



Faculty of Resource Science and Technology

**Effect of E' Food Grade Preservatives and Preservation Methods on
Physical, Chemical, and Microbiological Properties of Nipa Palm (*Nypa
fruticans*) Sap in Sarawak**

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2025**

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Chemical, and Microbiological Properties of Nipa Palm (*Nypa fruticans*) Sap
in Sarawak

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DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Malaysia Sarawak. Except where due acknowledgements have been made, the work is that of the author alone. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

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Signature

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Date :05/02/2025

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ABSTRACT

Nipa palm (*Nypa fruticans*) trees is one of the mangrove plants that grows wildly in coastal areas. Many products could be obtained from the tree, including nipa -sap (*air nira*), -syrup and -sugar (*Gula Apong*). Hence, it has a vast economic potential for Sarawak. However, its sap has a short shelf-life, which could contribute to low yield and bad quality of *Gula Apong*. This study aims to enhance the shelf-life of the sap in terms of controlling the pH, sugar content, organic acids content and its microbial growth using E' food grade preservatives – potassium sorbate and sodium metabisulfite. Different preservation methods were tested, applying preservatives before (pre-tapping) and after sap collection (post-tapping), and in solid (effervescent granules) or liquid (aqueous neutralizing solution) forms. The study was conducted at two locations: Kampung Pinggan Jaya (Site I, using plastic bottle) and Pusa (Site II, using bamboo vessel). Samples treated with effervescent granules (Sample X, Y, M and N) were collected at both sites, while samples treated with neutralizing aqueous solution (Sample A and B) were collected at Site I. The preservation methods tested were introducing the preservative during pre-tapping (Sample X, M and A); the preservative was added into the empty bottle before the tapping begins and post-tapping (Sample Y, N and B); the preservative was added after the sap has been collected. Changes in physical appearance has been evaluated using sensory evaluation method, meanwhile pH and sucrose content were measured on-site at every one-hour interval. Due to the remote location of Site II, further analysis could not be conducted. Only on-site experiments, such as pH, sensory evaluation, and sucrose content, were performed at Site II. The results showed that pre-tapping treatments preserved more sucrose (Sample X: 112.0 ± 0.4 g/L, Sample M: 110.0 ± 0.5 g/L, Sample A: 150.0 ± 0.1 g/L) than post-tapping treatments (Sample Y: 116.0 ± 0.4 g/L, Sample N: 105.0 ± 0.3 g/L, Sample B: 145.0 ± 0.4 g/L) after 24 hours of storage. Further analysis

showed that treated samples (both pre- and post-tapping) exhibited lower glucose and fructose concentrations compared to their controls. Sample A showed the lowest reducing sugar concentration (51.8 ± 2.4 g/L), followed by Sample B (51.3 ± 2.0 g/L), Sample X (67.6 ± 2.8 g/L) and Sample Y (63.6 ± 2.5 g/L). Sample A (0.95 g/L) and Sample B (0.97 g/L) had lower concentration of lactic acid compared to Sample X (1.15 g/L) and Sample Y (1.08 g/L). Sample A (0.08 g/L) and Sample B (0.07 g/L) also had lower acetic acid levels compared to Sample X (0.12 g/L) and Sample Y (0.09 g/L). The control sample experienced a rapid decline in RSA, dropping from $32.39 \pm 0.31\%$ at 8 hours to just $9.33 \pm 0.36\%$ at 24 hours. Pre-tapping samples (X and A) maintained higher antioxidant activity, with Sample X showing $37.32 \pm 1.27\%$ at 8 hours and $29.39 \pm 2.57\%$ at 24 hours, and Sample A showing $48.91 \pm 0.99\%$ at 8 hours and $45.38 \pm 1.30\%$ at 24 hours. In contrast, post-tapping samples (Y and B) showed lower antioxidant retention, with Sample Y at $32.55 \pm 7.93\%$ at 8 hours and $22.52 \pm 6.34\%$ at 24 hours, and Sample B at $39.95 \pm 1.07\%$ at 8 hours and $30.46 \pm 0.81\%$ at 24 hours. The study on antimicrobial activity showed that the pre-tapping treatments were more effective at reducing bacterial counts than post-tapping treatments. Sample A had the lowest bacterial counts, ranging from 1.47×10^3 CFU/mL at 10^{-1} to TFTC at 10^{-6} , showing the highest antimicrobial activity. This antimicrobial effect was comparable to the activity of VCO or its active compound, lauric acid, which was used as a reference for evaluating antimicrobial efficacy in the treated sap. Overall, the results suggested that the aqueous preservative reacts faster as it is already dissolved, which leads to a faster reaction rate compared to effervescent granules preservatives. Docking simulations showed that potassium sorbate binding strongly with glucose oxidase through hydrophobic interactions, with the most stable conformation showing a binding affinity of -4.9 kcal/mol.

Keywords: Nipa sap, shelf-life, potassium sorbate, sodium metabisulfite, antimicrobial

**Kesan Pengawet Gred Makanan E dan Kaedah Pengawetan terhadap Sifat Fizikal,
Kimia, dan Mikrobiologi Air Nira Nipah (*Nypa fruticans*) di Sarawak**

ABSTRAK

*Pohon nipah (*Nypa fruticans*) adalah salah satu tumbuhan bakau yang tumbuh secara liar di kawasan persisiran pantai. Pelbagai produk dapat dihasilkan termasuk sap nipah atau air nira, sirap nipah dan gula nipah (gula apong). Oleh itu, pohon nipah mempunyai potensi ekonomi yang besar untuk Sarawak. Walaubagaimanapun, sap nipah mempunyai jangka hayat yang pendek, yang menyumbang kepada hasil dan kualiti gula apong yang rendah. Penyelidikan ini bertujuan untuk meningkatkan jangka hayat sap nipah dengan mengawal perubahan pH, kandungan gula, asid organik dan pertumbuhan mikrob menggunakan bahan pengawet makan Gred E – kalium sorbat dan natrium metabisulfite. Pelbagai kaedah pengawetan yang sesuai telah diuji, dengan memasukkan pengawet sebelum (pra-penuaian) dan selepas (pasca-penuaian) sap dikumpulkan, serta menggunakan pengawet dalam bentuk pepejal (granul berbuak) dan juga cecair. Penyelidikan ini dijalankan di dua lokasi: Kampung Pinggan Jaya (Tapak I, menggunakan botol plastik) dan Pusa (Tapak II, menggunakan bekas buluh). Sampel yang dirawat dengan granul berbuak (Sampel X, Y, M and N) dikumpulkan dari kedua-dua tempat, manakala sampel yang dirawat menggunakan cecair peneutralan (Sampel A dan B) dikumpulkan di Tapak I. Kaedah pengawetan yang diuji adalah pra-penuaian (Sampel X, M dan A) dengan menambahkan pengawet ke dalam botol kosong sebelum proses torehan, dan juga pasca-penuaian (Sampel Y, N dan B) dengan menambahkan pengawet ke dalam botol yang berisi sap pohon nipah. Disebabkan lokasi Tapak II yang jauh, analisis lanjut tidak dapat dijalankan. Hanya penilaian eksperimen seperti pH, penilaian deria dan kandungan sukrosa dapat dijalankan. Hasil keputusan menunjukkan bahawa rawatan pra-penuaian mengandungi lebih banyak kandungan*

sukrosa (Sampel X: 112.0 ± 0.4 g/L, Sampel M: 110.0 ± 0.5 g/L, Sampel A: 150.0 ± 0.1 g/L) berbanding rawatan pasca-penuaian (Sampel Y: 116.0 ± 0.4 g/L, Sampel N: 105.0 ± 0.3 g/L, Sampel B: 145.0 ± 0.4 g/L) selepas 24 jam penyimpanan. Analisis lanjut menunjukkan bahawa sampel yang dirawat (pra dan pasca-penuaian) menunjukkan kepekatan glukosa dan fruktosa yang lebih rendah berbanding dengan sampel kawalan. Sampel A mengandungi kepekatan gula penurun yang paling rendah (51.8 ± 2.4 g/L), diikuti Sampel B (51.3 ± 2.0 g/L), Sampel X (67.6 ± 2.8 g/L) dan Sampel Y (63.6 ± 2.5 g/L). Sampel A (0.95 g/L) dan Sampel B (0.97 g/L) mempunyai kepekatan asid laktik yang lebih rendah berbanding Sampel X (1.15 g/L) dan Sampel Y (1.08 g/L). Sampel A (0.08 g/L) dan Sampel B (0.07 g/L) juga mempunyai tahap asid asetik yang lebih rendah berbanding Sampel X (0.12 g/L) dan Sampel Y (0.09 g/L). Sampel pra-penuaian (X dan A) mengekalkan aktiviti antioksidan yang lebih tinggi, dengan Sampel X menunjukkan $37.32 \pm 1.27\%$ pada 8 jam dan $29.39 \pm 2.57\%$ pada 24 jam, dan Sampel A menunjukkan $48.91 \pm 0.99\%$ pada 8 jam dan $45.38 \pm 1.30\%$ pada 24 jam. Manakala, sampel pasca-penuaian (Y dan B) menunjukkan penurunan aktiviti antioksidan, dengan Sampel Y pada $32.55 \pm 7.93\%$ pada 8 jam dan $22.52 \pm 6.34\%$ pada 24 jam, dan Sampel B pada $39.95 \pm 1.07\%$ pada 8 jam dan $30.46 \pm 0.81\%$ pada 24 jam. Penilitian berkait aktiviti antimikrob menunjukkan bahawa rawatan pra-penuaian lebih berkesan dalam mengurangkan bilangan bakteria berbanding rawatan pasca-penuaian. Sampel A mempunyai bilangan bakteria yang paling rendah, antara 1.47×10^3 CFU/mL pada 10^{-1} hingga TFTC pada 10^{-6} , menunjukkan aktiviti antimikrob yang tertinggi. Keseluruhan keputusan menunjukkan bahawa cecair pengawet peneutralan bertindak lebih cepat kerana ia telah larut, yang menyebabkan kadar tindak balas yang lebih cepat berbanding pengawet granul berbuak.

Kata kunci: Sap nipah, jangka hayat, kalium sorbat, natrium metabisulfit, antimikrob

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LIST OF ABBREVIATIONS

AAB	Acetic acid bacteria
Ca	Calcium
DPPH	2,2-Diphenyl-1-picrylhydrazyl
FDA	Food and Drug Administration
Fe	Iron
HPLC	High-performance liquid chromatography
K	Potassium
LAB	Lactic acid bacteria
LBDD	Ligand-based drug design
Mg	Magnesium
Na	Sodium
ROS	Reactive oxygen species
SBDD	Structure-based drug design
TBC	Total bacterial count
TFTC	Too few to count
TNTC	Too numerous to count
TDS	Total dissolved solids
VCO	Virgin Coconut Oil
Zn	Zinc

CHAPTER 1

INTRODUCTION

1.1 Study background

1.1.1 *Nypa fruticans* in Sarawak

The nipa palm (*Nypa fruticans*) has been a vital part of the local ecosystem and culture in Sarawak for centuries. They grow abundantly in the coastal area of Sarawak. It can be easily found on the swamp land and alongside the river. Nipa palms lessen the effects of storms, cyclones, and tsunamis, acting as the first line of defence (Widodo *et al.*, 2020). In Sarawak, nipa palm sap is a traditional beverage consumed by the locals, meanwhile nipa palm sugar or *gula apong* is a main ingredient to make some traditional desserts such as *sarang semut*, *kuih ros* and *kuih penyaram*.

Fresh nipa sap, which is also locally known as *air neera* or *air sadap*, has a sweet and fruity odour, without any presence of higher alcohols and acetic acid content (Minh, 2014). This *air sadap* is served either in a chilled or heated form, which is normally consumed after meals to refresh.

Besides, palm sugar is healthier than white and brown sugar as it is an unrefined sugar, meaning it is richer in vitamins and minerals, and no chemicals are used during processing. In comparison to sugarcane, palm sugar has higher nutrient content. Therefore, it could be employed as a healthy natural sweetener (Maryani *et al.*, 2021). It is unfortunate that traditionally harvested nipa palm sap remains fresh only for 1-2 hours due to lack of hygienic harvesting conditions.

1.1.2 Fermentation of *Nypa fruticans* sap

Fermentation process of nipa palm sap begins almost immediately after sap extraction, with enzymes such as invertase breaking down sucrose into glucose and fructose. These monosaccharides then serve as substrates for microorganisms, which convert them into organic acids, alcohols and gases such as carbon dioxide. Higher sugar concentrations provide more substrates for microbial metabolism, leading to increased production of ethanol, acids and other by-products (Tamunaidu *et al.*, 2013).

One of the most prominent microorganisms involved in nipa sap fermentation is *Lactobacillus* spp., a lactic acid bacteria (LAB) which play a significant role in the fermentation of nipa sap. LAB converts sugars into lactic acid, contributing to the sour flavour and lowering the pH of the sap. As the fermentation process progresses, the sap undergoes several undesirable changes including the reduction in sweetness, the development of tart taste, a white appearance and the release of unpleasant odour due to the accumulation of fermentation by-products (Lantemona *et al.*, 2023). These characteristics render the sap unsuitable for consumption and hinder its use in the production of traditional *gula apong* (palm sugar).

Nevertheless, its fermentation process can be controlled using various applications of chemicals or bacterial anti-ferments such as sodium metabisulfite, potassium metabisulfite, potassium sorbate, benzoic acid, citric acid calcium hydroxide and lime water (Dhansu *et al.*, 2023; Hebbar *et al.*, 2018). The chemical preservative helps to inhibit the growth of bacteria, moulds or yeast by disrupting cell division, permeability of cell membrane and activity. For instance, sodium metabisulfite is commonly used as a food preservative due to its antioxidant properties that help make the product less susceptible to degradation when exposed to various elements in its surrounding (Hariharan *et al.*, 2014).

Nipa palm sap contains high sucrose which makes it an ideal medium for bacterial growth under direct sunlight. The addition of preservative may help to slow down the sucrose damage, hence increasing the shelf-life of nipa palm sap and producing more derivative products (Oluwole *et al.*, 2023). Nipa palm sap with a good sap condition can be used to produce various products such as cosmetics, skincare, foods and beverages as well as other pharmaceutical products.

1.1.3 Impact of preservative on the shelf-life and economic viability of *Nypa fruticans* sap

The collection of nipa palm sap yields a substance with high sucrose content, a translucent appearance and sweet flavour. However, the sap is highly vulnerable to fermentation as soon as it is exposed to environmental microorganisms such as yeasts and bacteria. This microbial exposure triggers an immediate fermentation process, driven by the enzymatic breakdown of sucrose into glucose and fructose (Lantemona *et al.*, 2023; Minh, 2014; Tamunaidu *et al.*, 2013). As fermentation progresses, the sap undergoes several undesirable changes. The production of ethanol and organic acids results in a sour taste, an unpleasant odour and a whitish or cloudy appearance (Lantemona *et al.*, 2023). These changes making the sap unsuitable for consumption or further processing, leading to a short shelf-life of only a few hours. Consequently, the fermented sap can only be utilized for small-scale, domestic purposes, significantly limiting its commercial value and potential applications.

The introduction of preservative offers a solution to the rapid fermentation of nipa palm sap as shown in Figure 1.1. Various chemicals, including sodium metabisulfite, potassium sorbate, benzoic acid, calcium hydroxide and lime water, can be added to the sap (Dhansu *et al.*, 2023; Hebbar *et al.*, 2018). These preservatives work by inhibiting microbial growth, disrupting cell membranes and preventing enzymatic activities that lead to

fermentation. When preservatives are applied, they significantly slow down the fermentation process. The reduction in microbial activity results in a slower breakdown of sucrose, leading to a decreased production of ethanol and organic acids. As a result, the sap maintains its sweetness, odour, and yellowish appearance for a longer period. This extended shelf-life enhances the sap suitability for further processing and commercial use. The improved shelf-life allows for greater production capabilities and increased marketability of nipa palm sap products. Preserved sap can be effectively used in a variety of applications, including the manufacture of cosmetics, skincare products, foods, beverages and pharmaceuticals. This expanded usability opens up new commercial opportunities and supports the growth of nipa palm industry. The use of preservatives not only improve the shelf-life of nipa palm sap but also drive economic growth and industry advancement. By extending the shelf-life of nipa palm sap, preservatives contribute to increased economic benefits for small-scale entrepreneurs and local communities. The ability to process and market high-quality sap more effectively fosters industry growth and commercialization.

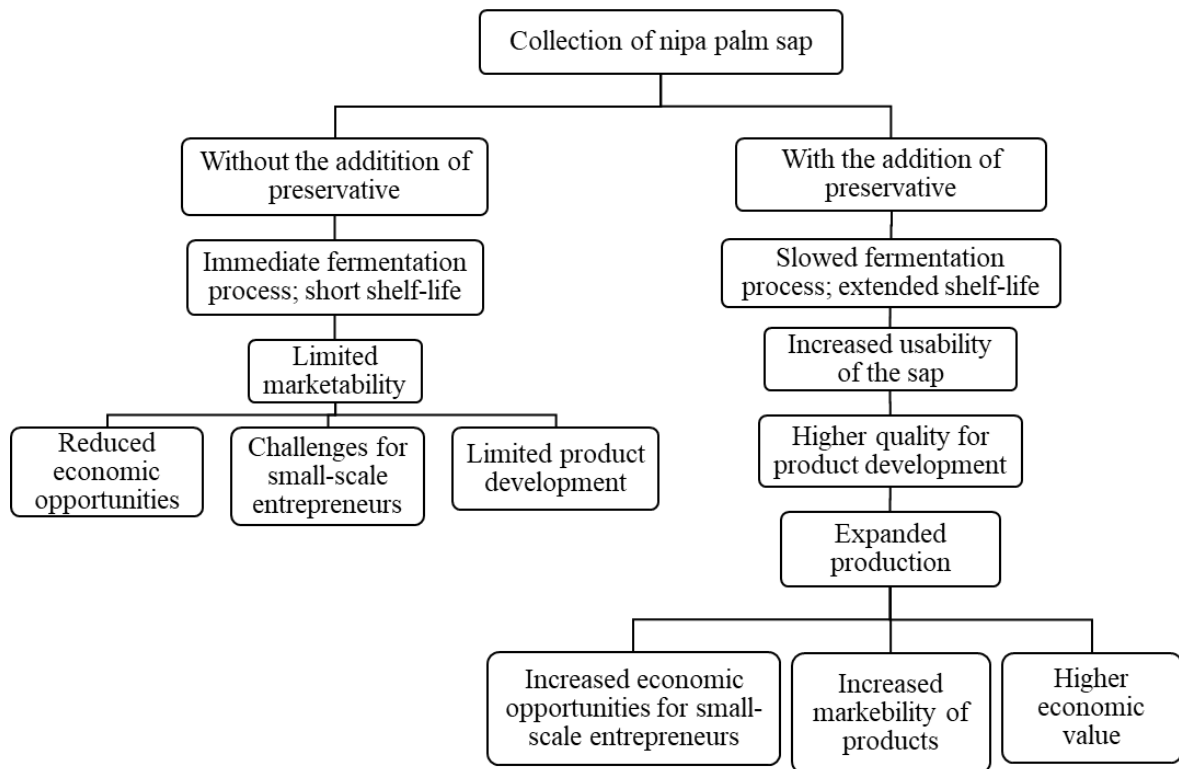


Figure 1.1: Comparison of the impact of preservative on nipa palm sap shelf-life and its industry viability

1.2 Problem statement

Nipa palm sap is rich in nutritional and therapeutic properties. However, it faces a significant challenge due to its rapid fermentation process, which begins within just an hour of being harvested. This natural fermentation is driven by microbial enzymatic activity in an anaerobic environment, leading to the degradation of carbohydrates. The resulting fermented sap develops a whitish appearance, a sour taste and unappealing odour, making it unappealing and unsuitable for local consumption or commercial trade. Once it reaches this state, the nipa sap becomes unusable for the production of traditional *gula apung* (palm sugar).

This rapid spoilage has severely hindered the exploitation and commercialization of nipa palm sap, limiting its use to small-scale, domestic production by local villagers. Nipa

palm sap has the potential for creating various products such as syrups, molasses, alcoholic beverages and traditional vinegar, the industry has not progressed beyond traditional methods. The ability to harness nipa palm sap for broader industrial purposes remains largely untapped, especially considering its potential to drive significant environmental, social and economic benefits, especially for rural communities.

The short shelf-life of nipa palm sap due to its active fermentation has deterred efforts to modernize its production and commercialization. This has had a direct impact on the *Apong* industry, restricting its growth and leaving its vast potential underutilized. To address this problem, this study aims to develop an effective preservative and preservation method for nipa palm sap, extending its shelf-life and enabling exploitation of this resource. This preservative or anti-fermentation product is safe and can be used in food and beverages. By extending the shelf-life of nipa palm sap, the full economic potential of the nipa palm industry can be unlocked. Thus, the nipa palm or *Apong* industry can be expanded to benefit small-scale entrepreneurs as well as local communities, fostering economic growth and transforming the nipa palm sap industry into a more commercially viable enterprise.

1.3 Objectives

The objectives of this study are:

- i. To prepare a preservative by mixing sodium metabisulfite and potassium sorbate at a certain ratio
- ii. To compare the efficiency between the two preservation methods
- iii. To study the antimicrobial and antioxidant activity of preservatives towards the effect of fermentation process

CHAPTER 2

LITERATURE REVIEW

2.1 Origin of *Nypa fruticans*

Nypa fruticans, commonly known as the nipa palm (Figure 2.1) or mangrove palm is a highly prevalent and valuable palm species found abundantly within the mangrove forests of Southeast Asia, extending to Europe, Africa and America. It can also be found within the tropical parts of northern Australia and parts of the Pacific Islands. This implies that the changes in climate conditions and/or the loss of adaptable genotypes capable of withstanding a wider range of environmental conditions. *Nypa fruticans* belong to the family of Arecaceae. This palm thrives in mangrove coastal areas, where it grows in brackish or saline waters along riverbanks. It is well-adapted to its unique environment, with its roots submerged in water and its trunk rising above the surface. Its extensive roots help stabilize riverbank and coastlines, acting as a barrier against storm surges and tidal waves. It has long, pinnate leaves and produces dense clusters of flowers that develop into large, round fruits containing seeds. Nipa palm also contributes to mangrove ecosystems by providing habitat and food for a variety of wildlife including fish, crustaceans and birds (Päivöke, 1985; Hossain & Islam, 2015; Kurniawan *et al.*, 2018; Saengkrajang *et al.*, 2021).



Figure 2.1: Nipa palm trees

Tsuji *et al.* (2011) reported that Malaysians distinguish between two varieties of nipa palm – *nipah gala* and *nipah padi*, which was based on the differences in the position of its leaflets. They also mentioned that a villager in Perak, Malaysia identified another two types of nipa palms called *nipah sawa* and *nipah tikus*. Hence, their observations suggest that the nipa palm population in Malaysia may exhibit greater morphological diversity, potentially comprising more than the two varieties initially described.

2.2 Utilization of *Nypa fruticans*

Nipa palm is among the most common and useful palms found in the mangrove forest as it has historically offered beneficial products to indigenous communities living near or within the estuaries and coastal areas of mangrove forest (Tsuji *et al.*, 2011, 2016).

2.2.1 Leaves

The leaves of nipa palm have numerous uses from thatching roofs for traditional dwellings to weaving baskets, mats, and hats. The matured leaves have significant cultural, economic and environmental importance as they provide a wide range of traditional uses

that are deeply rooted in coastal lifestyle. For example, coastal communities often use the matured nipa leaves for building and thatching of village huts or houses. They are also extensively used for weaving. The flexibility and strength of these leaves make them ideal for crafting a variety of woven products such as mats, baskets and hats. Besides, the leaves are also employed in culinary traditions, where they serve as natural food wrappers for steaming and cooking, contributing to a unique flavour and aroma to the dish (Päivöke, 1985; Tsuji *et al.*, 2011; Hossain & Islam, 2015; Harun *et al.*, 2021).

2.2.2 Flowers, buds and petals

The flower part of the nipa palm plays a crucial role in the production of sap and *gula apong*. However, it is also edible which usually serves as a dessert known as *mayang apong*. The use of nipa palm buds and petals are not as common compared to leaves and sap. The bud is edible (Tsuji *et al.*, 2011). In Kelantan, Malaysia, the buds are used to make a dish called *kerabu nipah*, meanwhile the petals can be brewed to make an aromatic coffee (Hossain & Islam, 2015).

2.2.3 Sap

The nipa palm produces copious quantities of sap, known as nipa sap which is also locally known as *air sadap* or *air nira*, obtained by incising either mature inflorescences or young inflorescences after the removal of their floral or fruit components (Tsuji *et al.*, 2011; Kurniawan *et al.*, 2018).

The nipa sap derived from the *Nypa fruticans* tree is a refreshing liquid characterized by its high sugar content, transparent appearance, and fruity aroma (Minh, 2014). This sap serves as a fundamental ingredient in the production of various products, including vinegar and alcoholic beverages since it can produce more ethanol compared to sugarcane. Nipa palm sap serves as a primary ingredient in the production distilled or fermented drink known

as "tuba" or "soom" in the Philippines, "arak" or "tuak" in Indonesia, and "toddy" in regions like Malaysia, India, and Bangladesh (Hamilton & Murphy, 1988). According to Halos (1981), the appeal of nipa palm as a promising source of biofuel lies in its potential for significantly increased alcohol production, which could reach up to 18,165 litres through modern management practices. This potential stems from the substantial volume of sap generated by nipa palm trees, which can be efficiently converted into alcohol. Because of its high capacity of sap production that can be converted to alcohol, nipa is also said to be useful for sources of biofuel owing to its sugar content that ranges from 14 to 17% of sucrose.

2.3 Tapping sap from *Nypa fruticans*

Tapping is a method for collecting sap from nipa palm, where timing plays a crucial role in the process. According to the local villagers, mature trees are selected for sap collection. The sap is extracted from the flower stalk or inflorescence of the nipa palm by cutting the stalk to allow the sap to flow (Hebbar *et al.*, 2018; Kurniawan *et al.*, 2018). However, before cutting the stalk, the flower stalks must be pre-treated by bending and kicking the stalk. This bending and kicking method are a traditional technique passed down through generations to loosen the tissues and facilitate the flow of sap. The pre-treatment helps to extract more sap or maximize the yield of the sap during the tapping process (Samarajeewa *et al.*, 1985; Tsuji *et al.*, 2011). Kurniawan *et al.* (2018) stated that the stalk is usually cut at an angle between 30-45° facing down as in Figure 2.2, to allow sap to flow into collection containers.



Figure 2.2: Stalk cutting process

This downward angle allows gravity to aid in the flow of sap downward into collection containers beneath, hence ensuring that the sap flows smoothly and efficiently. The tapping process is typically done in the evening. Cooler temperatures slow down natural fermentation processes which help to reduce the risk of rapid microbial growth and preserve the quality of the collected sap. Once collected, the nipa palm sap can be processed into various products such as palm sugar, vinegar or alcoholic beverages. The sap needs to be processed quickly to prevent fermentation and spoilage.

2.4 *Gula Apong* production in Sarawak

When it comes to preparing traditional cuisine, Malaysia has its own set of local sweeteners such as palm sugar (*gula melaka* and *gula apong*). In 1941, palm sugar was one of the foods people ate to survive during the Japanese invasion of Borneo (Abdullah *et al.*, 2022). Palm sugar is considered as a natural sugar as it undergoes minimal processing without the use of chemicals. Furthermore, it is rich in vitamins and minerals, which has contributed to its popularity as a substitute for white and brown sugar. Nipa palm sugar, locally known as *gula apong* is a famous product in Sarawak. It is a type of sugar that is derived from the nipa palm tree which grows abundantly in the coastal area of Sarawak. Nipa palm sugar is prepared by boiling the sap in a large pan. The pan is simply heated for several

hours, with the sap being continuously stirred to prevent burning and ensure even heating. This process continues until the sugar concentration in the sap increases. Eventually, the sap starts to thicken and form crystals. The heating process is continued until the sap thickens to the desired consistency and crystallized sugar form. Usually, this process lasts approximately three to four hours, resulting in the attainment of a high concentration of brown sugar (Ho *et al.*, 2007; Nguyen *et al.*, 2016; Hebbar *et al.*, 2018; Kurniawan *et al.*, 2018; Cherepanova & Nový, 2021; Abdullah *et al.*, 2022). As in Figure 2.3, the sugar crystals then are allowed to cool and solidify, making it ready for packaging.



Figure 2.3: Nipa palm sugar or *gula apong*

2.5 Factors affecting the growth and sap quality of *Nypa fruticans*

2.5.1 Environmental conditions

Environmental factors such as temperature, humidity and rainfall can affect the growth of nipa palm. In its growing areas, the average lowest temperature is 20°C, while the highest is 32-35°C. Adequate moisture provided by regular rainfall or proximity to water bodies is also essential for the growth of nipa palm. Nipa palm also prefers soft, muddy soils

with good drainage as they are rich in iron and manganese sulphides, calcium as well as other inorganic salts. For this reason, nipa palms thrive in coastal environments characterized by brackish or saline water such as estuaries, riverbanks and tidal areas (Tsuji *et al.*, 2011; Hossain & Islam, 2015; Saengkrajang *et al.*, 2021).

2.5.2 Time of tapping

As previously mentioned, tapping time plays a significant role during the sap collection process. The time and season during when harvested the sap can affect its quality. Sap is collected twice a day, often in the morning and evening. The sugar concentrations and/or taste of the sap may be different (Naknean *et al.*, 2010; Nguyen *et al.*, 2016; Wardani *et al.*, 2020). According to the locals, rainy or dry weather can also impact the production and quality of the nipa palm sap.

2.5.3 Tapping techniques

The method of tapping the nipa palm sap can impact the yield of the sap collected. Proper tapping methods are important for maximizing the yield without causing damage to the palm. Tappers often use bamboo knives or spiles to create incisions that allow sap to flow freely. Pre-treatment (bending, beating or kicking) before tapping is also necessary to stimulate the sap flow by breaking up the cellular structures and facilitating the release of sap. This pre-treatment technique is believed to be able to increase the yield of sap during tapping (Samarajeewa *et al.*, 1985; Tsuji *et al.*, 2011; Kurniawan *et al.*, 2018).

2.5.4 Storage and processing

The handling and storage of nipa palm sap after collection can affect its quality and shelf-life as well. Nipa palm sap has a highly fermentable nature that alters its properties including taste, aroma and quality (Jaraee *et al.*, 2023). Therefore, hygiene practices are essential to prevent contamination that can cause the nipa palm sap to spoil quickly.

2.5.5 Age of the palm

The age of the nipa palm can affect the yield of the sap produced. A healthy and mature nipa palm (9-12 years old stem) is more likely to yield higher quality of sap compared to a nipa palm with a stem of 15 years old (Nguyen *et al.*, 2016; Jaraee *et al.*, 2023).

2.6 Products made from palm sap

2.6.1 Palm wine

Palm wine, also known as palm toddy, is a fermented alcoholic beverage derived from the sap of various palm tree species, including the palmyra, coconut palm and date palm (Chandrasekhar *et al.*, 2012; Hebbar *et al.*, 2018; Cherepanova & Nový, 2021). The fermentation of palm sap is not only a natural process but also traditional practices in many cultures. This beverage is popular in many parts of Asia and Africa and is known by different names. For example, palm wine is known as tuba in Philippines, emu and ogogoro in Nigeria, kallu in South India, toddy in some parts in India and tuak in Borneo Island (Chandrasekhar *et al.*, 2012; Hebbar *et al.*, 2018; Jaraee *et al.*, 2023). Palm wine has a cloudy or whitish appearance with a sweet alcoholic taste, but also has a very short life of only one day (Chandrasekhar *et al.*, 2012; Jaraee *et al.*, 2023). This alcoholic beverage is produced by fermenting the palm sap, which is collected by tapping the young inflorescence or top of the trunk of these various palm trees (Lasekan *et al.*, 2007; Chandrasekhar *et al.*, 2012; Hebbar *et al.*, 2018; Cherepanova & Nový, 2021; Jaraee *et al.*, 2023).

The sap is high in sugar content, mainly sucrose, which is an excellent substrate for microbial growth (Chandrasekhar *et al.*, 2012; Jaraee *et al.*, 2023). It creates an ideal environment for yeast and bacteria to grow (Chandrasekhar *et al.*, 2012; Hebbar *et al.*, 2018; Cherepanova & Nový, 2021). This resulted in the natural fermentation to start almost immediately after being collected. Within a few hours, the sap can reach an alcohol

concentration up to 4%. Additionally, if the fermentation continues for more than a day, the palm wine begins to turn into palm vinegar (Lasekan *et al.*, 2007; Chandrasekhar *et al.*, 2012).

Chemical analysis of palm wine using Gas Chromatography-Mass Spectrometry (GC-MS) has identified 82 different chemical components. These include 47 esters, 9 alcohols, 9 hydrocarbons, 6 carbonyls, 5 acids, 4 terpenes and 2 acetals (Chandrasekhar *et al.*, 2012). Out of these chemical compounds, 41 were identified as odour active. Lasekan *et al.* (2007) reported further quantification and analysis of the odour activity values of 13 key odorants of palm wine revealed specific compounds that are responsible for the aroma of the palm wine. These odorants are the most potent and significantly influenced the distinctive aroma profile of palm wine. These include 3-methylbutyl acetate and ethyl hexanoate, which contribute to fruity scents of the sap; acetoin, which has a buttery aroma; 3-isobutyl-2-methoxypyrazine, which has an earthy smell; fruity and 2-acetyl-1-pyrroline, which smells like popcorn.

