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Effects of selected preservation techniques on the shelf-life of nipa sap

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Abstract

Nipa sap is a sweet, translucent sap from nipa palm (Nypa fruticans) tree. It is usually consumed as a refreshing beverage by local people. In Sarawak, Malaysia, nipa sap is a raw material to produce nipa sugar or gula apong, a semi-solid sweetener distinguished by its golden caramel colour. It is usually used for making traditional cakes, desserts, sauces and marinades. However, nipa sap usually spoils within 3 hours after collection due to natural fermentation. The present study aimed to enhance the shelf life of nipa sap using several selected preservation methods. The effectiveness of the preservation approaches applied was examined by assessing the physicochemical, microbiological and sensory qualities of nipa sap. The results revealed that high temperature, short time (HTST) pasteurisation and sodium benzoate preservative were able to extend the shelf-life of nipa sap up to 14 days at room temperature. The glucose level was able to be maintained throughout the storage while ethanol concentration was maintained low. For the sensory attributes, HTST showed better acceptance as compared to low temperature, long time (LTLT), sodium benzoate (SB) and citric acid (CA). Local producers can use HTST treatment to extend the shelf life of nipa sap, enabling them to better plan their daily activities and generate more income over an extended period. In addition, the findings will pave the way for the marketability of nipa sap.

1. Introduction

Nipa sap is a nutritious beverage collected from the Nypa fruticans palm tree. It is an important beverage in Southeast Asia, such as Thailand (Phetrit and Chaijan, 2020) Indonesia (Kurniawan et al., 2018), the Philippines (Aquino, 2019), and Malaysia (Radi et al., 2013). In Malaysia, nipa sap can be obtained in the northern region of Peninsular Malaysia (Radi et al., 2013) and coastal areas of Sarawak, East Malaysia (Jaraee et al., 2023). Nipa sap is usually consumed as a refreshing beverage, with wine and vinegar (Farid and Islam, 2015). Apart from that, in Sarawak, nipa sap is a raw material for nipa sugar locally known as gula apong production. The gula apong industry plays a significant role in the economic activities of the people of Sarawak. This industry provides employment opportunities and income for local people, especially people living in the coastal areas where the nipa palms grow in abundance.

Fresh nipa sap has a sweet taste, nice odour and clear colour. It contains various types of sugar, mainly sucrose, glucose and fructose (Jaraee *et al.*, 2023). However, due to its nature of high sugar content, it is

prone to undergo natural fermentation whereby its sugar is converted into alcohol. The natural fermentation of nipa sap is due to the microflora existence and unhygienic tapping practices. Once nipa sap becomes fermented, it has a sour taste, harsh odour and cloudy colour (Radi *et al.*, 2013). The nutrient in palm sap also degrades and the sensory quality of palm sap is greatly reduced (Tiepma *et al.*, 2013). It occurs due to the microbial activities and suspension existing in nipa sap. Thus, these conditions reduce the quality of nipa sap as a refreshing nutritious product (Hebbar *et al.*, 2018).

There are a few safety concerns that need to be addressed and intervened. The nipa sap was collected using a bamboo tube. These bamboo tubes are often not cleaned between uses (Jaraee *et al.*, 2023). Hence, during the sap collection, contamination of microbes, including pathogenic microbes occurred. The methods of tapping and collecting nipa sap influence the microbial content of the sap. Many problems arise from nipa sap collection and processing among local collectors, including a lack of good manufacturing procedures or hygiene codes, and a lack of knowledge of the fermentation process

(Karamako et al., 2012).

In addition, the short shelf life of nipa sap limits the local producers, especially in handling it as a raw material for gula apong production. They must boil nipa sap immediately right after collecting. Any delay in processing will spoil the quality of gula apong production, hence affecting their daily income. To date, researchers have studied different preservation technologies in palm sap. Few preservation approaches were adopted, consisting of thermal and non-thermal strategies. Locally, nipa sap has been reported to be preserved using the boiling method. Then, the boiled nipa sap is cooled before it is filled in a used or new polyethylene terephthalate (PET) bottle. By using the boiling method, the shelf-life of nipa sap increases by up to 2 days under ambient temperature and 4-5 days under refrigeration temperature.

