DEVELOPMENT AND EVALUATION OF READY TO SERVE BEVERAGES FROM RIPE PALMYRAH PALM FRUIT

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KERALA, INDIA

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KELAPPAJI COLLEGE OF AGRICULTURAL ENGINEERING AND FOOD TECHNOLOGY

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DECLARATION

I hereby declare that this thesis entitled **"DEVELOPMENT AND EVALUATION OF READY TO SERVE BEVERAGES FROM RIPE PALMYRAH PALM FRUIT"** is a bonafide record or research work done by us during the course of research and the thesis has not previously formed the basis for the award of any degree, diploma, associate ship, fellowship or other similar title of any other University or Society.

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Place: Tavanur

Date: 16/01/2025

CERTIFICATE

Certified that this project report entitled **"DEVELOPMENT AND EVALUATION OF READY TO SERVE BEVERAGE FROM RIPE PALMRAH PALM FRUIT"** is a Bonafide record of research work done independently by **Muhammed Thwahir (2021-06-013), Archana Bhuvandas (2021-06-015), Nandu M Vinod (2021-06-016), Alfiya Rahiman (2021-06-018), Afeefa M (2021-06-019)** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associate ship.

Place: Tavanur

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LIST OF SYMBOLS AND ABBREVIATIONS

WHO	: World Health Organisation
GRAS	: Generally Recognised as Safe
KMS	: Potassium Metabisulphite
Fig.	: Figure
TSS	: Total Soluble Solids
TPC	: Total Phenolic Content
BP	: Blood Pressure
CFU	: Colony Forming Unit
RPM	: Revolutions Per Minutes
ppm	: Parts per million
T	: Treatment
mL	: Millilitre:
w/v	: weight/volume
M	: Molar
L*	: Lightness or Darkness
a*	: Greenness or redness
b*	: Blueness or yellowness

V : Volume

K : Potassium

Ca : Calcium

Mg : Magnesium

RTS : Ready To Serve

KMS : Potassium Metabisulphate

SO_2 : Sulphur Dioxide

UV : UltraViolet

HPP : High Pressure Processing

PFP : Palmyrah Fruit Pulp

TPC : Total Plate Count

Na : Sodium

CHAPTER I

INTRODUCTION

The palmyrah palm (*Borassus flabellifer*), part of the Arecaceae family, thrives in the coastal regions of Northeast Sri Lanka, India, Southeast Asia, and various African countries. In India, there are about 85.9 million palmyrah palms, with Tamil Nadu alone accounting for around 51.9 million, especially in Thoothukudi district, which has the highest concentration (Sangheetha *et al.*, 2014).

These palms are dioecious, meaning they have separate male and female plants. The female plants produce fruit, classified as a type of stone fruit. Palmyrah offers numerous benefits, with every part of the tree being useful. It provides food in the form of sap, fruit, and tuber-based products, building materials from its timber and leaf petiole, and other resources such as fiber from the leaf sheath, ornamental items from tender leaves, and fertilizer from older leaves.

The palmyrah fruit is a highly nutritious tropical fruit, rich in carbohydrates, dietary fiber, and essential vitamins and minerals. It provides natural sugars like glucose and fructose for energy, along with fiber that supports digestive health and stabilizes blood sugar levels. The fruit is also a good source of vitamins C and A, which enhance immune function and promote skin and eye health. Additionally, it contains important minerals such as potassium, sodium, calcium, iron, and magnesium, which support bone health, oxygen transport, and muscle function. Palmyrah fruit has high water content, making it hydrating and refreshing, particularly in hot climates. It offers a small amount of protein and healthy fats, along with antioxidants that help combat oxidative stress.

Palmyrah pulp offers numerous health benefits, making it a valuable addition to a balanced diet. It is a good source of energy, thanks to its natural sugars, and has antidiabetic properties that help regulate blood sugar levels. Its cholesterol-lowering effects contribute to improved heart health, while its potential to reduce weight gain supports weight management. Rich in antioxidants, palmyrah pulp helps combat oxidative stress and protect cells from damage, and its anti-cancer activity highlights its potential in preventing certain types of cancers.

According to Sangheetha *et al.* (2014), in Sri Lanka, female palms yield about 20,000 tons of fruit pulp annually. Unfortunately, nearly 10,000 tons go to waste, often eaten by wildlife like pigs, which can also damage crops. The falling fruit can cause

destruction in cultivated areas. To address these issues, there's a push to utilize the fruit more effectively, creating value-added products for farmers. This initiative aims to reduce pulp waste, extend the availability of palmyrah based products beyond the fruiting season, and increase the income of those dependent on palmyrah. These products include panipanattu, palmyrah fruit and nut bars, panattu choco bars, palmyrah cordial, and fruit jelly and jam.

Ready-to-Serve (RTS) beverages are becoming increasingly popular in India, due to their convenience, refreshing taste, and wide range of flavors. These pre-packaged drinks are ready for immediate consumption, making them a favorite among people of all ages, especially in urban areas. As more consumers prioritize health, many are turning to fruit-based RTS beverages, which offer a healthier alternative to carbonated soft drinks.

The manufacturing process for palmyrah RTS beverages is simple and standardized. It involves blending preserved fruit pulp, sugar syrup, citric acid, preservatives, coloring agents, and flavors according to a specific formulation. The mixture is then homogenized, bottled, processed in heating retorts, and cooled. RTS fruit drinks must contain at least 10% fruit content, 10% total soluble solids, and approximately 0.3% acid, as per FSSAI regulations. These standards ensure that RTS beverages are safe for consumption and maintain consistent quality.

Palmyra RTS drinks are emerging as a unique option in this market. Made from the nutrient-rich palmyrah palm, these drinks are not only refreshing but also offer numerous health benefits. Their distinct, traditional flavor appeals to those seeking natural and indigenous options. With growing awareness of the nutritional value of palmyrah, these drinks are gaining popularity, particularly in regions like Tamil Nadu, where the palmyrah palm is widely cultivated. This rise in demand is also driven by initiatives to reduce post-harvest losses and utilize the fruit in innovative ways, thereby boosting local farmers' incomes and promoting sustainable agriculture.

The bitter taste in palmyrah fruit pulp is primarily attributed to the presence of a steroidal saponin, which poses a significant challenge in developing palmyrah based products. This bitterness, along with other factors such as the fruit's inherent astringency, variable pulp consistency, and short shelf life, has led to a general reluctance among producers to venture into creating products from palmyrah fruit.