Palm wine is highly valued in African cultures for its cultural and medicinal significance. It is commonly used in traditional naming and marriage ceremonies, incantations and folk medicines. Both health professionals and governments have acknowledged its role in treating malnutrition in some African regions (Cherepanova & Nový, 2021). A liter of palm wine contains approximately 300 calories, 0.5-2.0 grams of protein, and significant amounts of vitamins A, C, and K, which are beneficial for eyesight and overall health (Chandrasekhar *et al.*, 2012). Besides, palm wine is also known for its antioxidant properties and health-promoting benefits, which have been demonstrated in rat models (Djeni *et al.*, 2020).

2.6.2 Palm sugar

Palm sugar is a natural sweetener made from the sap extracted from palm trees. This sugar has been used in the South-east and South Asia such as Indonesia, Philippines, Malaysia, Thailand and India as traditional and alternative sweeteners. Palm sugar, a popular delicacy widely consumed across Asia, is used in a variety of applications, including cakes, desserts, food coatings, and beverages. It is produced by heating the sap from tropical coconut trees (*Cocos nucifera*) or palm trees such as *Arenga pinnata*, *Borassus flabellifer* and *Nypa fruticans* (Ho *et al.*, 2007; Hebbar *et al.*, 2018; Saputro *et al.*, 2019).

The process of making palm sugar is similar across various types of palm saps. The collected palm sap is filtered and poured into a large pan. Then, the sap is heated for 3 to 4 hours at $>100^{\circ}\text{C}$ until it becomes concentrated, brown, and highly viscous. After that, the hot palm sugar is poured into moulds made from bamboo or coconut shells to form solid palm sugar and allowed to cool. After an hour, the palm sugar is ready for consumption and packaging (Ho *et al.*, 2007; Nguyen *et al.*, 2016; Hebbar *et al.*, 2018; Kurniawan *et al.*, 2018; Cherepanova & Nový, 2021; Abdullah *et al.*, 2022). In the market, palm sugar is available as syrup, moulded sugar, or powdered sugar (Broberg, 2014 as cited in Saputro *et al.*, 2019). Palm sap syrup is made by boiling the sap until it reaches a specific Brix value and is then stored in glass or plastic containers. Meanwhile, to make powdered palm sugar, further heating with agitation is required until the sugar reaches its supersaturation point, followed by cooling to generate sugar crystals (Saputro *et al.*, 2019).

Polar side chain amino acids such as asparagine and glutamine can be found in palm sap and about 89.2% content in palm sap is consist of sugars like sucrose, glucose and fructose (Ho *et al.*, 2007; Hebbar *et al.*, 2018; Saputro *et al.*, 2019). The high sugar concentration could be fermented to make wine. During the heating process, the sugars in

palm sap undergo the Maillard reaction and caramelization. Previous researchers reported that during the Maillard reaction, the polar side chain amino acids were essential precursors for forming volatile compounds. This is because they release more free amino groups compared to other amino acids, which then will catalyse sucrose conversion into their reactive component of monosaccharides (Ho *et al.*, 2007; Saputro *et al.*, 2019). The use of an open pan evaporator promotes higher sucrose inversion and the formation of hydroxymethylfurfural, which reduces the glucose and fructose content in the final product (Kurniawan *et al.*, 2018). When palm sap is heated above 110 °C, Maillard reaction products, such as pyrazines, are released. The volatile components of palm sugar, such as pyrazines, furans, ketones, fatty acids, and organic acids, contribute to its sweet, toasty, and nutty caramel-like aroma (Hebbar *et al.*, 2018).

2.7 Chemical composition of *Nypa fruticans* sap

2.7.1 Sugars

Nipa palm sap is rich in sugars, primarily sucrose, glucose and fructose (Nguyen *et al.*, 2016; Hebbar *et al.*, 2018; Kurniawan *et al.*, 2018; Jaraee *et al.*, 2023). These sugars provide the nipa sap its sweet flavour and are the main precursor for the fermentation process that produces nipa palm sugar or alcoholic beverages. The presence of sugars causing the nipa palm sap to undergo the Maillard reaction and caramelization upon heating (Naknean *et al.*, 2010; Hebbar *et al.*, 2018).

2.7.2 Organic compounds

Like many natural plant fluids, nipa palm sap also contains some organic compounds that contribute to its unique flavour, nutritional value and can also influence the fermentation of the sap after tapping. For instance, various amino acids can be found in the nipa palm sap including lysine, leucine, valine as well as non-essential amino acids. These amino acids

contribute to the protein content of the sap and its overall nutritional composition (Saelee, 2022). Organic acids are also present in nipa sap such as acetic acid, lactic acid and succinic acid. These volatile compounds may be major odorants in both fresh and fermented nipa palm sap (Naknean *et al.*, 2010; Nguyen *et al.*, 2016; Saelee, 2022). Many kinds of vitamins like ascorbic acid, thiamine, riboflavin and niacin can also be found in the nipa palm sap which also contribute to the nutritional value of the nipa palm sap (Nguyen *et al.*, 2016; Saelee, 2022).

2.7.3 Inorganic compounds

Various inorganic compounds can be found in the nipa palm sap. Barh and Mazumdar (2008) reported that the nipa palm sap contains essential minerals such as K, Ca, Mg, Na along with other trace elements like Fe and Zn. High content of chloride ions is also present in nipa palm sap that grows near brackish water or seawater, most likely from the seawater salt. Si also can be found in nipa palm sap. It is one of the valuable trace elements that have various health benefits due to its anti-inflammatory properties (Nguyen *et al.*, 2016; Hebbar *et al.*, 2018; Nguyen *et al.*, 2019; Saengkrajang *et al.*, 2021).

2.7.4 Yeast and bacteria

Nipa palm sap contains yeasts and bacteria that contribute to its natural fermentation process and the production of traditional fermented products such as palm vinegar and palm wine. *Saccharomyces* yeast is naturally present in nipa palm sap and responsible for the conversion of sap into alcohol during nipa palm sap storage. Various bacteria species are also present in nipa palm sap such as acetic acid bacteria (*Acetobacter* sp.) and lactic acid bacteria (*Lactobacillus* sp.) which is commonly found in the sap. These bacteria contribute to the acidity and tangy taste of the fermented nipa palm products. The lactic acid bacteria are also responsible for changing the consistency and the colour of nipa palm sap from

translucent to whitish (Naknean *et al.*, 2010; Nguyen *et al.*, 2016; Jaraee *et al.*, 2023). Both acetic and lactic acid bacteria can produce glucose oxidase, an enzyme that catalyses the oxidation of glucose into gluconic acid. The production of gluconic acid further lowers the pH of nipa sap, increasing the acidity of the sap during natural fermentation process (Flores-Gallegos *et al.*, 2019).

2.8 Improving shelf-life of *Nypa fruticans* sap

The high sugar content of nipa palm sap makes it an ideal substrate for microbial growth. Once harvested, the sap begins to ferment almost immediately that leads to a rapid decline in quality. As a result, there has been a growing interest in the use of preservatives to extend the shelf-life of the sap. In order to enhance the shelf-life of nipa palm sap, many preservation techniques have been developed. In most of the experiments, the sap that was harvested by a traditional method was used which is unhygienic and partially fermented (Hebbbar *et al.*, 2018).

2.8.1 Preservatives

Amirpour *et al.* (2015) stated that preservatives such as benzoic and sorbic acid, as well as their sodium and potassium salts are commonly used to preserve the food. Food preservatives such as potassium sorbate, sodium metabisulfite, citric acid and benzoic acid also can be used to control the fermentation process of nipa palm sap. Sodium metabisulfite is a synthetic food preservative that has been widely used in wine industries to preserve and stabilize the wines due to its antioxidant and anti-bacterial properties that can inhibit microbes from growing, hence protecting the colour and flavour of the wine (Hariharan *et al.*, 2014). Potassium sorbate also can inhibit the growth of mold and helps in preserving the freshness of the products.

In Indonesia, *Millettia sericea* sp. root is commonly used to preserve the nipa palm sap. According to Filianty *et al.* (2006), the root helps in slowing down the fermentation process, hence preventing the sap turning into acid. Mangosteen bark also been widely used as nipa palm sap preservative as it has phytochemical components to slow the fermentation from taking place such as antioxidants, anti-bacterial, anti-fungal and other biological activities (Lantemona *et al.*, 2023). In addition, mangosteen peels can also be used as nipa palm sap preservative due to its bioactive compounds that are able to inhibit the sap from spoilage in a short time by maintaining the pH of the sap. It also consists of tannins which inhibit the activity of yeast, reducing the reaction of sucrose to reducing sugar via hydrolysis (Putra *et al.*, 2019). In some places, lime is dropped into the nipa palm sap bamboo or container to prevent the sap from damaging until processing phase (Hebbar *et al.*, 2018).

2.8.2 Hygienic collection and storage

Unhygienic collection practices can influence the fermentation of nipa palm sap as it exposes the sap to environmental contaminants such as plant debris, dust, insects and microorganisms from the surrounding area. This leads to faster pH reduction and production of lactic and acetic acids to be increased. As a result, pasteurization of nipa palm sap required a high processing temperature and a long processing period. Pasteurization of nipa palm sap is generally performed by heating at boiling temperature (Naknean, 2013). Proper hygiene during tapping, harvesting and handling the nipa palm sap are required to prevent the fermentation. Storing the sap at low temperatures can also help to prevent the fermentation from accelerating.

2.9 Food grade preservatives

Food preservatives can be classified into two types which are antimicrobials and antioxidants. Antimicrobial preservatives are designed to extend the shelf-life of food

products by protecting them from deterioration caused by pathogenic microbial agents. These preservatives are often combined with other preservation methods such as refrigeration, to inhibit the growth of spoilage and pathogenic microbes. Antioxidant preservatives, on the other hand, are compounds that prevent, or slow down food degradation caused by oxidation. It helps to inhibit autoxidation, which occurs when these substances interact with oxygen in the air at room temperature. By slowing down the autoxidation, they help prevent undesirable qualities like rancidity in foods (Berk, 2013; Dehghan *et al.*, 2018; Singh & Davidson, 2024). Food grade preservatives such as potassium sorbate, sodium metabisulfite, sodium benzoate, citric acid and many others has been used to extend the shelf-life of various products including palm sap, palm wine and its vinegar (Hebbbar *et al.*, 2018).

2.9.1 Potassium sorbate

Potassium sorbate (Figure 2.4), known as a potassium salt of sorbic acid and chemically known as potassium (2,4-hexa-2,4-dienoate) with a chemical formula of $\text{CH}_6\text{H}_7\text{KO}_2$, is designated as E-202 in the food industry (Dehghan *et al.*, 2018). It is a white, odourless crystalline substance in the form of granule or powder (Lück *et al.*, 2000 as cited in Dehghan *et al.*, 2018). This additive is widely used in processed foods such as ketchup, soy sauce, soda and fruit juice. This is due to the ability of potassium sorbate in inhibiting mold growth, preventing spoilage and preserving the freshness of the products (Lück, 1990; Ferrand *et al.*, 2000). Hence, it is commonly used to extend the shelf-life of commercial goods without affecting their texture, taste and aroma.

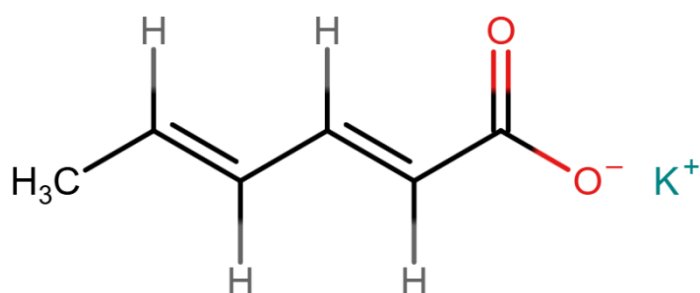


Figure 2.4: Chemical structure of potassium sorbate

Potassium sorbate is one of the synthetic additives that can break down into sorbitol, potassium, and sorbic acid (Samarajeewa *et al.*, 1985; Berk, 2013). Sorbic acid with a chemical formula of $\text{C}_6\text{H}_8\text{O}_2$ (Figure 2.5) is a straight-chained monocarboxylic acid which is naturally found in various plants, organism tissues as well as fermented products (Berk, 2013; Dehghan *et al.*, 2018).

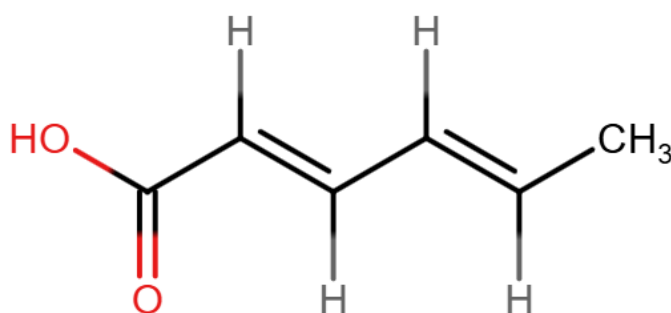


Figure 2.5: Chemical structure of sorbic acid

The antimicrobial effectiveness of potassium sorbate depends on the dissociated sorbic acid and the pH level (Berk, 2013). Sorbic acid and sorbates inhibit molds and yeasts more effectively than bacteria. However, the antimicrobial activities of sorbic acid and potassium sorbate have been studied broadly which shown that they have a high effect on growth rate of bacterial such as *Aspergillus niger*, *Candida albicans*, *Clostridium sporogenes*, *Escherichia coli*, *Klebsiella pneumonia*, *Penicillium notatum*, *Pseudomonas*

aeruginosa, *Pseudomonas cepacia*, *Pseudomonas fluorescens*, *Saccharomyces cerevisiae*, and *Staphylococcus aureus* (Stanojovic *et al.*, 2009 as cited in Dehghan *et al.*, 2018).

Factors like temperature, pH, food composition, water activity, various metals, and other additives can influence the stability of potassium sorbate (Ferrand *et al.*, 2000; Dehghan *et al.*, 2018). The U.S. Food and Drug Administration (FDA) recognizes it as generally safe when used appropriately. When ingested as a food additive, potassium sorbate passes through the body harmlessly as water and carbon dioxide without accumulating in the body. The acceptable daily intake is set at 3 mg per kg of body weight per day. This limit addresses concerns from animal research regarding reproductive and developmental toxicity. Rats fed 300 mg per day showed no adverse effects. This led the European Food Safety Authority (EFSA) to set a standard 100 times lower for added safety (EFSA Panel, 2015).

2.9.2 Sodium metabisulfite

Figure 2.6 showed a chemical structure of sodium metabisulfite. Sodium metabisulfite (E-223), also known as sodium pyrosulfite ($\text{Na}_2\text{S}_2\text{O}_5$), is a granulated powder that releases sulphur dioxide (E-220), SO_2 , when dissolved in liquid. It has been widely used in preserving food and beverages products (Davidson *et al.*, 2005). As a synthetic food additive, it is primarily used for its antioxidant and antibacterial properties (Samarajeewa *et al.*, 1985; Noorafshan *et al.*, 2013). The main benefit of sodium metabisulfite lies in its ability to prevent the growth of fungi and bacteria. It releases sulphur dioxide when dissolved in water, which is more effective against bacteria than against yeasts and molds (Grotheer *et al.*, 2005; Ough & Were, 2005). This makes it particularly useful in inhibiting spoilage and maintaining the shelf-life of various products. For example, sulphur dioxide in its free form is significantly more effective as an antimicrobial agent compared to its bound forms. In

grape juice, the bound forms of sulphurous acid exhibit only about 1/30 of the antimicrobial effectiveness of the free form (Cruess, 1912).

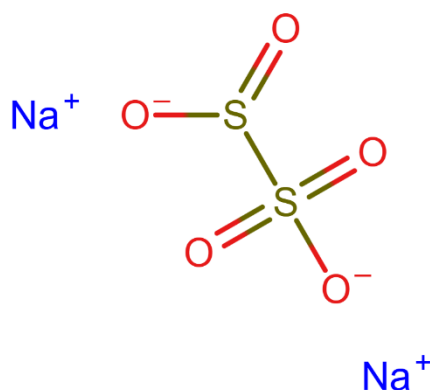


Figure 2.6: Chemical structure of sodium metabisulfite

Sodium metabisulfite is widely used in food and beverage industries for several purposes. In fruit preservation, sodium metabisulfite is used to destroy wild molds and bacteria on fruits thus extending their shelf-life (Grotheer *et al.*, 2005). This is because fruits are susceptible to rapid spoilage due to microbial contamination. Sodium metabisulfite has also been used in juices and concentrates as it helps prevent microbial growth and oxidation, thereby maintaining the quality and safety of the products (Ubigli *et al.*, 1982 as cited in Ough & Were, 2005). The use of sodium metabisulfite is also important in wine production. This is because sulphur dioxide is important in winemaking for controlling lactic acid bacteria such as *Lactobacillus*, *Leuconostoc*, *Pediococcus*, and *Oenococcus* (Amerine *et al.*, 1980 as cited in Ough & Were, 2005). These bacteria can cause spoilage in wines, particularly those with high acidity. The addition of sulphur dioxide helps maintain the stability of the wine and prevent unwanted fermentation and spoilage. Sodium metabisulfite is also used in pharmaceuticals to maintain the efficacy and stability of some medications (Knodel, 1977 as cited in Grotheer *et al.*, 2005).

The antioxidant properties of sodium metabisulfite are particularly valuable in preserving the quality and extending the shelf-life of various food products due to its ability to release sulphur dioxide when dissolved in water. Sulphur dioxide released from sodium metabisulfite can neutralize reactive oxygen species (ROS) by donating electrons. Thus, preventing them from initiating or propagating oxidative reactions (Davidson *et al.*, 2005). Sulphur dioxide also inhibits the polyphenol oxidase activity, which helps preventing the browning of the food products (Tan *et al.*, 2015). In wine production, sodium metabisulfite is used to prevent oxidation during fermentation and storage (Jacobs, 1976 as cited in Davidson *et al.*, 2005). Its antioxidant properties protect the wine from spoilage, preserve its colour and flavour, and extend its shelf-life. Meanwhile, in the preservation of fresh and dried fruits, sodium metabisulfite is used to prevent both enzymatic and non-enzymatic browning (Ahmadi *et al.*, 2021). This ensures that the fruits retain their natural colour, flavour, and nutritional value over time.

The FDA classifies sulphites, including sodium metabisulfite, as Generally Recognized as Safe (GRAS) when used in accordance with good manufacturing practices. However, their use is regulated to ensure consumer safety. For instance, the maximum allowable level of sulphur dioxide in wine is set at 350 mg/L by the regulating body for the U.S. alcoholic beverage industry. This ensures that the levels of sulphites in food and beverages remain safe for consumption while effectively preserving the products.

Research has shown that sodium metabisulfite is particularly effective against certain bacteria found in acid fruits and beverages. For example, a study by Cruess (1912) demonstrated that a sulphur dioxide concentration of 100 mg/L significantly reduced the population of *Acetobacter* sp., a common bacterium in palm sap, from 5.49 CFU/mL to 2.47 CFU/mL. Additionally, the work of Fornachon (1957) highlighted the importance of sulphur

dioxide and pH in controlling lactic bacteria in wines, emphasizing its role in maintaining the microbial stability of acidic beverages.

2.9.3 Citric acid

Citric acid (E-330) is an organic compound with the chemical formula $C_6H_8O_7$ (Figure 2.7). Citric acid naturally occurs in fruits like oranges, lemons, blackcurrants and strawberries. It imparts a sour and pungent taste to these fruits (Khatri & Shalini, 2008; Yilmaz *et al.*, 2008; Silva & Lidon, 2016; Abdel-Salam *et al.*, 2018). The FDA classifies citric acid as GRAS, making it a widely accepted additive in the food and beverage industry. It has been widely used in these industries as a food additive and shows no side effects (Inetianbor *et al.*, 2015 as cited in Silva & Lidon, 2016).

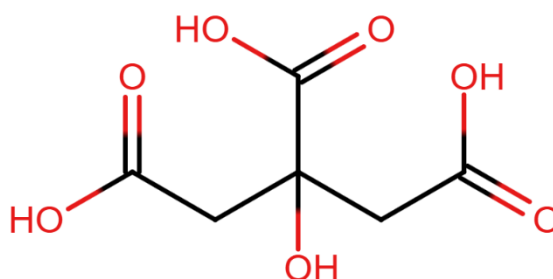


Figure 2.7: Chemical structure of citric acid

Most citric acid used in the processed food products is not extracted from citrus fruits, but via fermentation of fungus using *Aspergillus niger* mold and this citric acid is known as manufactured citric acid or MCA (Khatri & Shalini, 2008; Yilmaz *et al.*, 2008; Sherrell, 2023). One of the main functions of citric acid in food preservation is its ability to inhibit the growth of harmful microorganisms. Citric acid effectively slows the growth of bacteria, yeasts, molds and fungus by creating an unfavourable environment for these microorganisms. This antimicrobial property helps prevent spoilage, hence extending the

shelf-life of foods, beverages and cosmetic products. Citric acid can maintain the freshness of the products by retaining the original colour, flavour as well as the texture of the products, which is important for consumer's satisfaction and product quality (Khatri & Shalini, 2008; Sherrell, 2023).

In addition, citric acid also aids in balancing the pH level of foods and beverages. For example, in winemaking, citric acid is used to improve low acidity and enhance the flavour profile of the wine. This is due to its relatively weak acidity (pH 3-6), which makes it an ideal preservative or additive for improving the taste of various products such as ice cream, soft drinks, canned vegetable and fruit food, without overpowering their natural flavours (Yilmaz *et al.*, 2008; Sherrel, 2023).

Citric acid is also known for its antioxidant properties, which also helps in extending the shelf-life of the products. Ascorbic acid (E-300), which is a derived antioxidant, can be found in citric acid. It has been used widely to preserve soft drinks and meats (Silva & Lidon, 2016; Abdel-Salem *et al.*, 2018). For instance, sprinkling lemon juice, which contains citric acid, can prevent apples and bananas from turning brown.

While citric acid is generally safe, excessive consumption can erode tooth enamel over time. Lan *et al.* (1999) advises consuming foods containing citric acid in moderation and recommends rinsing the mouth after eating or drinking such products to protect dental health. This precaution ensures that consumers can enjoy the benefits of citric acid without adverse effects.

2.10 Molecular docking studies

Traditional drug discovery methods often began with isolating and purifying active ingredients from natural products or synthesizing compounds through a trial-and-error approach followed by in vivo screening. This process frequently lacked a clear scientific

rationale, as many compounds were synthesized without prior knowledge of their potential effectiveness. Consequently, it was time-consuming and inefficient, contributing significantly to the high costs associated with drug development (Rawlins, 2004; Bagchi, 2020). In modern drug designing, molecular docking assists in drug development and medicinal chemistry, as it provides insight about drug-receptor interaction. Molecular docking is a computational technique used to predict the interaction between two molecules, a small ligand and a larger biomolecule such as protein or nucleic acid. It offers valuable information about the binding orientation of small molecule drug candidates to their protein targets, helping to predict their binding affinity and activity (Lai-Yuen *et al.*, 2006; Vijesh *et al.*, 2013; Jakhar *et al.*, 2020). The main objective of docking is to accurately model the structure of a ligand within a receptor's binding site while estimating the binding strength. Docking methods are often paired with other computational approaches, such as molecular dynamics simulations, quantum mechanics, and pharmacophore modelling, to enhance the precision and predictive capability of drug discovery. These combinations enable a more detailed analysis of molecular interactions, accounting for factors like protein flexibility and solvent effects (Yuriev & Ramsland, 2013). Figure 2.8 illustrates the 'lock and key' analogy commonly used in molecular docking, where the ligand (key) specifically binds to the receptor (lock) based on structural and chemical complementarity.

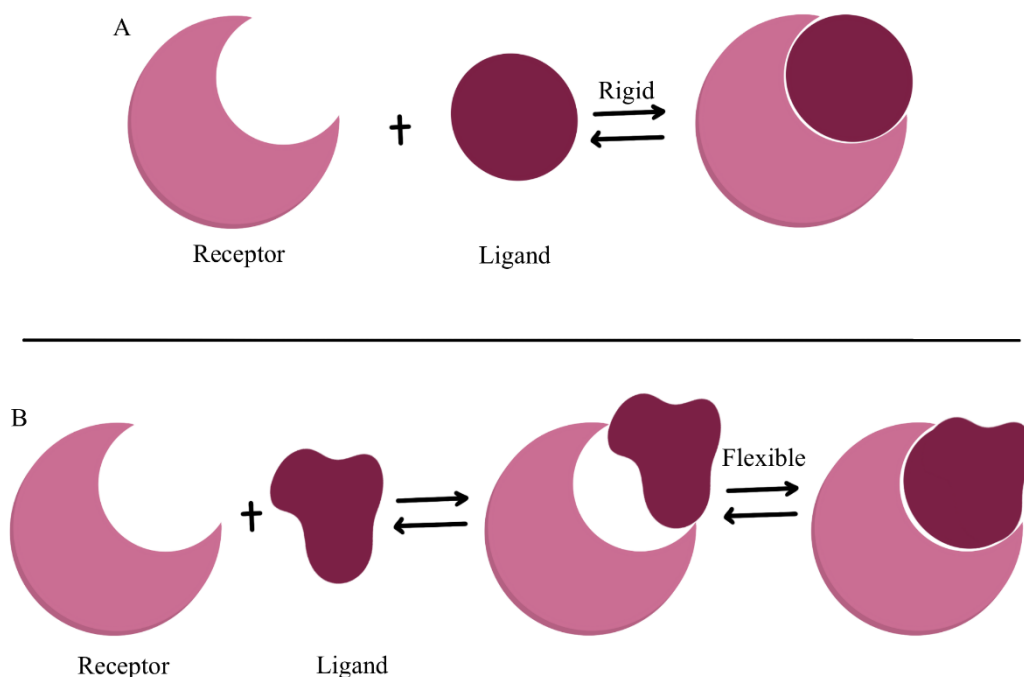


Figure 2.8: Molecular docking analogy. (A) A lock-and-key model. (B) Induced fit model

2.10.1 Computer-Aided Drug Design

Docking has become an important part of Computer-Aided Drug Design (CADD). CADD techniques are used by leading pharmaceutical companies and research groups during preliminary drug discovery studies to accelerate the development process, reduce costs, and minimize late-stage failures. By leveraging biological data to identify potential drug entities, CADD enables real-time visualization and manipulation of molecules in a virtual environment. This capability helps researchers better understand molecular interactions between target proteins and ligands, facilitating the design of novel drugs (Lai-Yuen *et al.*, 2006). Recent advancements in artificial intelligence (AI) and machine learning have further enhanced CADD by enabling the analysis of pharmaceutical big data, improving decision-making in drug discovery (Gurung *et al.*, 2021). CADD has been broadly divided into structure-based and ligand-based drug design. Both have been widely

adopted for identifying suitable lead molecules in drug discovery (Huang *et al.*, 2010). The structure-based drug design (SBDD) relies on the three-dimensional (3D) structure of the target receptor and its active sites to understand molecular interactions between the receptor and ligand. This approach typically begins with determining the binding site of the protein target and designing or optimizing ligands to interact effectively with it. Detailed structural information about the target, often obtained through experimental methods like X-ray crystallography or nuclear magnetic resonance (NMR) spectroscopy, is crucial to the success of SBDD. The process is iterative, involving cycles of screening and simulation to refine drug candidates before advancing them to clinical trials (Huang *et al.*, 2010; Bagchi, 2020; Pant *et al.*, 2022). In contrast, LBDD does not require the 3D structure of the macromolecular receptor or its ligand complex. Instead, it utilizes knowledge about known ligands that interact with the target to develop predictive models such as Quantitative Structure-Activity Relationship (QSAR) models, shape-based screening, and pharmacophore modelling. QSAR, in particular, employs statistical and analytical methods to explore the relationship between ligand structures and their biological effects (Huang *et al.*, 2010; Sharma *et al.*, 2021; Ajjarapu *et al.*, 2022). LBDD is especially effective in scenarios where the 3D structure of target is unknown, offering a systematic way to design new compounds by identifying key features of active ligands. This approach streamlines the drug development process, reduces costs, and enhances the biological activities of drug candidates (Ajjarapu *et al.*, 2022).

2.10.2 Auto Dock

Auto Dock, an open-source software suite developed by the Scripps Research Institute, is widely used for studying ligand-receptor interactions and has become a valuable tool in structure-based drug design (Goodsell & Olson, 1990). It predicts the optimal binding

pose of a ligand within the active site of a receptor by simulating various conformations and orientations. The setup and analysis of ligand-protein docking calculations using Auto Dock are facilitated by its graphical user interface, Auto Dock Tools (ADT). The Auto Dock scoring function is a subset of the AMBER force field that treats molecules using the United Atom model (Morris *et al.*, 2008). The Auto Dock scoring function, derived from a subset of the AMBER force field, utilizes the United Atom model to represent molecules (Morris *et al.*, 2008). To describe the receptor's three-dimensional structure, a grid-based approach is employed. The grid, generated using AutoGrid, calculates van der Waals and Coulombic interactions based on the AMBER force field (Huang *et al.*, 2010). Ligand preparation involves adding hydrogens, assigning charges, and introducing rotatable bonds to allow conformational flexibility. Auto Dock evaluates the binding energy of a ligand in different conformations within the receptor's binding site using its scoring function, which incorporates factors such as hydrogen bonding, hydrophobic interactions, van der Waals forces, and electrostatic interactions. The docking process is driven by optimization algorithms, with Auto Dock offering several options, including the Lamarckian Genetic Algorithm (LGA), Simulated Annealing, and Local Search Algorithm. The software generates multiple docking solutions, or "poses," ranked according to their binding energy. Researchers analyse these poses to identify the most stable configuration with the highest binding affinity. Docking studies using Auto Dock have shown strong quantitative correlations between the accuracy of docking results and the quality of the models used (Morris *et al.*, 2008; Huang *et al.*, 2010; Yuriev & Ramsland, 2013).

CHAPTER 3

MATERIALS AND METHODS

3.1 Overview

Citric acid, sodium bicarbonate, sodium metabisulfite, and potassium sorbate were purchased from DChemie Chemical Supplies, Skudai, Johor, while tartaric acid was obtained from Bischem Technology Sdn Bhd, Puncak Alam, Selangor. To prepare the preservative in effervescent granule form, sodium metabisulfite and potassium sorbate were ground into a fine powder using a blender. Then, sodium bicarbonate, citric acid, and tartaric acid were added and blended together with the initial mixture. The resulting powder was stored in an airtight container for future use. This granule preservative was tested on nipa palm sap samples collected from Kampung Pinggan Jaya and Pusa, Sarawak. For the aqueous preservative, potassium sorbate and sodium metabisulfite were dissolved in water using a double boiling method, with continuous stirring on a hot plate. Once thoroughly mixed, the solution was transferred to a conical flask and allowed to cool for 30 minutes before being stored in an airtight container. This aqueous preservative was tested only on sap samples from Kampung Pinggan Jaya.

Each experiment was conducted over three different days. On each day, 12 nipa trees were randomly selected, alongside three control sets (3 trees). Two methods were used to determine the most effective preservation technique. In the first method, the preservative was added to the sap collection vessel prior to sap tapping (pre-tapping). In the second method, the preservative was added to the collection vessel after the sap has been collected (post-tapping). There was a 12-hour interval between the preservative introduction and sap collection. The sap collection methods also varied between the two locations. At Kampung

Pinggane Jaya, a modern approach was used, where the sap was collected in 1.5-liter plastic bottles. Meanwhile, in Pusa, a traditional method was used, where sap was collected in 1.5-liter bamboo vessels.

At Kampung Pinggan Jaya, both effervescent and aqueous preservatives were tested. Acidity was measured using a pH meter, and sucrose content was determined with a refractometer. The samples were then transported to the lab for further analysis, including, reducing sugar, organic acids, antimicrobial properties and antioxidant activity. In Pusa, the effervescent granule preservative was tested, and on-site measurement of sap acidity and sucrose content were taken to compare with the data from Kampung Pinggan Jaya. A sensory analysis involving four panellist was also conducted at both locations. These on-site measurements were conducted for assessing the effectiveness of preservatives and determining the optimal timing for its application to preserve nipa sap.

3.2 Sampling area

Kampung Pinggan Jaya (Site I) and Pusa (Site II) were chosen as sampling sites for collecting nipa palm sap. Both locations, as shown in Figure 3.1 and 3.2, are known for their abundant *Nypa fruticans* palms. The selection of these locations was due to their different tapping techniques, which can affect the quality and preservation of nipa palm sap. In Pusa, traditional tapping methods were used, while Kampung Pinggan Jaya employed a slightly different technique.

