

**Thermo-Chemical Preservation of Date Palm (*Phoenix dactylifera*) Sap:
Effect on Physical, Chemical, and Sensory Properties**

A THESIS

BY

Julkifal Islam

Student ID: 1707059

Session: 2023-2024

Semester: January-June, 2024

MASTER OF SCIENCE

IN

FOOD PROCESSING AND PRESERVATION



**DEPARTMENT OF FOOD PROCESSING AND PRESERVATION
HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY
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ABSTRACT

Date palm sap, a nutritious and popular beverage, is highly prone to spoilage due to rapid fermentation, limiting its commercial potential. In order to prolong the shelf life of date palm sap, this study investigated the effectiveness of combining heat treatment (100°C) with chemical preservatives such as potassium metabisulfite (200 ppm), potassium sorbate (0.15 g), and citric acid (0.02 g) per 100 mL of sap. Freshly harvested sap was treated and concentrated into samples with total soluble solids (TSS) concentrations of 11%, 15%, and 19%, and then stored under refrigerated conditions of 4°C to assess the impact of these treatments on the sap's stability and quality. Throughout the storage period, the samples were evaluated for changes in physicochemical properties, such as pH, acidity, total soluble solids, and sugar content, as well as microbial load and sensory attributes. The results have shown that, while chemical preservatives assisted in preventing microbial development during the first 7 days of storage, heat processing was beneficial in concentrating the sap and enhancing its stability. Although, as time progressed, the effectiveness of these preservation techniques diminished, leading to eventual spoilage. Sensory evaluations indicated that the treated sap maintained favorable qualities during the first 7-day storage period, though some degradation in taste and appearance was observed later. This research highlights the potential for preserving date palm sap through a combination of heat treatment and chemical preservatives; of these, S2 (15% TSS) proved to be the most effective in retaining overall quality throughout storage. S2 showed a balance between microbial stability and sensory attributes, making it the most favorable for short-term preservation. However, further optimization of preservation methods such as, using Pulsed Electric Field (PEF) or High Hydrostatic Pressure (HHP) along with additional chemical preservatives is necessary to ensure long-term stability and offer promising prospects for the sap's commercialization.

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LIST OF ABBREVIATIONS

CFU : Colony Forming Unit

RI : Refractive Index

°C : Degree Celcius

CHAPTER I

INTRODUCTION

One of the world's oldest flowering plants is believed to be the palm (Redhead, 1989). Palm trees include bushes, trees, and perennial lianas. They are found from the 44 northern latitude to the 44 southern latitude and belong to the Arecaceae family (Wood, 2002). Both warm temperate and tropical regions are home to over 2600 different species of palm trees. A popular crop for its delicious sap and fruit, the date palm (*Phoenix dactylifera*) is the fifth-largest fruit crop in the world (FAOSTAT, n.d.).

The date palm is considered the oldest fruit crop and is rich in several therapeutic bioactive and functional substances, including vitamins, minerals, flavonoids, carotenoids, phytosterols, and phytoestrogens (Al-Alawi et al., 2017; Younas et al., 2020). Date palm, palmyra palm, and coconut palm are typical varieties of palm trees in Bangladesh. The wild date palm, sometimes called khejur or khajur, is widely distributed across Bangladesh, particularly in the districts of Jessore, Faridpur, Kustia, Khulna, and Rajshahi (Hussain et al., 2001; Shanta et al., 2021).

Date palm sap, a prized and highly valued product of the *Phoenix dactylifera*, is extracted by making an incision in the tree's vascular tissue, either from the trunk or inflorescence, typically during the winter season when sap flow is optimal. The sap is usually collected early in the morning to prevent fermentation due to its high sugar concentration (Khan et al., 2020). Date palm sap can be consumed in its raw form as a fresh (khejur ros) or fermented beverages such as tari, taddy, wine, and arak (Nath et al., 2015). Additionally, it may be processed to create a range of products, including concentrated syrups (honey), brown sugar (jaggery powder), and jaggery. Chocolate, cake, sweet soy sauce, food coating, ice cream, and other traditional Bengali delicacies are all made using these products.

Furthermore, the primary source of income for some Bangladeshi marginal areas is date palm sap, which is harvested by tapping the delicate tree trunk. About 200–250 L of sap (containing 12–15% sucrose) are collected per tree during the tapping season (winter season), from which 25–30 kg of molasses are produced (Hussain et al., 2001; Rana et al., 2009). Previous research investigations' findings have shown that the most common sugars in palm sugar are glucose, fructose, and sucrose, which make up 91.4 and 89.2% of the sugar, respectively (Apriyantono et al., 2002). Date palm sap has a high tendency of decaying in

both original and concentrated form concerning the flavor, vitamins, color, and nutrients in open conditions due to its high reducing sugars and total sugars (Ahmad et al., 2021; Kulkarni et al., 2010). That's why, most of the sap is used for "gur" (jaggery) production by the farmers. So, it is a prerequisite to developing the technique related to the preservation of juice and derivatives with optimal storage stability for long-term utilization. Processed date palm sap may pave the way for the commercial cultivation of date palm by the farmers. Thus, it can help to reinforce our economic growth by large-scale commercial production.

However, there is little to no information available regarding date palm sap, even if research data on the production and storage of date juice is available. Some studies have investigated strategies to extend the shelf life, improve taste profiles, and preserve the nutritional value of ready-to-serve (RTS) date palm beverages. Though, preserving raw date palm sap remains particularly challenging due to its rapid fermentation, and there has been relatively less focus on preservation techniques that maintain the sap in its natural or less processed state. The short shelf life of raw sap poses significant challenges for broader commercialization, making the development of effective preservation methods a research priority. Studies have explored methods for extending its shelf life through preservation techniques like pasteurization, fermentation, and the use of chemical preservatives (Hebbar et al., 2018; Naknaen & Meenune, 2016).

Thermal processing used to inactivate enzymes and reduce microbial activity. While it significantly enhances the formation of the Maillard reaction, it results in changes to color and sensory attributes, sometimes leading to undesirable outcomes. Furthermore, the volatile components of coconut and palm sugar, such as pyrazine, furan, ketones, fatty acids, and organic acids are what give these sugars their caramel-like sweetness, toasted notes, and nutty flavor (Sohn et al., 1995). When palm sap is heated over 110 °C, pyrazine is released (Barbara and Michael, 2004; Ho et al., 2007). Therefore, combining thermal processing with other preservation techniques, such as ultrafiltration, chemical preservatives and thermal processing is necessary to optimize quality.

