from 43.66 to 38.61, aligning with findings by Corona *et al.* (2016). The a* values remained negative throughout, indicating persistent greenish tones with minimal change, consistent with Randazzo *et al.* (2016) and Mahy *et al.* (1994). Meanwhile, b* values showed stronger yellow tones in F1 (9.35 to 8.32) than in F2 (2.09 to 2.53), likely due to carotenoids like beta-carotene present in the juice, as supported by Mahy *et al.* (1994) and Randazzo *et al.* (2016). These results reflect the influence of fermentation on the visual attributes of water kefir, as also reported in fruit-based kefir studies by Randazzo *et al.* (2016) and Corona *et al.* (2016)

3.2 Antioxidant properties of water kefir from a pineapple juice (F1) and pomace (F2)

The total phenolic content (TPC) of F1 and F2 changed during fermentation as shown in Table 2. Initially, F1 had significant (p<0.05) higher TPC (7.88 mg GAE/g) than F2 (2.86 mg GAE/g). After 24 hours, TPC increased in both F1 (11.60 mg GAE/g) and F2 (5.92 mg GAE/g) but decreased significantly (p<0.05) after 72 hours fermentation (F1: 6.29 mg GAE/g; F2: 2.90 mg GAE/g). The higher TPC in F1 may be due to more readily available phenolics in juice, while F2's phenolics are bound to fiber and released more slowly (Ferreira *et al.*, 2023). The decline over time is likely due to oxidative or enzymatic degradation (Yang *et al.*, 2019). Similar patterns were reported by Ozcelik *et al.* (2021), who reported an increment of TPC in fruit-based kefir, and by Septembre-Malaterre *et al.* (2018), who observed TPC decreases in aronia kefir. Despite lower TPC at further fermentation, antioxidant activity may still improve due to the formation of smaller, more potent antioxidant compounds (Tu *et al.*, 2019; Alrosan *et al.*, 2023).

The TFC of F1 and F2 showed different trends throughout fermentation as shown in Table 2. At 0 hours, F1 had a TFC of 0.06 mg QE/g, while F2 showed negligible levels. After 24 hours, F2's TFC increased significantly (p<0.05) to 0.15 mg QE/g but later declined significantly (p<0.05) to 0.04 mg QE/g at 72 hours.

These changes suggest that flavonoids are initially released during fermentation but may later degrade or be utilized by microbes, consistent with Agulló *et al.* (2020) and Abdel-Razek *et al.* (2017), who reported that microbial activity and prolonged fermentation affect flavonoid availability. The lower and delayed TFC in F2 is likely due to the fiber-bound nature of flavonoids in pomace, requiring more time for release compared to the readily available flavonoids in pineapple juice. This aligns with observations by Łopusiewicz *et al.* (2019) and Yirmibeşoğlu and Öztürk (2020), who noted similar flavonoid fluctuations in milk and non-dairy kefir. As highlighted by Prado *et al.* (2015), variations in TFC can also depend on substrate type, fermentation conditions, and microbial strains. Overall, these findings support the idea that flavonoid trends in fermented beverages are influenced by both the source and transformation of compounds during microbial fermentation.

During fermentation, the antioxidant properties of F1 and F2 showed distinct patterns, influenced by changes in TPC and TFC. In F1, both TPC and TFC increased initially due to microbial breakdown of compounds, followed by a decrease at 72 hours, possibly due to degradation or microbial utilization (Abdel-Razek *et al.*, 2017; Agulló *et al.*, 2020; Ferreira *et al.*, 2023). The antioxidant activity of F1, reflected by the DPPH IC₅₀ value, decreased slightly at 24 hours (from 435.09 to 500.50 mg/mL), then improved significantly ($p < 0.05$) at 72 hours with a lower IC₅₀ of 316.34 mg/mL, indicating enhanced radical scavenging as bioactive metabolites accumulated (Erskine *et al.*, 2023). Similarly, FRAP values in F1 slightly decreased at 24 hours and increased significantly ($p < 0.05$) at 72 hours, suggesting renewed antioxidant release (Dey *et al.*, 2016). In contrast, F2 had lower TPC and TFC overall, due to fiber-bound phenolics and flavonoids in the pomace that were less accessible. Its IC₅₀ values increased over time (from 675.38 to 731.39 mg/mL), indicating a continued decline in antioxidant activity, possibly due to limited compound release and ongoing degradation (Yang *et al.*, 2018). Though F2 had a higher FRAP value at 72 hours (3.91 mM Trolox/g), this may result from the slower release of antioxidants from its complex matrix, which eventually declined due to microbial use or oxidation (Tlais *et al.*, 2020). These findings emphasize how fermentation time and substrate type significantly affect the antioxidant profile of water kefir beverages (Prado *et al.*, 2015;

