PROCESSING AND SHELF-LIFE STUDIES OF COCONUT WATER

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TAVANUR-679573, MALAPPURAM

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THESIS

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KELAPPAJI COLLEGE OF AGRICULTURAL ENGINEERING AND TECHNOLOGY

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2024

DECLARATION

We hereby declare that this thesis entitled “Processing and Shelf-life Studies of Coconut Water” is a bonafide record of 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, associateship, fellowship or other similar title of any other University or Society.

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Place: Tavanur

Date: 24-01-2024

CERTIFICATE

Certified that this thesis entitled "**Processing and Shelf-life Studies of Coconut Water**" is a bonafide record of research work done independently by **Silpa O (2020-06-004), Nandana Vishnu (2020-06-008), Muhammad Siraj (2020-06-009) and Muhammed Arshad K C (2020-06-012)** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship, or associateship.

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DEDICATED TO

ALL FOOD TECHNOLOGISTS

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LIST OF SYMBOLS AND ABBREVIATIONS

WHO	: World Health Organisation
GRAS	: Generally Recognised as Safe
KMS	: Potassium Metabisulphite
CW	: Coconut Water
Fig.	: Figure
TSS	: Total Soluble Solids
TPC	: Total Phenolic Content
BP	: Blood Pressure
PNS	: Philippine National Standard
PE	: Polyethylene
FAs	: Fatty Acids
HDL	: High Density Lipoprotein
CFU	: Colony Forming Unit
MPN	: Most Probable Number
RPM	: Revolutions Per Minutes
g/L	: Grams per litre
PPO	: Poly Phenol Oxidase
POD	: Peroxidase
PET	: Polyethylene Terephthalate
K_{eq}	: Equilibrium constant
MAP	: Modified Atmospheric Storage

KINFRA	: Kerala Industrial Infrastructure Development Cooperation
ppm	: Parts per million
T	: Treatment
mL	: Millilitre
nm	: Nanometre
w/v	: weight/volume
M	: Molar
L*	: Lightness or Darkness
a*	: Greenness or redness
b*	: Blueness or yellowness
V	: Volume
TNTC	: Too Numerous to Count
TPC	: Total Plate Count
°	: Degree

INTRODUCTION

CHAPTER I

INTRODUCTION

Coconut Palm (Cocos nucifera) was one of the most important crops grown in humid tropics, cultivated for its multiple utilities; mainly nutritional and medicinal value. It belongs to Arecaceae family, the only living species of the genus Cocos. Since most of the components of coconut palm was getting transformed to useful products, it is referred to as the "Tree of life". It can provide various natural products for the development of medicines and some industrial products. The annual global production of coconut was around 62.4 million ton. Globally India ranks 3rd position with an average production of 11.5 million ton. The major conventional regions of coconut farming include the states of Tamil Nadu, Kerala, Puducherry, Karnataka, Odisha and the islands of Andaman and Nicobar and Lakshadweep.Area of cultivation of coconut in India during the year 2022 was 21 Lakh hectares with a productivity of 10,000 coconut per hectares. Though Kerala stands first in number of coconut trees, the statistics reveals that Tamil Nadu is the largest producer of coconut in India (Narmadha *et al.*, 2022).

Coconut water was a beverage appreciated worldwide. It is a drink with a slight sweet and acid flavour (pH 5.5), it was somewhat cloudy. Coconut water was a healthful beverage with numerous benefits. Not only is it a delicious and refreshing way to stay hydrated, but it also offers essential minerals like potassium, sodium, and magnesium, making it an excellent natural electrolyte source. The low-calorie content of coconut water makes it a preferable alternative to many sugary drinks, promoting hydration without excessive caloric intake. Its rich potassium content contributes to heart and muscle health, potentially aiding in blood pressure regulation. Moreover, the antioxidants found in coconut water help combat free radicals, supporting overall well-being by reducing oxidative stress and inflammation. With its fiber content, coconut water may assist in digestion and act as a mild laxative. Coconut water comprise approximately 94-95% water, it serves as a natural hydrator enriched with electrolytes, notably high levels of potassium, which is pivotal for maintaining cardiovascular and muscular health.

Nata de coco, a unique and popular delicacy, was a chewy and translucent jelly-like substance that originates from the fermentation of coconut water. This Filipino-originated treat has gained widespread popularity due to its distinct texture and versatility in various culinary applications. It was low in calories and fat, making it a guilt-free addition to snacks and desserts. Additionally, nata de coco was a good source of dietary fiber, promoting digestive health and contributing to a feeling of fullness. As a fermented food, it may also contain probiotics, which can have positive effects on gut health.

Coconut water was a highly nutritious beverage which favours microbial spoilage. The product was also susceptible to browning due to high oxido-reductase enzyme concentrations such as polyphenol oxidase and peroxidase.

The clarification process in coconut water was pivotal for enhancing its overall quality and desirability. Through the meticulous removal of impurities, debris and suspended particles, this process not only renders coconut water visually appealing but also significantly improves its taste. The resultant clarity achieved through filtration and centrifugation contributes to a transparent and aesthetically pleasing beverage that aligns with consumer expectations. Furthermore, the removal of microorganisms during pasteurization extends the shelf life of coconut water, ensuring its safety and nutritional consistency over time. The clarification process plays a vital role in reducing off-flavours and undesirable odours, promoting a more palatable and refreshing drink.

Potassium metabisulfite (KMS) plays a pivotal role in enhancing the shelf life of coconut water, contributing to both the preservation of its quality and the extension of its viability as a consumer beverage. Functioning as a potent antioxidant, KMS effectively inhibits oxidative reactions, thereby preventing colour deterioration and maintaining the beverage's appealing visual characteristics.

Citric acid serves as a valuable tool in enhancing the shelf life of coconut water, contributing to both its preservation and sensory appeal. It regulates the pH of coconut water, creating an acidic environment that inhibits the growth of spoilage microorganisms. The use of citric acid in the processing of coconut water, when carefully controlled, underscores its significance in achieving an optimal balance between preservation and sensory quality.

The effect of carbonation was also assessed, since it is generally known to provide a refreshing sensation and also because of a possible role in microbial growth inhibition. The introduction of carbon dioxide also creates an acidic environment.

In this background the project entitled “Processing and Shelf-life Studies of Coconut Water” was undertaken at Kelappaji College of Agricultural Engineering and Technology (KCAET), Tavanur, Kerala, India with the following objectives

1. To determine the physio chemical properties of raw coconut water.
2. To optimize the process parameters for preservation of coconut water.
3. Shelf-life studies of the optimised processed coconut water.

REVIEW OF LITERATURE

CHAPTER II

REVIEW OF LITERATURE

This chapter deals with literature reviews on coconut water and its composition, importance of coconut water and its utilisation in the industry. The variables which decide the quality parameters and functional properties relevant to the clarification of coconut water and its shelf-life studies have also been reviewed and discussed.

2.1 COCONUT WATER

Coconut (Cocos nucifera L.) has been described as the most important and extensively grown palm tree worldwide. It belongs to Arecaceae family. Every part of the plant was useful and, in many cases, human life would be impossible in its absence (Bourdeix,2006). The leaf and trunk provide building material, and the root was used as medicine (Ediriweera,2003). The fruit was the most marketable part; the envelope (mesocarp), called the husk, was processed into rope, carpets, geotextiles and growing media. The hard brown shell (endocarp) can be processed into very high-quality activated charcoal. The inner part of the nut (endosperm) was divided into two edible parts: a white kernel and a clear liquid: coconut water (Pieris,1971).

For more than a century, the coconut pulp or kernel has been considered as a cash crop because of its high fat content; however, nowadays, coconut was more than just an oil seed. Copra, the dried kernel, was a very important international commodity in the first part of the 20th century. Food and chemical industries processed the lauric oil extracted from copra into margarine or detergent. However, in the past 20 years, the volume of world trade in copra has decreased by 75% while the export of “fresh coconuts” has increased by 300%. The market for canned coconut milk, coconut cream and coconut juice/water are increasing considerably (Batugal *et al.,*1996). Coconut was no longer only an international oil commodity but was becoming a valuable fresh fruit.

Coconut water (CW) or coconut juice (not to be confused with coconut milk) is a sweet refreshing drink taken directly from the inner part of coconut fruits (Steiner *et al.,*2008). It differs from coconut milk, which was the oily white liquid extracted from the grated fresh kernel. In most cases, coconut water comes from small and scarce coconut tree plantations more related to "gardens". As a consequence, the coconut water remains a traditional and under-used resource which could thus be considered as an exotic beverage by most people living far from the coconut production area (Jordana,2000).

Coconut water was not only a tropical beverage but also a traditional medicine (Ediriweera,2003), a microbiological growth medium (Osazuwa *et al.,*1989) and a ceremonial gift (Rethinam *et al.,* 2001), and can be processed into vinegar (Sanchez,1985) or wine (Augustine,2007). These various uses are possible thanks to the original biochemical composition of the juice. The particular mineral composition and reasonable total sugar content make coconut water a natural isotonic liquid. The characteristics of coconut water make it an ideal rehydrating and refreshing drink after physical exercise (Saat *et al*., 2002).

Current research on coconut water was rare and mainly focuses on i) specific uses (10%), ii) biochemical composition (50%) and iii) preservation techniques (40%).

2.1.1 Uses of Coconut Water

As it was a sterile and pure liquid, coconut water has been a religious symbol for a long time. In Asia, and especially in India, tender, i.e., immature, coconuts are offered as ceremonial gifts and serve as purification media at traditional events (Rethinam *et al*., 2001).

Centuries ago, Polynesian, Melanesian and Micronesian mariners used coconut fruits as reserves of food and drink (Bourdeix,2006). Thanks to this "naturally canned" beverage, they survived on their journeys from one island to the next and colonised the entire Pacific Ocean. Nowadays, coconut water from immature nuts was still consumed as a refreshing drink by thousands of inhabitants

of tropical regions. The most developed market for coconut water was Brazil (Carvalho *et al*.,2006). The country's top-selling brand, which was produced by Amacoco, was acquired by PepsiCo in 2009 to complement "one of the fastest growing beverage categories due to its natural hydrating qualities, great taste and nutritional benefits" according to Massimo d'Amore, chief executive officer of PepsiCo Americas Beverages. The Coca-Cola Company has followed PepsiCo and become an investor in ZICO Beverages, a Californian company that sells coconut water.

Apart from its consumption as a natural drink, one of the most important uses of coconut water was medicinal (Kumar,1995). There are numerous references to medicinal uses of coconut in Sri Lanka, a country where coconut was consumed on a daily basis (Ediriweera,2003). Coconut water was traditionally prescribed for burning pain during urination, dysuria, gastritis, burning pain of the eyes, indigestion, hiccups or even expelling of retained placenta. In case of emergency in remote regions of the world and during World War II, coconut water was used as a short-term intravenous hydration fluid (Campbell *et al*.,2000).

In the early 1960s, coconut water was already known to favour microbial growth and especially "Nata de coco" bacterium (Alaban,1962). Nata de coco was bacterial cellulose naturally produced at the coconut water/air interface. Native to the Philippines, Nata de coco has become popular in many other Asian countries. The "Nata" bacterium was later identified as Acetobacter xylinum (Gallardo *et al.*,1973). Traditionally coconut water was also processed into wine (Augustine, 2007) or vinegar (Sanchez,1985) due to its sugar content and ability to ferment.

Coconut water (previously called "coconut milk") has been shown to induce division of mature cells (Overbeek *et al.,*1941). For example, the growth of spinach tissue on a medium supplemented with 10% to 15% (v/v) mature coconut water increased the weight of spinach callus after 5 weeks and accelerated shoot regeneration (4–5 weeks instead of 8– 12 weeks without) (Al-Khayri *et al.,*1992).

Many authors reported that coconut water contains a growth factor that stimulates different bacterial strains and in vitro culture of plants (Rethinam *et al*., 2001). For this purpose, coconut water from immature fruits was reported to produce better results than water from mature fruits.

2.1.2 Chemical Composition of Coconut Water

The coconut fruit is composed of five parts (Fig. 2.1). The refreshing and highly nutritious coconut water was in the centre of the coconut surrounded by five parts, i.e., kernel (coconut meat), testa, endocarp, mesocarp, and exocarp (inside out). The fruit was usually ovoid in shape, and the bunch of coconuts grows monthly. A single unit of coconut can grow up to 2 kg.

