

**SCREENING SUBSTRATES AND ADDITIVES FOR
ENHANCING YIELD AND QUALITY OF *NATA-DE-COCO***

By

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(2015-12-018)**

THESIS

**Submitted in partial fulfillment of the
Requirement for the degree of**

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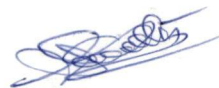


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2017**

DECLARATION

I hereby declare that the thesis entitled “**Screening substrates and additives for enhancing yield and quality of *Nata-de-coco***” is a bona fide record of research done by me during the course of study and the thesis has not previously formed the basis for the award of any degree, diploma, fellowship or other similar title, of any other University or Society.

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Certified that this thesis entitled “**Screening substrates and additives for enhancing yield and quality of *Nata-de-coco***” is a record of research work done independently by Geethumol Thankappan (2015-12-018) under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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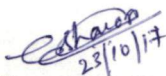
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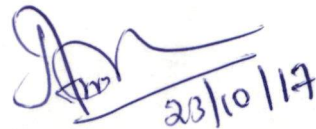
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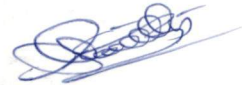
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Introduction

1. INTRODUCTION

Coconut (*Cocos nucifera* L.) is one of the most important and intensively grown palm trees worldwide. It is grown in more than 90 countries worldwide and is a popular plantation crop. Coconut palm is a unique plant and stands apart from all other palms because of its high degree of consistency and continuity in flowering and fruit production. They are permanent crops that bear fruit continuously for 60 to 80 years. Apart from the fruits, its by-products the roots, trunk, leaves, midrib and even flowers are useful to humankind and hence it is rightfully referred as *Kalpavriksha* (The Tree of Heaven). Hawaiians call coconut water *neolani* (*no-way lah-nee*), which means “dew from the heavens”.

The coconut palm is believed to have been introduced to India by at least 300 B.C (Prades *et al.* 2012). It is a well-acknowledged fact that India is one of the largest producers of coconut in the world. Coconut is cultivated mostly in the coastal regions of the country. Tamil Nadu, Andhra Pradesh, Karnataka, Kerala, Lakshadweep, Maharashtra, Assam, Orissa, West Bengal, Andaman and Nicobar Islands are the areas that have abundant coconut cultivation. The area under coconut cultivation in India is 2088.50 ha with a production of 22167.45 million nuts annually. Kerala accounts for 770.62 ha of area with a production of 7429.39 million nuts (CDB, 2016). Coconut palm exerts a profound influence on the rural economy of the areas where it is grown extensively and provides sustenance to more than 10 million people. The processing and related activities centred on the crop generate employment opportunities for over two million people in India.

Coconut has the advantage of having versatile uses, which no other crop can claim. Its products and by products can be commercially utilised for multiple purposes. Copra is the richest source of vegetable oil in the world, which is also used as hair oil, body oil and industrial oil throughout the country. Coconut oil is an ingredient in most of the superior cosmetic products. Coconut milk is an essential ingredient in many culinary preparations. It is a well-known beverage crop in many states in the country. The leaf

and trunk provide building material and the roots are used as dentifrice. Husk is processed into rope, carpets, geotextiles and growing media.

In the past, coconut was cultivated for limited items of use like copra, coconut oil that are used to meet the domestic requirements. Postharvest processing sector of coconut was mainly confined to manufacturing of copra and thereby coconut oil, which utilises 33 per cent of coconut production (Jackson *et al.*, 2004). Rest of the nut production is used directly as food either as mature nuts or as tender nuts. Thus, two main coconut products commercially traded are copra and oil. The price of raw coconut in the market is decided by the price of these two main commodities in the international market.

The copra production centres are situated in rural areas and utilise mature coconut, and the coconut water, which is an important by-product of copra, and oil industry is merely wasted. The unused coconut water is usually directly released into streams and paddy fields. The combined effect of low pH and high biological oxygen demand of coconut water causes adverse environmental effects.

Coconut water contains sugars, vitamins, amino acids, minerals that play different bio-functional roles in the human metabolic system and nutrient sources for microbial growth. Generally, people in tropical countries drink coconut water as a refreshing drink. The carbon and nitrogen sources present in coconut water can be utilised for the production of *Nata-de-coco*. Bacterial cellulose produced on the air liquid interphase of coconut water is popularly known as *Nata-de-coco* (Jagannath *et al.*, 2008)

To make coconut cultivation more remunerative, it is essential that all the products and by-products from the plant are fully exploited and commercialized. In this context, the present study entitled "Screening substrates and additives for enhancing yield and quality of *Nata-de-coco*" was undertaken with the objectives to evaluate the suitability of coconut water from tall, dwarf cultivars to improve yield and quality of *Nata-de-coco*, to evaluate the suitability of substrates for blending with coconut water for *Nata* production and to evaluate the suitability of additives to improve consumer acceptability of *Nata-de-coco*.

Review of literature

2. REVIEW OF LITERATURE

Bacterial cellulose produced by *Acetobacter xylinum* on the air liquid interface of coconut water is popularly known as *Nata-de-coco*. This unconventional product, based on coconut water is native to the Philippines and was developed locally first in 1949. Over the years, it has become popular in other countries like Japan, Korea and USA. *Nata-de-coco* is a gelatinous product, prepared from matured coconut water by the action of cellulose forming bacteria namely *Acetobacter aceti* subspecies *xylinum*.

The investigation entitled “Screening substrates and additives for enhancing yield and quality of *Nata-de-coco*” was undertaken during the period from September 2015 to May 2017 at the Department of Plantation Crops and Spices, College of Horticulture, Kerala Agricultural University, Vellanikkara, Thrissur. The objective of the study was to evaluate the suitability of coconut water from tall, dwarf cultivars to improve yield and quality of *Nata-de-coco*, to evaluate the suitability of substrates for blending with coconut water for *nata* production, and to evaluate the suitability of additives to improve flavour and consumer acceptability of *Nata-de-coco*.

Nata-de-coco is gaining popularity because of its high dietary fibre content and low calorific value. Many health benefits like prevention of colon cancer and heart attack have been attributed to *Nata*. It is excellent ingredient for ice creams, fruit cocktails, and other recipes. *Nata-de-coco* prepared using mature coconut water could serve as a healthy dietary fibre food. It has unique structure and texture when grown in sugar rich medium. Screening of different substrates and improving colour and flavour of *Nata-de-coco* by adding under exploited tropical fruits /vegetables and addition of natural flavours can go long way in improving palatability and consumer acceptability of the product. Therefore, the available literature on the product bacterial cellulose, coconut water, *Nata-de-coco* are reviewed hereunder and presented in this chapter.