Łopusiewicz *et al.*, 2019; Yirmibeşoğlu and Öztürk, 2020).

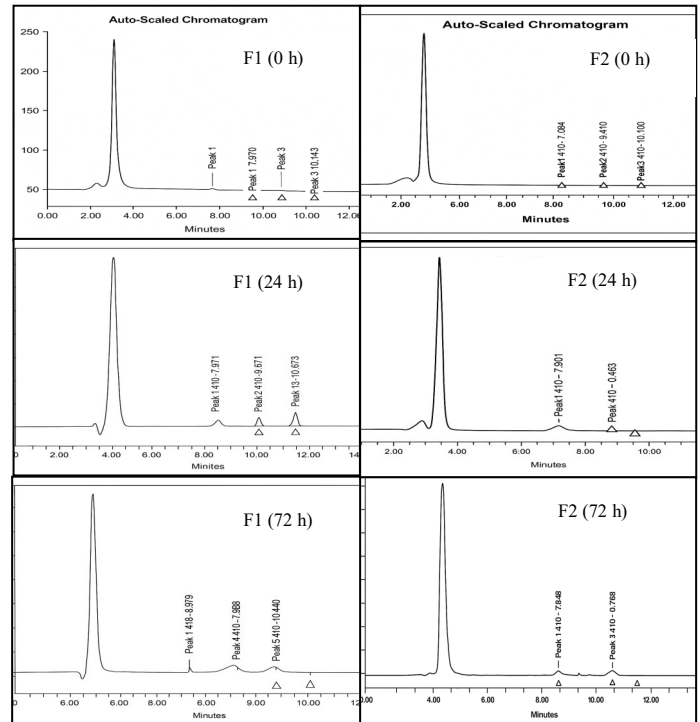


Figure 1. Chromatogram of glucose and fructose in kefir beverages from pineapple juice (F1) and from pineapple pomace (F2) at 0hrs, 24hrs and 72 h using HPLC-ELSD where (F) indicate peak for fructose while (G) indicate peak for glucose.

Sugar content in kefir beverages was analyzed using HPLC-ELSD, which detects peak areas corresponding to sugars such as glucose and fructose. Figure 1 show chromatograms of F1 and F2 at three fermentation hours: 0, 24, and 72 hours. The sugar content data are summarised in Table 3. In F1, fructose levels increased significantly ($p < 0.05$) from 0.51 mg/L at 0 hours to 2.28 mg/L at 72 hours, likely due to enzymatic breakdown of sucrose into monosaccharides such as fructose during fermentation (Guzel *et al.*, 2021). In contrast, F2 showed a less consistent trend in fructose: increasing to 0.81 mg/L at 24 hours, then decreased to 0.47 mg/L at 72 hours, possibly due to microbial utilization of fructose in later stages (Horvath *et al.*, 2020). The lower fructose levels in F2 are attributed to the lower initial sugar content of pineapple pomace compared to juice. Similarly, glucose levels in F1 rise significantly ($p < 0.05$) from 27.45 mg/L at 0 hours to 202.16 mg/L at 72 hours, suggesting disaccharide hydrolysis that may contribute to this accumulation (Kawada *et al.*, 2016). For F2, glucose

Table 3. Sugar content analysis of water kefir from pineapple juice (F1) and pomace (F2)

