BOTTLED TENDER COCONUT WATER: A CASE STUDY

A Dissertation submitted by

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DECLARATION

I, **FOUZIA M YOOSUF (VM23FPT009),** hereby declare that the thesis entitled **'IMPACT OF PASTEURIZATION AND POSTBIOTIC ADDITION ON TENDER COCONUT WATER: QUALITY, SENSORY, MICROBIAL ANALYSIS'** submitted to Human Resource Development Department, CSIR-CFTRI, Mysuru, 2025, for the partial fulfilment of Master of Vocational studies in Food Processing Technology has been written solely by me under the guidance of **Dr. Chetana R,** Principal Technical Officer, Traditional Foods and Applied Nutrition Department, CSIR-CFTRI, Mysuru.

I have not submitted this thesis to any other course or degree or university.

Place: Mysuru **Fouzia M Yoosuf**

Date:

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ABBREVIATION

TCW- Tender Coconut Water

TPC- Total Phenolic Content

TA- Titratable Acidity

TSS- Total Soluble Solids

FC- Folin-Ciocalteu

GAE- Gallic Acid Equivalent

DPPH- 2,2-diphenyl-1-picrylhydrazyl

HPLC- High-Performance Liquid Chromatography

CFU- Colony Forming Units

Ppm- Parts Per Million

Ao - Absorbance of control

A1 - Absorbance of sample

Fig- Figure

POD- Peroxidase

PPO-Polyphenol-oxidase

RSA- Radical Scavenging Activity

TPC – Total Plate Count

VRBA-Violet Red Bile Agar

DRBC- Dichloran Rose Bengal Chloramphenicol Agar

ABSTRACT

Tender coconut water (TCW) is a natural beverage and a main by-product of various coconut processing industries such as virgin coconut oil, coconut chips, coconut milk, etc. In spite of huge benefits, TCW's short shelf life limits its market potential. This study evaluates the impact of pasteurization, with and without the addition of the postbiotic bacteriocin (Nisin), on the physicochemical, microbial, and sensory characteristics of TCW stored at room temperature. Two treatment approaches were compared: (a) pasteurized TCW without Nisin and (b) pasteurized TCW with Nisin and sugar free additive, alongside untreated TCW as a control, in glass and PET bottles respectively. Key parameters analysed include pH, total soluble solids (TSS), titratable acidity, total sugar, transmittance, turbidity, colour, total phenolic content, DPPH antioxidant activity, and ascorbic acid content. No significant changes occurred in Brix (4.78), pH (4.7), and colour values and all remained stable throughout storage, while turbidity was increased. Ascorbic acid was decreased for all the samples. Microbial growth was more pronounced in PET bottles after 30 days, indicating that glass bottles are more suitable for preserving TCW at room temperature. Pasteurized TCW treated with Nisin and sucralose exhibited extended shelf life and maintained palatability, demonstrating the effectiveness of these treatments in improving TCW stability.

Chapter-1
INTRODUCTION

The coconut palm *(Cocos nucifera L.)* is a very important plant for tropical regions and the people living there. It was once grown on large plantations to produce oil but is now mostly farmed by small-scale farmers. This tree grows best in coastal areas within 20º north and south of the equator, from sea level up to 1,200 meters. It needs warm temperatures between 27-30ºC, high humidity, and soils that allow air to pass through easily to grow well. The coconut palm is the most widely grown and used palm tree in the world. It is a vital source of food and income for about 10 million families across over 80 countries. Many coconuts are used locally, showing how important this crop is for small communities and rural economies. Food, oil, and other items can be obtained from it. The fruit is referred to as a "wonder fruit" due to its rich macro and micronutrient profile for human health and nutrition (Mat, K et al., 2022).

Fruit juices are popular among customers as a nourishing and revitalizing beverage since they are high in nutrients, fibre, and antioxidants and may help the body regenerate. Coconut water is a natural, healthy, nutritious drink from coconut palm trees widely grown in tropical countries. Indonesia is the largest coconut growing country in the world. TCW has a therapeutic effect,4.5 containing various nutrients such as minerals, vitamins, antioxidants, amino acids, enzymes and growth hormones. TCW is rich in L-arginine, a free form amino acid, and vitamin C, which can prevent heart disease and lipid peroxidation. TCW also contains various important compounds for the body, such as magnesium, potassium, calcium, selenium, methionine, zinc, iodine, manganese, boron, molybdenum, tender coconut water can reduce oxidative stress and improve antioxidant status characterized by decreased MDA levels, increased levels of antioxidant enzymes.

Coconut water is a pure, non-fermented drink taken from the inside of a coconut. It is used in many industries and has become more popular because of its health benefits. It is a clear and refreshing liquid found inside young, green coconuts. Green coconuts have more water than ripe ones, which have less liquid but more thick white flesh (called the endosperm) and contain more lipids. Coconut water also has many helpful enzymes that aid digestion, as well as important nutrients like sugars, vitamin C, folic acid, amino acids, and several vitamins (B1, B2, B6, and pantothenic acid).

TYPE OF COCONUT

Although coconut comes in a variety of forms, it may generally be categorized into two groups are:

a**. dwarf coconut**: There are coconuts that produce nuts four to six years after they are planted.For example: ivory coconut (Eburnia variety), coconut king (Regia), and king malabar (Pretiosa) and quail (Pumila).

b. **The deep coconut** is a young coconut that bears fruit 15 years after it is planted. For instance, green coconuts (Viridis) and red coconuts (Rubescens) can grow up to 30 meters in height. The inter varietal crosses of dwarf deep coconut are called hybrids.

Tender coconut water is a natural, refreshing drink obtained from young green coconuts before they mature. The Coconut Water Tender, or TCW, is known as the "fluid of life". Coconut water contains the highest levels of water and sugar when the coconut is 6 to 7 months old, giving it its sweetest and most delicious taste. As the coconut matures further, the water and sugar content decrease. (Zulaikha et al., 2022). India, as a major producer, exports packaged coconut water to international markets, where it is increasingly recognized as a healthy alternative to sugary drinks.

The electrolytes (ionic minerals) in tender coconut water are comparable to those in human plasma, making it a refreshing beverage. More often used as a health supplement, this delicious beverage is packed with minerals which increase immunity and metabolism. Cytokinins are the components of coconut water that are significant and beneficial. The possible anti-cancer effects of particular cytokinins may offer fresh and promising insights into the search for treatments for various cancer types. The latest finding that coconut water has additional therapeutic benefits suggests that it has a promising future in enhancing human health.

Coconut juice, also referred to as tender coconut water (TCW), is the liquid component of coconut endosperm. TCW serves multiple purposes, including being a ceremonial gift, a traditional medicine, a growth medium for microorganisms, and a refreshing tropical drink. Additionally, it can be processed into vinegar or wine. TCW constitutes about 25% of a coconut's total weight. Naturally sterile, the liquid is enclosed within a sealed chamber has a slightly acidic and pleasantly sweet taste, and it is clear and translucent with total solids comprising approximately 5% of its weight (Prades et al., 2012).

The nutritional composition of TCW varies based on environmental conditions such as soil quality, geographical location, variety, and maturity of the coconut. Once extracted from the nut, TCW deteriorates quickly due to microbial contamination, physical pollutants, storage conditions, and packaging materials. Enzymes like polyphenol oxidase and peroxidase more active during handling, even in sterile conditions, leading to turbidity, off-flavours, discoloration, and nutrient degradation (Appaiah et al., 2015).

Similar to blood plasma in composition, TCW is a naturally occurring isotonic beverage. It has vital nutrients such vitamins, minerals, growth hormones, enzymes, amino acids, and antioxidants. Potassium, magnesium, selenium, calcium, zinc, methionine, manganese, iodine, molybdenum, boron, and phytohormones like as auxin, cytokinin, and gibberellin are main things of TCW. The main component of total soluble solids (TSS), which constitute around 5–8% of CW, is sugar (Burns et al., 2020).

The primary method for preservation and microbiological safety at present is pasteurization. However, some of the heat-sensitive aroma and other sensory qualities are impacted by pasteurization. So pasteurization with addition of nisin is a better method.

2 OBJECTIVES

1. To process the tender coconut water by pasteurization.
2. Effect of nisin addition on tender coconut water.
3. To conduct physio-chemical properties and microbiological studies on tender coconut water.

Chapter-3

REVIEW OF LITERATURE

This chapter deals with review of literature on processing of tender coconut water by pasteurization with nisin and storage parameters and their effect on physio-chemical and microbial parameters of processed tender coconut water.

Table-1: Taxonomical classification of coconut

(Source: Vikas et al., 2012)

Kingdom	Plantae-Plants
Sub kingdom	Tracheobionta -Vascular plants
Super division	Spermatophyta – Seed plants
Division	Mangnoliophyta – Flowering plant
Class	Liliopsida - Monocotyledons
Sub class	Arecida
Order	Arecales
Family	Arecaceae – Palm family
Genus	Cocos L – Coconut palm
Species	Cocos nucifera – Coconut palm

3.1 CULTIVATION

India is an agricultural country and most people are depending on it for their income and livelihood. Agriculture has always been a key part of the Indian economy. Coconut cultivation plays an important role in the social and economic lives of farmers. The price and market demand for coconuts and their by-products directly affect farmers' livelihoods. Tamil Nadu is the second-largest producer of coconuts after Kerala, with 60% of its farmers relying on coconut cultivation. Besides its cultural significance, coconut farming is also important for rural employment and income generation.

Around 70% of coconuts are consumed domestically, with more than half eaten fresh. Though initially found along sandy shores, it now grows in various soil types, from clay to sand, and can tolerate different pH levels. Its expansion inland is mainly due to human activity. Coconuts thrive in warm, humid climates and are easily recognized by their feather-like leaves and large fruit clusters.

In India, coconuts are widely grown along coastal regions and islands. They can be classified based on genetic markers, husk-to-nut weight ratio, and plant size. Most cultivated coconuts in India belong to the Niu Kafa group, known for their high husk content. While Western India is not a major coconut-growing region, a large-fruited variety was found in Maharashtra and studied for its unique traits. Coconut farming has a significant social and economic impact on cultivators. Market demand and pricing directly affect farmers' income. Kerala leads in coconut production, followed by Tamil Nadu, where 60% of farmers depend on it for their livelihood.

Tender coconut cultivation in India has gained importance due to its high demand as a natural, refreshing drink. India is one of the largest producers of coconuts, with major cultivation in Kerala, Tamil Nadu, Karnataka, Andhra Pradesh, Maharashtra, Odisha, and West Bengal. Coconut cultivation thrives in warm, humid tropical climates with temperatures between 27-35°C and an annual rainfall of 1500-2500 mm. The crop grows well in sandy loam, lateritic, and alluvial soils with good drainage and a pH range of 5.2 to 8.0. Among the best varieties for tender coconut production are Chowghat Orange Dwarf (COD), Malayan Yellow Dwarf (MYD), Malayan Green Dwarf (MGD), Gangabondam, and Kalpa Raksha. These dwarf varieties are preferred because they start bearing fruit early, within 3-4 years.

3.2 PRODUCTION

Coconut is a premium crop that support millions of people. Compared to other oilseed crops, coconuts are more productive and consistently produce more, and they are also less vulnerable to unusual weather patterns. It is projected that 61.7 million tons (MT) of coconuts are produced worldwide with an 5.2% output per hectare on average. With 75% of the world's production, the top three producers are India, the Philippines, and Indonesia. After basic processing, the fruit can be transformed into various products, such as milk, desiccated coconut, virgin oil, coconut water, and crude oil (Laura J Pham et al.,2016).

3.2.1 NATIONAL PRODUCTION STATUS

India is the leading country in coconut production and productivity, ranking third globally in terms of area under coconut cultivation. With a documented productivity of 9,346 nuts per hectare, India produced 20,309 million nuts in 2021–2022, accounting for more than 31% of the world's coconut production. The total area under coconut cultivation in the country is 21.73 lakh hectares, with four southern states—Kerala, Karnataka, Tamil Nadu, and Andhra Pradesh—accounting for approximately 90% of the total area and production.

Karnataka plays a significant role in India's coconut industry, with a cultivation area of 5.5733 lakh hectares. With a production of 10,581 nuts per hectare, the state produces 5,897.32 million nuts, significantly more than the national average of 9,123 nuts per hectare. Karnataka contributes 26.42% of the total coconut-growing area and 30.64% of the country's coconut production. Coconuts are consumed in various forms, including tender coconuts for drinking, mature coconuts for culinary use, and for religious purposes.

Table-2: Area, Production and Productivity in India

States/ Union Territories	Area(100 hectares)	Production(m nuts)	Productivity (nuts/ ha)
Andaman & Nicobar Islands	16.3	124.8	7,667
Andhra Pradesh	99.5	1,396.9	14,038
Gujarat	24.9	241.2	9,670
Karnataka	518.4	6,273.8	12,102
Kerala	807.1	8,452.1	10,472
Lakshadweep Islands	2.7	87.6	32,753
Maharashtra	27.0	127.9	4,743
Odisha	50.9	341.7	6,712
Pondicherry	1.8	27.8	15,357
Tamil Nadu	441.5	6,020.4	13,637
West Bengal	30.3	377.7	12,484

3.3 CHEMICAL COMPOSITION OF TCW

Fig 1 – Different stages of coconut

A coconut takes about 11 to 12 months to fully mature. Around the fifth month, a thin jelly-like layer of kernel starts forming inside the shell, which encloses the clear and sweet coconut water. At this stage, the water is under pressure, but as the fruit ripens, the pressure reduces, and the kernel gradually replaces the water by storing lipids. When a coconut reaches full maturity at 12 months, its water content ranges from 15% to 30% of its total weight; the amount of water in a nut varies depending on the variety and maturity stage, but on average, it is around 300 mL.