Previous studies have also explored the use of chemical preservatives like potassium metabisulfite (KMS) and sodium benzoate for preserving coconut or other palm syrup. While these preservatives extend the shelf life, they may negatively affect sensory qualities such as flavor, aroma, and color. Furthermore, scaling production while maintaining quality and meeting the demand for natural, less preservative beverage poses logistical and technical

challenges. In the study by Makhlouf-Gafsi et al., (2018), the researchers combined ultrafiltration and thermal processing to improve the biological properties and sensory qualities of date palm sap syrup.

The combined effect of these techniques on natural date palm sap remains underexplored. This research aims to fill this gap by investigating the effects of heat treatment and chemical preservatives (KMS, potassium sorbate, and citric acid) on the preservation of date palm sap. By analyzing parameters such as total soluble solids (TSS), titrable acidity, pH, sugar content, and microbial load, this study seeks to determine the optimal conditions for preserving the sap while maintaining its natural qualities.

The aim of the study-

1. To identify the effects of heat treatment and chemical preservatives for extending the shelf life of date palm sap while preserving its natural characteristics.
2. To examine the changes in physicochemical properties such as pH, acidity, total soluble solids, and sugar content in treated sap.
3. To monitor microbial load and analyze the sensory qualities of the sap over a refrigerated storage period.

CHAPTER II

REVIEW OF LITERATURE

2.1. Date Palm Tree (*Phoenix dactylifera*)

The date palm tree (*Phoenix dactylifera*) is one of the most ancient and widely cultivated fruit crops in the world. Its origins are traced back to Mesopotamia (modern-day Iraq), where it was cultivated as early as 4000 BCE. Today, it is grown extensively in arid and semi-arid regions, particularly in the Middle East, North Africa, and parts of South Asia. The date palm tree plays a crucial role in both the ecology and economy of these regions, providing food, shelter, and income to local populations.

2.1.1. Characteristics of the Date Palm Tree

- **Lifespan:** Date palms are perennial trees that can live for more than 100 years.
- **Fruit:** The date fruit, also known as a "khejur," is a rich source of energy due to its high sugar content (70-80% when ripe).
- **Sap Production:** In addition to producing dates, the palm tree is also tapped for its sap, especially in countries like Bangladesh and India, where it is a seasonal delicacy.

2.2. Date Palm Sap

Date palm sap, locally known as "khejur ros" in Bangladesh, is a clear, sweet liquid extracted from the tree during the winter season. The sap is collected by making an incision in the tree's trunk or inflorescence, allowing the sap to flow and be collected overnight. The sap is highly perishable, with rapid fermentation setting in soon after collection due to its high sugar content, making preservation an essential step for extending its shelf life.

2.3. Harvesting Sap from Date Palm in Bangladesh

2.3.1. Climbing Technique

Bamboo with side branches was attached with the palm trees for climbing. Sometimes, the climber used a rope for fastening his legs which helped in easy climbing the trees. This method is mainly used for palmyra palm tree and for date palms, old grooves of leaves are used. In this method harvesting was difficult, laborious and time consuming. Sometimes the

climber used spike shoes for easy climbing. Besides, ladder could be used for climbing dwarf trees. Generally, a climber can easily climb date trees by using the steps which were cut in the previous year. Climbing technique is shown in Figure 1 (Hussain, 2001).



Figure 1: Climbing Technique

2.3.2. Cutting and Sap Collection

Matured date palm trees were selected for collection of sap. After cleaning the tree, a V shaped surface was cut by cutting a thin slice on the head of the stem and a hollow circular bamboo stick was embedded into the lower angular point of the V shaped cut. A clean container (pitcher) was placed under outer end of the bamboo stick. The V shaped cut surface was periodically cleaned by cutting a very thin slice on the V shaped surface to allow sap flow into the earthen pot. Generally, sap was collected from one side of the tree in one year and the following years, sap must be collected from the opposite side of the previous years cut. Sometimes, one to two years of gap is given for attaining proper growth of the trees. The cutting and collecting method of sap from date palm are shown in Figure 2 (Hussain, 2001).



Figure 2: Cutting and sap collection

2.3. Quality Profiles of Sap

Palm sap is the phloem sap and known to be very rich in nutrients. The composition and quality of sap are varying with the place and duration of tapping (Borse et al., 2007). The sap has been found with low glycemic index of 35 as compared to refined white sugars with glycemic index of 65 (Trinidad et al., 2010). The fresh sap is rich source of phenolics, vitamins (Vitamin C), protein, essential elements (nitrogen, phosphorus, potassium, and magnesium), and micronutrients (zinc, iron, and copper). It helps in regulating high blood pressure and sugar metabolism due to the presence of potassium. It also acts as a potential antioxidant for overall production of immune system, respiratory system, cardiovascular, and reduction of inflammation. It also aids in electrolyte balance and acid/alkaline ratio (Hebbbar et al., 2015).

2.3.1. Carbohydrate

Generally, palm sap contains mostly sucrose, followed by glucose and fructose. The Ben Thabet et al., 2009; found 95.27% sucrose, 2.51% glucose, and 1.61% fructose in one of the palm species *Phoenix dactylifera*. Many studies have found that sugars with high sucrose

content are known to be good for health. Among the carbohydrate, sucrose causes less fattening effect than glucose or starch (Jentjens and Jeukendrup, 2005). Ruzzin et al., 2005, also reported 15% increase in energy consumption by sucrose without increase in weight gain. The total sugar content of coconut sap is high (16.19g/100mL), compared to its reducing sugar content (0.68). Further, sucrose- and fructose-rich sugars have low glycemic index (GI) and thus the coconut sap is reported to contain low GI (Trinidad et al. 2010). The extent of the sap's freshness can be easily measured by the composition of total and reducing sugars. If the sap is fresh and unfermented, the reducing sugar content should be less than 1.0g/100mL (Hebbbar et al., 2013).

2.3.2. Proteins and Amino Acids

Fresh palm sap is rich in amino acids; it is mainly composed of asparagine and glutamine, which are polar side chain amino acids. It is found that coconut sap contain about 17 amino acids which are the building blocks of proteins and also helps to maintain proper acid/alkaline balance (neutral pH) (Xia et al., 2011). Amino acid content of fresh sap is reported as 2.6gkg⁻¹. The reduction in amino acid of sap was observed on first day due to the production of microorganism. But, there as on for decrease in amino acid during second day might be due to degrade in protein content of sap. The significant decreases in amino acid was also observed on third day, but later it was insignificant reduction which may be due to the less amount of amino acid by death or autolysis of part of the yeast (Jolly et al., 2006). The polar side-chain amino acids are considered as essential precursors to the formation of volatiles during the production of sugar from palm sap, especially in the Maillard reaction, by releasing free amino acid groups (Sohn et al., 1995). The released amino acid substrate catalyzes sucrose into monosaccharides or undergoes retro aldol reactions to produce C2–C5 di carbonyl compounds, depending on cooking temperature and heating time (Martins et al., 2001; Carline and Van Boekel., 2003). All these di carbonyl compounds can react with amino acids to form α -aminoketones and aldehydes. Subsequently, in the later stage the chain reactions take place including dehydrations, cyclization, isomerizations, and retro-aldolizations, which ultimately lead to the formation of volatile compounds. These volatile substances are responsible for the characteristic aroma of palm sugar (Ho et al., 2007).