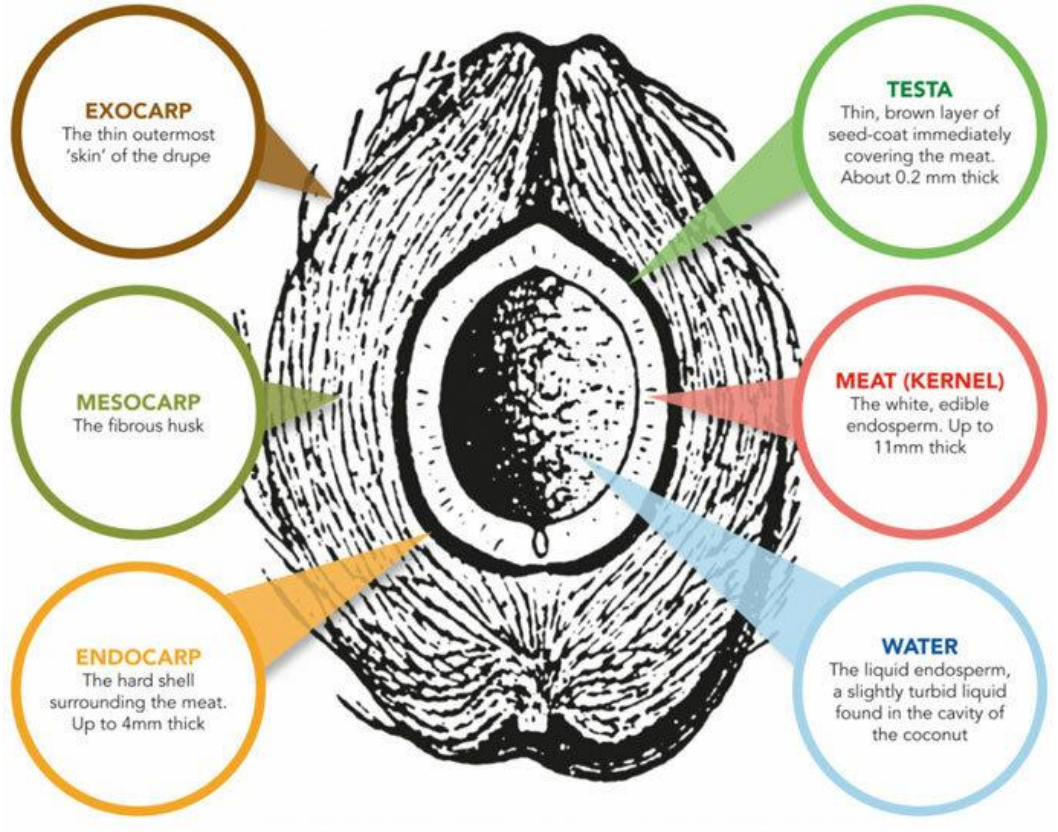

Fig 2.1 Parts of coconut (Parmar,2016)

The proximate composition of coconut water of the Malayan Tall coconuts was presented in Table 2.1

Table 2.1 Proximate Composition of Coconut Water

Physico-chemical properties	Maturity stage (months)		
	5–6 (Immature)	8–9 (Mature)	≥12 (Overly mature)
Volume of water (mL)	684 ± 27.0	518 ± 14.20	332 ± 19.90
Total soluble solids (TSS) (°brix)	5.60 ± 0.14	6.15 ± 0.21	4.85 ± 0.17
Titratable acidity (% malic acid)	0.09 ± 0.004	0.08 ± 0.01	0.06 ± 0.00
pH	4.78 ± 0.13	5.34 ± 0.12	5.71 ± 0.10
Turbidity	0.03 ± 0.013	0.34 ± 0.11	4.05 ± 0.32
Sugar content			
Fructose (mg/mL)	39.04 ± 0.82	32.52 ± 0.23	21.48 ± 0.21
Glucose (mg/mL)	35.43 ± 0.51	29.96 ± 0.24	19.06 ± 0.19
Sucrose (mg/mL)	0.85 ± 0.01	6.36 ± 0.06	14.37 ± 0.25
Minerals			
Potassium (mg/100 mL)	220.94 ± 0.32	274.32 ± 0.14	35.11 ± 0.13
Sodium (mg/100 mL)	7.61 ± 0.04	5.60 ± 0.02	36.51 ± 0.02
Magnesium (mg/100 mL)	22.03 ± 0.07	20.87 ± 0.02	31.65 ± 0.04
Calcium (mg/100 mL)	8.75 ± 0.05	15.19 ± 0.03	23.98 ± 0.05
Iron (mg/L)	0.29 ± 0.08	0.308 ± 0.01	0.32 ± 0.05
Protein (mg/mL)	0.04 ± 0.01	0.042 ± 0.00	0.22 ± 0.02
Total phenolic content (mg GAE/L)	54.00 ± 3.14	42.59 ± 0.83	25.70 ± 1.76

Source: Tan et al. (2014)

Table 2.2 Vitamin Content of Coconut Water

Vitamins	Value
Nicotinic acid	0.64µg/mL
Pantothenic acid	0.52µg/mL
Biotin	0.02µg/mL
Riboflavin	<0.01µg/mL
Folic acid	0.003µg/mL
Thiamine	Traces
Pyridoxine	Traces

Source: Priya and Ramaswamy, 2014.

2.1.2.1 *Total Soluble Solids*

Total soluble solids (TSS) indicate the richness of a liquid substance. It was found to be the highest in mature coconuts. TSS content of coconut water increases in the early months of maturing followed by a gradual decrease (Jackson *et al.,* 2004).

2.1.2.2 *Carbohydrates*

Principal sugars present in coconut are fructose, glucose, and sucrose which impart sweetness to the coconut and its products like coconut water, coconut milk, coconut cream, and coconut beverages (Campbell-Falck *et al.* 2000). Fructose was highest in coconut water followed by glucose and sucrose (Table 2.1). The sucrose content can reach up to as high as 90% of the total sugar content of coconut water (Solangi and Iqbal, 2011). It was evident from Table 2.1 that sucrose content of coconut water within the fruit increases with time which could be due to the formation of sucrose, a non-reducing sugar at an expense of monosaccharides, glucose, and fructose.

2.1.2.3 *Proteins*

Coconut water was not a rich source of protein (Table 2.1), but it should not be neglected. The presence of free amino acids such as lysine, tryptophan, glutamic acid, alanine, glycine, and aspartic acid along with reducing sugars may trigger Maillard reaction during its thermal processing for preparation of ready-to-drink coconut water. Maillard reaction in coconut water can lead to browning or discoloration of coconut water and subsequent impaired sensory properties (Jayalekshmy and Mathew, 1990).

2.1.2.4 *Vitamins*

Vitamins are very essential for a better healthy life. They are complex compounds and are not synthesized in the human body. Coconut water is a rich source of water-soluble vitamins particularly ascorbic acid (vitamin C), thiamine, riboflavin, niacin, pantothenic acid, pyridoxine, and folic acid.

2.1.2.5 *Minerals*

Generally, coconut water was rich in potassium and low in sodium content. The presence of magnesium, calcium, and iron though in a lesser amount than potassium and sodium make it a better replacement of sports drinks as it helps in fulfilling the loss of electrolytes lost during exercise (Saat *et al.*, 2002). More

evident from Table 2.1 was that coconut water of matured fruit has a more balanced mineral content than immature one which makes the former suitable replacement of rehydration and sports drinks.

2.1.2.6 *Total Phenolic Content (TPC)*

Aging causes changes in the total phenolic content of coconut water as well (Table 2.1). As coconut fruit ages, its TPC content decreases which was the measure of antioxidant properties. The reduction of TPC content coconut water reduces its free radical scavenging ability (Mantena *et al*., 2003).

2.1.2.7 *Titratable Acidity and pH*

The titratable acidity of coconut water was expressed as percentage malic acid as it was the major organic acid present in coconut water. Titratable acidity of coconut water decreases with time (Jackson *et al*., 2004). Similarly, as the maturity time of coconut progresses, pH also increases (Table 2.1).

2.1.3 Health Benefits of Coconut Water

Sailors in Melanesia, Micronesia, and Polynesia used coconut juice and fruit endosperm as food and drinking water reserves hundreds of years ago (Bourdeix, 2006). Coconut water was now popular as a refreshing drink, and thousands of people in the tropics consume the immature section of the nut. Coconut water was high in potassium, which plays an important role in both the inside and outside of the cell and helps to maintain osmotic pressure. Tender coconut water provides natural health benefits and can provide our bodies with energy (Ramesh, 2021).

Coconut water has many properties and was used mainly as a natural drink with variety of health benefits, but its primary function was to be utilized as a medicinal agent (Kumar, 1995). It is recognized for its Ayurvedic properties and can be used for Ayurvedic purposes. Coconuts are used in daily life in many areas. It was used for various types of health problems, such as urinary tract infections, eye irritation, stomach problems, placental problems, diarrhoea, etc.

Coconut water lowers blood pressure by lowering the systolic pressure factor (Bhagya *et al*.,2012). The research proved that if fresh coconut water was consumed about 300-400ml twice a day for 14-15 days it helps to brings down systolic blood but the same process of intake was not in diastolic blood pressure (Farapti,2013). According to research, the use of coconut water can reduce the heart rate of patients with severe hypertension. The systolic blood pressure and diastolic circulatory blood pressure (BP) of the test group were reduced by 10.6 mm Hg and 6.7 mm Hg, respectively (Zulaikhah, 2019).

Coconut water has been shown to lower glucose levels and increase other well-being indicators in diabetic patients. Diabetic patients treated with coconut water maintained better glucose levels than the control group; coconut water also had lower haemoglobin A1C levels, showing excellent long-term glucose control and improvements in glucose levels and oxidative pressure creators (Mathiowitz,1999).

Coconut water's antibacterial properties, the inclusion of lauric acid, and the ability to extract antimicrobial peptides Cn-AMP (Robinson and Lee 1987) from delicate coconut water have all drawn attention to its suitability for everyday use. An in-vitro study was conducted to determine the antimicrobial viability of delicate coconut water in its natural state against Streptococcus mutants (Rathbone,1996). With the delicate coconut water, which was fresh and sterile, and negative control (dimethyl formamide), there was no zone of restraint. Sure monitoring (0.2 %) chlorhexidine trace tests of thiamine B1 and pyridoxine B6 showed a zone of restraint. Coconut water also includes calories, sugar liquor, vitamin C, folic acid, free amino acids, phytohormones, chemicals, and growth factors. Coconut water was clean in its envelope and contains both organic and inorganic ingredients (practically all minerals found in food).

Kidney stone prevention necessitates adequate hydration. Despite the fact that plain water was an unthinkable choice, one study suggests that coconut water may be even better. Kidney stones form when calcium, oxalate, and other compounds combine to form gems which will be able to give shape as stone.

However, some gatherings are avoided to producing stone when coconut water intake was more (Robinson and Lee,1987).

2.2 NATA DE COCO

A large amount of mature coconut water was discarded during the course of coconut processing. Value addition of this nutritious liquid waste for the production of nata by static fermentation was considered beneficial not only for reducing pollution problem but also producing a natural dietary fiber. Bacterial cellulose produced by Gluconacetobacter xylinum (formerly Acetobacter xylinum) at the air liquid interface of coconut water was popularly known as nata-de-coco. Nata derived from Latin word natare which means "to float" from fermenting coconut water or fermenting rotting fruits. Nata can grow in coconut milk, an abundant domestic waste product or in a nutrient medium. It was a white, gelatinous food product popular in Philippines, Japan and Malaysia. Being microbial cellulose, it was highly hydrophilic, holding water over 100 times its weight. The water trapped in the cellulose matrix is highly useful for its application in food industry as a jelly like food. It has a distinct textural property like a firm chewy, soft and smooth surface and was rich in fibre. The production of nata is receiving a great attention because of its wide application possibilities (Keshk and Sameshima, 2006).

Chemically, the fiber contained in nata de coco was a cellulose fiber, known as bacterial cellulose. Bacterial celluloses have some advantages such as having a high purity without lignin, pectin, and hemicelluloses, which are commonly found in plant cellulose. Besides that, cellulose fibres or nata de coco fiber produced by Acetobacter Xylinum has certain physical properties which was different from plant cellulose (Yano *et al.,* 2008). The unique physical properties of cellulose derived from this bacterium was having a high purity, crystalline, mechanical strength, and porosity, and also having quite enough capacity to absorb water and easy to not get break down. This makes nata de coco fiber potentially to be developed further not only as ingredients of processed foods or beverages, but also can be used for important industries such as manufacturing of the transducer diaphragm for speakers and headphones, artificial skin to replace skin damaged by fire, membrane

separation, mixing materials in paper industry, producing carbon films electro-conductive, and materials for biomedical purposes (Watanabe *et al.,* 1995; Nakagaito *et al*., 2005; Fontana *et al.,* 1990; George *et al.,* 2005; Shibazaki, *et al.,* 1994; Mormino and Bungay, 2003; Serafica *et al*., 2002; Schumann *et al*., 2009).

2.2.1. Health Benefits of Nata De Coco

Nata de coco was a source of insoluble dietary fiber due to its cellulose content. Nata de coco contains about 98% water, 0.2% fat, 0.012% calcium, 0.002% phosphorus, 0.0017% vitamin B3, 51 mg/g sodium, potassium 280 mg/100 g, and 2.46 mg/100g vitamin C. This product has a high fiber content, including cellulose (2.5%), hemicellulose, lignin, and soluble fiber. Components of chemical compounds contained in nata de coco include: hexadecenoic acid (7.58%), benzene acetic acid (7.73%), 22-hydroxyhopane (3.96%), tetra decanoic acid (3.84%), 9-octadecenoic acid (3.65%), ρ-cresol (3.50%), 9-octadecenamide, (Z) (3.00%), phenol, 4-(2-aminoethyl) (2.73%), dodecanoic acid (2.21%), pentadecanoic acid (1.79%), 1-heptadecanecarboxylic acid (1.64%), indole (1.79%), hydrocinnamic acid (1.60%), heptadecanoic acid (1.54%), dan cyclohexanecarboxylicacid(1.47%). A functional food refers to a food product that contains nutrients but also has the potential to provide additional health benefits, either an improvement in one's health and well-being or a decrease in the risk of contracting disease . With regards to this definition, nata can be classified as a functional food.

