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The effect of soursop as fermentable substrate in formulating flavoured water kefir beverage

[1]Mat Ali N.N., [1,*]Abu Bakar F.I., [1]Abu Bakar, M.F., [1]Malik, N.H., [1]Muhammad, N., [1]Sabran, S.F., [1]Pa'ee, F., [1]Santoso, D.L., [2]Ramli, M.E., [3]Mohamad, A. and [3]Muhamad Hanafi, A.F.

[1]*Faculty of Applied Sciences and Technology, Universiti Tun Hussein Onn Malaysia, Hab Pendidikan Tinggi Pagoh, KM 1, Jalan Panchor, 84600 Muar, Johor, Malaysia*
[2]*Food Technology Division, School of Industrial Technology, Universiti Sains Malaysia, 11800 Minden, Pulau Pinang, Malaysia*
[3]*Success Vision Group Sdn. Bhd., Lot 3592 Kedai Tingkat 1, Taman D'Nik, Jalan Besar, Kampung Gong Badang, 22000 Jerteh, Terengganu*

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Abstract

Water kefir is a fermented sucrose solution from fruits or vegetable juices that produces acidic fermented beverages. This study aimed to explore the usage of soursop pulp as a fermentable substrate to formulate a non-dairy fermented beverage. In this study, fruit juice from soursop was fermented with water kefir grains for 48 hrs at 24°C. The first fermentation of sugary water with water kefir grains was carried out for four days and the second fermentation of the fermented water with soursop juice was carried out for two days at room temperature. The beverage formulations based on soursop concentrations were 25, 50, 75 and 100% (v/v). Results showed that the physicochemical analyses were in the range of (pH = 3.22 to 3.34, total soluble solid (TSS) = 4.59 to 10.42°Bx, and colour with lightness (L*) of 31.17 to 56.29, red and green intensity (a*) of -0.98 to -2.76 and yellow and blue intensity (b*) of -3.06 to 2.75). The alcohol content ranged from 0.43 to 0.62%. The total phenolic content decreased significantly (p<0.05) over time for each sample. Due to the process, it had heavily influenced the panellists during the sensory tests, in which favoured sample with 50% v/v soursop in the water kefir beverage. In conclusion, the utilization of soursop juice in the production of water kefir beverages can be introduced as a newly flavoured fermented drink using water kefir grains.

1. Introduction

The development of new functional beverages is rapidly growing in demand in the food industry. People are becoming more aware of the importance of their health and looking for the best functional drinks for their health. One functional beverage that is becoming increasingly popular is water kefir. Water kefir is a fermented sucrose through a symbiotic mixture of lactic acid bacteria (LAB) and yeasts (SCOBY) (Lynch *et al.,* 2021) from the genera *Lactobacillus* and *Pichia*, respectively. Brown sugar is typically used as the main substrate for water kefir fermentation (Guzel-Seydim *et al.,* 2021). Nowadays, various fruits and vegetables such as tiger nut (Satir, 2022), cashew nut (Araujo Filho *et al.,* 2023), cagaita (dos Santos *et al.,* 2024), coconut (Limbad *et al.,* 2024), and others have been used as substrate for fermentation. These alternatives of substrate offer diversification, nutritional enrichment and sensory improvement, making the products more attractive and accepted (Sanches *et al.,* 2024).

The water kefir fermentation is usually up to 96 hrs at temperatures between 20 and 25°C (Laureys and De Vuyst, 2014). The fermentation of beverages increases acidity, produces ethanol and carbon dioxide, and enhances the flavour of the product. The fermentation conditions, grain structure and substrate concentrations largely determine the beverage composition. Aligned with scientific and technological advancements, live symbiosis can have interesting biological functions for humans such as anti-inflammatory, hepaprotective, antihypertensive and others which have been recorded.