2.3.3. Minerals

Mineral elements are investigated in the sap originating from various palm species and reported about the predominant presence of potassium and some of the micronutrients. Mineral nutrients have importance in numerous metabolic functions. Indeed, zinc has a fundamental role in the brain, whereas calcium, iron, magnesium, etc., have physiological actions in muscular contraction, blood oxygenation, and coagulation, nervous impulses conduction, blood acid–base balance ensuring, and appropriate immune and heart functions. Some minerals, such as calcium, support the building of bone, teeth, or muscle tissues, while others are main components or activators of enzymes and hormone molecules. Mineral elements represent 4% human body weight and are provided by the food. N'guessan et al., (2015) reported that inflorescence sap produced from various coconuts (*Cocos nucifera* L.) cultivated in Cote d'Ivoire is rich in minerals and showed the varietal difference. Thirteen minerals comprising eight microelements (K, Cl, Si, Na, Mg, P, S, and Ca) and five oligo elements (Fe, Cu, Mn, Zn, and Br) were found in the coconut inflorescence sap. Macro elements in the sap ranged between 1.25 and 90.65 mg/100 g, and oligo elements varied from few traces to 0.70 mg/100 g.

2.3.4. Vitamins

Palm sap contains a wide range of vitamins such as vitamin C and B. It is found that in palmyrah sweet sap (*Borassus flabellifer*), vitamin C content is as high as 13.25 mg/ 100 cc, while for fresh coconut sap, it is 12.86 mg/100 cc. The vitamin C was observed to be decreased during first day of storage, but it increased to 20.7 mg/L during third day due to the activities of yeast during fermentation (Bremus et al., 2006).

2.3.5. Phenolics and Flavonoids

Phenolic compounds influence the color, sensory, nutritional, and antioxidant properties of foods. Free radical scavenging activity of the phenol moiety (hydroxyl substituent on the aromatic ring) is responsible for antioxidant property of phenolics. Generally, flavonoids constitute two third of dietary phenols and phenolic acid occupy remains one-third. These simple phenolic acids have aroused awareness and demand due to the potential antioxidant nature and health advantage. They also have other biological activities, such as blocking the biosynthesis of leukotrienes by caffeic acid (one of the most prominent naturally occurring cinnamic acids) which are involved in immunoregulation diseases, asthma, and allergic

reactions (Koshihara et al., 1984). The total phenolic content of fresh sap is about 0.33 g/L. During fermentation, the phenolic content starts increasing and reached the peak level of 1.24 g/L at 58 h and then there was no significant change (Xia et al., 2011). The reason for increased phenolic content may be due to binding of plant polyphenols with protein, sugar, starch, and cellulose, and formation of glucosidic bonds. The acids produced during fermentation degrade these glucosidic bonds and resulted in the production of phenolic components (Landbo and Meyer, 2004). Phenolic components are also produced due to the metabolism of some microorganism. Xia et al., 2011, reported the presence of caffeic acid, gallic acid, p-coumaric acid, protocatechuic acid, and galangin in coconut sap. Fresh sap was reported to have less amount of these five phenolic compounds than fermented sap. Gallic acid was dominant phenolic compound in fresh sap, and its content was 350 lg/L, whereas caffeic acid content (730 lg/L) was higher in fermented sap.

2.3.6. Volatiles

Fresh palm sap contains a non-objectionable odor and it changed into harsh odor, unpalatable in spite of nutritious during fermentation. Acids and volatiles present in the sap are believed to cause the astringency and harsh odor of fermented sap. Due to the abundant presence of polar side chain amino acids such as glutamine and asparagine and sucrose, volatile compounds are formed and more number of free amino acid groups are released during Maillard reaction in heating palm sap for sugar production (Sohn and Ho 1995). The major flavors such as ethyl lactate, ethyl lactate, 3-hydroxy-2-pentanone, farnesol, phenylethyl alcohol, 2-methyl tetrahydrofuran, and tetradecanone were found in fresh sap. But fermented sap contained 12 volatile compounds which represents more than 95% of the volatiles. Ethyl lactate, phenylethyl alcohol, and farnesol were among the seven compounds which are present in both fresh and fermented sap. The increased amounts of acids such as palmitoleic acid and dodecanoic acid (19.0 mg/L) and higher concentrations of ethyl alcohol and ethyl esters cause to astringency and harsh note to fermented sap (Borse et al., 2007).

2.4. Potential Food Products from Date Palm Sap

The versatile nature of date palm sap allows it to be processed into a variety of food products. These include:

1. **Date Palm Sugar:** When boiled down, the sap crystallizes into date palm sugar (also called jaggery or gur), a traditional sweetener used in many South Asian and Middle

Eastern dishes. The process involves concentrating the sap by heating, which caramelizes the sugars and produces a golden-brown sugar product rich in flavor (Figure 3).

2. **Palm Syrup:** The sap can be concentrated into a thick syrup, often referred to as "date syrup" or "palm honey." This syrup is used as a topping for desserts, pancakes, or as a natural sweetener in beverages (Figure 3).
3. **Fermented Beverages:** If allowed to ferment, date palm sap can be turned into alcoholic beverages such as *tari* (palm wine) or *arak*. Fermented sap is a traditional drink in several cultures, although the alcohol content makes it prohibited in certain regions.
4. **Palm Vinegar:** When the sap is further fermented, it can be processed into vinegar, which is used in cooking and as a natural preservative.
5. **Date Palm Jaggery Cake:** In regions like Bangladesh and India, palm jaggery is used to make traditional sweets like jaggery cakes and other confectioneries. These cakes are popular during festivals and offer a nutrient-rich alternative to refined sugar products.



Figure 3: Processed Food Products from Date Palm Sap (Gur and date syrup)

2.5. Historical Background of Date Palm Sugar

Date palm sugar, also known as jaggery or gur, has been used for thousands of years as a natural sweetener, long before the advent of refined white sugar. Its history can be traced back to ancient civilizations in the Middle East, South Asia, and Africa, where date palms have been a crucial agricultural resource.