2.0. NATA-DE-COCO: A VERSATILE PRODUCT

Nata-de-coco have a wide range of applications in food industry like spoonable and pourable dressings, sauces, gravies, icings, sour cream, cultured dairy products, aerated desserts and frozen dairy products.

According to Budhiono *et al.*, (1999), *Nata* has high market potential in South East Asian countries. It is a white, gelatinous food product popular in Philippines, Japan, and Malaysia. The word *Nata* is derived from Latin '*natar*' that means to float from fermenting coconut water or fermenting rotting fruits.

Iguchi *et al.*, (2000) opined that *nata* has high purity, unique strength, an ultra-fine structure and is biodegradable. Isolation and purification of this is also simple. These properties allow *Nata* to be used as a substitute for wood raw material in the high-quality paper industry, low-calorie foods and other materials.

Besides its use as a food, the gel-like substance is also considered to impart extraordinary mechanical strength when processed into film or sheets (Singh and Gopalakrishnan, 2002). They also reported that, commercial applications of bacterial cellulose such as *Nata* is in food products, audio head phone diaphragm, artificial skin for scalded or wound healing, ulcers and dental implants. In food industry, it has important applications in a variety of food formulations. It is especially used when lack of flavour interactions, foam stabilization and stability over a wide range of pH, temperature and freeze-thaw conditions are required. The same authors also opined that, the very high cost in the production of bacterial cellulose, limits the use of the same to eminent value added applications. The use of cheaper carbon and nutrient sources is an arousing strategy to surmount the limitation and therefore to increase the competitiveness of a unique material.

According to Sutanto (2004), *Nata* is white or bright grey in colour, transparent and sturdy, fibrous in cold situation and rather delicate in hot situation.

When the bacteria are inoculated in to coconut water medium containing sugar that will produce acetic acid and a white layer will float on the liquid medium. The white layer is called *Nata*.

Chowdary *et al.* (2005) opined that, bacterial cellulose produced from expensive culture media, containing glucose as carbon source and other nutrient sources results in very high production costs, which limit the use of material to a very high value added applications. The use of cheaper carbon and nutrient sources is an interesting strategy to overcome the limitation.

According to Keshk and Sameshima (2006), the production of *Nata* is gaining attention because of its wide application in food industry as a jelly like food. Apart from *Nata*, many potentially high value markets exist for this thin film bacterial cellulose, including acoustic diaphragms, artificial skin, artificial blood vessels, liquid loaded medical pads, super-sorbers, specialty membranes, and paper and pulp industry.

Huber (2006) reported that, *Nata de coco* is an organic high dietary fibre food product, produced by bacterial fermentation of coconut water. It is high in cellulose, low in fat and calories and contains no cholesterol. The water trapped in the cellulose matrix is highly useful for its application in food industry as a jelly like food. It is highly hydrophilic, holding water over hundred times of its weight. It has distinct textural properties like chewy, soft, and smooth surface. It has been thought capable of controlling weight, and protecting against diverticular diseases and cancer of the colon and rectum.

Mesomya *et al.* (2006) reported that health food from unpolished rice, hulled mung bean, *Nata-de-coco* and sweet corn may reduce serum triglycerides in hyperlipidemic patients because of the insoluble fibre in the supplement.

2.1.0 COCONUT WATER AS A WHOLSOME FOOD

For the production of bacterial cellulose, one of the commonly used culture medium is coconut water.

Shaw and Srivastava (1963) opined that, tender coconut water contains sugars, proteins, minerals, vitamins, amino acids and growth promoting factors. In all coconut-producing countries, it is used as a common drink.

The various products of coconut other than copra and coconut oil offer vast scope for further development, value addition and commercialisation. In tropical countries, tender coconut water is mainly consumed as a natural beverage. It contains greater amounts of minerals such as potassium, sodium, magnesium, chlorides, ascorbic acid and sugars (Sabapathy and Kumar, 1999).

Flack *et al.* (2000) reported that the mineral magnesium and potassium present in coconut water are known to help reduce high blood pressure. Several functional activities of tender coconut water are anti-carcinogenic, anti-oxidant, hepatoprotective, anti-aging and anti- thrombotic effects.

The nutrient composition of coconut water has been well documented. Now a days, it is one of the fast growing drinks due to natural hydrating qualities, enhanced taste, functional health properties and nutritional benefits (Campbell *et al.*, 2000).

Rethinam and Kumar (2001) reported that coconut water has been a religious symbol for a long time as it is a sterile liquid. In India, immature coconuts are offered as ceremonial presents and act as cleansing media in traditional events. It is considered as a 'fulsome, sweet liquid which increases semen and promotes digestion' in Indian Ayurvedic systems of medicine.

According to Jackson *et al.* (2004) coconut drink is gaining popularity in the beverage industry due to its high nutritional value and some potential therapeutic properties. This natural drink is believed to be useful in averting and relieving many health problems inducing dehydration, constipation, digestive problems, fatigue, heart stroke, diarrhea, urinary tract infections and kidney stones.

According to Jackson *et al.* (2004) in the early stages of maturity of tender coconut water, the most predominant sugars are glucose and fructose, which ranged from about 1.5 per cent to 5 per cent and then slowly decreased to 2 per cent at the stage of full maturity. In the case of tall coconut cultivars, total sugar content of coconut water increased from 5 to 7 months and subsequently total sugars rapidly decreased until it ripens at the age of 12 months. However, in dwarf varieties, changes in total sugar content differed slightly and it was high throughout the ripening process.

Tender coconut water is still consumed as a refreshing drink by thousands of inhabitants of Asian countries. In the tropics, coconut water is considered as both refreshing beverage and health tonic. It not only satisfies thirst but also invigorates the body and brings about a sense of well-being and renewed health. It has a long history of use as both a food and as a medicine. Women are encouraged to drink it when pregnant and nursing so their milk will provide all the nutrients essential for a healthy baby as reported by Alleyne *et al.* (2005).

According to Bourdeix *et al.* (2005) Polynesian, Melanesian and Micronesian mariners used coconut fruit as source of food and drink.

Unagul *et al.* (2007) suggested that tender coconut water is largely consumed in tropical countries. It is a clear, nutritive liquid obtained from the endosperm of coconuts.

According to Matsui *et al.* (2008), the main sugars in coconut water are sucrose, glucose, sorbitol and fructose. These sugars are the main fractions of soluble solids. Besides sugars, proteins and minerals are also present in coconut water.

Coconut water is discarded from many applications of agro-industries in Southeast Asian countries. Because, the residues still contain carbon and nitrogen sources, they could be utilised as a substrate for producing *Nata-de-coco* as reported by Kongruang, (2008; Kurosumi *et al.* (2009).