The soursop (*Annona muricata*) is a tropical fruit tree that belongs to the Annonaceae family. It is primarily consumed as fresh fruit due to its pleasant taste and aroma, giving it significant economic value. It is

*Corresponding author.
Email: *fazleen@uthm.edu.my*

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widely cultivated and consumed in tropical and subtropical regions such as South America, Central America and Asia including Malaysia (Pineda-Ramirez *et al.,* 2020). Soursop has garnered attention due to its being an excellent source of natural antioxidants, dietary fibres, and vitamin C, which are associated with significant potential health benefits, including the treatment of diarrhoea and intestinal parasitosis (Chang *et al.,* 2018; Selvanathan *et al.,* 2022).

Despite the numerous studies associated with water kefir has been done, no study has been found in the literature on the fermentation of water kefir and soursop. This study aimed to formulate the soursop extract as a new substrate for water kefir fermentation and provide unique insights into the physicochemical and sensory properties of newly formulated samples.

2. Materials and methods

2.1 Materials

Soursop (*Annona muricata L.*) was obtained from the local seller (Pagoh, Johor, Malaysia). Water kefir grains were obtained from My Kefir World company in Cheras, Malaysia. Brown sugar was obtained from the local market in Pagoh, Johor, Malaysia.

2.2 Production of water kefir beverages

Soursop fruit was washed thoroughly under running tap water to remove the impurities and hand peeled and the seed removed. The core and seeds were removed to obtain the pulp, blended and filtered through a plastic strainer. Then soursop juice was pasteurized at 78.8℃ for 5 mins and cooled at room temperature before stored at -18℃. For control, water kefir was inoculated into a brown sugar solution and incubated at 24℃ for four days to activate water kefir to form an active inoculum. Meanwhile, water kefir was inoculated into different concentrations of soursop juice at 25%, 50%, 75%, and 100% (v/v), coded as 1604, 1704, 1804, and 1904, respectively. The mixtures were then incubated at 24°C for two days.

2.3 Determination of pH

pH meter calibrated with buffer reference standards of pH7 (7±0.01), pH 4 (4.01±0.01) and pH 9.21 (9.21±0.01) was used to measure the pH of the formulation (Ozcelik *et al.,* 2021).

2.4 Determination of total soluble solid content

The total soluble solids (TSS) amount of the samples was measured using a refractometer (Atago, PAL-3, Japan) (Esatbeyoglu *et al.,* 2023).

2.5 Colour

The visual colour of the samples in terms of instrumental colour values L*, a* and b* were measured using a colourimeter (HunterLab Associates, USA) in which L* (lightness), a* (red-green) and b* (yellow-blue) are the coordinates of chromaticity (Esatbeyoglu *et al.,* 2023).

2.6 Determination of ethanol content

The dynamic changes of alcohol content in water kefir fermented with soursop were analyzed by gas chromatography-mass spectrometry (GC-MS) with the method described by Ebersole *et al.* (2017).

2.7 Phytochemical analysis

2.7.1 Determination of total phenolic content

The total phenolic content (TPC) was measured according to the Folin-Ciocalteu calorimetric method as described by McDonald *et al.* (2001). For quantification, 20 µL of the extracted sample was prepared in test tubes and added with 600 µL of 15% Na_2CO_3. The mixture was mixed with 2230 µL distilled water, and homogenized with vortexed. Then, 150 µL of Folin-Ciocalteu reagent was added and incubated for 2 hrs in the dark at 25°C. The absorbance value was measured by UV-Vis spectrophotometer at wavelength of 760 nm. Gallic acid was used as a standard and the result was expressed as milligram gallic acid equivalents per mL of soursop-flavoured water kefir samples (mg GAE/mL sample).