Despite the challenges, we are determined to develop a standardized Ready-to-Serve (RTS) beverage from palmyrah fruit. To achieve this goal, the project titled **"DEVELOPMENT AND EVALUATION OF READY-TO-SERVE BEVERAGE**

FROM RIPE PALMYRAH PALM FRUIT" was undertaken at Kelappaji College of Agricultural Engineering and Food Technology (KCAEFT), Tavanur, Kerala, India. The project aims to address the key challenges of palmyrah fruit utilization and focuses on the following objectives:

a) To evaluate the physicho chemical properties of plamyrah pulp
b) To standardize the composition of RTS beverage from plamyrah pulp
c) To evaluate the physicho chemical properties of RTS beverage

CHAPTER II

REVIEW OF LITERATURE

This chapter comprises of review of various research works done by various researchers related to palmyrah fruit, extraction of pulp, preparation of ready to serve beverages and preservation methods.

2.1 PALMYRAH FRUIT

The fruit of the palmyrah palm is its most valued component. The palmyrah palm, like every other species of Borassus, produces distinct blooms for males and females. Following pollination, fruits of a 10–18 cm diameter and a black to brown husk form. As seen in Fig.2.1, each fruit has one to three seeds. An immature fruit's seeds, or endosperm, can be consumed like a fruit. Because the fibrous section and hard rind must be removed before consumption, the unripe fruit of the palmyrah palm is quite challenging to eat. The fruit's fibrous outer shell, however, can also be consumed raw, roasted, or cooked when it is mature. About 51.07% of the ripe palmyrah palm's fruit is made up of the soft, orange-yellow mesocarp, which is its edible pulp (Fig. 2.1). Vitamin C and β-carotenoids are abundant in this sugary portion, which has a slightly bitter taste and contains 16.9 mg and 3 ppm of each 100 g of fruit pulp, respectively. Pectin, iron, phosphorus, calcium, magnesium, and antioxidants are also thought to be abundant in it (Sumonsiri *et al*., 2021).

By utilizing a pulp-to-water ratio of 1:1 or 1:2, the pulp can be removed manually or with a fruit pulp extractor. Cakes, jam, jelly, ice cream, soft drinks, toffee, sweets, cordials, and other delectable culinary items can all be made from it. Sadly, the palmyrah palm's high moisture content causes more than 60% of its yearly fruit supply to be lost within 10 days after harvesting, giving it a short shelf life and limiting the commercial use of ripe palm fruit. An annual waste of about 10,000 tons of fruit pulp is expected to occur (Sumonsiri *et al*., 2021).

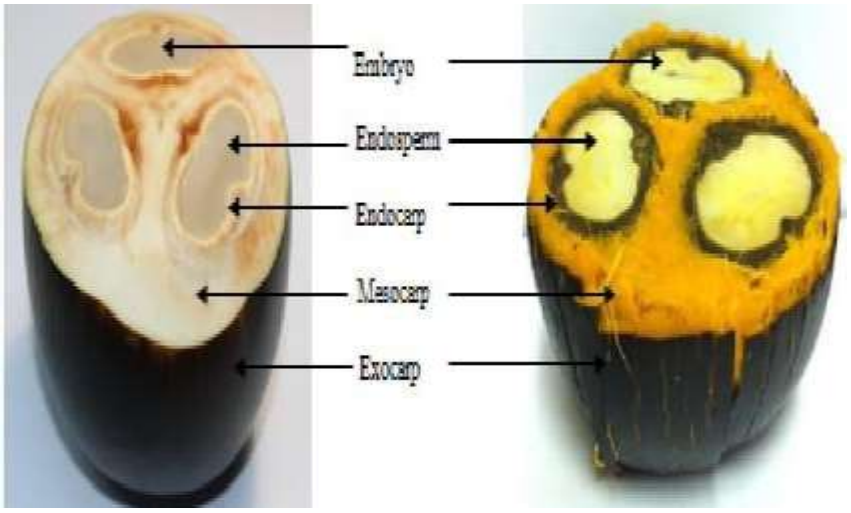

Plate 2.1 Segments of palmyrah fruit (Sumonsiri *et al*., 2021).

Based on the pigmentation of the fruit skin palmyrah palm can be broadly classified into two varieties;

2.1.1 Black skin fruits

Less red pigment is found on the fruit skin. Yield is less but superior seedlings with more starch content and less fibre content noticed. Pulp extraction process is easier. Alkaloids, minerals and free amino acids are lesser than red coloured fruits (Rao *et al*., 2021).

2.1.2 Red skin fruits

Variable amount of black pigment observed on the fruit skin. Fruit yield per tree is significantly high. Pulp, sugar and starch content are less when compared to black skin fruits. Both the black skin and red skin fruits are recorded for essential amino acids, Lysine and methionine. In view of the fruit characters and sap yield the red skinned fruit varieties are seemed to favour for selection for commercial exploitation (Rao *et al*., 2021).

Plate 2.2 Black skin and red skin palmyrah fruit (Rao *et al*., 2021)

2.2 NUTRITIONAL ATTRIBUTES OF PALMYRAH PULP

Typical fruit-based products are valuable economically and are also high in nutritious components, which have a major positive impact on human health. The time and place of collecting are two examples of the variables that affect the fruits and fruit-based products nutritional makeup. It is dependent on the palm's sex, genetic characteristics, and environmental elements including wind, temperature, rainfall and soil (Jansz *et al*., 2002).

2.2.1 Water content

The palmyrah fruit's water content varies from 74% to 81%, with an average of 79%. The remaining 21% of the pulp is made up of dry matter. One of the main factors that food processors use to assess the quality of fruit is the dry matter of the pulp's solid content. The majority of the fruit pulp's dry weight is made up of carbohydrates, which are found in complex forms including fiber and sugars. Palmyrah fruit pulp (PFP) has a total sugar content of 14– 15%, which includes 5–8% The primary components of lowering sugar are fructose (3.4%), glucose (3.5%) and sucrose (6.6%). The portion of food that cannot be broken down during digestion is called fiber. It can be separated into three types of fiber: soluble fibers, which dissolve rapidly in water, gummy fibers, and insoluble fibers, which do not dissolve in water. PFP has significant levels of both soluble (9.7–10.9) and insoluble fiber (12.8–16.8) (Seneviratne *et al*., 2005).

2.2.2 Proteins

Protein is divided into essential and non-essential amino acids. The former are unable to be produced by the body from other substances, while the latter can be obtained at a reasonable price from plants. Protein makes up 0.42 g/100 g of plamyrah fruit, primarily consisting of free amino acids such lysine, aspartate, glutamate, and phenylalanine (Vijayakumari *et al*., 2017).

2.2.3 Lipids

Lipids also known as crude fat, are present in relatively small amounts, neither their impact on obesity nor the energy value that PEP fat provides are significant. However, adding additional ingredients during processing can significantly alter the fat content (Mahilrajan *et al*., 2024)

2.2.4 Vitamins

PFP having a significant amount of vitamin A and C, processing may cause some of it to be lost. According to the studies, PFP contains both fat-soluble and water-soluble vitamins (Vijayakumi *et al.*,2017).