2.2.1.1. Antifungal and antimicrobial properties

Nata de coco was rich in a variety of fatty acids (FAs). FAs have been shown to be extremely promising for development as next-generation antibacterial agents for the treatment of a broad spectrum of bacterial infections. Benzene acetic acid (phenylacetic acid) was well-known for its antifungal properties. This compound possesses a broad antimicrobial spectrum and inhibited the growth of several soil-borne phytopathogenic fungi completely. Tetra decanoic acid and hexadecenoic acid have antimicrobial activities against multidrug-resistant bacteria.

2.2.1.2. Wound dressing

Bacterial cellulose, a naturally occurring jelly-like substance produced by A. xylinum, was widely used in wound dressings due to its high water-holding capacity and mechanical strength. Wound dressings can help to speed up the healing process of the wound by increasing the permeability and protection of the new tissue.

2.2.1.3. Control plasma cholesterol level

Tetra decanoic acid (myristic acid) was a long-chain saturated fatty acid composed of 14 C atoms. This acid was first extracted from the nutmeg plant. It was related to low plasma HDL cholesterol levels in the Mediterranean population. In hypercholesterolemic women, nata de coco consumption can lower total cholesterol levels in the blood.

2.3 FACTORS AFFECTING SPOILAGE OF COCONUT WATER

Jackson et al. (2004) studied the fungal spoilage of husked and dehusked coconut fruits stored at 10°C and 30°C for three months. The husked coconut fruits stored at both 10°C and 30°C and the dehusked coconut fruits stored at 10°C showed no evidence of microbial spoilage at the end of the three months storage period. However, dehusked coconut fruits stored at 30°C deteriorated. Aspergillus flavus and Aspergillus niger were the principal fungal agents associated with the spoilage. An investigation of the proximate composition of the dehusked fruits stored at 30°C indicated a marked significant difference in the percentage composition of moisture, protein, ascorbic acid, and carbohydrate content of 3.97±0.28, 3.98±0.07, 0.01±0.002 and 9.27±1.02 respectively as against 46.82±0.43, 3.77±0.05, 2.48±0.15 and 11.89±0.22 obtained for dehusked coconut fruits prior to storage. These results suggest that the deterioration in nutritional composition was due to breakdown of protein and carbohydrate by the spoilage fungi. Further tests confirmed the ability of the isolated spoilage fungi to utilize the different carbohydrate and nitrogen sources as source of carbon and energy.

Pilo *et al*. (2009) conducted a study to assess the quality of reconstituted fruit juices and coconut water sold for immediate consumption in bars, restaurants, and bakeries, and by street vendors in Belo Horizonte, Minas Gerais, Brazil. Microbial quality was determined by counting coliforms, yeasts, staphylococci, and salmonellae. Total titratable acidity, pH and total soluble solids of these beverages were recorded. For coconut water samples, the total reducing sugar content was also determined. The "juices" collected included reconstituted orange, cashew and grape-flavoured juice powders and concentrated cashew juice. Sixty samples of these juices and 45 samples of coconut water were collected. More than half (55%) of the juice samples did not comply with current Brazilian legislation, which states that there must be a total absence of coliforms in a 50mL sample. Sixteen percent of the coconut water samples exceeded the bacterial count limits defined in Brazilian law, with thermotolerant coliform densities above 102MPN/mL. The high levels of sugar and low pH found in the coconut water were 10 possibly related to the high yeast counts in most samples. Forty seven percent of coconut water samples showed staphylococcal counts above 103CFU/mL. The numbers of thermotolerant coliforms, yeasts and staphylococci found suggest unsatisfactory hygienic practices during the preparation of these beverages. Salmonella was not detected in any of the samples.

2.4. CLARIFICATION

In clarifier, clarification occurs due to microfiltration and centrifugation. A filter cloth acts as a medium for filtration.

The separation of solids from liquids or gasses by textile filter media was an essential part of countless industrial processes, contributing to purity of product, savings in energy, improvement in process efficiency, recovery of precious material and general improvement in pollution control. Complicated structure and thickness of Textile materials, particularly woven and non-woven, are suitable for filtration (Zerin and Datta, 2018).

Filtration resistance of a filter cloth may increase with warp and weft density and twist of fabrics. Porosity and air permeability of fabric was also plays important role in filtration process. (Anand and Horrocks, 2000) Textile Filtration was used in several ways, separating, and purifying liquids and solids, cleaning gases and effluents, absorbing dirt, fumes, and oil.

A centrifugal filter was a type of barrier that can be used to separate materials after they are spun in a centrifuge. When a centrifuge was outfitted with a filter, high density solids or liquids can be effectively removed from low density liquids. In many industries, a centrifugal filter was used to separate waste products from certain liquids, which can then be reused by the company. A centrifugal filter was usually used to separate solid matter from a liquid suspension. This allows the liquid portion of the material to be recycled. Solid matter that was removed from a liquid through a centrifugal filter usually escapes in the form of slurry. The wet material can then be dried, either in a special holding container within the centrifuge or in a separate container until it can be disposed. They are used by a number of different industries. They can be used to clarify waste oil, clean out glass or ceramic grinding fluids, or remove small particles of metal from liquids. The liquid remaining after the centrifugal filtration can often be used again once it was clean. This cuts down on waste materials and reduces a company's expenses.

Centrifugal force was used to provide the driving force in some filters. These machines are centrifuges fitted with a perforated bowl that may also have filter cloth on it. Liquid was fed into the interior of the bowl and under the centrifugal forces, it passes out through the filter material.

Centrifugal force was often used for industrial solid–liquid separation (Zhou, 2018), because it generates a powerful filtration driving force that separates solid and liquid phases rapidly. Centrifugation has been implemented for separating substances in industrial and laboratory settings for a long time, ever since it was first used for milk separation in farms. Currently, centrifugal separation was usually applied in biomedical technology. Fukuyama et al. discussed the consolidation

behavior of centrifugal dewatering with and without a supernatant (Fukuyama *et.al*., 2015).

Centrifugal filtration was one of the most efficient and economic methods for solid–liquid separation. Since the centrifugal field can generate a huge pressure drop through the filter cake, it has many advantages, such as high filtration rate, low cake moisture, etc. Therefore, it has been widely used in many chemical, food, environmental, and biochemical engineering processes. In these processes, the raw suspensions frequently contain multi-components, e.g., proteins, enzymes, microbial cells, and the other impurities co-existing in the cultivation products in fermentation. How to purify those mixtures was increasing its importance in the fine chemical and biochemical industries in recent years. However, rare researchers have paid their attentions on the separation mechanism of centrifugal filtration, especially for the purification of bio-mixtures. This fact causes process engineers to spend too much time and costs for searching the optimum operating conditions or efficient equipments.

Centrifugal filtration equipment was characterized by a high processing capacity and small size (Rathbone, 1996). Compared with other separators, the centrifuge not only produces a solid with a low water content and a high-purity supernatant, but it was also highly leak-proof. It can work continuously and can be extensively used in the refinement of petrochemicals; sludge thickening and the dewatering of sewage in treatment plants; the extraction of pesticides and biopharmaceuticals; and the medical, food, textile, metallurgical, and environmental protection industries.

The precipitation of sediment from a liquid by subjecting the mixture to the action of centrifugal force was finding application in a number of industries. In many instances such centrifugals have supplanted filters and are frequently spoken of as centrifugal filters, but there was nothing about such apparatus to justify the use of the word "filter," since the liquid is not passed through filter material. Such centrifugals properly belong to the class termed "clarifiers." It was to be understood, therefore, that this paper limits itself to centrifugals in which the liquid to be treated

is forced through filter material, although it may be stated in passing that clarification plays a more or less important part in nearly every type of centrifugal filter. Centrifugal filters may be divided into two general classes - Those in which the drum is perforated and those having an imperforate drum.

The former class has found but limited application in the commercial world outside of the sugar industry and the drying of crystals, etc. There was some doubt of the propriety of characterizing Centrifugals used in these instances as filters, for while the cake which forms in the periphery of the drum is probably more or less of a filtering medium, the function of the machine was more essentially that of a centrifugal extractor of the type used in laundries, for drying clothes. However, similarly constructed machines have been used rather extensively in an experimental way. The serious drawback to this class of filters is the fact that the action of the Centrifugal force constantly tends more firmly to pack the residue that has accumulated on the filter base, quickly rendering the mass impervious. We have here a vicious circle. The greater the force, the more densely the residue becomes packed against the periphery, and the more densely it was packed, the greater was the amount of centrifugal force necessary to force the liquid through. Furthermore, a filter of this class has no particular advantage over a standard type of plate press, and differs from it only in that the pressure was derived from centrifugal force instead of from gravity or a pump, both of which have a pronounced advantage over centrifugal force from the standpoint of expense and convenience.

2.5 THERMAL TREATMENT TO ENHANCE SHELF LIFE OF COCONUT WATER

The first paper on the preservation of tender coconut water was Indian (Srivatsa, 1995). Additives such as nisin, minimum heating, and packing in polymeric pouches and metal cans were cited as being used to achieve commercial sterility. A more detailed process to develop shelf-stable ready-to-serve green coconut water was described by Chowdhury et al. (Chowdhury *et. al.,* 2005). The authors filtered the freshly extracted coconut water, pasteurised it at 85 °C for 10 min and cooled

it. The coconut water was then poured into metal cans or glass bottles. Cans and bottles were sterilised at 121 °C for 30 min and at 100 °C for 15 min, respectively.

An experimental hot-fill process was also compared with other commercial coconut water subjected to cooling, freezing, aseptic filling of cartons and industrialised hot-fill processing. The experimental process consisted of filtration, addition of citric acid to reduce the pH to 4.5, addition of fructose to standardise the soluble solids content at 70 grams per litre and sodium metabisulphite (0.45 g/L), addition of sodium benzoate (1.24 g/L) and ascorbic acid (0.0013 g/L), pasteurisation at 90 °C for 2 min and pouring into 200-mL glass bottles. Samples were stored at ambient temperature (28 °C). The experimental hot-fill samples were acceptable even though they did not resemble other commercial samples in terms of physicochemical attributes.

In Taiwan, sterilisation was commonly used as a thermal treatment to stabilise coconut water and frequently causes non-enzymatic browning of the liquid (Tzeng and Chen, 1998). In order to remove the brown colour, active carbon, cation exchange resin, sulphur compounds such as sulphite, acetyl-cysteine, glutathione and cysteine were successfully tested.

2.5.1. Thermal Treatment and Microbiological Effects

The main objective of the thermal treatments was to stop or eradicate the microbiological load for consumer safety (Gabriel *et.al*., 2009). Thermal treatments were applied to buko, a mix of coconut water/distilled water (80/20) and, respectively, (60 and 20) g of macerated solid endosperm and refined sugar per litre of beverage. Glass test tubes were immersed in a hot water bath at (60, 70 and 80) °C for different lengths of time. According to the different temperatures and time treatments, a D value was determined, i.e. the time (in minutes) required for a 1 $\log_{10}$ reduction of the survival of the reference strain. The calculated D values for Escherichia coli on buko ranged from (0.26 ± 0.01) min at 80 °C to (0.56 ± 0.08) min at 60 °C.

2.5.2. Thermal Treatment and Thermophysical Properties

Before the design or adaptation of specific food processing equipment, an important step was often left out: assessment of the effect of temperature on the thermophysical properties of the raw material. Food composition and temperature are important factors which affect the thermal behaviour of a tropical fluid such as coconut water. The density, dynamic viscosity, thermal diffusivity, thermal conductivity and specific heat of the water of green Bahia coconuts (presumed to be the Brazilian Green Tall or Dwarf variety) bought from a local market in Brazil were measured using a range of temperatures from 5 °C to 80 °C (Fontan *et. al.,* 2009). Temperature significantly affected the above properties, which displayed linear trends, except for dynamic viscosity, which displayed an exponential curve.

2.5.3. Thermal Treatment and Enzymatic Browning Control

The major problem encountered in coconut water stabilisation was apparently not microbiological or chemical stability, since these objectives have already been partially achieved (Chowdhury, 2005), but the fact that enzymes need to be inactivated to stabilise the colour and taste of the final product. As it was true for many fruit juices, polyphenol oxidase (PPO) and peroxidase (POD) enzymes are present in young coconut water.

The consequence of PPO or POD activities in coconut water was discoloration. Yellow, brown or pink discoloration of the coconut water can occur a few minutes or a few hours after the nut was cracked. Discoloration can also occur after several weeks of storage of processed coconut water. Even though the mechanisms of PPO and POD activities are well described from a biochemical point of view (Prades *et. al.,* 2012), the same mechanisms remain to be explained during ripening of the fruit and post-harvest. A range of different factors affect the levels of activities of the enzymes and are often difficult to control (temperature, pH, mechanical impacts, oxygen concentration, etc.). To prevent the consequences of PPO and POD activities in coconut water, several authors suggested inactivating

the enzymes by thermal treatments either using conventional methods (pasteurisation, sterilisation) or by microwave heating.