2.8 Sensory analysis

The newly formulated water kefir with soursop was used for the sensory evaluation. The sensory were evaluated by a panel consisting of 100 untrained panellists from Universiti Tun Hussein Onn Malaysia, Johor, Malaysia. The panellists were asked to evaluate the appearance, odour, taste, acidity, and overall acceptability based on the visual assessment by assigning a liking score on a 0-point hedonic scale (1 = like extremely; 2 = like very much; 3 = like moderately; 4 = like slightly; 5 = neither like or dislike; 6 dislike slightly; 7 = dislike moderately; 8 = dislike very much; 9 = dislike extremely). The tests were accomplished in individual booths lightened with a white fluorescent lamp, and the samples were presented in 50 mL of soursop-flavoured water kefir fermented for 6 days and labelled with a 4-digit code to the panellists (Esatbeyoglu *et al.,* 2023).

2.9 Statistical analysis

The results obtained were performed in triplicate (n = 3). Results were expressed as mean ± standard deviation (SD). A one-way analysis of variance

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(ANOVA) test was used to analyze the data and the differences were compared by Tukey's test at a 95% level of significance using Statistical Package for the Social Sciences (SPSS).

3. Results and discussion

3.1 Physicochemical analysis

The pH of the food is an important parameter of food safety related to the structural changes of the microbiota and phytochemicals during fermentation. According to Table 1, the pH value of all samples reduced significantly (p<0.05) over the 6-day fermentation regardless of sample concentrations. The samples used the same water kefir base; hence the pH values were all identical on the 0^{th} day. On the 4^{th} day, a drop in the pH value of the samples is expected, relating metabolism of microorganisms which produce ethanol, volatile compounds and carbon dioxide in addition to producing acid during fermentation (Ahoussi-dahouenon *et al.*, 2013). Besides that, the changes in the pH of samples during storage may be due to the addition of glucose from soursop juice which promoted proton (H^+) concentration which eventually reduced the pH values.

The total soluble solid values in the samples ranged from 4.59 to 9.19°Bx as shown in Table 1. TSS values of all concentrations were significantly different where 25% had the lowest TSS value. According to Ozcelik *et al.* (2021), fermentable carbohydrates, like sugars, are converted to ethanol and carbon dioxide (CO_2) during fermentation and therefore, decrease the TSS value.

3.2 Ethanol content

In this study, as analyzed in the total soluble solid of all samples, the sugar decreased over time. Hence, it is thought that the alcohol was produced during the process of fermentation but decreased as it went to the fourth day since there might not have been enough sugar left in the environment for the growth of yeasts, and increased back after the addition of soursop, which added up extra sugars in the beverage. As seen in Table 2, the alcohol content range in this study was between 0.43 to 0.62% ABV. The highest alcohol produced was 0.62±0.01% ABV in 100% v/v soursop in the water kefir beverage. On the other hand, the sample with the least alcohol content is 25% with 0.43±0.00% ABV. The extra sugars from soursop juice that were added on the fourth day of fermentation might cause the level of alcohol to increase as it lets yeast break down the glucose into alcohol. The production of alcohol during the 72 hrs of fermentation was said to be similar to the production of lactic acid, but opposite to that of the amount of sugar (Pertiwi *et al.*, 2013). However, between the samples, there is a significant difference (p<0.05).

Table 2. Alcohol content (%ABV) of soursop-flavoured water kefir

Sample	Alcohol content (%ABV)
25%	0.42±0.02^a
50%	0.49±0.02^b
75%	0.55±0.00^c
100%	0.63±0.01^d

Values are presented mean±SD of triplicate determinations. Values with different superscripts within the same column are statistically significantly different (p<0.05).

3.3 Colour analysis

For the colour parameters, Undoubtedly, the fermentation of soursop juice caused the colour changes of kefir water and influenced the organoleptic properties. The utilization of soursop in the water kefir beverage caused changes to the colour values. Soursop pulp is creamy and white in colour (Sabokbar and Khodaiyan, 2016) whereas the water kefir is brown, due to the brown sugar used as fermentable substrates. The lightness values (L*) of the different concentrations of soursop juice were significantly reduced (p<0.05) after 6 days of fermentation (Table 3), which might be due to the partial degradation of nutrients like fat (Satir, 2022). Besides that, the negative value of a*, indicated the greenish colour observed in fermented products may be attributed to the riboflavin content of kefir grains.