There are three main types of coconut varieties: tall (which cross-pollinates), dwarf (which self-pollinates), and hybrid (a mix of the two, usually with a dwarf mother and a tall father). Coconut water is naturally sterile inside the shell and contains both organic and inorganic nutrients, including almost all essential minerals. Coconut water has a lower dry weight than other fruit juices, ranging from 5% to 6%, whereas apple juice has 12% to 15%. Its main components are soluble sugars, along with proteins and salts **(** Alexia PRADES et al.,2012).

A number of natural and beneficial enzymes, such as diastase, catalase, dehydrogenase, peroxidase, and acid phosphatase, are present in coconut water and may greatly facilitate the digestion of nutrients that are essential to human health, such as sugars, vitamins C, B1, B2, and B6, free amino acids, auxin, folic acid, and pantothenic acid. The minerals potassium (at a high concentration), sodium, calcium, magnesium, and phosphorus are also present in coconut water (Yong et al., 2009).

The vitamin C content varies from 20 to 40 mg.L-1.This level is not high compared with orange, acerola, but it is not sufficient to prevent oxidation. Sugars are the main soluble solids present in tender coconut water. Minerals are also present, it is about 0.4% to 1% to the liquid volume. The distinct flavour and taste of coconut water differs from the familiar fragrance of the coconut kernel. Volatile molecules add to the fresh liquid's aroma, just as organic acids made up of malic, succinic, citric, acetic, and tartaric acids do to the flavour of coconut water.

2.3.1 Sugars (Natural Sweetness): The sweetness in tender coconut water comes from glucose and fructose in the early stages. As the coconut matures, sucrose (table sugar) increases while glucose and fructose decrease. Young coconuts have about 5–5.5% sugar, but this drops to 2% in fully mature coconuts. In a fully mature coconut, 90% of the sugar is sucrose.

2.3.2 Minerals (Electrolytes for Hydration): Contains essential minerals like potassium, sodium, calcium, phosphorus, iron, copper, sulphur and chlorides. Potassium makes up more than half of the minerals and helps increase urine output. Potassium level is influenced by how much potassium fertilizer the coconut tree gets.

2.3.3 Protein (Amino Acids for Nutrition): Has a small amount of protein, but it contains important amino acids like arginine, alanine, cystine, and serine. These amino acids are found in higher amounts than in cow's milk. Since it doesn't have complex proteins, it's safe for patients because it doesn't cause allergic reactions or shock.

2.3.4 Vitamins (Nutrients for Health): Contains Vitamin C (ascorbic acid) and B vitamins. Vitamin C levels range from 2.2 to 3.7 mg per ml, but decrease as the coconut matures and its outer layer hardens.

Table-3: Composition of TCW

Moisture	95.2
Sugars	6.6
Ash	0.2
Protein	0.2

Table-4: Amino Acid Component

Amino Acid	**mg**
Alanine	10
Arginine	32
Lysine	10
Glycine	10

Table-5: Mineral Component

Minerals	**mg/100g**
Calcium	12
Potassium	186
Magnesium	11.0
Phosphorous	9.0
Copper	0.220
Iron	0.4

Table 6: Vitamin Component

Vitamins	mg/100g
Thiamine	0.01
Riboflavin	0.02
Niacin	0.10

(Source: Prabhakar Reddy et al.,2014)

Endosperm develops in three main ways: nuclear, cellular, with coconut following the nuclear mode. At first, the endosperm is a liquid filled with free nuclei, formed as the primary endosperm nucleus divides multiple times without splitting the cell (cytokinesis). Later, cytokinesis begins from the outer layer and moves inward, creating a cellular structure. Initially, this layer is jelly-like and translucent, but as the coconut matures, it hardens into the white flesh known as coconut meat. It has anti-ageing and anti-carcinogenic effect.

3.4 PRODUCTS FROM TCW

The coconut tree is a versatile tropical palm that significantly benefits human life. It serves as an excellent example of how every part of its fruit is utilized—whether as food or as an ingredient in various nutritious products rich in protein and essential nutrients. Beyond its edible components, the tree's inedible parts are also valuable, being used for energy production and a wide range of industrial applications that support daily life. Coconut-derived products encompass an extensive assortment of items, celebrated for their adaptability and nutritional benefits. The study of coconut products highlights both traditional and innovative uses, underscoring their significance across industrial, culinary, and health sectors.

Tender coconut water

Coconut water is the liquid endosperm of the nut, making up about 25% of its total weight. It is a clear, slightly sweet, and mildly acidic liquid (pH 5.6) found inside young green coconuts, containing around 5% total solids. Naturally, it exists in a sterile environment within the nut. Due to growing consumer awareness of the negative health effects of carbonated drinks, there is a high demand for bottled coconut water that retains its fresh taste while having a longer shelf life. It have great low-calorie (17.4 kcal/100g) alternative to sports drinks, as it contains essential nutrients like vitamins C and B, minerals such as potassium, calcium, magnesium, and sodium, as well as beneficial bioactive compounds. However, its nutritional content varies depending on factors like maturity, variety, location, and soil conditions. Rich in nutrients, coconut water is a delicious, thirst-quenching, and healing natural beverage. Because of its salt content, it is also regarded as a naturally occurring isotonic beverage. Diarrhea, dehydration, myocardial infarction, kidney problems, urinary tract infections, weariness, digestive troubles, antiaging issues, and many other health-related conditions have been inhibited and alleviated by it. Coconut water reduces feelings of fullness and nausea as compared to regular drinking water. It is a good growth-promoting medium for a variety of beneficial microbes in addition to being fit for human consumption (Mohan Naik et al.,2022).

Fig 2 - TCW

Coconut water concentrates

The evaporation method can be used to create coconut water concentrate. From fresh coconut water, frozen coconut water is made. Water from fresh coconuts extracted under sanitary conditions from a recently opened shell. While the minerals were extracted from the centrifuged coconut water by passing it through an ionic resin-packed column to provide a sweet taste, the suspended particles and oils were eliminated by centrifugation prior to concentration. Depending on the level of concentration, the concentrate's shelf life ranged from six to twenty-four months.

TCW powder

It can be obtained by spray drying or freeze-drying technique. One popular technique for producing particles is spray drying, which involves turning a fluid substance into dried particles. A heated atmosphere is used to eliminate moisture from the feed product as part of the spray-drying operation. Three main phases—atomization, droplet-to-particle conversion, and particle collection—can be used to characterize the process. By pumping a solution to an atomizer, the liquid supply is divided into a thin droplet spray. After that, the droplets are sent into a drying gas chamber, where the moisture vaporization process takes place and dry particles are produced. Lastly, the dried particles are separated from the drying media using a suitable device, and they are then collected in a tank.

3.5 USES OF TCW

3.5.1 As a religious symbol

Coconut water has long been regarded as a religious symbol, particularly in Asia, and especially in India, due to its natural sterility and purity. In traditions, tender coconuts are considered sacred and are often used in religious ceremonies as offerings to deities. The water inside remains untouched until the shell is broken, symbolizing divine purity and blessings.

3.5.2 As a natural beverage

Mariners from Polynesia, Melaneasia, and Micronesia used coconut fruits as food and drink stores for centuries. They survived their travels from One island to the next by drinking a "naturally canned" beverage. Uses, composition, and properties of coconut water, which spread over the Pacific Ocean. Thousands of people still enjoy the delicious beverage of coconut water, which is prepared from immature nuts, in tropical regions. The most developed market for coconut water is in Brazil. Tender coconut water's hydration qualities, nutritional advantages, and pleasant flavor make it a popular natural beverage.

It is a rich source of electrolytes, including potassium, magnesium, and calcium, making it an excellent alternative to artificial energy drinks. Naturally low in calories and free from added sugars or preservatives, it serves as a healthy choice for hydration, especially in hot climates. Its mild sweetness and light texture make it a preferred drink for athletes, individuals recovering from illness, and those looking for a natural way to replenish lost fluids.

3.5.3 As medicine

One of the most significant applications of coconut water, aside from its use as a natural beverage, is medicine. Traditionally, coconut water is recommended for burning pain when urinating, gastritis, indigestion, hiccups, burning pain in the eyes, dysuria, or even the ejection of a retained placenta. Its high potassium content supports heart health by regulating blood pressure and reducing the risk of cardiovascular diseases. Coconut water also acts as a natural diuretic, aiding in kidney function by preventing the formation of kidney stones and flushing out toxins. It has antioxidant and anti-inflammatory properties, which help reduce oxidative stress, boost immunity, and promote overall well-being. Additionally, coconut water is known to aid digestion, relieve bloating, and prevent acid reflux by neutralizing stomach acids. It also has antimicrobial properties, making it useful in fighting infections and improving skin health when applied topically (Manuel DORNIER et al.,2012).

3.6 HEALTH BENEFITS OF TCW

3.6.1 Natural Isotonic Drinks

Coconut water's high potassium content is essential for preserving the osmotic pressure both inside and outside. Plasmolysis is the process by which water in a cell escape when it is submerged in a solution with a greater osmotic pressure (hypertonic), causing the cell to wrinkle. On the other hand, plasmoptysis is the process by which water from the outside enters the cell and causes it to enlarge when it is submerged in a solution with a lower osmotic pressure (hypotonic). For cells to stay balanced, the pressure inside and outside must be the same (isotonic).

3.6.2 Prevent Oxidative Stress

Triglycerides, free fatty acids, and blood pressure can all be lowered with the aid of tender coconut water (TCW). TCW increased antioxidant activity and decreased MDA, a sign of cell damage, in mice given a high-fructose diet. This implies that TCW could reduce oxidative stress and strengthen the body's defence against free radical damage. According to certain research, it had a beneficial impact on preventing blood clots and lessened the oxidative stress brought on by isoproterenol. Vitamin C and L-arginine are abundant in TCW. Vitamin C is a potent antioxidant that protects against tissue damage, while L-arginine aids in the reduction of free radicals. L-arginine can also lessen cell damage and prevent the generation of dangerous free radicals.

3.6.3 Anti-oxidant Activity

Tender coconut water can boost antioxidant enzyme levels. Coconut water vinegar helped reduce liver damage caused by acetaminophen by restoring antioxidant activity and reducing inflammation. Other studies suggest that coconut water has antioxidant effects. For example, giving coconut water (6 mL per 100 g of body weight) to female rats exposed to carbon tetrachloride increased antioxidant enzymes SOD and CAT (catalase) and reduced cell damage. Coconut water is rich in L-arginine, which lowers free radicals and acts as an antioxidant, as well as vitamin C (ascorbic acid), which helps prevent cell damage in rats.

3.6.4 Improve Blood Pressure

Tender coconut water lowers free fatty acid and triglycerides. Coconut water helps to lower systolic pressure. Fresh tender coconut water (300 ml) taken twice daily for 14 consecutive days was shown to lower systolic blood pressure, though it did not affect diastolic blood pressure. TCW enhances HDL levels while lowering LDL, triglyceride, and total cholesterol levels. Coconut water can lower HDL and lower LDL, triglycerides, and total cholesterol. TCW may also lower serum levels of triglycerides, LDL, and total cholesterol. In male rats, giving them coconut water (4 mL/100 g body weight) offset the rise in these chemicals brought on by feeding them cholesterol.

3.6.5 Diarrhea

Coconut water contains enzymes such as reductase, polyphenol oxidase (PPO), and peroxidase (POD), which are responsible for the brownish colour it develops when exposed to air for a long time. Due to its beneficial properties, coconut water has been used to treat diarrhea in both children and adults, as well as gastroenteritis. It is also used for dissolving urinary stones, providing short-term intravenous hydration, and protecting against gastrointestinal infections (Thomas Zulaikhah et al.,2019).

3.6.6 Anti-Diabetic Effect

The coconut kernel protein has been found to have potent anti-diabetic properties, primarily due to its ability to support pancreatic β-cell regeneration and regulate carbohydrate metabolism. One of the key components responsible for this effect is arginine, an amino acid present in coconut protein. In diabetes, the pancreatic β-cells, which are responsible for producing insulin, often get damaged, leading to improper glucose regulation. Studies suggest that arginine stimulates insulin secretion and promotes β-cell regeneration, helping restore normal pancreatic function. Additionally, coconut kernel protein helps reverse glycogen depletion in the liver and muscles, ensuring that glucose is properly stored and utilized.

Furthermore, it plays a crucial role in modulating carbohydrate-metabolizing enzymes such as:

- Glucokinase – Enhances glucose uptake by cells.
- Hexokinase – Helps in the first step of glucose metabolism.
- Phosphofructokinase – Regulates sugar breakdown for energy production.
- Glucose-6-phosphatase – Helps control blood sugar levels by regulating glucose release from the liver (Manisha Deb Mandal et al.,2011).

3.7 PREVIOUS STUDIES IN THE PRESERVATION OF TCW

The best approach to preserve coconut water is still to use young, sensitive coconuts (in their natural container), but the nuts cannot be kept for over six days at room temperature. Young, fragile coconuts can be kept secure and fresh for three weeks at (13 to 15) °C and 70% relative humidity if you follow their preparation recommendations (see containers). A similar supply chain for young, sensitive coconuts has been established from the field to the terminals and retailers owing to an initiative supported by the Indian Coconut Development Board. The minimal amount of processing is to dip partially husked nuts in a 0.5% potassium and 0.5% citric acid solution. The finished product can be kept at 5 to 7 °C for up to 24 days when wrapped in polypropylene cling film.