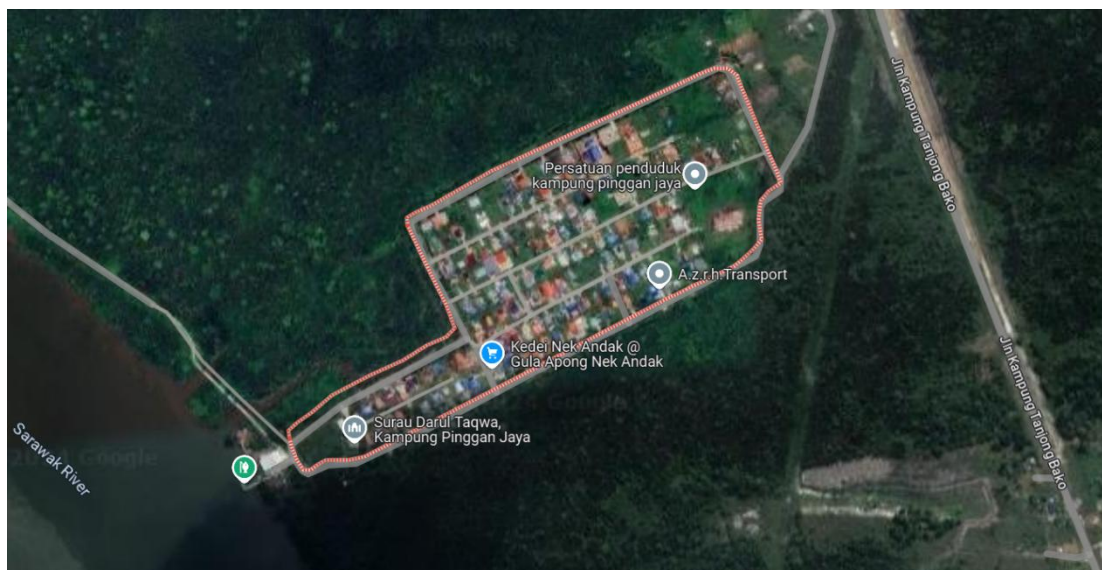


Figure 3.1: Location of Kampung Pinggan Jaya, Sarawak

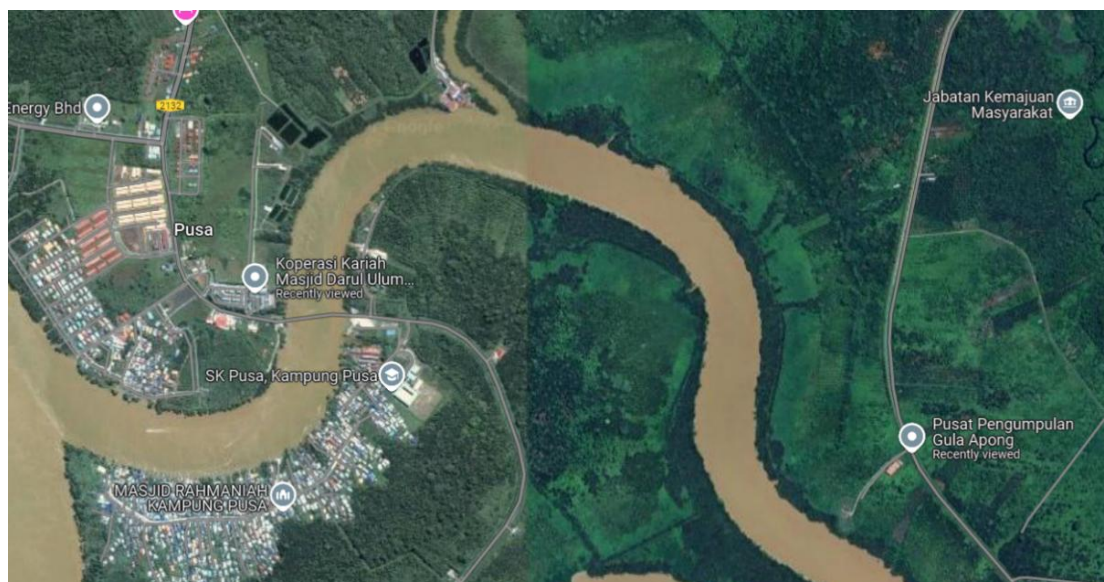


Figure 3.2: Location of Pusa, Sarawak

3.3 Preparation of preservatives

3.3.1 Effervescent granules

The formulation for preparing nipa palm sap preservative in effervescent granules involved the mixing of E' food grade preservatives – potassium sorbate (E202) and sodium metabisulfite (E223) with sodium bicarbonate, citric acid and tartaric acid that acts as the effervescent base (Grajang & Wahyuningsih, 2019; Rani *et al.*, 2021). Effervescent

technique was used so that the preservative could dissolve completely and faster in nipa palm sap. A blend of tartaric acid and citric acid was selected as the effervescent basis using either acid individually. Tartaric acid alone tends to produce chalky, granular granules, while citric acid alone results in a sticky mixture that is difficult to granulate. The formulation for nipa sap preservative in effervescent granules was shown as in Table 3.1 (Asaruddin, 2020).

Table 3.1: Composition of nipa palm sap effervescent fermentation retardant granules

Ingredients	Weight (g)
Potassium sorbate	18
Sodium metabisulfite	2
Sodium bicarbonate	68
Citric acid	24
Tartaric acid	36

Note: Patent No. PI2022006670, Asaruddin, M.R., “Fermentation retardation of *Nypa fruticans* sap”, 2020-11-25.

Different composition may affect the preservation and taste of the sap differently. The ingredients were weighed accurately according to the formula. Sodium bicarbonate as the main effervescent agent was added first into the mixer. Next, citric acid and tartaric acid which serve to provide acidity and flavour to the granules were carefully incorporated into the mixture. The potassium sorbate and sodium metabisulfite were then added to the mixture. Once all the ingredients were in the mixer, the blending process began. The mixing continued until a homogenous blend was achieved, with all ingredients evenly dispersed throughout the mixture. After the mixing process was completed, any lumps or clumps in the mixture were broken down or further blended to achieve a smooth powder. The blended mixture was transferred to an airtight packaging for further use as in Figure 3.3.



Figure 3.3: Effervescent granules nipa palm sap preservatives

3.3.2 Aqueous solution

The preparation process of the neutralizing aqueous nipa palm sap preservative is shown in Figure 3.4 below.

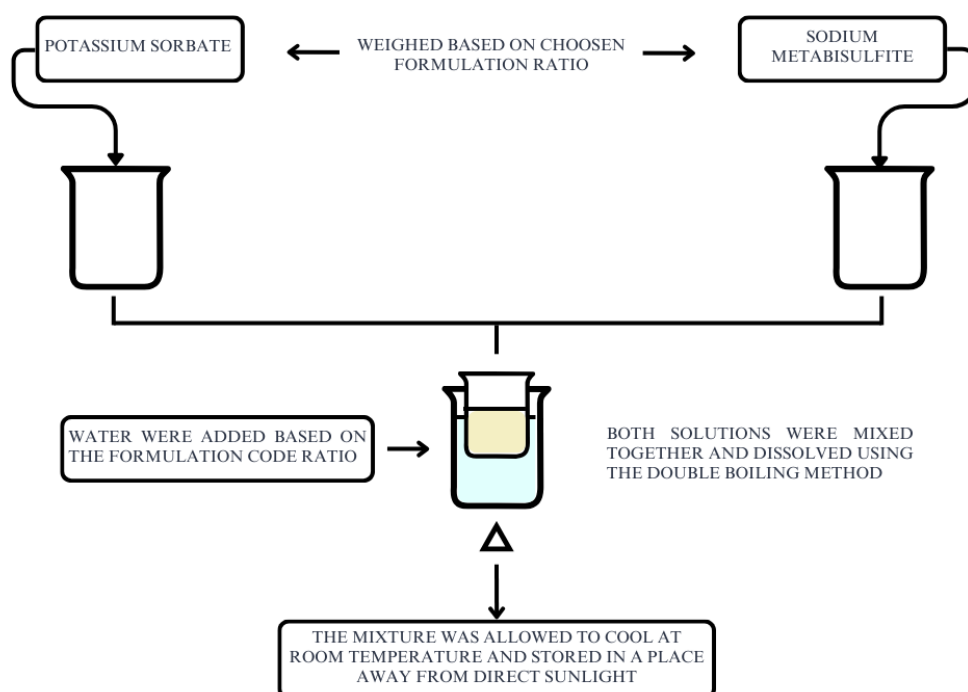


Figure 3.4: Flow diagram of preparing neutralizing aqueous nipa palm sap preservative

First, neutralizing aqueous solution for high quality or the preservation of nipa palm sap was prepared in the different stoichiometric ratios. In order to control or slow down the spontaneous fermentation in the nipa palm sap, E' food grade preservatives – potassium sorbate (E202) and sodium metabisulfite (E223) were used. In this formulation, water served to ensure both potassium sorbate and sodium metabisulfite mix thoroughly. Additionally, sorbic acid is formed when potassium sorbate dissolves in water (Berk, 2013).

Formulations F1, F2 and F3 were the code for the palm sap preservative with different stoichiometric ratios. Table 3.2 below shows the stoichiometric ratios for each formulation code, meanwhile Table 3.3 below shows the formulation for each formulation code (Abidin et al., 2023).

Table 3.2: Composition of preliminary formulation (ratio)

Formulations	Potassium sorbate	Sodium Metabisulfite	Water
F1	8	1	40
F2	16	2	40
F3	4	0.5	40

Table 3.3: Different concentrations of neutralizing aqueous solution for high quality or the preservation of nipa palm sap

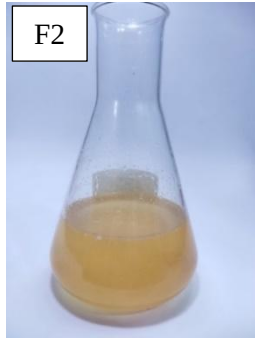
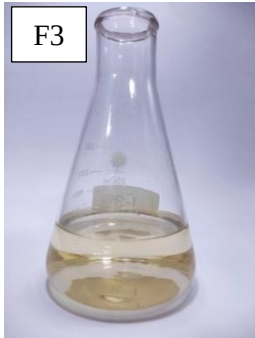
Ingredients (g)	Formulation		
	F1	F2	F3
Potassium Sorbate	15.99	27.10	8.79
Sodium Metabisulfite	2.05	3.47	1.13
Water	81.96	69.43	90.08

Note: Patent No. PI2023004076, Abidin *et al.*, “Aqua Palm Sap Preservative”, 2023-07-26.

Based on the formulation above, the total weight of the ingredients comprising the preservative amounts to 100 g. Potassium sorbate, sodium metabisulfite and water were weighed accurately with analytical balance and were mixed using a double boiling method with continuous stirring on a hot plate. The mixture was then placed in a conical flask and allowed to be cool for 30 minutes. Table 3.4 shows the characteristics of the preservative for each formulation code.

Various compositions of nipa palm sap preservatives described above could result in preservatives with varying degrees of effectiveness in preserving the sap and influencing its taste thereafter. Based on the characteristics above, F3 was preferable to be the most suitable formulation for controlling the natural fermentation of nipa palm sap in this study. It was slightly acidic; hence, it should be able to effectively inhibit the growth of spoilage microorganisms, thereby extending the shelf life of the sap and maintaining its quality for producing nipa palm sugar. The lower the pH, the greater the antimicrobial activity of the preservatives. Furthermore, the colourless appearance of F3 could minimize the risk of altering the appearance of the sap and ensuring that the nipa palm sap remains visually appealing.

Table 3.4: Characteristics of neutralizing aqueous solution for high quality or the preservation of nipa palm sap

Formulation Characteristics	F1	F2	F3
Physical Appearance			
pH	6.94	7.31	6.86
TDS (ppt)	10	10	10
Conductivity, EC (µs/cm)	20	20	20
Salinity (g/kg)	0	0	0
Bulk density (g/mL)	5.047 g / 4.6 mL = 1.097	5.053 g / 4.4 mL = 1.148	5.099 g / 5 mL = 1.0198
Tapped density (g/mL)	1.097	1.148	1.0198
Hausner's ratio	1	1	1
Preservative Cessation time (s)	1	1	1

3.4 Sample preparation

3.4.1 Effervescent fermentation retardant granules

The effect of effervescent fermentation retardant granules on the shelf-life of nipa palm sap was studied at both sites. At Site I, 10 g of nipa palm sap effervescent fermentation retardant granules was added into three separate empty plastic bottles of 1.5 litres size before tapping process and labelled as X₁, X₂ and X₃. After 12 hours of tapping, the samples were collected. An additional six samples were also collected (post-tapping sample). Of these, three samples labelled as Y₁, Y₂ and Y₃ had 10 g of effervescent granules added and were gently shaken to ensure even distribution of the preservative. The remaining three samples were served as an experimental control for comparison, labelled as Z₁, Z₂ and Z₃.

The experiment was repeated at Site II. However, the sap samples were collected using traditional bamboo vessels. Pre-tapping samples were labelled as M₁, M₂ and M₃, meanwhile the post-tapping samples were labelled as N₁, N₂ and N₃. Another three samples that did not been treated with preservative has also been collected to serve as an experimental control and labelled as O₁, O₂ and O₃.

3.4.2 Neutralizing aqueous solution preservative

The effect of neutralizing aqueous solution preservative on the shelf-life of nipa palm sap was investigated only at Site I. As before, 15 g of the preservative was added to three empty plastic bottles of 1.5 litres size, labelled as A₁, A₂ and A₃, prior to sap collection, and allowed to sit overnight for 12 hours duration. Following this overnight sap collection, six additional samples were obtained. The preservative was then added to samples labelled B₁, B₂ and B₃, with gentle shaking to ensure even distribution of the preservative throughout each sample. The remaining samples, labelled as C₁, C₂ and C₃, were left untreated to serve as experimental control.

3.5 Sensory analysis

Sensory analysis was done with a total of four untrained panellists, each evaluating one sample of nipa palm sap from different groups using a 4-point rating scale. Thus, the score “1” indicated the strong disagreement (extremely dislike), “2” implied disagreement without full rejection (dislike), “3” showed the acceptance without full appreciation (like), and “4” revealed the panellist full acceptance with enjoyment (extremely like). Parameters evaluated included colour, aroma and taste of the nipa palm sap.

3.6 Determination of pH

The pH of nipa palm sap was determined using a pH meter (on-site) after calibration with a standard buffer at room temperature. The pH reading was measured and recorded. A graph of data was generated based on its average.

3.7 Determination of total soluble solids (TTS)

The on-site sugar analysis was determined as °Brix, using a refractometer dissolved sucrose brix meter. The brix also represents the percentage of sucrose in a solution (Toledo, 2014, as cited in Abidin *et al.*, 2024). A drop of sap sample was placed on the sample zone of the refractometer, and the TTS measurement in °Brix was recorded. The average °Brix from each sample group was calculated, and a graph has been plotted. The correlation between °Brix and sucrose content is presented as follows (Elewa *et al.*, 2020, as cited in Abidin *et al.*, 2024):

$$1\text{ }^{\circ}\text{Brix} = \frac{1\text{ g of sucrose}}{100\text{ g of solution}}$$

3.8 Determination of reducing sugar contents

3,5-dinitrosalicylic acid (DNS) method was used to determine the reducing sugar contents in nipa palm sap, using the method by Madigal *et al.* (2020) with a slight

modification. In this experiment, one sample was selected from each group for analysis. A series of standard solution for both glucose and fructose were prepared at concentrations of 0, 0.2, 0.4, 0.6, 0.8 and 1.0 (1 mg/mL). 1 mL of each sap sample was placed in separate test tube and then mixed with 2.5 mL of DNS reagent and heated for 5 minutes. After heating, the samples were allowed to cool at room temperature. Absorbance was measured at 540 nm using a UV-VIS spectrophotometer. Each sample was subjected to three independent measurements, the mean was calculated, and the results were expressed in g/L.

3.9 Determination of organic acids

High-performance liquid chromatography (HPLC) was performed to analyse the organic acids (lactic and acetic acid) content in nipa sap after 24 hours of storage. One sample was selected from each group for analysis. According to Jaraee *et al.* (2023), a 0.005 M of sulfuric acid (H_2SO_4) was used as the mobile phase with 0.8 mL/min flow rate at column temperature of 60°C. The analysis was done in triplicate.

3.10 Antioxidant activity

The antioxidant activity of nipa palm sap has been measured using the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay method with a slight modification (Haryanti *et al.*, 2023). 4 mg of DPPH was weighed and transferred into 100 mL volumetric flask. Then, it was diluted with methanol up to 100 mL in a volumetric flask. The solution was then wrapped with aluminium foil and kept in cool, dark place. Following that, 7.5 mL of 0.1 mM DPPH•methanol solution was then transferred into a test tube containing 2.5 mL of nipa palm sap sample and vigorously shaken. The blank solution was prepared in the same manner but with methanol, instead of the sap sample. The solution was incubated at room temperature for 30 minutes. The absorbance of each sample was measured three times using UV-VIS spectrophotometer at 517 nm. The DPPH activity was then calculated against the

blank solution of reagent at 517 nm. The following equation was used to evaluate the antioxidant activity as a percentage of radical scavenging activity (RSA):

$$RSA (\%) = \frac{A_o - A_s}{A_o} \times 100\%$$

where:

A_o = absorbance of the blank

A_s = absorbance of the sample at 515 nm after 30 minutes

3.11 Antimicrobial activity

To study the antimicrobial activity of nipa palm sap, the total bacterial count (TBC) method with a slight modification was used (Jaraee *et al.*, 2023). This plates agar count method allowed for the comparison of microbial growth between untreated (control) and treated nipa sap samples (both pre-tapping and post-tapping). A sample was selected from each group for analysis, and the standard spread plate method was done for surface plating. A 7 g of nutrient agar was weighed and transferred into a Duran bottle containing 300 mL of distilled water. The bottle was swirled to mix the content and then the media was subjected to the autoclave at 121°C and 15lbs for 15 minutes. After autoclaving, the hot media was poured into sterile petri plates, and it was allowed to cool and solidify for about 10 minutes. After the agar becomes solid, it was stored in the incubator at 37°C to avoid any contamination. Bend glass rod was sterilised using ethanol (70%) and burned using Bunsen burner. A 0.85% w/v of saline solution was used as a buffer by weighing 8.5 g of sodium chloride and dissolving it in 1 litre of distilled water. 9 mL of buffer solution was transferred into six falcon tubes and the diluent was subjected to autoclave at 121°C and 15lbs for 15 minutes. 1 mL of pre-tapping sap sample was serially diluted with saline solution to prepare a 1:10 dilution (10⁻¹) and another appropriate dilution (10⁻², 10⁻³, 10⁻⁴, 10⁻⁵ and 10⁻⁶) was also prepared. By using micropipettes, 0.1 mL of each dilution was poured and spread plated on

respective agar plates. Each dilution was plated in triplicate. The agars were incubated at 37°C for 24 hours. After that, the colonies in incubated plates were counted. Two additional experiments were conducted by replacing the pre-tapping sap sample with post-tapping sap and control samples, to compare the antimicrobial activity across different treatments. The results were compared to the antimicrobial potential of virgin coconut oil (VCO) and amoxicillin in controlling microbial growth in nipa palm sap.

3.12 Statistical analysis

The data were analysed using mean $\pm$ standard deviation (SD) and/or one-way analysis of variance (ANOVA). A significant difference exists when the F-count value is greater than the F-table. When $p < 0.05$, differences were considered significant.

3.13 Molecular docking

The molecular docking process as described by Li *et al.* (2023) was used with slight modifications. The three-dimensional structure of potassium sorbate and its active form (sorbic acid), along with lauric acid and the reference drugs (amoxicillin, ibuprofen and aspirin) were obtained from PubChem in SDF format and converted to PDB format using Open Babel software (Figure 3.5). The ligand was further processed in Auto Dock Tools (Figure 3.6), where its rotatable bonds were identified. The prepared ligand file was then saved in PDBQT format for docking. The protein structure of glucose oxidase was downloaded from the Protein Data Bank (PDB) with the accession code 3QVR and saved in PDB format. The protein structure was prepared for docking using the Auto Dock Tools platform through a series of pre-processing steps.

First, all water molecules and non-protein components, such as inhibitors, were removed to eliminate interference during docking. Polar hydrogen atoms were added to optimize potential hydrogen bonding interactions, followed by the addition of Kollman

charges. The processed protein was then saved in PDBQT format. Then, a grid box was defined around the active site of glucose oxidase to confine the docking search space. The size and coordinates of the grid box were adjusted to encompass all potential binding sites while maintaining computational efficiency. For the molecular docking process, the Lamarckian Genetic Algorithm (LGA), together with the grid-based energy evaluation method, was chosen. This approach ensured efficient and accurate exploration of potential binding poses. The best docking results were generated in DLG format, which was further analysed and visualized using Auto Dock.

Molecular docking was then performed using the Auto Dock platform, generating 10 distinct binding poses for the potassium sorbate-glucose oxidase complex. From the 10 docking results, the binding pose with the lowest binding energy or highest binding affinity was selected for further conformational and interaction analysis, and the best ligand-protein complex was saved in PDBQT format. For docking analysis, the selected docking result was converted back to PDB format using Open Bable. The Protein-Ligand Interaction Profiler (PLIP) (<https://plip-tool.biotec.tu-dresden.de/plip-web/plip/index>) was used to analyse and clearly visualize the binding position of the ligand with the protein and its residues in 3D view.

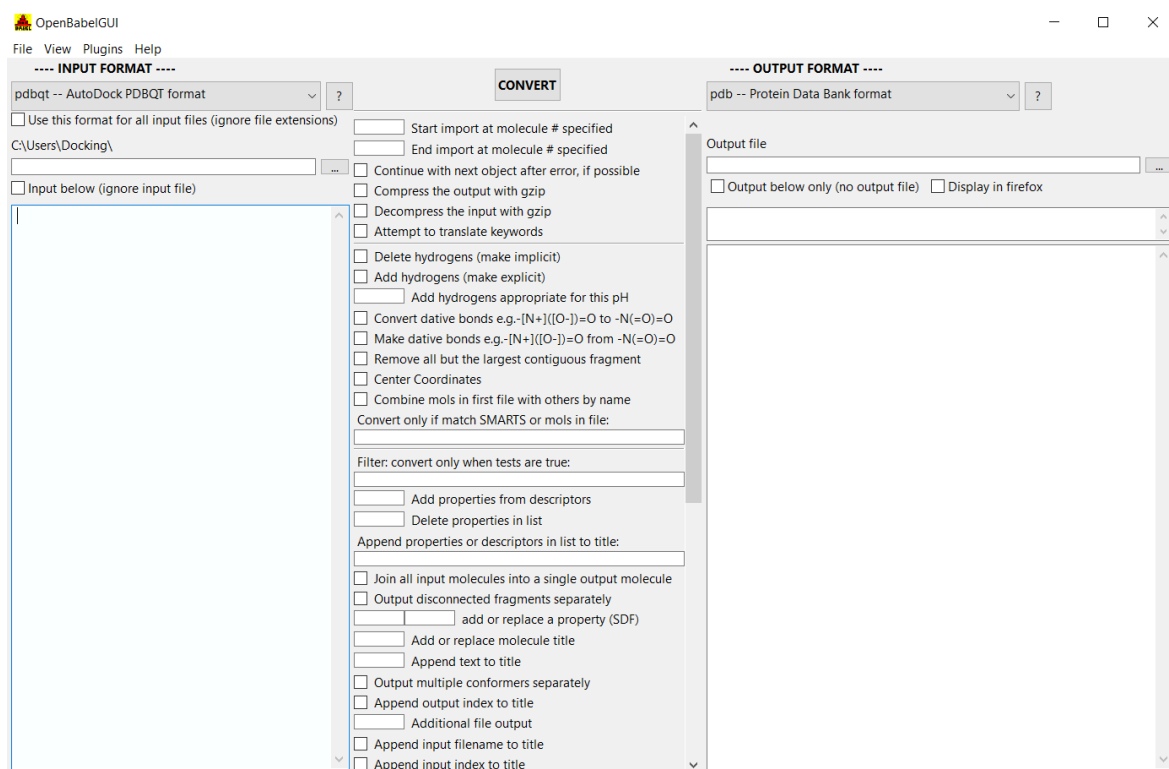


Figure 3.5: Open Babel software programme

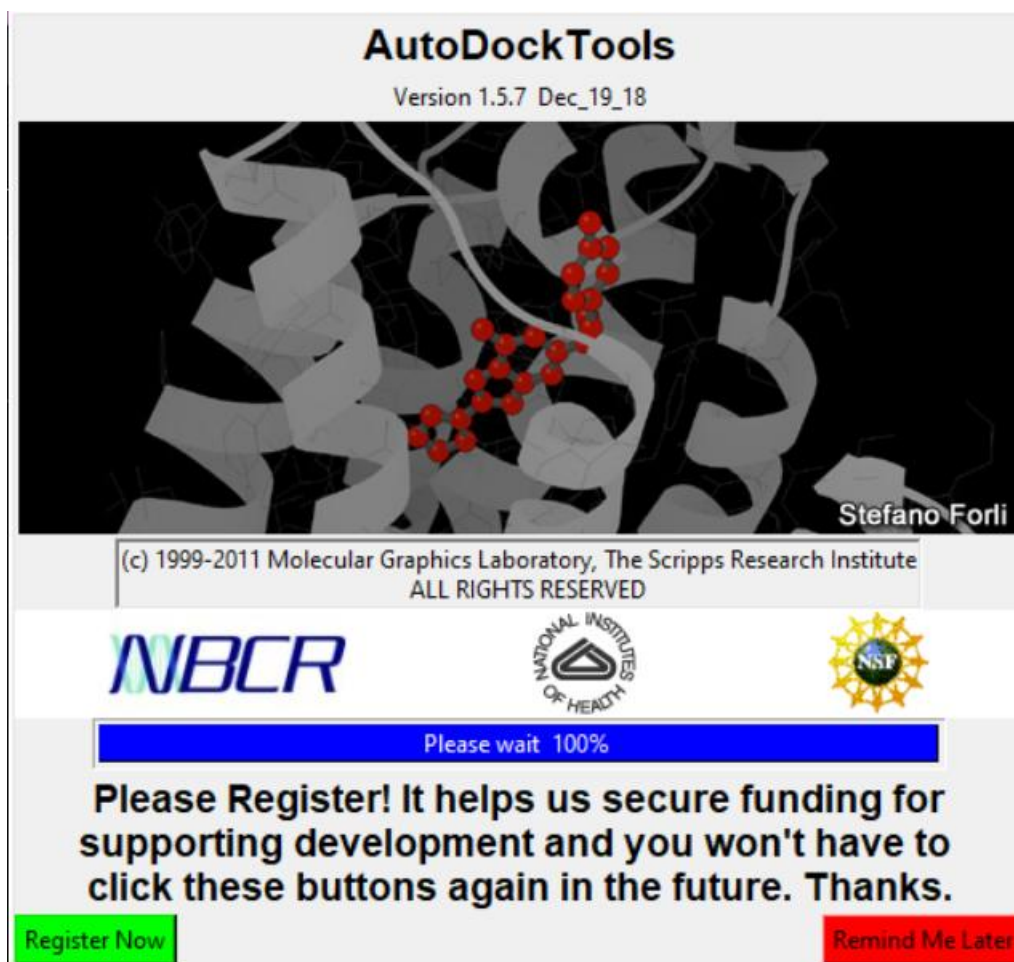


Figure 3.6: Auto Dock Tools software programme.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Overview

4.1.1 Sensory evaluation

According to Hebbar *et al.* (2018), the stage of fermentation of nipa palm sap can be determined by its appearance and flavour. Natural fermentation of nipa palm sap causes lactic acid bacteria (LAB) and acetic acid bacteria (AAB) to produce organic acids which affect the quality of nipa palm sap. Thus, sensory evaluation was done to see how the use of preservatives might impact the physical characteristics of nipa palm sap. Panellists were asked to rate the parameter of the sap when the sap samples were first collected and after 2 hours of storage using scores from 1 until 4. Table 4.1 shows the results of sensory evaluation of nipa palm sap with preservatives (sample A, B, M, N, X and Y) and without preservatives (control Z, O and C) after 12 hours of storage.

Table 4.1: Result of sensory evaluation

Sample	Colour		Odour		Taste	
	0 hour	12 hours	0 hour	12 hours	0 hour	12 hours
Control Z	3.75 ^a	1.42 ^b	3.67 ^a	1.75 ^b	3.83 ^a	1.75 ^b
Sample X	3.75 ^a	3.50 ^a	3.67 ^a	3.58 ^a	3.75 ^a	3.58 ^a
Sample Y	3.75 ^a	3.50 ^a	3.67 ^a	3.58 ^a	3.75 ^a	3.58 ^a
Control O	3.67 ^a	1.25 ^b	3.33 ^a	1.25 ^b	3.67 ^a	1.17 ^b
Sample M	3.67 ^a	2.75 ^b	3.42 ^a	2.00 ^b	3.67 ^a	2.50 ^b

Table 4.1 continued

Sample N	3.67 ^a	2.50 ^b	3.42 ^a	2.00 ^b	3.67 ^a	2.50 ^b
Control C	3.75 ^a	1.42 ^b	3.75 ^a	1.75 ^b	3.92 ^a	1.50 ^b
Sample A	3.83 ^a	3.67 ^a	3.75 ^a	3.67 ^a	3.75 ^a	3.67 ^a
Sample B	3.75 ^a	3.67 ^a	3.75 ^a	3.67 ^a	3.92 ^a	3.75 ^a

Note: Means with different letters in the same column are significantly different ($p < 0.05$) based on the Fisher test. Sample Z, O, and C serve as control samples for their respective locations. Samples X and M were treated with effervescent granules preservative before the tapping process, while Samples Y and N received the same preservative after sap collection. Meanwhile, Sample A was treated with a neutralizing aqueous preservative before tapping, and Sample B was treated with the same preservative after collection.

4.1.1.1 Colour

The colour of the sap was used to analyse the effect of preservatives on the physical characteristic of nipa palm sap. According to Nur Aimi (2023), the yellowish colour of the nipa palm sap indicates a good sugar quality and the freshness of the sap. Upon collection, the highest acceptance score for colour parameter was found in Sample A (3.83) as can be seen in Table 4.1. However, it is not significant difference ($p > 0.05$) compared to other samples. This may have happened because the panellist had trouble distinguishing the colour between each sample. Each sample showed a similar appearance which is yellowish in colour.

Despite that, the samples collected using bamboo vessels (Sample M and N), were rated the least favourable in terms of colour, with a score of 3.67. Therefore, the study suggests that the choice of collection vessel can significantly influence the physical characteristics of the sap. The contact between the sap and bamboo walls may cause a slight darkening of the sap, especially if the vessels are reused or not properly cleaned (Abidin *et*

al., 2024). When the sap samples were collected in bamboo vessel, the unhygienic bamboo vessels might alter its appearance. The yellowish colour of fresh nipa palm sap may shift to a slightly duller, making it less appealing to the panellists.

After 12 hours, significant changes in the colour of both treated and untreated nipa palm sap samples were observed. The control or untreated samples collected using plastic bottles, labelled as Control Z and Control C, received a colour score of 1.42. Meanwhile, the control sample collected using bamboo vessel, Control O, received the lowest score (1.25). All of the control samples changed into cloudy white which indicates that the fermentation took place after 12 hours.

In contrast, the treated samples showed a better colour retention. Sample A and B which were collected using plastic bottles and treated with an aqueous preservative solution, maintained the highest score of 3.67 after 12 hours. Similarly, Sample X and Y, treated with powder preservative, retained a slightly lower but still favourable score of 3.50. The slight difference in effectiveness between the aqueous and powder forms of preservative in maintaining the fresh colour of nipa palm sap could be due to uneven distribution of powder preservative in the sap samples compared to the aqueous preservative solution.

Overall, the results are aligned with that of Victor and Orsat (2018), Azis (2021) as well as Jaraee *et al.* (2023), who found that the *Nypa fruticans* sap would turn cloudy or creamy white at low pH, meanwhile a fresh sap would appear yellowish and translucent at higher pH levels. According to previous studies, sulphur dioxide that possessed antioxidant property was released when sodium metabisulfite reacts with liquid. It helps to inhibit the polyphenol oxidase that catalyse the oxidation of phenolic compounds, resulting in brown pigments (Davidson *et al.*, 2005; Tan *et al.*, 2015). Therefore, the addition of sodium

metabisulfite in the sap sample helps maintain its fresh appearance, which is crucial for consumer acceptance.

4.1.1.2 Odour

Table 4.1 showed that the addition of preservatives had no significant effect ($p>0.05$) on the aroma of nipa palm sap during the initial storage period (0-hour). A strong aroma of palm sap can still be detected by the panellists even with the addition of preservatives. The scores were slightly differing from each other which might be due to its sap concentration. The sap concentration of each palm is different as it might be influenced by its tree age. Yanto and Handayani (2021) stated that a sap with a higher concentration would produce a stronger aroma. However, panellists describing that the sap samples collected using bamboo vessels – Sample M and N, has a little smoky odour. The smoke odour in nipa palm sap collected using bamboo vessels likely results from the traditional practice of smoking the inside of the bamboo containers. This method is often used to dry and disinfect the bamboo to prevent microbial contamination, which can spoil the sap (Myint, 2011). The smoke particles and compounds from the charring process can adhere to the bamboo's surface and subsequently be transferred to the sap, imparting a smoky odour.

After 12 hours, the highest acceptance score for odour parameter was found in Sample A and B (both score 3.67). It was not significantly different ($p>0.05$) compared to other treated samples, but it was significantly different ($p<0.05$) compared to the untreated samples. The data showed that the natural fermentation in nipa palm sap was slowed down by the preservatives. Panellists found that a fermented nipa palm sap produced a quite strong unpleasant smell. Fermenting microbes secrete various of enzymes into the food matrix which then would produce unpleasant and malty aroma. The activity of LAB and AAB in the nipa palm sap can produce volatile organic compounds (VOCs) such as alcohols, fatty

acids, aldehydes, ketones and organic compounds which contribute to the aroma of fermented nipa palm sap during the fermentation process (Smid & Kleerebezem, 2014; Behera *et al.*, 2020).

However, fermented nipa palm sap is also popular in some tropical regions of Asia, Africa and South America as it becomes alcohol after a few hours of tapping. They are known locally as palm wine. Meanwhile, traditional palm vinegar can be produced by AAB fermenting the alcohol beverage further (Nguyen *et al.*, 2016; Jaraee *et al.*, 2023; Oluwole *et al.*, 2023).