Matsui *et al.* (2009) opined that, wide applications of coconut water could be attributed to its unique chemical composition. It is rich in potassium, sodium, phosphorous, chloride, magnesium, vitamins, sugars, proteins, free amino acids and growth promoting factors.

According to Chowdhury *et al.* (2009), processing of coconut water is essential to reduce the transportation volume and cost associated with whole fruit and to improve the shelf life.

According to Markose and Pothuval (2009), glucose and fructose form an important constituent of coconut water. Its characteristic flavour is contributed by delta lactones and it serves as a mineral drink with therapeutic properties that help in regaining the vitality of human body.

Chang and Wu, (2011) reported that catechins present in the coconut water possess antioxidant, antimicrobial and anticancer activities.

Prades *et al.* (2012) suggested coconut water is progressively becoming a natural healthy drink and is considered as simple tropical refreshment or occasionally as a medicine and its flavour is sensitive to temperature.

Sangamithra *et al.* (2014) reported that the composition and physiochemical properties of coconut water vary with maturity of the nut. Potassium is the main mineral element in coconut water. The main sugars in mature coconut water are sucrose, sorbitol, glucose and fructose, followed by minor sugars like galactose, xylose and mannose. Compared to tender coconut water, protein content in mature coconut water is high. Mature coconut water is often regarded as a by-product of copra and oil industry which appeared to have great potential to be developed into value added products.

Most of the copra production centres are situated in rural areas and coconut water is usually released in to streams and paddy fields. The combined effect of lower pH and high biological oxygen demand (BOD) causes damage to paddy fields, water sources and consequently adverse environmental effects. Therefore, it is highly important that the wastage of this nutritionally rich, multidimensional by-product has to be reduced by using it for producing value added products such as vinegar and *Nata-de-coco* as reported by Reddy and Lakshmi (2014). They also opined that dwarf coconut cultivars with high volume of water, more sugar contents and good organoleptic scores are the most desirable cultivars to obtain a sweet and tasty product.

According to Gayathry. (2015), coconut water is a tasty and refreshing drink. It consists primarily of sugars, minerals, protein nitrogen and vitamins but is slightly acidic, with pH around 5.5, transparent, non-sticky and slightly sweet taste. These features make the coconut water an excellent substrate for microbial growth.

2.2.0 EFFECT OF SUBSTRATES AND ADDITIVES ON YIELD AND QUALITY OF NATA-DE-COCO

Raw *Nata* is transparent without any flavour. In order to enhance the consumer acceptability, improving colour and flavour is very important. Use of different substrates for production of *nata* and storing in different fruit juices have been reported to not only improve yield but also flavour and consumer acceptability. The available literature on this aspect is reviewed and presented here under.

According to Lapuz *et al.* (1967), *Acetobacter xylinum* is widely distributed in nature and is a common contaminant in industrial production of vinegar by *Acetobacter aceti*. *Acetobacter sub species xylinum* has been isolated from rotting fruits vegetables and fermenting coconut water as reported by Gallardo *et al.* (1971).

Okiyama *et al.* (1993) opined that *nata* has endowed *Kamaboko* (processed Japanese seafood) with better stiffness and brittleness, almost eliminating the springiness. This modified *Kamaboko* could better endure the aging process.

According to Vandamme *et al.* (1998) the use of acetic acid to bring down the pH of tender coconut water had a better effect compared to the other acids on the growth of *Acetobactor xylinum* and *Nata* formation.

According to Iguchi *et al.* (2000) one of the first uses of bacterial cellulose is the manufacturing of a Filipino traditional dessert with a smooth mouth feel that is called *Nata-de-coco*, where by coconut water is fermented for the biosynthesis of *nata*, and then it is chopped into minute sections and immersed in sugar syrup.

Setiaji *et al.* (2002) studied the influence of mixing of coconut water and sucrose concentration to coconut milk skim as substrate of *Nata- de - coco*. The result showed that coconut milk skim could be used as substrate for *Nata -de -coco* along with 50 percentage coconut water and sucrose.

Singh and Gopalakrishnan, (2002) studied the effect of various flavouring agents like almond, banana, lemon, strawberry, and vanilla with *Nata-de-coco*. They reported that these flavouring agents improved the acceptability of *Nata* produced.

Illiaskutty, (2004) tried three different base materials for preparation of *nata-de-coco*, such as plain coconut water, blends of coconut water with pineapple juice and blends of coconut water with soya milk. *Nata* product obtained from coconut water with blends of pineapple juice was found the best organoleptically.

Keshk and Sameshima, (2006) reported that there is a tendency to use many different substrates /agricultural by products (cane molasses, beet molasses, pineapple skin, cheese whey permeate, high solids potato effluents for the manufacture of *Nata-de-coco*. Coconut water may be preferred for a better fermentation of *Acetobactor xylinum* than other agro-wastes. However, taken together, abundant pineapple fruit waste in addition to coconut water should be further investigated for their best formulation in conjunction with cultivation methods for production of bacterial cellulose.

Jagannath *et al.* (2008) studied the effect of pH, sucrose and ammonium sulphate concentrations on the production of *Nata-de-coco*. The study showed that *Acetobacter xylinium* could effectively use sucrose as the sole carbon source in coconut water medium and that cellulose production was more dependent on pH than either sucrose or ammonium sulphate concentrations. Maximum thickness of *Nata* was obtained at pH 4.0 with 10 per cent sucrose and 0.5 percent ammonium sulphate concentrations. These conditions also produced good quality *Nata-de-coco* with a smooth surface and soft chewy texture.

Wonganu and Kongruang, (2010) stated that, vegetarian meat may be prepared by using *Nata* in combination with *Monascus* (red pigmented mould). The composite is stable against change in colour and morphology and its flavour is much like natural meat.

Phisalaphong and Chiaoprakobkij, (2012) reported that *Nata-de-coco* has turned in to a very well liked food and is now quickly spreading worldwide in the form of a dessert or candy.

Afreen and Lokeshappa, (2014) studied the production of *Nata* using coconut water, papaya juice and musk melon juice with and without addition of sugar. In this, papaya juice gave the highest yield of *nata* followed by coconut water.

The stickiness of creamy condiment could be noticeably improved upon addition of *Nata* so that it could be easier to serve quantitatively using a spoon as reported by Shi *et al.*, (2014).

Ullah *et al.*, (2016) reported that addition of *Nata* increases the gel strength of *Tofu* (food made by coagulating and pressing soymilk), providing fitness and better texture. They also suggested that addition of *Nata* to chocolate drink prevented the precipitation of cocoa due to the retention properties of *Nata*.