Types of Sugar	Formulation 1 (F1)			Formulation 2 (F2)		
	Fermentation time (hour)					
	0	24	72	0	24	72
Fructose (mg/L)	0.51 ± 0.1 ^{bA}	0.87 ± 0.1 ^{bA}	2.28 ± 0.1 ^{aA}	0.34 ± 0.1 ^{aA}	0.81 ± 0.2 ^{bA}	0.47 ± 0.1 ^{aB}
Glucose (mg/L)	27.45 ± 15.9 ^{cA}	74.45 ± 17.3 ^{bA}	202.16 ± 12.7 ^{aA}	5.57 ± 0.3 ^{cB}	32.0 ± 3.1 ^{bB}	10.66 ± 0.9 ^{aB}

Values were the mean ± standard deviation of three replicates analysis. The different small letter a-c is significantly different at ($p < 0.05$) between samples and the different capital letters A-B are significantly different between fermentation time.

Table 4. Percentage (%) of volatile compounds in water kefir from pineapple juice (F1) and pomace (F2).

Compounds	Formulation 1 (F1)			Formulation 2 (F2)		
	Fermentation time (hour)			Fermentation time (hour)		
	0	24	72	0	24	72
Phenylethyl Alcohol			7.12 ± 0.02 ^A	2.03 ± 0.01 ^b	0.51 ± 0.02 ^c	7.71 ± 0.08 ^{AA}
Acetic acid	0.31 ± 0.02 ^B			0.51 ± 0.03 ^{CA}	0.92 ± 0.01 ^b	1.09 ± 0.01 ^a
2,5 - dihydroxybenzoic acid	0.41 ± 0.03 ^B			1.09 ± 0.06 ^{CA}	19.88 ± 0.11 ^b	39.54 ± 0.21 ^a
5 - hydroxymethylfurfural		9.58 ± 0.21 ^a	2.74 ± 0.04 ^c			
Eicosane		0.21 ± 0.04 ^c	1.95 ± 0.02 ^a	3.52 ± 0.02		
Heptadecane		4.18 ± 0.07 ^a	3.84 ± 0.14 ^a	3.52 ± 0.11		
Cyclohexene		0.16 ± 0.01				
Octadecamethyl				0.09 ± 0.01 ^c	3.16 ± 0.03 ^b	43.14 ± 0.13 ^a
2 - phenylethyl ester	0.31 ± 0.02					3.01 ± 0.03
Cyclononasiloxane	1.20 ± 0.04 ^A			0.09 ± 0.01 ^{CB}	3.16 ± 0.02 ^b	43.14 ± 0.21 ^a
Squalene	41.67 ± 0.17					

Values were the mean ± standard deviation of three replicates analysis. The different small letter a-c is significantly different at (p<0.05) between samples and the different capital letters A-B are significantly different between fermentation time.

increased modestly from 5.57 mg/L to 10.66 mg/L, indicating limited sugar availability due to the pomace's higher fiber and lower free sugar content (Korkmaz, 2022).

At 0 hours, both F1 and F2 had low sugar concentrations, reflecting their unfermented state. At 24 hours, F1 showed notable increases in sugar levels due to active fermentation. After 72 hours, F1's sugar levels peaked, while F2's fructose declined and glucose plateaued, suggesting microbial consumption and limited sugar liberation. Overall, F1 consistently showed higher sugar concentrations than F2, highlighting pineapple juice's richer sugar content and better suitability for fermentation. Pineapple pomace, being more fibrous and less sugary, is a less favourable substrate for sugar release (Jose *et al.*, 2022).

3.3 Volatile compounds analysis of water kefir from a pineapple juice (F1) and pomace (F2)

The volatile compounds identified in F1 and F2 at different fermentation times, as shown in Table 4, significantly influence sensory properties such as aroma, flavour, and overall acceptability. These compounds include alcohols, acids, aldehydes, alkanes, alkenes, esters, and hydrocarbons. Among alcohols, phenylethyl alcohol which is known for its floral and honey-like aroma (Horvath *et al.*, 2020) was found at 7.12% in F1 at