The short shelf-life of nipa sap is one of the biggest challenges in marketing nipa sap as beverages by cottage food producers. Nipa sap is highly perishable, beginning to ferment within a few hours after being extracted from the nipa palm tree. If not processed and consumed quickly, the sap can spoil, which can affect the taste and quality of the beverage. Other than that, nipa sap is a delicate product that requires careful handling and storage to maintain its freshness and quality. This can be challenging, especially if the sap is being transported long distances or stored for an extended period of time. The short shelf life of nipa sap can pose significant challenges for producers and manufacturers, impacting their income and profitability. However, with proper planning, investment in equipment and facilities, and development of value-added products, it is possible to overcome these challenges and create a sustainable and profitable industry. Therefore, the aim of this study was to explore and assess different preservation approaches, that are practical and applicable to cottage food producers of nipa sap in Sarawak. These findings will help in the marketability of nipa sap produced by cottage food producers.

2. Materials and methods

2.1 Nipa sap collection

Nipa sap was collected using a sterile bottle from local collectors in sterile screw-capped autoclavable containers and kept at 4°C during the transportation to the Faculty of Resource Science and Technology, UNIMAS. The nipa sap was processed as soon as possible to prevent degradation.

2.2 Treatment to enhance nipa sap shelf-life pasteurisation

In this study, the nipa sap was pasteurised at 60°C for 1 hr (low temperature, long time [LTLT]) and 98°C for 10 s (high temperature, short time [HTST]) as described by Ramachandran *et al.* (2017). Then, the treated nipa sap was filled aseptically into polypropylene plastic bottle (Buvé *et al.*, 2018).

2.3 Addition of chemical preservatives

Fresh nipa sap was filled aseptically into polypropylene plastic bottle. Then, 0.1% (w/w) of sodium benzoate (SB) and 0.5% (w/w) of citric acid (CA) were added separately in different nipa sap samples was added to the nipa sap (Mishra *et al.*, 2011).

2.4 Microbial analysis of treated nipa palm sap

To conduct a test for *Escherichia coli*, the treated nipa sap was enriched with nutrient broth at 37°C for 24 hrs. Then, a loopful of broth was streaked on EMB agar, and the plates were kept in an incubator at 35±0.5°C for 18-24 hrs. Any potentially suspicious *E. coli* colonies were characterised as dark-centred and flat with or without a metallic sheen. If found, up to five colonies were transferred from each plate to PCA slants and incubated at 35±0.5°C for 18-24 hrs for further Gramstaining and biochemical tests. On the other hand, for *Staphylococcus aureus*, a loopful of broth was streaked on tryptic soy agar at 37°C for 48 hrs. Potential *S. aureus* colonies were tested for gram-staining and biochemical tests.

2.6 Shelf-life analysis

All the juice samples, fresh and pasteurised with and without preservatives were stored at ambient temperature (30°C) for 30 days. The nipa sap samples were analysed every 2 days for pH, sugar content, alcohol content total bacterial count and sensory attributes.

2.7 pH

The pH of nipa sap was determined in triplicates using a pH meter (Biobase, China) after calibration with a standard buffer. The pH value was taken every 2 days.

2.8 Sugar and ethanol profiles

The samples were centrifuged at 10,000 rpm for 10 mins and supernatants were filtered through a $0.45~\mu m$ millipore membrane filter (EMD Millipore, Billerica, MA). Sugar (sucrose, glucose, and fructose), organic acids (lactic and acetic acid), and ethanol analyses were performed using high-performance liquid chromatography (Shimadzu, Japan), Index (RID-10A)

detector with a column of prominence CTO-20A (Shimadzu, Japan) and $0.005~M~H_2SO_4$ as mobile phase at 0.8~mL/min flow rate for glucose, ethanol and lactic acid determination at $60^{\circ}C$ column temperature.

