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Influence of Soursop Fruit Tea Concentration on the Fermentation and Quality Attributes of Water Kefir Beverages

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ABSTRACT: Soursop is a seasonal and highly perishable fruit with a short shelf life after harvest. Therefore, fermentation with water kefir could be a great combination to develop a novel probiotic and dairy-free alternative beverage with distinctive flavors all year round. Therefore, this study aimed to formulate and characterize fermented beverages utilizing soursop fruit tea and water kefir microorganisms. An evaluation of physical parameters, antioxidant capacity, microbiological profiles, and sensory attributes was conducted after the fermentation process of the soursop water kefir. The result indicated that pH and °Brix values remained in the acceptance range for consumption. According to microbiological results, all water kefir samples provided a high number of lactic acid bacteria and yeasts, although no significant differences were observed in microbial counts. Furthermore, the organoleptic acceptability of the fruit water kefirs was encouraging, particularly at a 1% initial soursop fruit tea concentration, with favorable ratings for taste and overall acceptability.

KEYWORDS: *A. muricata,* soursop tea, water kefir, fermented beverage, total polyphenol content, total flavonoid content, antioxidant capacity, probiotic

I. INTRODUCTION

Soursop (*Annona muricata* Linn.), a member of the Annonaceae family, is a tropical and subtropical fruit renowned for its unique flavor and potential medicinal properties. Traditionally used in various cultures, soursop is rich in bioactive compounds, including annonaceous acetogenins, flavonoids, alkaloids, phytosterols, tannins, and phenolics (Coria-Téllez et al., 2018; Orak et al., 2019; Osei et al., 2023). These compounds have various health benefits, such as anti-inflammatory, antioxidant, antitumoral, analgesic, antidiabetic, and hypolipidemic activities. Additionally, soursop contains essential minerals (K, Ca, Na, Cu, Fe, Mg) and vitamin C, making it a valuable source of nutrients. Despite its nutritional benefits, soursop's high moisture content contributes to its susceptibility to post-harvest losses, reaching up to 40-60% due to improper handling and processing (Gustavsson et al., 2011; Okigbo & Obire, 2008). Various methods have been applied to address this issue and enhance soursop's organoleptic acceptability and commercial value. These efforts have led to the development of a diverse range of soursop-based products, including yogurt, frozen desserts, kombucha, wine, and instant spray-dried powder (Leite Neta et al., 2019; Okigbo & Obire, 2008; Virgen-Ceceña et al., 2019). Tan et al. (2020) investigated the effects of various storage conditions on soursop kombucha, which revealed significant impacts on the metabolic profile of the fermented beverage, resulting in enhanced antioxidant and antimicrobial activities and a substantial increase in phenolic content.

Water kefir is a sparkling, carbonated fermented beverage produced by the symbiotic association of lactic acid bacteria (LAB), acetic acid bacteria (AAB), bifidobacteria, and yeasts through the fermentation of kefir grains and various substrates, including sucrose, fruits, and molasses. This fermentation process produces lactic acid, carbon dioxide, ethanol, acetic acid, and various volatile aromatic compounds, resulting in a distinctive acidic and low-alcohol flavor profile (Laureys & De Vuyst, 2014; Moretti et al., 2022). Water kefir possesses a range of functional properties and potential health benefits. These benefits include antibacterial, anti-inflammatory, anti-cancer, anti-diabetic, antimicrobial, antioxidant, hepatoprotective, and wound-healing effects. Moreover, water kefir may be particularly beneficial for individuals with lactose intolerance due to its immune-boosting, digestive-enhancing, cholesterol-lowering, and prebiotic properties (Laureys & De Vuyst, 2014; Moretti et al., 2022; Romero-Luna et al., 2020).

Influence of Soursop Fruit Tea Concentration on the Fermentation and Quality Attributes of Water Kefir Beverages

There is increasing interest in novel probiotic beverages using non-dairy substrates such as fruit and vegetable juices and other substrates apart from sugary kefir. Therefore, the aim of this study was to develop and characterize fermented beverages using soursop fruit tea and water kefir microorganisms. Evaluation of physical changes, antioxidant capacity, microbiological parameters, and sensory analysis after the fermentation process of the fermented soursop water kefir was performed.

II. MATERIALS AND METHODS

A. Materials

Water kefir was purchased from Green House – Water Kefir, Milk Kefir, and Kombucha (Binh Thanh District, HCMC). Soursop tea was purchased in Vinh Long province, Vietnam. The chemicals used for analysis included 2,2-diphenyl-1-picrylhydrazyl (DPPH), quercetin, and gallic acid purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A). Folin Ciocalteu phenolic reagent Merck (Darmstadt, Germany). All the chemicals used were of analytical grade.