72 hours, whereas in F2, it peaked at 7.71%. This suggests that fermentation supports greater alcohol production due to differing microbial activities and substrate compositions. Acetic acid, typically produced by acetic acid bacteria, was detected highest in F2 (0.92% at 24 hours and 1.09% at 72 hours), possibly due to substrate-specific microbial metabolism (Surek *et al.*, 2022). A notable increase was observed in 2,5-dihydroxybenzoic acid in F2, rising from 1.09% to 39.54%, indicating enhanced antioxidant potential (Mandha *et al.*, 2022). Aldehyde content, represented by 5-hydroxymethylfurfural, declined in F1 from 9.58% to 2.74% over time, suggesting microbial utilization during fermentation (Meena *et al.*, 2021). Heptadecane and eicosane levels also decreased in F1, which reflects lipid oxidation, although their sensory impact is minimal. Esters, such as octadecamethyl and 2-phenylethyl ester, which are important for fruity and floral aromas and were more prominent in F2 at 72 hours, likely due to the complex organic makeup of pomace that supports ester formation (Nan *et al.*, 2021). This suggests that pomace fermentation encourages ester development through diverse microbial pathways, in contrast to the simpler profiles seen in juice-based kefir. Hydrocarbons like squalene and heptasiloxane were initially higher in F1 at 0 hours but disappeared later, likely due to microbial degradation. Meanwhile, cyclononasiloxane accumulated

Table 5. 9-Point hedonic scale of sensory acceptability of water kefir from pineapple juice (F1) and pomace (F2).

Attributes	Formulation 1 (F1)			Formulation 2 (F2)		
	Fermentation time (hour)			Fermentation time (hour)		
	0	24	72	0	24	72
Colour	6.970 ± 0.273 ^{AA}	6.470 ± 0.335 ^{AA}	2.770 ± 0.294 ^{BB}	4.200 ± 0.350 ^{BB}	3.270 ± 0.362 ^{CB}	6.070 ± 3.280 ^{AA}
Aroma	5.800 ± 0.397 ^{AA}	4.770 ± 0.417 ^{AA}	3.200 ± 0.402 ^{BA}	4.200 ± 0.400 ^{AB}	3.230 ± 0.367 ^{BB}	4.270 ± 0.395 ^{AA}
Taste	6.700 ± 0.333 ^{AA}	4.600 ± 0.479 ^{BA}	2.470 ± 0.317 ^{CB}	4.570 ± 0.394 ^{AB}	2.630 ± 0.344 ^{BB}	4.100 ± 0.497 ^{AA}
Viscosity	6.330 ± 0.319 ^{AA}	5.600 ± 0.391 ^{BA}	4.070 ± 0.383 ^{CA}	4.770 ± 0.428 ^{AB}	3.730 ± 0.426 ^{BB}	5.170 ± 0.424 ^{AA}
Overall Acceptability	6.970 ± 0.354 ^{AA}	4.670 ± 0.422 ^{BA}	2.730 ± 0.253 ^{CB}	4.970 ± 0.388 ^{BB}	2.770 ± 0.278 ^{CB}	5.570 ± 0.725 ^{AA}

Values were the mean ± standard deviation of three replicates analysis. The different small letter a-c is significantly different at (p<0.05) between samples and the different capital letters A-B are significantly different between fermentation time.

in F2 at 72 hours, indicating compound-specific retention during pomace fermentation. Overall, F1, with its sugar-rich substrate, initially contained more hydrocarbons and esters, contributing to sweet and fruity aromas during fermentation. Conversely, F2 showed greater production of acids and esters, especially at later stages, reflecting more complex fermentation kinetics and a broader range of volatile compounds that may enhance antioxidant properties and aroma diversity.