The study examined the antibacterial properties of nisin and its effectiveness in inactivating microorganisms in fruit juices. However, this preservation technique may impact the sensory attributes of the product. The approval of bacteriocins as food additives is restricted, particularly in plant-based foods. Nisin concentration remained stable in cashew, soursop, peach, mango, passion fruit, orange, guava, and cupuassu juices for at least 30 days at both room and refrigerated temperatures, without causing significant changes in their physicochemical characteristics (Adelson et al.,2015).

Fresh coconut water was collected from healthy green coconuts, filtered, and pasteurized at 85°C for 10 minutes before being cooled. The effects of citric acid, potassium metabisulfite ($K_2S_2O_5$), beta-carotene, ascorbic acid, and green colour were tested. Changes in colour, flavour, total soluble solids (TSS), acidity, gas formation, and fungal growth were monitored. The samples were stored at room temperature (27-35°C) and refrigerated (4-6°C), with observations made every four months for up to 12 months. The colour and flavour of green coconut water remained stable throughout storage. Adding acid and preservatives improved transparency. No gas formation was detected in either the canned or bottled samples. Pasteurized coconut water without citric acid was highly acceptable. The canned coconut water remained fresh for up to 12 months in the refrigerator and 10 months at room temperature.

Mahindru (2000) reported that nisin up to 5000 I.U is allowed in prepared TCW. Srivatsa and Sankaran (1995) studied how to preserve tender coconut water in 200 mL plastic pouches and 200 mL/350 mL aluminium cans. Since tender coconut water is easily affected by heat, they used minimal heating and added nisin (a natural preservative) to keep it commercially sterile. This method helped maintain its natural pH (4.9-5.2) instead of dropping below 4.5, which would have made it less appealing. They tested the microbial, chemical, and taste quality of the stored coconut water for three months at room temperature. The results showed that the product remained safe and acceptable for consumption.

3.8 TENDER COCONUT WATER PROCESSING

The processing of tender coconut water involves several steps to ensure its quality, safety, and extended shelf life while retaining its natural nutrients and flavour. Initially, fresh tender coconuts are carefully selected, cleaned, and sanitized to remove any dirt or contaminants. The water is then extracted under hygienic conditions to prevent microbial contamination. Filtration is carried out to remove any solid particles, followed by pasteurization or other thermal/non-thermal processing methods such as high-pressure processing (HPP) to eliminate harmful microorganisms while preserving its natural taste and nutritional value. In some cases, preservatives or antioxidants may be added to prevent oxidation and spoilage. The processed coconut water is then packaged in sterile containers, such as bottles, tetra packs, or cans, using aseptic packaging techniques to maintain freshness. Proper storage and cold chain management are essential to ensure that the final product retains its health benefits and remains safe for consumption (Alexia PRADES et al.,2012).

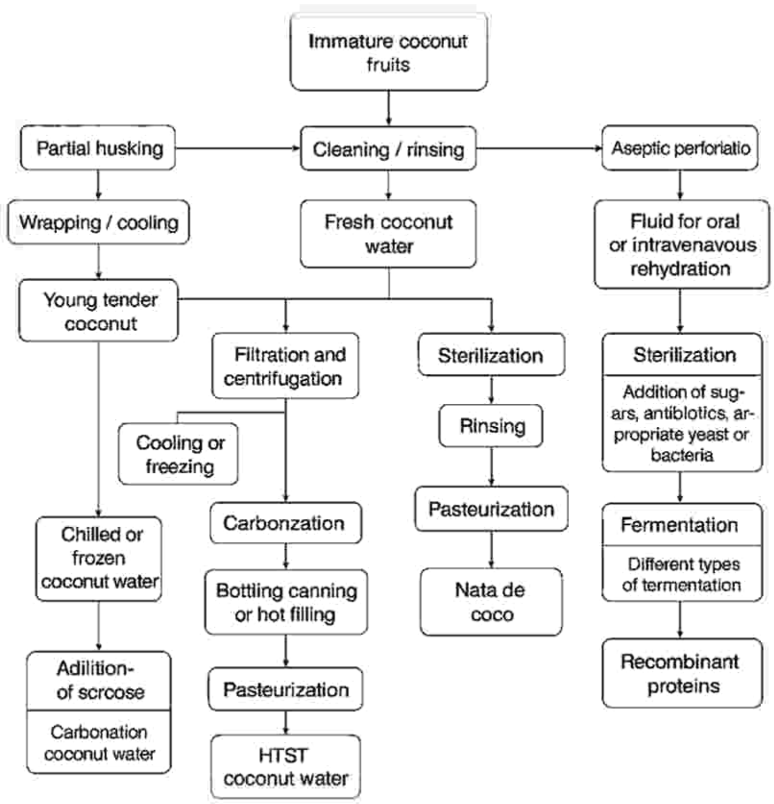


Fig 3: Processing of TCW

3.8.1 Non-thermal treatment

Non-thermal technologies such as ultrasound, high-pressure processing (HPP) will shelf life of TCW. Most TCW industries use ineffective thermal treatments and preservatives for storage and processing. Therefore, it is essential to optimize treatment conditions to prevent the loss of heat-sensitive biochemical compounds. Non-thermal methods for preserving delicate, aromatic fruit juices include membrane filtration. Since the flavour of coconut water is temperature-sensitive, microfiltration (MF) may be able to pasteurize the product at room temperature while maintaining its fragrance. A few writers attempted to remove PPO, POD from coconut water by ultrafiltration right after microfiltration in order to avoid enzymatic browning.

"Pinking" of coconut water is prevented by using food grade resins include polyvinyl polypyrrolidone (PVPP), gelatin, and calcium bentonite, which are frequently utilized in the production of wine and beer. Although none of the resins that were examined were able to effectively control discolouration, more research is necessary to enhance the experimental design. A very new technique called continuous dense-phase CO2 (DPCD) was likewise failed to stop discolouration at room temperature.

Therefore, microfiltration seems to be a good method of stabilizing coconut water from a microbiological perspective, but it has no influence on enzyme activity because the enzymes are able to traverse the membrane. In ultrafiltration, PPO and POD enzymes are retained (Rajashri et al.,2022).

3.8.2 Thermal treatments

Sterilization, pasteurization and combined methods are some thermal treatments done in TCW. This will preserve the TCW and remove or kill the microorganisms present in it. Thermal treatment is defined by specific combination of temperature and time. It will enhance flavour, texture and sensory attributes. However, when transferring the laboratory treatments to an industrial setting, the combinations of treatments might not work as planned, particularly in terms of the quality of the finished output. This is mostly because of the variations in heat distribution consistency that occur during the upscaling process. The fact that numerous researchers have proposed different temperature (80–130℃) and time (8–900 s) combinations for the therapy of TCW (Naik, Sunil, Rawson, & Venkatachalapathy, 2020). Therefore, in order to replicate the results even on an industrial scale, it is essential to investigate the thermal distribution behaviour of the packaging material used to process TCW.

Main aim of thermal treatment is to reduce the growth of microbial growth in TCW. D-value can be determined based on time taken and temperature applied for the treatments. The D-value (decimal reduction time) is the time required to reduce the population of microorganisms by 90% (1 log10) at a specific temperature.

Other than microbial growth, there is a major problem encountered with TCW stabilization. Enzymes need to be inactivated to stabilize the colour and flavour. PPO and POD are present in TCW. A few minutes or a few hours after the nut is cracked, the coconut water may get discoloured in a yellow, brown, or pink manner. After storing processed coconut water for a few weeks, discoloration may also happen. Temperature, pH, mechanical effects, oxygen level, and other parameters all have an impact on the enzymes activity levels and are frequently hard to regulate. In order to mitigate the effects of PPO and POD activity in TCW, thermal inactivation can be achieved by pasteurization and sterilization (Manuel Dornier et al.,2012).

Sterilization

Sterilization is one of the most effective food preservation method. It is the process of eliminating all forms of microorganisms including bacteria, fungi, spores. Steam sterilization, also known as moist heat sterilization, uses pressurized steam to kill microorganisms by denaturing their proteins. Auto-clave is a moist heat sterilization method that uses pressurized steam to kill microorganisms and their spores. It involves the use of controlled heat and pressure to ensure safe and effective preservation while maintaining the quality of the product. It is faster than dry heat sterilization and is commonly done using an autoclave. The TCW samples are placed inside the autoclave. For a predetermined amount of time, the product is in direct contact with steam that is hot and pressurized. The high temperatures required to swiftly kill germs are achieved through the use of pressure. After sterilization, liquids are cooled slowly to prevent boiling over. Although sterilization is used to get rid of all microbes and heat-resistant spores, it typically results in significant product quality losses. The food industry continues to depend extensively on traditional sterilization methods, such as thermal sterilization, today. Developing TCW with a very long shelf life is one benefit of thermal sterilization. The nutritional content of TCW is lost during thermal sterilization, and the end goods colour, flavour, and other characteristics are altered (Xiang Li et al.,2016).

Pasteurization

In the food sector, it is one of the most popular thermal techniques. This heat method preserves the quality of food and beverages while eliminating dangerous bacteria. The process is heating the product to a particular time and temperature, then cooling rapidly. It will kill the vegetative cell of bacteria that cause food borne illness and this will increase the shelf life of product. For TCW, pasteurization will be in an autoclave at 90℃ ,1.5Bar for 10min. It is important to understand that not all organisms are completely eliminated by pasteurization (Davies, 1975); For example, the majority of bacterial spores will survive, though the extent to which pasteurization can lower their numbers depends on the organism's type and heat tolerance in addition to the original quantities.

Low-Temperature, Long-Time (LTLT): 63°C (145°F) for 30 minutes.

High-Temperature, Short-Time (HTST): 72°C (161°F) for 15-20 seconds.

Ultra-High Temperature (UHT): above 135°C (275°F) for 1-2 seconds, allowing for extended shelf life without refrigeration.

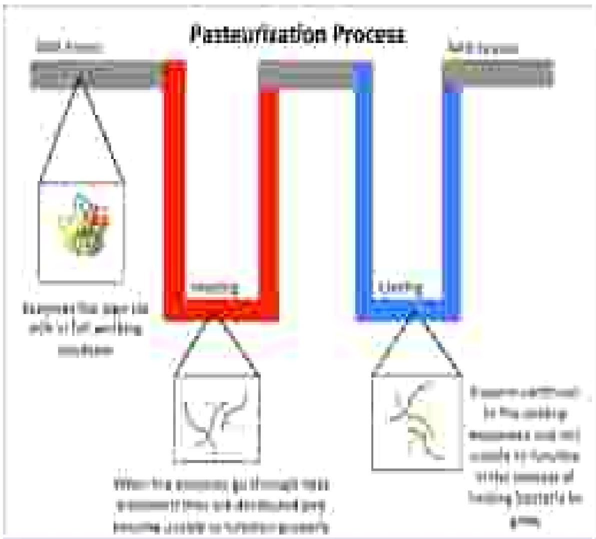


Fig 4 – Pasteurization process

It is the process of heating the entering product, usually with the holding tube's pasteurized product, final heating with steam or hot water to reach the pasteurization temperature; holding for a predetermined amount of time to provide the intended microbiological impact; and cooling the product with water or refrigerant.

The process consists of 4 stages, it includes:

- The incoming product is preheated using previously pasteurized product from the holding tube. This step helps reduce energy consumption by utilizing residual heat.
- The product is then heated to the pasteurization temperature using hot water or steam to ensure proper microbial inactivation.
- The product is held at the pasteurization temperature for a fixed time to achieve the desired microbiological effect, ensuring the elimination of harmful bacteria.
- After pasteurization, the product is rapidly cooled using water or a refrigerant to prevent further heat exposure and maintain quality.

Impact of pasteurization in tender coconut water

Pasteurization is a common thermal process applied to tender coconut water (TCW) to ensure its microbiological safety and extend shelf life. Typically, this involves heating the tender coconut water to temperatures below 90°C. While effective in inactivating harmful microorganisms and enzymes like peroxidase (POD) and polyphenol oxidase (PPO), which are responsible for undesirable changes such as browning and off-flavours, pasteurization can also impact the sensory and nutritional qualities of TCW. Studies have shown that heat treatments can lead to alterations in flavour, aroma, and nutrient content, potentially diminishing the fresh-like characteristics that consumers prefer. Consequently, there is growing interest in non-thermal preservation methods, such as microfiltration, ultrafiltration, and pulsed light treatment, which aim to maintain the natural sensory and nutritional attributes of TCW while ensuring its safety and extending shelf life (V. Prithviraj et al.2021).

Microbial Safety and Enzyme Inactivation :-

The primary purpose of pasteurization is to kill harmful bacteria, yeast, and moulds that could cause spoilage. It also inactivates enzymes like polyphenol oxidase (PPO) and peroxidase (POD), which contribute to browning and deterioration of flavour. This helps maintain the clarity and appearance of the coconut water for a longer time. Pasteurization effectively reduces microbial load in TCW. For instance, a study utilizing pulsed light (PL) treatment achieved significant reductions in *Escherichia coli*, *Bacillus cereus*, and *Listeria monocytogenes* populations.

Thermal pasteurization can inactivate enzymes like polyphenol oxidase (PPO) and peroxidase (POD), which are responsible for undesirable changes in TCW. Research indicates that PPO and POD have relatively high heat resistance, necessitating adequate thermal processing to ensure their inactivation. However, excessive heat can adversely affect the nutritional quality of TCW.