4.1.1.3 Taste

After 12 hours of storage period, Sample B received the highest acceptance score for taste parameter, but it was not significantly different ($p > 0.05$) from Sample A, X and Y. This highlights that both preservatives are effective in preserving the natural sweetness of the sap. The preservatives cause the environment to be less favourable for microbial activity and growth. On the other hand, Sample M and N, collected in bamboo vessels showed a significant taste degradation ($p < 0.05$) as indicated by the lower taste scores compared to other treated samples. As stated by Abidin *et al.* (2024), bamboo vessels allowed more exposure to air, insects and microbes which accelerating fermentation and causing a faster deterioration of sugary taste of nipa palm sap.

The results also showed that the use of preservatives had a significant effect ($p < 0.05$) on the sugary taste of nipa palm sap after 12 hours of storage. Panellists found that the untreated samples became sour compared to when they were first collected. Without preservatives to inhibit microbial growth, the sucrose in the sap would convert to alcohol and acids, resulting in a sour or fermented taste. Jaraee *et al.* (2023) stated that nipa palm sap is susceptible to microbial contamination due to its high sucrose content. The microbial

activity would produce various organic acids as well as ethanol that metabolised away the pleasant sugary taste of the fresh nipa palm sap in a short period of time, increasing its acidity and giving it a sour taste (Nur Aimi *et al.*, 2013; Hebbar *et al.*, 2018; Flores-Gallegos *et al.*, 2019; Jaraee *et al.*, 2023;). Hence, it influences the acceptability of fermented nipa palm sap. The finding agrees with the result of Mcfeeters (2004), who observed that the interaction between the active enzymes in the raw material and the metabolic activities of microorganisms causing the changes in taste during fermentation. Moreover, nipa palm sap contains various sugars, phenolic compounds and other organic molecules that are susceptible to oxidation that can lead to off-flavours. The antioxidant properties of sodium metabisulfite scavenges ROS and other free radicals, which prevents them from initiating oxidative reactions that would spoil the sap (Davidson *et al.*, 2005). By inhibiting these oxidative reactions, sodium metabisulfite helps maintain natural flavour of the sap. In addition to its antioxidant properties, sodium metabisulfite also has antimicrobial effects. It inhibits the growth of bacteria, yeasts, molds, and other microorganisms that could ferment the sugars in the sap, leading to spoilage (Grotheer *et al.*, 2005; Ough & Were, 2005; Noorafshan *et al.*, 2013). In this study, sodium metabisulfite complemented potassium sorbate that has a limited antioxidant property. When used together, they preserve the sensory value of the sap and ensures that nipa palm sap remains a high-quality product throughout its storage and distribution.

In a previous study by Levi and Oruchec (1957), sulphite, propionic acid and benzoate were used to preserve the palm sap. Iwuagwu and Izuagbe (1985) also able to preserve the oyokpo beer using the combinations of sodium benzoate and pasteurization. But their attempt resulted in a change of taste due to the activity of fermenting microbes. In contrast, the data in this study showed that the preservatives used had no significant effect

on the flavour characteristics of the nipa palm sap as potassium sorbate and sodium metabisulfite were tasteless and odourless since they were used in small quantities.

4.2 Chemical characteristics of *Nypa fruticans* sap during fermentation

4.2.1 The effect of preservatives on the acidity of *Nypa fruticans*

This study investigated the impact of effervescent granules and a neutralizing aqueous solution on the acidity and shelf-life of *Nypa fruticans* sap. Yeast, LAB and AAB play the most important roles in the natural fermentation of nipa palm sap. These microorganisms were known to be essential for the growth of bacteria, thus the presence of these elements in nipa palm sap may aid in the fermentation process (Huzir *et al.*, 2023; Jaraee *et al.*, 2023).

The pH of fresh coconut sap should be approximately 7, but the average pH was lower in this study. This may be due to the fact that the nipa palm sap was collected after harvesting for 12 hours, during which the sap most likely underwent partial fermentation (Adisetya & Krisdiarto, 2022). However, the results showed that the E' food grade preservatives used were effective against the bacteria and able to extend the shelf-life of nipa palm sap.

4.2.1.1 Effervescent granules

a. Sap samples collected at Site I

All samples were collected 12 hours after harvesting. Sample X were gently shaken upon collection to ensure uniform distribution of the added effervescent. Preservatives were added to Sample Y immediately after they were collected, and it was also shaken distribute them evenly. The pH levels of nipa palm sap samples were monitored over a 24-hour storage period. Table 4.2 and Figure 4.1 show the effects of using effervescent granules to control

the acidity of nipa palm sap at Kampung Pinggan Jaya during pre-tapping (Sample X) and post-tapping (Sample Y) collection.

Table 4.2: pH levels of nipa palm sap collected from Site I and treated with effervescent granules preservatives

Sample pH	Control Z				Sample X				Sample Y			
	1st	2nd	3rd	$\bar{x}$	1st	2nd	3rd	$\bar{x}$	1st	2nd	3rd	$\bar{x}$
0	5.66	5.58	5.58	5.61	6.10	5.58	5.53	5.74	6.09	5.61	5.51	5.74
1	5.52	5.43	5.41	5.45	5.91	5.32	5.52	5.58	5.89	5.53	5.32	5.58
2	5.40	5.32	5.30	5.34	5.70	5.34	5.34	5.46	5.66	5.26	5.10	5.34
3	5.15	5.10	5.04	5.10	5.65	5.23	5.21	5.36	5.61	5.21	4.98	5.27
4	4.40	4.36	4.34	4.37	5.61	5.12	5.10	5.28	5.57	5.14	4.87	5.19
5	4.27	4.02	3.96	4.08	5.52	5.08	5.07	5.22	5.49	5.10	4.81	5.13
6	3.93	3.74	3.76	3.81	5.45	5.08	5.07	5.20	5.42	5.03	4.76	5.07
7	3.64	3.62	3.64	3.63	5.38	5.02	4.97	5.12	5.34	4.96	4.73	5.01
8	3.58	3.56	3.58	3.57	5.31	4.96	4.94	5.07	5.31	4.93	4.66	4.97
9	3.52	3.54	3.54	3.53	5.29	4.96	4.85	5.03	5.25	4.87	4.62	4.91
10	3.49	3.45	3.45	3.46	5.23	4.92	4.83	4.99	5.21	4.87	4.58	4.89
11	3.46	3.42	3.42	3.43	5.17	4.90	4.80	4.96	5.13	4.82	4.58	4.84
12	3.40	3.37	3.36	3.38	5.11	4.86	4.77	4.91	5.07	4.81	4.54	4.81

Table 4.2 continued

13	3.35	3.34	3.32	3.38	5.04	4.84	4.72	4.87	4.93	4.75	4.51	4.73
14	3.31	3.27	3.27	3.28	4.97	4.81	4.69	4.82	4.86	4.70	4.51	4.69
15	3.26	3.24	3.22	3.24	4.97	4.76	4.65	4.79	4.82	4.64	4.47	4.64
16	3.24	3.21	3.17	3.21	4.94	4.71	4.62	4.76	4.75	4.59	4.41	4.58
17	3.21	3.19	3.14	3.18	4.91	4.71	4.62	4.75	4.72	4.53	4.36	4.54
18	3.18	3.14	3.12	3.15	4.85	4.66	4.59	4.70	4.70	4.47	4.33	4.50
19	3.13	3.11	3.07	3.10	4.83	4.64	4.59	4.69	4.66	4.44	4.25	4.45
20	3.11	3.05	2.97	3.04	4.78	4.63	4.53	4.65	4.62	4.42	4.19	4.41
21	3.07	2.96	2.91	2.95	4.78	4.60	4.49	4.62	4.58	4.42	4.16	4.39
22	3.05	2.94	2.87	2.95	4.76	4.58	4.45	4.60	4.58	4.38	4.06	4.34
23	2.97	2.88	2.85	2.90	4.75	4.58	4.42	4.58	4.57	4.34	4.04	4.32
24	2.91	2.87	2.83	2.87	4.74	4.58	4.40	4.58	4.55	4.30	3.98	4.28

Note: $\bar{x}$ represents the mean of pH value, calculated as the average of all measured pH readings. Control Z serves as control sample. Sample X was treated with preservative before tapping, while Sample Y was treated with preservative after sap collection.

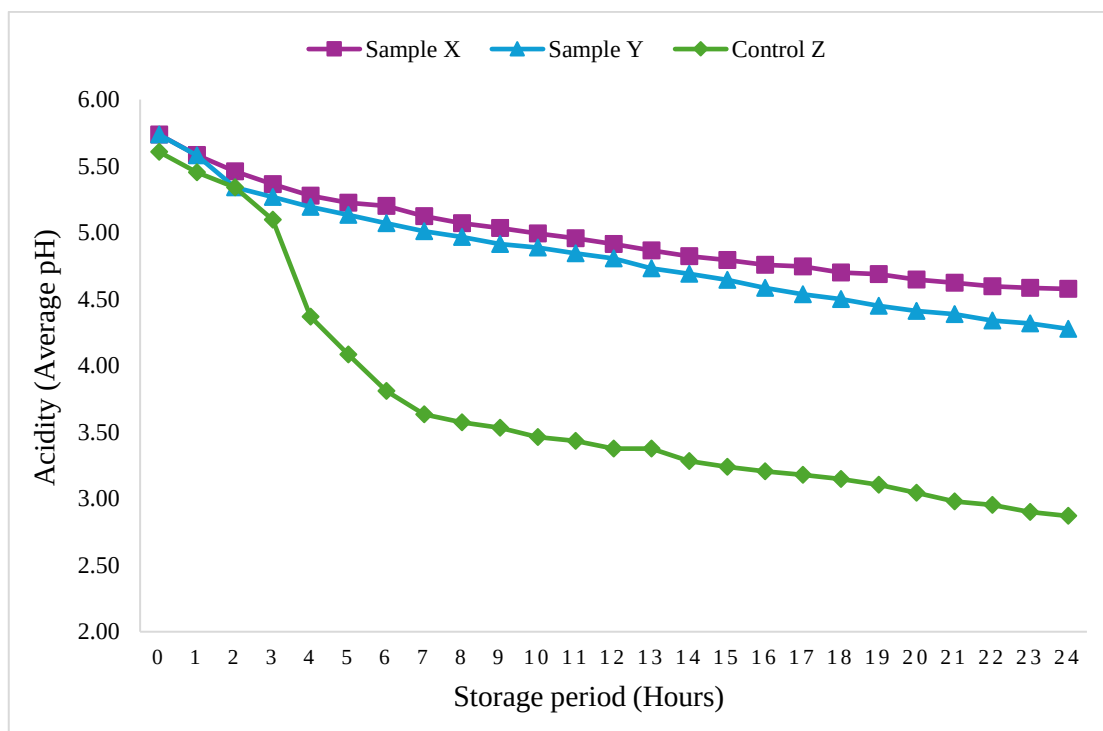


Figure 4.1: Changes of acidity in control, sample X and sample Y of nipa palm sap during 24 hours of storage

Both treated samples, despite timings of preservative application, initially had the same pH of 5.74. This might be due to variations in sap composition from different nipa palm trees, which may result in similar starting acidity even with different preservative application timings. In contrast, the control sample, which had no preservative added, showed a slightly lower pH of 5.61 ± 0.05 at the beginning of the experiment. Over time, differences in pH between the treated samples became more apparent. After 2 hours, the pH of Sample Y had decreased to 5.34 ± 0.29 , while Sample X showed a slightly less pH decrease (5.46 ± 0.21). This indicates that the pre-tapping treatment of Sample X was more effective in slowing the pH decline, likely due to better inhibition of microbial activity. After 8 hours of storage, pH of Sample X remained more stable at 5.07 ± 0.21 , while Sample Y which was treated with preservatives after the sap was tapped and collected, had a lower pH of 4.97 ± 0.33 .

By the 12-hour mark, the pH values of both treated samples continued to decrease with Sample X still showed a higher pH (4.91 ± 0.18) compared to Sample Y (4.81 ± 0.27). The earlier application in Sample X continued to provide better control over microbial growth as this trend persisted through the 24-hour storage period, with final pH values of 4.58 ± 0.18 for Sample X and 4.28 ± 0.29 for Sample Y. Additionally, the difference in pH highlights the impact of preservative application before tapping may initially buffer the acidity of the sap more effectively than adding it after the nipa sap was collected. Adding preservatives before tapping may have inhibited microbial growth more effectively from the start. In contrast, when preservatives were added after sap collection, the sap had already been exposed to microbial activity for several hours, leading to faster acidification (Samarajeewa *et al.*, 1985; Karseno & Yanto, 2019). This could have resulted in a slower decrease in pH of Sample X compared to Sample Y. The more significant decrease in Sample Y suggests that microbial fermentation was more aggressive in this sample due to the delay in applying preservatives.

On the other hand, the pH of control sample drops from 5.61 ± 0.05 to 3.37 ± 0.04 by the end of the storage period as this sample did not receive any preservative treatment. This rapid acidification is consistent with the natural fermentation of nipa palm sap when left untreated. Without preservatives to inhibit the microbial growth, the sugars in the sap are rapidly fermented and producing organic acids that drastically reduce the pH (Naknean, 2013; Jaraee *et al.*, 2023).

As shown in Table 4.3, the use of effervescent granules had a significant effect on the acidity of nipa palm sap as the F count was larger (30.09701) than the F-table. The addition of effervescent granules stabilizes the nipa palm sap, thus slowing the natural acidification that would occur due to microbial fermentation. The effervescent granules

released the active ingredients which effectively inhibited microbial growth and slowed the fermentation process.

Table 4.3: Analysis of pH variance for samples collected at Site I, and treated with effervescent granules and its control

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	22.35865	2	11.17932	30.09701	3.16E-10	3.123907
Within Groups	26.7439	72	0.371443			
Total	49.10255	74				

b. Samples collected at Site II

The tapping methods in Pusa differ from those in Kampung Pinggan Jaya, as the locals still use the traditional techniques by collecting the sap in bamboo vessels. Table 4.4 and Figure 4.2 show the changes in pH of nipa palm sap harvested from Pusa over 24 hours for three samples. Sample M, where the preservative was added before the sap was tapped (pre-tap); Sample N, where the preservative was added after 12 hours of harvesting (post-tap); and a control, where no preservative was added. The comparison between these samples highlights the role of preservatives in delaying the natural process of fermentation, and how timing plays a critical role in their effectiveness.

In the control sample, where no preservative was applied, the pH drops rapidly from 4.63 ± 0.06 at the start to 2.03 ± 0.19 at the 24-hour mark. This sharp decline in pH indicates an aggressive fermentation process, where microorganisms break down sucrose and other sugars in the sap, converting them into organic acids like lactic and acetic acid. The acid

buildup in turn accelerates the decrease in pH, signifying that the sap is becoming more acidic and less stable for storage or processing (Naknean, 2013; Jaraee *et al.*, 2023). This is a typical outcome in untreated sap, where the absence of any microbial inhibitor leads to rapid spoilage.

Table 4.4: pH levels of nipa palm sap collected from Site II and treated with effervescent granules preservatives

Sample pH	Control O				Sample M				Sample N			
	1st	2nd	3rd	$\bar{x}$	1st	2nd	3rd	$\bar{x}$	1st	2nd	3rd	$\bar{x}$
0	4.56	4.67	4.67	4.63	4.91	4.86	5.02	4.93	4.63	4.69	4.83	4.72
1	4.11	4.27	4.22	4.20	4.91	4.86	5.02	4.93	4.50	4.61	4.83	4.65
2	3.42	3.56	3.53	3.50	4.83	4.76	4.86	4.82	4.49	4.54	4.81	4.61
3	3.11	3.51	3.47	3.36	4.76	4.65	4.75	4.72	4.35	4.32	4.78	4.48
4	3.03	3.47	3.38	3.29	4.74	4.57	4.69	4.67	4.24	4.20	4.76	4.40
5	2.95	3.47	3.30	3.24	4.69	4.53	4.63	4.62	4.10	4.06	4.51	4.22
6	2.85	3.38	3.20	3.14	4.40	4.51	4.57	4.49	4.03	3.90	4.47	4.13
7	2.78	3.33	3.17	3.09	4.51	4.47	4.49	4.49	3.81	3.76	4.44	4.00
8	2.68	3.28	3.11	3.02	4.00	4.44	4.44	4.29	3.71	3.69	4.34	3.91
9	2.61	3.20	3.11	2.97	3.91	4.38	4.40	4.23	3.63	3.57	4.26	3.82
10	2.54	3.16	3.10	2.93	3.91	4.35	4.36	4.21	3.57	3.48	4.17	3.74
11	2.47	3.03	2.97	2.82	3.87	4.27	4.28	4.14	3.51	3.43	4.03	3.66

Table 4.4 continued

12	2.43	2.93	2.87	2.74	3.87	4.09	4.11	4.02	3.48	3.43	3.58	3.50
13	2.38	2.81	2.76	2.65	3.82	4.09	4.11	4.01	3.46	3.43	3.56	3.48
14	2.31	2.76	2.70	2.59	3.78	4.04	4.07	3.96	3.42	3.40	3.56	3.46
15	2.27	2.73	2.68	2.56	3.71	3.97	4.07	3.92	3.40	3.38	3.55	3.44
16	2.23	2.62	2.58	2.48	3.68	3.91	3.93	3.84	3.39	3.38	3.52	3.43
17	2.14	2.57	2.51	2.41	3.68	3.85	3.87	3.80	3.39	3.36	3.52	3.42
18	2.07	2.51	2.46	2.35	3.65	3.81	3.85	3.77	3.35	3.36	3.48	3.40
19	2.03	2.46	2.40	2.30	3.61	3.74	3.76	3.70	3.34	3.34	3.46	3.38
20	2.03	2.40	2.34	2.26	3.57	3.69	3.74	3.67	3.33	3.31	3.46	3.37
21	1.93	2.35	2.26	2.18	3.56	3.69	3.71	3.65	3.31	3.31	3.44	3.35
22	1.90	2.29	2.21	2.13	3.55	3.65	3.68	3.63	3.31	3.29	3.41	3.34
23	1.84	2.21	2.17	2.07	3.53	3.60	3.68	3.60	3.30	3.29	3.38	3.32
24	1.81	2.16	2.11	2.03	3.53	3.57	3.68	3.59	3.30	3.29	3.34	3.31

Note: $\bar{x}$ represents the mean of pH value, calculated as the average of all measured pH readings. Control O serves as control sample. Sample M was treated with preservative before tapping, while Sample N was treated with preservative after sap collection.

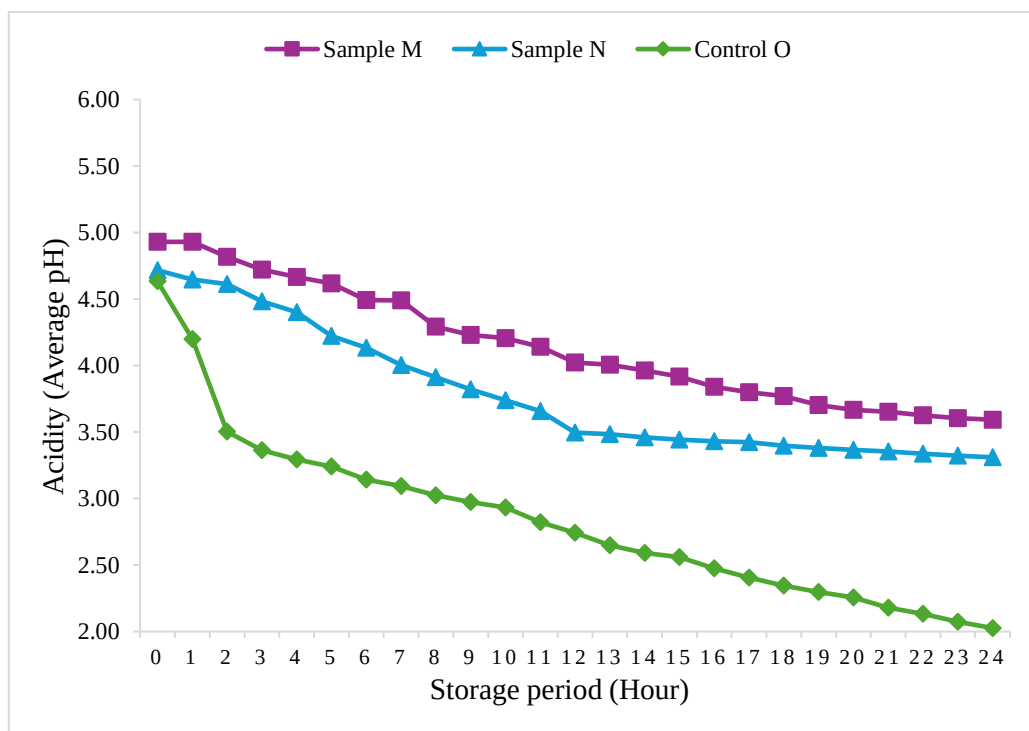


Figure 4.2: Changes of acidity in control, sample M and sample N of nipa palm sap during 24 hours of storage

In contrast, Sample M, where the preservative was added before the sap was collected shows a much slower decrease in pH. Starting at a pH of 4.93 ± 0.08 , the acidity of the sap decreases gradually, reaching 3.59 ± 0.08 after 24 hours. This indicates that introducing the preservative early, before the sap is exposed to the environment, is highly effective in controlling microbial growth and fermentation, even when traditional bamboo vessels are used. By inhibiting the microorganisms from the outset, the preservatives significantly slow the breakdown of sugars into acids, preserving the quality of the sap for a longer period (Samarajeewa *et al.*, 1985; Karseno & Yanto, 2019).

Sample N, where the preservative was introduced 12 hours after sap collection falls between the control and Sample M in terms of pH behaviour. The pH drops from 4.72 ± 0.10 to 3.31 ± 0.03 over the 24-hour period. Even though the preservatives were eventually introduced to Sample N, microbial activity and enzymatic reactions would have already started. The delayed introduction of preservative causing the sap to be exposed to

fermentation for a considerable time before the preservative was applied. During those 12 hours, microorganisms had already begun to ferment the sugars, producing acids and lowering the pH (Naknean, 2013; Jaraee *et al.*, 2023).

Bamboo vessels are prone to introducing microbes (Abidin *et al.*, 2024), hence without immediate preservative treatment, the sap begins fermenting shortly after collection. The preservatives act as microbial inhibitors, preventing the early breakdown of sugars and subsequent acid buildup. As shown in Table 4.5, the use of effervescent granules had a significant effect ($p < 0.05$) on the acidity of nipa palm sap as the F count was larger (40.58597) than the F-table. Therefore, although the preservative slows further fermentation after it is added, the acidity of the sap is already higher than in Sample M, where the preservative was added into the empty bamboo vessels before tapping the sap.

Table 4.5: Analysis of pH variance for samples collected at Site II, and treated with effervescent granules and its control

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	22.82452	2	11.41226	40.58597	1.58E-12	3.123907
Within Groups	2.024549	72	0.281187			
Total	43.07001	74				

4.2.1.2 Neutralizing aqueous solution

The aqueous preservatives appear to be more effective than the effervescent granules at maintaining a higher pH, particularly in Sample A, where the pH remained relatively stable even after 24 hours. This suggest that the neutralizing aqueous solution may have a stronger buffering effect to prevent rapid acidification.

As shown in Table 4.6 and Figure 4.3, Sample A which treated with preservatives before sap collection, had the highest initial pH of was 6.33 ± 0.29 . This indicates that early preservative application successfully delayed the fermentation and inhibited microbial activity from the very beginning. In contrast, Sample B and control sample had a lower initial pH of 5.52 ± 0.03 and 5.52 ± 0.27 , respectively, indicating that fermentation had already begun. The delay in applying preservative to Sample B, along with the absence of preservatives in the control, exposed the sap to environmental contaminants such as dust, insects and dirt during the collection process. These factors contributed to the introduction of microbes, which initiated fermentation (Hebbar *et al.*, 2015).

Over the first hour, Sample A showed no change in pH, remaining stable due to the early application of preservatives. The preservatives effectively prevented microbial activity in the sap from the moment it begins flowing, allowing the sap to maintain its initial pH. On the other hand, Sample B and control sample showed a slight decrease in pH to 5.41 ± 0.02 and 5.25 ± 0.26 , respectively. The pH drops in Sample B might be due to the rapid shift in pH as the preservative began to inhibit microbial activity and stabilize the pH, resulting in a more stable pH level during the remaining storage period.

By the 12-hour mark, Sample A maintained a relatively high pH of 5.57 ± 0.04 . This aligns with the finding by previous researchers, who demonstrate the importance of early preservative application in controlling microbial growth (Samarajeewa *et al.*, 1985; Karseno & Yanto, 2019). In contrast, Sample B showed a pH of 5.17 ± 0.02 at the same period. The control sample had the most significant pH drop ($p < 0.05$), reaching 3.26 ± 0.14 by the 12-hour mark. This sharp decline in pH indicates rapid fermentation as organic acids were produced by microorganism, further lowering the pH and accelerating spoilage (Jaraee *et al.*, 2023).

By the end of the 24-hour storage period, the pH levels of all samples decreased significantly, indicating ongoing fermentation and acid production by the microorganisms present in the nipa palm sap (Naknean, 2013). The control sample continued to undergo rapid fermentation, with its pH dropping to 2.87 ± 0.17 . Sample A had the most stable pH decrease, reaching 5.48 ± 0.02 , while Sample B dropped to 5.10 ± 0.03 . The higher pH levels in both treated samples indicate that the preservatives were effective in controlling microbial activity and slowing fermentation process. The timing of preservative application also significantly affects the effectiveness of controlling the acidity of the sap as applying preservative before sap collection provide a better defence against fermentation compared to applying preservative after sap collection, though it still offers protection compared to untreated samples. Without preservatives, fermentation proceeds rapidly, leading to sap spoilage within 24 hours.

Table 4.6: pH levels of nipa palm sap collected from Site I and treated with neutralizing aqueous preservatives

Sample pH	Control C				Sample A				Sample B			
	1st	2nd	3rd	$\bar{x}$	1st	2nd	3rd	$\bar{x}$	1st	2nd	3rd	$\bar{x}$
0	5.67	5.67	5.21	5.52	6.42	6.56	6.01	6.33	5.67	5.67	5.61	5.65
1	5.42	5.38	4.95	5.25	6.42	6.56	6.01	6.33	5.40	5.40	5.44	5.41
2	5.15	5.11	4.87	5.04	6.38	6.48	5.97	6.28	5.40	5.43	5.35	5.39
3	4.42	4.38	4.21	4.34	6.27	6.33	5.93	6.18	5.40	5.43	5.35	5.39
4	4.11	4.07	4.00	4.06	6.16	6.20	5.88	6.08	5.41	5.42	5.31	5.38
5	3.81	3.57	3.47	3.62	6.01	6.01	5.88	5.97	5.39	5.41	5.30	5.37

Table 4.6 continued

6	3.77	3.51	3.41	3.56	5.88	5.88	5.74	5.83	5.38	5.38	5.28	5.35
7	3.71	3.47	3.34	3.51	5.81	5.76	5.69	5.75	5.35	5.35	5.26	5.32
8	3.65	3.42	3.30	3.46	5.76	5.71	5.63	5.70	5.32	5.32	5.25	5.30
9	3.58	3.36	3.27	3.40	5.71	5.63	5.62	5.65	5.25	5.25	5.20	5.23
10	3.53	3.33	3.22	3.36	5.71	5.62	5.62	5.65	5.21	5.20	5.17	5.19
11	3.47	3.25	3.17	3.30	5.62	5.57	5.60	5.60	5.20	5.18	5.16	5.18
12	3.41	3.21	3.15	3.26	5.57	5.53	5.60	5.57	5.19	5.17	5.15	5.17
13	3.35	3.17	3.15	3.22	5.55	5.52	5.57	5.55	5.19	5.17	5.15	5.17
14	3.33	3.15	3.11	3.20	5.54	5.52	5.55	5.54	5.18	5.17	5.14	5.16
15	3.31	3.13	3.10	3.18	5.54	5.52	5.55	5.54	5.17	5.16	5.12	5.15
16	3.27	3.11	3.10	3.16	5.54	5.51	5.54	5.54	5.17	5.15	5.11	5.14
17	3.27	3.11	3.05	3.14	5.53	5.51	5.54	5.53	5.16	5.15	5.10	5.14
18	3.24	3.08	3.03	3.12	5.53	5.50	5.53	5.53	5.15	5.14	5.10	5.13
19	3.21	3.03	2.97	3.07	5.52	5.50	5.53	5.52	5.15	5.13	5.09	5.12
20	3.14	2.94	2.91	3.00	5.52	5.49	5.51	5.52	5.14	5.12	5.08	5.11
21	3.14	2.91	2.84	2.96	5.52	5.48	5.50	5.51	5.13	5.11	5.08	5.11
22	3.11	2.86	2.80	2.92	5.51	5.48	5.49	5.50	5.13	5.10	5.07	5.10
23	3.11	2.81	2.77	2.90	5.51	5.47	5.48	5.49	5.12	5.10	5.07	5.10
24	3.06	2.81	2.74	2.87	5.50	5.46	5.47	5.48	5.12	5.10	5.07	5.10

Note: $\bar{x}$ represents the mean of pH value, calculated as the average of all measured pH readings. Control C serves as control sample. Sample A was treated with preservative before tapping, while Sample B was treated with preservative after sap collection.

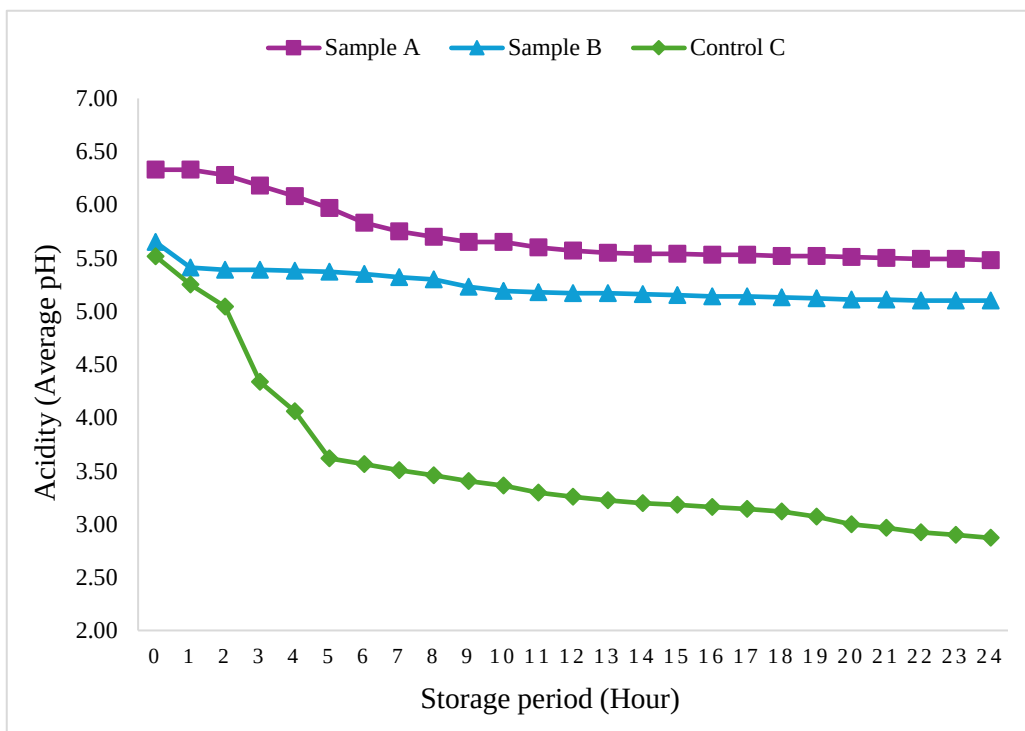


Figure 4.3: Changes of acidity in control, sample A and sample B of nipa palm sap during 24 hours of storage

The analysis of variance (Table 4.7) showed that the F-count value (152.7128) was greater than the F-table value (3.123907). Thus, the use of preservatives significantly affects ($p < 0.05$) the pH levels and shelf-life of nipa palm sap. Neutralizing aqueous solution as palm sap's preservative was able to prevent the pH of nipa palm sap from dropping too quickly while it was being stored. The antimicrobial effects of these preservatives inhibited microbial activity, thereby reducing the rate of fermentation.

Table 4.7: Analysis of pH variance for samples collected at Site I, and treated with neutralizing aqueous solution and its control

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	65.84434	2	32.92217	152.7128	1.25E-26	3.123907
Within Groups	15.52193	72	0.215582			
Total	81.36627	74				

4.2.1.3 Comparison between effervescent granules and neutralizing solution in controlling acidity of nipa palm sap

Table 4.8 and Figure 4.4 show the comparison of acidity (pH) of nipa palm sap in Sample X (treated with effervescent granules) and Sample A (treated with an aqueous neutralizing solution) over a 24-hour storage period. Both samples were tapped at the same location and treated with preservatives before tapping process, but the form of the preservatives differed.