At a low temperature (90 °C), total inactivation was obtained after 550 s for PPO and after 310 s for POD (Campos *et. al.,* 1996). At a temperature of 139 °C for 10 s combined with 200 mg/L of ascorbic acid, PPO was entirely inactivated, whereas POD was still active at 40% of its original level (Campos *et. al.,* 1996). Contrary to Campos et al., who underlined the fact that PPO was more resistant than POD to pasteurisation (Campos *et. al.,* 1996), Abreu and Faria concluded that POD was inversely more thermostable using sterilisation.

2.6.CARBONATION OF COCONUT WATER

2.6.1. Properties of Carbon Dioxide

Carbon dioxide can be derived in solid, liquid, gas, or supercritical state. Gaseous CO2 was the most popular and well-known form compared to the others. CO2 was a colourless gas with a faintly pungent odour and has an acid taste. It was 1.53 times heavier than air at normal temperature and pressure. CO2 has several special properties such as non-oxidizing quality, inhibitive and partial disinfecting action on certain bacteria, and ability to stimulate taste sensation, and thus, CO2 has found applications in various food and processing industries (Jones,1923).

2.6.2 Effect of Carbonation on Microbial Load Reduction

Carbon dioxide was effective for extending the shelf-life of perishable foods by retarding bacterial growth. The overall effect of carbon dioxide was to increase both the lag phase and the generation time of spoilage microorganisms; however, the specific mechanism for the bacteriostatic effect was not known. Displacement of oxygen and intracellular acidification were possible mechanisms that were proposed, then discounted, by early researchers. Rapid cellular penetration and alteration of cell permeability characteristics have also been reported, but their relation to the overall mechanism was not clear. Several researchers have proposed that carbon dioxide may first be solubilized into the liquid phase of the treated

tissue to form carbonic acid (H_2C0_3), and investigations by the authors tend to confirm this step, as well as to indicate the possible direct use of carbonic acid for retarding bacterial spoilage. Most recently, a metabolic mechanism has been studied by a number of researchers whereby carbon dioxide in the cell has negative effects on various enzymatic and biochemical pathways. The combined effect of these metabolic interferences was thought to constitute a stress on the system, and result in a slowing of the growth rate. The degree to which carbon dioxide was effective generally increases with concentration, but high levels raise the possibility of establishing conditions where pathogenic organisms such as Clostridium botulinum may survive. It was thought that such risks can be minimized with proper sanitation and temperature control, and that the commercial development of food packaging systems employing carbon dioxide will increase in the coming years.

The first observations regarding the effect of carbon dioxide on retarding bacterial growth were made nearly 100 years ago (Frankel, H. R. 1889). Since then, the potential for retarding spoilage through application of carbon dioxide has been explored in relation to a number of commodities. A partial list includes: fruits, vegetables, eggs, carbonated beverages, pork, poultry, beef, and seafoods (Gray, R. J., D. G. Hoover, and A. M. Mvir. 1983). Through these, and other studies, carbon dioxide has been shown to be effective for foods whose spoilage flora was dominated by gram-negative, aerobic, psychotropic bacteria. For this reason, as well as economic considerations, recent research has centred on use of carbon dioxide with fresh meats, poultry, and seafoods. The observation that high concentrations of carbon dioxide can cause darkening in tissues by combining with myoglobin to form metmyoglobin (Brown, W. D., and L. B. Mebine 1969) has discouraged use of the technique with meats containing high levels of myoglobin, and has further focused attention on its use with poultry and seafoods. In general, application of carbon dioxide increases both the lag phase and the generation time in the growth cycle of microorganisms. These effects vary with the concentration of carbon dioxide, incubation temperature, organism, and water activity of the medium (Wodzinski, R. J., and W. C. Frazier. 1961). Despite over 100 years of

study, and numerous publications exploring the effect of carbon dioxide on foods and bacterial cultures researchers have been unable to determine conclusively the method(s) by which carbon dioxide exerts an inhibitory effect on bacterial growth.

2.6.3. Mechanism of Carbondioxide Action

2.6.3.1. Displacement of oxygen

One of the first explanations for the action of carbon dioxide was that it displaced some or all of the oxygen available for bacterial metabolism, thus slowing growth by a proportional amount. This possibility was discounted early in the study of this system by experiments which showed that anaerobic bacteria were also inhibited by carbon dioxide atmospheres. Callow confirmed these findings by replacing the bacterial growth atmosphere with 100% nitrogen. He did not observe the degree of inhibition equal to that of when carbon dioxide was present. Although reducing available oxygen may have some effect on bacterial growth, it does not appear to be the most limiting factor (Callow, E. H.1932)

2.6.3.2.Influence on pH

Most studies on carbondioxide atmospheres and bacterial growth make the observation that the pH of the growth medium is decreased (Becker, Z. E. 1933).

2.6.4. Application of Carbondioxide for Food Preservation

Two aspects concerning use of carbon dioxide need to be addressed in regard to food preservation. One was the concentration of carbon dioxide needed to produce optimal inhibition of bacterial growth; the other was the potential for growth of pathogenic bacteria under high carbon dioxide concentrations. With regard to optimal concentration, there is considerable ambiguity among various researchers as to reported values, as well as methodologies for approaching the question.

Valley reported that concentrations only slightly above atmospheric can actually stimulate bacterial growth, but that in still higher concentrations bacterial growth was inhibited (Valley, G. 1928). Haines reported that concentrations as low as 10

to 20% were sufficient to inhibit growth of Pseudomonas and Achromobacter (Haines, R. B. 1933). Shewan recommended concentrations between 30 and 40% for improving the quality of whitefish. Tarr recommended a minimum of 40 to 50% to derive maximum benefit in the storage of fresh fish. Coyne, in one of the original studies on using carbon dioxide atmospheres to prolong fish quality, recommended concentrations between 40 and 60%, and suggested that above the upper concentration no additional benefits could be derived. (Coyne, F. P. 1932).

Although this author makes no recommendations as to optimal level or commercial applications, it represents the extreme of the numerous concentrations that can be found in the literature. There was some evidence to support the observation that the bacterial inhibition increases with the concentration of carbon dioxide present in the system. King and Nagel controlled the various growth factors for pure cultures of Pseudomonas aeruginosa, and found a linear relationship between generation time and carbon dioxide level. This relationship was more recently confirmed by Blickstad *et al.,* who concluded that the bacteriostatic/preservative effect of carbon dioxide increases with increasing concentration. While it may be impossible to prove that in C02-rich atmospheres the potential for contamination with toxin from Clostridium botulinum does not exist, there was evidence that it may not present a significant risk provided proper sanitation and temperature controls are employed (Blickstad, E., S-0 Enfors, and G. Molin. 1981).

2.7. SHELF-LIFE STUDIES OF COCONUT WATER

Ediriweera(1996) has conducted shelf life studies of coconut water.Prior to the development of treated coconut water,the physico-chemical and processing parameters of coconut water was determined. Coconut water was analyzed for sugar, minerals and vitamin C content based on reviewed methods. Studies were conducted to identify the optimum pH, total soluble solid (TSS), type of acidulant and pasteurization conditions based on sensory properties of the product using semi trained panelists. Processed coconut water was packed in three different packages and stored at refrigerated (4°C) condition for two months. Changes in

pH, TSS and titratable acidity were evaluated in two weeks intervals throughout the storage period. The results showed that optimum pH, TSS, type of acidulant and pasteurization conditions were 4.4, 9, malic acid and 70˚C/15 sec., respectively. The product was microbiologically (less than 50 CFU/ml) safe for consumption even after 8 weeks of storage. These storage studies revealed that the changes in pH, TSS and titrable acidity of coconut water packed in three packaging materials had no significant difference ($p<0.05$) during refrigerated storage (Ediriweera, 1996).

MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHODS

This chapter deals with different materials and methodologies for the clarification of raw coconut water, addition of preservatives and carbonation process for the development of treated coconut water. The procedures adopted for the evaluation of physiochemical, microbial and sensory qualities of stored coconut water are also explained in detail.

3.1 RAW MATERIAL AND PRETREATMENTS

Coconut water (CW) required for the study were collected from the M/s Sarayu Oil Mill, Edappal, Kerala. The coconut water was stored in sterile PET cans. The coconut water collected was stored under refrigerated condition (4±2°C) until the conduct of experiments. Nata de coco was obtained from M/s Nata nutrico coconut food products, KINFRA industrial parks, Kannur, Kerala.

3.2 PHYSICOCHEMICAL PROPERTIES OF RAW COCONUT WATER

The physicochemical properties of raw coconut water were determined using the standard procedures. Properties namely carbohydrate, protein, TSS, pH, colour, titrable acidity and ascorbic acid were estimated.

The procedures to find out the properties are furnished below

3.2.1. Carbohydrate

Carbohydrates was found out using anthrone reagent method and the steps performed are given. First, 0.5 ml and 0.1 ml of the sample was taken as the test solution. The standards were prepared by taking 0, 0.2, 0.4, 0.6, 0.8 and 1 ml of the glucose working standard. In all the test tubes, volume was made up to 1 ml using distilled water. Then, 4ml anthrone reagent was added to all tubes and the tubes were heated for 8 minutes. Then, the tubes were cooled rapidly and read at 630 nm. Then, a standard graph was drawn by plotting concentration of the standard against

absorbance and the amount of carbohydrate in sample was calculated using the graph.

$$Amount\ of\ carbohydrate\ present\ in\ 100mg\ of\ sample = \frac{mg\ of glucose}{volume\ of\ test\ sample} * 100$$

…3.1.

3.2.2. Protein

Protein was estimated using Lowry Protein Assay Method.

First the test solutions should be prepared as follows:

a) Solution A: 2% (w/v) sodium carbonate in 0.1 M sodium hydroxide.

b) Solution B: 1% (w/v) copper sulphate

c) Solution C: 2% (w/v) sodium potassium tartrate.

d) Solution D: Copper reagent- Mix 0.5 volume of solution B, 0.5 volume of solution C and 50 volumes of solution A.

e) Solution E: Folin-Ciocalteau reagent is diluted to 1M according to the supplier's instruction.

Protein estimation using Lowry Protein Assay method was done as described. First, to 1 mL of the test solution, 5mL of Solution D was added (Copper reagent), then it was mixed thoroughly by vortexing and made to stand at room temperature for 10 min. Then, 0.5 mL of solution E was added to the above solution (Folin-Ciocalteau reagent), and mixed rapidly, and incubated for 30 min at room temperature.

The absorbance was measured at 600 nm against reagent blank not containing protein. The concentration was estimated by referring to a standard curve obtained at the same time using known concentrate of bovine serum.

3.2.3. Total Soluble Solids (TSS)

Total soluble solids (TSS) was found using hand refractometer. First, a small quantity of the test solution (2-3drops) was transferred to the surface of the fixed prism of the refractometer & immediately the movable prism was adjusted. Then, the field of view was illuminated suitably and readings were taken.

Plate 3.1 Refractometer

3.2.4. pH

pH was determined using pH meter. Initially, the pH meter was turned on and adequate time was given for the meter to warm up (30 minutes). Then the electrode was taken out of its storage solution & rinsed with distilled water. An empty beaker was kept to collect the waste water used for washing. Then, the electrode was blot dried using a tissue paper. The buffer solutions with pH 7 & one with pH 4 (for acidic solutions) were prepared using pH capsule. Then, the electrode was placed in the buffer with pH value 7 & and allowed the reading to stabilize by letting it sit for 1-2 minutes. The reading was then set to 7 using calibration knob. Then, the electrode was rinsed using distilled water. The above steps were also repeated with the second buffer. Then, the electrode was placed in the sample. The reading was measured. Finally, the electrode was rinsed with distilled water & blot dried using tissue paper. The electrode was then kept in the storage solution.

Plate 3.2 pH meter

3.2.5. Titrable Acidity

For determining titrable acidity, first, 10 ml of sample was taken and it was made up to 100ml. Then, 10 ml solution was taken from the above sample and to that 2-3 drops of phenolphthalein indicator was added. 0.1 N NaOH was prepared by dissolving 0.4g NaOH in 100ml distilled water and was taken in the burette. Then the blank and sample was titrated. The titre values were obtained. Titrable acidity was then found by substituting the values in the given equation.

$$\%\ Acidity = \frac{Ew.of\ acid * Titer\ value * N * Vol.made\ up}{1000 * Vol.of\ aliquot\ taken\ for\ titration * vol.of\ sample} * 100 \quad \ldots 3.2.$$

- Eq. wt. of Malic acid = 67
- Eq. wt. of citric acid = 64

3.2.6. Colour

Colour was measured using hunter lab colour flex meter. A cuvette filled with the sample was placed in the sample holding port and reading was taken. The cuvette should be washed with distilled water before and after taking the sample.

Plate 3.3 Colorimeter

3.2.7. Ascorbic Acid

First, dye solution was prepared by dissolving 52mg of 2,6 dichlorophenol indophenol and 42 mg of sodium bicarbonate in 200ml distilled water. Then, the following solutions were prepared.