Nutritional Changes :-

Tender coconut water is rich in vitamins (like vitamin C), amino acids, and bioactive compounds. However, exposure to heat during pasteurization can degrade heat-sensitive nutrients, particularly vitamin C, leading to a reduction in its antioxidant properties. Minerals like potassium, sodium, and magnesium are more stable under heat, so their concentration is less affected.

Flavour and Aroma Alterations :-

The delicate, refreshing taste of fresh tender coconut water is due to volatile compounds such as aldehydes and esters. Pasteurization can cause a loss of these volatile compounds, leading to a slightly "cooked" or caramelized taste, which consumers may find less appealing (Joseph Adubofuor et al.,2016).

Shelf-life and quality of processed TCW

It is clear that non-thermal and thermal treatments are not sufficient to enhance shelf-life of TCW. Additives such as nisin want to be incorporated to preserve the TCW and this additive will increase shelf-life and quality of product. Then only pasteurized TCW can preserve for 2-3 months under ambient temperature.

Microfiltration did not prevent pink coloration in clarified coconut water. Pink coloration was observed in microfiltered coconut water from dwarf varieties but not from tall varieties when stored at ambient temperature. Discoloration did not occur in samples stored at 9-10°C immediately after processing. However, samples from dwarf varieties remained pink after cold storage at ambient temperature.

3.9 Nisin

Nisin is a one class I bacteriocin that is frequently used. Nisin was first made available for sale in England in 1953, and throughout the following decades, it was authorized for use in more than 48 nations. Nisin was deemed safe for use in food by the World Health Organization's (FAO/WHO) Joint Food and Agriculture Organization Expert Committee on Food in 1969. Nisin was added to the European list of food additives in 1983 with the number E234, and the US Food and Drug Administration (FDA) authorized its use in pasteurized goods.

Nisin is an antimicrobial peptide produced by the bacterium Lactococcus lactis. It is widely used in the food industry as a preservative due to its effectiveness against a range of bacteria, including gram-positive pathogens. It is a 34-amino acid polypeptide that contains odd amino acids like dehydroalanine and lanthionine. When used in tender coconut water, nisin offers a way to enhance its shelf life and safety while maintaining its natural characteristics. Tender coconut water, being nutrient-rich and slightly acidic, is highly susceptible to microbial spoilage, particularly from gram-positive bacteria and spore-formers like Clostridium and Bacillus. It is a preservative, is a chemical compound used to maintain the quality and freshness of food products. Bacteriocins are antimicrobial peptides produced by certain bacteria, usually as part of their natural defence mechanisms. They kill or inhibit the growth of other bacteria, often in a highly specific manner.

Temperature, substrate type, and pH all have a significant impact on nisin's solubility, stability, and biological activity. At thermal, it remains stable and can be autoclaved for 90°C for 10min. Gram-positive and Gram-negative bacteria, fungi, and viruses are among the many species that AMP (Anti-microbial propertites) have been shown to be effective against. Higher organisms use AMPs as a first line of defence against dangerous microorganisms and to support innate immunity mechanisms, and may rise in response to human inflammation and injury. Nisin is also a postbiotics. Postbiotics are metabolites or cellular components (non-living) derived from probiotics that confer health benefits to the host.

The current development of multi-drug-resistant bacteria has prompted to a greater seek for innovative treatment strategies. Bacteria are efficient because they release peptides known as bacteriocins. That are effective against viruses, fungi, and bacteria (Alexis, Kamel, & Raphel, 2020). These compounds, which are distinguished from antibiotics by their ribosomal synthesis, protein-like structure, and generation during the first growth phase, are well-known for their antibacterial qualities. Digestive enzymes are known to make a number of them non-toxic to humans when employed as preservatives. Their manufacturing processes and modes of action are different. Due to its strong particular action against harmful microorganisms, it is utilized as a natural preservative in the food business and as an antibacterial peptide in the pharmaceutical industry.

Bacteria produce peptides or proteins called bacteriocins, which either inhibit or kill other bacteria that are closely related. Bacteriocin's producer strain is naturally immune since it has immunity protein. Numerous characteristics, including the strain's development, specific defence and killing mechanisms, genetics, molecular weight, synthesis type, etc., are used to classify bacteriocins. Bacteria that are Gram-positive are classes I, II, and III for bacteriocin.

Both Gram-positive and Gram-negative bacteria produce bacterial AMPs, also known as bacteriocins. While eukaryotic AMPs require micromolar concentrations to function, bacteriocins are frequently quite strong, functioning at pico- to nanomolar concentrations. Food can contain bacteriocins in three different ways: (i) by inoculating it with lactic acid bacteria strains that produce bacteriocins; (ii) by adding purified or semi-purified bacteriocin; and (iii) by adding a fermented component that contains bacteriocinogenic bacterial strains. Nisin is the intensively studied and used bacteriocin to extend the shelf-life of products (Sumonsiri, N., 2019).

3.9.1 Mechanism of action

Nisin exhibits little to no activity against viruses, fungi, or Gram-negative bacteria, although it has antimicrobial effects against a wide range of Gram-positive bacteria and spore germination. It has antibacterial activity against Listeria monocytogenes, Staphylococcus aureus, and other harmful bacteria, including Lactobacillus acidophilus, Enterococcus faecalis, Streptococcus mutans, and Streptococcus sanguinis.

Nisin functions primarily through two mechanisms: interfering with the synthesis of cell walls in vegetative (active) cells and creating pores in cell membranes [**pore formation** in cell membranes and interference with **cell wall synthesis** in vegetative (active) cells]. In pore formation mechanism, nisin has both positively charged and hydrophobic (water-repelling) parts. It interacts with the cell membrane's negatively charged components, specifically with phosphate groups, via electrostatic interactions. Lipid II, a critical molecule involved in cell wall synthesis, serves as a "docking site" for nisin. This targeted binding facilitates pore formation in the bacterial membrane. The pores formed by nisin are small (2–2.5 nm in diameter), allowing essential small molecules (like potassium ions, ATP, and amino acids) to escape from the cell. This loss disrupts the cell's membrane potential, which is essential for energy and nutrient balance. As a result, the cell's barrier function breaks down, leading to a halt in cellular activities and eventually cell death.

When nisin interference with cell wall synthesis in vegetative cells, nisin also disrupts the synthesis of the cell wall, preventing bacteria from maintaining structural integrity. By targeting lipid II, it blocks the addition of building blocks to the cell wall, weakening the bacterial structure.

The outcome of combined effect of pore formation and disruption of cell wall synthesis results in rapid bacterial death. The mechanism of pore formation is similar to that of other antimicrobial peptides known as lantibiotics, such as lacticin 3147, subtilin, and epidermin. These lantibiotics share structural and functional similarities with nisin.

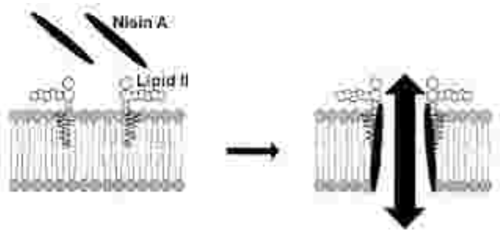


Fig 5 – Mechanism of nisin

Research on toxicology indicates that nisin has no negative effects on the human body. When taken orally, its LD50(Lethal Dose 50) was 6950 mg/kg, which is equal to table salt. Studies on the oral microbiota demonstrated that one minute following chocolate ingestion, saliva containing nisin showed only 1/40 of the original nisin activity. The US has approved it as the sole bacteriocin to be used as a preservative in a range of food items. The WHO recommends 33,000 international units (IU) (0.825 mg) per kilogram as the Acceptable Daily Intake (ADI), the maximum amount of additive that could be consumed daily without endangering the health of consumers, based on the "no effect" level seen in toxicological evaluations of animals and permitted for humans.

3.9.2 Application of nisin in food industry

The necessity for food safety is more widely recognized in society as a result of the numerous health issues caused by food additives. It is applicable in wide range of solids and liquid food item, can be stored at ambient and cold temperature. Because bacteriocin is derived from a natural source, using it in this circumstance is harmless. It's utilized in the food industry in a number of ways, including additives, food packaging, and more (Rogers, 1928; Yang, Lin, Sung, & Fang, 2014). The safety and effectiveness of a number of bacteriocins that have been expressly permitted for use in food are presently being closely examined (Bisht, Das et al., 2024).

In foods including coconut water, tender coconut water, cheese, butter, canned alcoholic beverages, sausages, pasteurized liquid eggs, and salad dressings, among others, nisin is used as a natural preservative by itself.

Nisin for food preservation

Nisin, the first antimicrobial peptide identified in LAB, is released by Lactococcus lactis during food processing. A food preservative called Nisaplin® is used to stop microbiological diseases. Unlike synthetic preservatives, it does not significantly alter the taste or texture of food, making it an attractive natural alternative. However, its activity is pH-dependent and primarily targets Gram-positive bacteria, which may require combination with other hurdles such as organic acids or high-pressure processing for broader protection (Cotter et al., 2005). Despite these limitations, nisin remains one of the most studied and commercially used bacteriocins in food preservation due to its efficacy and consumer acceptance [Delves-Broughton, J. et al.,2005].

Because the bacteriocin can be better or more uniformly disseminated in liquid or homogenous foods, nisin functions better in them than in solid or heterogeneous items. Because fat is hydrophobic, it may prevent food from being distributed or make it unavailable for use (Jung et al., 1992).

3.9.3 Impact of nisin in tender coconut water

Mahindru 2002 reported that nisin is allowed up to 5000 U.I in prepared coconut water. Nisin will act as a preservative against different pathogenic and spoilage microorganisms. Nisin will be stable at coconut water at room temperature for at-least 30 days of storage [Adelson Alves de Oliveira Junior].

Nisin did not significantly alter the physical or chemical properties of TCW, suggesting minimal impact on the taste, colour, or texture of tender coconut water. Nisin was effective in reducing bacterial contamination in fruit juices, particularly against Alicyclobacillus acidoterrestris, Bacillus cereus, and Staphylococcus aureus. If applied to tender coconut water, nisin could help control microbial growth, extending shelf life. Nisin helped preserve vitamin C, which suggests it could help retain the natural nutrients in tender coconut water (Adelson et al.,2015).

The effectiveness of nisin in tender coconut water (TCW) depends on whether it is added before or after pasteurization. When added before pasteurization, nisin enhances microbial inactivation by weakening bacterial cells, allowing for a lower pasteurization temperature, which helps preserve heat-sensitive nutrients and flavour. However, high temperatures may partially degrade nisin, reducing its antimicrobial effectiveness. Additionally, while it improves bacterial kill rates, it may not be sufficient to eliminate heat-resistant spores like *Bacillus* spp. On the other hand, adding nisin after pasteurization ensures its full antimicrobial activity, preventing post-processing contamination and extending shelf life. This approach is particularly useful in controlling spoilage bacteria that may survive or re-contaminate TCW after heat treatment.

3.10 Sucralose

Sucralose is an artificial sweetener. It is about 600 times sweet as sugar but zero calories. Sucralose is a high potent sweetener used in food industries. Sucralose is made from sucrose by a chemical procedure, which will enhance sweetness, maintain a pleasant sugar like taste. It is a highly versatile sweetener that can be used in different food and drinks in a permissible rate.

However, its sweetness can vary based on factors like temperature, acidity (pH), how sweet the final product should be, and the presence of other ingredients. To match the sweetness of sugar (sucrose) in a food or drink, only a tiny amount of sucralose is needed. For example, to make a beverage as sweet as one containing 9–10% sugar (which is similar to many soft drinks), you only need about 200 mg (0.2 g) of sucralose per litre instead of 90–100 g of sugar per litre.

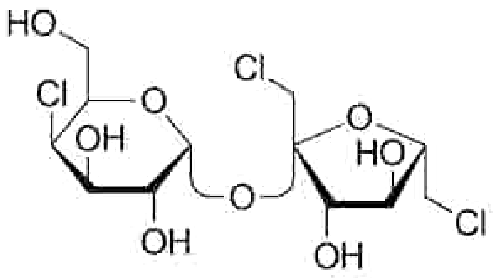


Fig 6 - Sucralose

APPLICATIONS

Sucralose is a highly versatile sweetener that may be used in a wide variety of culinary applications due to its sweetness quality and exceptional durability. Sucralose is stable across a broad pH range and resistant to high food temperatures.

Beverage Industry

One of the largest area use of sucralose is in soft drinks. It is 600 times sweetener as sugar. Sucralose is very stable and provides high-quality sweetness, so it can be used alone or blended with other sweeteners to adjust flavour. It also works well with sugar to create great-tasting, reduced-sugar drinks, allowing up to 30–40% sugar reduction without affecting taste.

It dissolves easily in water, sucralose can be quickly mixed without acid and does not cause foaming. It remains stable in low-pH and heat-treated drinks like pasteurized or UHT beverages. Compared to other sweeteners like acesulfame K and aspartame, sucralose is one of the most stable options for beverages.

Table 7: Usage level of sucralose for beverages

BEVERAGES	USE LEVEL (%)
Carbonated soft drinks	0.015-0.022
Flavoured waters	0.008-0.015
Flavoured drink mixes	1.500-3.500
Isotonic drinks	0.010-0.020
Still beverages	0.012-0.018

[Source: Samuel V. Molinary et al.,2012]

SAFETY AND REGULATORY STATUS

Government and international organizations evaluated sucrose's safety as a non-caloric sweetener. Based on long-term research, the Joint Expert Commission for Food Additives (JECFA) established an acceptable daily intake (ADI) of 0–15 mg/kg body weight per day. Despite not being a regulatory authority, JECFA's recommendations helped sucralose gain approval in more than 100 nations, including the US and the EU. Sucralose was also accepted by the Codex Alimentarius, which enabled the food, beverage, and pharmaceutical sectors to produce more delicious, high quality low-sugar goods for consumers (Molinary, S. V et al., 2012).