B. Production of soursop tea water kefir beverage and experimental setup

To prepare the tea solution, the dry pulp soursop tea at different concentrations (0, 1, 3, 5, and 10% w/v) was weighed into 1000 mL of hot filtered water, which was then brewed for around 30 minutes at room temperature. Then, the mixture was filtered, sugar was added in hot condition, stirred until smooth, and cooled to room temperature of 20±1 ^{o}C. Water kefir grains, previously activated for 72 h at 25 ^{o}C, were added to the soursop tea mixture at a rate of 5% (w/w), and fermentation was carried out at 28 ◦C for 48 h. Water kefir samples were centrifuged before further analysis. Total soluble solid content, pH, total phenolic compound, and DPPH radical scavenging activity were determined. The initial mixture's total soluble solid content and pH were adjusted to reach 10 ± 0.5 Brix and 4.5 ± 0.05, respectively.

C. Analytical methods

Physical Properties

The pH was measured before and after the fermentation using a calibrated electric pH meter (HANNA HI98107, Romania). The total soluble solids (TSS) were measured using a refractometer (Atago RX-5000α, Tokyo, Japan).

Total phenolic content

The Folin–Ciocalteu colorimetric method was used to measure the total phenolic content in fermented soursop water kefir according to Singleton & Rossi (1965) with some modifications. Briefly, 0.5 mL of soursop water kefir was mixed with 0.5 mL of distilled water, 0.5 mL of Folin-Ciocalteu reagent, and 0.5 mL of Na2CO3 solution (7.5% w/v). Then, the mixture was incubated in the dark for 30 min at ambient temperature; the absorbance of the solution was later measured at 765 nm UV-Vis spectroscopy. A standard curve was constructed using gallic acid standards ranging in concentration from 0 to 0.1 mg/mL. The total phenolic content was expressed as mg gallic acid equivalent/ 100 mL of fermented soursop water kefir (mg GAE/100mL).

Antioxidant activity

The antioxidant activity of fermented soursop water kefir was determined according to the DPPH method (Shekhar, 2014) with a slight modification. Radical scavenging activity % = (Acontrol-Asample) / Acontrol*100, where A represents the absorbance values of the control (samples with no extracts) and the test samples, respectively.

Microbiological analyses

Ten milliliters of each sample was diluted with 90 mL of 0.1% peptone water under aseptic conditions. Samples were appropriately diluted and pour-plated using de Man-Rogosa-Sharpe (MRS) Agar at 30°C for 72 h to enumerate *Lactobacillus* spp. and Potato Dextrose Agar (PDA) to enumerate yeasts at 35°C for 72 h. Microbiological analysis results were expressed as log colony-forming unit CFU/mL (Şafak et al., 2023; Spencer & Spencer, 2008).

Sensory analysis

30 panelists were asked to evaluate each product on a 9-point hedonic scale (9 = like extremely; 1 = dislike extremely) based on the appearance, aroma, taste, and acceptability of the water kefir beverages. Refrigerated samples were served randomized in glasses marked with three-digit random numbers.

Statistical Analysis

All experiments were performed in triplicate, and the results were expressed as mean ± standard deviation. Data analysis was conducted using Minitab version 17. Statistical variances between the samples and controls were assessed using a one-way analysis of variance (ANOVA) with Fisher's LSD for significant comparisons ($P < 0.05$).

III. RESULTS AND DISCUSSION

A. Physico-chemical analyses

Table 1 shows the pH and total solid content analyses of soursop tea water kefir. All samples after fermentation show a pronounced drop in pH with the symbiotic activity of lactic acid bacteria, acetic acid bacteria groups, and yeast. The result of this study followed the report from (Laureys & De Vuyst, 2014), which stated that 24 hours of incubation time was enough for sucrose, the major substrate of the fermentation, to be completely converted. The pH of 1, 3, and 5% soursop tea showed no significant difference. The initial pH of soursop fruit tea was 4.5, which support the growth of water kefir grain. After 24 hours, all pH values ranged from 3.37 to 3.76. During the fermentation, the decrease in pH was discovered to be inversely proportional to the increment of lactic acid content after fermentation (Sin et al., 2024). The total solid content significantly differed from 0% (the control) to 10% of the initial soursop fruit tea. The observed decrease in total soluble solids (TSS) was attributed to the consumption of sugars by the metabolic activities of yeast and lactic acid bacteria, producing carbon dioxide and ethanol. This trend of decreasing TSS typically happens throughout the fermentation process (Esatbeyoglu et al., 2023; Moretti et al., 2022)

Table 1. pH and of fermented soursop fruit beverage using water kefir

Concentrations (%)	pH	TSS
0 (Control)	3.37 ±3.45^{b}	7.24±0.75^{c}
1	3.45±0.21^{ab}	8.54±0.14^{b}
3	3.45±0.1 ^{ab}	8.73±0.37^{b}
5	3.49±0.11 ^{ab}	9.15±0.36^{b}
10	3.76±0.14^{a}	10.39±0.14^{a}

^{a,b,c} Different superscripts within the same column demonstrate significant differences ($p<0.05$)

Figure 1. The fermented soursop water kefir at 0, 1, 3, 5, and 10% of the soursop tea concentrations (from left to right)

B. Total phenolic content (TPC) and antioxidant activity

The differences in TPC and DPPH of water kefir made with soursop fruit tea are given in Table 2. Significant differences were found between the TPC of soursop fruit tea water kefir at various initial soursop concentrations ($p < 0.05$). The DPPH assay was used to investigate the antioxidant activities of the different fruit tea water kefir were significantly different ($p < .05$). There was an increment in total phenolic content, and % DPPH scavenging activities followed the increase in the concentration of fruit tea. The highest phenolic content and DPPH achieved in 10% fruit tea concentration, 83.49 ± 0.50 (mg GAE/100ml) and 24.65±4.08 (%), respectively. These results of this study was in accordance with study from Do et al. (2024).