Materials and methods

3. MATERIALS AND METHODS

The present study entitled “Screening substrates and additives for enhancing yield and quality of *Nata-de-coco*”, was conducted in the Department of Plantation crops and Spices, College of Horticulture, Kerala Agricultural University, Vellanikkara, Thrissur, during October 2015- July 2017. The objective of the study was to evaluate the suitability of substrates, additives and flavour to improve yield and consumer acceptability of *Nata-de-coco*. The methodology adopted for the study is presented in this chapter.

3.1. 0 THE EXPERIMENTAL MATERIAL

The experimental materials used in the present study are as follows. The experimental materials consisted of

3.1.1: Substrates: Various substrates include

- a) Coconut water from tall (WCT) cultivar
- b) Coconut water from dwarf (COD) cultivar
- c) Coconut water from copra Mills
- d) Sucrose
- e) Ammonium sulphate
- f) Jackfruit juice
- g) Beetroot juice
- h) Mango juice
- i) Pineapple juice
- j) Papaya juice
- k) Lovi-lovi juice
- l) Watermelon juice.

3.1.2: Acidulants: Acidulants such as acetic acid, bilimbi juice were used in the study.

3.1.3: Bacterial Inoculum: Sources of bacterial inoculum used in the study include

- a) *Acetobactor xylinum* culture (NCIM 2526)
- b) Mother liquor

3.1.4: Additives: Various additives used in the study include the following

- a) Sugar syrup
- b) Rose apple syrup
- c) West Indian Cherry syrup

3.1.5: Flavours: Various flavours used in the study include the following

- a) Ginger extract
- b) Vanilla extract

3.1.6: Containers for preparation of *Nata-de-coco*: Plastic trays measuring 22cm length x13cm width were used for inoculation of substrates with bacterial culture and mother liquor.

3.1.7: Containers for storing *Nata-de-coco*: The *Nata –de-coco*, after production were stored in the glass bottles obtained from Department of Community Science, College of Horticulture, Vellanikkara, Thrissur.

3.2.0: COLLECTION OF EXPERIMENTAL MATERIALS: The materials such as substrates, bacterial inoculum, additives, flavours and glass bottles were collected as follows.

3.2.1: Substrates

a) Nut water of tall (WCT) cultivar was collected from the nuts of WCT (12 month old) collected from the CDB coconut farm, at the Central Nursery, Vellanikkara during September 2016 to March 2017.

b) Nut water (12 month old) of dwarf (COD) cultivar was collected from the nuts of COD collected from farmers' field in the Krishibhavan, Nattika, Thrissur, during January 2017 to March 2017.

c) **Coconut water from Copra mills** are collected from the Chempakassery Oil Mills, Mudikode, Thrissur, during January 2017 to March 2017.

d) **Sucrose:** Sucrose was collected from the Chemind Thrissur,

e) **Ammonium sulphate:** Ammonium sulphate was collected from the Chemind Thrissur. Substrates from (f) to (l) were collected from the orchard, College of Horticulture, Vellanikkara and nearby vegetable markets and farmers' field

3.2.2: Acidulants: Acidulants such as acetic acid was collected from the Chemind, Thrissur. Fruits for bilimbi juice were collected from the Plantation Crops and Spices farm, Vellanikkara and nearby farmers' field.

3.2.3. Bacterial Inoculum: Sources of bacterial cultures used include

a) *Acetobacter xylinum* culture (NICM 2526). This was collected from the National Collection of Industrial Microorganisms (NCIM), Pune, Maharashtra, during April 2016.

b) Mother liquor: Mother liquor was collected from the Sevashram, Angamalay, Ernakulum district.

3.2.4: Additives; Additives such as sugar syrup, rose apple syrup and west Indian cherry syrup were prepared by fruits collected from nearby farmers' field and nearby markets during January to May 2017.

3.2.5: Flavours: Flavouring materials such as ginger extract was collected from the ginger oleoresin prepared in the Department of Plantation Crops and Spices. Vanilla extract was purchased from the Elite super market, Thrissur.

3.2.6: Containers: Containers for preparation and storing of *Nata -de-coco* were collected from nearby markets and the Department of Community Science, College of Horticulture, Vellanikkara, Thrissur.

3.3.0: PREPARATION OF EXPERIMENTAL MATERIALS:

Substrates, acidulants, bacterial inoculums, additives and flavours used in the study were prepared as detailed below.

3.3.1: Preparation of substrates:

a) Nut water from WCT cultivars: Mature nuts of WCT were cut opened and approximately 1.0 litre nut water collected, TSS (° Brix) measured, sterilised in an autoclave at 121°C for 15 minutes.

b) Nut water from COD cultivars: Mature nuts of COD were cut opened, 1.0 litre of nut water collected TSS (°Brix) measured, sterilized by boiling in an autoclave at 121°C for 15 minutes.

c) Coconut water from tall cultivars were collected from Copra mills (1.0 litre), TSS (°Brix), sterilized in an autoclave at 121°C for 15 minutes.

d) Sucrose: Sucrose 10 % was prepared and added into the coconut water

e) Ammonium sulphate: Ammonium sulphate 0.5% was prepared and added into the coconut water.

Substrates from (f) to (l) were prepared by washing, peeling, cutting, coring, pitting wherever required and edible portion were cut into pieces and juice was extracted and sieved through a muslin cloth. TSS (°Brix) of juice were noted.

3.2.2: Acidulants: Acidulants such as acetic acid was prepared by diluting glacial acetic acid (98 per cent). Bilimbi juice was prepared by grinding, extracting the juice through a muslin cloth, pH was noted.

3.3.3: Preparation of bacterial inoculum: *Acetobacter xylinum* culture (NCIM 2526) collected sub cultured and then starter culture was prepared from sub culture.

a) Preparation of *Acetobacter xylinum* sub culture: *Acetobacter xylinum* culture (NCIM 2526) sub culture was prepared as suggested by National Collection of Industrial Microorganisms (NCIM), Pune, as shown below.

Composition of *Acetobacter suboxydans* media

Ingredients	Quantity
Sorbitol	5.0g
Yeast extract	0.5g
Distilled water	100ml
Agar	2g
pH of the medium	6.2

Acetobacter xylinum culture was inoculated into the above medium. Growth of colonies were observed after 4 days of inoculation.

b) Maintenance of *Acetobacter xylinum* subculture

Acetobacter xylinum (NCIM 2526) culture was maintained on tomato agar slants as suggested by Jagannath *et al.* (2008). Two hundred gram of fresh tomatoes and 500 ml of distilled water was boiled for 30 minutes. This tomato infusion was filtered and mixed with 100 g yeast extract, 50 g sucrose, 2.5 g peptone and 20 g agar. The volume was made up to 1000 ml with distilled water and sterilized at 121° C for 15 minutes. *Acetobacter xylinum* was streaked on the slants and incubated at 30° C for 7 days.

c) Preparation of *Nata-de-coco* starter

Acetobacter xylinum maintained on tomato agar slant was inoculated to Hestrin and Shramm media as shown below.