Chapter-4

MATERIALS AND METHODS

4.1 Raw Material

Fresh tender coconut was purchased from the local market (Mysore, Karnataka, India) just before the experimental trials. Nisin (Bacteriocin) procured from liable sources. Tender coconut with good quality and without any disease infected were selected for the process. Water from tender coconut was extracted before the sterilization process. Muslin cloth and sieve were used to remove the solid particles from the extracted tender coconut water (TCW).

To the filtered TCW, 0.0 1% nisin and sucralose was added. Then it was portioned into 200ml sterile glass & PET bottles. The sample bottles were pasteurized in a pilot scale autoclave with specified parameters that includes 2 bar pressure for 5 to 10mins.

Fig 7: Tender Coconut

NISIN: Nisin is a polypeptide which can be used as a preservative in TCW against Gram-positive bacteria, including Clostridium botulinum and B. cereus, as well as some bacterial spores. It can be produced by Lactococcus lactis subspecies lactis. In India, nisin is allowed up to 125ppm in TCW.

SUCRALOSE: Sucralose is an artificial sweetener. It is about 600 times sweet as sugar but zero calories. TCW can be made sweeter without adding calories by adding sucralose, which appeals to consumers who are health-conscious.

4.2 OPTIMISATION STUDIES

Trials and optimisation were done to determine the stability of TCW.

4.2.1 Composition of TCW with Gums

TCW along with nisin, intense sweetener, thickeners like guar gum and gum acacia were incorporated into it. Sweetener amount was calculated and added based on Total Soluble Solids (TSS) of TCW.

Table 8– Composition of TCW with gums

Ingredients	Fresh	Without additives	With additives
TCW	1L	1L	1L
Nisin	-	-	0.001%
Sweetener	-	-	3.98×10^{-6} %
Gum acacia	-	-	0.04%
Guar gum	-	-	0.04%

TCW made with gum were hot-filled and autoclaved at 90°C in glass bottle and PET bottle. This formulation was rejected because of the presence of microbial growth, which showed low stability at room temperature.

Fig 8- Hot-filling process

Fig 9- Autoclave

4.2.2 Composition of TCW with citric acid

Table 9 – Composition of TCW with citric acid

Ingredients	Fresh	Without nisin	With Nisin
TCW	1L	1L	1L
Nisin	-	-	0.2%
Bulk Sweetener	-	-	3.98×10^{-6} %
Citric acid	-	-	0.5%

TCW made with citric acid were hot-filled and autoclaved at 90°C**.** This formulation was rejected due to the presence of pink colour which indicates oxidation and enzymatic activity**.**

Fig 10: TCW samples with citric acid (a) Autoclaved TCW (b) Hot-filled TCW

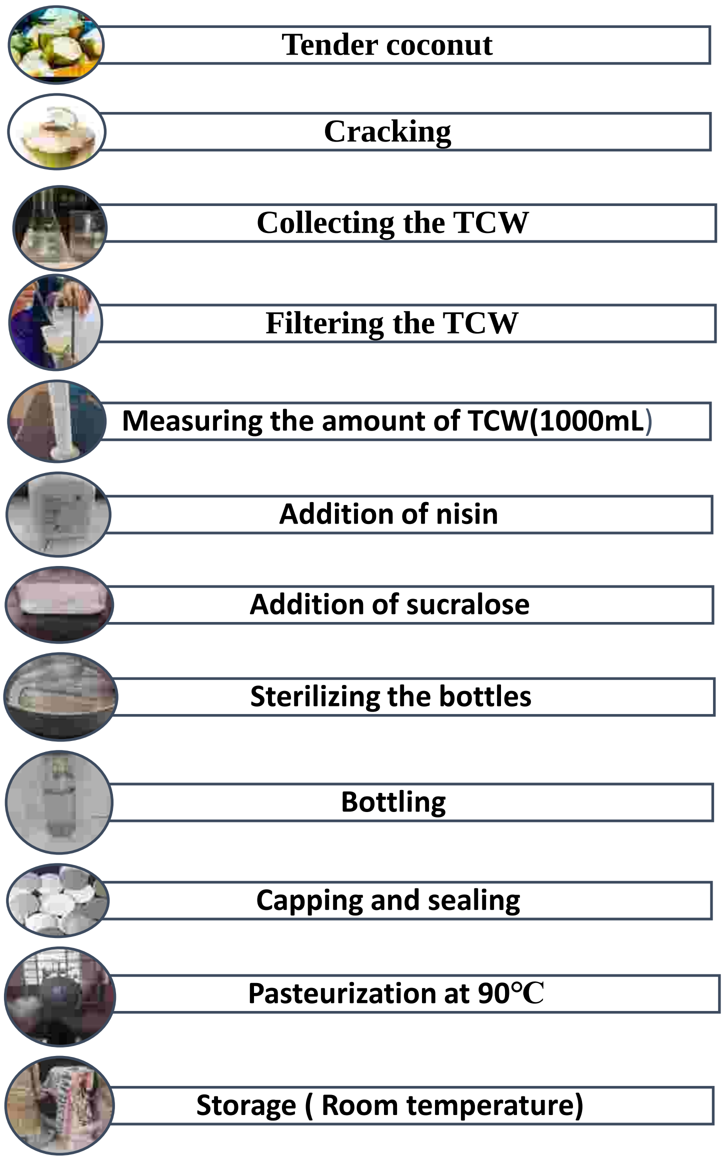
4.2.3 Standardized Formulation of TCW [1 L]

TCW with nisin shows highest storage stability at room temperature. Therefore, it was chosen as the best composition for pasteurization of TCW.

Table 10– Composition of TCW with gums

Ingredients	Fresh	Without Nisin	With Nisin
TCW	1L	1L	1L
Nisin	-	-	0.001%
Sweetener	-	-	3.98×10^{-6} %

Table 11 – Flow chart of processing of TCW



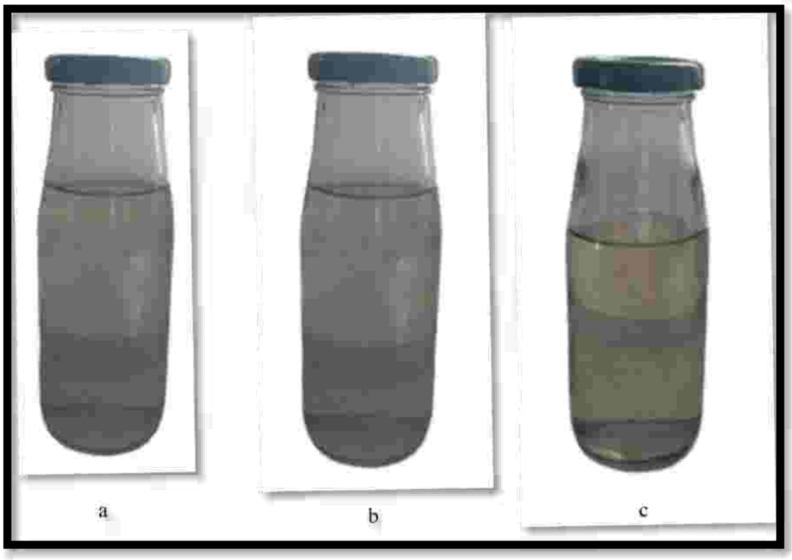


Fig 11 - Samples of TCW in glass bottle (a)Fresh TCW; (b) Without nisin; (c) Nisin TCW

(a) (b) (c)

Fig 12 - Samples of TCW in PET bottle (a)Fresh TCW; (b) Without nisin; (c) Nisin TCW

4.3 PHYSICOCHEMICAL PROPERTIES

4.3.1 Determination of pH

pH is essential for quality control and product development in beverage production [Andre'es-Bello et al., 2013]. pH meter is a statistical tool that monitors the hydrogen-ion activity in water-based samples. pH measures the acidity or alkalinity of solutions. Samples with a pH higher than 7.0 is considered as basic and above 7.0 is considered as acidic. A pH of 7.0 is neutral. A pH electrode connected to a pH meter was used to measure the pH of TCW. A well calibrated pH meter was used for pH assessment (Hanna instrument, USA) (Raj Kumar Maguluri et al., 2023). The electrode had been immersed in the thoroughly mixed TCW samples placed in a beaker, and after it stabilized, the value was recorded.

Fig 13 – pH Meter

4.3.2 Determination of TOTAL SOLUBLE SOLIDS

Total Soluble Solids (TSS) is defined as the amount of dissolved substances in a liquid, including sugars, acids, proteins and minerals. It is expressed as % Brix was determined by a digital source refractometer (Hanna instrument, USA). It will show the º Brix. TSS are measured by placing a drop of TCW on the prism of refractometer, and it displays the value within 2 seconds. A refractometer measures a liquid's refractive index for determining characteristics like sugar content (Brix).

Fig 14 – Refractometer

4.3.3 Determination of Titratable Acidity

The titratable acidity (TA) of a solution is a measure of its total acid content. The TA of fresh TCW, without nisin, and nisin-treated TCW was determined using the titration method. A 10 mL sample was transferred to a 250 mL conical flask and diluted to 90 mL, followed by the addition of 3–4 drops of phenolphthalein as an indicator. The mixture was then titrated with 0.1N NaOH until a pale pink colour appeared (AOAC 962.12) (AOAC, 1998) (Thuan-Chew Tan et al.,2014). TA was expressed as malic acid (W/V) using the following equation;

$$\textbf{Titratable Acidity (\%)} = V \times N \times M \times 100 / V \times 1000$$

where, V is the volume of NaOH

N is the Normality of NaOH

M is the malic acid factor (67.05)

V is the volume of sample taken

Fig 15 - Titration

4.3.4 Determination of Ascorbic Acid

The estimation of ascorbic acid (Vitamin C) using the metaphosphoric acid method involves a titration technique with 2,6-dichlorophenolindophenol (DCPIP) dye as the titrant. In this method, DCPIP, which appears blue in an alkaline solution and red in an acidic solution, is reduced by ascorbic acid to a colourless form.

To prepare the standard ascorbic acid solution, a known quantity of pure ascorbic acid (10 mg) is dissolved in 100 mL of a 3% metaphosphoric acid solution, yielding a concentration of 100 µg/mL. A series of dilutions are then prepared to obtain concentrations of 10 µg/mL, 20 µg/mL, 30 µg/mL, 40 µg/mL, and 50 µg/mL. For each dilution, 5 mL of the solution is transferred into separate conical flasks and titrated with DCPIP solution until a faint pink colour persists for 15 seconds. The volume of DCPIP required for titration is recorded for each standard, as it is directly proportional to the ascorbic acid concentration.

For the sample, 10 mL of tender coconut water is collected and mixed with a metaphosphoric acid solution in a 1:4 dilution ratio (2 mL of metaphosphoric acid with 8 mL of coconut water). For titration, 5 mL of the prepared sample is transferred into a conical flask and titrated with DCPIP solution until a faint pink colour persists for 15 seconds (Ranganna, 1986). Ascorbic acid is calculated using the following equation:

$$\textbf{Ascorbic acid (mg/100mL)} = C \times Vs/Vt \times 100/W$$

Where, C= concentration of ascorbic acid from curve (mg/mL)

Vs= Volume of DCPIP used for the samples (mL)

Vt=Volume of DCPIP used for the standard solution with a known concentration.

W= Volume of the sample (mL)

4.2.4 Determination of Total Sugars

The total sugar content was determined using the phenol-sulfuric acid method. A working solution (0.1 mg/mL) was prepared by diluting the stock solution (1 mg/mL) to obtain final concentrations of 0.2, 0.4, 0.6, 0.8, and 1.0 mg/mL in Milli-Q water, making up a total volume of 1 ml. To each sample, 1 mL of 5% phenol solution was added, followed by the addition of 5 mL of 96% concentrated sulfuric acid directly onto the liquid surface to ensure proper mixing. The mixture was then incubated for 10 minutes, allowing for the development of a pale-yellow colour. The absorbance was measured at 490 nm using a spectrophotometer to determine the total sugar concentration. The calibration curve developed with standard glucose was used to compute total sugar ((Dubios et al., 1956).

4.4 ANTIOXIDANT PROPERTIES

4.4.1 Determination of Total Phenolic Content (TPC)

Principle

The total phenolic content (TPC) assay is widely used to quantify the phenolic compounds in a sample. The principle involves a Folin-Ciocalteu (FC) reagent that will react with phenolic compound to produce a colour change. It can be measured by spectrophotometry. FC reagents contain phosphomolybdic-phosphotungstic acid complex. Under alkaline condition phenolic compound, phenolic compound in the sample will get reduced. This results in blue coloured complex whose intensity is proportional to the concentration of phenolic compounds.

Procedure

With some modifications, the Folin-Ciocalteu method was used to calculate the total phenolic content (TPC) of tender coconut water (Rajkumar,2024). A 0.5 mL sample of TCW was placed in a test tube, followed by the addition of 2.5 mL of distilled water, 0.5 mL of Folin & Ciocalteu's phenol reagent. The mixture was incubated for 5 minutes before adding 0.7 mL of 7.5% (W/V) sodium carbonate. The mixture was then incubated for 30 minutes at room temperature. Absorbance was measured at a wavelength of 765 nm using a UV-Visible spectrophotometer (Maguluri, R. K et al., 2023).