- Preparation of Standard solution: Adding 100 mg of ascorbic acid to 100 ml 4% oxalic acid.
- Preparation of working standard solution: 10 mL of standard solution was pipetted out and was diluted to 100 ml using 4% oxalic acid.

Then, the 5ml coconut water 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 10ml of 4% oxalic acid was added and titrated against the dye. The end point was the appearance of pink colour. Amount of dye consumed is equal to the amount of ascorbic acid. Then, 10 ml of the sample extract was pipetted out to which 10 mL of 4% oxalic acid was added. It was then titrated against the dye.

$$Dye\ factor = \frac{0.5}{Titratable\ acidity\ (V1)} \quad \ldots 3.3.$$

$$Ascorbic\ acid\ in\ \frac{mg}{100g} = \frac{0.5mg}{V1\ mL} * \frac{V2}{5mL} * \frac{100mL}{wt.of\ sample} * 100$$

…3.4.

V_1 = Amount of dye consumed by ascorbic acid present in the working standard solution.

V_2 = Amount of dye consumed by the liquid sample.

3.3. EXPERIMENTAL DESIGN

3.3.1 Independent Variables

1. Composition of preservatives
   - Citric acid – 100 ppm, 200 ppm, 300 ppm
   - KMS – 50 ppm, 100ppm, 150 ppm
   - No preservative
2. Addition of Nata de coco
3. Pasteurization at 80℃ for 10 minutes
4. Carbonation

3.3.2. Dependent Variables

1. Ascorbic acid

2. Titrable acidity

3. Total soluble solids

4. Color

5. pH

6. Microbial count

7. Sensory analysis

3.4 SAMPLE CODES

The sample code for each sample based on the treatments used are provided below

Table 3.1. Sample codes for different treatments.

Sl. no.	Preservatives	Quantity	Non-pasteurised samples		Pasteurised samples	
			Coconut water	Coconut water with nata de coco	Coconut water	Coconut water with nata de coco
1	Citric acid	100 ppm	T_1	T_8	T_{15}	
2	Citric Acid	200 ppm	T_2	T_9	T_{16}	T_{22}
3	Citric Acid	300 ppm	T_3	T_{10}	T_{17}	
4	KMS	50 ppm	T_4	T_{11}	T_{18}	
5	KMS	100 ppm	T_5	T_{12}	T_{19}	T_{23}
6	KMS	150 ppm	T_6	T_{13}	T_{20}	
7	No preservatives	-	T_7	T_{14}	T_{21}	

3.5 PREPARATION OF SAMPLES

3.5.1 Preparation of Non-Pasteurised Coconut Water Samples

The samples T_1 to T_7 varies only in the quantity of preservatives added. They are prepared as follows:

First the required quantity of coconut water was measured using a measuring cylinder.

1. TSS of coconut water was adjusted to 10.2° Brix by adding 52g sugar per 1000ml of coconut water.
2. The required quantity of preservative was added according to the type of treatment as shown in table 3.1.
3. Coconut water was then clarified using the clarifier.
4. The clarified coconut water was filled in 200 mL capacity PET bottles and stored at 4℃ in the chiller.

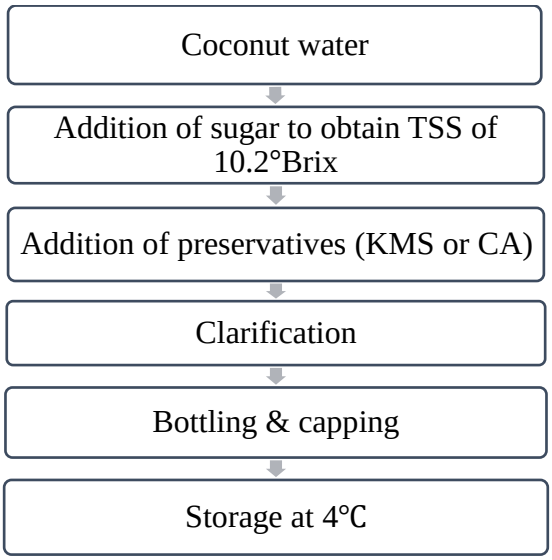

Fig 3.1. Preparation of non-pasteurised coconut water samples

3.5.2 Preparation of Non-Pasteurised Coconut Water Samples with Nata de coco

The samples T_8 to T_{14} contains Nata de coco. It also varies in the quantity of preservatives added. They are prepared as follows:

1. Required quantity of coconut water was measured using a measuring cylinder.
2. TSS of coconut water was adjusted to 10.2° Brix by adding 52g sugar per 1000ml of coconut water.

3. The required quantity of preservative was added according to the type of treatment as shown in table 3.1.
4. The coconut water was then clarified using the clarifier.
5. The clarified coconut water is filled in 200 mL capacity PET bottles containing 20g of Nata de coco each and is stored at 4℃ in the chiller.

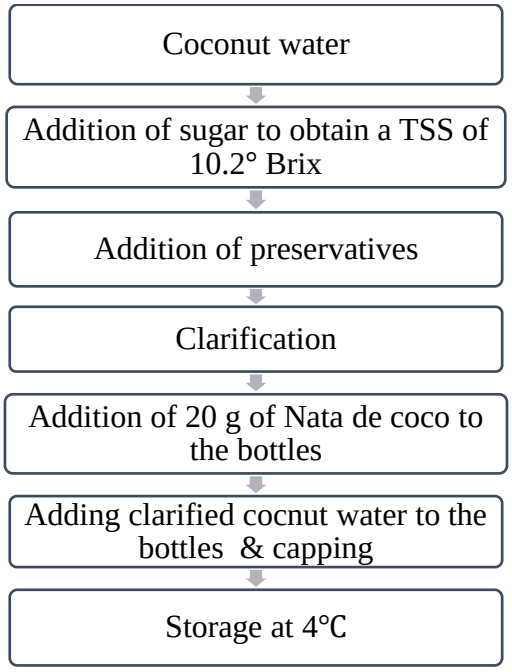

Fig 3.2. Preparation of non-pasteurised coconut water samples with nata de coco

3.5.3 Preparation of Pasteurised Coconut Water Samples

The samples T_{15} to T_{21} are pasteurised. It also varies in the quantity of preservatives added. They are prepared as follows:

1. Required quantity of coconut water was measured using a measuring cylinder.
2. Then the TSS of coconut water was adjusted to 10.2°Brix by adding 52g sugar per 1000ml of coconut water.
3. The coconut water was clarified using the clarifier.
4. The clarified coconut water was pasteurised at 80℃ for 10 minutes.
5. Then preservatives are added as mention in table 3.1 to each sample.

6. The coconut water was filled in 200 mL capacity PET bottles and stored at 4℃ in the chiller.

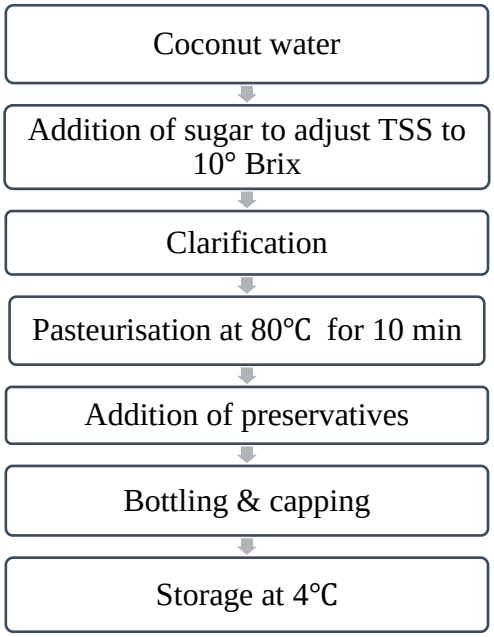

Fig 3.3. Preparation of pasteurised coconut water samples

3.5.4 Preparation of Pasteurised Coconut Water Samples with Nata de Coco

The samples T_{22} and T_{23} contains Nata de coco. It also varies in the type of preservatives added. They are prepared as follows:

1. Required quantity of coconut water was measured using a measuring cylinder.
2. Then the TSS of coconut water was adjusted to 10.2° Brix by adding 52g sugar per 1000ml of coconut water.
3. The coconut water was clarified using the clarifier.
4. The clarified coconut water was pasteurised at 80℃ for 10 minutes.
5. Then preservatives are added as mention in table 3.1 to each sample.
6. Nata de coco was added to each PET bottle, 20g each.
7. The coconut water was filled in 2 bottles and stored at 4℃ in the chiller.

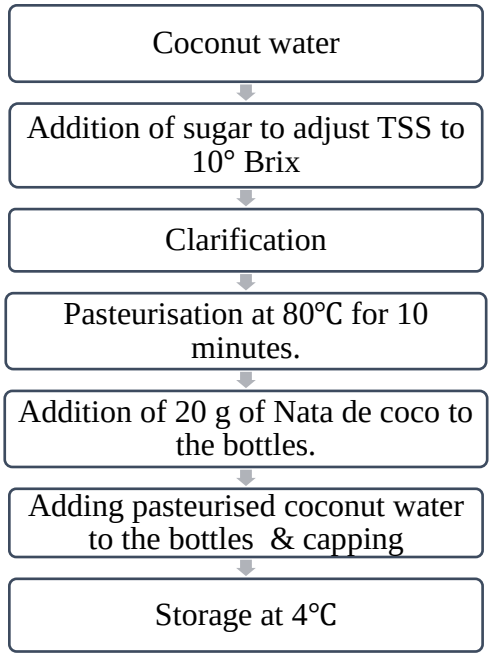

Fig 3.4 Preparation of pasteurised coconut water samples with nata de coco

3.5.5. Carbonation

Carbonation was done at the final stage after bottling and before capping. Carbonation was done using a soda maker.

Plate 3.4. Soda maker

3.6 OPERATION OF CLARIFIER

3.6.1 Clarification Equipment (Laboratory Setup)

Clarifier was used to do the clarification process of coconut water with the help of centrifugal force in order to remove impurities and to reduce microbial load. The figure of clarifier setup is shown in Fig 3.1. It can be also called as centrifugal filter. As filtration occurs with the aid of centrifugal force. The clarifier consists of a cylindrical casing in which the filter medium is to be placed. Inside the cylindrical casing another perforated hollow cylinder which is open at the top is present. It has provisions to attach the filter cloth. This cylinder will rotate to produce the desired centrifugal force required for separation. It is also connected to a fixed speed motor. On the top of the clarifier, an inlet was provided to give the feed. At the bottom an outlet is provided to obtain the clarified product. The whole set up was made up of food grade stainless steel. The filter medium used was a filter cloth. The filter cloth used has a pore size of 5 micrometres. The filter cloth should be properly placed inside the clarifier for effective clarification.

Plate 3.5 Clarifier apparatus

3.6.2 Cleaning Protocol Before and After Operation of the Plant

3.6.2.1. Cleaning procedure before operation

1. The filter cloth should be washed in normal water first. Then it should be soaked in warm water for 5 minutes and was again washed in the same water. Then after draining off water completely, it was dried inside a dryer for 1 hour at 80℃.
2. The clarifier was first washed using normal water. Then it was rinsed using alcohol. Then again it was rinsed and washed using hot water for 2-3 times.

3.6.2.2. Cleaning procedure after operation

1. After the operation, the filter cloth should be taken out. It was then soaked in water for 1 hour and is washed in normal water. Then it was dried.
2. The clarifier should be washed using normal water for 2-3 times by operating it by giving water as feed.

3.6.3 Standard Operation Procedure of Clarifier for Conducting Experiments

1) Open the lid of the Clarifier.
2) Clean the Clarifier using hot water.
3) Wipe off water with clean Muslin cloth (Cotton cloth).
4) Keep the filter medium (Filter cloth) in the center position and close the lid.
5) Clarifier was operated without adding the sample to remove the remaining water.
6) Switch on the main power.
7) Switch on power by pressing the green button.
8) When water was removed completely from the outlet, add the sample through the inlet to the clarifier.
9) Clarified sample will be obtained through the outlet.
10) Switch off the power by pressing red button.
11) Switch off the main power supply.

3.7 OPTIMIZATION OF PROCESS PARAMETERS FOR PRESERVATION OF COCONUT WATER

Based on the review of literature and preliminary studies conducted 23 treatments were selected for this study. The optimization of process parameters was done based on the quality parameters, microbial analysis and sensory analysis of treated coconut water.

3.7.1. Quality analysis of Treated Coconut Water

The following physicochemical properties of treated coconut water samples was estimated.

1. TSS
2. pH
3. Titrable Acidity
4. Colour
5. Ascorbic acid

The tests were conducted using procedures described in section 3.2

3.7.2. Microbiological Analysis

The microbiological quality characteristics of the coconut water samples were determined both for fresh and treated samples at different storage periods. The microbial growth was estimated through standard plate count method and serial dilution agar plate technique.