Composition of Hestrin and Shramm media

Ingredients	Quantity
Glucose	20 g
Yeast extract	5g
Peptone	5g
Di potassium hydrogen phosphate	2.7g

pH was adjusted to 4.2 with acetic acid. The inoculated media was incubated for 7 days at ambient temperature.

All these works were done in Department of Agricultural Microbiology, College of Horticulture, Vellanikkara, Thrissur.

3.3.3.1: Mother liquor:

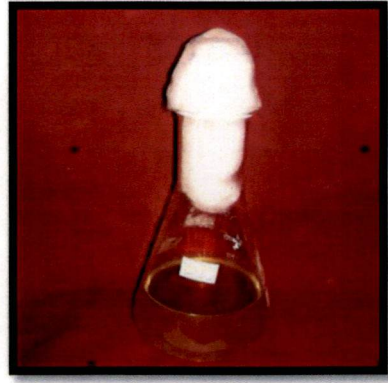
Mother liquor collected was kept in refrigerator and added into the coconut water along with bacterial culture. Plate 1 shows sources of bacterial culture and media.

3.3.3.2: Preliminary study

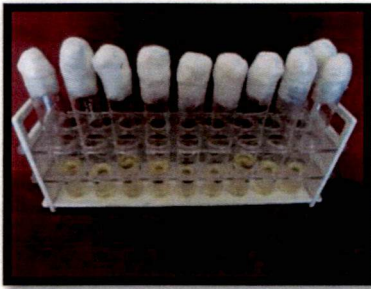
Preliminary lab studies were conducted to find out the amount of starter culture (*Acetobacter xylinum* -NCIM 2526) and mother liquor to be added to the substrates used in the present study as shown below



a. *Acetobacter xylinum*
(NCIM 2526) culture



b. *Acetobacter suboxydans*
media



c. *Nata-de-coco* starter media



d. Mother liquor

Plate. 1 Bacterial culture and media used for the production of *Nata-de-coco*

Thickness of *Nata* formed using various amount of starter culture and mother liquor.

Treatment	Coconut water (ml)	Starter culture (ml)	Mother liquor (ml)	Thickness of <i>Nata</i> (mm)
1	100	10	10	1
2	150	15	15	2
3	250	25	25	4
4	500	50	50	6
5	1000	100	100	8

From the preliminary study done, it was found that starter culture and mother liquor used in the 1:1 proportion, improved the thickness of *Nata*. The experiments in the present study were conducted by using the following ingredients given below.

Composition of media for *Nata-de-coco* production

Ingredients	Quantity
Coconut water	1000 ml
Sucrose	100g
Ammonium sulphate	5g
Acetic acid/ bilimbi juice	3ml / 25 ml
Starter culture	100ml
Mother liquor	100ml

3.3.4: Additives: Sugar syrup and fruit syrups were prepared as suggested by Jothi *et al.*, (2014).

a) Preparation of sugar syrup

100 ml water was poured in to a thick bottomed sauce pan. Upon heating sugar was added until all the sugar crystals were dissolved in water. Final TSS of sugar syrup was maintained as 65° Brix.

b) Preparation of fruit juice syrup

Freshly harvested Rose apple and West Indian cherry fruits free from diseases and bruises were washed thoroughly in cold water and juices were extracted by grinding with a pestle and mortar. The extracted juices were strained through a muslin cloth. Filtered fruit juices were mixed with sugar syrup after cooling. TSS of fruit syrup was maintained at 65° Brix.

3.3.5. Flavours: Ginger oleoresin was diluted in distilled water and 0.1ml of diluted oleoresin was added into the *Nata-de-coco*. From the vanilla extract, 0.1ml was taken and added into the *Nata-de-coco*.

3.4.0 DESIGN AND LAYOUT OF THE EXPERIMENTS

The study consisted of three experiments viz. Experiment No. 1: Screening of coconut water from tall and dwarf cultivars for *Nata-de-coco* production, Experiment No. 2: Screening suitability of different substrates for *Nata-de-coco* production and Experiment No. 3: Improving colour and flavour of *Nata-de-coco* by using additives.

3.4.1. SCREENING COCONUT WATER FROM TALL (WCT) AND DWARF (COD) CULTIVARS FOR NATA –DE-COCO PRODUCTION

The experiment was laid out using Complete Randomised Design (CRD) with 8 treatments as follows and three replications as shown below.

1. Sources of coconut water
 - a. Mature coconut water from tall cultivar (WCT)
 - b. Mature coconut water from dwarf cultivar (COD)
2. Addition of sucrose
 - a. Coconut water was added with sucrose (10%)
 - b. Coconut water was added without sucrose.
3. Sources of acidulant:
 - a. Acetic acid
 - b. Bilimbi juice

Accordingly different treatments are as follows

- T₁: Coconut water from tall cultivar (WCT) added with sucrose and acetic acid
T₂: Coconut water from tall cultivar (WCT) added with sucrose and bilimbi juice
T₃: Coconut water from tall cultivar (WCT) added without sucrose and acetic acid
T₄: Coconut water from tall cultivar (WCT) added without sucrose and bilimbi juice
T₅: Coconut water from dwarf cultivar (COD) added with sucrose and acetic acid
T₆: Coconut water from dwarf cultivar (COD) added with sucrose and bilimbi juice
T₇: Coconut water from dwarf cultivar (COD) added without sucrose and acetic acid
T₈: Coconut water from dwarf cultivar (COD) added without sucrose and bilimbi juice.

The coconut water from WCT and COD cultivars were collected and added with sucrose (10 per cent) and without sucrose. pH was adjusted to 4 with acetic acid / bilimbi juice. All the treatments were added with ammonium sulphate 0.5 per cent. The substrate was inoculated with *Acetobacter xylinum* culture (NCIM 2526), mother liquor and kept for the growth of bacteria. *Nata* sheet formed was harvested when it was firm with uniform thickness. Harvested *nata* sheets were washed thoroughly in distilled water, cut in to cubes and boiled for 15 minutes to remove the sourness. Cut *Nata* cubes were kept in sugar syrup (65° Brix) and stored in glass bottles. Evaluation of *Nata* was done on physico-chemical parameters.

3.4.2 SCREENING SUITABILITY OF DIFFERENT SUBSTRATES FOR *NATA* –*DE-COCO* PRODUCTION

The experiment was laid out using Complete Randomised Design (CRD) with 8 treatments and 3 replications as follows.