1. **Origins in Mesopotamia and Ancient Egypt:** The earliest records of date palm sugar use date back to ancient Mesopotamia and Egypt, where date palm sap and sugar were used not only as food but also for religious rituals and medicinal purposes. It was considered a symbol of fertility and abundance.
2. **Use in South Asia:** In South Asia, date palm sugar has a long history of being used as a sweetener in traditional medicine and food preparations. It was favored for its unrefined, natural quality, and its health benefits compared to processed sugar. The production of jaggery from date palm sap remains a thriving industry in regions like West Bengal, Bangladesh, and certain parts of India.
3. **Cultural Importance:** In many cultures, date palm sugar is associated with harvest festivals and is often consumed during winter, when the sap is harvested. Its production is often done by local farmers using traditional methods, and it plays an important role in the economy of rural communities.

2.6. Health Benefits of Date Palm Sap and its Products

Date palm sap and its derivatives have numerous health benefits, making them more than just sweeteners. These include:

- **Rich Source of Energy:** Due to its high sugar content, date palm sap provides an immediate energy boost, making it a popular drink in the morning.
- **Improved Digestion:** The fiber and natural sugars in date palm sap aid digestion and may help alleviate constipation.
- **Anemia Prevention:** The iron content in date palm sap and jaggery is beneficial in preventing anemia by boosting hemoglobin levels.
- **Antioxidant Protection:** The polyphenols and flavonoids present in the sap act as antioxidants, protecting cells from oxidative stress and inflammation.

2.7. Challenges in Preservation of Date Palm Sap

Previous research highlights the inherent instability of date palm sap under ambient conditions. The high levels of reducing sugars, combined with microbial contamination, accelerate fermentation, leading to spoilage within hours of extraction (Kulkarni et al., 2010). To mitigate these issues, various preservation techniques, such as thermal treatments, chemical preservatives, and ultrafiltration, have been explored (Hebbbar et al., 2018). However, maintaining the sap's natural qualities, including its flavor and nutritional profile, remains a challenge.

2.7.1. Thermal Processing for Preservation

Thermal processing is commonly used to inactivate enzymes and microorganisms responsible for spoilage. Studies have shown that heat treatments, such as pasteurization, can effectively extend the shelf life of sap and other palm-based products by reducing microbial load and slowing fermentation (Naknaen & Meenune., 2016). However, excessive heat can alter the sap's sensory properties, as evidenced by the formation of Maillard reaction products that affect color and flavor (Sohn and Ho., 1995). In particular, the release of volatile compounds like pyrazines at temperatures above 110°C imparts a caramelized, nutty flavor that may be undesirable in natural sap (Barbara & Michael., 2004).

2.7.2. Chemical Preservatives

The use of chemical preservatives, including potassium metabisulfite (KMS) and potassium sorbate, has been widely studied for the preservation of palm-based products. KMS, in particular, is effective in controlling microbial growth while maintaining the sensory qualities of sap (Makhlouf-Gafsi et al., 2018). Potassium sorbate, another common preservative, inhibits yeast and mold growth, prolonging the sap's shelf life (Chadare et al., 2021). Despite their efficacy, these preservatives can sometimes negatively impact the flavor and aroma of the sap (Salvia-Trujillo et al., 2011). Therefore, balancing the concentration of preservatives to maintain both microbial safety and sensory acceptability is critical.

2.7.3. Combined Preservation Techniques

Recent studies emphasize the effectiveness of combining preservation techniques to optimize the quality and safety of palm sap. For example, a combination of ultrafiltration and thermal processing has been shown to improve both the biological properties and sensory qualities of

sap-based syrups (Hebbbar et al., 2018). Additionally, the combined use of heat treatment and chemical preservatives, as explored in your study, provides a promising approach to maintaining the sap's natural properties while extending its shelf life. Further exploration of these combined techniques is essential to overcome the limitations of individual methods.

2.8. Gaps in Existing Research

While several studies have investigated the preservation of palm sap derivatives like syrup and jaggery, there is a paucity of research specifically addressing the preservation of raw date palm sap in its natural state. The rapid fermentation of raw sap poses significant challenges for broader commercialization, as most studies focus on processed sap. Furthermore, the long-term effects of combined preservation methods on the sensory and nutritional attributes of date palm sap require further investigation, especially in the context of maintaining its raw form.

CHAPTER III

MATERIALS AND METHODS

The study was conducted in the Food and Process Engineering Lab-1 and Microbiology laboratory under the Faculty of Engineering, Hajee Mohammad Danesh Science and Technology University, Dinajpur.

3.1. Collection of Date Palm Sap

During the winter season, traditional methods were employed by local collectors to extract the sap from date palm trees. This practice involves skillfully peeling the upper portion of the date palm tree to initiate the natural flow of sap, which gradually begins to drip from the exposed area. The sap was meticulously harvested by harvesters using jars to collect the droplets overnight.

In this study, fresh date palm sap was collected during the early morning hours to ensure optimal freshness. The harvested sap was immediately transferred into sanitized containers, adhering to stringent hygiene protocols to maintain its quality. To remove any particulate matter, the sap was filtered using a sterile cloth. This initial filtration step was crucial in preserving the natural purity of the sap, ensuring it was in the best possible condition for subsequent experimental treatments.

3.2. Chemicals, Solvents and Ingredients

Chemicals and solvents used in the study were of AR grade, and water was glass distilled unless specified otherwise. Additional raw materials were acquired from the local marketplace, and necessary components were taken from the laboratory's inventory.

3.3. Treatment and Preparation of Processed Date Palm Sap

Fresh and clean date palm sap was analyzed for various physical, chemical and microbiological properties. Then it was heated at boiling temperature and concentrated on preparing three processed sap samples, S₁, S₂ and S₃, containing 11% 15% and 19% TSS. Following that, 200 ppm of potassium metabisulfite (KMS), 0.15 gm potassium sorbate and 0.02 gm of citric acid are added per 100 ml of each sample. Then the date palm sap samples

were preserved in sterilized bottles. Processed sap samples were taken for physical, chemical, and subjective analysis and the investigation of shelf-life.

3.4. Physico-Chemical Characterization of Fresh and Processed Date Palm Sap

3.4.1. Determination of pH

A digital pH meter (Jenco 6177M Benchtop Digital pH meter, USA) was used to determine the pH value of the sample. 10 ml date palm sap samples were taken in a 50 ml beaker. Then the electrode of the pH meter was dipped in the beaker. The reading was taken when the value was stable on the display of the pH meter. Previously the meter was calibrated with two known buffer solutions (pH 4 and pH 7).