Table 4.8: Comparison between effervescent granules and neutralizing solution in controlling acidity of nipa palm sap

Sample pH	Sample X				Sample A			
	1st	2nd	3rd	$\bar{x}$	1st	2nd	3rd	$\bar{x}$
0	6.10	5.58	5.53	5.74	6.42	6.56	6.01	6.33
1	5.91	5.32	5.52	5.58	6.42	6.56	6.01	6.33
2	5.70	5.34	5.34	5.46	6.38	6.48	5.97	6.28
3	5.65	5.23	5.21	5.36	6.27	6.33	5.93	6.18

Table 4.8 continued

4	5.61	5.12	5.10	5.28	6.16	6.20	5.88	6.08
5	5.52	5.08	5.07	5.22	6.01	6.01	5.88	5.97
6	5.45	5.08	5.07	5.20	5.88	5.88	5.74	5.83
7	5.38	5.02	4.97	5.12	5.81	5.76	5.69	5.75
8	5.31	4.96	4.94	5.07	5.76	5.71	5.63	5.70
9	5.29	4.96	4.85	5.03	5.71	5.63	5.62	5.65
10	5.23	4.92	4.83	4.99	5.71	5.62	5.62	5.65
11	5.17	4.90	4.80	4.96	5.62	5.57	5.60	5.60
12	5.11	4.86	4.77	4.91	5.57	5.53	5.60	5.57
13	5.04	4.84	4.72	4.87	5.55	5.52	5.57	5.55
14	4.97	4.81	4.69	4.82	5.54	5.52	5.55	5.54
15	4.97	4.76	4.65	4.79	5.54	5.52	5.55	5.54
16	4.94	4.71	4.62	4.76	5.54	5.51	5.54	5.54
17	4.91	4.71	4.62	4.75	5.53	5.51	5.54	5.53
18	4.85	4.66	4.59	4.70	5.53	5.50	5.53	5.53
19	4.83	4.64	4.59	4.69	5.52	5.50	5.53	5.52
20	4.78	4.63	4.53	4.65	5.52	5.49	5.51	5.52
21	4.78	4.60	4.49	4.62	5.52	5.48	5.50	5.51
22	4.76	4.58	4.45	4.60	5.51	5.48	5.49	5.50

Table 4.8 continued

23	4.75	4.58	4.42	4.58	5.51	5.47	5.48	5.49
24	4.74	4.58	4.40	4.58	5.50	5.46	5.47	5.48

Note: $\bar{x}$ represents the mean of pH value, calculated as the average of all measured pH readings. Both samples were collected at Site I and treated with preservative before the tapping process. Sample X was treated with effervescent granules preservative, while Sample A was treated with neutralizing aqueous preservative.

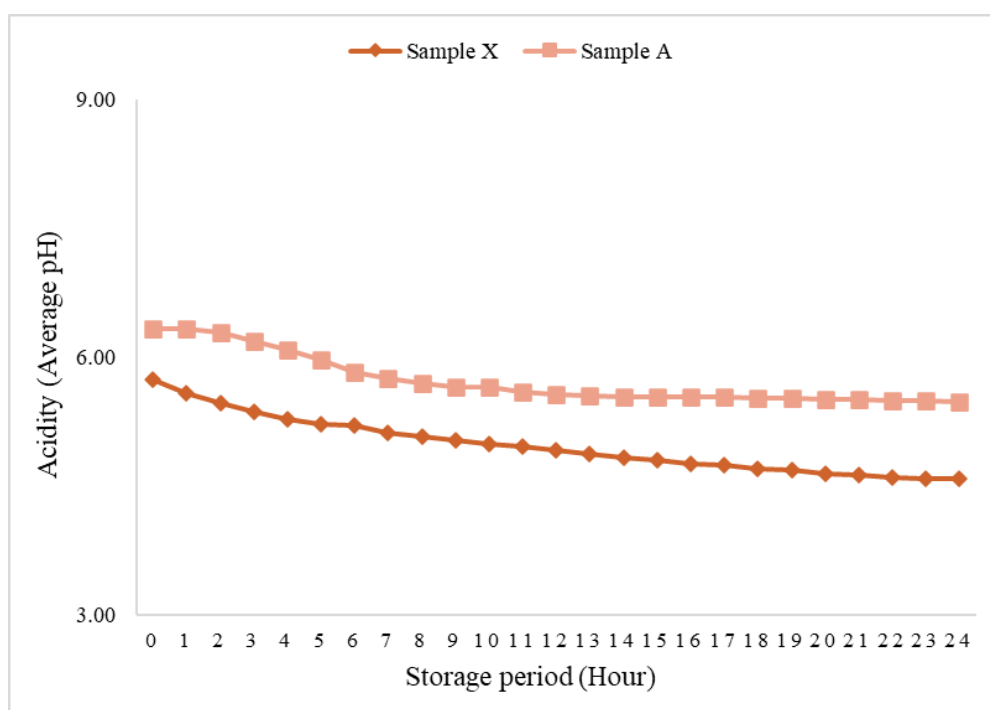


Figure 4.4: Comparison between effervescent granules (Sample X) and neutralizing aqueous solution (Sample A) in controlling acidity of nipa palm sap

At the beginning of storage period, Sample A exhibited a higher pH (6.33 ± 0.29) compared to Sample X, which had a pH of about 5.74 ± 0.32 . This difference in initial pH could be attributed to the form of preservative used. The study suggests that the liquid preservative in Sample A might have provided a more immediate and uniform distribution within the sap, leading to higher initial neutralization of acidity. Effervescent granules, which release their active ingredients through a reaction with liquid (sap) may not have

dissolved and dispersed as rapidly as liquid preservative. This could have caused the initial pH in Sample X to be slightly lower, as the neutralizing effect took longer to fully integrate into the sap.

Both Sample X and Sample A showed a gradual decrease in pH over time, indicating increasing acidity as the storage period progressed. However, the rate of pH decline in Sample A was slower compared to Sample X. After 12 hours, Sample X had a pH of 4.91 ± 0.18 , while Sample A maintained a higher pH of 5.57 ± 0.04 . These results highlight the efficacy of the liquid preservative in controlling microbial growth and delaying acidification, which is important for preserving the quality of the sap during storage.

The final pH values recorded after 24 hours further highlight the advantages of using a liquid preservative. The pH of Sample A remained at 5.48 ± 0.02 , meanwhile pH of Sample X dropped to 4.58 ± 0.18 . This difference in pH stability suggests that the liquid preservatives have more consistent and immediate effects as they are fully dissolved upon application. It also suggests that the effervescent granules were less effective in maintaining the pH stability of the nipa palm sap. The more rapid acidification in Sample X could be attributed to the slower release and uneven distribution of the granules within the sap, leading to a delayed neutralizing effect. Besides, the delayed effect of the granules might allow microbial activity to proceed for a longer period before the preservative takes full effect, further accelerating the acidification or fermentation process.

Overall, the final pH values of nipa palm sap samples treated with neutralizing aqueous solution showed a lesser pH reduction – Sample A (from 6.33 ± 0.29 to 5.48 ± 0.02) compared to the nipa palm sap samples treated with effervescent granules – Sample X (5.74 ± 0.32 to 4.58 ± 0.18). The pH reduction of Sample A observed in this study was higher compared to previous studies that introduced the combination of nisin with pasteurization

(Naknean, 2013) and calcium hydroxide with pasteurization (Sarma *et al.*, 2021). Our approach focused on preserving the nipa palm sap without any heat treatment nor proper packaging. This difference highlights the efficacy of our preservative method in maintaining sap quality solely through the combination of E' food-grade preservatives (potassium sorbate and sodium metabisulfite), without relying on pasteurization to inhibit microbial activity and acid formation.

4.2.2 The effect of preservatives on sucrose content of *Nypa fruticans* sap

There are various types of damage that can affect the shelf-life and quality of nipa palm sap, but it is generally considered damaged when its sucrose content is inverted into reducing sugars, with glucose and fructose present in the same ratio. The damage of sap influenced by factors such as excessive temperature, imbalance pH (either too low or high) and its microbial activity (Sardjono, 1988 as cited in Putra *et al.*, 2019). Preservatives extend the shelf-life of nipa palm sap by inhibiting the microbial growth which reduces the rate of sugar being metabolized into acids and alcohol, thereby helping to maintain the sugar concentration of the nipa palm sap for a longer period. In addition, preservatives can also suppress the enzymatic activities that degrade sugars. In the presence of preservatives, hydrolysis of sucrose into glucose and fructose became less active which further contributes to maintaining the sugar content during storage period (Hebbar *et al.*, 2018; Lantemona *et al.*, 2023). After harvesting, natural enzymatic processes and microbial activity can lead to the fermentation of sugars, resulting in a decrease in sugar concentration. This fermentation process not only alters the taste and sweetness of the sap but also reduces its nutritional value over time (Hebbar *et al.*, 2018).

4.2.2.1 Effervescent granules

a. Samples collected at Site I

Table 4.9 and the stacked line graph (Figure 4.5) show changes in sucrose content for nipa palm sap samples collected from Kampung Pinggan Jaya over a 24-hour storage period. The data indicate a gradual decline in sucrose levels as time progresses, suggesting that natural enzymatic activity and microbial fermentation are breaking down sucrose into simpler sugars.

Table 4.9: Sugar level (%Brix) of nipa palm sap collected from Site I and treated with effervescent granules

Sample °Brix	Control Z				Sample X				Sample Y			
	1st	2nd	3rd	$\bar{x}$	1st	2nd	3rd	$\bar{x}$	1st	2nd	3rd	$\bar{x}$
0	15.2	15.0	14.8	15.0	15.0	14.8	14.6	14.8	15.2	15.0	14.8	15.0
1	15.1	15.0	14.8	15.0	15.0	14.8	14.6	14.8	15.1	15.0	14.8	15.0
2	14.7	14.6	14.6	14.6	14.8	14.6	14.4	14.6	15.0	14.8	14.6	14.8
3	14.3	13.9	13.9	14.0	14.8	14.6	14.3	14.6	14.8	14.6	14.4	14.6
4	14.0	13.9	13.6	13.8	14.2	14.0	13.9	14.0	14.8	14.6	14.4	14.6
5	13.9	13.8	13.6	13.8	14.2	14.0	13.9	14.0	14.6	14.3	13.8	14.2
6	13.6	13.5	13.2	13.4	14.0	13.8	13.6	13.8	14.0	13.9	13.6	13.8
7	13.6	13.3	13.4	13.4	13.8	13.6	13.4	13.6	14.0	13.9	13.6	13.8
8	13.3	12.8	12.8	13.0	13.8	13.6	13.4	13.6	13.8	13.6	13.4	13.6
9	13.3	12.8	12.8	13.0	13.3	13.2	13.0	13.2	13.8	13.6	13.4	13.6
10	12.9	12.6	12.2	12.6	13.3	13.2	13.0	13.2	13.6	13.3	13.4	13.1

Table 4.9 continued

11	12.6	12.5	12.0	12.4	12.2	12.0	11.8	12.0	12.8	12.4	12.0	12.4
12	11.1	11.0	10.8	11.0	12.1	12.0	11.8	12.0	12.6	12.2	11.8	12.2
13	11.1	11.0	10.8	11.0	12.1	12.0	11.8	12.0	12.6	12.1	11.8	12.2
14	11.1	11.0	10.8	11.0	12.0	12.0	11.7	11.9	12.5	12.1	11.7	12.1
15	11.0	11.0	10.7	10.9	12.0	11.9	11.7	11.9	12.5	12.0	11.7	12.1
16	10.9	10.8	10.7	10.8	11.8	11.8	11.6	11.7	12.4	12.0	11.7	12.0
17	10.8	10.7	10.6	10.7	11.8	11.8	11.4	11.7	12.4	11.9	11.6	12.0
18	10.7	10.6	10.5	10.6	11.7	11.7	11.4	11.6	12.3	11.9	11.6	11.9
19	10.6	10.5	10.4	10.5	11.7	11.6	11.3	11.5	12.3	11.8	11.5	11.9
20	10.5	10.5	10.4	10.5	11.6	11.5	11.2	11.4	12.2	11.8	11.4	11.8
21	10.4	10.4	10.3	10.4	11.6	11.4	11.1	11.4	12.2	11.7	11.3	11.7
22	10.3	10.4	10.2	10.3	11.5	11.4	11.0	11.3	12.1	11.6	11.2	11.6
23	10.3	10.3	10.1	10.2	11.5	11.3	10.8	11.2	12.0	11.6	11.2	11.6
24	10.1	10.3	10.0	10.1	11.5	11.3	10.7	11.2	12.0	11.6	11.2	11.6

Note: $\bar{x}$ represents the mean of Brix value, calculated as the average of all measured Brix readings. Control Z serves as control sample. Sample X was treated with preservative before tapping, while Sample Y was treated with preservative after sap collection.

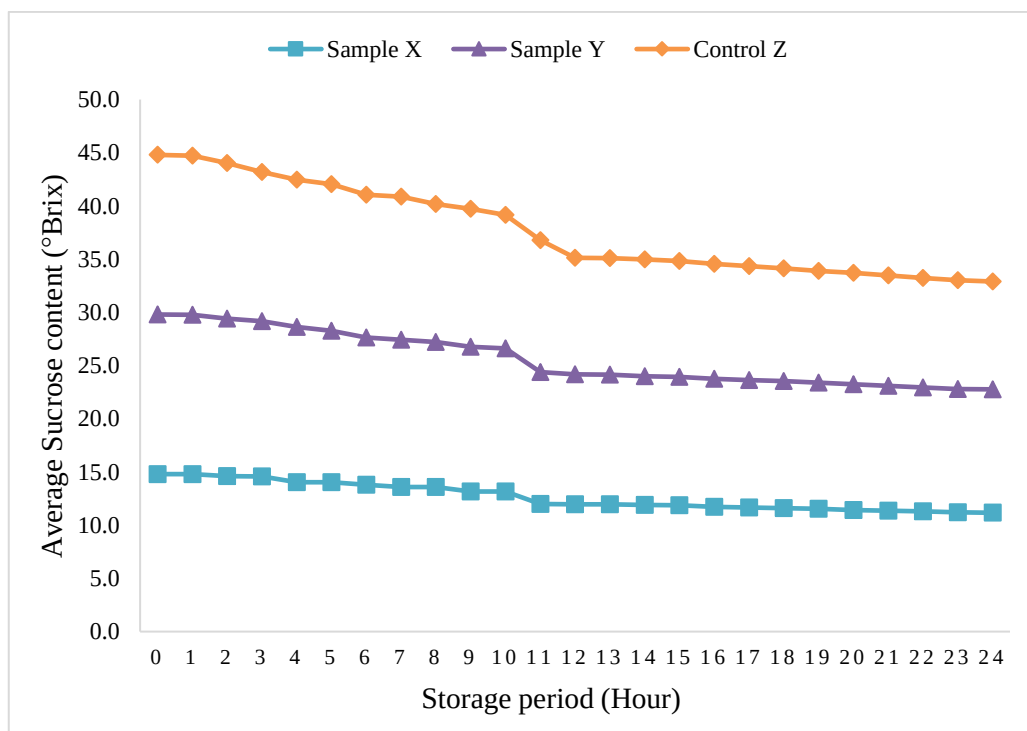


Figure 4.5: Sucrose degradation in samples treated with effervescent granules at Site I

Based on Figure 4.5, it was observed that fresh *Nypa fruticans* sap was initially rich in sucrose content and then decreased due to enzymatic reduction. The control sample with no preservative added begins with the highest initial sucrose content (45.0 ± 0.2 °Brix). However, over the 24-hour storage period, it exhibits the most decline in sucrose content. In the first few hours, the decline is relatively slow, but after 12 hours, a steep drop is observed, with the sucrose content eventually falling to around 35.0 ± 0.2 °Brix at the end of storage. This rapid decline in sucrose content in the control sample is due to the natural fermentation process. The sugars in the sap are broken down by microbial activity. Without the presence of preservatives to inhibit the microbial growth, fermentation accelerates. This makes the sap unsuitable for further sugar production.

Sample X which was treated with preservative before the sap was tapped, begins with a lower initial sucrose content (15.0 ± 0.2 °Brix). This initial difference suggests that the preservative may altered the sap composition. However, Sample X showed a slower and

gradual decline in sucrose content compared to the control sample. By the end of the storage period, the sucrose content in Sample X remains at approximately 10.0 ± 0.4 °Brix. Although the preservative does not completely prevent the loss of sugar, it significantly slows the degradation process. This indicates that early treatment of the sap with preservative provides an effective barrier against microbial and fermentation. According to Dehghan *et al.* (2018), potassium sorbate is not entirely stable, especially in the presence of moisture. Therefore, the study suggested that when the effervescent granules was added to the sap's collection vessel, potassium sorbate may have begun to oxidize. This oxidation may reduce the ability of effervescent granules preservative to inhibit microbial and enzymatic activity in nipa palm sap over time, leading to a gradual increase in microbial and enzyme activities in Sample X, resulting in a reduction in sucrose content.

Sample Y, treated with preservative after the sap was collected, begins with an initial sucrose content of 30.0 ± 0.2 °Brix. Like Sample X, Sample Y also experiences a gradual decline in sucrose content over the 24-hour storage period, however its rate of decline is slightly faster than Sample X. By the end of the storage period, the sucrose content in Sample Y falls to approximately 15.0 ± 0.4 °Brix. This final value is higher than the control sample but lower than Sample X, suggesting that while preservatives applied after collection are effective at slowing down sucrose degradation even though they are not as effective as preservatives applied prior to tapping. This difference in efficacy may be due to the preservative taking longer to penetrate the sap after it has already been exposed to microbial activity.

The faster rate of sucrose degradation in Sample Y compared to Sample X implies that applying preservatives after collection allows some initial microbial activity to occur, potentially leading to a more rapid breakdown of sucrose. Method of applying the

preservatives before tapping the sap offers a more controlled fermentation process, reducing the risk of spoilage and maintaining the quality of sap for a longer duration, hence extending its shelf-life.

Sucrose degradation in the treated samples may have been slower than the control sample, but their analysis of variance (Table 4.10) shows that there was no significant difference ($p>0.05$) in the final sucrose content between treated and untreated samples. This suggests that the preservatives used (sodium metabisulfite and potassium sorbate) did not significantly impact sucrose content of the nipa palm sap during storage. The data also implies that the microbial activity and enzymatic activities were not substantially inhibited by the preservatives. The potential oxidation of preservatives during storage likely reduced their effectiveness. This aligns with the hypothesis that potassium sorbate may have been oxidised, diminishing its antimicrobial and enzymatic inhibition properties (Lück. 1990; Dehghan *et al.*, 2018). Besides, the method of delivering the preservative in effervescent granules might not have provided uniform distribution. It required dissolution through the effervescent reaction, which might take times to properly dissolve and become effective.

Table 4.10: Analysis of sucrose content for samples treated with effervescent granules collected in plastic bottle

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	9.902341	2	4.95117	2.464469	0.092202	3.123907
Within Groups	144.6495	72	2.009021			
Total	154.5519	74				

b. Samples collected at Site II

As shown in Table 4.11 and Figure 4.6, the control sample showed a rapid decline in sucrose (from 14.6 ± 0.4 °Brix to 6.1 ± 0.1 °Brix) over time due to the absence of any preservative. In contrast, both Sample M and Sample N, which were treated with preservatives had a higher sucrose content upon initial collection. Within the first 8 hours of storage, both samples showed a minimal sucrose degradation with Sample M decreased from 14.8 ± 0.3 to 13.8 ± 0.2 °Brix, and Sample N 14.8 ± 0.3 to 13.4 ± 0.2 °Brix. By the end of storage period, the sucrose content in Sample M and Sample N further declined to 11.0 ± 0.5 °Brix and 10.5 ± 0.3 °Brix, respectively. These results highlighted the impact of preservative timing on sucrose preservation in nipa sap as pre-tapping application offers a more immediate protective effect. Pre-tapping preservative application helped maintain higher sucrose content due to immediate inhibition of microbial growth, while post-tapping application allowed fermentation processes to initiate before the preservative took effect resulting to greater sucrose loss over time.

Table 4.11: Sugar level (%Brix) of nipa palm sap collected from Site II and treated with effervescent granules

Sample °Brix	Control O				Sample M				Sample N			
	1st	2nd	3rd	$\bar{x}$	1st	2nd	3rd	$\bar{x}$	1st	2nd	3rd	$\bar{x}$
0	14.2	14.6	15.0	14.6	14.6	14.6	15.1	14.8	15.3	14.6	14.5	14.8
1	14.5	14.2	14.0	14.2	14.6	14.6	14.9	14.7	15.0	14.3	14.4	14.6
2	14.0	13.6	13.8	13.8	14.6	14.6	14.9	14.7	15.0	14.3	14.4	14.6
3	14.0	13.2	13.6	13.6	14.4	14.4	14.5	14.4	14.8	14.0	14.2	14.3
4	13.6	12.7	13.6	13.3	14.4	14.4	14.5	14.4	14.6	14.0	14.2	14.3

Table 4.11 continued

5	13.2	12.7	13.4	13.1	14.2	14.2	14.2	14.2	14.4	14.0	14.0	14.1
6	12.8	12.7	13.0	12.8	14.2	14.0	14.2	14.1	14.2	13.8	14.0	14.0
7	12.4	12.4	12.6	12.5	14.0	14.0	14.0	14.0	14.0	13.6	13.8	13.8
8	12.4	12.1	12.4	12.3	13.8	13.6	14.0	13.8	13.6	13.2	13.4	13.4
9	12.0	11.6	12.2	11.9	13.6	13.4	13.8	13.6	13.6	13.2	13.4	13.4
10	12.0	11.6	11.7	11.8	13.6	13.4	13.8	13.6	13.2	13.0	13.2	13.1
11	11.8	11.4	11.7	11.6	13.2	13.0	13.6	13.3	13.2	13.0	13.0	13.1
12	11.5	10.1	11.0	10.9	13.0	12.7	13.2	13.0	12.8	12.6	12.8	12.7
13	11.3	10.0	10.6	10.6	12.8	12.4	13.2	12.8	12.8	12.2	12.6	12.5
14	10.1	9.6	10.6	10.1	12.6	12.0	13.0	12.5	12.4	12.0	12.3	12.2
15	10.1	9.6	10.4	10.0	12.4	12.0	12.7	12.4	12.2	11.6	12.1	12.0
16	10.1	9.1	10.0	9.7	12.0	12.0	12.4	12.1	12.2	11.6	12.1	12.0
17	9.4	8.7	10.0	9.4	12.0	11.7	12.4	12.0	11.8	11.5	11.7	11.7
18	8.4	7.2	8.4	8.0	11.6	11.7	12.0	11.8	11.8	11.6	11.4	11.5
19	7.4	7.2	7.4	7.3	11.6	11.7	12.0	11.8	11.4	10.8	11.4	11.2
20	7.0	6.8	7.0	6.9	11.4	11.0	11.8	11.4	11.1	10.8	11.4	11.1
21	6.5	6.1	6.4	6.3	11.1	10.8	11.8	11.2	10.6	10.8	11.1	10.8
22	6.2	6.0	6.4	6.2	11.1	10.5	11.4	11.0	10.4	10.4	10.8	10.5
23	6.2	6.0	6.2	6.1	11.1	10.5	11.4	11.0	10.4	10.2	10.8	10.5

Table 4.11 continued

24	6.2	6.0	6.2	6.1	11.1	10.5	11.4	11.0	10.4	10.2	10.8	10.5
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Note: $\bar{x}$ represents the mean of Brix value, calculated as the average of all measured Brix readings. Control O serves as control sample. Sample M was treated with preservative before tapping, while Sample N was treated with preservative after sap collection.

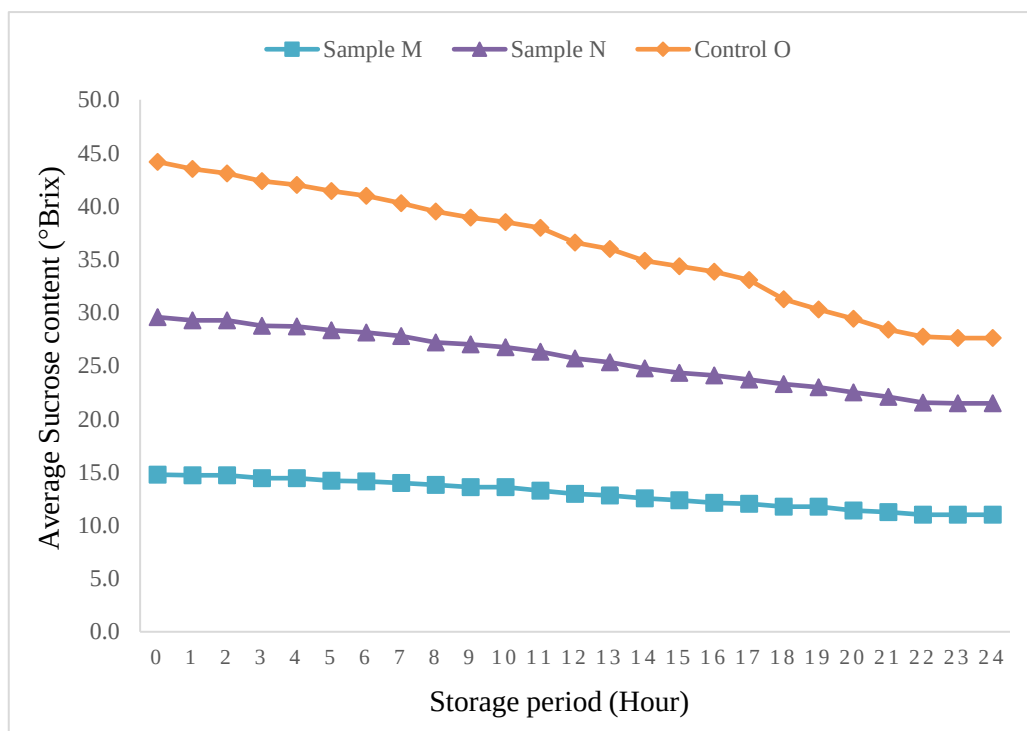


Figure 4.6: Sucrose degradation in bamboo-sap samples treated with effervescent granules at Site II

Table 4.12 showed that the use of effervescent granules had a significant effect ($p < 0.05$) on the sucrose content sap samples collected in bamboo as the F count was larger (11.20431) than the F-table (3.123907). The results indicate that the preservative treatment has a positive effect in preserving sucrose content in both Sample M and N when compared to their control.

However, the samples collected using bamboo vessels showed a faster reduction in sucrose content compared to those collected in plastic bottles (Figure 4.5). This is because bamboo vessels provide a favourable environment for microbial growth as they are porous

and typically left uncovered, allowing foreign particles to easily enter and contaminate the sap. This traditional collection method tends to facilitate fermentation and microbial activity, which accelerates the sugar breakdown despite the presence of preservatives due to increased microbial exposure. In contrast, the use of plastic bottle for sap collection provides a more controlled and sealed environment. Therefore, samples collected in plastic bottles showed a better preservation of sucrose. This finding aligns with the study by Abidin *et al.* (2024), who reported that sap collected using plastic bottles showed lower contamination levels than sap collected in bamboo vessels. The air-tight seal of plastic bottles enhanced the preservative action by reducing the exposure to insects, pests, debris and other contaminants.

Table 4.12: Analysis of sucrose content for bamboo-sap samples treated with effervescent granules

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	86.95327	2	43.47664	11.20431	5.48E-05	3.123907
Within Groups	279.3852	72	3.880351			
Total	366.3385	74				

4.2.2.2 Neutralizing aqueous solution

Table 4.13 and Figure 4.7 illustrate the sucrose degradation in *Nypa fruticans* sap samples collected at Site I, both with and without preservatives. The control sample showed a steep decline in sucrose content, from 50.0 ± 0.2 to 40.0 ± 0.1 °Brix over 24-hour period. The resulting acidic conditions facilitated the inversion of sucrose into reducing sugars (glucose and fructose), thereby decreasing the sucrose content. This decline correlated with a drop in pH from 5.52 ± 0.27 to 2.87 ± 0.17 (Figure 4.3), likely due to microbial

contamination during the 24-hour tapping process. This acidity not only soured the sap but also affected its suitability for nipa palm sugar production.

Table 4.13: Sugar level (%Brix) of nipa palm sap collected from Site I and treated with neutralizing aqueous preservative

Sample °Brix	Control C				Sample A				Sample B			
	1st	2nd	3rd	$\bar{x}$	1st	2nd	3rd	$\bar{x}$	1st	2nd	3rd	$\bar{x}$
0	17.2	16.9	17.0	17.0	16.7	16.6	16.6	16.6	16.1	16.2	16.1	16.1
1	17.0	16.6	16.8	16.8	16.6	16.6	16.5	16.6	16.1	16.2	16.1	16.1
2	16.4	16.2	16.4	16.3	16.4	16.5	16.4	16.4	16.0	16.1	16.0	16.0
3	15.7	15.5	15.5	15.6	16.4	16.5	16.4	16.4	15.8	16.0	15.8	15.9
4	15.5	15.1	15.3	15.3	16.4	16.4	16.3	16.4	15.8	15.8	15.7	15.8
5	15.1	15.0	15.0	15.0	16.1	16.1	16.1	16.1	15.6	15.3	15.6	15.5
6	15.1	15.0	15.0	15.0	16.1	16.1	16.0	16.1	15.5	15.3	15.6	15.5
7	15.0	14.6	14.7	14.8	16.0	15.8	16.0	15.9	15.3	15.1	15.5	15.3
8	14.7	14.0	14.3	14.3	15.9	15.6	15.8	15.8	15.2	15.0	15.5	15.2
9	14.3	13.7	14.0	14.0	15.8	15.5	15.7	15.7	15.0	14.9	15.2	15.0
10	13.7	13.5	13.7	13.6	15.8	15.5	15.6	15.6	15.0	14.8	15.1	15.0
11	13.3	13.1	13.1	13.2	15.6	15.4	15.5	15.5	14.9	14.6	15.1	14.9
12	13.1	13.1	12.8	13.0	15.6	15.3	15.5	15.5	14.8	14.6	15.0	14.8
13	13.0	12.6	12.6	12.7	15.5	15.3	15.4	15.4	14.6	14.5	15.0	14.7

Table 4.13 continued

14	12.7	12.4	12.4	12.5	15.4	15.2	15.2	15.3	14.4	14.2	14.9	14.5
15	12.5	12.1	12.0	12.2	15.3	15.2	15.1	15.2	14.3	14.2	14.8	14.4
16	12.1	11.5	11.7	11.8	15.2	15.2	15.1	15.2	14.2	14.0	14.6	14.3
17	11.9	11.5	11.5	11.6	15.2	15.1	15.1	15.1	14.0	13.8	14.5	14.1
18	11.7	11.4	11.5	11.5	15.1	15.1	15.0	15.1	14.0	13.7	14.3	14.0
19	11.3	11.1	11.2	11.2	15.1	15.1	15.0	15.1	13.8	13.6	14.1	13.8
20	11.1	10.6	10.8	10.8	15.1	15.0	14.9	15.0	13.7	13.5	14.1	13.8
21	10.8	10.4	10.5	10.6	15.0	14.9	14.8	14.9	13.7	13.4	14.0	13.7
22	10.5	10.4	10.4	10.4	14.8	14.7	14.7	14.7	13.5	13.3	13.9	13.6
23	10.3	10.2	10.3	10.3	14.7	14.6	14.5	14.6	13.3	13.1	13.7	13.4
24	10.1	10.0	10.0	10.0	14.7	14.6	14.5	14.6	13.1	13.0	13.6	13.2

Note: $\bar{x}$ represents the mean of Brix value, calculated as the average of all measured Brix readings. Control C serves as control sample. Sample A was treated with preservative before tapping, while Sample B was treated with preservative after sap collection.

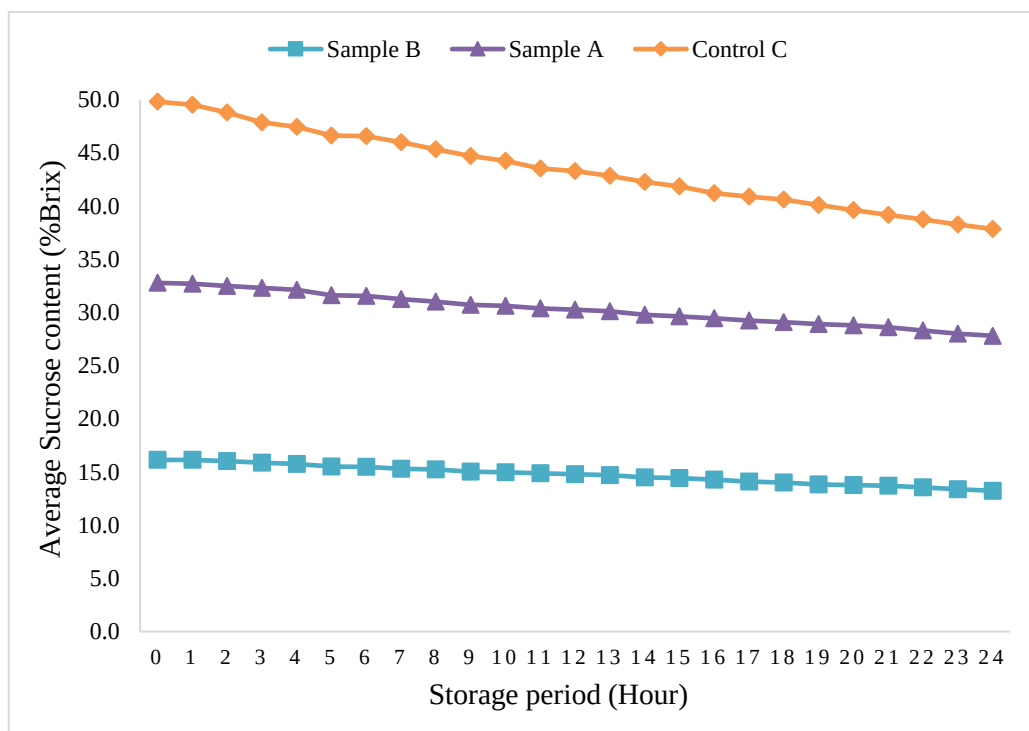


Figure 4.7: Sucrose degradation in sap samples treated with neutralizing aqueous solution at Site II

High sap quality correlates with elevated sucrose content, influencing the texture of resultant brown sugar; inverted sucrose hinders solidification (Fernando, 2014 as cited in Putra *et al.*, 2019). Sample A which treated with neutralizing aqueous solution prior to tapping process resulted in stable sucrose levels. Its sucrose content slowly decreased from approximately 32.0 ± 0.1 to 31.0 ± 0.1 °Brix. This preservation method effectively mitigated fermentation and acidity, thus maintaining a higher sucrose level during storage period by slowing down the fermentation.