3.4 Sensory evaluation analysis of water kefir from a pineapple juice (F1) and pomace (F2)

Table 5 presents the sensory evaluation results for F1 and F2 at different fermentation times (0, 24, and 72 hours), revealing notable differences influenced by both formulation and fermentation duration. Initially, F1 was the most preferred at 0 hours with a score of 6.97, while F2 scored lowest (2.77) at 24 hours. However, by 72 hours, F2 showed improved acceptability with a score of 5.57, while F1 dropped significantly to 2.73. These findings are consistent with Santos *et al.* (2021), who reported high acceptability in grape juice-based kefir for attributes such as colour, acidity, aroma, and overall preference. Colour acceptability declined in F1 at 72 hours, possibly due to enzymatic reactions and changes in phenolic content during fermentation, as reported by Corona *et al.* (2016) and Randazzo *et al.* (2016), who also noted a loss of brightness in fermented beverages over time. Aroma scores decreased for both F1 and F2 at 72 hours, particularly for F1, likely due to the accumulation of fermentation by-products like lactic acid and ethanol, which can create sour or yeasty notes and an observation supported by Randazzo *et al.* (2016) and Santos *et al.* (2021). Taste acceptability also declined, especially in F1 at 72 hours (score: 2.47), reflecting sugar consumption and acid production during fermentation, consistent with findings from Randazzo *et al.* (2016). In terms of viscosity, F2 showed higher and more stable values, attributed to its greater fiber content, which enhances beverage thickness; this trend aligns with Aly *et al.* (2021), who emphasized the role of fruit type and fiber in determining viscosity. These results demonstrate that sensory characteristics such as colour, aroma, taste, and viscosity are significantly affected by ingredient composition and fermentation time factors that are critical for consumer acceptance and the commercial viability of fruit-based kefir products.

Conclusion

In conclusion, this study showed that different types of pineapple-based water kefir and fermentation times give different benefits. Water Kefir with Pineapple Pomace (F2) at 24 hours had the best texture, pH, and sugar level, making it the most stable and consistent. Water Kefir with Pineapple Juice (F1) at 24 hours had

the highest antioxidant activity, making it great for health benefits. For taste and smell, Water Kefir with Pineapple Juice (F1) at 0 hours was the most liked, with a fresh, sweet, and pleasant flavour. The study also found that sugar breaks down differently in each type: in juice-based kefir (F1), sugars increase and lead to fruity flavours, while in pomace-based kefir (F2), sugar is used more slowly, creating more acids and complex flavours. This shows that the type of ingredient used affects the taste, health benefits, and quality of water kefir.

Conflict of interest

The authors declare no conflict of interest.

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Declaration of generative AI in scientific writing process

This study acknowledges the use of generative artificial intelligence (AI) tools to assist in the writing process. OpenAI's ChatGPT was utilized to support language refinement, grammar correction, paraphrasing, and organizing of scientific content. All data analysis, interpretation, and critical thinking were conducted by the author. The final content was thoroughly reviewed and verified to ensure accuracy, originality, and compliance with academic standards. The author(s) assume full responsibility for the accuracy and integrity of the content presented in this manuscript.

References

- Abdel-Razek, A., Badr, A. and Shehata, M. (2017). Characterization of Olive Oil By-products: Antioxidant Activity, Its Ability to Reduce Aflatoxigenic Fungi Hazard and Its Aflatoxins. *Annual Research and Review in Biology*, 14(5), 1–14. <https://doi.org/10.9734/arrb/2017/35065>
- Adebamowo, S.N., Spiegelman, D., Flint, A.J., Willett, W.C. and Rexrode, K.M. (2015). Intakes of Magnesium, Potassium, and Calcium and the Risk of Stroke among Men. *International Journal of Stroke*, 10(7), 1093–1100. <https://doi.org/10.1111/ijss.12516>
- Agulló, V., Domínguez-Perles, R. and García-Viguera, C. (2020). Sweetener influences plasma concentration of flavonoids in humans after an acute intake of a new (poly) phenol-rich beverage. *Nutrition, Metabolism and Cardiovascular Diseases*, 31(2), 930-938. <https://doi.org/10.1016/j.numecd.2020.11.016>
- Alrosan, M., Tan, T.C., Easa, A.M., Gammoh, S., Alu'datt, M.H., Aleid, G.M. and Maghaydah, S. (2023). Evaluation