3.7.2.1 *Total bacterial count in coconut water samples*

The bacterial population in coconut water samples were analysed by different microbiological methodologies, that includes enumeration of the microorganism in selective media for different dilutions of sample, incubation of plates and counting the number of colonies present. The media generally used for enumeration bacteria was nutrient agar medium. The coconut water of 1 mL was pipetted using a sterile pipette into a test tube containing 10mL of sterile water

which gave a l:10 (10) dilution. The test tubes were shaken well for 10-15 minutes for uniform distribution of microbial cell in the water blank. Then 10 dilution was prepared by pipetting out 1mL of (10) dilution to 9mL of sterile water in test tube with a sterile one mL pipette, the process was repeated up to 10^{-6} dilutions with a serial transfer of the dilutants. One millilitre of aliquot from 10^{-5} dilution was transferred to the sterile petri dishes for the enumeration of bacteria.

Approximately, 15-20mL of molten and cooled (45°C) agar medium was added to each petri dish 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. After the incubation period, the colonies were counted and the number of organisms (total bacteria) per gram of sample was calculated by using the equation

$$No.of\ Colony\ Forming\ Units\ (CFU) per\ gram\ of\ the\ sample = \frac{no.of\ colonies * \ dilution\ factor}{volume\ of\ sample\ taken}$$

…3.5.

3.7.3 Sensory Analysis

Sensory analysis was a scientific study used to measure, analyse, and interpret reactions to those characteristics of foods as they are perceived by the senses of sight, smell, taste, touch, and hearing. In general, sensory quality of liquid food was the consumer's reaction to the physical nature and chemical constituents of the food in its prepared and formulated form. Organoleptic evaluation was carried out by a panel of ten untrained judges for appearance, colour, taste, odour, mouthfeel and overall acceptability using nine-point hedonic scale. Sensory analysis was conducted separately for carbonated and non- carbonated samples. Based on the sensory analysis four samples were optimized. These samples were only selected for further studies.

SENSORY SCORE CARD FOR COCONUT WATER

Date:

Name of panelist:

You are requested to assess the product in terms of general acceptability on a 9-point hedonic scale.

Score system

Like extremely – 9

Like very much – 8

Like moderately – 7

Like slightly – 6

Neither like nor dislike – 5

Dislike slightly – 4

Dislike moderately – 3

Dislike very much – 2

Dislike extremely – 1

Sample code	Appearance	Colour	Taste	Odour	Mouthfeel	Overall acceptability
A						
B						
C						
D						
E						
F						
G						
H						
I						

Name:

Signature:

Fig 3.5 Sensory score card

3.8.SHELF-LIFE STUDIES OF THE OPTIMIZED TREATED COCONUT WATER

Four samples were selected finally based on the sensory analysis. These samples were subjected to storage studies for a period of two months. 200 mL of samples were filled in PET bottles and kept under refrigerated condition (8℃). The quality analysis of the stored samples were conducted at every 15-day intervals.

RESULTS AND DISCUSSION

CHAPTER IV

RESULTS AND DISCUSSION

In this chapter the details of the results of the effect of clarification and preservatives added on Coconut Water (CW) at different treatment conditions to enhance the shelf life have been presented. Physio-chemical properties of the treated coconut water and its shelf-life studies are also included in this chapter.

4.1. PHYSIO-CHEMICAL PROPERTIES OF FRESH COCONUT WATER

The physio-chemical properties of fresh coconut water are shown in table 4.1. Carbohydrate, protein TSS, pH, titrable acidity, colour and ascorbic acid values of fresh sample are presented in table 4.1:

Table 4.1 Values of physio chemical properties of fresh coconut water.

Sl. No.	PROPERTIES (UNIT)		VALUE
1	Carbohydrate (g/100mL)		3.2
2	Protein (g/100mL)		0.5
3	TSS		3.4
4	pH		5.25
5	Titrable acidity (%)		0.134
6	Colour	L*	50.4
		a*	3.2
		b*	6.3
7	Ascorbic acid (%)		5.74

The physio-chemical properties of fresh coconut water are shown in table 4.1. The fresh coconut water had a Carbohydrate content 3.2g/100mL, protein content of 0.5 g/100mL, TSS value of 3.4, pH of coconut water was found to be 5.25, titrable acidity of coconut water was estimated as 0.134, colour values of L*, a* and b* was observed as 50.4, 3.2, 6.3, respectively.

4.2. PHYSIO-CHEMICAL PROPERTIES OF TREATED SAMPLES

The raw coconut water was subjected to clarification using a clarifier apparatus. The clarified coconut water was then added with preservatives (citric acid and KMS at permissible level). 23 treatments were selected in this study. The physio-chemical properties of treated samples namely TSS, pH, titrable acidity, colour and ascorbic acid are tabulated in table 4.2.

Table 4.2. Values of physio chemical properties of the treated samples.

Sample	TSS	pH	Titrable acidity (%)	Colour				Ascorbic acid (%)
				L*	a*	b*	c*	
T_1	10.2	5.12	0.134	69.3	4.1	5.5	6.9	3.444
T_2	10.2	5.02	0.067	67.3	3.9	6.6	7.7	4.592
T_3	10.2	4.86	0.134	70.4	4.2	4.9	6.5	4.592
T_4	10.2	5.10	0.067	70.0	4.2	4.9	6.5	4.592
T_5	10.3	5.00	0.067	70.4	4.2	5.0	6.5	3.444
T_6	10.2	5.03	0.1005	70.0	4.1	5.6	6.9	5.74
T_7	10.2	5.29	0.1005	68.2	3.9	6.4	7.5	5.74
T_8	10.2	5.08	0.134	70.1	4.3	4.5	6.2	5.74
T_9	10.2	5.04	0.134	62.6	3.4	7.6	8.3	4.592
T_{10}	10.2	4.86	0.067	69.5	3.8	5.1	6.3	3.444
T_{11}	10.2	5.10	0.134	67	4.7	7.9	9.2	4.592
T_{12}	10.2	5.14	0.1005	71.6	4.1	4.0	5.8	5.74
T_{13}	10.2	5.18	0.1005	66.3	4.5	6.2	7.7	4.592
T_{14}	10.2	5.30	0.1005	69.1	4.4	5.7	7.2	4.592
T_{15}	10.2	5.08	0.1005	69.5	5.3	6.2	8.1	4.592
T_{16}	10.2	4.90	0.1005	69.2	5.6	5.5	7.8	6.888
T_{17}	10.2	4.84	0.1005	69.4	5.1	3.8	6.4	4.592
T_{18}	10.2	5.32	0.1005	69.7	4.9	4.4	6.6	4.592

T_{19}	10.2	5.32	0.1005	69.6	4.8	4.5	6.6	3.444
T_{20}	10.2	5.32	0.1005	70.2	4.9	4.6	6.7	6.888
T_{21}	10.2	5.33	0.067	69	4.9	5.7	7.5	4.592
T_{22}	10.2	4.93	0.1005	69.2	5.2	5.3	7.4	4.592
T_{23}	10.2	5.33	0.067	70.2	4.8	5.5	7.3	4.592

Table 4.2 depicts the physicochemical properties of treated coconut water. The TSS of the sample ranged from 10.2 to 10.3, similarly pH, titrable acidity and ascorbic acid values of the treated samples varied from 4.8 to 5.3, 0.06 to 0.13 and 3.4 to 6.8, respectively. Hence from the table it was understood that there was no significant difference among treated coconut water samples.

Fig 4.1 Treated coconut water samples packed in PET bottles

Fig 4.2. Coconut water before and after clarification

4.3 OPTIMIZATION OF PROCESS PARAMETERS FOR PRESERVATION OF COCONUT WATER

Based on the microbial studies and sensory evaluation of treated sample 23 treatments were selected in this study. The optimization of process parameters was done based on the microbial analysis of treated samples.

4.3.1. Microbiological Studies

The treated samples were stored for 14 days under refrigerated condition (8°C). Microbiological studies were conducted at 0^{th} day and 14^{th} day and the results are tabulated in tables below.

Table 4.3. Results of microbial analysis conducted on first day.

Sample	1^{st} day reading (in CFU/mL)	3^{rd} day reading (in CFU/mL)
Fresh CW	4.5×10^5	4.5×10^5
T_2	2×10^5	3×10^5
T_5	0.5×10^5	4×10^5
T_7	2.5×10^5	3.5×10^5
T_9	1×10^5	1×10^5
T_{12}	0.5×10^5	0.5×10^5
T_{14}	1.5×10^5	1.5×10^5
T_{16}	0	0
T_{19}	0.5×10^5	0.5×10^5
T_{21}	0	0
T_{22}	1.5×10^5	2.5×10^5
T_{23}	0	0

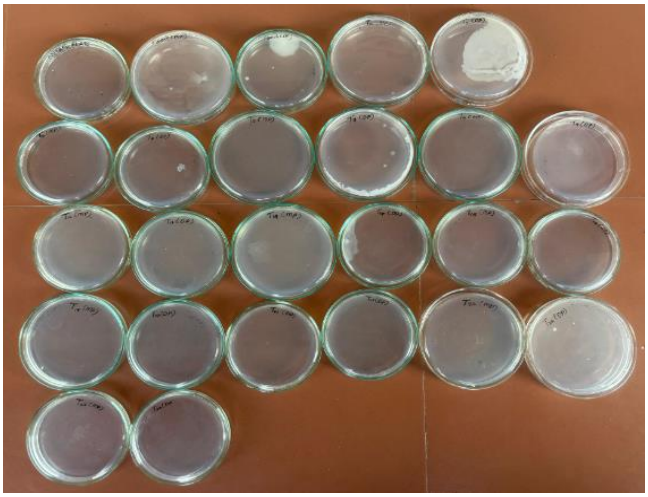

Fig 4.3. Microbiological analysis conducted on first day

Table 4.4. Results of microbial analysis conducted on 14th day

Sample	1st day reading (CFU/mL)	3rd day reading (CFU/mL)
T_1	2×10^5	TNTC
T_3	TNTC	TNTC
T_4	1.5×10^5	2×10^5
T_5	1.5×10^5	7×10^5
T_6	3×10^5	3.5×10^5
T_7	TNTC	TNTC
T_8	TNTC	TNTC
T_9	TNTC	TNTC
T_{11}	2.5×10^5	13×10^5
T_{12}	0	0
T_{15}	0	0
T_{16}	0	0
T_{18}	0.5×10^5	6.5×10^5
T_{19}	0	1×10^5
T_{21}	0	0
T_{22}	0.5×10^5	TNTC
T_{23}	0	0

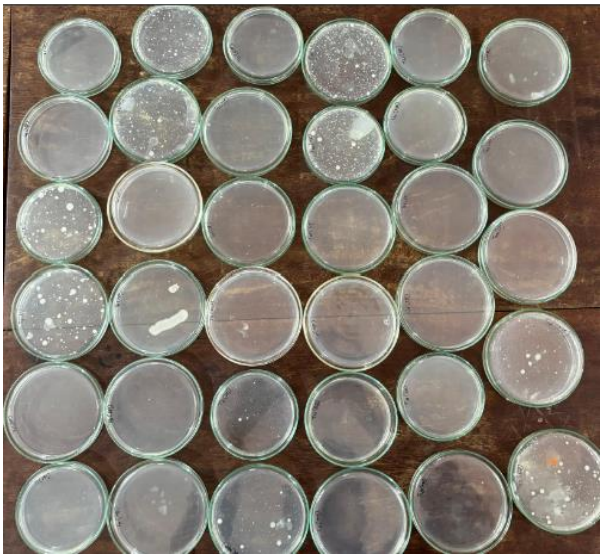

Fig 4.4. Microbial analysis conducted on 14th day

The initial optimisation was done based on the microbiological analysis. The samples with zero readings were selected. Treatments T_{12}, T_{15}, T_{16}, T_{18}, T_{19}, T_{21}, T_{22} and T_{23} were the samples selected initially as they were microbiologically safe. Rest of the samples were contaminated. The selected samples were then carbonated. Then both the carbonated and non-carbonated samples were subjected to conduct sensory analysis.

4.3.2 Sensory Evaluation

Sensory evaluation of treatments was carried out for consumer acceptance and preference using 10 untrained panellists selected at random. Appearance, colour, taste, odour, mouthfeel and overall acceptability of the samples was rated using a nine-point Hedonic scale where nine and one represent like extremely and dislike extremely respectively. Sensory evaluation was carried out at ambient conditions in a comfortable and quiet area without disturbance. Water was supplied to cleanse palate between samples. Sensory analysis was carried out separately for carbonated and non-carbonated samples.

Fig 4.5 Sensory evaluation of treated coconut water

4.3.2.1. Sensory Evaluation of Non-Carbonated Samples

Treatments T_{12}, T_{15}, T_{16}, T_{18}, T_{19}, T_{21}, T_{22} and T_{23} were selected for sensory evaluation,100 ppm of KMS were added to all selected samples and stored in PET bottles with random codes.