In contrast, Sample B, which preservatives were added after sap has been collected, begins with a sucrose content at approximately 16.1 ± 0.1 °Brix and reduces to 13.2 ± 0.3 °Brix by the end of storage period. Although the initial sucrose level was slightly lower than Sample A, both methods still effective in preventing further fermentation after the addition of preservatives. The preservative clearly helped reduce the rate of sucrose degradation due

to fermentation. These findings also highlight the effect of preservation timing on sucrose retention, with both samples demonstrating effective sucrose preservation.

As shown in Table 4.14, the aqueous form of potassium sorbate and sodium metabisulfite significantly impacted ($p < 0.05$) sucrose content in the sap samples. The F-count (25.07512) was greater than F-table (3.123907), highlighting its efficacy in preserving the quality of nipa palm sap for sugar processing.

Table 4.14: Analysis of sucrose content for samples treated with neutralizing aqueous solution

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	87.40003	2	43.70001	25.07512	5.44E-09	3.123907
Within Groups	125.4790	72	1.742764			
Total	212.8791	74				

4.2.2.3 Comparison between effervescent granules and neutralizing aqueous solution in controlling sucrose level in nipa palm sap at the same site

As shown in Table 4.15 and Table 4.16, neutralizing aqueous solution was more effective in preserving sucrose levels in nipa palm sap. Both Sample A and Sample B, which were treated with neutralizing aqueous solution showed a gradual decrease in sucrose content, from 16.6 ± 0.1 to 14.6 ± 0.1 °Brix and 16.1 ± 0.1 to 13.2 ± 0.3 °Brix, respectively, as shown in Figure 4.8 and Figure 4.9. This slow decline indicates that the aqueous solution effectively inhibited microbial activity, thereby slowing the fermentation process and maintaining higher sucrose levels. Moreover, this consistency between Sample A and Sample B highlights the stability and efficacy of the neutralizing aqueous solution, regardless of when it was applied.

Table 4.15: Comparison between effervescent granules (Sample X) and neutralizing aqueous solution (Sample X) in controlling sucrose level in nipa palm sap when applying before tapping

Sample °Brix	Sample X				Sample A			
	1st	2nd	3rd	$\bar{x}$	1st	2nd	3rd	$\bar{x}$
0	15.0	14.8	14.6	14.8	16.7	16.6	16.6	16.6
1	15.0	14.8	14.6	14.8	16.6	16.6	16.5	16.6
2	14.8	14.6	14.4	14.6	16.4	16.5	16.4	16.4
3	14.8	14.6	14.3	14.6	16.4	16.5	16.4	16.4
4	14.2	14.0	13.9	14.0	16.4	16.4	16.3	16.4
5	14.2	14.0	13.9	14.0	16.1	16.1	16.1	16.1
6	14.0	13.8	13.6	13.8	16.1	16.1	16.0	16.1
7	13.8	13.6	13.4	13.6	16.0	15.8	16.0	15.9
8	13.8	13.6	13.4	13.6	15.9	15.6	15.8	15.8
9	13.3	13.2	13.0	13.2	15.8	15.5	15.7	15.7
10	13.3	13.2	13.0	13.2	15.8	15.5	15.6	15.6
11	12.2	12.0	11.8	12.0	15.6	15.4	15.5	15.5
12	12.1	12.0	11.8	12.0	15.6	15.3	15.5	15.5
13	12.1	12.0	11.8	12.0	15.5	15.3	15.4	15.4
14	12.0	12.0	11.7	11.9	15.4	15.2	15.2	15.3

Table 4.15 continued

15	12.0	11.9	11.7	11.9	15.3	15.2	15.1	15.2
16	11.8	11.8	11.6	11.7	15.2	15.2	15.1	15.2
17	11.8	11.8	11.4	11.7	15.2	15.1	15.1	15.1
18	11.7	11.7	11.4	11.6	15.1	15.1	15.0	15.1
19	11.7	11.6	11.3	11.5	15.1	15.1	15.0	15.1
20	11.6	11.5	11.2	11.4	15.1	15.0	14.9	15.0
21	11.6	11.4	11.1	11.4	15.0	14.9	14.8	14.9
22	11.5	11.4	11.0	11.3	14.8	14.7	14.7	14.7
23	11.5	11.3	10.8	11.2	14.7	14.6	14.5	14.6
24	11.5	11.3	10.7	11.2	14.7	14.6	14.5	14.6

Note: $\bar{x}$ represents the mean of pH value, calculated as the average of all measured pH readings. Both samples were collected at Site I and treated with preservative before the tapping process. Sample X was treated with effervescent granules preservative, while Sample A was treated with neutralizing aqueous preservative.

Table 4.16: Comparison between effervescent granules (Sample Y) and neutralizing aqueous solution (Sample B) in controlling sucrose level in nipa palm sap when applying after tapping

Sample °Brix	Sample Y				Sample B			
	1st	2nd	3rd	$\bar{x}$	1st	2nd	3rd	$\bar{x}$
0	15.2	15.0	14.8	15.0	16.1	16.2	16.1	16.1
1	15.1	15.0	14.8	15.0	16.1	16.2	16.1	16.1

Table 4.16 continued

2	15.0	14.8	14.6	14.8	16.0	16.1	16.0	16.0
3	14.8	14.6	14.4	14.6	15.8	16.0	15.8	15.9
4	14.8	14.6	14.4	14.6	15.8	15.8	15.7	15.8
5	14.6	14.3	13.8	14.2	15.6	15.3	15.6	15.5
6	14.0	13.9	13.6	13.8	15.5	15.3	15.6	15.5
7	14.0	13.9	13.6	13.8	15.3	15.1	15.5	15.3
8	13.8	13.6	13.4	13.6	15.2	15.0	15.5	15.2
9	13.8	13.6	13.4	13.6	15.0	14.9	15.2	15.0
10	13.6	13.3	13.4	13.1	15.0	14.8	15.1	15.0
11	12.8	12.4	12.0	12.4	14.9	14.6	15.1	14.9
12	12.6	12.2	11.8	12.2	14.8	14.6	15.0	14.8
13	12.6	12.1	11.8	12.2	14.6	14.5	15.0	14.7
14	12.5	12.1	11.7	12.1	14.4	14.2	14.9	14.5
15	12.5	12.0	11.7	12.1	14.3	14.2	14.8	14.4
16	12.4	12.0	11.7	12.0	14.2	14.0	14.6	14.3
17	12.4	11.9	11.6	12.0	14.0	13.8	14.5	14.1
18	12.3	11.9	11.6	11.9	14.0	13.7	14.3	14.0
19	12.3	11.8	11.5	11.9	13.8	13.6	14.1	13.8
20	12.2	11.8	11.4	11.8	13.7	13.5	14.1	13.8

Table 4.16 continued

21	12.2	11.7	11.3	11.7	13.7	13.4	14.0	13.7
22	12.1	11.6	11.2	11.6	13.5	13.3	13.9	13.6
23	12.0	11.6	11.2	11.6	13.3	13.1	13.7	13.4
24	12.0	11.6	11.2	11.6	13.1	13.0	13.6	13.2

Note: $\bar{x}$ represents the mean of Brix value, calculated as the average of all measured Brix readings. Both samples were collected at Site I and treated with preservative the sap has been collected. Sample Y was treated with effervescent granules preservative, while Sample B was treated with neutralizing aqueous preservative.

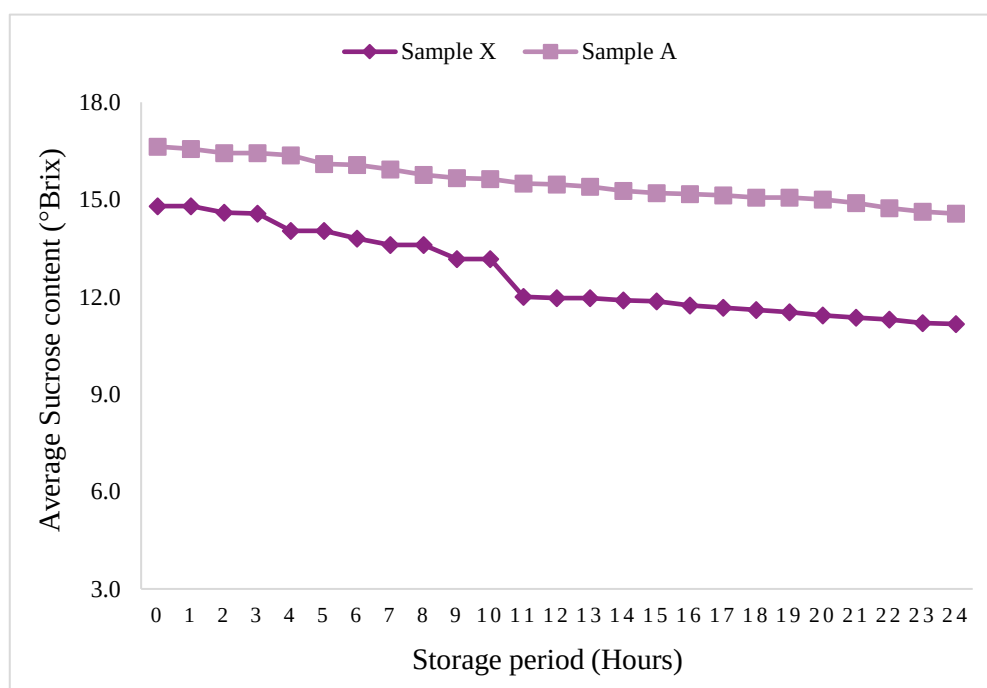


Figure 4.8: Sucrose content of nipa palm sap in pre-tapping samples

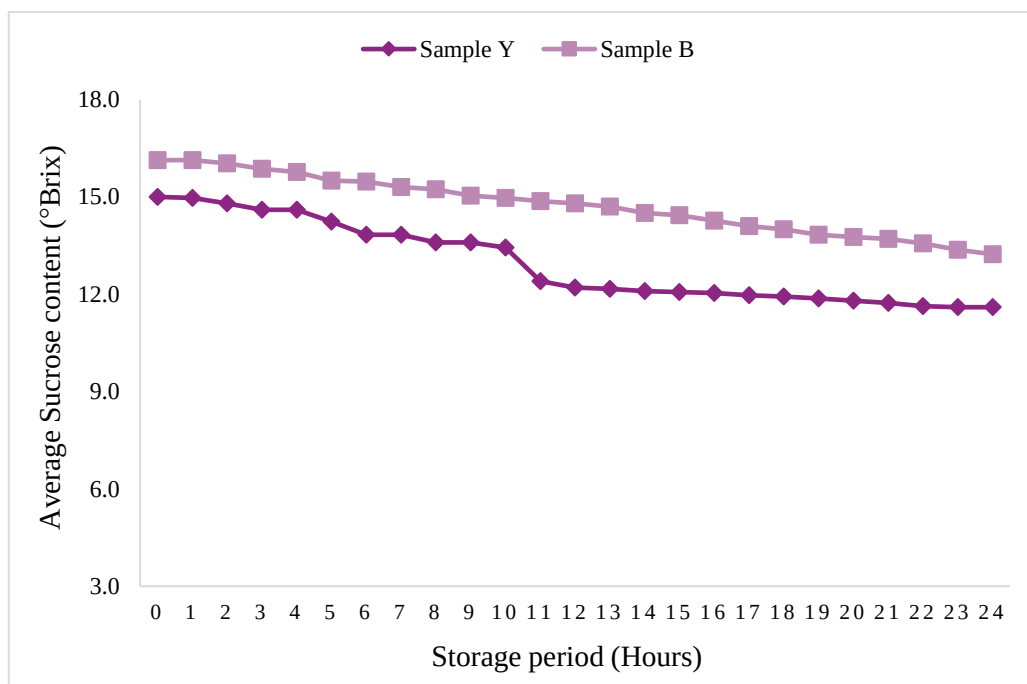


Figure 4.9: Sucrose content of nipa palm sap in post-tapping samples

In contrast, Sample X and Sample Y which were treated with effervescent granules decreased more rapidly. Sample X begins at sucrose content of 14.8 ± 0.2 °Brix, then decreased to 11.2 ± 0.4 °Brix by the end of storage period. Meanwhile, sucrose content in Sample Y decreased from 15.0 ± 0.2 to 11.6 ± 0.4 °Brix. The faster rate of sucrose decline in these samples suggests that the effervescent granules were not efficient as neutralizing aqueous solution in preventing sucrose breakdown. This may be due to the slower dissolution of the granules within the sap that leads to a weaker preservation efficacy.

According to Lück (1990), sorbates are more preferable when it is used in liquid or aqueous form, where potassium sorbate dissolves into sorbic acid in water. Sorbic acid is an unsaturated aliphatic monocarboxylic fatty acid, inhibits molds, yeasts, and fungi in which it can be used as antifungal and antimicrobial preservatives in a variety of food, cosmeceutical, and pharmaceutical products. Therefore, in this study, sorbic acid can be used in the form of potassium sorbate to prevent fermentation of the sap, thus preserving the sugar content. When the preservative comes in contact with the sap, the sorbic acid is distributed

uniformly in the sap, thus protecting the sap from microbial contamination (Lück, 1990; Beck, 2013; Dehghan *et al.*, 2018). Meanwhile, when sodium metabisulfite is dissolved in water, it produces sodium bisulfite and sulphur dioxide. It has been observed that bacteria are substantially more susceptible to sulphur dioxide than yeast and molds, limiting microbial activity and impeding fermentation (Ough & Were, 2005). In addition, Okafor (1975) investigated the effect of preservatives (sorbic acid and sodium metabisulfite) and pasteurization on palm wine. He reported that pasteurizing palm sap at 70 °C for 30 minutes was more effective in reducing the microbial population than using preservatives alone, which could further enhance the preservation of palm sap.

4.2.3 Effects of preservatives on chemical composition in *Nypa fruticans* sap

The decreased in sugar content indicated that a large proportion of sugars had been fermented, especially in the early stages of storage as the microbes could be introduced into the nipa palm sap via unhygienic tapping tools, procedures and collection (Naknean *et al.*, 2010; Jaraee *et al.*, 2023). The decrease in sucrose content may be resulted from an inversion reaction catalysed by invertase activity and an acidic condition caused by microbial metabolic activity (Flores – Gallegos *et al.*, 2019) and preservative was used to minimize the inversion. Table 4.17 and 4.18 showed the chemical composition of nipa palm sap samples treated with effervescent granules- and neutralizing aqueous solution-preservative after 24 hours of storage, respectively. The chemical composition analysis of untreated and treated nipa palm sap samples after 24 hours of storage show that both effervescent granules and neutralizing aqueous solution work effectively as preservatives, particularly in inhibiting fermentation and stabilizing sugar content in nipa palm sap.

Table 4.17: The chemical composition of nipa palm sap samples treated with effervescent granules after 24 hours of storage

Parameters	Control Z*	Sample X*	Sample Y*
pH	2.87 ± 0.04	4.58 ± 0.18	4.28 ± 0.29
Total sugar (g/L)	172.2 ± 2.9	179.6 ± 2.9	179.6 ± 2.6
Sucrose (g/L)	101.0 ± 0.2	112.0 ± 0.4	116.0 ± 0.4
Glucose (g/L)	29.1 ± 2.1	28.9 ± 1.9	27.4 ± 1.7
Fructose (g/L)	42.1 ± 2.0	38.7 ± 2.1	36.2 ± 1.9
Lactic acid (g/L)	1.19 ± 0.02	1.15 ± 0.04	1.08 ± 0.04
Acetic acid (g/L)	0.17 ± 0.04	0.12 ± 0.04	0.09 ± 0.03

Note: * means that values are presented as mean ± SD from triplicate measurements. Sample Z serve as control sample. Samples X was treated with effervescent granules preservative before the tapping process, while Samples Y received the same preservative after sap collection.

Table 4.18: The chemical composition of nipa palm sap samples treated with neutralizing aqueous solution after 24 hours of storage

Parameters	Control C*	Sample A*	Sample B*
pH	2.87 ± 0.17	5.48 ± 0.02	5.10 ± 0.03
Total sugar (g/L)	164.8 ± 3.6	201.8 ± 2.4	196.2 ± 2.1
Sucrose (g/L)	100.0 ± 0.1	150.0 ± 0.1	145.0 ± 0.4
Glucose (g/L)	26.7 ± 3.1	19.7 ± 1.4	19.4 ± 1.8
Fructose (g/L)	38.1 ± 1.8	32.1 ± 2.0	31.8 ± 0.9
Lactic acid (g/L)	1.21 ± 0.03	0.95 ± 0.02	0.97 ± 0.04
Acetic acid (g/L)	0.17 ± 0.05	0.05 ± 0.03	0.07 ± 0.02

Note: * means that values are presented as mean $\pm$ SD from triplicate measurements. Sample C serve as control sample. Samples A was treated with neutralizing aqueous preservative before the tapping process, while Samples B received the same preservative after sap collection.

The untreated control samples – Control Z and Control C, had lower pH values of 2.87 ± 0.04 and 2.87 ± 0.17 , respectively, which indicates an acidic environment in fresh nipa sap due to microbial growth and fermentation. In treated samples, both preservatives significantly ($p < 0.05$) increased the pH of the sap. Samples treated with effervescent granules had a pH value of 4.58 ± 0.18 (Sample X) and 4.28 ± 0.29 (Sample Y), meanwhile samples treated with aqueous solution reached even higher pH values of 5.48 ± 0.02 (Sample A) and 5.10 ± 0.03 (Sample B). This increased in pH in these treated samples suggest that both preservatives neutralized or reduce the sap's acidity, with the aqueous solution showing a greater pH rise, which can inhibit microbial activity and delay fermentation, potentially prolonging the shelf-life of the sap.

After 24 hours, the total sugar contents in both Control Z and Control C samples were measured as 172.2 ± 2.9 g/L and 164.8 ± 3.6 g/L, respectively, the lowest among all samples. This low in total sugar content correlated with the low pH in these untreated samples, which lead to the rapid breakdown of sucrose into reducing sugars via invertase activity. This reduction in sugar content aligns with findings by Naknean *et al.* (2010), who observed that acidic environments promote invertase activity and inversion process. Although the sugar composition of untreated samples of this study correlated with other previous research, the total sugar composition in this study was lower than that reported by Jaraee *et al.* (2023) but higher than that in other studies in Peninsular Malaysia (Tamunaidu *et al.*, 2013), Thailand (Phetrit *et al.*, 2020) and Indonesia (Tomomatsu *et al.*, 1996). This

difference is likely due to differences in the tapping process, environmental conditions and the presence of preservatives, which can affect the sugar composition of nipa palm sap from one location to another. In the treated samples, there was an increase in total sugar content for both methods, with the effervescent granule treatment had a total sugar content of 179.6 ± 2.9 g/L (Sample X) and 179.6 ± 2.6 g/L (Sample Y). While samples treated with aqueous solution showed even higher total sugar content after 24 hours, with values of 201.8 ± 2.4 g/L for Sample A and 196.2 ± 2.1 g/L for Sample B. These higher sugar contents indicate that both preservatives were highly effective in preserving the sugar content of nipa sap, especially neutralizing aqueous solution.

Based on Table 4.17, the control sample exhibits the highest reducing sugar content (71.2 ± 2.9 g/L), followed by Sample X (67.6 ± 2.8 g/L) and Sample Y (63.6 ± 2.5 g/L). The reducing sugar content in the control sample close to the levels found in fermented nipa palm sap, as reported by Jaraee *et al.* (2023). This suggests that without preservatives, the nipa palm sap naturally tends toward fermentation, changing its sugar composition significantly within 24 hours. Table 4.19 demonstrates that the use of effervescent granules preservatives significantly affects ($p < 0.05$) reducing sugar content in nipa palm sap. The F-value of 8.413972 was greater than the critical value of 5.143253, indicating a statistically significant impact. This data suggests that the addition of preservatives, whether before or after the tapping process, able to preserve the original sugar content of the sap, hence delaying fermentation.

Table 4.19: Analysis of variance of reducing sugar content in nipa palm sap treated with effervescent granules preservative

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	86.72	2	43.36	8.413972	0.018157	5.143253
Within Groups	30.92	6	5.153333			
Total	117.64	8				

Based on the data in Table 4.18, the control sample has the highest reducing sugar content (64.8 ± 3.6 g/L), followed by Sample A (51.8 ± 2.4 g/L) and Sample B (51.3 ± 2.0 g/L). The reduction in reducing sugar content in the treated samples indicates that the preservative successfully minimized the enzymatic breakdown of sucrose into glucose and fructose. The F-value of 37.42016 was greater than the critical value of 5.143253, indicating a statistically significant impact (Table 4.20). This data suggests that the addition of neutralizing aqueous solution preservatives, whether before or after the tapping process, can significantly extend the shelf-life of nipa palm sap by preserving sucrose and reducing the formation of reducing sugars.

Table 4.20: Analysis of variance of reducing sugar content in nipa palm sap treated with neutralizing aqueous solution

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	351.5	2	175.75	37.42016	0.000409	5.143253
Within Groups	28.18	6	4.696667			
Total	379.68	8				

As these sugars are metabolized, they are converted into organic acids, resulting in an increase in the lactic acid and acetic acid concentrations. Additionally, LAB and AAB carry out oxidative fermentation which transform various substrates into valuable products, such as converting ethanol to acetic acid via oxidation (Manel *et al.*, 2011; Jaraee *et al.*, 2023). In untreated samples, the concentrations of both lactic and acetic acids were higher, with lactic acid concentrations at 1.19 ± 0.02 g/L in Control Z, and 1.21 ± 0.03 g/L in Control C. Meanwhile, acetic acid concentrations in Control Z and Control C were 0.17 ± 0.04 g/L and 0.17 ± 0.05 g/L, respectively. These higher organic acids content in untreated samples suggests ongoing fermentation, where microorganisms break down sugar and produce organic acids that lead to lower pH levels. The low pH in untreated samples accelerates the inversion of sucrose into glucose and fructose, hence increasing their acid production. This correlated with findings from Naknean *et al.* (2010), Manel *et al.* (2011), Tamunaidu *et al.* (2013) and Jaraee *et al.* (2023), which highlight that lower pH levels would cause inversion reaction of sugars to become more rapid, leading to an increased in organic acid levels, and consequently accelerating the spoilage of the sap. In contrast, treated samples had lower lactic and acetic acid concentrations. Samples treated with effervescent granules showed lactic acid concentration of 1.15 ± 0.04 g/L in Sample X and 1.08 ± 0.04 g/L in Sample Y, and acetic acid concentration at 0.12 ± 0.04 g/L and 0.09 ± 0.03 g/L, respectively. Samples treated with aqueous solution had the lowest lactic acid and acetic acid concentrations. Sample A had 0.95 ± 0.02 g/L lactic acid and 0.05 ± 0.03 g/L acetic acid, while Sample B had a lactic acid and acetic acid concentrations at 0.97 ± 0.04 g/L and 0.07 ± 0.02 g/L, respectively. These results indicate that the treatment effectively inhibited fermentation, as well as acid formation. The lower organic acid concentrations in treated samples align with the higher (more alkaline) pH, which is less favourable for fermentative bacteria. Thus,

reducing acid production through the application of preservatives is important for maintaining the stability of nipa palm sap. Although the addition of preservatives effectively reduced the initial rate of sugar conversion, it did not halt the process entirely as organic acids continue to be produced. This occurs because some spoilage microorganisms can survive the preservative treatment. As a result, a gradual increase in organic acid concentrations in the treated samples can still occur over time (Naknean *et al.*, 2014).

4.3 Antioxidant activity of nipa palm sap

Table 4.21 presents the DPPH radical scavenging activities (RSA) obtained from the antioxidant activities of the nipa palm sap samples, based on three analytical replicates. The percentage of DPPH radical scavenging activity of nipa palm sap decreased as the storage period increased.

Table 4.21: Percentage of DPPH radical scavenging activity (% RSA) of nipa palm sap throughout storage period

Sample Storage period	Antioxidant Activity (% RSA)		
	8 Hour	12 Hour	24 Hour
Control sample (without preservative)	32.39 ± 0.31	24.37 ± 0.74	9.33 ± 0.36
Sample X	37.32 ± 1.27	36.91 ± 1.59	29.39 ± 2.57
Sample Y	32.55 ± 7.93	31.20 ± 4.09	22.52 ± 6.34
Sample A	48.91 ± 0.99	48.25 ± 1.00	45.38 ± 1.30
Sample B	39.95 ± 1.07	37.36 ± 0.81	30.46 ± 0.81

Note: The values are expressed in mean ± SD from three replications. Samples X was treated with effervescent granules preservative before the tapping process, while Samples Y received the same preservative after sap collection. Samples A was treated with neutralizing

aqueous preservative before the tapping process, while Samples B received the same preservative after sap collection.

As shown in Table 4.21, the aqueous preservative was more effective than effervescent granules in preserving the antioxidant activity of sap, particularly when applied prior to sap collection. The control sample, which was not treated with any preservative showed a significant decline in antioxidant activity over the 24-hour period. Initially, it had the antioxidant activity (% RSA) at 32.39 ± 0.31 % but dropped to 9.33 ± 0.36 % by the 24-hour mark. This highlights the rapid degradation of antioxidant properties in untreated nipa palm sap, which is consistent with previous findings that fresh nipa palm sap is highly susceptible to oxidation and microbial fermentation if left unprotected.

In contrast, the use of effervescent granules in Samples X and Y showed moderate effectiveness in preserving antioxidant activity, though their performance varied depending on the timing of application. Sample X started with a slightly higher RSA of 37.32 ± 1.27 % at 8 hours and retained 29.39 ± 2.57 % RSA by 24 hours. Conversely, Sample Y showed lower antioxidant activity throughout the storage period, with RSA was 32.55 ± 7.93 % at 8 hours, which decreased to 22.52 ± 6.34 % by 24 hours. The lower initial value and more pronounced decline in Sample Y suggest that applying the preservative after sap has been collected may have allowed for some initial degradation of antioxidant compounds before the preservative could take effect. This indicates that pre-tapping application of effervescent granules provides some protection against oxidation, likely by immediately inhibiting microbial activity and minimizing exposure to oxygen during sap collection.

Sample A consistently exhibited the highest antioxidant activity throughout the entire storage period. It began with an RSA of 48.91 ± 0.99 % at 8 hours and only decreased slightly to 45.38 ± 1.30 % by 24 hours. The relatively stable antioxidant activity suggests that the

aqueous preservative was highly effective at protecting the sap from oxidative degradation, likely due to its ability to create a protective barrier that prevents oxygen exposure and inhibits microbial activity right from the point of tapping. Although the preservative in Sample B was applied after sap collection, it still outperformed the effervescent granules. Sample B had an RSA of 39.95 ± 1.07 % at 8 hours, which was lower than Sample A but still higher than both Samples X and Y. By 24 hours, Sample B retained 30.46 ± 0.81 % RSA, significantly higher than the effervescent granule-treated samples. This indicates that aqueous preservatives offer better protection for nipa palm sap's antioxidant properties, even when applied post-tapping. Additionally, the DPPH scavenging activity of nipa palm sap treated with aqueous preservative (Sample A) after 24-hour of storage period was higher than the report of Phetrit *et al.* (2019), who found the values of 40.03 ± 0.99 $\mu\text{mol TE/g}$ in nipa palm sap with kiam wood as its preservative.

Our findings indicate that both type of preservative and the timing of its application significantly affect the antioxidant activity of nipa palm sap. It has been reported that sodium metabisulfite dissociates in water to release sulphur dioxide which is a potent antioxidant (Grotheer *et al.*, 2005; Ough & Were, 2005). Sulphur dioxide reacts with oxygen and other reactive, reducing their availability for oxidative reactions. It acts as a scavenger for free radicals, which are highly reactive molecules that can cause oxidative damage to the components of the sap, including sugars, proteins, and phenolic compounds (Tan *et al.*, 2014 as cited in Tan *et al.*, 2015; Ahmadi *et al.*, 2021). By protecting against oxidative degradation, sodium metabisulfite also helps retain the nutritional quality of the sap, including its vitamins and mineral contents (Ahmadi *et al.*, 2021). Hence, this study suggests that metabisulfite not only reduce oxidative damage but also protects the antioxidant

capacity of the sap. Sodium metabisulfite helps to maintain these antioxidant levels, thus ensuring that the quality of the sap and its shelf-life are preserved.

4.4 Antimicrobial activity of *Nypa fruticans* sap

This study analysed the antimicrobial effects of two preservative formulations on nipa palm sap to extend its shelf life. Sodium metabisulfite and potassium sorbate were the active components in both preservatives, applied either before tapping or after sap collection. TBC (CFU/mL) was used in this study to evaluate the effectiveness of these preservation methods. The data in Table 4.22 showed the differences in bacterial suppression based on both preservative type and the timing of application after 24 hours of storage.

Table 4.22: Total bacterial count (CFU/mL) of nipa palm sap at different concentrations

Sample Dilution	Average of Total Bacterial Count (CFU/mL)					
	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
Control sample	TNTC	TNTC	2.36 (± 0.06) $\times$ 10^5	1.89 (± 0.09) $\times$ 10^6	1.54 (± 0.07) $\times$ 10^7	1.32 (± 0.06) $\times$ 10^8
Sample X	1.58 (± 0.09) $\times$ 10^3	1.48 (± 0.07) $\times$ 10^4	1.25 (± 0.06) $\times$ 10^5	1.15 (± 0.07) $\times$ 10^6	0.86 (± 0.07) $\times$ 10^7	0.73 (± 0.04) $\times$ 10^8

Table 4.22 continued

Sample Y	1.61 (± 0.05) $\times$ 10^3	1.59 (± 0.05) $\times$ 10^4	1.33 (± 0.04) $\times$ 10^5	1.21 (± 0.05) $\times$ 10^6	1.05 (± 0.08) $\times$ 10^7	0.95 (± 0.08) $\times$ 10^8
Sample A	1.47 (± 0.05) $\times$ 10^3	1.16 (± 0.04) $\times$ 10^4	1.02 (± 0.05) $\times$ 10^5	0.69 (± 0.04) $\times$ 10^6	0.42 (± 0.06) $\times$ 10^7	TFTC
Sample B	1.53 (± 0.04) $\times$ 10^3	1.37 (± 0.05) $\times$ 10^4	1.17 (± 0.04) $\times$ 10^5	0.86 (± 0.04) $\times$ 10^6	0.62 (± 0.05) $\times$ 10^7	0.48 (± 0.05) $\times$ 10^8
Sample treated with VCO	1.36 (± 0.03) $\times$ 10^3	1.12 (± 0.05) $\times$ 10^4	1.02 (± 0.06) $\times$ 10^5	0.66 (± 0.04) $\times$ 10^6	0.42 (± 0.05) $\times$ 10^7	TFTC
Sample treated with amoxicillin	2.15 (± 0.08) $\times$ 10^3	1.79 (± 0.04) $\times$ 10^4	1.57 (± 0.05) $\times$ 10^5	1.28 (± 0.03) $\times$ 10^6	1.17 (± 0.05) $\times$ 10^7	0.95 (± 0.03) $\times$ 10^8

Note: The values are expressed in mean $\pm$ standard deviation from three replications. Any plate which has more than 250 colonies is designated as “too numerous to count” (TNTC), while any plate which has fewer than 25 colonies is designated as “too fewer to count” (TFTC). Samples X was treated with effervescent granules preservative before the tapping process, while Samples Y received the same preservative after sap collection. Samples A was treated with neutralizing aqueous preservative before the tapping process, while Samples B received the same preservative after sap collection.

In the control sample, the bacterial counts were too numerous to count (TNTC) at dilution 10^{-1} and 10^{-2} , indicating a high initial microbial load. This is due to the absence of any preservatives or antimicrobial agents in the sample that impede the growth and proliferation of bacteria which naturally occur in the nipa palm sap. Without the presence of any preservatives, the control sample was highly susceptible to microbial contamination during collection and storage, which accelerated the process of fermentation and spoilage. Therefore, the total bacterial count in the control sample was higher in the treated samples. As stated by Oluwole *et al.* (2023), nipa palm sap is high in fermentable sugars, which is conducive to bacterial colonization. Other studies further explain that these nutrients act as a readily available energy source for microorganisms, promoting rapid growth and reproduction (McFeeters, 2004; Hebbar *et al.*, 2018; Jaraee *et al.*, 2023). Microbes consumed the sugars in the sap, producing acidic by-products that lowered the pH. The decrease in pH creates an acidic environment that further stimulates microbial activity and resulting in a cycle of fermentation and spoilage (Jaraee *et al.*, 2023).

Samples treated with effervescent granule preservative had lower bacterial counts than the control, although not as much as in samples treated with the neutralizing aqueous preservative. Sample X, treated prior to tapping showed bacterial counts lower than the control across all dilutions. For instance, at the 10^{-4} dilution, the bacterial count was $1.15 (\pm 0.07) \times 10^6$ CFU/mL, lower than the control at $1.89 (\pm 0.09) \times 10^6$. Sample Y, treated after sap collection showed a slightly higher bacterial counts than Sample X, with $1.21 (\pm 0.05) \times 10^6$ CFU/mL at 10^{-4} dilution and $1.21 (\pm 0.05) \times 10^7$ CFU/mL at 10^{-5} dilution. The presence of higher bacterial counts in Sample Y implies that bacteria may have already proliferated in the sap before preservative application. These results also suggest that applying the

effervescent granule preservative after the sap has been collected may be less effective in controlling bacterial growth compared to the pre-tapping method.