2.2.5 Minerals

The typical amount of the mineral material known as ashes in PFP ranges from 0.5% to 5%. Different rates of element uptake from the soil and climate conditions cause variations in the mineral composition of the PFP (Vijayakumari *et al*., 2017).

Table 2.1 Nutritional composition of palmyrah fruit

PARAMETERS	COMPOSITION (%)
Water content	79
Total sugar	11-14
Total protein	2.58
Lipids	1.2
Vitamin C	28.5

Source: (Mahilrajan *et al*., 2024)

PFP is composed of a variety of simple sugars and oligosaccharides. Specifically, sucrose is found at a concentration of 6.6 g per 100 g, while glucose and fructose are present at 3.5 g and 3.4 g per 100 g, respectively. Additionally, oligosaccharides account for 1.5 g per 100 g, with traces of rhamnose detected. Pectin levels are reported to be between 4.4 g and 6.7 g per 100 g. In terms of carotenoids, the initial reported level was 3.2 mg per 100 g, but subsequent studies have shown significant variation, with levels ranging from 1 to 10 mg and more recently reported as high as 2 to 253 mg per 100 g. The carotenoids identified include lycopene and zeta-carotene (both non-pro vitamin A) as well as alpha-carotene and zeacarotene (which are pro-vitamin A). Spectral analysis indicates that these carotenoids exhibit maximum absorption at wavelengths between 422 and 428 nm, suggesting a mixture with varying compositions. Regarding minor components, the sample contains vitamin C at a concentration of 28 mg per kilogram. The macrometallic ion content includes potassium (K) at 5.7 g/kg, calcium (Ca) at 0.7 g/kg, sodium (Na) at 0.2 g/kg, and magnesium (Mg) at 0.6 g/kg (Aman, 2017). The flabelliferins are a family of chemicals that gained notoriety after one of them was found to be the source of bitterness. Additional research revealed a variety of at least 14

flabelliferins, including one that has antimicrobial properties. Because of the specific name flabellifer, the term flabelliferin was created to refer to these substances, which were steroidal saponins. Since then, there have been reports of numerous research directions aimed at finding and isolating flabelliferins as well as bioactivity studies (Jansz *et al*., 2002). Additionally, it seems that the flabelliferins are crucial in determining how PFP will be used in the future.

2.3 DEBITTERING

This specifically relates to the elimination of bitterness. This is often accomplished by cooking the palmyrah fruit on hot coals, proximal end up. When the froth is eliminated, some of the bitterness is lost. Up to now, the sole scientific approach of debittering has been enzymatic, utilizing heat-stable ∝-amylase or naringinase (which has rhamnosidase and glycosidase activity). Naringinase is the sole enzyme that can hydrolyze a less bitter molecule, but both enzymes can hydrolyze one extremely bitter chemical (Jansz *et al*., 2002). A crucial first step in using PFP more extensively in the form of jams and cordials is debittering. Note that some other non-bitter flabelliferins are also hydrolyzed as a result of debittering.

2.4 HEALTH BENEFITS OF PALMYRAH FRUIT

Palmyrah products are a rich source of carbohydrates, which are essential for human energy. They contain steroidal saponin flabelliferin II, which contributes to the bitter taste and can help slow down the absorption of simple sugars, making energy boosts last longer. Palmyrah products have been found to reduce the occurrence of diabetes, with mice fed with palmyrah fruit pulp showing reduced weight gain and blood glucose levels. The high dietary fiber content in palmyrah fruit pulp helps to lower total cholesterol levels, which can be attributed to its ability to binding bile salts and preventing their reabsorption (Mahilrajan *et al*., 2024). Palmyrah fruit pulp also contains antioxidant properties, such as phenolic compounds, carotenoid, and vitamins, which help to scavenge unstable molecules and inhibit oxidative mechanisms that contribute to chronic diseases like cancer and heart disease. The compound extracted from palmyrah fruit pulp has shown anti-cancer activity, with compounds like vinca alkaloids and vinblastine being used as effective chemotherapeutic agents.

2.5 SCOPE OF PALMYRAH FRUIT

With the increasing population, industrialization and urbanization, India facing serious challenges in food security. In order to sustain the agricultural production and adress the challenges of food and livelihood security, agricultural diversification has to be adopted through the concept of the value addition by minimizing the dependency on main staple crops. Introduction of the new species in the agricultural production system in India is the need of the hour to increase the resiliency of agriculture. In this context, palmyrah palm is one such under exploited crop which have received less attention from agricultural research workers, probably on account of the fact that is very slow growing palm found mostly in the wild state inspite of having a good number of produces in fresh form (palm neera, nungu) as well as in value added form with a capacity to provide high nutritional value which is having the potential to overcome the problem of malnutrition in developing countries like India (Jansz *et al*., 2002).

2.6 VALUE ADDED PRODUCTS

Panipanattu is a condiment food product made by incorporating spices into palmyrah fruit leather, with treacle acting as a preservative. The fruit is cut into pieces, ground into powder, and mixed with roasted gingilly, roasted parboiled rice, and cumin. The palmmyrah treacle is then de-limed and filtered to remove suspended particles. The clear liquid is siphoned out and concentrated, forming a thick dark syrup with 65 g of treacle. The mixture is allowed to soak for 3 days before being ready for use.

Panattu fruit and nut bars are optimized, containing 25g of palmyrah fruit, 10% gelatin, 60g milk powder, 40g condensed milk, 17.5g peanut, 17.5g soybeans, and 64g treacle. The desert contains 6.22g moisture, 3.62g ash, 21.8g crude protein, 15.68g crude fat, 0.36g crude fiber,
3.89g reducing sugar, total sugar, 170 mg phosphorous, and 472.82 Kcal energy in 100 bar (Sulakshan *et al*., 2015).

Panattu choco bars are prepared with three different bitter reducing agents, such as sugar, salt, and citric acid, in different ratios. The ready-to-serve (RTS) product contains 12% fruit pulp, 12.5% sugar, and a pH of 4.0 with citric acid as acidulant. The cordial is formulated and improved using palmyrah pulp (Pavithra *et al.*, 2021).

Palmyrah fruit jelly, jam, wine, sauce, ice cream and yoghurt are all products made from palmyrah fruit pulp. Jeyar ajasingam *et al.* 2016, prepared palmyrah fruit jelly using 40 g of fruit pulp, 50 g of sugar, 0.5 g of seaweed extract, and 0.5 g of citric

acid. The jelly contained 29.40% moisture, 6.48% reducing sugar, 56.05% total sugar, 1.67% protein, and 0.89% ash content. Thabira, S., 2017, formulated palmmyrah jam using palmyrah fruit pulp with other 20% orange fruit juice. Sobini *et al*., 2018, studied the formation of superlative wine using pectinase treated palmyrah fruit pulp, which is rich in sugar lycopene and vitamin C. Palm sauce was prepared using palmmyrah fruit pulp with sugar, vinegar, salt, and water. Parameswaran *et al.,* 2016, developed palm ice cream incorporating palm fruit pulp, which contained 66.01% moisture, 9.89% fat, 1% ash, 0.77% dietary fiber, 21.36% total sugar, 4.3% reducing sugar, 17.38% non-reducing sugar, 101.50 mg per 100 g vitamin C, 49.75 mg per 100 g calcium, 36.74 mg per 100 g sodium, and 183.67 mg per 100 g potassium.

