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The Nutritional, Physico-chemical, and Antioxidant Changes during the Production of Soursop Vinegar Influenced by Yeast and Aeration

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ABSTRACT

This study aims to produce soursop vinegar and evaluate how its nutritional composition changes during production. This process offers a solution to convert acidic fruits like soursop into a nutritious product with a longer shelf life. Fresh soursop juice was extracted using a mechanical press, followed by alcoholic and acetous fermentation under different aerobic and anaerobic conditions, with some samples aerated. The nutritional analysis included proximate composition, vitamin C content, acetic acid, pH, and soluble solids (TSS or ºBrix). Results during fermentation showed increased moisture content (90.73–93.99%) compared to the control (78.56%) and a decrease in protein content (0.5–0.61%) when *Saccharomyces cerevisiae* was present (1.53–1.84% without it). Acetic acid and vitamin C levels remained relatively stable, while ºBrix values significantly decreased (4.83–7.00 ºBrix) compared to the initial 15 ºBrix of the control during fermentation. Aeration during production improved the vinegar's antioxidative capacity, with the highest enhancement observed when *Acetobacter* sp. was added. Overall, this study highlights the potential of soursop vinegar to retain the nutritional composition of the fruit, resulting in a healthier, natural product with an extended shelf life.

Keywords: Antioxidant, fermented food, food waste, functional food, postharvest, soursop, vitamin

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INTRODUCTION

Vinegar, known for its sour taste due to the presence of acetic acid, has a long history dating back to ancient times. Babylonians produced wine vinegar from palm as early as 5000 BC (Jamaludin et al., 2017), while cereal vinegar was found in China around 3000 BC (Wu et al., 2012). Today, vinegar comes in various types, each with its colors, tastes, and smells, derived from different ingredients, resulting in unique flavors. It comes from a plant-based source and is rich in flavonoids, phenolic acids, and aldehydes, making it a functional food (Bakir et al., 2016; Shin Yee et al., 2021).

Although synthetic methods exist for acetic acid production, bacterial fermentation is the preferred process for food intended for human consumption due to its safety and suitability for food products (Faizal et al., 2023; Hajar-Azhari, Wan-Mohtar et al., 2018; Khudair et al., 2023; Wan-Mohtar et al., 2019). Additionally, The Malaysia Food Regulation 1985 (Regulation 334) stated that the minimum acetic acid content in vinegar should be not less than 4% by weight. It ensures the vinegar has the characteristic acidic taste and properties of this product (Jamaludin et al., 2017).

Commercial vinegar production employs both slow and fast fermentation processes. Slow fermentation, a traditional method, takes several months to years, resulting in a rich taste and aroma (Abd Rahim et al., 2023). Fast fermentation, on the other hand, is more commonly used and involves a double fermentation process. Yeast converts sugar to alcohol, which is then transformed into acetic acid by acetic acid bacteria, known as the "mother" of vinegar (*Acetobacter* sp.) (Ho et al., 2017). This faster process can be completed within a week, making it ideal for commercialization.

Soursop fruit, scientifically known as *Annona muricata*, is a popular local fruit in Malaysia. It has a green outer skin and a creamy white pulp with a sour taste. Locals believe it has high nutritional value and benefits health (Badrie & Schauss, 2010). However, soursop has a short shelf life and reduced production due to climate change, making it prone to rapid spoilage and chilling injury (Badrie & Schauss, 2010). Diseases like black canker (*Phomopsis anonnaccarum*) and diplodia rot (*Botryodiplodia theobramae*) further contribute to fruit spoilage (Sanusi & Abu Bakar, 2018).

This study focuses on producing soursop vinegar using a double fermentation method, with or without aeration, to address the rapid spoilage and preserve its nutritional content. The double fermentation method involves variations in the presence or absence of yeast *Saccharomyces* sp. Thus, a "double" fermentation as the subsequent fermentation will be performed by *Acetobacter* sp. The objective is to investigate the nutritional changes during the vinegar production process and compare them to the nutritional profiles of fresh soursop juice (negative control 1, SJ) and Bragg's organic raw and unfiltered Apple Cider vinegar (positive control 2, AC).

By utilizing the double fermentation process, the study aims to create soursop vinegar that retains its nutrients and enhances its shelf stability. Overall, this research highlights the potential of soursop vinegar as a value-added product, offering a practical and sustainable solution to prevent rapid spoilage while maximizing its nutritional benefits.