The aqueous preservative formulation showed greater effectiveness in bacterial suppression compared to effervescent granule preservative, which may be due to its better dispersion and immediate interaction with the sap. Sample A had the lowest bacterial counts among all treated samples. For example, at dilution of 10^{-4} , the bacterial count was $0.69 (\pm 0.04) \times 10^6$ CFU/mL, significantly lower than the corresponding counts in both the control and effervescent granule-treated samples. Additionally, the bacterial count at the 10^{-6} dilution was too few to count (TFTC), indicating a strong inhibitory effect on bacterial growth, which indicates a substantial reduction in microbial load. Sample B also showed a reduced microbial load compared to the control, but was slightly less effective than Sample A. At 10^{-4} dilution, the bacterial count was $0.86 (\pm 0.04) \times 10^6$ CFU/mL and at 10^{-5} , it was $0.62 (\pm 0.05) \times 10^7$ CFU/mL. The lower bacterial counts in Sample A compared to Sample B highlights the efficacy of pre-tapping treatment in preventing bacterial proliferation. Post-tapping treatment with aqueous preservatives is still effective in suppressing bacterial growth, though less effectively than pre-tapping treatment.

This finding revealed that aqueous preservatives had a better bacterial suppression than effervescent granules due to the more uniform distribution and immediate interaction of the aqueous solution with the sap. Furthermore, pre-tapping applications (Sample X and A) resulted in lower bacterial counts than post-tapping applications (Sample Y and B). This observation suggests that introducing preservatives at the beginning of the tapping process provides a more effective barrier against bacterial contamination. This approach most likely causes the microbial growth to be inhibited at an early stage and any contaminants that might have occurred during the collection process are immediately encountered by the

antimicrobial agents. In contrast, in the post-tapping samples, there was a delay in preservative application, allowing the sap to be exposed to the environment without any antimicrobial protection. The gap in time allows the possible contamination and growth of bacteria during the collection process, resulting in higher number of bacterial counts.

The effectiveness of sodium metabisulfite in reducing bacterial counts has been demonstrated in previous studies. Okafor (1975) treated sap with sodium metabisulfite and reported bacterial counts of 6.31 log CFU/mL. This level of bacterial reduction was higher than the bacterial counts observed in this study. This difference suggests that the combined use of potassium sorbate and sodium metabisulfite may provide stronger antimicrobial protection than sodium metabisulfite alone. Potassium sorbate is known for its effectiveness against various bacteria, often complementing the antimicrobial action of sodium metabisulfite (Lück, 1990; Ferrand *et al.*, 2000). Hariharan *et al.* (2014) also found that toddy samples collected in pots and treated with sodium metabisulfite had a total bacterial load of 0.38 CFU/mL upon collection, demonstrating its immediate inhibitory effect. However, the bacterial counts increased to 1.593 log CFU/mL after 24 hours, indicating that the effectiveness of sodium metabisulfite reduce over time, despite its initial effectiveness. These limitations are addressed by the combination of sodium metabisulfite and potassium sorbate in this study, which providing both immediate and sustained antimicrobial effects. Sodium metabisulfite, which releases sulphur dioxide in solution, work effectively to inactivate microorganisms by disrupting cellular functions, including enzyme inhibition (Davidson *et al.*, 2005; Grotheer *et al.*, 2005; Ough & Were, 2005). Potassium sorbate, on the other hand, is particularly effective at low pH and may creating a less favourable condition for microbial adaptation and regrowth especially for high-nutrient substrates like nipa palm sap that promote rapid microbial growth (Berk, 2013). Moreover, in the presence

of liquid, potassium sorbate converts to sorbic acid, which has strong antifungal and antimicrobial properties, enhancing the preservative effect against microbes responsible for nipa palm sap spoilage (Das *et al.*, 2010). For instance, in this study, samples treated with aqueous preservative formulation (Sample A) had a lower bacterial count of $0.69 (\pm 0.04) \times 10^6$ at the 10^{-4} dilution after 24 hours. This count was lower than what was observed in the earlier study, suggesting that the addition of potassium sorbate helped maintain the microbial suppression more effectively than sodium metabisulfite alone.

The nipa palm sap sample treated with VCO showed a bacterial suppression, with bacteria counts lower than the control, and the samples treated with aqueous and effervescent preservatives. At the 10^{-3} dilution, the bacterial count was $1.02 (\pm 0.06) \times 10^5$ CFU/mL, while at the 10^{-4} dilution, the bacterial count was $0.66 (\pm 0.04) \times 10^6$ CFU/mL. VCO is known to contain antimicrobial agents such as lauric acid, which disrupt bacterial membranes and inhibits microbial growth (Srivastava *et al.*, 2018). The lower bacterial counts suggest that VCO has potential as a natural antimicrobial agent, but its effectiveness may be limited due to its oil-based nature. Since nipa sap is an aqueous solution, the dispersion of VCO is less uniform, leading to inconsistent contact with bacterial cells and reduced overall efficacy in inhibiting microbial growth. The sample treated with amoxicillin also showed a reduction in bacterial counts, highlighting its antimicrobial potential as a standard antibiotic. However, it was less effective in protecting nipa sap compared to the aqueous and effervescent preservatives. At the 10^{-1} dilution, the bacterial count was $2.15 (\pm 0.08) \times 10^3$ CFU/mL, while at the 10^{-6} dilution, the bacterial count was $0.95 (\pm 0.03) \times 10^8$ CFU/mL. The limited efficacy of amoxicillin may be due to its specificity to certain bacterial strains (Jeet *et al.*, 2022). Moreover, using antibiotics like amoxicillin in food preservation raises concerns about residual antibiotics in food products and the development of antibiotic resistance, making it

a less desirable option compared to food-grade preservatives. Both VCO and amoxicillin were effective in reducing bacterial counts compared to the control, but their antimicrobial activity was not as robust as the combination of potassium sorbate and sodium metabisulfite. VCO offers a natural alternative with mild antimicrobial effects, while amoxicillin provides some bacterial suppression but is unsuitable for food preservation due to regulatory and health concerns. This highlights the advantage of using food-grade preservatives, which balance effectiveness, safety, and practicality for extending the shelf life of nipa palm sap.

4.5 Ligand-Based Structure Drug Design

4.5.1 Docking studies

Glucose oxidase catalyses the oxidation of glucose into gluconic acid, a process that later producing hydrogen peroxide, H_2O_2 (Castro-Muñoz *et al.*, 2022). This reaction can lead to an accumulation of gluconic acid and a reduction in sap pH, creating an environment conducive to microbial fermentation and spoilage (Abidin *et al.*, 2024). Glucose oxidase indirectly supports the breakdown of sucrose by creating conditions favourable to microbial invertase (Flores-Gallegos *et al.*, 2019). By interacting with glucose oxidase, potassium sorbate and its active form may inhibit this enzymatic activity, preventing these undesirable changes, hence extending the freshness of the sap.

The molecular docking of potassium sorbate with glucose oxidase (PDB ID: 3QVR) was conducted using Auto Dock Tools to elucidate its potential role in preserving the shelf-life of nipa palm sap by inhibiting enzymatic activity or microbial growth (Figure 4.10). The docking result confirmed the validity of the simulation, indicating that potassium sorbate interacts with glucose oxidase through strong hydrophobic interaction. The ligand was observed to bind within the active site of 3QVR, specifically interacting with the hydrophobic residue, PHE 564 (Figure 4.11). This residue stabilizes the binding through

interaction with the hydrophobic benzene region of the ligand. Hydrophobic interaction is a reaction that causes protein structures to compact to prevent any contact with water or aqueous conditions. The docking simulations generated 10 distinct conformations, with binding affinities ranging from -4.9 to -4.72 kcal/mol. The conformation with the lowest binding energy of -4.9 kcal/mol was identified as the most stable and indicative of the strongest binding interaction. The results suggest that potassium sorbate forms favourable interactions with glucose oxidase, which may contribute to its preservative effect by interfering with the functionality of enzyme. By interfering with enzymatic activity in nipa palm sap, potassium sorbate may hinder processes essential for microbial proliferation, thereby extending the shelf life of nipa palm sap.

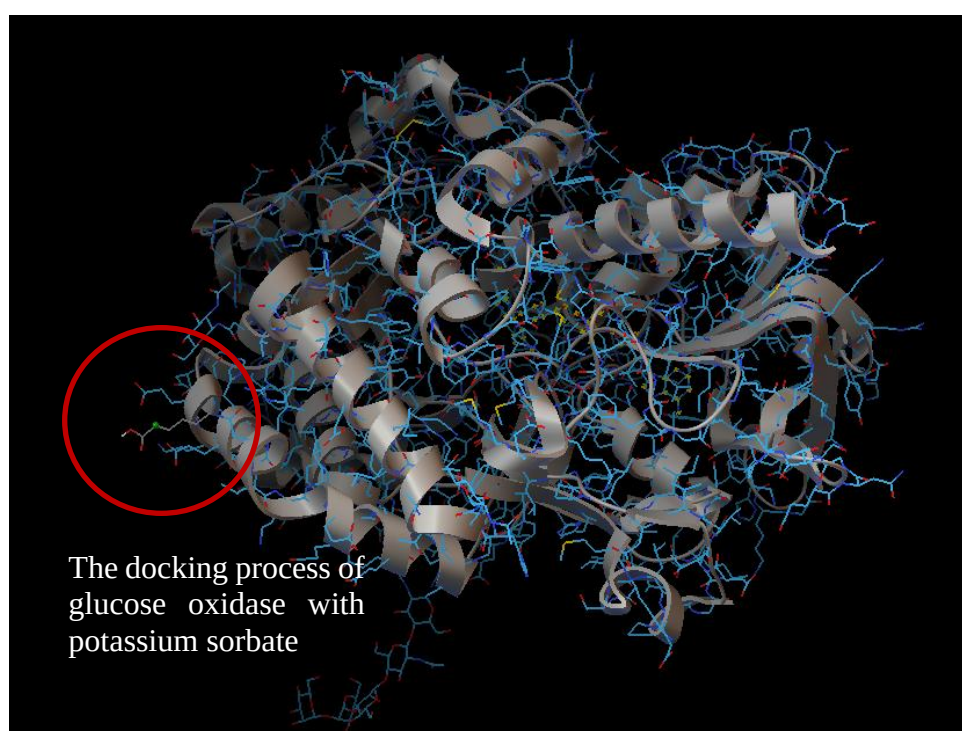


Figure 4.10: Molecular docking of glucose oxidase with potassium sorbate at binding site

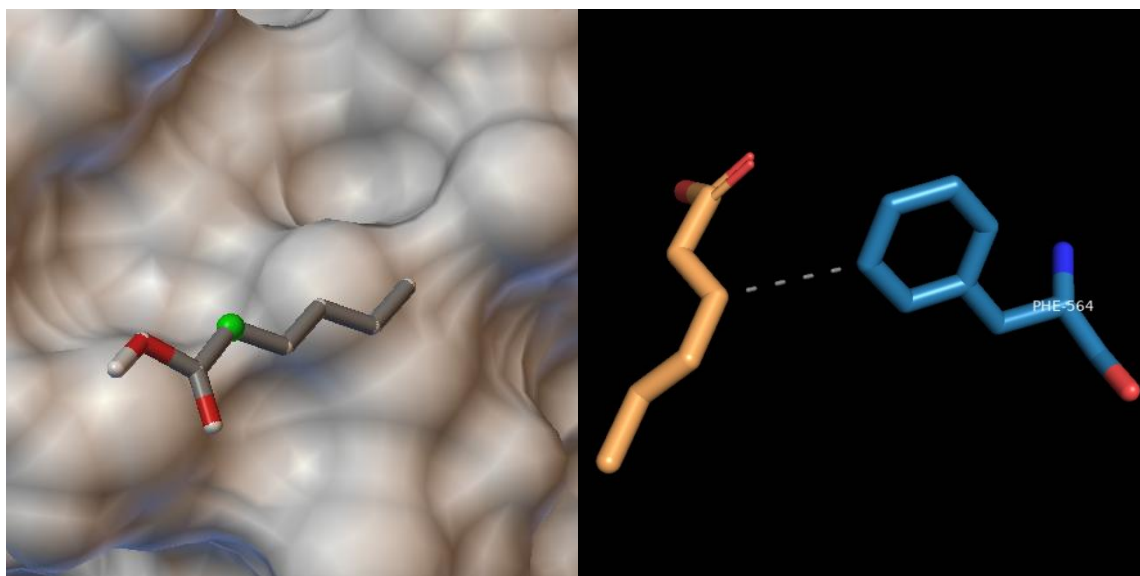


Figure 4.11: The interaction of glucose oxidase protein with binding site of potassium sorbate

To validate the docking interaction, the active site of potassium sorbate, known as sorbic acid, was re-docked into the 3QVR structure (Figure 4.12) using the Auto Dock Tools. The conformation with the highest binding affinity, -5.26 kcal/mol, was selected further analysis. This result suggests a strong and favourable interaction, involving two strong hydrogen bonds. The hydrogen bond interactions between the protein and docked compounds were assessed to determine the stability of the best dock position of these compounds by highlighting the main residues engaged in hydrogen bond formation. According to Jack *et al.* (2022), hydrogen bonds are classified as strong when their distance falls within 2.5–3.1 Å, and weak when the distance is between 3.1–3.55 Å. Similarly, Suh *et al.* (2004), mentioned that the normal hydrogen bond length is between 2.8–2.9 Å. In this study, SER 291 and GLY 549 were identified as the polar residue (Figure 4.13) involved in the interaction of sorbic acid with 3QVR structure, forming hydrogen bonds distance of 2.02 Å and 3.31 Å, respectively. Previous study suggested that short hydrogen bonds, which have a length of less than 2.6 Å, are frequently observed in enzymes when an –OH (or –NH) bond involves H-bonding with a nearby oxyanion. These bonds, often referred as “short strong

hydrogen bond” as it plays a crucial role in enzyme processes (Suh *et al.*, 2004). The hydrogen bonds formed between the backbone carbonyl group of sorbic acid and the hydroxyl groups (–OH) of SER291 and GLY549 provide additional stabilization to the inhibitor within the binding pocket. This stabilization enhances the inhibitory effect of sorbic acid on glucose oxidase activity. By inhibiting glucose oxidase, sorbic acid disrupts the microbial processes that produce glucose oxidase, hence preserving sucrose levels in the treated sap samples. Moreover, glucose oxidase can lead to an accumulation of gluconic acid, which lowers the pH of the sap, creating an environment conducive to microbial fermentation and spoilage (Abidin *et al.*, 2024). By limiting gluconic acid production, sorbic acid helps maintain the pH of the sap, reducing conditions that favour microbial fermentation. This dual action, preservation of sucrose and maintenance of pH stability highlights the effectiveness of sorbic acid in extending the shelf life of nipa palm sap.

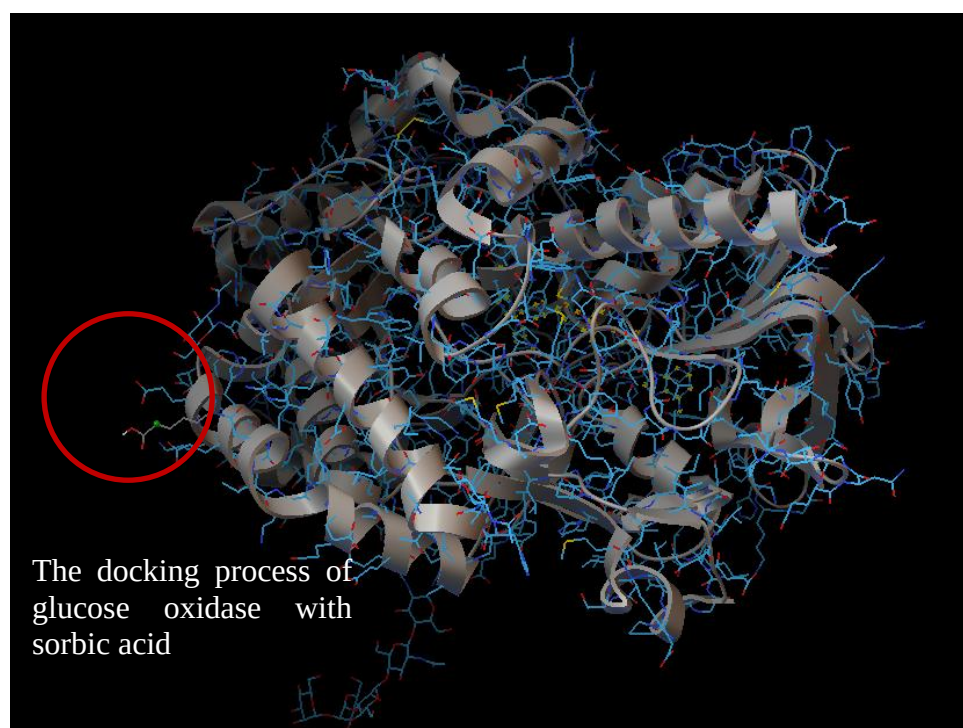


Figure 4.12: Molecular docking of glucose oxidase with sorbic acid at binding site

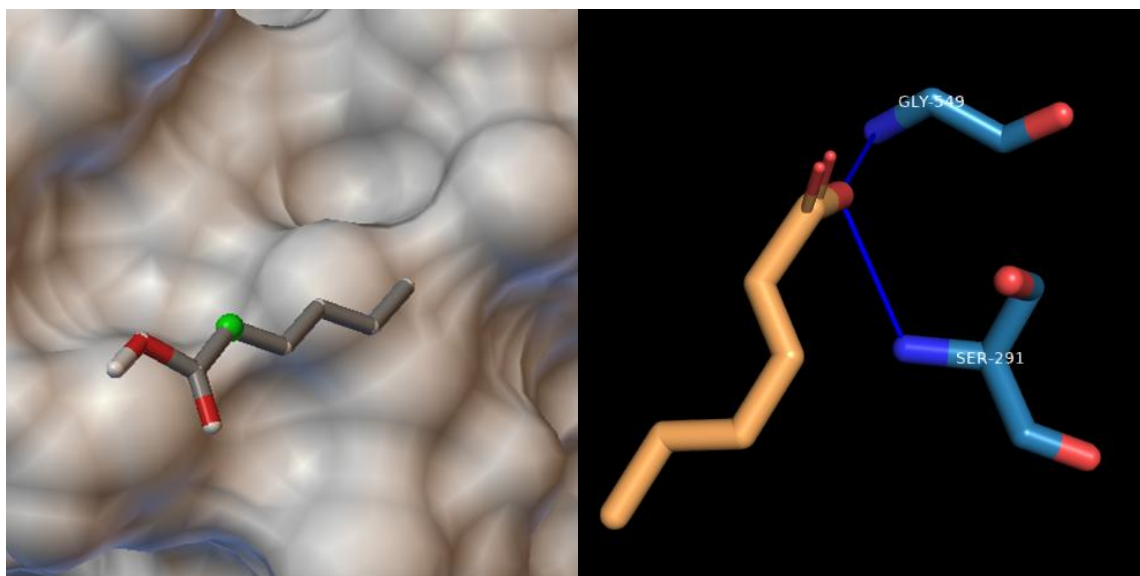


Figure 4.13: The interaction of glucose oxidase protein with binding site of sorbic acid

Figure 4.14 shows the molecular docking of glucose oxidase with lauric acid in VCO using the Auto Dock Tools. The results showed that VCO had a binding affinity of -6.75 kcal/mol, indicating strong interaction with glucose oxidase. This suggests that VCO can be used as a natural preservative with antibacterial properties. It was reported that the lauric acid in the VCO can convert into monolaurin, which destroys the lipid membrane of bacteria (Srivastava *et al.*, 2018). The PLIP results (Figure 4.15) show that lauric acid forms hydrophobic interactions with residue ILE 94 and PHE 564 with bond distances of 3.47 Å and 3.34 Å, respectively. These higher binding affinities indicate greater specificity and potent inhibitory effects on microbial enzymes (Jack *et al.*, 2022). On the other hand, the molecular docking of glucose oxidase with amoxicillin (Figure 4.16) showed a binding affinity of -2.74 kcal/mol. The weaker binding affinity of amoxicillin indicates a less favourable interaction with glucose oxidase, although it still exhibited hydrogen bonding and hydrophobic interactions with the enzyme (Figure 4.17). The hydrophobic interactions involved amino acid residues of TYR 80, ALA 292 and VAL 518 with a bond distance of

2.76 Å, 3.00 Å and 3.56 Å, respectively, while the hydrogen bonds were form with SER 96, SER 103, SER 291 and GLY 517.

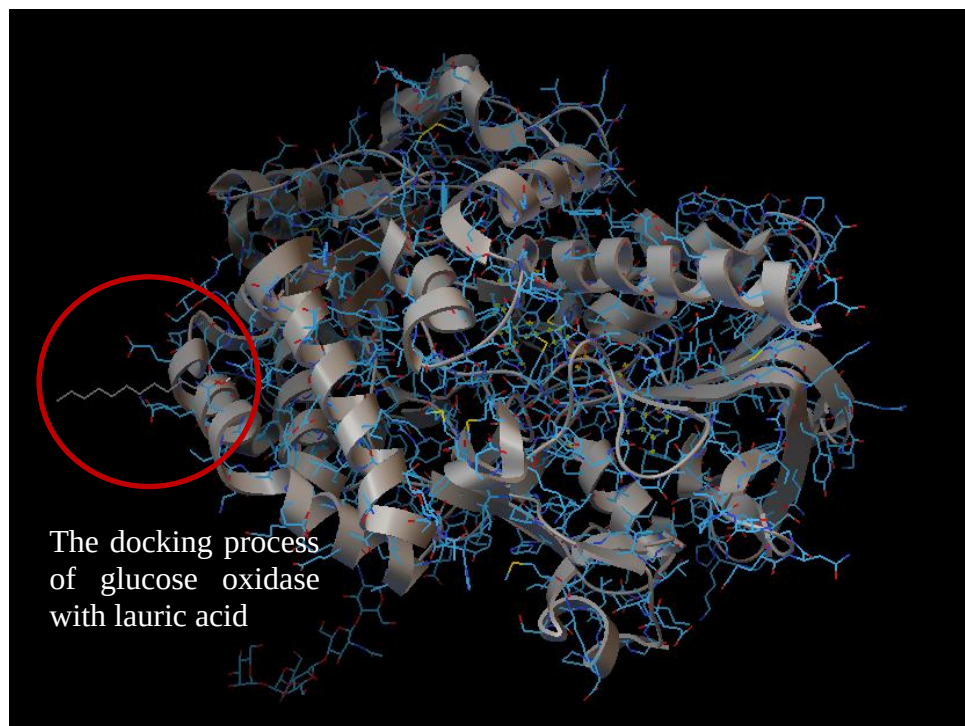


Figure 4.14: Molecular docking of glucose oxidase with lauric acid at binding site

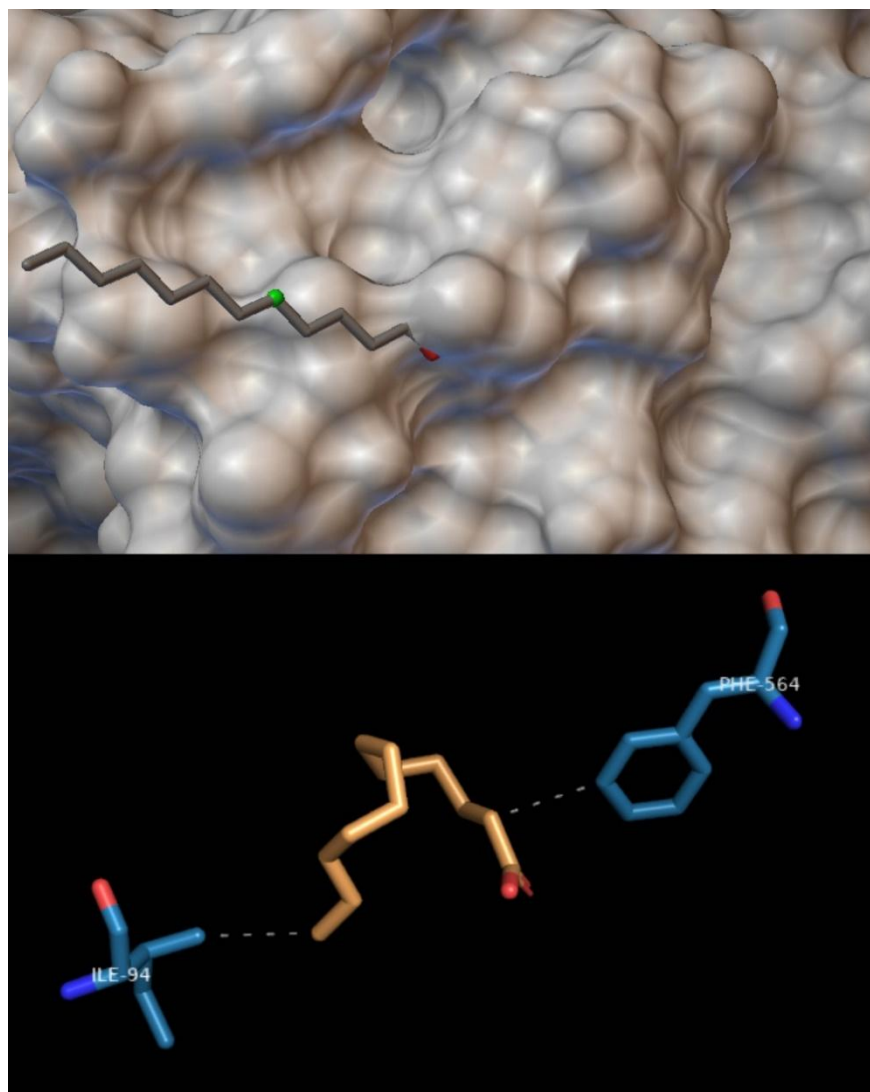


Figure 4.15: The interaction of glucose oxidase protein with binding site of lauric acid

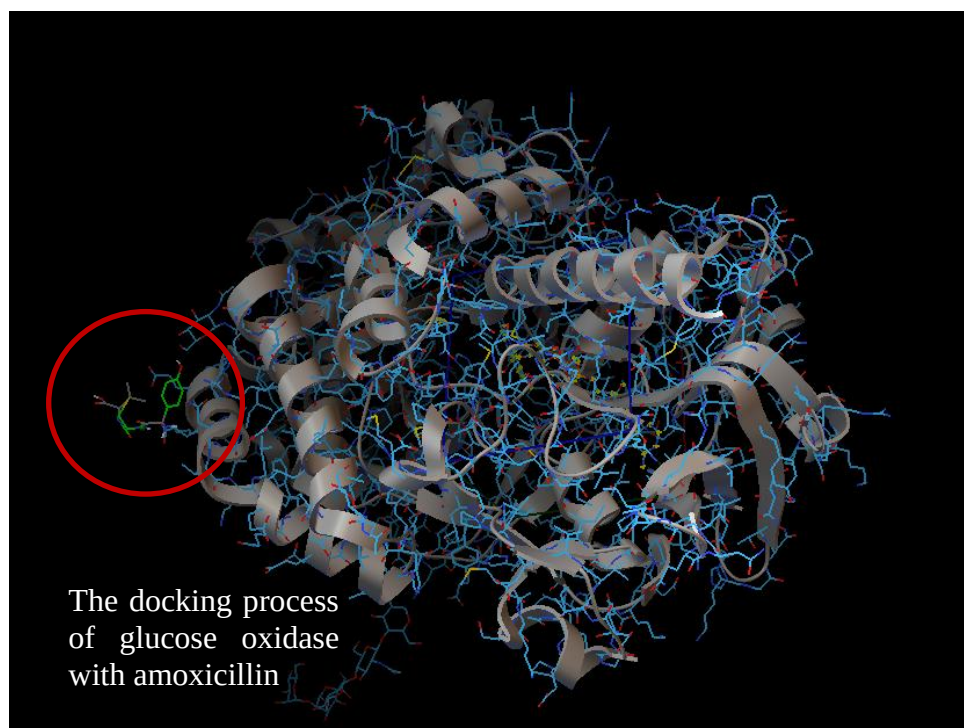


Figure 4.16: Molecular docking of glucose oxidase with amoxicillin at binding site

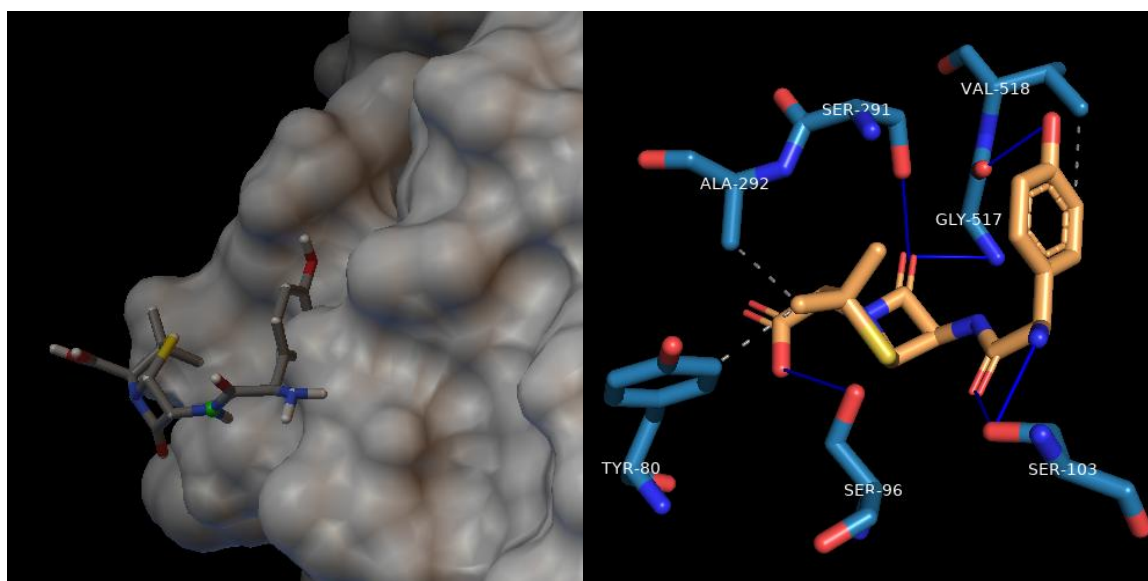


Figure 4.17: The interaction of glucose oxidase protein with binding site of amoxicillin

For comparison, the molecular docking of glucose oxidase with other reference drugs such as ibuprofen (Figure 4.18) and aspirin (Figure 4.19), was also conducted using the Auto Dock Tools. Both ibuprofen and aspirin showed strong binding results with the glucose oxidase receptor, with energy binding affinities of -6.85 kcal/mol and -8.43 kcal/mol,

respectively. These higher binding affinities indicate greater specificity and potent inhibitory effects on microbial enzymes (Jack *et al.*, 2022).

The ligand–receptor complex from ibuprofen docking showed interactions such as salt bridge formation, hydrophobic interactions and hydrogen bond (Figure 4.20). Based on the result via PLIP, the ibuprofen-glucose oxidase complex formed hydrogen bonds with TYR 515 at a bond distance of 1.92 Å, along with hydrophobic interaction with amino acid residues of TYR 80, ALA 292, VAL 293, VAL 518, and PHE 564. Besides hydrophobic interactions and hydrogen bonds, salt bridge might also play vital roles in the complex. Salt bridge formation is defined as having two ionized molecules, namely, one with a hydrogen bond and the other with an electrostatic to react in a non-covalent way (Jack *et al.*, 2022). Ibuprofen has salt bridge interaction with amino acid residue of glucose oxidase, HIS 516. Similarly, aspirin formed hydrogen bonds with SER 103 and TYR 515 with bond distance of 2.29 Å and 1.96 Å, respectively (Figure 4.21). Aspirin was also found forming hydrophobic interaction involving amino acid residues such as VAL 518, VAL 560 and PHE 564.