- of quality and protein structure of natural water kefir-fermented quinoa protein concentrates. *Food Chemistry*, 404, 134614. <https://doi.org/10.1016/j.foodchem.2022.134614>
- Aly, E., Darwish, A.A. and Tawfek, M. (2019). Quality characteristics of sweet whey-based fruits beverages fermented with *Lactobacillus plantarum*. *Egyptian Journal of Food Science*, 47, 141–254. <https://doi.org/10.21608/ejfs.2019.17601.1024>
- AOAC. (1992). Official methods of analysis, 14th edn. Association of Official Analytical Chemists, Washington, DC.
- Benzie, I. F. and Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: the FRAP assay. *Analytical Biochemistry*, 239(1), 70-76. <https://doi.org/10.1006/abio.1996.0292>
- Cai, Y., Luo, Q., Sun, M. and Corke, H. (2004). Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. *Life Sciences*, 74(17), 2157–2184. <https://doi.org/10.1016/j.lfs.2003.09.047>
- Campbell-Falck, D., Thomas, T., Troy, M.F., Narco, T. and Kathleen, C. (2000). The intravenous use of coconut water. *The American Journal of Emergency Medicine*, 18, 108–111. [https://doi.org/10.1016/S0735-6757\(00\)90062-7](https://doi.org/10.1016/S0735-6757(00)90062-7)
- Corona, O., Randazzo, W., Miceli, A., Guarcello, R., Francesca, N., Erten, H., Settanni, H., Cufaoglu, G. and Erdinc, A.N. (2023). 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>
- Cufaoglu, G. and Erdinc, A. N. (2023). An alternative source of probiotics: Water kefir. *Food Frontiers*, 4(1), 21-31. <https://doi.org/10.1002/fft2.200>
- Dey, T.B., Chakraborty, S., Jain, K.K., Sharma, A. and Kuhad, R.C. (2016). Antioxidant phenolics and their microbial production by submerged and solid state fermentation process: A review. *Trends in Food Science and Technology*, 53, 60–74. <https://doi.org/10.1016/j.tifs.2016.04.007>
- Erskine, E., Ozkan, G., Lu, B. and Capanoglu, E. (2023). Effects of fermentation process on the antioxidant capacity of fruit byproducts. *ACS Omega*, 8(5), 4543–4553. <https://doi.org/10.1021/acsomega.2c07602>
- Esatbeyoglu, T., Fischer, A., D.S. Legler, A.E., Oner, M.F., Wolken, H., Köpsel, M., Ozogul, Y., Özyurt, G., De Biase, D. and Ozogul, F. (2023). Physical, chemical, and sensory properties of water kefir produced from *Aronia melanocarpa* juice and pomace. *Food Chemistry*, 4, 100683. <https://doi.org/10.1016/j.fochx.2023.100683>
- Guzel-Seydim, Z.B., Gökirmaklı, Ç. and Greene, A.K. (2021). A comparison of milk kefir and water kefir: Physical, chemical, microbiological and functional properties. *Trends in Food Science and Technology*, 113, 42-53. <https://doi.org/10.1016/j.tifs.2021.04.041>
- Hampton, J., Tang, C., Jayasree Subhash, A. and Serventi, L. (2021). Assessment of pear juice and puree as a fermentation matrix for water kefir. *Journal of Food Processing and Preservation*, 45(3), e15223. <https://doi.org/10.1111/jffpp.15223>