MATERIALS AND METHODS

Materials

The soursop fruit used in this study was purchased from Mawai, Kota Tinggi, Johor, Malaysia. Instant yeast powder (*Saccharomyces cerevisiae*) of Mauri-Pan (Malaysia) was obtained from Rakanda Enterprise Sdn. Bhd. in Seri Kembangan, Selangor, Malaysia. The "mother" of vinegar (*Acetobacter* sp.) was obtained directly from Bragg (USA)'s organic raw and unfiltered Apple Cider (AC) vinegar. The experiment used fresh soursop juice (SJ) and commercial AC vinegar as controls.

Preparation of Soursop Vinegar

The soursop pulp was collected and stored in a freezer at -20°C to prepare soursop vinegar. Thawed pulp was mechanically pressed to obtain the juice. Two variables were considered: the presence of yeast and the aerated environment (Figure 1). The first stage involved alcoholic fermentation for 7 days at 30°C. Subsequently, acetous fermentation was initiated by adding the "mother" of vinegar (*Acetobacter* sp.), and the samples were subjected to aeration in an incubator shaker at 30°C and 60 rpm for 1 day. Samples without aeration were fermented for 15 days at 30°C to reach an adequate level of acetic acid. All experiments were performed in triplicate.

Analytical Procedures

Total soluble solid (TSS) was measured by using the Brix value. The Brix value is expressed in degrees Brix (ºBx), with 1 degree Brix being 1 g of soluble solids content in 100 g of solution. The Brix value of samples was determined using a hand refractometer. A pH meter determined the pH value (Abd Rahim et al., 2022). The acetic acid content was determined by adding 2 ml of sample with 20 ml of distilled water to ensure compliance with the Malaysia Food Regulation 1985 (Regulation 334). After proper mixing, 3 to 5 drops of phenolphthalein (R&D Chemicals, Malaysia) were added as an indicator. The solution was then titrated with standard 0.1 M sodium hydroxide (NaOH, Sigma-Aldrich, USA). The burette reading was measured and used to calculate acetic acid content.

For vitamin C determination, 2 cm^3 of 1% vitamin C solution (Sigma-Aldrich, USA) was prepared in a conical flask. The 1% 2,6-dichlorophenolindophenol solution (DCPIP, Sigma-Aldrich, USA) was added drop by drop into the conical flask and gently shaken until the blue color disappeared. The amount used for the DCPIP solution was recorded. Then, the procedure was repeated by substituting the vitamin C solution with the soursop vinegar samples. The vitamin C

content is calculated in the standard solution in mg/cm^3.

The antioxidant properties of the samples were assessed using modified versions of the 1,1-diphenyl-2-picrylhydrazyl (DPPH) and ferric-reducing antioxidant power (FRAP) methods. The antioxidant assays were conducted following the procedure suggested by Jasmi et al. (2020) and Mansor et al. (2020). For the DPPH assay, 100 mM Tris-hydrochloric acid buffer (Tris-HCl, pH 7.4, R&D Chemicals, Malaysia) was combined with 400 µl of the samples or blank. This mixture was added with 2 ml of 500 µM DPPH solution (Sigma-Aldrich, USA) in absolute ethanol. The resulting mixture was vigorously mixed and then incubated at room temperature for 20 min. The absorbance of the solution was measured at 517 nm using a UV-VIS spectrophotometer.

The FRAP assay involved the preparation of the FRAP solution by combining three solutions in a 10:1:1 ratio. These solutions included 10 mM 2,4,6-Tri(2-pyridyl)-s-triazine (TPTZ, Sigma-Aldrich, USA), 300 mM acetate buffer (pH 3.6, Sigma-Aldrich, USA), and 20 mM iron(III) chloride ($FeCl_3$, Sigma-Aldrich, USA). Subsequently, 3 ml of the FRAP reagent (Sigma-Aldrich, USA) was mixed with the samples and heated at 37°C for 10 min. The FRAP value was determined by measuring the difference in absorbance between the samples and the blank solution.