2.9 Sensory evaluation

Sensory testing was performed by panels, comprising twenty panels from the Faculty of Resource Science and Technology, UNIMAS. They evaluated the sensory properties based on taste, aroma, texture, acceptance and overall preferences using five points of the hedonic rating scale. To calculate the score for each product, each descriptor was assigned a score value: like very much = 5, like moderately = 4, neither like nor dislike = 3, dislike moderately = 2, dislike very much = 1. The data were analysed statistically by ANOVA using SPSS version 14.0 and the means were separated by Duncan's Multiple Range Test (Liang *et al.*, 2022).

3. Results and discussion

3.1 Effect of different collection methods on nipa sap quality

In this study, the impact of different collection methods on the quality of nipa sap was assessed. Figure 1 shows the pH of nipa sap collected in bamboo tube (BT) and sterilized bottle (SB). There was a significant difference (p<0.05) in the pH of the two samples. On Day 1 (the day of collection) the pH of SB and BT was 6.00±0.02 and 5.11±0.01, respectively. Both samples were slightly acidic due to the fermentation process that already started during 12 hrs of the collection period (Hebbar et al., 2018). Several species of organic acids were found in nipa palm sap, including succinic acid, tartaric acid, malic acid, pyroglutamic acid, citric acid, lactic acid and fumaric acid (Tomokatsu et al., 1999; Radi et al., 2013; Phetrit et al., 2020; Saengkrajang et al., 2021). Based on Figure 1, the pH of nipa sap collected in sterilised bottle was higher compared to pH of nipa sap collected in bamboo tube. Notably, the pH of both nipa saps gradually decreased over time, showing that the nipa saps slowly turned acidic. It was due to the conversion of sugar by the existing microorganism to ethanol (Khaire et al., 2021), explaining the decreasing trend of total sugar in Figure 2 and the increasing trend of ethanol in Figure 3.

Despite the decreasing trend of total sugar concentration in both types of collection methods, it was clearly shown that sterilised bottle consistently outperforms bamboo tubes. On each day, the total sugar content of nipa sap in the sterilised bottle is consistently higher than that in the bamboo tube. On day 1, it was observed that nipa sap collected in sterilised bottle was 204.66±7.31 g/L, which was enhanced by 1.5-fold than

that in bamboo tube. On day 5, the total sugar content of nipa sap collected in a sterilised bottle was approximately 2.03 times higher than that in a bamboo tube. In addition, nipa sap collected in sterilised bottles showed promising improvement in reducing ethanol production during nipa sap collection. Across all five days, the ethanol content in nipa sap collected in sterilised bottles consistently appears to be lower than that in bamboo tubes. This reveals that ethanol production for nipa sap collected in sterilised bottles is almost 100 times lower than that in bamboo tubes.

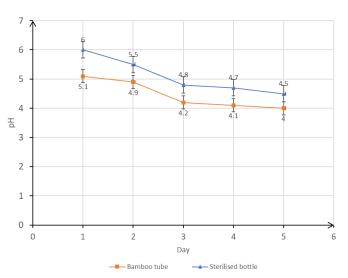


Figure 1. pH profile of nipa sap collected in bamboo tube (BT) and sterilized bottle (SB).

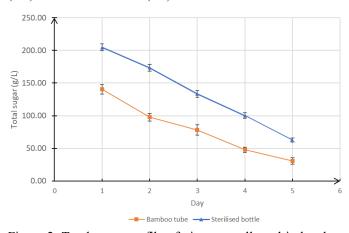


Figure 2. Total sugar profile of nipa sap collected in bamboo tube (BT) and sterilized bottle (SB).