Chathuranga *et al*., (2021) developed a value addition for Palmyrah Fruit with the formulation of Palmyrah Wattalappam (PW), which contained palmyrah jaggery (PJ), eggs, coconut milk, and spices. PW was more stable in transparent polypropylene and had a good texture, flavor, aroma, and shelf life of one month. Outschoorn *et al*., (2022) formulated palmyrah fruit pulp (25%) incorporated with yeast extract and vegetable extract, showing a pH of 4.76 ±0.01 and titratable acidity of 1.69 ± 0.04 g/100ml. The bread spread was developed using palmyrah fruit pulp (52.5%), peanut (25%) coco powder, 2.5% sugar, or palmyrah treacle (15%) and coconut oil (5%).

A study was conducted to increase the value addition to Palmyrah fruit using acid and heat management to lessen or mask its bitterness. The fruit pulp added milk beverage was developed, containing 15% of fruit pulp, 6% palmyrah sugar, and 0.2% carboxymethyl cellulose stabilizer. The palmyrah pulp and coconut milk incorporated drink showed a nutritional content of 83.29, 3.4, 1.63, 23.49, 0.21, and 1.55%, respectively (Mahilrajan *et al*., 2024). Spray-dried palmyrah fruit pulp incorporated with skim milk was prepared by adding maltodextrin and gum Arabic at 170°C temperature, containing total sugar, protein, and Vitamin C. The 100 g powder contained total sugar, protein, and Vitamin C, with 78.30 mg GA/100 g and 70.42 % solubility with 46.47% yield.

2.7 READY TO SERVE (RTS) BEVERAGES

This is a type of fruit beverage which contains at least 10% fruit juice and 10% total soluble solids besides about 0.3% acid as per FSSAI. It is not diluted before serving; hence it is known as ready-to-serve (RTS). They are widely preferred due to their convenience, uniform taste, and nutritional value. Fruit Juice: At least 10% fruit juice

content, which imparts the natural flavor and nutrients of the fruit. Total soluble solids (TSS) At least 10% total soluble solids, which include sugars and other dissolved components. This ensures a balanced sweetness and texture. Acidity approximately 0.3% acid (usually from citric acid or naturally occurring fruit acids), which enhances the taste and helps in preservation by reducing microbial growth. To prepare RTS beverages, extract juice or pulp from fresh fruits using appropriate methods. Filter the juice to remove impurities, then add sugar syrup, citric acid, and preservatives to adjust sweetness and acidity. Homogenize the mixture for uniform consistency, followed by pasteurization to ensure safety and shelf stability. Finally, package the beverage in sterilized containers and store in a cool, dark place.

2.7.1 Preservation methods of ready-to-serve (RTS) beverages

Preservatives are added to RTS beverages to increase their shelf life by preventing microbial growth, spoilage, and chemical degradation. These can be classified into chemical and physical methods, with their allowable quantities regulated by food safety standards.

Chemical Preservatives, these substances directly inhibit the growth of microorganisms or delay chemical reactions that lead to spoilage. Sodium Benzoate, Permissible Limit Up to 120 ppm (parts per million). Effective in acidic conditions (pH below 4.0). Inhibits the growth of molds, yeasts, and some bacteria. Potassium Metabisulfite (KMS), Permissible Limit Up to 70 ppm of sulfur dioxide (SO_2). Releases sulfur dioxide, which prevents browning and microbial growth. Citric Acid (or natural acids), Depends on the desired acidity, but generally 0.2-0.3%. Reduces pH, enhancing the effectiveness of other preservatives. Physical Preservatives include, Pasteurization, Ultraviolet (UV) Radiation, Cold Sterilization (High-Pressure Processing - HPP) etc. Pasteurization is a widely used preservation method in the food and beverage industry. It involves the controlled application of heat to destroy harmful microorganisms, extend shelf life, and retain the quality of the product. Pasteurization Kills or inactivates pathogenic microorganisms (e.g., bacteria, yeasts, molds). Reduces enzymatic activity that causes spoilage. Retains most of the product's sensory and nutritional qualities.

2.8 FRUIT BASED READY TO SERVE BEVERAGES

The Department of Horticulture, College of Agriculture, Junagadh Agricultural University, Junagadh, conducted an experiment in 2007 to standardize the recipe for

making a ready-to-serve beverage from mango (*Mangifera indica L.*). Three repeats of the experiment were set up in a completely randomized design. Mango, lime, and cardamom juices, both fresh and blended, were used to make the 8%, 10%, and 12% juice.

The overall acceptance of mango RTS beverages decreased as storage time increased. Among the tested formulations, T11, which contained 10% blended juice (mango, lime, and cardamom with 12% TSS), consistently had the highest overall acceptance values at each storage interval (range from 44.61 to 40) and the smallest reduction in acceptability with time. T1 (8% mango juice, 10% TSS) had the lowest acceptance scores (from 33.855 at month 0 to 28.075 at month 4) and the biggest drop in acceptability, which was consistent with the other treatments with 8% juice, 12% TSS, or 12% juice, 10% TSS. These findings are consistent with previous studies by Rao *et al* (1979), who discovered that blends containing acid lime and Rangpur lime performed the best in terms of flavor and consistency. The overall acceptance of mango RTS beverages decreased as storage time increased. Among the tested formulations, T11, which contained 10% blended juice (mango, lime, and cardamom with 12% TSS), consistently had the highest overall acceptance values at each storage interval (range from 44.61 to 40) and the smallest reduction in acceptability with time. T1 (8% mango juice, 10% TSS) had the lowest acceptance scores (from 33.855 at month 0 to 28.075 at month 4) and the biggest drop in acceptability, which was consistent with the other treatments with 8% juice, 12% TSS, or 12% juice, 10% TSS. These findings are consistent with previous studies by Rao et al. (1979), who discovered that blends containing acid lime and Rangpur lime performed the best in terms of flavor and consistency.

Fully mature, firm hill lemons were washed, peeled, sliced, and juice extracted with a screw extractor. The juice was filtered, pasteurized at 90°C for 10 seconds, chilled, and preserved with700 ppm KMS before being stored in PET bottles. Based on taste evaluations, an RTS beverage with 85% hill lemon and 15% ginger juice, sugar, sorbitol-sugar (50:50), and stevia-sugar (75:25) was chosen for the storage stability test. The RTS was packed in PET bottles (with 70 ppm SO_2) and glass bottles (heat processed), and stored at ambient (26–33°C) and refrigerated (3–7°C) conditions. Stability was assessed at 0, 30, 60, and 90 days. Following packaging, PET and glass bottles were stored for up to three months at room temperature (26℃ to 33℃) and in a refrigerator (3℃ to 7℃) (Reddy, 2024).