Proximate Analysis

The moisture content of the samples was determined according to the Association of Official Agricultural Chemists (AOAC) method (AOAC, 2005). The crucible, along with its lid, was cleaned and dried in an oven for 30 min, followed by cooling in a desiccator. The weight of the crucible and lid was repeatedly measured until a constant reading was obtained. Next, 1–2 g of the sample was weighed into the crucible, and with the lid covered, it was heated at 105°C until a constant reading was achieved.

About 3–5 g of the sample was placed in a cleaned and dried crucible, which was then covered with its lid to determine the ash content. The crucible was heated in a muffle furnace at 550°C for 3 hr or until no black particles were present and a constant weight was obtained. Subsequently, the crucible with the ash was cooled in the desiccator and weighed until a constant reading was achieved.

The protein content was measured using the Kjeldahl method. A 0.5 g sample was mixed with 0.8 g of Kjeldahl catalyst (Merck, USA), and 2.5 ml of concentrated sulfuric acid (H_2SO_4, Merck, USA) was added. The mixture was slowly heated for 10 min under a fume hood until it became clear. The temperature was then gradually reduced to 40°C, and 10 ml of distilled water was added to the distillation tube. Next, 10 ml of 45% 0.313N NaOH solution (Sigma-Aldrich, USA) was slowly added to the distillation tube to form a two-layered solution. Prior to distillation, 10 ml of 2% boric acid (Sigma-Aldrich, USA) with a few drops of indicator (Merck, USA) was added to a 50 ml conical flask. The resulting

green-colored product was gently swirled, and the unreacted boric acid was titrated with 0.05 N H_2SO_4 (Merck, USA) until a purple color appeared, indicating the determination of Kjeldahl nitrogen (Hajar-Azhari, Shahruddin, et al., 2018).

The different methods determined the carbohydrate content, assuming the fat and crude fiber content to be 0 (Okokon & Okokon, 2019).

Data Analysis and Interpretation

Data analysis was performed using one-way analysis of variance (ANOVA), with a confidence interval of 95%. Mean differences were tested, and the nutritional values of soursop vinegar were compared between treatments (A, B, C, and D) and the control (SJ and commercialized AC vinegar).

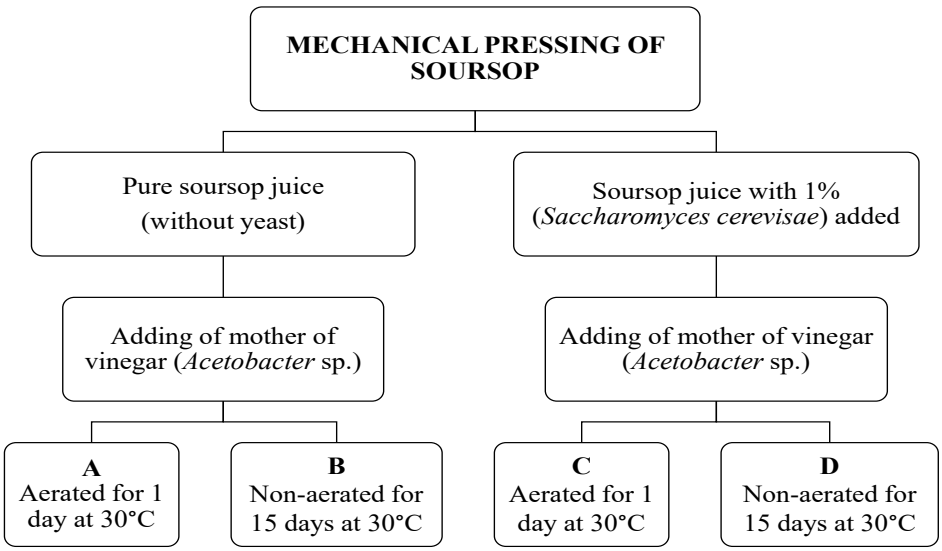

Figure 1. The experimental design to produce soursop vinegar involved four different conditions: A = Soursop vinegar with yeast under aerobic conditions; B = Soursop vinegar with yeast under anaerobic conditions; C = Soursop vinegar without yeast under aerobic conditions; and D = Soursop vinegar without yeast under anaerobic conditions

RESULTS AND DISCUSSION

Table 1 illustrates the results and the impact of yeast and aeration conditions on the nutritional composition of soursop vinegar, soursop juice, and AC vinegar. Significant changes (p<0.05) were observed for all nutritional components except vitamin C. The preservation of vitamin C from soursop juice is a promising finding, indicating that the production of soursop vinegar results in a product with a higher nutritional value compared to commercially available synthetic vinegar. Fruits are known for their vitamin C content because they naturally synthesize and accumulate this nutrient for their growth and development. Vitamin C

is synthesized in the fruit's tissues through enzymatic processes, primarily ascorbic acid.