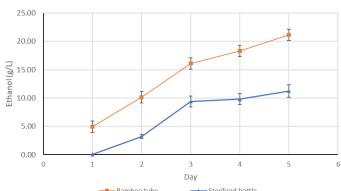


Figure 3. Ethanol concentration of nipa sap collected in bamboo tube (BT) and sterilized bottle (SB).

Lowered ethanol production indicates a lower rate of microbial metabolism of sugar in nipa sap (Tamunaidu *et al.*, 2013). The findings reported by Saengkrajang *et al.* (2021) and Naknean *et al.* (2013) suggest that maintaining hygienic conditions has the potential to decelerate the fermentation process.

Nipa sap fermentation has been reported to be an alcoholic, lactic and acetic fermentation (Ouoba *et al.*, 2012; Santiago-Urbina *et al.*, 2013; Amoa-Awua *et al.*, 2017). Due to this, further fermentation of the sap by acetic acid bacteria would result in the production of vinegar. Hence, the pH value gradually decreased. This resulted in a sour taste which made nipa sap unsuitable for consumption as refreshing beverages. Overall data suggest that SB could improve the quality of nipa sap by minimising the physiological changes of nipa sap. It may be possible to enhance the quality, stability, and marketability of nipa sap, providing a more appealing product to consumers.

3.2 Different preservation strategies to enhance shelf-life of nipa sap

A preliminary study was conducted to assess the effectiveness of various preservation approaches to improve the shelf life of nipa sap. In this study, a few preservation approaches were involved such as high temperature short time (HTST), low temperature long time (LTLT), the addition of sodium benzoate (SB) and addition of citric acid (CA). There were variations in the shelf stability of nipa sap depending on the preservation approach used. Based on Figures 4 and 5, the glucose concentration of nipa sap treated with HTST and SB did not significantly change from Day 1 to Day 14. On the other hand, both LTLT and CA treatments were unable to extend the shelf life of nipa sap beyond that of the control nipa sap, that did not undergo any treatment.

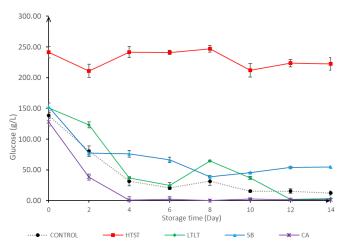


Figure 4. Profile of glucose concentration in nipa sap under different preservation approaches. HTST: High temperature short time, LTLT: Low temperature long time, SB: Sodium benzoate, CA: Citric acid

The concentration of ethanol remained low even though the sap was stored at room temperature after HTST treatment. This high ethanol residual concentration in nipa sap also raised halal concerns among Muslim consumers as it is also consumed as an alcoholic drink. According to JAKIM, the Islamic authority in Malaysia who stipulated the law for halal certification, the maximum amount of permissible amount of ethanol in food and beverages is 1% v/v (Ghani and Ismail, 2010).

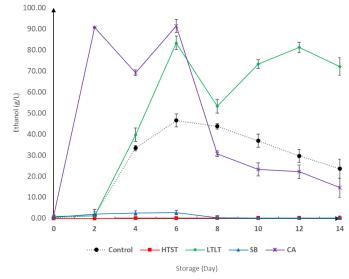


Figure 5. Profile of ethanol concentration in nipa sap under different preservation approaches. HTST: High temperature short time, LTLT: Low temperature long time, SB: Sodium benzoate, CA: Citric acid.

Apart from that, this study explored the potential of chemical preservatives, such as sodium benzoate and citric acid as preservatives for nipa sap. Sodium benzoate is commonly used in food and beverages to extend the life span. It has been categorized as Generally Recognize as Safe (GRAS) food additive (Talasila et al., 2012). Food and Drug Administration (FDA) controls the amount of food additives allowed in foods or other commodities to ensure safety and decrease the danger of overconsumption (Pongsavee, 2015). The FDA approves the use of benzoate groups, such as sodium benzoate and potassium benzoate, in dairy products, such as ice cream, pudding and yoghurt, at 300 mg/kg. Sodium benzoate has been utilised for decades as a preservative because of its excellent stability and solubility in water (Bruna et al., 2018).