2.9 RTS FROM PALMYRAH PALM FRUIT

Formulation of a milk beverage based on palmyrah pulp The SLS Standard 917: 1991 specification for beverages containing milk was consulted during the formulation process. By varying the pulp percentage in three different levels (12%, 15%, and 18%), adding two different kinds of sweeteners (cane sugar and palmyrah sugar), and adding two different kinds of stabilizers (pectin and CMC), a total of twelve treatments were created.

Development of a milk beverage made from palmyrah pulp, made using the following technique. Palmyrah fruit pulp was mixed with preheated milk (72°C), followed by the addition of stabilizer and sugar. The mixture was homogenized at 10,000 rpm for seven minutes, then heated in a water bath at 85°C for 20 minutes, with temperature monitored using a thermocouple. Sterile 200 ml glass bottles, pre-sterilized at 160°C for two hours, were used for packaging. The heat-treated mixture was filled into the bottles, sealed, and stored at 4°C. The pH of fresh milk ranged from 6.5 to 6.7, while the acid-treated pulp had a pH of 4.7 (Schonbrun, 2002). The titratable acidity of the finalized milk sample and the commercially accessible milk sample differs significantly. This is due to the fact that commercially available milk has less acidity than pulp, which has an acid concentration of 0.33%. The total soluble solid content of the samples varies significantly as well. Since pulp's total soluble solid content of 16.5 contributes to the total soluble solids of the prepared drink, its addition to the milk may be the cause of the final milk sample's higher total soluble solids value compared to the commercially available milk sample. 1.2g of ash, 7.74g of total sugar, 30.74 mg of sodium, 341.85 mg of potassium, 266.83 mg of phosphorous, and 18.95 mg of calcium are all present in 100 g of pulp.According to the microbiological analysis results, the yeast and mold count was zero, while the overall plate count did not surpass 10/0.1 ml, even after four weeks of refrigeration.

Getting the pulp from palmyrah fruits the fruits were maintained at 200°C until the soap-like foam stopped forming. After the pulp was extracted using a 1:1 water ratio. The drink was then prepared by homogenizing and blending the pulp. Compared to the beverage made with preserved fruit pulp, the one made with fresh fruit pulp had a comparatively superior flavor and taste. Through the initial sensory evaluation, treatment number 15 (pulp 12%, sugar 12.5%, and pH 4.0) was chosen from among the 18 treatments in order to maximize the constituents in the formulae.

Sugar, pectin, and palmyrah pulp were combined and pasteurized for one minute at 90°C. After adding citric acid, the mixture was put into glass bottles that had been sterilized and pasteurized for 20 minutes at 96°C. Bottles of the prepared RTS beverage were stored at room temperature. Fruit pulp and sugar levels in the range of 10–20% were assessed in order to determine the amounts of ingredients to include in the beverage, while other ingredients were kept constant across all products. Using a preliminary sensory evaluation, pectin was chosen as a stabilizer and citric acid as an acidulant for the RTS drink. The pH of the RTS (Ready-to- Serve) beverage was 3.89. 14.75°Brix was the total soluble solids (TSS) concentration. The measured titratable acidity was 0.11 ± 0.01%. Moreover, 86.41 ± 8.8 ppm of benzoic acid was present. All of these factors show that the RTS has the potential to be a tasty and stable product. The pectin was optimized between 0.60 and 0.69 percent. The created Palmyra fruit RTS Drink has a minimum shelf life of 60 days at room temperature because no yeast, mold, or bacterial colonies were found until nine weeks (Sivarajah *et al*., 2018).

CHAPTER III

MATERIALS AND METHODS

This chapter describes the materials used and the methodology adopted for the development ready to serve beverage from palmyrah fruit pulp. The procedures adopted for the evaluation of the physiochemical, microbial and sensory qualities of the palmyrah fruit based RTS are also explained in detail.

3.1 PROCUREMENT OF RAW MATERIALS AND CHEMICALS

Fresh and fully ripened palm fruits (*Borassus flabellifer* L) were procured directly from farmers in Palakkad district, Kerala. The procured samples were brought to the Food Processing laboratory of Department of Processing and Food Engineering, KCAEFT,Tavanur, Malappuram.

3.2 EXTRACTION OF PULP

The preparation of ripe palmyrah fruit ready to serve (RTS) beverage involves several steps to ensure a high-quality and safe product. The ripe palm fruits were thoroughly washed under hot water at about 50ºC to remove dirt, debris, and any surface contaminants. Properly washed fruit's outer skin was carefully peeled manually, to expose the soft, fleshy part of the fruit, ensuring all fibrous outer layers are removed. After peeling, the pulp extracted from the fruit using different strainers to separate the pulp from the seeds. Transfer the collected pulp in a clean bowl. This extracted pulp was stored in well sterilised air tight stainless-steel barrels at below -18±2°C and then used for treatments as required.