3.4.2. Determination of Titratable Acidity (TA)

Sadler & Murphy (2010) outlined a method for determining titratable acidity, wherein 1ml of the samples were accurately weighed and dissolved in 20 mL of distilled water. The solution was then titrated with 0.1 N sodium hydroxide (NaOH) using phenolphthalein as an indicator until a persistent pink endpoint was reached. The titratable acidity was calculated as the percentage of citric acid equivalent, based on the volume of NaOH used for neutralization. The results were expressed as %, following the standard procedure established in their study.

$$\% \text{ Acidity} = \frac{\text{Titre value} \times \text{Normality of NaOH} \times \text{Acid factor}}{\text{Weight of sample}} \times 100$$

Where, Normality of NaOH = 0.1; Acid factor (Equivalent weight of citric acid) = 64.04 g/mol

3.4.3. Determination of Total Soluble Solids (TSS)

TSS were determined using a refractometer (HI-96801, Italy) expressed in %brix. Distilled water was used to tare the refractometer. After that, sample spread on the sample chamber and the reading was taken.

3.4.4 Determination of Sugar by Lane & Eynon Method (1923)

Titration of Reducing Sugar: 10ml of Fehling's solution was taken in a 250ml conical flask 50ml distilled water was added to it heated to boiling point and titrated with plant extract sugar solution. At the end point brick like red ppt. will appear.

$$\% \text{ Reducing Sugar} = \frac{\text{Fehling's factor} \times \text{dilution made up} \times 100}{\text{Titrate value} \times \text{wt.of sample} \times 1000}$$

Preparation of Sample for Titration of Total Invert Sugar: 50ml of the previously prepared samples was taken in a 250ml Beaker with 5g of citric acid and 50ml of distilled water was added to it and boiled for 10 minutes then cooled and neutralized by 1N NaOH solution then the solution transferred in a 250 ml volumetric flask and volume up to the mark with distilled water.

Titration: 10ml of Fehling's solution was taken in a 250ml conical flask 50ml distilled water was added to it heated at the boiling point and titrated with an unknown sugar solution.

$$\% \text{ Total Invert Sugar} = \frac{\text{Fehling's factor} \times \text{dilution made up} \times 100}{\text{Titrate value} \times \text{wt.of sample} \times 1000}$$

$$\% \text{ Non-Reducing Sugar} = \% \text{Total Invert sugar} - \% \text{Total reducing sugar.}$$

$$\% \text{ Total Sugar} = \% \text{Reducing sugar} + \% \text{ Non-Reducing sugar}$$

The fresh and heat-treated date palm juice was analyzed for total soluble solids (TSS) by refractometer, pH by digital pH meter (cyber scan pH 510, Eutech), total sugar (%), reducing sugar (%), and titratable acidity (as % citric acid) were estimated as per the method of (Ranganna, 1986).

3.5. Mineral Quantification

The determination of micro- and macro-minerals was performed following (Rybicka et al. 2017) with minor modifications. Briefly, 0.5 mL samples were mixed with 15 mL of nitric acid and perchloric acid solution (2:1) and heated to 205 °C until the sample was completely digested. The samples were then filtered and diluted to a volume of 100 mL with deionized water. The levels of P, Ca, Mg and Fe were determined by atomic absorption spectroscopy (SpectrAA-7000, Shimadzu, Kyoto, Japan). The data was presented as ppm of sample.

3.6. Microbial Examination

To monitor the microbial quality of date palm sap throughout various storage stages, a Standard Plate Count (SPC) was performed to assess the total bacterial load, along with a yeast and mold count to quantify fungal contamination. The microbial enumeration method was conducted following the guidelines outlined in the American Public Health Association (APHA) Compendium of Methods for Microbiological Examination of Foods, 5th edition (2015).

3.6.1. Sample Preparation

Date palm sap from all treatments was sampled at different storage stages. For each sample, decimal dilutions were prepared to ensure accurate microbial enumeration. The dilutions aimed to reduce the microbial load to a countable range on the culture media, making it easier to quantify the microbial populations.

3.6.2. Standard Plate Count (SPC)

Decimal dilutions from 10^{-1} to 10^{-8} were prepared using sterile distilled water. 1 mL aliquots of each dilution was transferred to sterile Plate Count Agar (PCA) plates using the spread plate technique, where the sample is evenly distributed across the agar surface. The plates were incubated at 37°C for 24–48 hours to promote the growth of aerobic bacteria. After incubation, colonies were counted, and results were expressed as log CFU/mL (Colony Forming Units per millilitre) of the sap. The logarithmic transformation of the colony counts simplifies the data analysis, especially for large ranges of microbial counts.

3.6.3. Yeast and Mold Count

For the enumeration of yeasts and molds, decimal dilutions from 10^{-1} to 10^{-8} were prepared using a peptone physiological salt solution (which provides nutrients and osmotic balance for accurate microbial growth). 1 mL of each dilution was spread on Potato Dextrose Agar (PDA), a medium specifically designed to support the growth of yeasts and molds. Plates were incubated at 25°C for 72 hours, a temperature optimal for fungal growth, allowing slow-growing yeasts and molds to develop visible colonies. After incubation, yeast and mold colonies were counted and reported as log CFU/mL.

3.7. Storage studies

Fresh and processed date palm sap samples were stored at a refrigerated temperature (4° C). The different parameters for assessing deterioration and spoilage of processed date palm sap samples were observed at regular intervals of time. The pH, acidity, TSS, Total Sugar, Reducing Sugar, Non-Reducing Sugar, microbial load (standard plate count) and physical quality were monitored weekly during the storage period of 2 weeks.

3.8. Sensory evaluation

Sensory evaluations of fresh and all the processed date palm sap were done by a taste-testing panel of 15 panelists. At first, fresh and three processed date palm juice samples were presented to the panelist with a score sheet. They were asked to evaluate the processed date palm juice for color, flavor, taste, sweetness and overall acceptability by scoring a 9 points hedonic scale. The hedonic scale was as follows: 9 = Like extremely; 8 = Like very much; 7 = Like moderately; 6 = Like slightly; 5 = Neither like nor dislike; 4 = Dislike slightly; 3 = Dislike moderately; 2 = Dislike very much; and 1 = Dislike extremely. The preference differences were evaluated by statistical analysis of data for variance and consequently quantified. The sensory evaluation tests were conducted according to established ethical guidelines. By maintaining all regulations, consent was obtained from all participants before the experiment.

3.9. Statistical Analysis

All results were presented as average value $\pm$ standard deviation (SD). Data were analyzed by One-way analysis of variance (ANOVA) of the SPSS 22 software (Chicago, IL, USA). Mean separations were performed by Duncan's multiple-range test. Differences were considered significant at $p < 0.05$.

Chapter IV

Results and Discussions

4.1. Changes in pH and Titratable Acidity of Fresh and Processed Date Palm Sap During Storage:

Table 1 demonstrates the preservatives and thermal treatment affect the pH and titratable acidity in fresh and processed date palm sap during storage.