The present study showed that the addition of sodium benzoate can retard the fermentation process. Their presence created unfavourable environment for the microorganism to grow and produce spores. This can cause physiological and metabolic changes in microorganisms, eventually leading to their death (Chipley, 2020). However, the addition of citric acid showed no sign of fermentation retardation in nipa sap.

According to past studies, pathogenic microbes, such as Staphylococcus aureus and Escherichia coli, had been reported to be found in the sap of various palms, such as coconut and palmyra (Atputharajah et al., 1986; Karamoko et al., 2012; Santiago-Urbina et al., 2013). Therefore, in this study, S. aureus and E. coli were used as indicators to evaluate the effectiveness of proposed pasteurisation. Escherichia coli is a common inhabitant of the intestinal tract of humans and animals and is considered an indicator of faecal contamination in food. Escherichia coli can be easily disseminated in different ecosystems through the food chain. Staphylococcus aureus is an important foodborne pathogen because of its ability to produce a wide range of extracellular protein toxins and virulence factors that contribute to the pathogenicity of the organism. The inactivation of Grampositive S. aureus and Gram-negative E. coli by thermal addition pasteurization, and the of preservatives are depicted in Table 1. It was found that the complete inhibition of E. coli and S. aureus was detected in HTST and SB treatment.

Table 1. The effect of different treatments towards inactivation of Gram-positive *S. aureus* and Gram-negative *E. coli*.

	S. aureus	E. coli
Control	+	+
Fresh nipa sap	+	+
High temperature short time	-	-
Low temperature long time	-	-
Sodium benzoate	-	-
Citric acid	+	+

⁺ detected, - not detected

3.3 Pasteurisation of nipa sap

The pasteurisation process employs a heat transfer mechanism (Cheng et al., 2020). The aim of pasteurisation is mainly to kill pathogenic organisms in liquid foods, such as milk and juice, and to extend the shelf-life of the products (Deak, 2014). Pasteurisation process has been adapted by the local villagers to extend the shelf-life of nipa sap. It is produced at small scale and sold at local small shops. Usually, traditionally pasteurised nipa sap can maintain its quality for two days in ambient temperatures and 4-5 days in refrigerated temperatures.

After the pasteurisation process, the number of microorganisms was generally reduced (Kaavya *et al.*, 2019). Nevertheless, nipa sap may have a limited shelf-life due to several factors. To begin with, the surviving microorganism may spoil nipa sap. There were some microorganisms that were heat resistant and able to survive the pasteurisation process. Even though all

microbes were destroyed during the pasteurisation process, there were possibilities that the microorganisms were introduced to the beverage during the packaging process due to inadequate sanitation and poor hygienic practices by the producers (Jaraee *et al.*, 2023). Food contamination due to microorganisms may occur throughout the food production chain, from farm to fork (Lianou *et al.*, 2016). The potential sources of contamination are not only raw materials but also environment and food handlers.

In this study, preservation approaches were chosen based on their practicality and applicability for local and small-scale nipa sap producers. The feasibility of these approaches has been considered for implementation by small-scale producers who may have limited access to advanced technologies and equipment. Figure 6 illustrates the pasteurization process used in this study and how it can be applied by local producers. The proposed pasteurisation treatments of nipa sap hold two promising avenues for potential applications that could significantly benefit local producers of nipa sap beverages and nipa sugar. Firstly, this proposed pasteurisation offers the opportunity to produce nipa sap beverages with extended shelf-life. Currently, nipa sap beverages have a limited shelf-life of 1-2 days. In contrast, the proposed pasteurisation method could potentially prolong the duration to up to 14 days, which increases the profitability for the local producers.