Among the samples, the soursop juice (control) exhibited the highest TSS content at 15.0 ºBrix, while AC vinegar displayed the lowest TSS content at 3.90 ºBrix. Notably, a significant reduction in the percentage of carbohydrates was observed in all treatments, with values ranging from 4.62 to 7.61% (and AC vinegar at 1.06%), in contrast to the soursop juice (control), which had a carbohydrate content of 18.89%. This reduction can be attributed to fermentation, which converts the sugar substrate into alcohol (Hajar-Azhari, Wan-Mohtar, et al., 2018), subsequently transforming it into acetic acid. While fruit sugars are generally preferable to refined sugars, a decrease in sugar content is desirable, especially for reducing the risk of human metabolic diseases (Hajar-Azhari et al., 2020, 2021).

Table 1
Nutritional value of soursop juice (SJ), Apple Cider commercial control (AC), and soursop vinegar produced from fermentations (denominated from A–D)

Parameters	SJ	AC	A	B	C	D
TSS (º Brix)	15.00 ± 0.00^{a}	3.90 ± 0.00^{c}	6.33 ± 0.17^{bc}	5.89 ± 0.38^{bc}	7.00 ± 2.55^{b}	4.83 ± 1.43^{bc}
pH	3.67 ± 0.26^{b}	2.98 ± 0.13^{c}	4.27 ± 0.06^{a}	4.59 ± 0.05^{a}	4.34 ± 0.07^{a}	4.33 ± 0.09^{a}
Moisture (%)	78.56 ± 0.36^{c}	98.46 ± 0.10^{a}	92.99 ± 1.07^{ab}	91.92 ± 2.65^{b}	90.73 ± 4.73^{b}	93.99 ± 1.46^{ab}
Ash (%)	1.45 ± 0.63^{a}	0.32 ± 0.04^{b}	0.69 ± 0.16^{ab}	0.65 ± 0.13^{b}	1.06 ± 0.07^{ab}	0.90 ± 0.20^{ab}
Protein (%)	1.09 ± 0.32^{b}	0.16 ± 0.08^{d}	0.61 ± 0.10^{c}	0.50 ± 0.01^{cd}	1.53 ± 0.06^{a}	1.84 ± 0.11^{a}
Carbohydrate (%)	18.89 ± 0.38^{a}	1.06 ± 0.08^{c}	4.79 ± 1.13^{bc}	5.59 ± 2.77^{bc}	7.61 ± 4.74^{b}	4.62 ± 1.31^{bc}
Vitamin C (mg/cm^{3})	0.61 ± 0.00^{a}	0.40 ± 0.18^{a}	0.48 ± 0.06^{a}	0.22 ± 0.08^{a}	0.61 ± 0.17^{a}	0.51 ± 0.29^{a}
Acetic Acid (%)	NT	4.59 ± 1.75^{a}	3.98 ± 1.85^{b}	2.96 ± 0.67^{b}	4.90 ± 0.66^{a}	3.39 ± 0.32^{b}

Note. A = Aerated for 1 day at 30°C without yeast; B = Non-aerated for 15 days at 30°C without yeast; C = Aerated for 1 day at 30°C with yeast; D = Non-aerated for 15 days at 30°C with yeast. Values are expressed as mean ± standard deviation of triplicate measurement. Superscripts with different letters significantly differ at $p<0.05$ in the same row. NT = Not tested

TSS and moisture content generally have an inverse relationship, which can be attributed to the conversion of sugar to alcohol and acetic acid during fermentation (Deshmukh et al., 2014). This relationship is demonstrated in Table 1, where higher Brix values correspond to lower moisture content. The AC vinegar sample displayed the highest moisture content of 98.46%, attributed to the proper filtering process that

removes most residues from the solution. While adding external yeast is beneficial, the absence of yeast does not significantly impact alcoholic fermentation, as yeast naturally exists on the surface of fruits (Aneja et al., 2014). However, the absence of yeast may reduce fermentation efficiency, requiring more days to achieve the desired alcohol level.