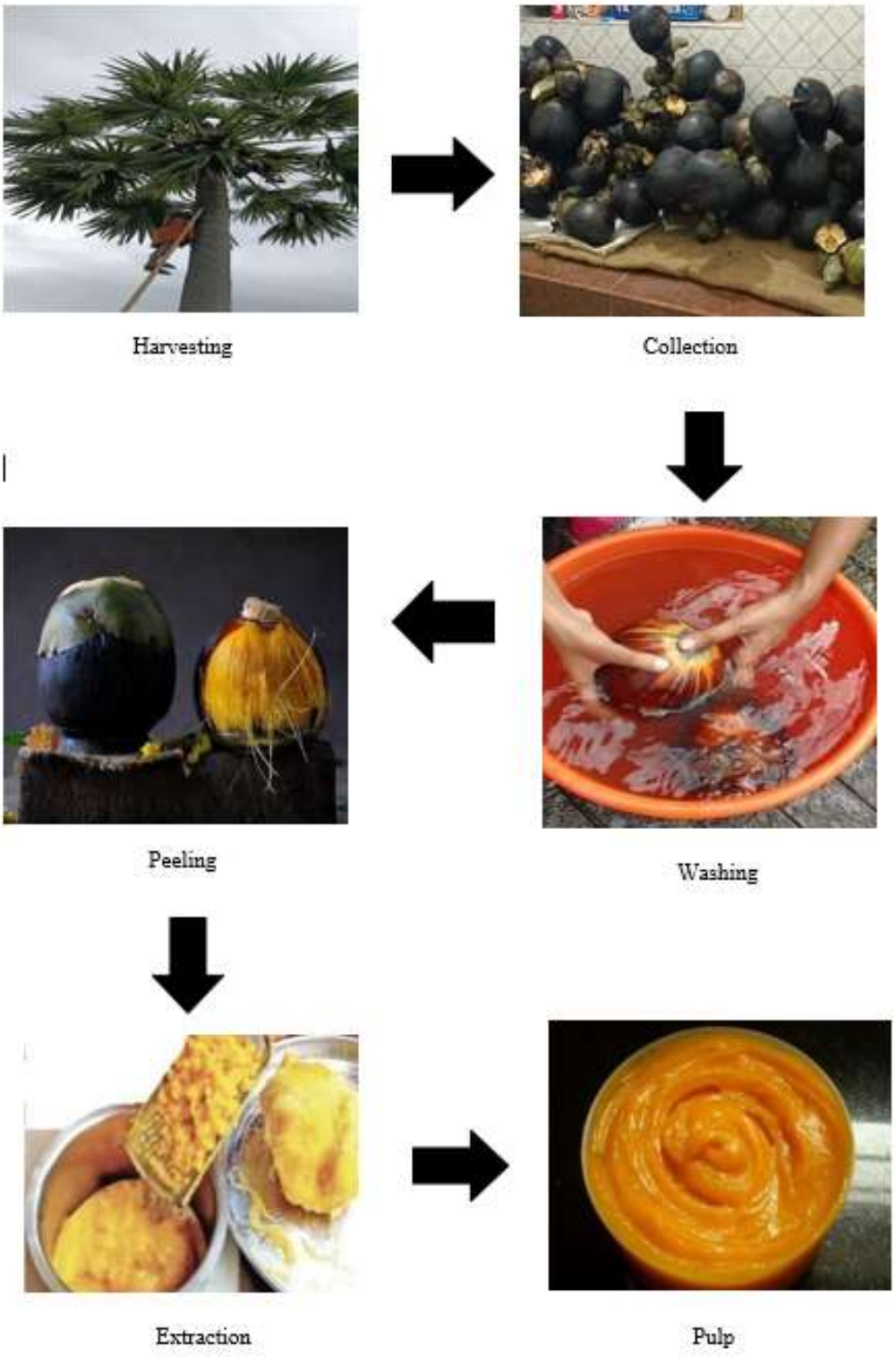

Plate 3.1 Different step in pulp extraction

3.3 PHYSICO CHEMICAL CHARACTERISTICS OF PULP

3.3.1 Total soluble solids (TSS)

The total soluble solids in ripe palm fruit pulp was determined with the help of hand refractometer (ERMA make) of 0-32 °B and results expressed in degree Brix (°B) (Ranganna, 2017). The prism of the refractometer was washed with distilled water and wiped dry before every reading. The observation for TSS was taken by placing 2 drops of pulp on the prism and reading was expressed in the term of degree Brix.

Plate 3.2 Digital brix refractometer

3.3.2 pH

The pH of ripe palm fruit pulp was determined by using digital pH meter. Before estimation of pH of sample, the pH meter was calibrated with different buffers of pH 4.0 and pH 7.0 and operating instructions were followed. The electrode was dipped in beaker containing sample to be tested and reading was recorded (AOAC, 2000).

Plate 3.3 Digital pH meter

3.3.3 Titrable acidity

Total acidity of pulp was determined and expressed in malic acid equivalent percentage (AOAC, 2000). Five millilitres of samples were collected in 250 ml conical flask containing 100 ml of distilled water. Few drops of phenolphthalein were added to

the solution as an indicator and shaken well. The burette was filled with 0.1 N NaOH. The solution was titrated against the solution in burette until the sample solution showed a faintest discernible pink colour which persisted for 30 seconds. Acidity was estimated using the following equation:

$$\text{Acidity}(\%\ \text{malic acid}) = \frac{\text{Volume of titrant(ml)} \times \text{Normalityof titrant} \times 0.067}{\text{Sample weight(g)} \times 100}$$

3.3.4 Moisture content

The infrared moisture meter determines the moisture contents of samples by emitting infrared radiation on the sample surface. The moisture content is determined based on the interaction between the infrared radiation and the sample. Before starting the actual measurement, the infrared moisture meter needs to be calibrated. Then prepare the sample and it should be finely ground or homogenized to ensure consistent results. After that, turn on the infrared moisture meter and allow it to warm up as per the manufacture instructions. Make sure the instrument is clean and free from any contaminants that could affect the accuracy of the measurements. Place the prepared sample in the sample holder or container provided by the moisture meter. Ensure that the sample is spread evenly and covers the measurement area completely. Then activate the moisture meter to initiate the measurement process. The moisture meter will display the moisture content value on its screen digitally. Repeat measurements to ensure accuracy and precision.

Plate 3.4 Infrared moisture meter

3.3.5 Color

The optical property of a material is the color which is determined on the basis of three values; L*, a* and b* using a tintometer. L* value indicates the darkness to whiteness (0 to 100), a* value from green (-) to red (+) and b* value from blue (-) to

yellow (+). Color of the palmyrah pulp was measured using a Lovibond Tintometer (Model: LC100), in which the sample is filled in the cuvette provided and placed on the port of tintometer and closed with the opaque cover. The opaque cover acts as a light trap to block out the outside light interference. The colour was measured from all four sides and the mean was recorded.

Plate 3.5 Lovibond tintometer

3.3.6 Yellowness index

Yellowness index (YI) indicates the degree of yellowness. Yellowness is one of the important color characteristics in the case of fruit juices. This value represents the color variation due to scorching, soiling, and general product degradation by light, chemical exposure and processing. Yellowness indices are used mainly to quantify these types of degradation with a single value. They can be used when measuring clear, near-colorless liquids or fruit juices. The yellowness index is estimated from the L* and b* values.

$$\text{Yellowness index} = \frac{142.86\ b*}{L*}$$

3.3.7 Ascorbic acid

Ascorbic acid content in fruit pulp was estimated using the 2, 6-dichlorophenol indophenols titrimetric method as described by Sadasivam and Manickam (1992). Dye solution was prepared by dissolving 52 mg of 2, 6 dichloro phenol indophenols, and 42 mg of sodium bicarbonate in 200 ml distilled water. Standard solution was prepared by adding 100 mg of ascorbic acid to 100 ml of 4% oxalic acid. To prepare working standard solution, 10 ml of standard solution was pipetted out and was diluted to 100 ml using 4% oxalic acid. The 5 ml fruit juice samples were made up to 50 ml using 4 percent oxalic

acid. To find dye factor, 10 ml of working standard solution was pipetted out into a 50 ml conical flask and 10 ml of 4% oxalic acid was added and titrated against the dye. The end point was the appearance of pink colour which persisted for a few minutes. The titration was repeated to get concordant values. The amount of dye consumed was equal to the amount of ascorbic acid present in the working standard solution (V_1). Ten millilitre of sample extract was pipetted out to which 10 ml of 4% oxalic acid was added. It was then titrated against the dye. The titration was replicated for each sample until the concordant values were obtained (V_2).

$$Dye\ factor = \frac{0.5}{Titrable\ value(V_1)}$$

$$Ascorbic\ acid\ \frac{mg}{100g} = \frac{0.5\ mg}{V_1\ ml} \times \frac{V_2}{5\ ml} \times \frac{100\ ml}{wt.\ of\ \ the\ sample} \times 100$$

V_1 - Amount of dye consumed by ascorbic acid present in the working standard solution, ml.