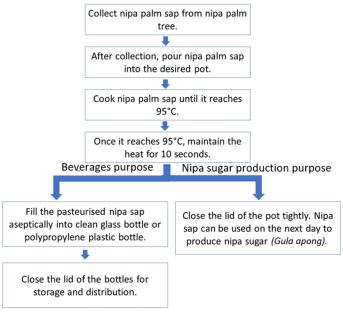


Figure 6. Flow chart of pasteurisation process of nipa palm sap.

Secondly, the proposed pasteurisation also benefits nipa sugar (gula apong) producers. During specific periods, notably in hot seasons, the yield of nipa sap is reduced. However, due to the nature of nipa sap that are easily fermented, it must be processed immediately to prevent spoilage. This leads to the wasting of the resources such as firewood, labour and time. Hence, by leveraging the proposed pasteurisation method, local producers can pasteurise the nipa sap and produce nipa sugar the next day, with the addition of a new batch of nipa sap. This strategy not only mitigates resource wastage but also optimises production efficiency within the constraints of fluctuating nipa sap availability.

On the other hand, Table 2 provides a comparison between the pasteurisation process commonly used by local producers and the process proposed in this study. The proposed method used sterilised bottles during nipa sap collection, which provides a controlled environment that minimises bacterial contamination (Naknean *et al.*, 2013). In addition, the proposed method emphasises immediate packaging, aiming to minimise the exposure of the sap to contaminants, thereby preserving its freshness and preventing undesired fermentation. Apart from that, the proposed method used polypropylene plastic (PP), which was heat resistant compared to polyethylene terephthalate (PET) bottle (Hisham, 2016). On the other hand, both methods took 1 hr delay before pasteurisation due to logistics and transportation.

Table 2. Comparison of traditional pasteurisation process and proposed pasteurisation process.

Traditional	Proposed
12 hr	12 hr
Bamboo tube	Sterilised bottle
1 hr	1 hr
0.5 to 1 hr	Immediate
PET bottle	Polypropylene plastic bottle.
	12 hr Bamboo tube 1 hr 0.5 to 1 hr

Table 3 shows the comparison of the physiological properties of pasteurised nipa sap purchased from local sellers and laboratory pasteurised nipa sap. Both nipa saps were freshly pasteurised during collection and analysis. Based on the table, there were substantial changes after one week of storage between the nipa sap acquired from the local villagers and the nipa sap from this study. The pH of the nipa sap obtained from the villagers was 3.50±0.04, but the pH of the nipa sap examined was 5.01±0.02. This shows that the nipa sap produced from this study is less acidic than the nipa sap obtained from the villagers.

Furthermore, the glucose content of the nipa sap purchased from the villager was 3.41 ± 0.06 brix w/w%, which was much lower than the glucose content of this study's nipa sap (20.3 ± 0.14 brix w/w%). The ethanol concentration of the nipa sap obtained from the villager was 26.1 ± 1.21 g/L, while the nipa sap from this study only had a trace amount of ethanol at 0.05 ± 0.00 g/L. This suggests that the nipa sap from this study has a

lower ethanol content compared to the nipa sap purchased from the villagers.

The nipa sap from this study had a non-detectable level of both bacteria and yeast/mold. In contrast, the nipa sap purchased from the villager had a high total bacterial count of 9.61±0.75 log CFU/mL and a high total yeast and mould count of 7.36±0.27 log CFU/mL. This suggests that after one week of storage, the nipa sap from this study has better microbial quality than the nipa sap bought from the villager.

Table 3. The comparison between nipa sap purchased from a local villager and nipa sap in this study after being stored for one week.