It is important to note that there were no significant differences in pH among the different treatments, except for the control samples, which demonstrated significantly lower pH. According to the Malaysia Food Regulation 1985 (Regulation 334), vinegar must contain a minimum of 4% weight per volume of acetic acid, excluding other mineral acids. In the case of treatments A, B, and D, this requirement was not fully met, indicating that a longer fermentation period may be necessary to achieve the desired concentration of acetic acid. Interestingly, the non-aerated treatments (B and D) showed lower levels of acetic acid than aerated treatment, suggesting that oxygen may play a crucial role in vinegar production. The absence of oxygen in these treatments may have restricted the growth and activity of acetic acid bacteria, as previously demonstrated (Vikas et al., 2014). The combination of aeration (hence, higher dissolved oxygen) and yeast has shown satisfactory acetic acid content (4.9%) in sample C, comparable to commercial control.

It is also interesting to note that the pH values do not correspond to the acetic acid levels produced by the soursop vinegar. For instance, although treatment B showed a low percentage of acetic acid, its pH was not significantly different from treatments A, C, and D. This observation suggests that there may be other acids present in the soursop juice, either naturally or as a result of fermentation processes (Bhat & Paliyath, 2016). Furthermore, the increased pH observed in the treatments compared to the soursop juice control indicates that yeast might have caused the breakdown of citric acid during fermentation, which can be beneficial during the acetous stage of vinegar production (Schwan & Wheals, 2004).

The presence of yeast had a minimal (not significant) impact on the ash content and protein content of the samples. Treatments C and D (with yeast) exhibited slightly higher ash content, with 1.06 and 0.90%, respectively, but not significant to treatments A and B (without yeast). Notably, the ash content of the treatments with yeast is closer to that of AC control, likely because yeast is utilized in both treatments. The higher ash content in treatments C and D could be attributed to the presence of yeast during fermentation. Yeast is a rich source of macro- and micro-minerals, such as sodium, potassium, and magnesium, providing more than 80% of the daily recommended intake per 100 g (Jaeger et al., 2020).

The significantly higher protein content observed in treatments C and D can also be attributed to the higher presence of yeast. Yeast is known to be a rich source of protein. During fermentation, the nitrogen values can increase due to the presence of microbial

biomass or the concentration of protein as sugars are consumed (Day & Morawicki, 2018), which resulted in the higher protein content observed in those samples.

Antioxidant Capacities of Soursop Vinegar

In addition to the essential nutrients, antioxidants in functional foods are widely recognized for their importance. While the human body possesses its internal antioxidative enzymes, the supplementation of antioxidants from external sources, particularly through dietary intake, is highly beneficial (Amirah et al., 2020; Ramli et al., 2016; Salleh et al., 2022; Tan et al., 2021). Antioxidants play a crucial role in counteracting the harmful effects of free radicals, both internally and externally, thus preventing chain reactions that can lead to cellular damage (Abadl et al., 2022; Mediani et al., 2014; Nawawi et al., 2023). Plant-based juices, such as sugarcane, often contain abundant beneficial antioxidants (Abd Rahim et al., 2022; Jasmi et al., 2020; Mohd Zaini et al., 2023).

Natural antioxidants in soursop vinegar, derived from the fruit's inherent compounds, enhance its potential health benefits. By incorporating soursop vinegar into their diet, individuals can obtain an additional supply of antioxidants, which aids in the neutralization of free radicals and offers protection against oxidative stress-related harm in the body (Halim et al., 2023; Kadum et al., 2019). The assessment of FRAP value serves as an indicator of the antioxidant content, with higher values representing a greater concentration of antioxidants. Conversely, a lower DPPH value signifies enhanced antioxidant activity.