V_2 - Amount of dye consumed by the liquid sample, ml.

3.3.8 Total phenolic content

Total phenolic content of ripe palmyrah fruit RTS was determined by Folin-ciocalteu method as described by Sadasivam and Manickam (1992). The production of blue coloured complex during the reaction of phenols with phosphomolibdinic acid in presence e of Folin- ciocalteu reagent in alkaline medium is the basis of the estimation. One millilitre of fruit juice samples was diluted with 10 times volume of 80% ethanol and then centrifuged at 10000 rpm for 20 minutes. The supernatants were collected and re-extracted with five times volume of 80% ethanol. The supernatant is then centrifuged at 20000 rpm for 20 minutes and were collected. About 0.2 ,0.5 and 1ml aliquots of palm fruit RTS were pipetted out in to the test tubes and the volume was made up to 1 ml using distilled water. The stock solution was prepared by dissolving 100 milligram gallic acid in 100 millilitre distilled water. To make the working standard 10 ml stock was made upto 100 millilitre. Add 5 ml Folic- ciocalteu and 4 ml sodium carbonate to 1 ml aliquot of dilute sample. Vortex the mixture and incubate for 2 hours under dark. Measure the absorbance at 750 nm. A standard curve of gallic acid was plotted with different

concentration of tannic acid from 0.2 -1 ml at interval of 0.2 ml. The phenol content of the sample was measured as the gallic acidequivalent.

Plate 3.6 Spectrophotometer

3.4 PREPARATION OF RTS

The preparation of RTS from palmyrah palm fruit involves a systematic process where the pulp concentration, sugar concentration, and treatment type are varied, while citric acid is kept constant at 0.28 g. The various combinations required for preparation of RTS is given in table 3.1

The fruit pulp stored in the freezer at -18ºC were gradually cooled to room temperature and used for the preparation of Ready to serve beverages. Different fruit pulp concentrations such as 10, 14 and 18 % were selected for preparation of RTS beverages.

For preparation of 100 ml RTS, three different concentration of sugar syrups was prepared with diluting the sugar in appropriate quantity of water (heated) as explained in Table 3.1. After cooling the sugar syrup to room temperature, various concentration of fruit pulps was mixed with respective sugar syrups. About 0.28 g citric acid was added in to each pulp sugar blend. These mixtures are thoroughly blended to prepare RTS beverages.

The prepared RTS was categorized in to three different equal portions. One portion of the RTS was filled in sterilized PET bottles and stored at 4ºC in refrigerated condition without any further treatment. The second portion of RTS was added with edible chemical preservative potassium meta bisulphate at a concentration of 70 ppm (as specified in FSSAI). Then packed in sterilized PET bottles and stored at 4°C in refrigerated condition. The remaining portion of RTS were filled in sterilized PET bottles and pasteurized by heating them to 80°C for 20 minutes keeping in temperature controlled hot water tank. The pasteurized samples were allowed to cool to room temperature

gradually and stored at 4ºC in refrigerated condition.

Table 3.1 Combinations of RTS preparation

Treatment	**Samples**	**Sugar concentration(g)**	**Citric acid (mg)**	**Water (ml)**	**Pulp concentration (g)**
Treatment	T1	10	0.28	100	10
Normal	T2	10	0.28	100	10
Pasteurized	T3	10	0.28	100	10
Addition of KMS	T4	14	0.28	100	14
Normal	T5	14	0.28	100	14
Pasteurized	T6	14	0.28	100	14
Addition of KMS	T7	18	0.28	100	18
Normal	T8	18	0.28	100	18
Pasteurized	T9	18	0.28	100	18

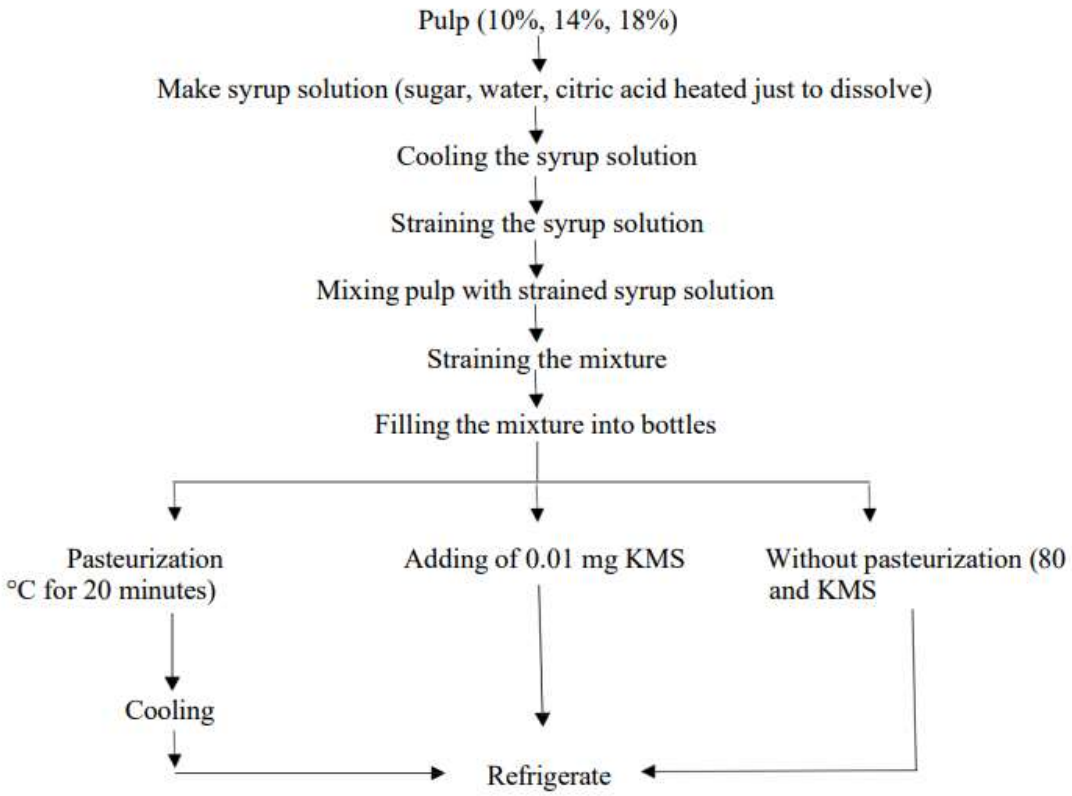

Fig. 3.1 Flowchart for preparation of RTS

3.5 PHYSICOCHEMICAL CHARACTERISTICS OF RTS

The RTS beverage samples were analysed for various physiochemical properties such as pH, TSS, titrable acidity, ascorbic acid and total sugars as described in section 3.3.