3.4 Total phenolic content

The total phenolic content of the samples is shown in Table 4. The TPC content was increased on day 4 of fermentation and later decreased on the 6th day. Among the samples, 100% showed the highest TPC value

Table 1. pH values and TSS of soursop-flavoured water kefir beverage

Sample	pH			TSS		
	0^{th}	4^{th}	6^{th}	0^{th}	4^{th}	6^{th}
25%	3.97±0.01^a	3.59±0.01^b	3.22±0.02^b	9.19±0.06^a	6.54±0.02^d	4.59±0.03^a
50%	3.97±0.01^a	3.62±0.01^a	3.23±0.02^b	9.19±0.06^a	7.42±0.07^c	5.83±0.01^c
75%	3.97±0.01^a	3.62±0.01^a	3.32±0.01^a	9.19±0.06^a	8.11±0.02^b	8.64±0.05^b
100%	3.97±0.01^a	3.59±0.00^b	3.34±0.02^a	9.19±0.06^a	10.55±0.0^a	10.42±0.04^a

Values are presented mean±SD of triplicate determinations. Values with different superscripts within the same column are statistically significantly different (p<0.05).

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Table 3. Colour analysis of soursop-flavoured water kefir beverage.

Sample	L*			a*			b*		
	0^{th}	4^{th}	6^{th}	0^{th}	4^{th}	6^{th}	0^{th}	4^{th}	6^{th}
25%	43.58 ± 1.75^{a}	30.55 ± 4.31^{c}	31.17 ± 1.42^{d}	0.30 ± 0.16^{a}	-1.06 ± 0.77^{a}	-0.98 ± 0.03^{a}	18.85 ± 0.73^{a}	-1.04 ± 2.33^{b}	-3.06 ± 0.13^{b}
50%	43.58 ± 1.75^{a}	39.04 ± 5.6^{bc}	40.22 ± 2.77^{c}	0.30 ± 0.16^{a}	-1.48 ± 0.63^{a}	-1.98 ± 0.09^{b}	18.85 ± 0.73^{a}	0.33 ± 1.65^{ab}	-3.07 ± 0.15^{b}
75%	43.58 ± 1.75^{a}	45.19 ± 3.47^{b}	47.02 ± 0.44^{b}	0.30 ± 0.16^{a}	-1.96 ± 0.22^{a}	-2.52 ± 0.20^{c}	18.85 ± 0.73^{a}	-0.1 ± 0.43^{b}	-2.54 ± 0.35^{b}
100%	43.58 ± 1.75^{a}	59.69 ± 2.90^{a}	56.29 ± 1.47^{a}	0.30 ± 0.16^{a}	-1.78 ± 0.582^{a}	-2.76 ± 0.03^{c}	18.85 ± 0.73^{a}	4.24 ± 1.45^{a}	2.755 ± 0.13^{a}

Values are presented mean±SD of triplicate determinations. Values with different superscripts within the same column are statistically significantly different ($p<0.05$).

Table 4. Total phenolic content of soursop-flavoured water kefir beverage.

Phytochemical contents	Sample	Fermentation time		
		0^{th}	4^{th}	6^{th}
TPC (mg GAE/g)	25%	$6.58\times10^{-5}\pm0.00^{a}$	$5.27\times10^{-5}\pm0.00^{a}$	$2.63\times10^{-5}\pm0.00^{a}$
	50%	$6.58\times10^{-5}\pm0.00^{a}$	$6.53\times10^{-5}\pm0.00^{b}$	$3.92\times10^{-5}\pm0.00^{b}$
	75%	$6.58\times10^{-5}\pm0.00^{a}$	$7.97\times10^{-5}\pm0.00^{c}$	$4.92\times10^{-5}\pm0.00^{c}$
	100%	$6.58\times10^{-5}\pm0.00^{a}$	$8.18\times10^{-5}\pm0.00^{c}$	$7.99\times10^{-5}\pm0.00^{d}$

Values are presented mean±SD of triplicate determinations. Values with different superscripts within the same column are statistically significantly different ($p<0.05$).