Parameters	Nipa sap Parameters purchased from local villager	
pH	3.50 ± 0.04	5.01±0.02
Glucose (brix wt/wt%)	3.41 ± 0.06	20.3±0.14
Ethanol (g/L)	26.1±1.21	0.05±0.00
Total bacterial count (log CFU/mL)	9.61±0.75	n.d
Total yeast and mould count (log CFU/mL)	7.36±0.27	n.d

n.d: not detected

There could be several reasons for the observed differences between the nipa sap purchased from the local villagers and the nipa sap from this study. First, proper handling and sanitation during the collection and processing of the nipa sap influenced the quality of nipa sap (Hebbar et al., 2015; Madigal et al., 2020) which prevents cross-contamination after the pasteurisation process (Ebert, 2008). Furthermore, PP packaging plays a major role in extending the shelf-life of pasteurised nipa sap. Their non-toxic properties and flexibility make them a crucial component of the packaging industry (Panigrahi et al., 2021). The study showed that pasteurised sugarcane juice was processed and packed using three different types of bottles: glass, PET and PP. The results showed that the juice packed in glass and PP bottles had good quality, and there were significant effects of packaging materials on the sensory properties of the juice. Although glass bottles are considered to be the best option for preserving the quality of nipa sap, they can be challenging to transport and store due to their fragility. As a result, PP bottles are often preferred because of their versatility and ease of handling. It is also more cost-effective.

3.4 Sensory evaluation

The effects of the different preservation approaches on the sensory attributes of nipa sap are shown in Table 4. It was found that the appearance, taste and aroma characteristics of normal nipa sap (control) scored 4.75,

4.50 and 4.45, respectively, with the score of overall acceptability being 4.55. The pasteurisation treatment applied to nipa sap did not significantly impact its taste. However, the treatment did affect the appearance of the sap, particularly in the case of HTST treatment, which caused a slight brownish colouration. This discolouration may have been due to the Maillard reaction. Maillard reaction is a non-enzymatic browning reaction that involves carbonyl compounds, particularly reducing sugars, reacting with compounds that have a free amino group, such as amines, protein and amino acids (Saputro et al., 2020). The addition of citric acid significantly impacted the taste, appearance, and aroma of nipa sap. The sourness of the sap increased due to the addition of citric acid, resulting in a decrease of 17% in the overall acceptability of the sap. Furthermore, the altered taste and aroma caused by the addition of citric acid may not be preferred by consumers, highlighting the importance of considering the impact of additives on sensory characteristics when developing nipa sap-based products.

4. Conclusion

In conclusion, the study showed that the method of collecting nipa sap significantly influenced its quality. Nipa sap collected in a sterilised bottle had a higher pH compared to that collected in a bamboo tube. The gradual decrease in pH value suggested further fermentation of nipa sap, which could result in the production of vinegar. In addition, the HTST and SB treatments were effective in inhibiting the growth of microbes in nipa sap, helping to maintain its glucose level and prevent the accumulation of ethanol. On the other hand, the LTLT and CA treatments did not improve the shelf life of nipa sap. The addition of sodium benzoate showed retardation in fermentation, but citric acid did not show any significant effect. Furthermore, this study explored practical preservation approaches, such as HTST and SB, to improve the shelflife of nipa sap for small-scale producers with limited access to advanced technologies and equipment. In terms of sensory attributes, pasteurisation treatment did not significantly impact the taste of nipa sap but affected its appearance. The use of the HTST method without additives is a practical approach that can be easily applied by local nipa sap producers, thus benefiting their economy. However, for larger-scale and commercial

production, further research can be done to explore more advanced preservation technologies that are suitable and applicable to these entities.

Conflict of interest

The authors declare no conflict of interest.

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Table 4. Sensory attributes of treated nipa sap.

Treatments / Sensory attributes	Appearance	Taste	Aroma	Overall acceptability
Control	4.75	4.50	4.45	4.55
High Temperature short time	4.50	4.45	4.10	4.35
Low Temperature long time	4.70	4.50	4.35	4.40
Sodium benzoate	4.30	3.80	4.10	3.75
Citric acid	3.60	3.75	3.80	3.70

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