3.6 SENSORY EVALUATION

The sensory evaluation of the prepared RTS beverages was conducted using a 9-point hedonic scale to assess consumer acceptability. A panel of 17 semi-trained judges, aged between 20 to 45 years, including faculty members and postgraduate students, was carefully selected. The panel evaluated the beverages for sensory attributes such as color, taste, flavor, appearance, astringency, and overall acceptability. Nine samples of RTS beverages with different pulp concentrations (10%, 14%, and 18%) and a control sample were presented to the judges. Plain water was provided to rinse their mouths between the evaluations of samples to minimize taste carryover. Sensory scores were recorded on individual sensory cards, and the standardization of one optimal concentration was based on the sensory evaluation results.

3.7 CHARACTERISTICS OF RTS BEVERAGES

The RTS sample secured highest score in the sensory evaluation were further analysed for various physicho-chemical characteristics such as phenolic content, tannin, anti-oxidant activities, mineral content (potassium and sodium), titrable acidity, pH, ascorbic acid, moisture content, color measurement, yellowness index and browning index.

3.7.1 Measurement of anti – oxidant activity

A spectrophotometer was used to measure the antioxidant capacity of the fruit juices. A stock solution of DPPH (2, 2-Diphenyl-1-picrylhydrazyl) was prepared by dissolving approximately 15 mg DPPH in 100 ml methanol and stored at −20 ºC until further use. The working solution was prepared by mixing 10 ml of stock solution with 45 ml of methanol to adjust absorbance at 517 nm wavelength to unity, which was then kept in dark. The change in colour of the DPPH solution from purple to yellow, resulting from the addition of different quantities of ascorbic acid standards and ethanolic extract of juices (20 to 200 µl) was measured at 517 nm after allowing the solution to stand in the dark for 20 min. The decrease in absorbance of DPPH after 20 min was calculated and expressed as mg of ascorbic acid equivalents antioxidant capacity per 100 g. The control sample was prepared as above without any sample extract and methanol was used for the baseline correction. The inhibition rate is calculated by using equation given below (Nazir *et al.,* 2013).

$$\text{Percentage radical scavenging activity (\%)} = \frac{Absorbance\ of\ control - Absorbance\ of\ sample}{Absorbance\ of\ conrol}$$

3.7.2 Minerals

Minerals in the liquid foods were estimated using the Atomic Absorption Spectrophotometer (AAS) (Plate 3.17) as per AOAC (2000). Five hundred milligram of sample was taken in a pyrex flant bottomed flask and digested with a mixture of concentrated nitric acid, sulphuric acid and perchloric acid (9:2:1 v/v). Initial digestion was carried out in a cold state and then digested over sand both until an ashy white digest was obtained. The digest was filtered and made up to a known volume. The triple acid aliquot was used for the estimation of minerals.

Flame atomic absorption technique was used for analysis of minerals. The liquid is injected in to the inlet of the AAS. The liquid samples were aspirated, aerosolized, and

mixed with combustible gases such as acetylene and nitrous oxide. The mixture was ignited in a flame whose temperature ranges from 2100 to 2800˚C. During combustion, atoms of the element of sample were reduced to free unexcited state atoms, which absorb light at characteristic wavelength. The amount of light absorbed would be measured against a standard curve. The minerals such as calcium, potassium, and sodium were determined by comparing the atomic spectroscopic signal for each with that for standard solution of same ion.

3.8 STORAGE STUDY

A storage study was conducted to evaluate the stability and quality of RTS beverages prepared from ripened palmyrah fruit pulp with different pulp concentrations (10%, 14%, and 18%). Each concentration was divided into three samples: one with normal RTS, the second added with potassium metabisulphite (KMS), and the third subjected to pasteurization (80°C for 20 minutes), resulting in a total of nine samples. The prepared beverages were stored under refrigerated conditions (4℃ ± 2), and quality parameters were analysed at intervals of 7 days over the storage period. The analyses included titratable acidity, ascorbic acid (vitamin C) content, total soluble solids (TSS), pH, color attributes, and microbial load, to assess changes in physicochemical, sensory, and microbiological properties during storage. This systematic evaluation helped determine the impact of pulp concentration and preservation methods on the shelf life and quality of the beverages.

3.9 MICROBIOLOGICAL ANALYSIS

The microbiological quality characteristics of both fresh ripe palm fruit pulp and RTS samples were determined. The growth of bacteria and fungi were found through standard plate count method.

Enumeration of the total bacterial count and yeast and mould count in fruit juices. The bacterial and fungi population in fruit juices were analyzed by different microbiological methodologies, that includes enumeration of the microorganism in selective media for different dilutions of samples, incubation of plates and counting the number of colonies present. The media generally used for enumeration of bacteria is nutrient agar medium, whereas, for fungi enumeration chloramphenicol yeast glucose agar media was used (Allen, 1953). The fruit juice sample of 1 ml was pipetted using a sterile pipette into a test tube containing 9 ml of sterile water which gave

a 1:10 (10^{-1}) dilution. The test tubes were shaken well for 10-15 minutes for uniform distribution of microbial cell in the water blank. Then 10^{-2} dilution was prepared by pippetting out 1 ml of (10^{-1}) dilution to 9 ml of sterile water in test tube with a sterile one ml pipette. The process was repeated up to 10^{-6} dilutions with the serial transfer of the dilutants. One millilitre of aliquots from 10^{-5} and 10^{-6} dilutions were transferred to the sterile petri dishes for the enumeration of bacteria and one millilitre aliquots from 10^{-4} and 10^{-3} dilutions were transferred to the sterile petri dishes for the enumeration of fungi. The dilutions were so selected based on the preliminary studies conducted and a thorough review of literature. The experiments were carried out in duplicate and the mean value is reported.

Approximately, 15-20 ml of molten and cooled (45ºC) respective agar medium was added to each petridish containing the sample dilutions and the plates were rotated in clockwise and anticlockwise direction for thorough mixing of the dilutants and the medium. The plates were then incubated at 35°C (room temperature) for 24-48 hours for bacteria and at 20 to 25°C for two to four days for yeast and mould, respectively. After the incubation period, the colonies were counted and the number of organisms (total bacteria and yeast and mould) per gram of sample was calculated by using the equation given below;

$$\text{Number of colony forming units(cfu)per gram of the sample} = \frac{\text{Mean number of cfu x Dilution factor}}{\text{Quantity of sample on weight basis}}$$