Different treatments are shown below

- T₁. Coconut water alone
T₂. T₁+ Jackfruit juice
T₃. T₁+ Beetroot juice
T₄. T₁+ Mango juice
T₅. T₁+ Pineapple juice

T₆. T₁+ Papaya juice

T₇. T₁+ Lovi-lovi juice

T₈. T₁+ Watermelon juice

Coconut water of tall cultivars from copra mill and fruit/ vegetables were used in this study. Coconut water + fruit /vegetable juices were added in the proportion of 70:30 as suggested by Illaikkutty (2004), which formed the substrates for *Nata-de-coco* production.

The substrates (Plate. 2) were added with sucrose (10%), ammonium sulphate (0.5%) and pH was adjusted to 4 with acetic acid and autoclaved at 120° C for 20 minutes. Sterilised media was poured in to washed and U.V sterilized trays measuring 22 cm length x 13 cm width. The substrates were inoculated with starter culture and mother liquor. Trays were covered with muslin cloth and kept for the formation of *Nata*. The *Nata* sheets formed were harvested when it was firm with uniform thickness. Harvested nata sheets were washed thoroughly in distilled water, cut in to cubes and boiled for 15 minutes to remove the sourness. Cut *Nata* cubes were put in sugar syrup (65° Brix) and stored in glass bottles. Evaluation of *Nata* was done on physico chemical parameters and sensory qualities.

3.4.3. IMPROVING COLOUR AND FLAVOUR OF *NATA-DE-COCO* BY USING ADDITIVES

The experiment was laid out using Complete Randomised Design (CRD) with 12 treatments and 2 replications as follows.

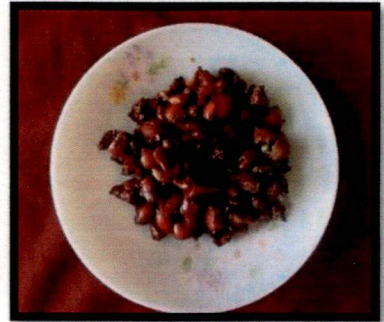
Nata-de-coco formed from coconut water of WCT and COD cultivars as substrate were put in three syrups namely

1. Sugar syrup
2. Rose apple syrup
3. West Indian Cherry syrup

Then these were flavoured using two flavours such as



Jack fruit



Lovi-lovi



Papaya



Water melon

Plate 2. Various substrates used for blending with coconut water



Pineapple



Beetroot



Mango

Plate 2. (Continued) Various substrates used for blending with coconut water

1. Vanilla extracts

2. Ginger extracts

Accordingly there were 12 treatments as shown below.

- T₁: *Nata* formed using coconut water from WCT packed in sugar syrup, flavoured with ginger extract.
- T₂: *Nata* formed using coconut water from WCT packed in sugar syrup, flavoured with vanilla extract.
- T₃: *Nata* formed using coconut water from WCT packed in rose apple syrup, flavoured with ginger extract
- T₄: *Nata* formed using coconut water from WCT packed in rose apple syrup flavoured with vanilla extract
- T₅: *Nata* formed using coconut water from WCT packed in West Indian cherry syrup flavoured with ginger extract
- T₆: *Nata* formed using coconut water from WCT packed in West Indian cherry syrup flavoured with vanilla extract
- T₇: *Nata* formed using coconut water from COD packed in sugar syrup flavoured with ginger extract
- T₈: *Nata* formed using coconut water from COD packed in sugar syrup flavoured with vanilla extract
- T₉: *Nata* formed using coconut water from COD packed in rose apple syrup flavoured with ginger extract
- T₁₀: *Nata* formed using coconut water from COD packed in rose apple syrup flavoured with vanilla extract
- T₁₁: *Nata* formed using coconut water from COD packed in West Indian cherry syrup flavoured with ginger extract
- T₁₂: *Nata* formed using coconut water from COD packed in West Indian cherry syrup flavoured with vanilla extract.

Nata-de-coco produced from water of tall (WCT) and dwarf (COD) cultivars were used in this study. Harvested *Nata* sheet was cut in to cubes, washed thoroughly

in distilled water and boiled for 15 minutes to remove the sourness. *Nata* cubes were packed in sugar/fruit syrup in 1:1 ratio, ginger and vanilla extracts (0.1ml) were added and stored in glass bottles and sensory evaluation was conducted.

3.5.0. COLLECTION OF EXPERIMENTAL DATA

Nata-de-coco obtained from Experiment no.1 and 2 subjected to physico-chemical analysis

3.5.1 Physical parameters

Harvested *Nata* sheets after several washes were analysed for physical parameters as shown below.

3.5.1.1 Days to *Nata* formation (Number of days)

Nata was ready to harvest when it was firm enough to be pulled out as a single sheet. The number of days taken by each treatment from the day of inoculation till the formation of a firm *Nata* sheet was counted and expressed as number of days.

3.5.1.2 Thickness of *Nata* (mm)

Thickness of *Nata* sheet was measured at four different positions by using a vernier caliper. Values were averaged and expressed as mm.

3.5.1.3 Weight of *Nata* (g)

Harvested *Nata* sheets were washed in distilled water thoroughly and recorded the weight using a weighing balance and weight is expressed in grams.

3.5.1.4 Moisture content (% w/w)

Harvested, washed *Nata* sheets were cut in to pieces and the moisture content was determined as per A. O.A.C (1984). To determine the moisture content of *Nata*, 5.0 g of sample was taken in a petridish and dried at 60°C to 70°C in a hot air oven, cooled in a desiccator for 30 minutes and weighed. Dry weight recorded until three consecutive weight were the same. The moisture content of the sample was calculated from the loss in weight during drying and expressed in percentage.

$$\text{Moisture content} = \frac{\text{Initial weight} - \text{final weight}}{\text{Initial weight}} \times 100$$

3.5.2 Chemical characters

3.5.2.1 Acidity (%)

Acidity of *Nata-de-coco* was determined by titration with standard sodium hydroxide (0.1N) and expressed as percent of acetic acid as detailed by Ranganna (1997).

One gram of *Nata* was grinded and made up to 100ml in a standard flask. One drop of phenolphthalein indicator was added and titrated against NaoH (0.1 N). End point of titration was noted as appearance of light pink colour of solution in the beaker. Acidity was expressed in percentage.

$$\text{Acidity (\%)} = \frac{\text{Titre Value} \times \text{Normality} \times \text{Equivalent weight of acetic acid} \times \text{Volume made up} \times 100}{\text{Weight of sample taken} \times \text{volume pipetted out} \times 1000}$$

3.5.2.2 Total Soluble Solids (TSS) (°Brix)

The Total Soluble Solids (TSS) of *nata* was measured using a hand held refractrometer as detailed as detailed (Alvarez *et al.*, 2003). The harvested *Nata* was washed, cut in to pieces and crushed using mortar and pestle until a fine paste was obtained. To measure the TSS, the day light plate was lifted and the *nata* content was placed on the top of prism assembly. The refractrometer was held in the direction of natural light and viewed through the eyepiece. TSS was directly recorded as ° Brix.