Table 4.5. Sensory score for non – carbonated samples

Sample	Appearance	Colour	Taste	Odour	Mouthfeel	Overall acceptability
FRESH	5.4	5.3	4.4	4.9	5.9	5.5
T_{12}	8.3	8.3	8.15	6.3	8.3	8.35
T_{15}	8.2	8.2	7.3	6.9	7.6	7.7
T_{16}	8.4	8.5	7.45	7.3	7.5	7.95
T_{18}	8.7	8.45	7.9	7.6	7.8	8.3
T_{19}	8.3	8.2	7.8	7.65	8	8.1
T_{21}	8.8	8.8	7.7	7.4	8.9	7.8
T_{22}	8.4	8.8	7.3	7	7.6	7.95
T_{23}	8.5	8.8	7.8	7.35	8	8.45

From the sensory analysis it could be observed that all the processed samples showed a significant increase in the acceptability of sensory attributes. Sample T_{23} got highest score for overall acceptability. Sample T_{12} got high scores for taste and mouthfeel. Also, it had the second highest score for overall

acceptability. So, it could be observed that non-pasteurised sample had a better flavour retention. So, samples T_{12} and T_{23} were selected.

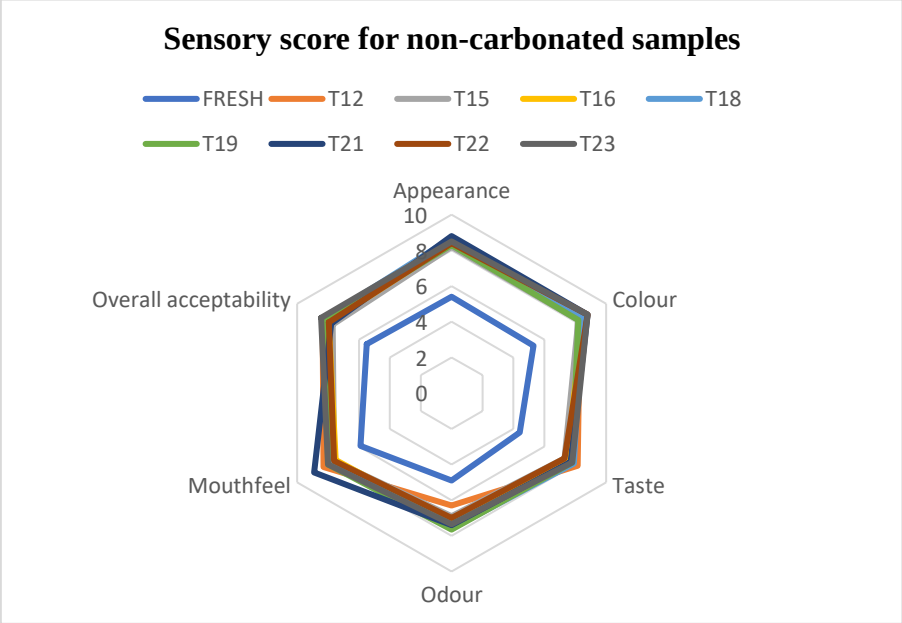

Fig 4.6. Sensory score for non-carbonated coconut water samples

4.3.2.2. Sensory Evaluation of Carbonated Samples

Treatments T_{12}, T_{15}, T_{16}, T_{18}, T_{19}, T_{22} and T_{23} were selected for sensory evaluation, 100 ppm of KMS was added to all the selected samples then the samples were carbonated using a soda maker and were stored in PET bottles with random codes.

Table 4.6. Sensory score for carbonated samples

Sample	Appearance	Colour	Taste	Odour	Mouthfeel	Overall acceptability
FRESH	7.6	7.6	6.2	6	6.4	6.6
T_{12}	8.7	8.9	7.9	7.4	8.1	8.75
T_{15}	8	8.05	7.4	7.3	7.7	8
T_{16}	8.2	8.3	7.4	7.1	7.6	7.7
T_{18}	8.5	8.3	7.8	7.2	7.7	8.1
T_{19}	8.2	8.15	7.6	6.9	7.7	8.2
T_{22}	8.1	8.3	7.2	6.8	7.3	7.6
T_{23}	8.6	8.6	7.95	7.4	8	8.3

From the sensory analysis it could be observed that all the processed samples show a significant increase in the acceptability of sensory attributes. Sample T_{12} got high scores for appearance, colour, taste, odour, mouthfeel and overall acceptability. Sample T_{23} got highest score for taste and mouthfeel. Hence samples T_{12} and T_{23} were only selected for further studies.

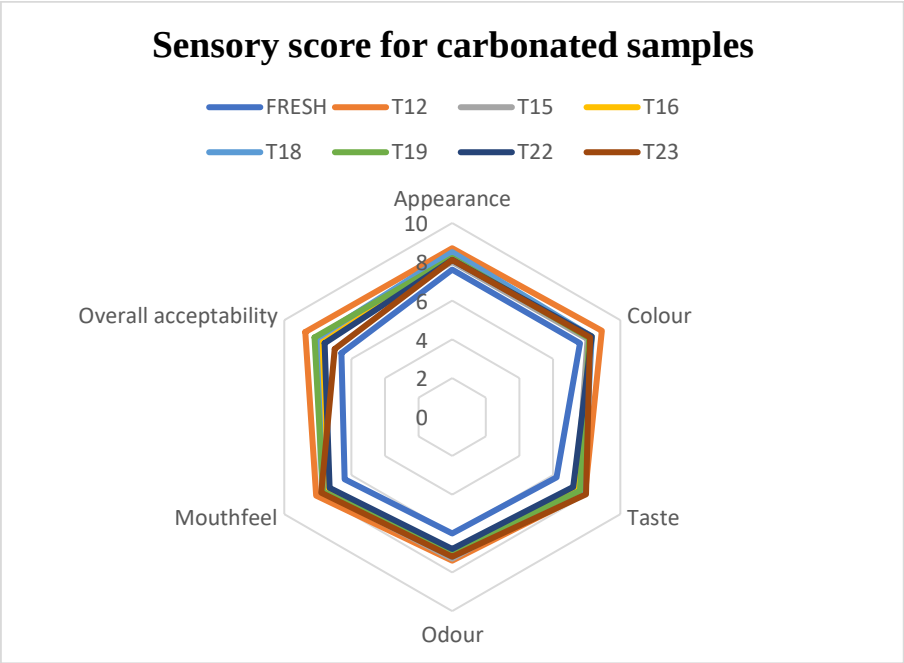

Fig 4.7. Sensory score for carbonated coconut water samples

The second optimisation was done based on the sensory analysis. A pair of samples from both carbonated and non-carbonated treatments were selected. Treatments T_{12} and T_{23} were selected from both treatments. These two samples were then used to conduct the shelf-life study.

So, from both set treatments T_{12} and T_{23} was selected for shelf-life study. Treatment T_{12} contained 100ppm KMS as preservative, it contains Nata de coco. Both carbonated and non-carbonated samples were prepared from T_{12} for shelf-life study. Treatment T_{23} also contains 100ppm KMS as preservatives but it was pasteurised. It also contains nata de coco. Both carbonated and non-carbonated samples were prepared from T_{23} for conducting shelf-life study.

4.4. SHELF-LIFE STUDIES OF OPTIMIZED TREATED COCONUT WATER

The optimized coconut water samples (T_{12} & T_{23}) were subjected to storage studies.100 ppm KMS were added as preservatives. Then the samples were carbonated using a soda maker and filled in PET bottles. The storage studies were conducted for a period of 45 days under refrigerated condition (8°C). The shelf-life studies were conducted based on the microbial stability of the samples during storage.

The result of microbial study during storage are tabulated in table 4.7. Table showed that there was no microbial contamination up to 30 days for both samples. TPC count of the samples after 45 days was observed as 2.5×10^5 and 1.5×10^5 respectively for T_{12} and T_{23} non- carbonated samples and as 1.5×10^5 and 1×10^5 respectively for T_{12} and T_{23} carbonated samples. From the results it was revealed that both the treated samples were microbiologically safe for 30 days. So, the samples were found to have a shelf life of one month under refrigerated condition (8°C).

Table 4.7. Total Plate Count of optimised treated samples

No. of days	Total Plate Count			
	Non-carbonated		Carbonated	
	T_{12}	T_{23}	T_{12}	T_{23}
0	0	0	0	0
15	0	0	0	0
30	0	0	0	0
45	2.5×10^4	1.5×10^5	1.5×10^5	1×10^5

From the table 4.7 it is revealed that all the treatments were found to have a shelf life of 1 month.

SUMMARY AND CONCLUSION

CHAPTER V

SUMMARY AND CONCLUSION

Coconut was considered a good source of energy and it has nutrients which are essential to our body. It contains protein, vitamins and several essential minerals. Coconut contains 90 percent saturated fatty acids and rest unsaturated fatty acids. Manganese was rich in coconut, which was necessary for bone strength and the metabolism of carbohydrates, little cholesterol, and proteins. Also, it was high in iron and copper, which helps to generate red blood cells as well as antioxidants like selenium that keeps our cells protected. Coconut water (CW) was a refreshing and light beverage, composed of bioactive enzymes that enhance fat metabolism and aid with digestion. Also, coconut water was high in potassium balancing out the level of sodium we consume.

Coconut water was obtained as a byproduct during milling of coconut to obtain coconut oil and also during other processing of coconut. The preservation of coconut water was difficult as it has high amount of nutrients and also as it gets fermented easily. So, it was necessary to prevent fermentation of coconut water to extend its shelf life.

Prior to the development of treated coconut water, the physiochemical properties of raw coconut water were estimated using the standard procedure. From the reviews of literature and preliminary experiments 23 treatments were selected. The various unit operations like clarification, addition of preservatives, carbonation etc., were done for the development of treated coconut water.

The optimization of process variables was done based on the physiochemical analysis of treated coconut water, microbial analysis and sensory analysis. The optimised samples (4 no.) were subjected to storage studies for a duration of 45 days under refrigerated condition (8°C). Storage studies was done based on the microbial analysis of the stored CW.

The result of the work done was summarised as follows.

The physiochemical properties of the fresh CW were done based on the standard procedures. The fresh CW had a carbohydrate content of 3.2 g/100mL, protein 0.5 g/100mL, TSS value of 3.4, pH of CW was found to be 5.25, titrable acidity of CW was estimated as 0.134, colour value of L*, a and b* was observed as 50.4, 3.2 and 6.3 respectively.

After the development of treated CW samples, the physiochemical properties of all the treated CW samples were tested based on standard procedures. There were no significant changes in the physiochemical properties of the treated CW samples as compared to the physiochemical properties of fresh CW.

Initially, the treated CW samples were optimised based on the result of microbial analysis conducted on 14^{th} day of preparation of samples. Total plate count of all the samples were obtained. From the 23 samples, 8 samples were selected as they were found to be microbially safe. The 8 samples were T_{12}, T_{15}, T_{16}, T_{18}, T_{19}, T_{21}, T_{22} and T_{23}.

Then, the optimisation was done based on sensory analysis. Two sets (Carbonated and non-carbonated) of 8 samples selected earlier was used to conduct sensory analysis. From the sensory analysis it was clear that the treatments used has helped to increase the sensory quality of fresh CW. Samples T_{12} and T_{13} were selected from both set of samples.

So finally, four samples were selected for storage studies for a period of 45 days. Both carbonated and non-carbonated samples of T_{12} and T_{13} were the samples selected for storage studies. From the storage studies conducted, all the samples were found to have a shelf life of one month.

REFERENCE

REFERENCE

Alaban, C. A. (1962). Studies on the optimum conditions for nata de coco bacterium or nata formation in coconut water. *Philipp. Agric*, *45*, 490-516.

Al-Khayri, J. M., Huang, F. H., Morelock, T. E., & Busharar, T. A. (1992). Spinach tissue culture improved with coconut water. *HortScience*, *27*(4), 357-358.

Anand, S., & Horrocks, A. R. (Eds.). (2000). *Handbook of technical textiles*. CRC Press/Woodhead Pub.

Augustine, S. (2007). *U.S. Patent Application No. 11/570,144*.

Batugal, P. A., Ramanatha Rao, V., & Bong, C. (1996). Promoting multi-purpose uses and competitiveness of the coconut. *IPGRI, Chumphon, Thailand*, 23-25.

Becker, Z. E. (1936). A comparison between the action of carbonic acid and other acids upon the living cell. *Protoplasma*, *25*, 161-175.

Bhagya, D., Prema, L., & Rajamohan, T. (2012). Therapeutic effects of tender coconut water on oxidative stress in fructose fed insulin resistant hypertensive rats. *Asian Pacific journal of tropical medicine*, *5*(4), 270-276.

Blickstad, E., Enfors, S. O., & Molin, G. (1981). Effect of hyperbaric carbon dioxide pressure on the microbial flora of pork stored at 4 or 14 C. *Journal of Applied Bacteriology*, *50*(3), 493-504.

Bourdeix, R., Konan, J. L., & N'Cho, Y. P. (2006). *Coconut: a guide to traditional and improved varieties*. Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD).

Brown, W. D., & Mebine, L. B. (1969). Autoxidation of oxymyoglobins. *Journal of biological Chemistry*, *244*(24), 6696-6701.

Callow, E. H. (1932). Gas storage of pork and bacon. Part I. Preliminary experiments. *Journal of the Society of Chemical Industry*, *51*, 116-119.

Campbell-Falck, D., Thomas, T., Falck, T. M., Tutuo, N., & Clem, K. (2000). The intravenous use of coconut water. *The American journal of emergency medicine*, *18*(1), 108-111.

Campos, C. F., Souza, P. E. A., Coelho, J. V., & Glória, M. B. A. (1996). Chemical composition, enzyme activity and effect of enzyme inactivation on flavor quality of green coconut water 1. *Journal of food processing and preservation*, *20*(6), 487-500.