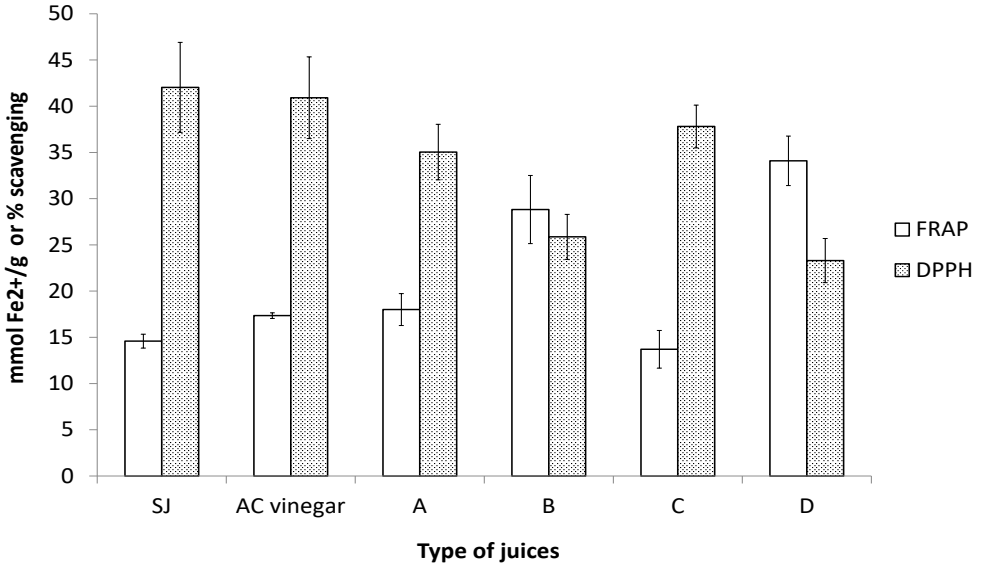

Figure 2. The antioxidant activities of SJ (soursop juice), AC (Apple Cider) vinegar, and soursop vinegar

Note. A = Aerated for 1 day at 30°C without yeast; B = Non aerated for 15 days at 30°C without yeast; C = Aerated for 1 day at 30°C with yeast; D = Non aerated for 15 days at 30°C with yeast

Figure 2 visually represents the antioxidant content and activity, as assessed by FRAP and DPPH measurements, respectively. Based on the figure, converting soursop juice into vinegar successfully retained or enhanced its antioxidant properties. Notably, treatments B and D, produced under anaerobic/non-aerated conditions, exhibited a significant increase in antioxidant content, surpassing both control samples. Treatment B demonstrated an impressive 97.60% higher FRAP content and a significantly lower DPPH value of 38.46% compared to the SJ control. Similarly, treatment D exhibited significantly higher FRAP and DPPH values, with increases of 133.65 and 44.58%, respectively.

The findings from this study suggest that the anaerobic/non-aerated vinegar production process, exemplified by treatments B and D, resulted in a noteworthy enhancement in antioxidant content compared to the soursop juice control. It indicates the potential for these vinegars to effectively neutralize free radicals and combat oxidative stress, positioning them as functional food products with higher antioxidant properties. It is worth noting that acetic acid bacteria such as *Acetobacter* sp. typically require oxygen to convert ethanol to acetic acid. Hence, the lower acetic acid content observed in vinegar B and D can be attributed to the absence of oxygen in the anaerobic conditions. However, the mechanisms underlying the observed increase in antioxidants under oxygen-limited conditions remain partially understood.

One possibility is that the anaerobic pathway of acetic acid production, which involves the recycling of nicotinamide adenine dinucleotide (NAD^+) through pyruvate reduction, may also lead to the formation of other derivatives that contribute to the heightened antioxidant capacity. Similar phenomena have been observed in anaerobic fermentation processes involving Baker's yeast and lactic acid bacteria, where the conversion of ferulic and caffeic acids resulted in increased amounts of their derivatives, such as sinapic acid (Verni et al., 2019). These findings highlight the promising potential of anaerobic vinegar production in yielding vinegar with enhanced antioxidant properties, further enhancing its value as a functional food with potential health benefits.

CONCLUSION

The production of soursop vinegar presents an excellent opportunity to add value to overripe soursop fruit, reducing waste while preserving or enhancing its nutritional content. Soursop vinegar stands out due to its elevated levels of vitamin C and protein and its reduced sugar content compared to the original fruit. Additionally, including non-nutritive components, such as antioxidants, has shown promising outcomes in soursop vinegar.

Utilizing aeration or adding specific microorganisms during the production process has proven to be effective in significantly boosting the vinegar's antioxidant content. It emphasizes the significance of selecting appropriate

processing methods, including aeration, to optimize the production of nutritious soursop vinegar. Ultimately, the transformation of soursop fruit into vinegar allows for the utilization of excess or overripe fruit and results in a vinegar product with improved nutritional properties.

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