The fresh sap had the lowest pH value of 7.78 and the lowest titratable acidity of 0.048%. On the other hand, S₃ shows a progressive rise in pH to 8.42 and a higher titratable acidity rate of 0.106% because of the heating treatment and concentration effect on date palm sap. It has been reported by Kumar et al., 2023 that the titratable acidity of pomelo juice increases with an increase in the heat treatment time.

During storage, pH value of palm sap decreased slightly after 7 days. In Table 1, it can be seen that, the decrease in the pH value of S₁, S₂ and S₃ from 8.33 to 7.84, 8.29 to 7.82 and 8.42 to 7.9 respectively. In contrast, acidity increases in a minimal rate throughout the storage of 7 days. Thermal treatment and preservatives inhibited microbial development throughout the first week, steadily reducing pH and increasing acidity. Even though, the fresh date sap spoiled during the time, because of its highly decaying characteristics (Dhansu et al., 2023; Hebbar et al., 2018). After 14 days of storage, the pH value of palm sap decreased significantly ($p < 0.05$) for every sample. Where the acidity increased with the similar pattern during the storage of 14 days. Fermentation of processed date palm sap resulted in decreased pH and increased acidity (Hebbar et al., 2015; Ansar et al. 2023).

Table 1. Changes in pH and Acidity of Fresh and Processed Date Palm Sap During Storage

Sample	pH			Acidity (%)		
	0Day	7Days	14Days	0Day	7Days	14Days
Fresh	7.48±0.012 ^d	Spoiled		0.048±0.003 ^d	Spoiled	
S1	^A 8.33±0.01 ^b	^B 7.84±0.01 ^b	^C 5.91±0.01 ^c	^C 0.065±0.002 ^c	^B 0.07±0.002 ^c	^A 0.077±0.002 ^c
S2	^A 8.29±0.01 ^c	^B 7.82±0.01 ^c	^C 5.94±0.005 ^b	^B 0.077±0.003 ^b	^B 0.082±0.003 ^b	^A 0.089±0.003 ^b
S3	^A 8.42±0.011 ^a	^B 7.9±0.01 ^a	^C 5.96±0.01 ^a	^B 0.106±0.004 ^a	^{AB} 0.110±0.003 ^a	^A 0.116±0.004 ^a

All results are expressed as Mean Standard Deviation.

^{a-d} different superscript small letter indicates difference among the sample in specific storage day at $p<0.05$.

^{A-C} different superscript Capital letter indicates difference among the sample during storage day at $p<0.05$.

Where, S1= Sample containing 11% TSS; S2= Sample containing 15% TSS; S3= Sample containing 19% TSS.

4.2. Changes in TSS, Total Sugar, Reducing Sugar and Non-Reducing Sugar of Fresh and Processed Date Palm Sap During Storage:

After thermal processing, the TSS values increased compared to the fresh sap, reflecting the concentration effect of heat treatment. The fresh sample had a TSS of 9.92%, while S₁, S₂, and S₃ had 11.10%, 15.04%, and 19.06%, respectively. This increase can be attributed to water loss during heat treatment, leading to a concentration of sugars and other dissolved solids in the sap (Figure 2).

TSS significantly decreased for all samples after 14 days due to fermentation activity. This reduction can be explained by the conversion of sugars into alcohol and other metabolites by microorganisms, which reduces the overall soluble solids in the sap (Kasture & Kadam, 2018; Hebbar et al., 2018).

Fresh sap had a total sugar content of 9.80%, while the processed samples (S₁, S₂, and S₃) showed elevated total sugar levels of 10.18%, 14.50%, and 18.91%, respectively (Table 2). This indicates that the heat treatment concentrated the sugars and other dissolved solids in the sap by lowering its water content (Table 2).

As a result of fermentation, a gradual reduction in the total sugar content was noted throughout storage. Microbes use sugars to make ethanol and other byproducts (Medeiros et al., 2014). The reduction in total sugar was slower in the first 7 days due to the preservatives slowing down microbial activity (Table 2).

Table 2 demonstrates that the fresh sap had a 3.72% reducing sugar content (glucose and fructose), whereas the processed samples had higher amounts of reducing sugar after heat treatment. This is presumably due to the breakdown of sucrose into glucose and fructose during the heat treatment process (Nadeem et al., 2015).

As sucrose continued to hydrolyse into glucose and fructose over the storage period, reducing sugars increased slightly in the first 7 days (e.g., in S₃, from 9.08% to 9.46%). Then, because of microbial fermentation, reducing sugar levels to slow or even slightly decline. This suggests that the reducing sugars are initially synthesized and subsequently consumed by microbes during fermentation (Kim et al., 2007; Hebbar et al., 2018).

In the fresh sap, non-reducing sugars (mostly sucrose) accounted for 6.08%. During heat treatment, partial sucrose hydrolysis caused a slight decrease in the non-reducing sugar levels in the processed samples. Table 2 shows that, in S₃ the non-reducing sugar content dropped to 9.82% (Divya & Archana Kumari, 2009; Sharma et al., 2014).

From Table 2, a notable reduction in non-reducing sugar was observed during storage, especially after 14 days. Non-reducing sugar fell from 5.97% to 4.85% in S₁ and 9.82% to 8.59% in S₃. This reduction indicates that the non-reducing sugars were hydrolyzed into reducing sugars, which were subsequently fermented by microorganisms (Kim et al., 2007; Fasreen et al., 2017).

Table 2. Changes in TSS, Total Sugar, Reducing Sugar, and Non-Reducing Sugar of Fresh and Processed Date Palm Sap During Storage

Sample	TSS			% Total Sugar (v/v)			% Reducing Sugar (v/v)			% Non-Reducing Sugar (v/v)		
	0	7	14	0	7	14	0	7	14	0	7	14
Fresh	9.92±0.01 ^d	-	-	9.8±0.02 ^d	-	-	3.72±0.01 ^d	-	-	6.08±0.01 ^c	-	-
S1	^A 11.10±0.02 ^c	^B 10.77±0.01 ^c	^C 10.13±0.01 ^c	^A 10.18±0.02 ^c	^B 9.91±0.02 ^c	^C 9.38±0.03 ^c	^C 4.21±0.01 ^c	^B 4.36±0.01 ^c	^A 4.54±0.03 ^c	^A 5.97±0.01 ^d	^B 5.55±0.02 ^c	^C 4.85±0.03 ^c
S2	^A 15.04±0.01 ^b	^B 14.82±0.01 ^b	^C 14.22±0.01 ^b	^A 14.50±0.03 ^b	^B 14.34±0.03 ^b	^C 13.81±0.02 ^b	^C 6.48±0.03 ^b	^B 6.61±0.03 ^b	^A 6.77±0.02 ^b	^A 8.02±0.03 ^b	^B 7.73±0.02 ^b	^C 7.04±0.02 ^b
S3	^A 19.06±0.01 ^a	^B 18.83±0.01 ^a	^C 18.2±0.01 ^a	^A 18.91±0.02 ^a	^B 18.73±0.02 ^a	^C 18.05±0.02 ^a	^C 9.08±0.03 ^a	^B 9.29±0.03 ^a	^A 9.46±0.02 ^a	^A 9.82±0.02 ^a	^B 9.45±0.02 ^a	^C 8.59±0.02 ^a

All results are expressed as Mean Standard Deviation.