Gallic acid was used as the standard, and the 0.1 mg/mL working standard solution was used to prepare aqueous gallic acid standard solutions (10, 20, 40, 60, and 80 mg/L), which provided appropriate concentrations for the standard curve. Prior to absorbance measurement at 765 nm, all of the stock standard solutions were treated the same as the TCW samples. Gallic acid equivalents (GAE) were used to express TPC, with units in mg/L (mg GAE/L).

4.4.2 Determination of Radical Scavenging Activity (DPPH)

Principle

The DPPH method is commonly used in antioxidant studies to measure how well a sample can neutralize free radicals. It is done by using DPPH(2,2-diphenyl-1-picrylhydrazine). DPPH, a synthetic stable free radical, appears as a purple solution. The compound's antioxidants scavenge the free radical, resulting in reduction in colour intensity. Since gallic acid is a naturally occurring polyphenol with potent antioxidant and radical scavenging properties.

Procedure

Gallic acid is used as the standard. 10 ug/ml gallic acid solution and 0.1mM DPPH solution were prepared. A diluted sample was made for the experiment with different concentrations. These samples were then mixed with a DPPH methanol solution and vortexed vigorously. They were then let to rest at room temperature in the dark for 10 minutes to allow for the reaction. The absorbance was measured at 517nm against a blank using spectrophotometer.

Radical scavenging activity (%) = (Ao – A1)/ Ao ×100

Where, Ao is the absorbance of the control

A1 is the absorbance of the sample

Fig 16 - Spectrophotometer

4.5 Turbidity and Transmittance

Turbidity was determined by using a spectrophotometer (Shimadzu) by wavelength of 610nm using a transparent cuvette (Campos et al., 1996). The turbidity was measured at an interval of 15 days until the end of 30 days storage period. Absorbance of the sample was read in relation to distilled water and the transmittance and respective turbidity were calculated by the following equation:

Transmittance (T)=100 × (10 -Abs)

Where, Abs is the adsorption at wavelength of 610nm.

Turbidity = 100 – T

T is the transmittance at wavelength of 610nm.

4.6 Colour Analysis

Colourimetry was performed using a spectrophotometer according to the CIE L*a*b*scale. The instrument was calibrated prior to use. The colour measurement resulted in CIE L*a*b* values for lightness (L*, L = 100 is white and L = 0 is black), redness (a*, + red to green component) and yellowness (b*, + yellow to blue component). The evaluation of the colour of the coconut water was determined using colorimeter. The coconut water was pipetted into a quartz cell. The D65 illuminant was used for calibration, and a 10-mm optical path recorded the parameters.

Fig 17 - Colorimeter

4.7 Microbial Studies on processed TCW

4.7.1 Total Plate Count Agar (TPCA)

Total Plate Count Agar, also referred to as Standard Plate Count (SPC) Agar, is a general-purpose medium used to estimate the total viable count in food products.

TPC agar is used to examine the viable microorganisms in a TCW sample. To prepare the media for Total Plate Count (TPC), dissolve 6g of Plate Count Agar in 200mL distilled water, mix well, and autoclave at 121°C for 15 minutes. After autoclaving, pipette 1mL of diluted sample (10^{1}) to the sterile petri dish and pour the warm media into it and allow it to solidify. Incubate the plates at 37°C for 24 hours, then examine, count the colonies, and record results in CFU/mL.

Composition of TPCA

1. Peptone: Provide nitrogen and essential growth factors for bacterial growth.
2. Agar: Solidifying agent that support the growth of bacteria
3. Salt: Maintain the pH and osmotic balance of the medium.
4. Neutral pH: Support the growth of a wide variety of bacteria without inhibiting any particular group (Standard AOAC).

4.7.2 Violet Red Bile Agar (VRBA)

VRBA is a selective medium used to detect and enumerate Coliform bacteria, especially Escherichia coli in food and water sample. It is designed to inhibit the growth of gram-positive bacteria while promoting the growth of coliforms.

VRBA is used to identify the coliform bacteria in the sample. Prepare media by dissolving 8.30g of agar in 200mL of distilled water and autoclave at 121°C for 15 minutes. Dilute 100 µL of the sample in 900 µL of sterile distilled water and performed serial dilutions. After autoclaving, pour the warm media into sterile Petri dishes and pipette 100 µL of sample on the agar surface. Then the samples are spread evenly with the use of a sterile L-shaped rod and allow it to solidify. Incubate the plates at 37°C for 24 hours, then examine, count the colonies, and record results in CFU/mL (Standard AOAC).

4.7.3 Dichloran Rose Bengal Chloramphenicol Agar (DRBCA)

DRBC agar is a specialized medium designed for counting yeasts and moulds in food and environmental samples. It restricts the growth of rapidly spreading fungi, ensuring clearer colony separation for more precise enumeration.

DRBC (Dichloran Rose Bengal Chloramphenicol Agar**)** is used to detect the fungi (yeast, mould) in the sample**.** To prepare the media for DRBCA, dissolve 6.32g of Plate Count Agar in 200mL distilled water, mix well, and autoclave at 121°C (15 psi pressure) for 15 minutes. It was cooled, mixed well, poured in petri plates and allow it to solidify. Pipette 0.1mL of diluted sample (10^1) to the sterile petri dish and and with the help of spreader the sample was spread and kept for incubation. Incubate the plates at 37°C for 48 hours, then examine, count the colonies, and record results in CFU/mL (Standard AOAC).

The Colony Forming Units (CFU) per mL is calculated using the formula:

$$\mathbf{CFU/mL} = \frac{\textbf{Number of colonies}}{\textbf{Dilution} \times \textbf{Volume plated}}$$

Fig 18- Media

4.8 Sensory Evaluation

Ten randomly chosen untrained panelists were used to sensory evaluate samples for consumer acceptance and preference. According to the standard proforma, the samples' appearance, flavour, and general acceptability were evaluated using a 9-point Hedonic scale, where 9 and 1 stand for "like extremely" and "dislike extremely," respectively. Sensory evaluation was carried out at ambient conditions in a comfortable and quiet area without disturbance under fluorescent lighting. Water was supplied to cleanse palate between samples.

9	Like extremely
8	Like very much
7	Like moderately
6	Like slightly
5	Neither like nor dislike
4	Dislike slightly
3	Dislike moderately
2	Dislike very much
1	Dislike extremely

Chapter-5

RESULT AND DISCUSSION

5.1 Physicochemical Properties

Physio-chemical properties of TCW samples in PET, Glass bottles were analysed. TCW sample with nisin and without nisin were pasteurized at 90℃ in an autoclave.

Table 12: Physio-chemical properties of Fresh, without and nisin treated tender coconut water samples in PET, Glass bottles.

Properties			**Pasteurized@90℃**			
	Fresh TCW		**Without nisin**		**With-nisin**	
	PET	**Glass**	**PET**	**Glass**	**PET**	**Glass**
pH	4.7	4.5	4.8	4.7	4.82	4.7
Titratable acidity (%)	0.067±0.0	0.065±0.05	0.070 ±0.05	0.074±0.0	0.078±0.05	0.075±0.05
TSS (º Brix)	4.5	4.7	4.6	4.7	4.7	4.8
Total sugars (g/100mL)	4.5	4.3	4.6	4.83	5.6	5.9
Ascorbic acid(mg/100mL)	5.5	5.5	3	3.6	2.5	2.9

5.1.1 Determination of pH

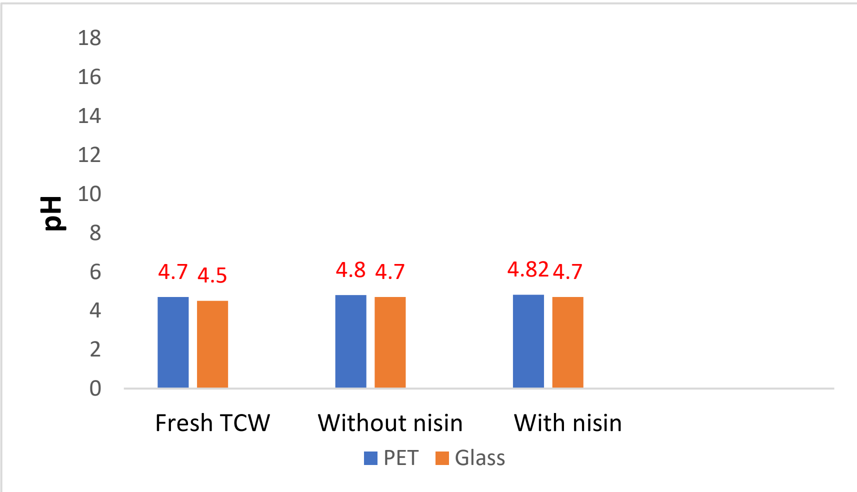


Fig 19- pH level in TCW samples

A pH of 7 is neutral, below 7 are acidic and above 7 are basic. The pH has a significant impact on processes like protein denaturation, enzyme activity, and the kinetics of microbial inactivation. All the TCW samples have almost same pH range. Pasteurization did not affect the pH of TCW in PET, glass bottles. For the PET bottles, pH ranges from 4.7 to 4.82 and for glass bottle it is from 4.5 to 4.7.

5.2.1 Determination of Total Soluble Solids

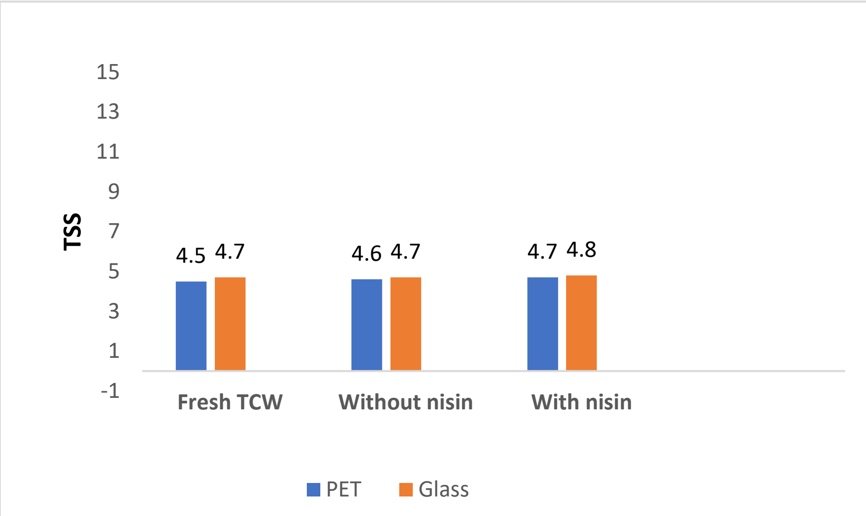


Fig 20- TSS of TCW samples

Total Soluble Solids (TSS) is defined as the amount of dissolved substances in a liquid, including sugars, acids, proteins and minerals. TSS indicates the sweetness of TCW. It is measured in degrees brix using a refractometer. Pasteurized TCW (without nisin and with nisin) have more TSS than fresh sample. This is attributed by pasteurization process, where breakdown of polysaccharides causes, increase in sugar content. In glass bottles, TSS ranges from 4.7 to 4.8, whereas for PET bottles, it is from 4.5 to 4.7.

5.1.3 Determination of Titratable Acidity (TA)

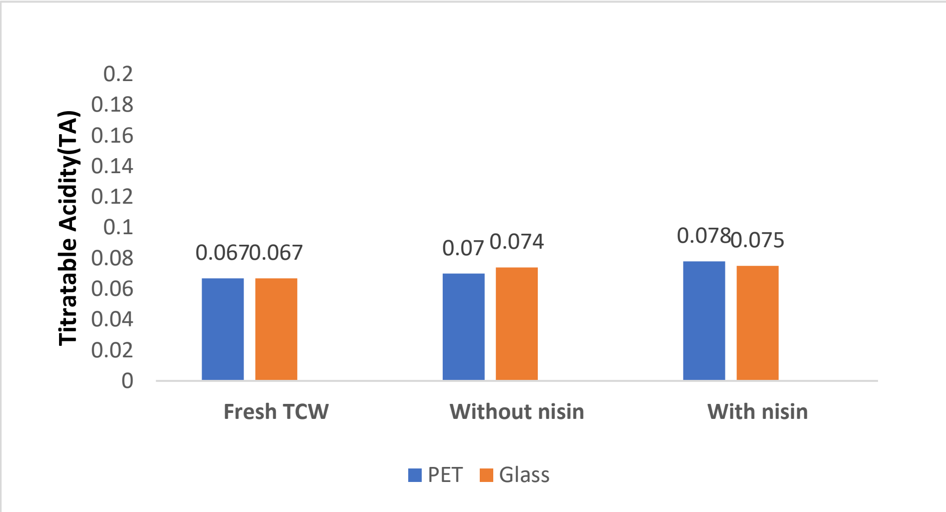


Fig 21 – Titratable acidity of TCW samples

Titrable acidity, which is represented as a percentage of malic acid in tender coconut water, quantifies the overall amount of acids in a liquid (Santoso et al ., 2009). TA depends on various factors like storage condition, maturity and processing. TA of TCW samples in different condition was found to be in the range of 0.067 to 0.078. In both bottles (PET, Glass), there is not much significant difference in TA of the samples.