- Heaney, R.P. (2004). Sodium, Potassium, Phosphorus, and Magnesium. Humana Press EBooks, 327–344. https://doi.org/10.1007/978-1-59259-740-6_20
- Horváth, B.O., Sárdy, D.N., Kellner, N. and Magyar, I. (2020). Effects of High Sugar Content on Fermentation Dynamics and Some Metabolites of Wine-Related Yeast Species *Saccharomyces cerevisiae*, *S. uvarum* and *Starmerella bacillaris*. *Food Technology and Biotechnology*, 58(1), 76–83. <https://doi.org/10.17113/ftb.58.01.20.6461>
- Jose, M., Himashree, P., Sengar, A.S. and Sunil, C.K. (2022). Valorization of food industry by-product (Pineapple Pomace): A study to evaluate its effect on physicochemical and textural properties of developed cookies. *Measurement: Food*, 6, 100031. <https://doi.org/10.1016/j.meafoo.2022.100031>
- Kawada-Matsuo, M., Oogai, Y. and Komatsuzawa, H. (2016). Sugar Allocation to Metabolic Pathways is Tightly Regulated and Affects the Virulence of *Streptococcus mutans*. *Genes*, 8(1), 11. <https://doi.org/10.3390/genes8010011>
- Korkmaz, A. (2022). Changes in volatile compounds, sugars and organic acids of different spices of peppers (*Capsicum annum* L.) during storage. *Food Chemistry*, 311, 125910. <https://doi.org/10.1016/j.foodchem.2019.125910>
- Kumari, A., Pareek, N., Jain, N. and Mishra, S. (2020). Nutraceutical Potential of Pineapple Pomace and Its Utilization in Development of Value-Added Bread. *International Journal of Recent Scientific Research*, 11 (6), 38868- 38870. <http://dx.doi.org/10.24327/ijrsr.2020.1106.5392>
- Lee, H.J., Nagappan A., Park, H.S., Hong, G.E., Yumnam, S., Raha, S., Saralamma, V.V.G., Lee, W.S., Kim, E.H. and Kim, G.S. (2015). Flavonoids isolated from *Citrus platymamma* induce mitochondrial-dependent apoptosis in AGS cells by modulation of the PI3K/AKT and MAPK pathways. *Oncology Reports*, 34(3), 1517–1525. <https://doi.org/10.3892/or.2015.4122>
- Łopusiewicz, L., Drozłowska, E., Siedlecka, P., Meżyńska, M., Bartkowiak, A., Sienkiewicz, M. and Kwiatkowski, P. (2019). Development, characterization, and bioactivity of non-dairy kefir-like fermented beverage based on flaxseed oil cake. *Foods*, 8(11), 544. <https://doi.org/10.3390/foods8110544>
- Mahy, M., Van Eycken, L. and Oosterlinck, A. (1994). Evaluation of uniform color spaces developed after the adoption of CIELAB and CIELUV. *Color Research and Application*, 19(2), 105–121. <https://doi.org/10.1111/j.1520-6378.1994.tb00070.x>
- Mandha, J., Shumoy, H., Devaere, J., Schouteten, J.J., Gellynck, X., De Winne, A., Matemu, A.O. and Raes, K. (2022). Effect of Lactic Acid Fermentation on Volatile Compounds and Sensory Characteristics of Mango