Table 5. Sensory results for each attribute in different samples of soursop-flavoured water kefir beverage.

Attributes	Sample			
	25%	50%	75%	100%
Appearance	5.50 ± 1.86^{b}	6.42 ± 1.53^{a}	6.22 ± 1.52^{a}	5.96 ± 1.67^{ab}
Odour	5.61 ± 1.65^{a}	6.06 ± 1.44^{a}	5.99 ± 1.68^{a}	6.03 ± 1.57^{a}
Taste	5.11 ± 1.84^{b}	6.08 ± 1.35^{a}	5.90 ± 1.814^{a}	6.05 ± 1.89^{a}
Acidity	5.02 ± 1.77^{b}	5.92 ± 1.52^{a}	5.89 ± 1.65^{a}	5.92 ± 1.70^{a}
Overall acceptance	5.08 ± 1.73^{b}	6.24 ± 1.66^{a}	6.04 ± 1.55^{a}	6.16 ± 1.84^{a}

Values are presented mean±SD of triplicate determinations. Values with different superscripts within the same column are statistically significantly different ($p<0.05$).

Table 6. Sensory results for each attribute in different samples of soursop-flavoured water kefir beverage.

Attributes	Sample			
	25%	50%	75%	100%
Appearance	5.50 ± 1.86^{b}	6.42 ± 1.53^{a}	6.22 ± 1.52^{a}	5.96 ± 1.67^{ab}
Odour	5.61 ± 1.65^{a}	6.06 ± 1.44^{a}	5.99 ± 1.68^{a}	6.03 ± 1.57^{a}
Taste	5.11 ± 1.84^{b}	6.08 ± 1.35^{a}	5.90 ± 1.814^{a}	6.05 ± 1.89^{a}
Acidity	5.02 ± 1.77^{b}	5.92 ± 1.52^{a}	5.89 ± 1.65^{a}	5.92 ± 1.70^{a}
Overall acceptance	5.08 ± 1.73^{b}	6.24 ± 1.66^{a}	6.04 ± 1.55^{a}	6.16 ± 1.84^{a}

Values are presented mean±SD of triplicate determinations. Values with different superscripts within the same column are statistically significantly different ($p<0.05$).

whereas 25% showed the lowest content of TPC on the 6th day of fermentation. According to Sabokbar and Khodaiyan (2016), the TPC content increased during fermentation due to the metabolic activities of microorganisms in kefir grains. More studies have shown that fermentation by lactic acid bacteria is capable of increasing the content of phenolic compounds in fermented (Dordević *et al.*, 2010; Coda *et al*., 2012).

3.5 Sensory analysis

The results of the sensory evaluation of soursop-flavoured water kefir were evaluated in terms of appearance, odour, taste, acidity and overall acceptance as shown in Table 5. The samples showed satisfactory scores in all sensory attributes assessed where the concentration of 50% scored the best values of overall acceptance at 6.24, followed by 100%, 75% and 25% with the scores 6.16, 6.04 and 5.08, respectively. There was a decreasing trend in sensory scores in parallel with the soursop concentrations.

4. Conclusion

In this study, water kefir fermented with health-promoting ingredients, soursop was evaluated by physicochemical parameters, total phenolic content and sensory analysis. The result demonstrated that the pH, colour, and total phenolic decreases over time for all samples throughout the fermentation process. Besides that, the incorporation of 50% soursop exerted the highest acceptability from panellists during sensory analysis. Therefore, it can be concluded that the utilization of soursop juice in the production of water kefir beverages can help further research into the development of functional beverages.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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