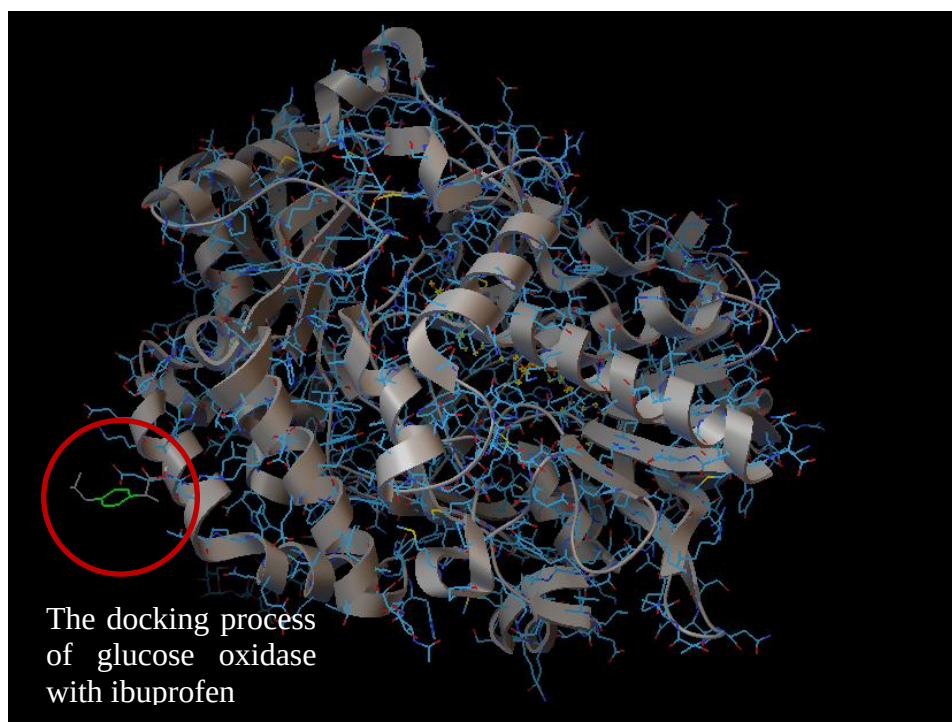


Figure 4.18: The interaction of glucose oxidase protein with binding site of ibuprofen at binding site

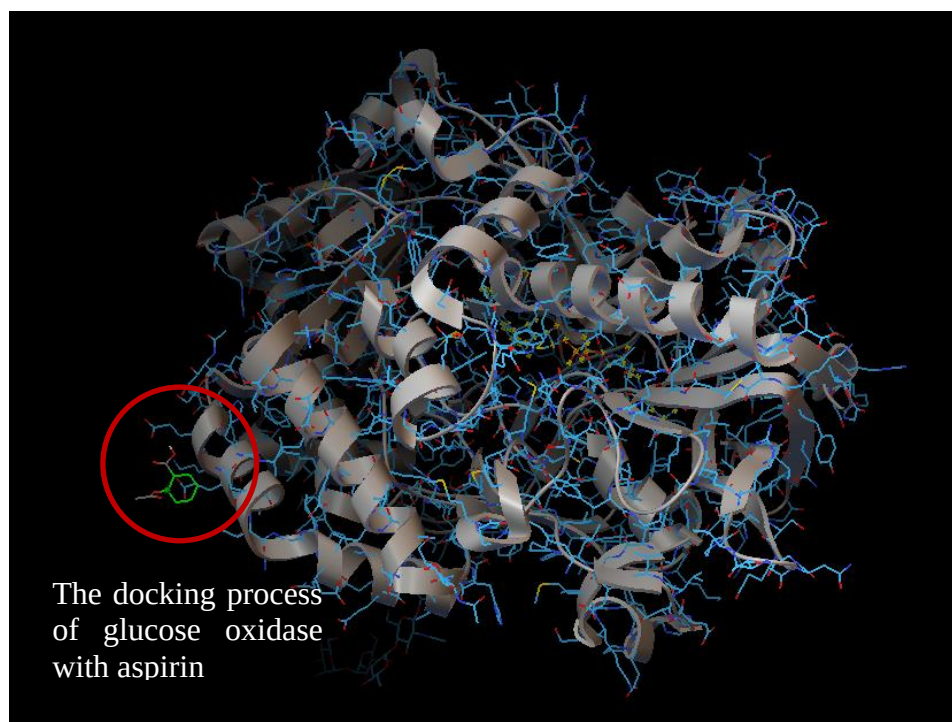


Figure 4.19: The interaction of glucose oxidase protein with binding site of aspirin at binding site

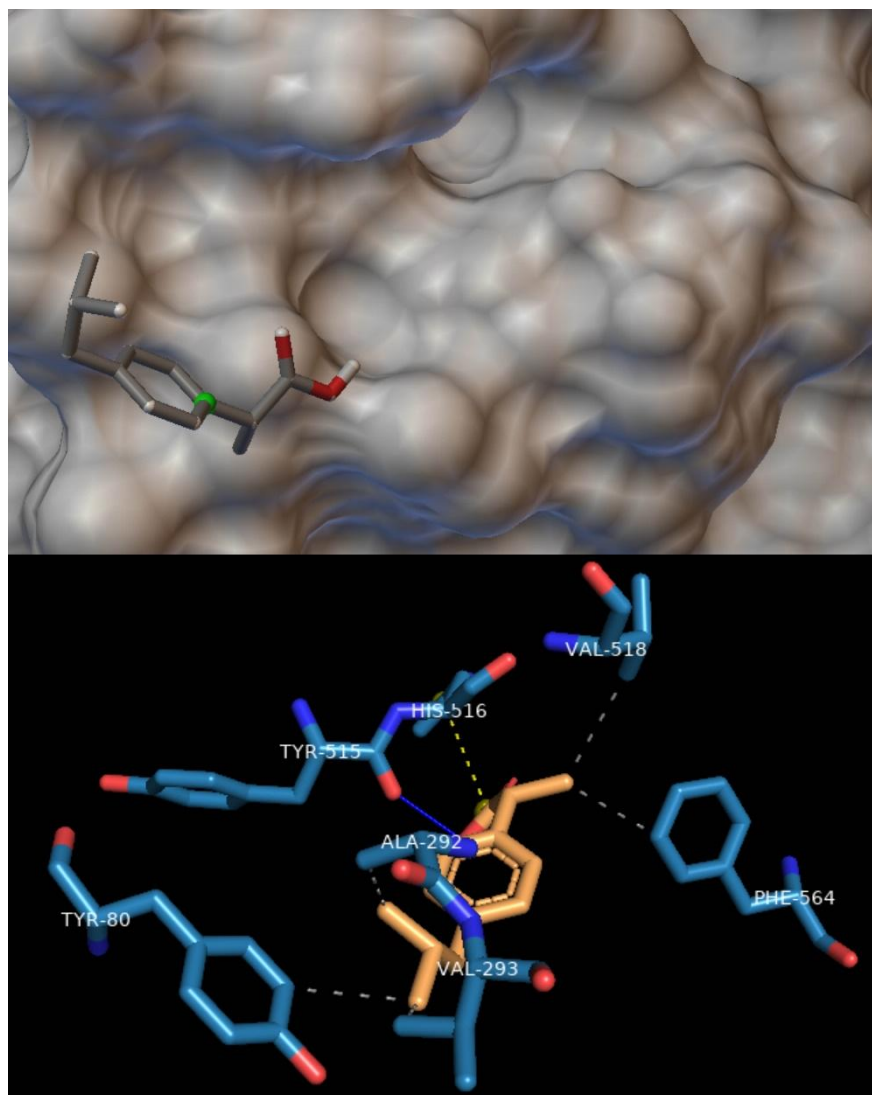


Figure 4.20: The interaction of glucose oxidase protein with binding site of ibuprofen

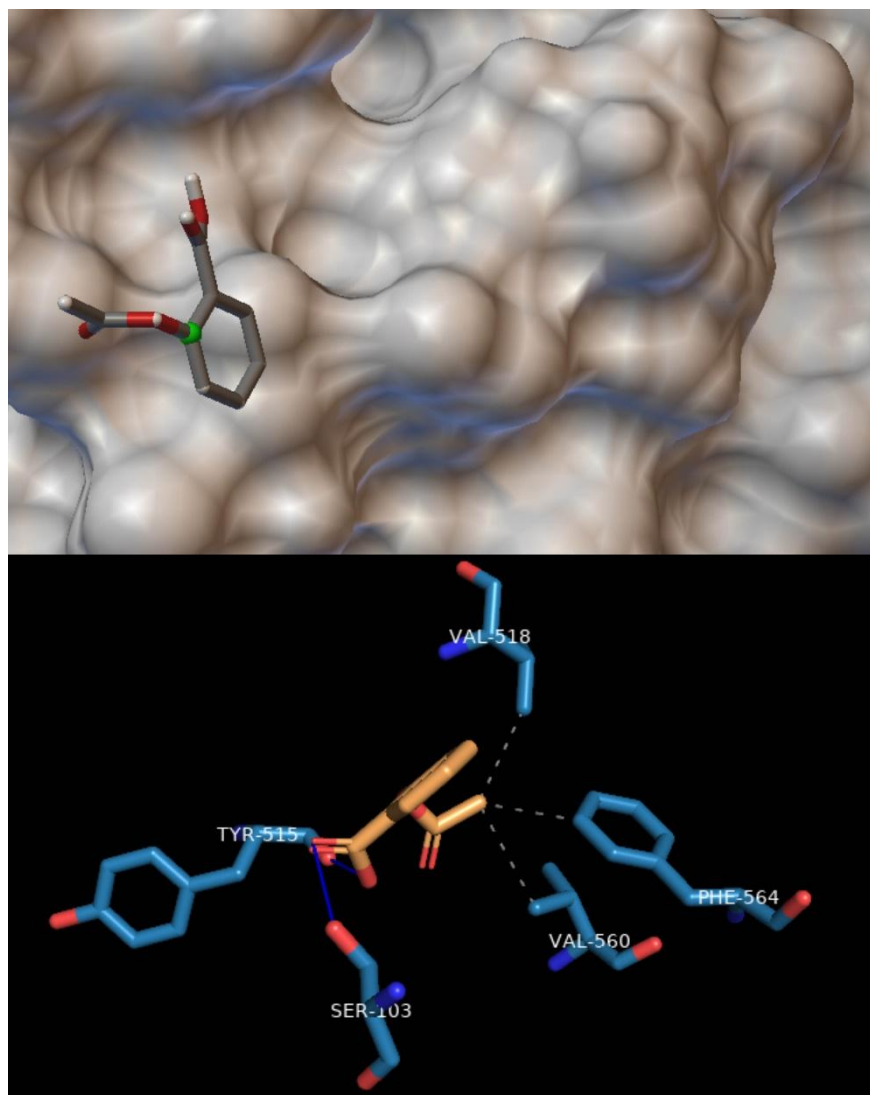


Figure 4.21: The interaction of glucose oxidase protein with binding site of aspirin

Although the binding affinity of potassium sorbate and its active form are weaker compared to VCO (lauric acid), aspirin and ibuprofen, its primary role as a preservative is to inhibit enzymatic reactions indirectly, reducing microbial growth and spoilage. By targeting glucose oxidase, potassium sorbate slows enzymatic activity that facilitates microbial spoilage, thereby complementing its antimicrobial properties. Additionally, the inhibitory effects of potassium sorbate on microbial enzymes further enhance its role as a preservative, creating a synergistic effect in extending the shelf-life of the sap (Pereira *et al.*, 2013). Although antibiotics like aspirin and ibuprofen exhibit higher binding affinities and

stronger antimicrobial actions, their use in food preservation is limited due to potential health concerns and regulatory limitations. Their primary role as a pharmaceutical antibiotic raises potential health risks, such as allergic reactions and the development of antibiotic resistance, making them impractical for food preservation purposes (Kirchhelle, 2018; Wang *et al.*, 2024). Besides, the cost of VCO compared to synthetic food-grade preservatives like potassium sorbate may make it less practical for large-scale or commercial applications. Thus, potassium sorbate serves as a safer and more practical alternative for preserving food products like nipa palm sap. Its ability to maintain sap quality through the inhibition of glucose oxidase and preservation of sucrose levels, coupled with its role in pH stabilization, makes it an effective solution for extending the shelf life of nipa palm sap.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

This study demonstrated the possible use of E' food grade preservative which is potassium sorbate (E202) and sodium metabisulfite (E223). It can be concluded that both effervescent fermentation retardant granules and neutralizing aqueous solution of nipa palm sap preservative can improve the shelf-life of nipa palm sap up to 8 hours by maintaining its sensory qualities, pH and sugar content. Sodium metabisulfite exhibits antioxidant properties by acting as a reducing agent. It reacts with oxygen, thus protecting the sap from oxidative damage. This helps maintain the sensory qualities and nutritional content of the sap while preventing microbial growth in the sap. This study also highlights the effectiveness of using these preservatives combination in enhancing the shelf-life of nipa palm sap compared to the use of these preservatives in chemical powder alone. Sugar content can also be maintained properly by using this preservative as it only has a minimal impact on the chemical composition of nipa palm sap. Neutralizing aqueous preservatives may offer better stability and effectiveness over time as they remain dissolved and active in the liquid phase. This is due to the efficacy of preservatives in effervescent granules that may depend on the dissolution rate and interaction with other components in the sap. Moreover, being in liquid form, neutralizing aqueous solution can be easily mixed into the nipa palm sap, ensuring uniform distribution of the preservatives throughout the sap which may aid in inhibiting microbial growth and fermentation. It can be concluded that the choice between aqueous preservatives and effervescent granules for preserving nipa palm sap depends on factors such as desired shelf-life extension, preservation efficacy and ease of application. This study also

provided a better understanding of the preservation method of nipa palm sap which is important in order to improve the nipa sugar industry in Sarawak. The method of adding preservative inside the empty vessel before the tapping process ensures immediate protection for the nipa palm sap as any microbial activity that may initiate during the tapping process is inhibited. Additionally, the antioxidant activity of sodium metabisulfite complements the preservative effect of potassium sorbate, creating a stable environment that preserves the quality of the sap. By preventing both microbial growth and oxidative reactions, this combination helps maintain the high sucrose content and low reducing sugar levels in the sap, which are important indicators of quality in nipa palm sap. Furthermore, through molecular docking studies, potassium sorbate demonstrated favourable interactions with glucose oxidase, including hydrophobic and hydrogen bond interactions, which contribute to its inhibitory effects. Despite its binding affinity is lower than VCO (lauric acid) and reference drugs like aspirin and ibuprofen, potassium sorbate can be role as a safe and practical preservative makes it ideal for extending the shelf life of food products. By maintaining sucrose levels and preventing spoilage, potassium sorbate highlights its potential as a reliable preservative for nipa palm sap.

5.2 Recommendations

This study helped to clarify how pH level, sugar concentration, reducing sugar concentration and microbial growth of *Nypa fruticans* sap are impacted by the preservatives applied to the fresh sap. This study showed the trend of pH on the fresh nipa palm sap at different times after preservative application. It is possible to investigate the effect of preservatives on palm sugar which may impact the product's quality. To maintain the quality of nipa palm sap during transportation, this research may be able to reduce the microbial development. To raise the quality standard of fresh nipa palm sap, a sensory analysis study

with a higher number of panels could be conducted as this will help in improving consumer acceptability, hence raising the economic value of *air nira*. It is important for future studies to continue exploring and comparing various preservatives and preservation methods. This includes investigating alternative formulations, concentrations and application techniques to optimize the preservation of nipa palm sap for extended periods without affecting its quality or taste. Future studies should explore sustainable and environmental-friendly preservative options as it may offer new insights into inhibiting fermentation and extending the shelf-life of the sap.

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APPENDICES

Appendix A: Copy of accepted journal article for publication in BioMed Central (BMC)



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Effect of preservative and preservation methods on physical, chemical and microbiological properties of nipa palm (*Nypa fruticans*) sap

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Short Report

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Abstract

Objectives

Nipa palm sap that has undergone fermentation is creamy white in colour with an unpleasant aroma and taste, which makes it unacceptable for consumption. This study aimed to evaluate the effectiveness of the E' food grade preservative – potassium sorbate (E202) and sodium metabisulfite (E223) to extend the shelf-life of nipa palm sap, hence determining the effects of different preservation methods on its sucrose content and total bacterial count (TBC).

Results

The result showed that samples during post-sap collection were found more susceptible to microbial growth and contamination than those samples for pre-sap collection. Upon collecting, the initial pH of Sample A was higher (6.33) than Sample B (5.52) and control sample (5.52). Meanwhile, the TBC were the highest in the control sample (2.83×10^5), followed by Sample B (1.14×10^5) and the lowest TBC were in Sample A (1.03×10^5).

Conclusion

Compared to utilizing either potassium sorbate or sodium metabisulfite alone, the combination of the two proved to be far more successful in preserving the palm sap and adding it during pre-sap collection was found more effective to slow down the natural fermentation, rendering longer keeping quality up to 8 hours ensuring colour, flavour and aroma.

Introduction

Nipa palm sap has a short shelf-life and is easily fermented. Nevertheless, its fermentation process can be controlled using various applications of chemicals or bacterial anti-ferments such as sodium metabisulfite, potassium sorbate, benzoic acid, calcium hydroxide and lime water [1, 2]. The chemical preservative helps to inhibit the growth of bacteria, moulds or yeast by disrupting cell division, permeability of cell membrane and activity [3].

Nipa palm sap contains high sucrose which makes it an ideal medium for bacterial growth under direct sunlight. The addition of preservative may help to slow down the sucrose damage, hence increasing the shelf-life of nipa palm sap and producing more derivative products [4]. The natural fermentation of palm sap occurs when microbial activities break down carbohydrates in an anaerobic condition, producing ethanol and various organic acids. Fermented palm sap will produce unpleasant aroma, sour taste and its pH becomes more acidic, which is not suitable for consumption. These properties will hinder the production of palm sugar [5].

Methods

Preparation of nipa palm sap preservatives

In order to control or slow down the spontaneous fermentation in the nipa palm sap, E' food grade preservatives – potassium sorbate (E202) and sodium metabisulfite (E223) were used. In this formulation, water served to ensure both potassium sorbate and sodium metabisulfite mix thoroughly. Additionally, sorbic acid is formed when potassium sorbate dissolves in water [6]. Table 1 and Table 2 below shows the formulation and characteristics of the preservative for each formulation code.

Table 1
Different concentrations of neutralizing aqueous solution for high quality of nipa palm sap

Ingredients (g)	Formulation		
	F1	F2	F3
Potassium Sorbate	15.99	27.10	8.79
Sodium Metabisulfite	2.05	3.47	1.13
Water	81.96	69.43	90.08

Table 2
Characteristics of neutralizing aqueous solution for high quality of nipa palm sap

Formulation	F1	F2	F3
Characteristics			
Physical appearance	Yellowish	Dark Yellow	Pale Yellow
pH	6.94	7.31	6.86
TDS (ppt)	10	10	10
Conductivity, EC ($\mu\text{S}/\text{cm}$)	20	20	20
Salinity (g/kg)	0	0	0

Potassium sorbate, sodium metabisulfite and water were weighed accurately with analytical balance and were mixed using a double boiling method with continuous stirring on a hot plate. The mixture was then placed in a conical flask and allowed to be cool for 30 minutes. F3 was preferable to be the most suitable formulation for controlling the natural fermentation of nipa palm sap. It was slightly acidic; hence, it should be able to effectively inhibit the growth of spoilage microorganisms, thereby extending the shelf life of the sap and maintaining its quality for producing nipa palm sugar. The lower the pH, the greater the antimicrobial activity of the preservatives.

Collection and preservation of nipa palm sap

The nipa palm saps used were collected from Kampung Pinggan Jaya, Sarawak. 7 different palms were randomly chosen. The nipa palm sap was obtained by cutting the stalk of matured nipa fruit and collected using a plastic bottle. All of the samples were obtained 12 hours after it was tapped in the evening. At least 15 g of nipa palm sap preservative was placed into three separate sap collection vessels of 1.5-2 litres (labelled as A1, A2 and A3) prior to collection, allowing it to sit overnight (12 hours duration). After overnight sap collection, an additional four samples (labelled as B1, B2, B3 and control) were obtained. The preservative was added to samples B1, B2 and B3, followed by gentle shaking to ensure even distribution of the preservative within the sample. The components in the nipa palm sap were able to dissolve fully due to the use of an aqueous solution. Control sample was collected without adding any preservative in the collection vessels.

Determination of pH

The pH of nipa sap was determined using a pH meter after calibration with a standard buffer at room temperature. The pH was measured in triplicates for all samples and a graph of data was generated based on its average.

Determination of Dissolved Sugar Content

The dissolved sugar content (%), specifically sucrose was determined using a refractometer dissolved sucrose brix meter. The average was calculated, and a graph has been plotted.

Determination of Microbial Growth Activity

With a slight modification, the total bacterial count (TBC) was determined according to the method adopted by Jaraee et al. [7]. The microbial growth of untreated and treated nipa sap was determined by using plates agar count. A surface plating using the standard spread plate method was done. 1 mL of treated nipa sap containing preservative was serially diluted with saline solution to prepare some appropriate dilutions (10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} , 10^{-5} and 10^{-6}). 0.1 mL of each dilution was poured and spread plated on respective agar plates. Each sample was done in triplicate. The agars were incubated at 37°C for 24 hours. After that, the colonies in incubated plates were counted. Another experiment was done by replacing the treated nipa sap with untreated nipa sap.

Discussion

Figure 1: Fresh nipa palm sap that contains preservative

From the results, it was observed that the addition of preservative did not affect the resulting colour (as in Figure 1), taste and aroma of the nipa palm sap as the nature of potassium sorbate as well as sodium metabisulfite were odourless. The colour of sample without preservative that acts as the control changed into creamy white within 2 hours, indicates that the fermentation process took place. The taste also turns sour and produces an unpleasant aroma. In contrast, the colour of Sample A was slightly darker than the control, yet after 2 hours, it still retained a pleasant sweetness in both taste and aroma. For Sample B, the preservative was applied upon the collection. Initially, its physical appearance was the

same as the control. However, after 2 hours, its colour, aroma and taste remained unchanged. According to the local villagers, the yellowish colour of the nipa palm sap indicates a good sugar quality and the freshness of the sap. In this study, sodium metabisulfite works as a preservative by inhibiting the growth of yeast, molds and bacteria in the sap which contributes to undesirable smells [7]. It also possesses antioxidant properties that help in preventing oxidation reactions which can alter the aroma and degrade the colour of the sap. As reported by Nur Aimi et al. [8], fermented nipa palm sap contains ester, diacetyl, acetoin and alcohol which contribute to the development of unpleasant and malty aromas.

Figure 2: Changes of acidity in control, sample A and sample B of nipa palm sap during 12 hours of storage.

The effect of nipa palm sap preservative on the pH as well as the shelf-life of nipa palm sap was investigated. From Figure 2, it can be deduced that the preservative used was effective against the bacteria. It was observed that the initial pH value of nipa palm sap in Sample A was the highest (6.33). The preservative used may possess microbicidal activity that kill the bacteria or hinder the bacteria from growing, hence improving the shelf-life of the nipa palm sap. Upon collecting, the initial pH of Sample A was higher than Sample B (5.52). The difference was due to the preservative being introduced first inside the container of Sample A before the tapping began. In contrast, for Sample B, preservative was added 12 hours later which was during the sap collection or harvesting process. These two methods were applied to determine the optimal time to add the preservative, hence identifying the most effective approach. From the results, it can also be observed that the pH value of Sample A remained unchanged, meanwhile the pH value of Sample B decreased to 5.41 during the first hour after collecting the samples. As for the control, the pH starts declining slowly during the first 2 hours, followed by a more significant drop thereafter. After 12 hours of fermentation process, the pH value of nipa palm sap that acts as the control was the lowest (4.21), followed by Sample B (5.17) and Sample A (5.54). The decrease in pH indicates an increase in the production of lactic and acetic acids during the fermentation of nipa palm sap, consequently leading to an increase in ethanol production [7].

Figure 3: Changes of sugar content (% Brix) in nipa palm sap during 12 hours of storage.

Figure 4: Relationship between pH and sugar content of nipa palm sap

Based on Figure 3, it was observed that fresh nipa palm sap was initially rich in sugar content but decreased due to the enzymatic reduction. The bacteria degraded the sucrose into glucose and fructose, causing the sugar content to decrease significantly during fermentation. The results here were concurrent with those reported by Jaraee et al. [7]. The decrease in sugar content also indicates the increase in reducing sugar content. The fermentation causes the sugar content to be decreased as the sap was converted to alcohol [2]. It was also suggested that microbes and other potential contaminants could be introduced during the unhygienic tapping or collecting process causing the sucrose content to drop. The decrease in sucrose content may be resulted from an inversion reaction catalysed by invertase activity and an acidic condition caused by microbial metabolic activity [9]. As shown in Figure 4, the control sample of pH 5.52 showed the highest sugar concentration which was 17% Brix and reduced to

about 11% Brix at pH 4.21. The amount of sucrose decreased rapidly without the use of preservatives. The addition of preservatives showed a significant effect on the sugar content of nipa palm sap as it slowed down the fermentation process and reduced the acidic substances in nipa palm sap, thus pH decreases can be maintained [10]. Besides, sodium metabisulfite helps stabilize the sugar which minimizes or prevents the sugar inversion that can occur due to the acidic conditions [11]. TBC was done to determine the microbial quality of nipa palm sap after 24 hours of storage. The microbial growth of the nipa palm sap was analysed after 24 hours of incubation as shown in Table 3.

Table 3: Average of Total Bacterial Count (cfu/ml) of nipa palm sap at different concentrations

Sample	Average of Total Bacterial Count (cfu/ml)					
Dilution	10 ⁻¹	10 ⁻²	10 ⁻³	10 ⁻⁴	10 ⁻⁵	10 ⁻⁶
Control Sample	TNTC	TNTC	2.83 x 10 ⁵	1.89 x 10 ⁶	1.51 x 10 ⁷	1.30 x 10 ⁸
Sample A	1.56 x 10 ³	1.18 x 10 ⁴	1.03 x 10 ⁵	TFTC	TFTC	TFTC
Sample B	TNTC	1.30 x 10 ⁴	1.14 x 10 ⁵	0.83 x 10 ⁶	0.40 x 10 ⁷	TFTC

The main objective of adding a preservative was to inhibit the growth of bacteria and to extend the shelf-life of nipa palm sap. Results obtained showed that the preservative, composed of potassium sorbate and sodium metabisulfite, works effectively against bacteria. It acts as a protective agent, preventing the spoilage and deterioration of the sap during storage. In the formulation, water was used to ensure potassium sorbate and sodium metabisulfite dissolve and mix properly, but it also aids in converting potassium sorbate to sorbic acid. Sorbic acid possesses antifungal and antimicrobial properties. Therefore, the combination of sodium metabisulfite and sorbic acid kills or inhibits growth of microbes responsible for spoiling nipa palm sap [12]. A higher number of TBC was observed in the control sample as it does not have any substances that impede the growth and proliferation of bacteria which naturally occur in the nipa palm sap. Therefore, as the control sample lacked microbial inhibitory measures, its total bacterial count was higher than that of sample A and B, which contained a preservative. The control sample was susceptible to microbial contamination during collection and storage period without the presence of preservative. According to Oluwole *et al.* [4], nipa palm sap is rich in nutrients and serves as an ideal medium for the growth of bacteria. The natural sugars and other components in the sap provide a favourable condition for microbial colonization and reproduction. Both sample A and sample B contain preservatives, however the number of TBC in sample A was lower than sample B. The time of the application of preservative influencing the microbial proliferation in the nipa palm sap. In sample A, the preservative was introduced before starting the tapping process. This approach most likely causes the microbial growth to be inhibited at an early stage and any contaminants that might have occurred during the collection process are immediately encountered by the antimicrobial agents. Conversely, in Sample B, the preservative was added after the nipa palm sap was collected. The gap in time allows the possible contamination and growth of bacteria during the collection process, resulting in higher number of TBC in

sample B compared to sample A. From the results, it was clearly shown that applying preservative prior to the tapping process effectively minimizes the risk of microbial growth.

Limitation

This study's limitation is that environmental factors such as temperature, humidity and exposure to light can impact microbial growth rates and enzymatic activities, hence affecting the overall preservation process.

Declarations

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Author contributions

DNA did the research proposal writing, data collection, data analysis, data interpretation and was a major contributor in writing the manuscript. MRA contributed to data collection, data interpretation and supervision. SAB provided supervision and validation to the project. AFS and KSJ were involved in the sampling collection, processing and data analysis. All the authors read and approved the final manuscript.

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Availability of data and materials

All data generated or analysed during this study are included in this published article.

Ethics approval and consent to participate

Not applicable.

Consent for publication

All authors give consent for the publication of the manuscript.

Competing interests

The authors declare that they have no competing interests.

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Figures



Figure 1

Fresh nipa palm sap that contains preservative

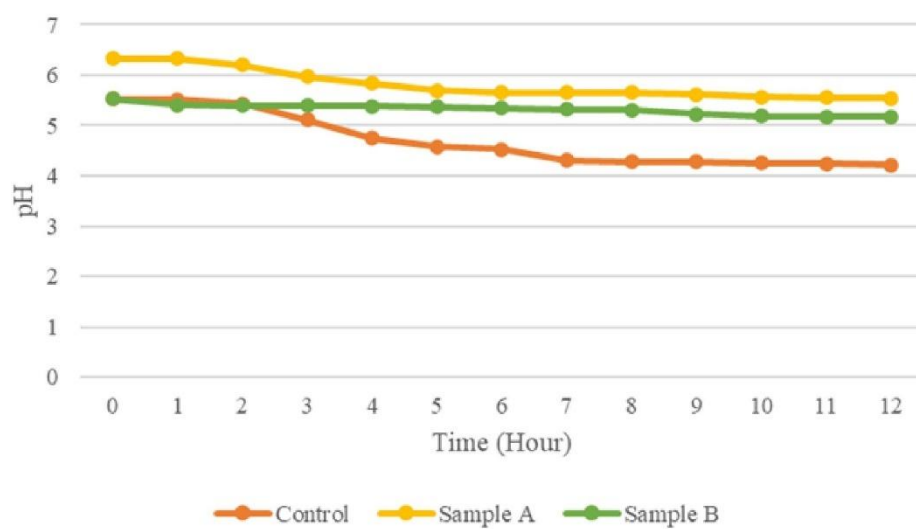


Figure 2

Changes of acidity in control, sample A and sample B of nipa palm sap during 12 hours of storage.

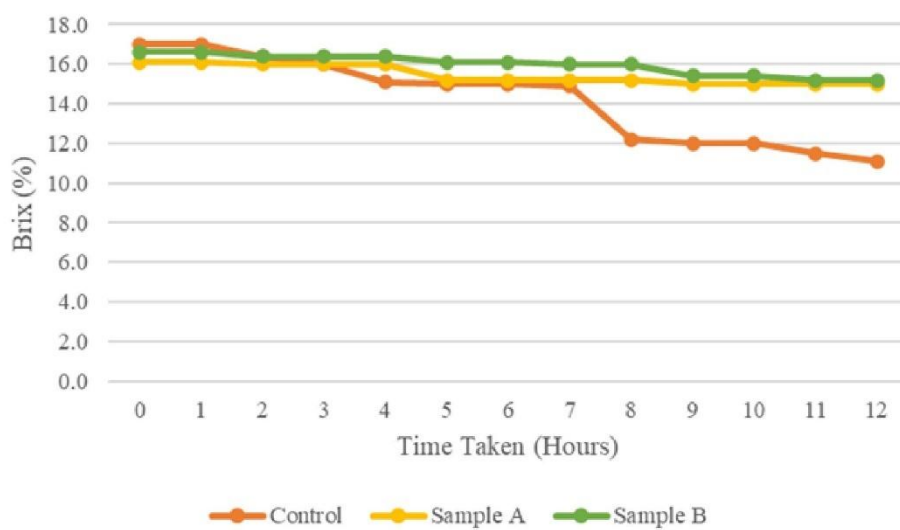


Figure 3

Changes of sugar content (% Brix) in nipa palm sap during 12 hours of storage.

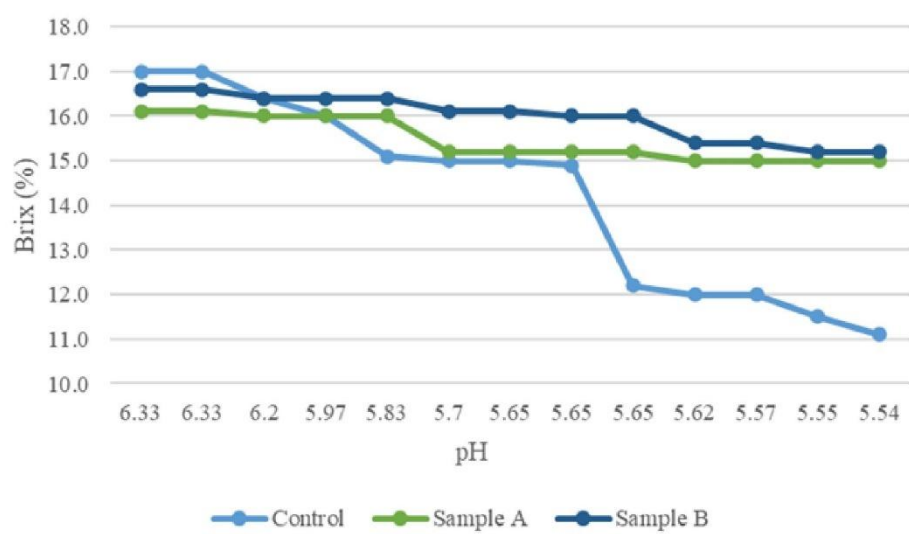


Figure 4

Relationship between pH and sugar content of nipa palm sap

Appendix B: Nipa palm (*Nypa fruticans*) sap and its fruits



Appendix C: Fresh nipa palm sap collected using plastic bottles



Appendix D: Bamboo vessels used to collect nipa palm sap



Appendix E: Measuring the pH of the nipa palm (*Nypa fruticans*) sap samples on-site



Appendix F: Process of determining the total bacterial count in samples



Appendix G: With a local *Gula Apong* producer, En. Bujang



Appendix H: Antimicrobial test for nipa palm sap treated with VCO and amoxicillin

For each 200 mL of nipa sap, 10 mL of VCO and 500 mg of amoxicillin was added to individual bottles and shaken. A 1 mL of sample was serially diluted with saline solution to prepare a 1:10 dilution (10^{-1}) and another appropriate dilution (10^{-2} , 10^{-3} , 10^{-4} , 10^{-5} and 10^{-6}) was also prepared. Using micropipettes, 0.1 mL of each dilution was poured and spread plated on respective agar plates. Each dilution was plated in triplicate. The agars were incubated at 37°C for 24 hours, after which the colonies were counted.