Carvalho, J. M. D., Maia, G. A., Sousa, P. H. M. D., & Maia Jr, G. A. (2006). Água-de-coco: Propriedades nutricionais, funcionais e processamento. *Semina ciênc. agrar*, 437-452.

Chowdhury, M. M., Aziz, M. G., & Uddin, M. B. (2005). Development of shelf-stable ready-to-serve green coconut water. *Biotechnol*, *4*, 121-125.

Coyne, F. P. (1933). The effect of carbon dioxide on bacteria growth. *Proceedings of the Royal Society of London. Series B, Containing Papers of a Biological Character*, *113*(782), 196-217.

Daniels, J. A., Krishnamurthi, R., & Rizvi, S. S. (1985). A review of effects of carbon dioxide on microbial growth and food quality. *Journal of food protection*, *48*(6), 532-537.

Ediriweera, E. R. H. S. S. (2003). Medicinal uses of coconut (Cocos nucifera L.). *Cocoinfo Int*, *10*, 11-21.

Ediriweera, N.D. (1996). King coconut. *CORD XII*, 2, 43-47

Fanestil, D. D., Hastings, A. B., & Mahowald, T. A. (1963). Environmental CO2 stimulation of mitochondrial adenosine triphosphatase activity. *Journal of Biological Chemistry*, *238*(2), 836-842.

Farapti, F., Sayogo, S., & Siregar, P. (2013). Effect of tender coconut water on systolic and diastolic blood pressure in prehypertensive women. *Health Science Journal of Indonesia*, *4*(2), 64-68.

Fontan, R. D. C. I., Santos, L. S., Bonomo, R. C. F., Lemos, A. R., Ribeiro, R. P., & Veloso, C. M. (2009). Thermophysical properties of coconut water affected by temperature. *Journal of food process engineering*, *32*(3), 382-397.

Fontana, J. D., De Souza, A. M., Fontana, C. K., Torriani, I. L., Moreschi, J. C., Gallotti, B. J., ... & Farah, L. F. X. (1990). Acetobacter cellulose pellicle as a temporary skin substitute. *Applied biochemistry and biotechnology*, *24*, 253-264.

Fukuyama, R., Jami, M. S., Tanaka, T., & Iwata, M. (2015). Consolidation behaviour of thick suspension in centrifugal dewatering with and without supernatant. *Separation and Purification Technology*, *150*, 223-228.

Gabriel, A. A., Cruz, K. G. S., & De Guzman, J. A. D. (2009). Thermal death times of Escherichia coli in young coconut endosperm beverage. *Journal of Food Processing and Preservation*, *33*, 136-144.

Gallardo De Jesus, E. M. M. A., Andres, R. M., & Magno, E. T. (1973). Study on the isolation and screening of microorganisms for production of diverse-textured nata. *Philippine J Sci*.

George, J., Ramana, K. V., Sabapathy, S. N., & Bawa, A. S. (2005). Physico-mechanical properties of chemically treated bacterial (Acetobacter xylinum) cellulose membrane. *World Journal of Microbiology and Biotechnology*, *21*, 1323-1327.

Gray, R. J. H., Hoover, D. G., & Muir, A. M. (1983). Attenuation of microbial growth on modified atmosphere-packaged fish. *Journal of Food Protection*, *46*(7), 610-613.

Jackson, J. C., Gordon, A., Wizzard, G., McCook, K., & Rolle, R. (2004). Changes in chemical composition of coconut (Cocos nucifera) water during maturation of the fruit. *Journal of the Science of Food and Agriculture*, *84*(9), 1049-1052.

Jansz, E.R., Rabel, C.S., Marikar, F.A. (1992). Some factors affecting the development of pink colour in coconut water. *Journal National Science Council, Sri Lanka*, 20(1), 107-113

Jayalekshmy, A., & Mathew, A. G. (1990). Changes in the carbohydrates and proteins of coconut during roasting. *Food Chemistry*, *37*(2), 123-134.

Jordana, J. (2000). Traditional foods: challenges facing the European food industry. *Food Research International*, *33*(3-4), 147-152.

Kaliyan, N., Morey, R. V., Wilcke, W. F., Alagusundaram, K., & Gayathri, P. (2007). Applications of carbon dioxide in food and processing industries: current status and future thrusts. In *2007 ASAE Annual Meeting* (p. 1). American Society of Agricultural and Biological Engineers.

Keshk, S., & Sameshima, K. (2006). The utilization of sugar cane molasses with/without the presence of lignosulfonate for the production of bacterial cellulose. *Applied Microbiology and Biotechnology*, *72*, 291-296.

Mantena, S. K., Jagadish, Badduri, S. R., Siripurapu, K. B., & Unnikrishnan, M. K. (2003). In vitro evaluation of antioxidant properties of Cocos nucifera Linn. water. *Food/Nahrung*, *47*(2), 126-131.

Mathiowitz, E., Chickering III, D. E., & Lehr, C. M. (Eds.). (1999). Bioadhesive drug delivery systems: fundamentals, novel approaches, and development.

Mormino, R., & Bungay, H. (2003). Composites of bacterial cellulose and paper made with a rotating disk bioreactor. *Applied microbiology and biotechnology*, *62*, 503-506.

Nakagaito, A. N., Iwamoto, S., & Yano, H. (2005). Bacterial cellulose: the ultimate nano-scalar cellulose morphology for the production of high-strength composites. *Applied Physics A*, *80*, 93-97.

Nanda Kumar, T. B. (1995). Tender coconut water: nature's finest drink. *INDIAN COCONUT JOURNAL-COCHIN-*, *26*, 42-45.

Narmadha, N., Karunakaran, K. R., Anjugam, M., Palaniswami, N. V. and Vasuki, R. 2022. An economic analysis on Indian scenario of coconut production: Trends and prospects. New Delhi Publishers. J. of Trop. Med. 67 (3),263- 269.

Osazuwa, O. E., & Ahonkhai, I. (1989). Coconut water as growth medium for micro-organisms. *Niger. J. Palms Oil Seeds*, *10*(11), 91-95.

Overbeek, J., Conklin, M. E., & Blakeslee, A. F. (1941). Factors in coconut milk essential for growth and development of very young Datura embryos. *Science*, *94*(2441), 350-351.

Parmar, P. T., Singh, A. K., & Borad, S. G. (2016). Coconut (Cocos nucifera). *Oilseeds: Health Attributes and Food Applications*, 163-189.

Pieris, W. V. D. (1971). Coconut water, the liquid component of the coconut endosperm. a note on terminology. *Oleagineux*.

Pilo, F. B., Pereira, N. O., Miranda, A. N., do Carmo, L. S., Gomes, F. D. C. O., Nardi, R. M. D., & Rosa, C. A. (2009). Microbiological testing and physical and chemical analysis of reconstituted fruit juices and coconut water/Avaliacao microbiologica e caracterizacao fisico-quimica de refrescos e agua de coco. *Alimentos e Nutricao (Brazilian Journal of Food and Nutrition)*, *20*(4), 523-533.

Prades, A., Dornier, M., Diop, N., & Pain, J. P. (2012). Coconut water preservation and processing: a review. *Fruits*, *67*(3), 157-171.

Priya, S. R., & Ramaswamy, L. (2014). Tender coconut water-Natures elixir to mankind. *International Journal of Recent Scientific Research*, *5*(8), 1485-1490.

Ramesh, S. V., Pandiselvam, R., Shameena Beegum, P. P., Saravana Kumar, R. M., Manikantan, M. R., & Hebbar, K. B. (2021). Review of Cocos nucifera L. testa-derived phytonutrients with special reference to phenolics and its potential for encapsulation. *Journal of Food Science and Technology*, 1-10.

Rathbone, M. J. (1996). Oral mucosal drug delivery. *(No Title)*.

Reddy, N. R., Armstrong, D. J., Rhodehamel, E. J., & Kautter, D. A. (1991). Shelf-life extension and safety concerns about fresh fishery products packaged under modified atmospheres: a review. *Journal of Food safety*, *12*(2), 87-118.

Rethinam, P., & Nanda Kumar, T. B. (2001). Tender coconut-an overview. *INDIAN COCONUT JOURNAL-COCHIN-*, *32*(1), 2-22.

Robinson, J. R., & Lee, V. H. (1987). Controlled drug delivery Marcel Dekker. *Inc. New York, Basel*.

Saat, M., Singh, R., Sirisinghe, R. G., & Nawawi, M. (2002). Rehydration after exercise with fresh young coconut water, carbohydrate-electrolyte beverage and plain water. *Journal of physiological anthropology and applied human science*, *21*(2), 93-104.

Sanchez, P. C. (1985). Village-level technology of processing coconut water vinegar. *Phil. Agr.*, *68*, 349-448.

Schumann, D. A., Wippermann, J., Klemm, D. O., Kramer, F., Koth, D., Kosmehl, H., ... & Salehi-Gelani, S. (2009). Artificial vascular implants from bacterial cellulose: preliminary results of small arterial substitutes. *Cellulose*, *16*, 877-885.

Serafica G., Mormino R., Bungay H. 2002. Inclusion of Solid particles in Bacterial Cellulose. Appl Microbiol Biotechnol, 58:756-760.

Shibazaki, H., Kuga, S., & Onabe, F. (1994). Mechanical properties of papersheet containing bacterial cellulose. *Japan Tappi Journal*, *48*(12), 1621-1630.

Solangi, A. H., & Iqbal, M. Z. (2011). Chemical composition of meat (kernel) and nut water of major coconut (Cocos nucifera L.) cultivars at coastal area of Pakistan. *Pakistan Journal of Botany*, *43*(1), 357-363.

Srivatsa, A. N. (1995). Preservation of tender coconut water in polymeric pouches and metal cans.

Steiner, I., & Desser, A. (2008). Coconut water–composition, properties and processing. *Ernähr*, *32*, 513-516.

Tan, T. C., Cheng, L. H., Bhat, R., Rusul, G., & Easa, A. M. (2014). Composition, physicochemical properties and thermal inactivation kinetics of polyphenol oxidase

and peroxidase from coconut (Cocos nucifera) water obtained from immature, mature and overly-mature coconut. *Food Chemistry*, *142*, 121-128.

Tzeng, E., & Chen, H. E. (1998). Preventing nonenzymatic browning in coconut water during sterilization. *Food Sci.(Taiwan)*, *25*, 304-313.

Valley, G. (1928). The effect of carbon dioxide on bacteria. *The quarterly review of Biology*, *3*(2), 209-224.

Watanabe, K., & Yamanaka, S. (1995). Effects of oxygen tension in the gaseous phase on production and physical properties of bacterial cellulose formed under static culture conditions. *Bioscience, biotechnology, and biochemistry*, *59*(1), 65-68.

Wodzinski, R. J., & Frazier, W. C. (1961). Moisture requirements of bacteria IV: Influence of temperature and increased partial pressure of carbon dioxide on requirements of three species of bacteria. *Journal of Bacteriology*, *81*(3), 401-408.

Yano, S., Maeda, H., Nakajima, M., Hagiwara, T., & Sawaguchi, T. (2008). Preparation and mechanical properties of bacterial cellulose nanocomposites loaded with silica nanoparticles. *Cellulose*, *15*, 111-120.

Zerin, I., & Datta, E. (2018). A review article on applications of filter cloth. *Int. J. Cloth. Sci*, *5*(1), 1-6.

Zhou, F., Sun, G., Han, X., Zhang, Y., & Bi, W. (2018). Experimental and CFD study on effects of spiral guide vanes on cyclone performance. *Advanced Powder Technology*, *29*(12), 3394-3403.

Zulaikhah, S. T. (2019). Health benefits of tender coconut water (TCW). *Int J Pharm Sci Res*, *10*(2), 474-80.

PROCESSING AND SHELF-LIFE STUDIES OF COCONUT WATER

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ABSTRACT

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ABSTRACT

Coconut water was a highly nutritious beverage with low calorific value. Due to its high nutrition content and high chance of fermentation, the shelf life of coconut water was very less. So, it was usually discarded during the processing of coconut. In this project our objectives were to evaluate the physicochemical properties of coconut water, to optimise the process parameters for preservation of coconut water and to conduct shelf-life studies of treated and processed coconut water.

Here we have initially studied about the nutrition content of fresh coconut water by estimating its carbohydrate content and protein content. Then the various physicochemical properties of fresh coconut water such as TSS, pH, titrable acidity, colour and acidity was estimated. 23 treatments were selected based on review of literature and preliminary experiments. The various unit operations like clarification, addition of preservatives, carbonation etc., were done for the development of treated coconut water. On analysing the physicochemical properties of treated coconut water it was understood that there was no significant difference among treated coconut water samples.

The optimisation of processed variables for preservation of coconut water was done in terms of microbial analysis and sensory analysis. Out of the 23 treatments under study, 4 treatments (carbonated and non-carbonated samples of T_{12} and T_{13}) were optimised. These samples were subjected to storage studies for a duration of 45 days under refrigerated condition (8°C). From the storage studies conducted all the samples were found to have a shelf life of one month under refrigerated condition (8°C).