3.5.2.3 Protein (%)

The protein content of *Nata-de-coco* was determined by Lowry's method as given by Sadasivam and Manickam (1992).

Reagent A: Two per cent sodium carbonate in 0.1 N sodium hydroxide

Reagent B: 0.5% copper sulphate in 1 per cent potassium sodium tartarate

Reagent C: Mix 50ml of Reagent A and 1 ml of Reagent B prior to use

Reagent D: Folin- Ciocalteau Reagent

Stock standard: 50mg of bovine serum albumin was weighed and dissolved in distilled water and made up to 50ml in a standard flask.

Working standard: 10ml of the stock solution was diluted to 50ml with distilled water in a standard flask.

Nata-de-coco sample (0.50g) was ground well in a mortar with 5 to 10ml of phosphate buffer. It was centrifuged and supernatant was used for protein estimation. Working standards 0.2, 0.4, 0.6, 0.8, and 1ml were pipetted out in a series of test tubes. Sample extract (0.1ml) was pipetted out in another test tube. Tubes with 1.0 ml water was served as blank.

To each test tube including blank, Reagent C (5.0ml) was added. It was mixed well and allowed to stand for 10 minutes. To all the test tubes Reagent D (0.50 ml) was added, mixed well and incubated at room temperature in dark for 30 minutes till blue colour was developed. Absorbance was recorded in a spectrophotometer at 660 nm. A standard graph was drawn plotting the concentration of working standards in X axis and absorbance in Y axis.

3.5.2.4 Fibre (%)

Crude fibre content was estimated by acid alkali digestion method as suggested by Sadasivam and Manickam (1992).

Two grams of *Nata-de-coco* was dried, defatted and boiled with 200ml of 1.25 per cent sulphuric acid for 30 minutes. This was filtered through muslin cloth and washed with boiling water and again boiled with 200ml of 1.25 per cent sodium hydroxide solution for thirty minutes. Again it was filtered through muslin cloth and washed with 25ml of sulphuric acid (1.25 per cent), 50 ml portion of water and 25ml of alcohol. The residue was taken in a previously weighed ashing dish (W_1) dried at 130°C in an oven for 2 hours. The ashing dish was cooled in a desiccator and weighed

(W₂). The residue was ignited in muffle furnace at 600°C for 30 minutes, cooled in a desiccator and reweighed (W₃).

$$\text{Crude fibre (\%)} = \frac{(W_2 - W_1) - (W_3 - W_1)}{\text{Weight of sample}} \times 100$$

3.5.2.5 Total sugar (g)

The total soluble sugar content of *Nata-de-coco* was determined by Phenol sulphuric acid method as given by Nielsen (2010).

Standard glucose stock: 100mg in 100ml water

Working standard: 10 ml stock solution diluted to 100ml with distilled water.

Homogenized 500mg of *Nata-de-coco* sample with hot eighty per cent methanol. Centrifuged the extract. Repeated the extraction and made to 50ml with 80 per cent methanol. Pipetted out 0.2, 0.4, 0.6, 0.8 and 1.0ml of working standards and 0.50ml of sample extract in to a series of test tubes. Made up the volume in each tube to 1.0ml with distilled water. To each test tube, 1.0 ml of phenol solution (5.0%) was added followed by 5.0 ml of 96 per cent sulphuric acid. The tubes were shaken well and kept for 10 minutes. It was then placed in a water bath at 25-30°C for 20 minutes to develop a light yellowish brown colour. Test tubes were cooled and absorbance was read at 490nm. A standard graph was drawn plotting the concentration of working standards in X axis and absorbance in Y axis.

3.5.3 Total microbial population (cfu/g)

The total microbial population of *Nata* was enumerated after harvest of the product. The method used for the evaluation was serial dilution and plate count method as described by Agarwal and Hasija (1986). 1g of sample was added to 90ml sterile water and agitated for 20 minutes. One ml of solution was transferred to a test tube containing 9ml sterile water to get 10⁻² dilution and similarly 10⁻³, 10⁻⁴, 10⁻⁵ and 10⁻⁶ dilutions were also prepared.

Enumeration of total microbial count was carried out by using Nutrient agar media for bacteria, Rose Bengal agar for fungus and Sabouraud's dextrose agar media for yeast. The dilution used for bacteria was 10^{-6} and for fungus and yeast 10^{-3} . Colony counts were taken and colony forming units (cfu) per gram of sample was calculated as shown below

$$\text{cfu/g of sample} = \frac{\text{Number of colonies} \times \text{Dilution factor}}{\text{Volume of sample plated}}$$

3.5.4.0 Organoleptic Evaluation

Organoleptic evaluation of *Nata* was conducted using score card by a panel of fifteen judges.

3.5.4.1 Selection of judges

A series of organoleptic trials were carried out using simple triangle test at laboratory level to select a panel of fifteen judges between the age group of 18 to 35 years as suggested by Jellinek (1985).

3.5.4.2 Preparation of score card

Score card containing eight quality attributes such as appearance, colour, flavour, texture, taste, after taste, chewiness and overall acceptability was prepared for organoleptic evaluation of *Nata* based products. Each of the above mentioned qualities were assessed by a nine point hedonic scale. The score card used for organoleptic evaluation of the products is given in Appendix I and II.

3.6.0 Analysis of data

The observations recorded were tabulated and the data was analysed statistically by using Web Based Agricultural Statistics Software Package (WASP). Correlation analysis was done using OPSTAT statistical software. The scores of organoleptic evaluation were analysed by Kendall's coefficient of confidence.

Results

4. RESULTS

The investigation entitled “Screening substrates and additives for enhancing yield and quality of *Nata-de-coco*” was undertaken in three experiments namely, Screening of coconut water from tall and dwarf cultivars for *Nata-de-coco* production, Screening suitability of different substrates for *Nata-de-coco* production and Improving colour and flavour of *Nata-de-coco* by using additives. The data was statistically analysed to find out the suitability of cultivars, substrates and additives used to improve the yield and quality of *Nata-de-coco*. The results of the above three experiments are analysed and presented in this chapter.

4.1.0 EFFECT OF COCONUT WATER FROM TALL (WCT) AND DWARF (COD) CULTIVARS FOR *NATA-DE-COCO* PRODUCTION

In the present study, coconut water from tall (WCT) and dwarf (COD) cultivars were used by adding 10 per cent sucrose and without sucrose. Acetic acid /bilimbi juice were used as acidulant sources for maintaining a pH of 4.0. The experiment consisted of the following treatments.