5.1.4 Determination of Ascorbic Acid

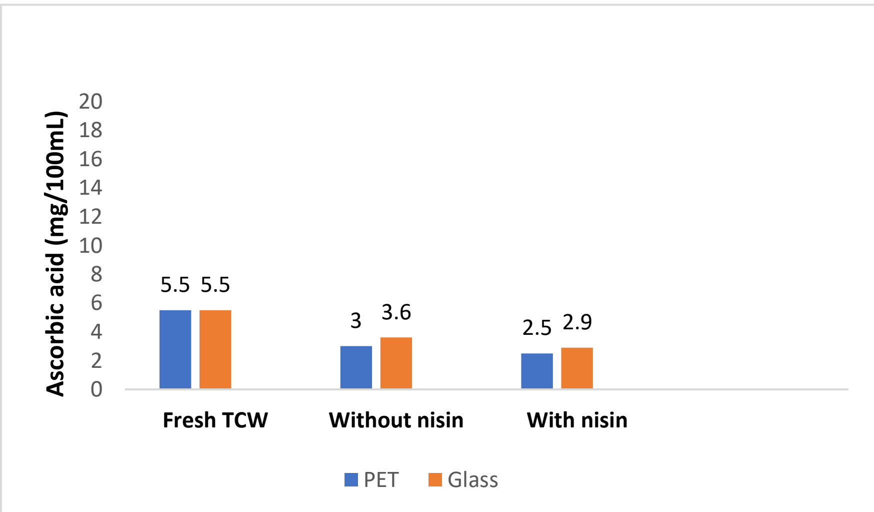


Fig 22 – Ascorbic acid of TCW samples

Ascorbic acid (vitamin C) is a key antioxidant in tender coconut water (TCW), and its levels can be affected by processing, storage, and preservation treatments. Ascorbic acid is a heat-sensitive bioactive compound that plays a vital role in human health and can act as an antioxidant. The highest ascorbic acid content is observed in fresh tender coconut water. This is expected because vitamin C is highly sensitive to oxidation and degradation over time. The results indicate that fresh tender coconut water has the highest ascorbic acid content, while both varied conditions that is without nisin and with nisin samples exhibit a slight decrease due to processing/storage effects.

Heating causes ascorbic acid (vitamin C) to break down when exposed to oxygen because it is sensitive to heat. Additionally, ascorbic acid can degrade due to free hydroxyl radicals formed through light-driven chemical reactions linked to oxidation. Similar findings were reported by Goh et al. (2012). Higher temperatures during heating speed up the loss of ascorbic acid. Compared to PET bottles, glass bottle, reduction is slower because they are more robust under temperature fluctuation and minimizing ascorbic acid degradation.

5.1.5 TOTAL SUGARS

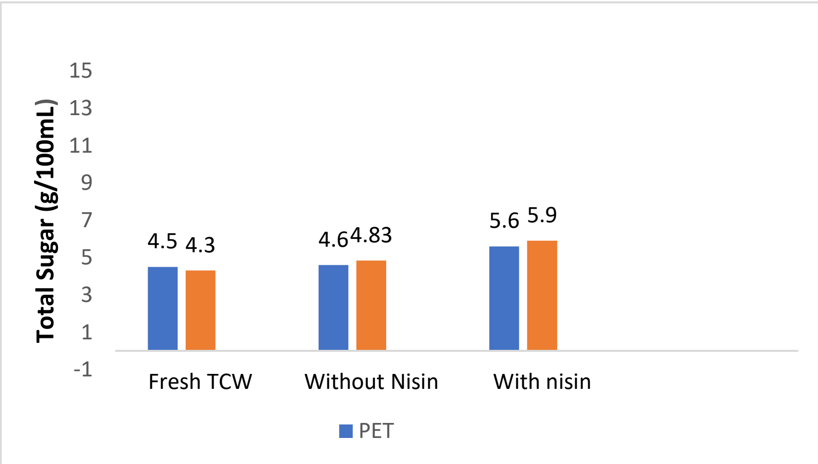


Fig 23-Total sugar in TCW samples

The primary sugars that contribute to the sweet taste in tender coconut water are fructose, glucose, and sucrose. These sugars are naturally present in tender coconut water and are responsible for its mildly sweet flavour.

The results indicate that the nisin treated TCW had the highest value for Glass and PET bottle which is 5.9 g/100mL 5.6 g/100mL.During pasteurization the added sucralose will dissolve completely and increase the sweetness in tender coconut water.

Total sugar for PET bottle ranges from 4.5 g/100mL for fresh tender coconut and for glass is 4.3g/100mL. After pasteurization of tender coconut water there is not much difference in total sugar content. For without nisin pasteurized TCW have 4.6 and 4.83 g/100mL sugar.

5.2 Determination of Antioxidant Activity

Table 13: Antioxidant assay of TCW samples in Glass, PET Bottles

SAMPLES	ANTIOXIDANT PROPERTIES			
	TPC (mg/L)		RSA (%)	
	PET	Glass	PET	Glass
Fresh TCW	11.34	17.104	72	73.38
Without Nisin	11.5	18.493	74.5	75.47
With Nisin	11.9	20.92	71.8	74.08

5.2.1 Total Phenolic Content

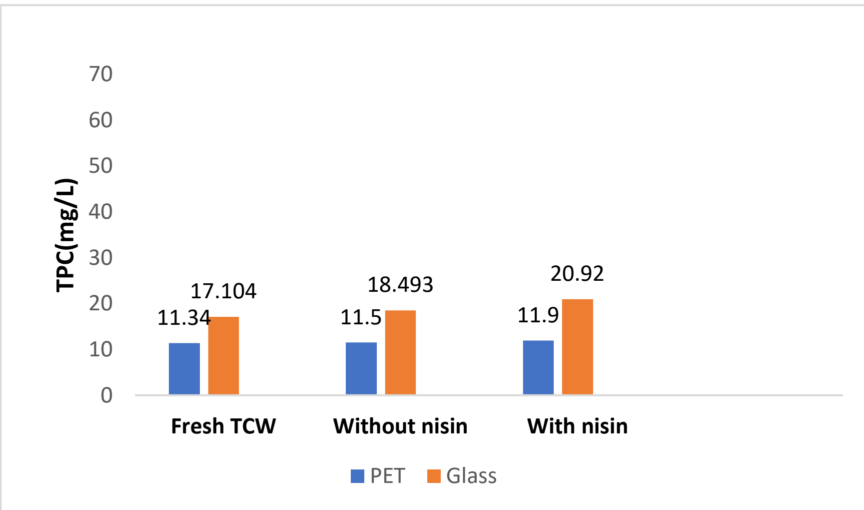


Fig 24- Total Phenolic Content in TCW samples

Phenolic compounds are beneficial compounds found in plants. Mostly due to their antioxidant properties, they have been implicated to a decrease in degenerative diseases in humans.

With the addition of nisin to TCW, total phenolic content is little more than fresh TCW in both glass and PET bottles. In glass, polyphenolic content ranges from17 to 20.9 mg/L, while in PET bottles it ranges from 11.34 to 11.9 mg/L. Nisin enhance antioxidant stability by preventing microbial spoilage, which helps retain phenolic compound. Not disturbing the polyphenolic content. So polyphenols are good.

While comparing the glass and PET bottle, values are different because glass is a non-reactive material so it does not interact with the phenolic compound. While PET bottle will have slight interaction during heat and storage condition, so degradation occurs in phenolic content.

5.2.2 DPPH

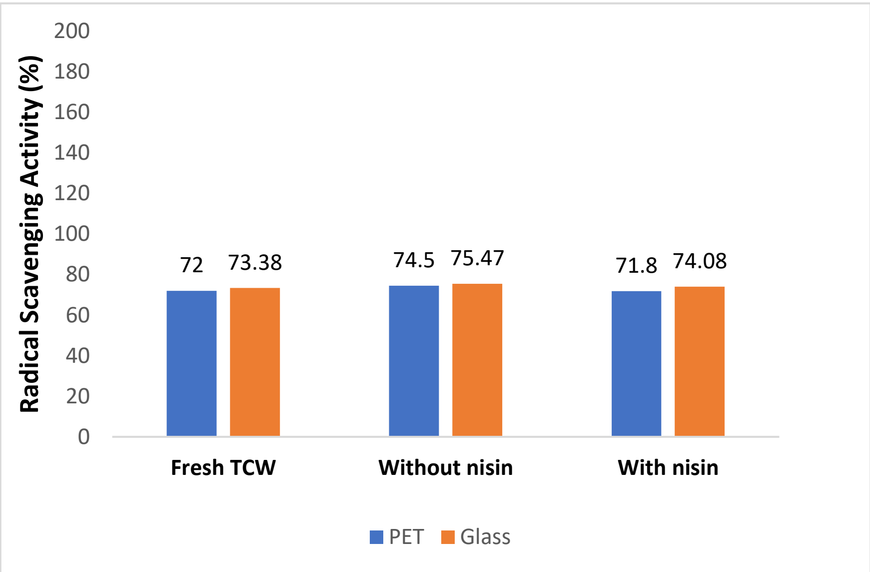


Fig 25 – RSA in Samples

2,2-Diphenyl-1-picrylhydrazyl, or DPPH, is a chemical molecule that is frequently used to determine the antioxidant activity of a variety of substances, including tender coconut water. The color of this stable free radical is deep purple. The effectiveness of antioxidants was tested by checking how well they could donate hydrogen atoms to DPPH, a stable free radical. According to (Soare et al., 1997), DPPH becomes stable by gaining an electron or a hydrogen atom. The antioxidant power of the sample was measured by the decrease in absorbance at 517 nm. A lower absorbance means the sample has a stronger ability to neutralize free radicals. Without nisin shows the greatest ability to scavenge radicals among them. Scavenging capacity for fresh TCW is (73.38%) and for the nisin pasteurized TCW have (72.20%). Fresh TCW, which is ranked slightly lower than without nisin, demonstrated a noteworthy ability to scavenge radicals. For glass and PET bottle, radical scavenging activity was similar. Both bottles help to preserve the antioxidant activity in TCW samples.

5.3 Turbidity and Transmittance

Table 14: Turbidity in fresh, control and nisin treated tender coconut water in Glass, PET bottle.

		Fresh TCW		Without nisin		With nisin	
		PET	GLASS	PET	GLASS	PET	GLASS
TURBIDITY	0th Day	0.156±0.0	0.156±0.0	0.166±0.0	0.168±0.0	0.166±0.0	0.112±0.0
	15th Day	0.366±0.0	0.308±0.0	0.191±0.0	0.183±0.0	0.198±0.0	0.149±0.0
	30th Day	0.456±0.0	0.482±0.0	0.550±0.0	0.282±0.0	0.439±0.0	0.192±0.0
TRANASMITTANCE	0th Day	69.89±0.0	69.89±0.0	68.89±0.0	67.92±0.0	68.07±0.0	77.26±0.0
	15th Day	43.05±0.0	49.02±0.0	64.4±0.0	65.6±0.0	63.38±0.0	70.95±0.0
	30th Day	34.99±0.0	32.96±0.0	28.1±0.0	52.2±0.0	36.3±0.0	64.26±0.0