Table 2. Changes in TPC, TFC, and DPPH of fermented soursop fruit beverage using water kefir

Concentrations (%)	Antioxidant parameters	
	TPC (mg GAE/100ml)	DPPH (%inhibition)
0 (Control)	3.38± 1.31^{e}	1.80±0.90^{c}
1	15.67±1.02^{d}	6.44±0.80^{c}
3	39.33± 3.63^{c}	6.83±1.31^{c}
5	54.56± 1.42^{b}	16.34±4.57^{b}
10	83.49 ± 0.50^{a}	24.65±4.08^{a}

a,b,c,d, e Different superscripts within the same column demonstrate significant differences ($p<0.05$)

C. Microbiological analyses

Water kefir grain primarily consists of lactic acid bacteria and yeasts. Maintaining kefir grains determines a well-balanced lactic acid bacteria and yeast ratio in the final beverage. After 24 hours of fermentation with water kefir grains, the *Lactobacillus* spp and yeast count reached log 6.71-6.93 CFU mL^{-1} and 4.71-4.81 CFU mL^{-1}, respectively (Table 3). Nonetheless, no significant variation in colony-forming units per milliliter of lactic acid bacteria and yeast counts was observed among all samples. The obtained results were aligned with previous studies (Alves et al., 2021; Gökırmaklı et al., 2024; GÜLHAN, 2023; Güzel-Seydim et al., 2023; Laureys & De Vuyst, 2014; Limbad et al., 2023). Sin et al. (2024) proposed that the optimal incubation duration for probiotic proliferation from water kefir fermentation lies within 30-42 hours.

Table 3. Microbiological contents of fermented soursop fruit water kefir beverage

Concentrations (%)	Microbiological analyses (log CFU/ml)	
	Lactic acid bacteria	Yeast
0 (Control)	6.93± 0.08 ^{a}	4.71±0.04 ^{a}
1	6.88±0.07 ^{a}	4.77±0.03 ^{a}
3	6.84± 0.14 ^{a}	4.81±0.10 ^{a}
5	6.72± 0.16 ^{a}	4.80±0.08 ^{a}
10	6.71 ± 0.50 ^{a}	4.74±0.13 ^{a}

Different superscripts within the same column demonstrate significant differences ($p<0.05$)

D. Sensory analysis

The sensory evaluation of soursop water kefir included assessments of appearance, aroma, taste, and general acceptability (Table 4). In general, the sample of 1% of initial soursop fruit tea received a significantly higher score for all attributes. Moreover, the sensory score control sample and the sample of 1% of initial soursop fruit tea were not significantly different ($p < .05$). In comparison, the sample of 10% of initial soursop fruit tea received the lowest scores in all criteria. The reason might be that the distinct smell and color of soursop were still present in the final fermented product, negatively impacting its sensory properties. Customers might not be familiar with plant-based extract products, especially dried soursop tea. Sensory analysis of craft beer made with soursop also showed relatively high scores (6.9-7.6), which was in line with the result of this study.

Table 4. Sensory evaluation of fermented soursop fruit beverage using water kefir

Concentrations (%)	Attributes			
	Appearance	Aroma	Taste	Acceptability
0 (Control)	7.20±0.84^{ab}	6.00±0.71^{ab}	6.80±1.30^{a}	6.60±1.32^{ab}
1	7.60±0.89^{a}	7.04±0.89^{a}	7.00±1.23^{a}	7.20±0.84^{a}

3	5.80±.1.3^{bc}	6.05±0.76^{ab}	6.00±1.41^{a}	6.20±1.30^{abc}
5	5.40±0.89^{c}	5.40±0.55^{b}	5.20±0.45^{ab}	5.20±1.45^{bc}
10	5.00±0.71^{c}	4.80±0.84^{b}	3.80±0.84^{ab}	4.20±1.14^{c}

^{a,b,c} Different superscripts within the same column demonstrate significant differences ($p<0.05$)

IV. CONCLUSION

This study investigated the benefit of using soursop fruit tea to develop fermented beverages using water kefir microorganisms. In conclusion, soursop fruit tea is considered a novel and a good substitute for producing water kefir with promising polyphenol, antioxidant activity, and sensory acceptance, especially as a dairy-free alternative.

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