^{a-d} different superscript small letter indicates difference among the sample in specific storage day at $p < 0.05$.

^{A-C} different superscript Capital letter indicates difference among the sample during storage day at $p < 0.05$.

Where, S1= Sample containing 11% TSS; S2= Sample containing 15% TSS; S3= Sample containing 19% TSS.

4.3. Changes in Microbial Load and Physical Observation of Fresh and Processed Date Palm Sap During Storage:

The microbial load and physical condition of both fresh and processed date palm sap showed a significant variation ($p < 0.05$) during the storage period. On day 0, the fresh sap had a bacteria and yeast mold count of 4.60 log CFU/ml. However, the microbial loads of all treated samples (S1, S2, S3) were noticeably reduced. Such as S1 had 3.34 log CFU/ml, S2 had 3.21 log CFU/ml, and S3 had 3.10 log CFU/ml. The use of heat treatment and preservatives (potassium metabisulfite, potassium sorbate, and citric acid) was responsible for the decreased microbial counts in the treated samples. The microbial activity was successfully controlled in the beginning by the measures mentioned above (Gironés-Vilaplana et al., 2016; Salvia-Trujillo et al., 2011).

On day 7, the fresh sap had gone decaying, and the microbiological counts were too high to quantify. In contrast, the processed samples showed a rise in bacteria and yeast mold count, but they were still within acceptable limits. S1 displayed a load of 4.21 log CFU/ml, whereas S2 and S3 showed loads of 4.10 and 3.93 log CFU/ml, respectively. All processed samples were in suitable physical condition despite the rise in microbial activity. This demonstrates how chemical preservatives maintain their protective effects (Chadare et al., 2021).

After 14 days, a certain level of spoiling was seen in all samples. The microbial load in the processed samples continued to rise significantly ($p < 0.05$). S1 had the highest microbial count at 5.44 log CFU/ml, followed by S2 at 5.36 log CFU/ml, and S3 at 5.14 log CFU/ml. Every treated sample has some physical deterioration. This indicates that although the preservatives inhibited the development of microorganisms, their efficacy decreased over time (Sperber, 2009).

Table 3. Changes in Microbial Load and Physical Observation of Fresh and Processed Date Palm Sap During Storage

Storage	Samples	Microbial load log CFU/ ml	Physical Observations
0 Days	Fresh	4.60±0.12 ^a	Good Condition
	S1	^C 3.34±0.13 ^b	Good Condition
	S2	^C 3.21±0.08 ^{bc}	Good Condition
	S3	^C 3.10±0.05 ^c	Good Condition
7 Days	Fresh	-	Spoiled
	S1	^B 4.21±0.03 ^a	Good Condition
	S2	^B 4.10±0.08 ^a	Good Condition
	S3	^B 3.93±0.09 ^b	Good Condition
14 Days	Fresh	-	Spoiled
	S1	^A 5.44±0.06 ^a	Slightly Spoiled
	S2	^A 5.36±0.07 ^c	Slightly Spoiled
	S3	^A 5.14±0.04 ^b	Slightly Spoiled

All results are expressed as Mean Standard Deviation.

^{a-d} different superscript small letter indicates difference among the sample in specific storage day at $p < 0.05$.

^{A-C} different superscript Capital letter indicates difference among the samples during storage day at $p < 0.05$.

Where, S1= Sample containing 11% TSS; S2= Sample containing 15% TSS; S3= Sample containing 19% TSS.

4.4. Mineral Content of Fresh and Processed Date Palm Sap:

As shown in Table 4, the fresh date palm sap had a phosphorus content of 20.95 mg/L. All samples showed a noticeable increase in phosphorus levels after processing. S1 sample increased slightly to 21.45 mg/L, whereas S2 and S3 showed larger increases to 27.4 mg/L and 32 mg/L respectively. The action of heat treatment in concentrating certain minerals may be the cause of this rise. These findings indicate that processing increases the bioavailability of phosphorus in date palm sap (Cilla et al., 2011).

Calcium content in fresh sap was 525.75 mg/L, which increased significantly ($p < 0.05$) with processing. The highest calcium concentration was observed in sample S3 (826.25 mg/L), followed by S2 (799.15 mg/L) and S1 (723.05 mg/L). The substantial rise in calcium content post-processing may be due to mineral concentration as water evaporates during heat treatment (Anema, 2009). This suggests that the thermal processing retains or enhances calcium content, making processed sap a richer source of this mineral.

Magnesium content followed a similar trend, starting from 108.15 mg/kg in fresh sap and increasing progressively through the processed samples. S1 contained 133.65 mg/L, S2 increased to 176.15 mg/L, and S3 reached 216.65 mg/L. The rise in magnesium levels can be attributed to the concentration effect during processing, where water loss might lead to higher mineral density (Morales-De La Peña et al., 2011). Magnesium, being heat-stable, is less likely to degrade during thermal treatments, ensuring its retention or slight enhancement.

The iron content showed a marked increase from 2.565 mg/kg in fresh sap to 5.825 mg/L in S3. Intermediate values were observed in S1 (3.215 mg/L) and S2 (4.375 mg/L). This sharp increase in iron concentration could be due to the concentration effect during heat treatment, and iron may also react with other ingredients, such as citric acid, which could enhance its bioavailability (Morales-De La Peña et al., 2011; Zhang et al., 2007). The higher iron content in processed samples indicates a potential nutritional advantage for processed date palm sap, especially for addressing iron deficiency.

Table 4. Mineral Content of Fresh and Processed Date Palm Sap

Sample	Phosphorus (mgL ⁻¹)	Calcium (mgL ⁻¹)	Magnesium (mgL ⁻¹)	Iron (mgL ⁻¹)
Fresh	20.95±1.06 ^c	525.75±33.44 ^b	108.15±3.89 ^c	2.565±1.48 ^c
S1	21.45±0.21 ^c	723.05±6.29 ^a	133.65±3.74 ^c	3.215±0.98 ^{ab}
S2	27.40±0.28 ^b	799.15±64.55 ^a	176.15±6.71 ^b	4.375±0.46 ^{ab}
S3	32.00±0.28 ^a	826.25±79.12 ^a	216.65±18.03 ^a	5.825±0.60 ^a

All results are expressed as Mean Standard Deviation.