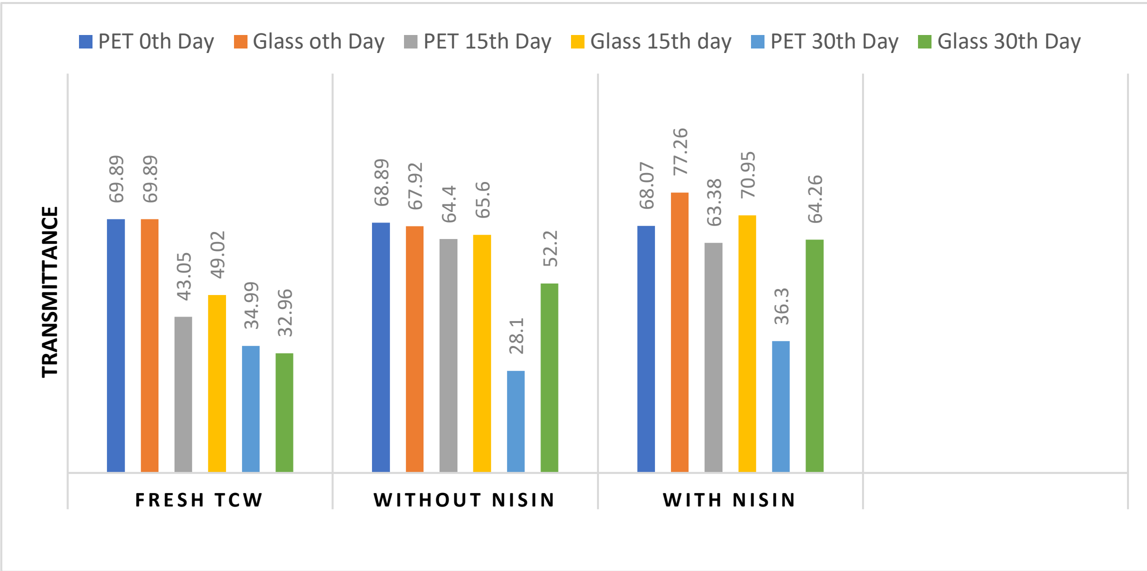


Fig 26–Transmittance in TCW Samples

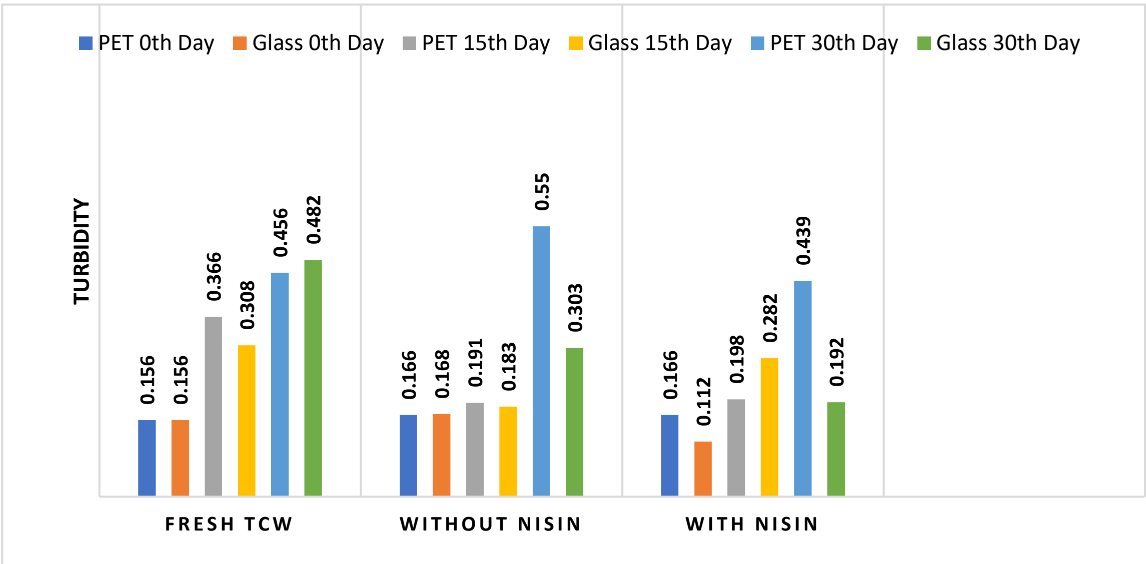


Fig 27 –Turbidity in TCW Samples

The term "turbidity" describes how opaque a liquid is as a result of microscopic particles, such as dirt or microbes, floating in it. Turbidity, which is commonly expressed in Nephelometric Turbidity Units (NTU), increases with the number of particles present. On the other hand, transmittance quantifies the amount of light that can flow through a liquid. More light can flow through a clear liquid because of its high transmittance, but more light can be blocked or scattered by a cloudy liquid because of its poor transmittance. The turbidity of the tender coconut was assessed by measuring light transmission at a wavelength of 610 nm using a spectrophotometer. There is an inverse relationship between turbidity and light transmission values.

The turbidity of TCW samples was found to increase during storage. The turbidity was found to be more for fresh TCW. This might be due to the interaction between the chemical compounds which may accelerate by fermentation process during storage. Faster fermentation process would have taken place and breakdown of sugars would have led to high turbidity in fresh TCW. Clarity of coconut water is one of the important attributes. Nisin treated TCW have low turbidity because it will prevent microbial spoilage and maintain the clarity.

So, transmittance value decreased for fresh, without nisin and nisin pasteurized TCW samples after 30 days of storage in glass and PET bottles.

5.4 Colour Analysis

Colour plays a crucial role in shaping the appearance of product and fulfils various purposes such as identification, safety, overall acceptability.

Table 15: Colour analysis of TCW samples in Glass, PET Bottle.

	SAMPLES	L*(D65)	a*(D65)	b*(D65)	dE
GLASS BOTTLE	Fresh TCW	102.593± 0.0	-0.13± 0.0	-0.053± 0.0	2.6±0.0
	Without nisin	101.43± 0.0	-0.33± 0.0	4.33± 0.0	2.61±0.005
	With Nisin	101.26± 0.0	-0.23 0.0	4.87± 0.0	4.58±0.0
PET BOTTLES	Fresh TCW	103.72±0.0	0.15±0.0	1.01±0.02	3.93±0.05
	Without nisin	103.07±0.0	0.14±0.0	0.73±0.01	3.16±0.01
	With nisin	101.94±0.0	0.04±0.0	0.87±0.03	4.07±0.02

The colour measurement results on table 12 suggest that the L* values (lightness) remained stable across fresh, without nisin and nisin-treated tender coconut water (TCW), indicating minimal changes in overall brightness. The a* values, which represent the red-green spectrum, showed marginal or non-significant variations, suggesting that there was no major shift in the reddish or greenish hues. However, an increase in b* values (yellow-blue spectrum) was observed, which may be attributed to enzymatic or non-enzymatic reactions leading to a slight yellowing of the TCW.

This increase in b* values could be due to polyphenol oxidation, Maillard reactions, or pigment breakdown during storage. Nisin treatment, while effective in microbial control, might not have fully prevented such colour changes, especially those linked to enzymatic browning. a* values for glass bottle are good as they show slightly greenish tones because of less oxidation. While a* values for PET bottle indicating a small shift towards red tone possibly due to minor oxidation.

5.5 Microbiological Analysis

Table 16: TPC of TCW samples

TOTAL PLATE COUNT (TPC)						
DAYS OF STORAGE	**PET BOTTLE**			**GLASS BOTTLE**		
	FRESH TCW	**WITHOUT NISIN**	**WITH NISIN**	**FRESH TCW**	**WITHOUT NISIN**	**WITH NISIN**
0	ND	ND	ND	ND	ND	ND
30	TNTC	TNTC	TNTC	TNTC	TNTC	ND

[TNTC= too numerous to count, ND= not defined]

On the 0th day, the bacterial count was lower in TCW samples without nisin. However, after 30 days of storage, bacterial growth significantly increased in all TCW samples stored in PET bottles in both nisin-treated and untreated TCW samples, making them unsafe for consumption. PET bottles are permeable to gases than glass bottles, allow oxygen to enter and create favourable environment for bacterial growth. In PET bottles, nisin's antimicrobial effect may be reduced due to interactions with the plastic, making it less effective in controlling bacterial growth.

Nisin-treated TCW samples stored in glass bottles had a lower bacterial count. Nisin-treated TCW stored at room temperature in glass bottles remained edible even after 30 days, with no bacterial growth detected. This indicates that glass bottles provide a better barrier against contamination and help maintain the sterility of TCW samples

Fig 28- PCA in nisin treated TCW samples in glass bottles

Fig 29- PCA in fresh, without nisin, with nisin TCW samples in PET bottles

Table 17: Fungal count of TCW samples

Di-chloran Rose Bengal Agar (DRBC)						
DAYS OF STORAGE	**PET BOTTLE**			**GLASS BOTTLE**		
	FRESH TCW	**WITHOUT NISIN**	**WITH NISIN**	**FRESH TCW**	**WITHOUT NISIN**	**WITH NISIN**
0	ND	ND	ND	ND	ND	ND
30	TNTC	TNTC	TNTC	TNTC	TNTC	ND

[TNTC= too numerous to count, ND= not defined]

On 0th day, fungal count indicated no growth in all samples in PET and glass bottles. After 30th day PET bottle with fresh TCW have gone bad because of fungal growth. Fungi can produce harmful toxins like mycotoxins, which will cause food poisoning and serious health issues. So, TCW samples in PET bottles are not edible. After 30th day, nisin treated sample in glass bottle was better as it does not indicate any fungal growth.

Fig 30- DRBC agar in nisin treated TCW samples in glass bottles

Fig 31- DRBC agar in fresh, without nisin, with nisin TCW samples in PET bottles

Table 18: E-Coli count of TCW samples

Violet Red Bile Agar (VRBA)						
DAYS OF STORAGE	**PET BOTTLE**			**GLASS BOTTLE**		
	FRESH TCW	**WITHOUT NISIN**	**WITH NISIN**	**FRESH TCW**	**WITHOUT NISIN**	**WITH NISIN**
0	ND	ND	ND	ND	ND	ND
30	TNTC	TNTC	TNTC	TNTC	TNTC	ND

[TNTC = too numerous to count, ND= not defined]

On the 0th day, the E-Coli count in TCW samples both in PET, glass bottles was not defined. However, after 30 days of storage, E-coli growth significantly increased in TCW samples stored in PET bottles in both nisin-treated and untreated TCW samples, making them unsafe for consumption. PET bottles are permeable to gases than glass bottles, allow oxygen to enter and create favourable environment for bacterial growth.

Fresh and without nisin treated TCW have E-coli in glass bottle. Nisin-treated TCW samples stored in glass bottles had no E-coli count after 30^{th} day. This sample is remained edible even after 30 days. This means nisin remained effective in preventing bacterial growth in glass bottles. This indicates that glass bottles provide a better barrier against contamination and help maintain the sterility of TCW samples

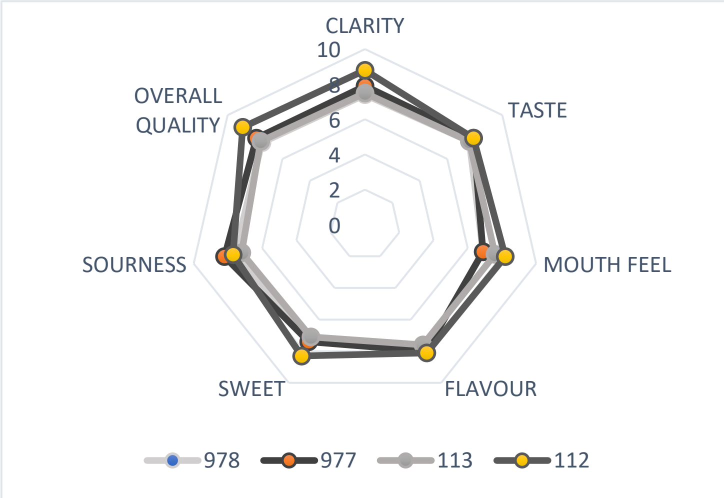
Fig 32- VRBA agar in nisin treated TCW sample in glass bottles

Fig 33- VRBA agar in fresh, without nisin, with nisin TCW samples in PET bottle

5.6 Sensory Evaluation of TCW Samples

Table -19 Sensory evaluation of fresh, treated and nisin treated tender coconut water samples.

	BEFORE TREATMENT		AFTER TREATMENT	
	PET (978)	GLASS (977)	PET (113)	GLASS (112)
Clarity/Colour	7.4±0.5	7.9±0.6	7.5±0.70	8.8±0.42
Taste	7.6±0.6	7.9±0.63	7.6±0.84	7.9±0.42
Mouth feel	6.9±0.81	6.9±0.87	7.6±0.69	8.2±0.42
Flavour	8.0±0.5	8.0±0.56	7.6±0.84	8.1±0.42
Sweet	7.2±0.42	7.4±0.52	7.1±1.70	8.3±0.94
Sourness/ Acid note	7.6±2.11	8.2±2.11	7.2±2.09	7.7±1.82
Overall Quality	7.5±0.5	7.9±0.5	7.6±0.84	8.9±0.31



PET (978), GLASS (977), PET (113) GLASS (112)

Fig 34- Sensory analysis of TCW samples.

The levels of overall acceptance between the fresh and TCW treated with nisin at room temperature were evaluated by untrained panelists with 9-point hedonic scale. According to Table 21, in case of clarity, treated TCW sample in glass bottle scored highest and untreated samples in PET bottle scored less. Both fresh and treated sample obtained overall acceptance scores above '7' or 'moderately like'. Sourness of TCW samples were similar. Nisin treated in glass bottle have overall acceptability with colour and other properties. It can be concluded that the thermal treatment, the concentration of the preservative, and the storage temperature significantly influence the shelf life and quality of TCW.

Chapter – 6
CONCLUSION

CONCLUSION

A study on preservation of Tender Coconut Water using pasteurization at 90℃ for 10 minutes and preservative nisin at a concentration of 5000 I.U was undertaken with an aim to develop process technology and improve the shelf life of glass and PET bottles. The samples were bottled and kept at room temperature for 30 days to assess the change in physio-chemical, microbiological qualities.

Tender coconut water (TCW) is a highly nutritious and refreshing natural beverage known for its rich electrolyte content, low calorie profile, and health benefits. It is widely consumed in tropical regions and has gained global popularity as a functional drink due to its hydrating properties and bioactive compounds. However, its high perishability presents a significant challenge, necessitating effective preservation techniques such as pasteurization, the use of natural preservatives like nisin, and proper packaging in glass or PET bottles.

Different composition of TCW samples were made in both glass and PET bottles.

1. TCW with the addition of nisin and sugar alternative.
2. TCW concentrate with sugar alternative and citrulline.

The following conclusion were drawn from the study:

Ascorbic acid content in both PET and glass bottle decreased during storage due to oxidation and enzymatic activity.

pH and TSS does not change significantly but titratable acidity will increase due to organic acid formation from residual microbial activity or slow chemical reactions.

Turbidity is more for PET bottle than glass bottle. Nisin-treated TCW exhibited lower turbidity (high transmittance), suggesting effective microbial control, while untreated samples were more turbid, highlighting the benefits of pasteurization and nisin as a preservative.

Microbial analysis for PET bottle indicated by fungal and bacterial growth after 15 days of storage for without nisin and nisin pasteurized TCW samples. So, PET bottles after 15 days of storage have gone bad in room temperature. But nisin pasteurized TCW samples in glass bottles are good after 30 days of storage.

Colour analysis indicated a slight yellowing of TCW over time, likely due to enzymatic or non-enzymatic reactions such as polyphenol oxidation and Maillard reactions. Glass-bottled TCW showed better colour stability, with minimal oxidation.

It could be concluded that pasteurized nisin and sucralose added TCW sample in glass bottle are better for over 30 days of storage in room temperature based on physio-chemical, microbiological and sensory data. The thermal process, concentration of chemical preservative and storage temperature have a significant impact on extent of the shelf life and quality of TCW.

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