- (*Mangifera indica*) Juices. *Foods*, 11(3), 383. <https://doi.org/10.3390/foods11030383>
- Meena, L., Sengar, A.S., Neog, R. and Sunil, C.K. (2021). Pineapple processing waste (PPW): bioactive compounds, their extraction, and utilisation: A review. *Journal Food Science Technology*, 59, 4152-4164. <https://doi.org/10.1007/s13197-021-05271-6>
- Mohd Ali, M., Hashim, N., Abd Aziz, S. and Lasekan, O. (2020). Pineapple (*Ananas comosus* L.) By-Products Valorization: Novel Bio Ingredients for Functional Foods. *Molecules*, 26(11), 3216. <https://doi.org/10.3390/molecules26113216>
- Nan, L., Liu, L., Li, Y., Huang, J., Wang, Y., Wang, C. and Wang, Z. (2021). Comparison of Aroma Compounds in Cabernet Sauvignon Red Wines from Five Growing Regions in Xinjiang in China. *Journal of Food Quality*, 2021(1), Article ID 5562518. <https://doi.org/10.1155/2021/5562518>
- Ozcelik, F., Akan, E. and Kinik, O. (2021). Use of Cornelian cherry, hawthorn, red plum, roship and pomegranate juices in the production of water kefir beverages. *Food Bioscience*, 42, 101219. <https://doi.org/10.1016/j.fbio.2021.101219>
- Ozogul, Y., Özyurt, G., De Biase, D. and Ozogul, F. (2023). Physical, chemical, and sensory properties of water kefir produced from *Aronia melanocarpa* juice and pomace. *Food Chemistry: X*, 18, 100683. <https://doi.org/10.1016/j.fochx.2023.100683>
- Prado, M.R., Blandón, L.M., Vandenberghe, L.P.S., Rodrigues, C., Castro, G.R., Thomaz-Soccol, V. and Soccol, C.R. (2015). Milk kefir: Composition, microbial cultures, biological activities, and related products. *Frontiers in Microbiology*, 6, 1177. <https://doi.org/10.3389/fmicb.2015.01177>
- Randazzo, W., Corona, O., Guarcello, R., Francesca, N., Germanà, M.A., Erten, H., Moschetti, G. and 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>
- Rathnavelu, V., Alitheen, N.B., Sohila, S., Kanagesan, S. and Ramesh, R. (2016). Potential role of bromelain in clinical and therapeutic applications. *Biomedical Reports*, 5(3), 283–288. <https://doi.org/10.3892/br.2016.720>
- Rocha-Gomes, A., Escobar, A., Soares, J.S., Silva, A.A. da, Dessimoni-Pinto, N.A.V. and Riul, T.R. (2018). Chemical composition and hypocholesterolemic effect of milk kefir and water kefir in Wistar rats. *Revista de Nutrição*, 31(2), 137–145. <https://doi.org/10.1590/1678-98652018000200001>
- Ferreira, R.M., Costa, A.M., Pinto, C.A., Silva, A.M.S., Saraiva, J.A. and Cardoso, S.M. (2023). Impact of Fermentation and Pasteurization on the Physico-Chemical and Phytochemical Composition of *Opuntia ficus-indica* Juices. *Foods*, 12(11), 2096. <https://doi.org/10.3390/foods12112096>
- Santos, D.I., Martins, C.F., Amaral, R.A., Brito, L., Saraiva, J.A., Vicente, A.A. and Moldão-Martins, M. (2021). Pineapple (*Ananas comosus* L.) By-Products Valorization: Novel Bio Ingredients for Functional Foods. *Molecules*, 26(11), 3216. <https://doi.org/10.3390/molecules26113216>
- Septembre-Malaterre, A., Remize, F. and Poucheret, P. (2018). Fruits and vegetables, as a source of nutritional compounds and phytochemicals: Changes in bioactive compounds during lactic fermentation. *Food Research International*, 104, 86–99. <https://doi.org/10.1016/j.foodres.2017.09.031>
- Surek, M., Sartori, D. and Spinosa, W.A. (2022). Role of Acetic Acid Bacteria in Food and Beverages. *Food Technology and Biotechnology*, 61(1), 85–103. <https://doi.org/10.17113/ftb.61.01.23.7811>
- Tlais, A.Z., Da Ros, A., Filannino, P., Vincentini, O., Gobbetti, M. and Di Cagno, R. (2020). Biotechnological re-cycling of apple by-products: A reservoir model to produce a dietary supplement fortified with biogenic phenolic compounds. *Food Chemistry*, 336, 127616. <https://doi.org/10.1016/j.foodchem.2020.127616>
- Tu, C., Azi, F., Huang, J., Xu, X., Xing, G. and Dong, M. (2019). Quality and metagenomic evaluation *Turkish Journal of Veterinary and Animal Sciences*, 44(4), 774–781. <https://doi.org/10.3906/vet-2001-82>
- Yang, C., Han, Y., Tian, X.L., Sajid, M., Mehmood, S., Wang, H. and Li, H. (2022). Phenolic composition of grape pomace and its metabolism. *Critical Reviews in Food Science and Nutrition*, 64(15), 4865-4881. <https://doi.org/10.1080/10408398.2022.2146048>
- Yang, X., Zhou, J., Fan, L., Qin, Z., Chen, Q. and Zhao, L. (2018). Antioxidant properties of a vegetable–fruit beverage fermented with two *Lactobacillus plantarum* strains. *Food Science and Biotechnology*, 27(6), 1719–1726. <https://doi.org/10.1007/s10068-018-0411-4>
- Yirmibeşoğlu, S.S.S. and Öztürk, B.E.T. (2020). Comparing microbiological profiles, bioactivities, and physicochemical and sensory properties of donkey milk kefir and cow milk kefir. *Turkish Journal of Veterinary and Animal Sciences*, 44(4), 774–781. <https://doi.org/10.3906/vet-2001-82>