^{a-d} different superscript small letter indicates difference among the sample in specific storage day at $p<0.05$.

Where, S1= Sample containing 11% TSS; S2= Sample containing 15% TSS; S3= Sample containing 19% TSS.

4.5. Organoleptic Acceptability of Fresh and Processed Date Palm Sap:

Fresh and processed date palm sap samples were subjected to sensory evaluation. The colour, flavour, taste, sweetness, and overall acceptability of these sap samples were evaluated by a panel of 15 panellists.

The sensory evaluation of fresh (F) and processed date palm sap samples (S1, S2, S3) revealed consistent improvements across multiple parameters, including flavor, taste, sweetness, and overall acceptability with processing.

Across all parameters, fresh sap (F) received the lowest scores, while processed samples such as S2 and S3 scored higher. Comparing the flavor and sweetness to the processed and fresh sap (F) samples, significant variation ($p < 0.05$) was observed. Fresh sap scored 7 for flavor and 7.09 for sweetness, while S3 had the highest flavor score of 7.82 and sweetness of 8 (Figure 4).

In terms of color and taste, there were no significant variations ($p \leq 0.05$). S2 being rated the most visually appealing and palatable by rated 7.64 and 7.9 respectively. This indicating that processing had minimal impact on the sap's appearance.

S2 scored significantly higher ($p < 0.05$) than the other samples for overall acceptability. This suggests that the panelists found S2 to be the most well-balanced and pleasing in terms of its sensory attributes, such as flavor, taste, and sweetness. The higher score for S2 also indicates that the specific combination of processing and preservatives used in this sample was particularly effective in enhancing its overall appeal, making it the top choice among the panelists.

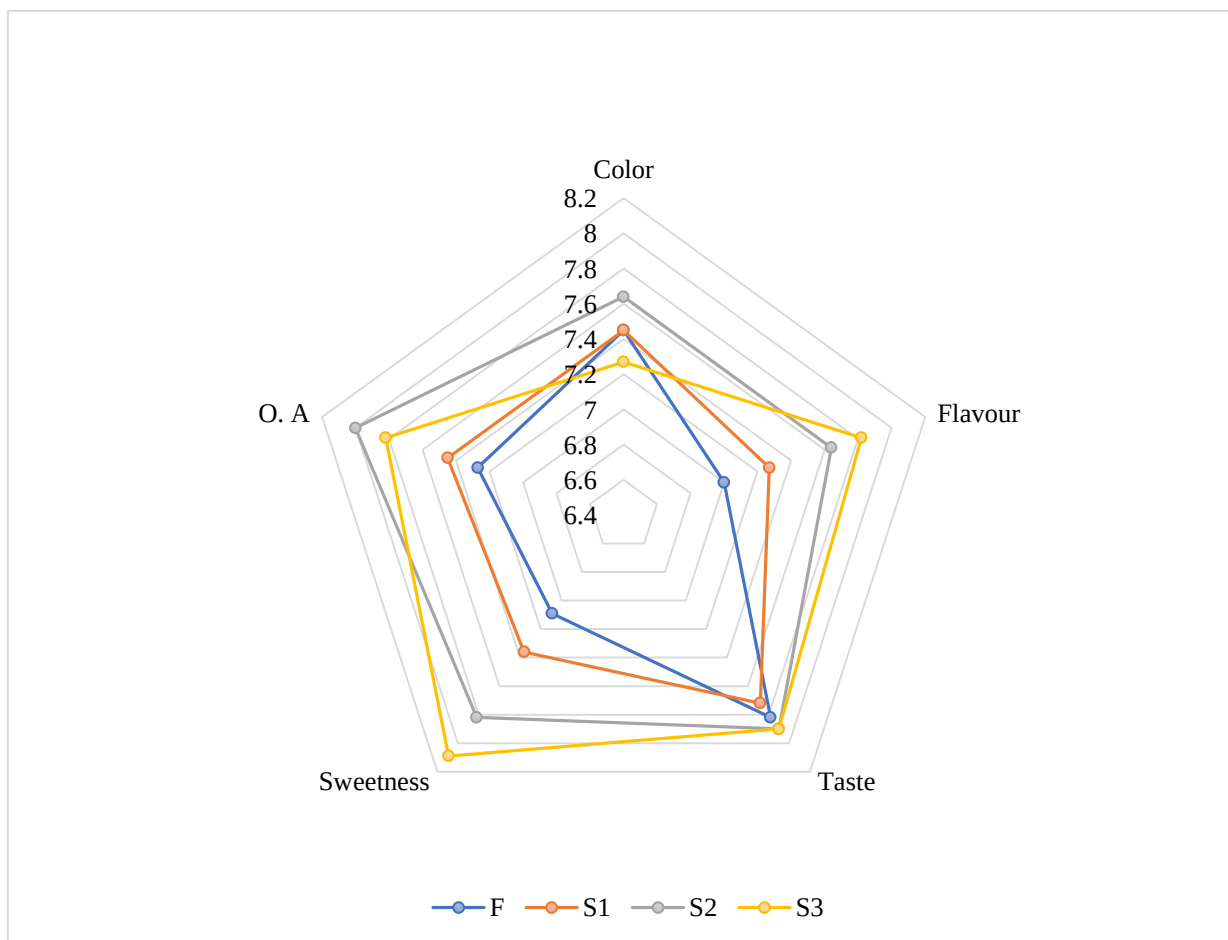


Fig. 4: Organoleptic Properties of Fresh and Processed Date Palm Sap.

F= Fresh Sap; S1= Sample containing 11% TSS; S2= Sample containing 15% TSS; S3= Sample containing 19% TSS.

CHAPTER V

CONCLUSION

This research demonstrates that a combination of heat treatment and chemical preservatives such as potassium metabisulfite, potassium sorbate, and citric acid can effectively extend the shelf life of date palm sap. The application of thermal treatment increased total soluble solids (TSS) and sugar concentrations, which enhanced the sap's stability. Microbial activity was effectively suppressed during the first 7 days of storage, allowing the sap to be preserved in good condition during this period.

The study found that while chemical preservatives helped in controlling microbial growth and preserving the sensory attributes in the short term, their effectiveness diminished over time, suggesting that further refinements are needed for long-term preservation. The findings underline the potential for commercializing date palm sap through improved preservation techniques, providing economic benefits while maintaining its nutritional and sensory qualities. Overall, this study contributes to the body of knowledge on sap preservation and opens avenues for future research on optimizing storage stability through innovative preservation methods.

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