- T₁: Coconut water from tall (WCT) cultivar added with sucrose and acetic acid
- T₂: Coconut water from tall (WCT) cultivar added with sucrose and bilimbi juice
- T₃: Coconut water from tall cultivar (WCT) added without sucrose and acetic acid
- T₄: Coconut water from tall cultivar (WCT) added without sucrose and bilimbi juice
- T₅: Coconut water from dwarf cultivar (COD) added with sucrose and acetic acid
- T₆: Coconut water from dwarf cultivar (COD) added with sucrose and bilimbi juice
- T₇: Coconut water from dwarf cultivar (COD) added without sucrose and acetic acid
- T₈: Coconut water from dwarf cultivar (COD) added without sucrose and bilimbi juice.

Ammonium sulphate 0.5 per cent was used as source of nitrogen for the growth of the bacteria. The substrates were inoculated with starter culture and mother liquor and kept for bacterial growth. The *Nata* formed were analysed for various physico chemical parameters.

4.1.1 Days to *Nata* formation

Table. 1 shows the effect of tall (WCT) and dwarf (COD) cultivars on the physico-chemical properties of *Nata-de-coco*. There were significant differences with respect to the days to *nata* formation among treatments. Coconut water from tall (WCT) cultivar added with sucrose and acetic acid-T₁, recorded lowest days for *nata* formation (15.50) which was on par with T₂- coconut water form WCT cultivar added with sucrose and bilimbi juice (16.00). Highest number of days for production of *nata* (17.50) were observed in T₆ -coconut water from dwarf cultivar (COD) added with sucrose and bilimbi juice which was on par with T₅- coconut water from dwarf cultivar (COD) added with sucrose and acetic acid (17.00). There were no *nata* formation in the treatments T₃, T₄, T₇ and T₈ that were inoculated without sucrose. (Plate. 3)

4.1.2 Thickness of *Nata* (mm)

Significant differences were observed among treatments with respect to thickness of the *nata* formed. Highest thickness (8.75) of *nata* was observed in T₅- coconut water from COD added with sucrose and acetic acid, which was on par with T₆- coconut water from COD added with sucrose and bilimbi juice (8.50). Coconut water from WCT added with sucrose and bilimbi juice -T₂, recorded lowest thickness (7.25) which was on par with T₁-coconut water from WCT added with sucrose and acetic acid (8.00). There were no *Nata* formation in the treatments T₃, T₄, T₇ and T₈ that were inoculated without sucrose.



a. *Nata* formed in T₁



b. *Nata* formed in T₂



c. *Nata* formed in T₅



d. *Nata* formed in T₆

Plate. 3 *Nata-de-coco* formed using coconut water from WCT and COD cultivars

4.1.3. Weight of *Nata* (g)

From the Table.1, it is clear that coconut water from WCT and COD cultivars showed significant difference with respect to weight of the *Nata* formed. Highest weight (378.50) of *Nata* was found in T₅.coconut water from COD added with sucrose and acetic acid, which was on par with T₆.coconut water from COD added with bilimbi juice (377.75), followed by T₁. coconut water from WCT added with sucrose and acetic acid (372.00). Coconut water from WCT added with sucrose and bilimbi juice -T₂ showed significantly less weight (352.70) compared to all other treatments.

4.1.4 Total soluble solids (TSS) (°Brix)

It was observed that the TSS (° Brix) of *Nata* varied significantly among treatments. Highest TSS content (8.72) was recorded in T₅- coconut water from COD added with sucrose and acetic acid. T₅ was on par with T₆. coconut water from COD added with sucrose and bilimbi juice (8.62). The lowest mean value for TSS (7.35) was recorded in T₂. coconut water from WCT added with sucrose and bilimbi juice, followed by T₁. coconut water from WCT added with sucrose and acetic acid (8.17).

4.1.5 Acidity (%)

Various treatments recorded no significant effect on the acidity (%) of *Nata* as shown in Table.1. The mean value for acidity of *Nata* was 0.60 % in all the treatments.

4.1.6 Total sugar (g/100g)

The total sugar (g) of *Nata* found to vary significantly among the treatments as shown in the Table.1. *Nata* with highest total sugar (1.26g) was formed in T₅. coconut water from COD added with sucrose and acetic acid, followed by T₆. coconut water COD added with sucrose and bilimbi juice (1.25) which were on par. The lowest sugar content (0.97) was noted in T₂. coconut water from WCT added with sucrose and bilimbi juice, followed by T₁.coconut water from WCT added with sucrose and acetic acid (1.03g), which were significantly different.

Table 1: Physico chemical characters of *Nata-de-coco*

Treatment	Days to <i>Nata-de-coco</i> formation	Thickness of <i>Nata</i> (mm)	Weight (g)	TSS (°Brix)	Acidity (%)	Total sugar (g 100g ⁻¹)	Protein (%)	Fibre (%)	Moisture (%)
T ₁	15.50	8.00	372.00	8.17	0.60	1.03	0.13	2.84	71.94
T ₂	16.00	7.25	352.70	7.35	0.60	0.97	0.12	2.95	70.76
T ₃	-	-	-	-	-	-	-	-	-
T ₄	-	-	-	-	-	-	-	-	-
T ₅	17.00	8.75	378.50	8.72	0.60	1.26	0.05	2.75	72.34
T ₆	17.50	8.50	377.75	8.62	0.60	1.25	0.06	3.03	72.27
T ₇	-	-	-	-	-	-	-	-	-
T ₈	-	-	-	-	-	-	-	-	-
CD (0.05)	1.09	0.94	15.24	0.86	NS	0.05	0.01	NS	NS

T₁: Coconut water from WCT added with sucrose and acetic acid
T₂: Coconut water from WCT added with sucrose and bilimbi juice
T₃: Coconut water from WCT added without sucrose and acetic acid
T₄: Coconut water from WCT added without sucrose and bilimbi juice
T₅: Coconut water from COD added with sucrose and acetic acid
T₆: Coconut water from COD added with sucrose and bilimbi juice
T₇: Coconut water from COD added without sucrose and acetic acid
T₈: Coconut water from COD added without sucrose and acetic acid

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4.1.7 Protein (%)

The protein content (%) of *nata* found to vary significantly among the treatments as shown in the Table.1. The mean value for protein content was recorded highest (0.1) in T₁- coconut water from WCT added with sucrose and acetic acid, which was on par with T₂ coconut water from WCT added with sucrose and bilimbi juice (0.12). Coconut water from COD added with sucrose and acetic acid (T₅) noted the lowest mean value for protein (0.05), followed by T₆- coconut water from COD added with sucrose and bilimbi juice (0.06), which were on par.

4.1.8 Fibre (%)

Various substrates recorded no significant effect in the fibre content (%) of the *nata* as shown in Table.1. The mean value for fibre content of *nata* ranged from 2.75 (T₅-coconut water from COD added with sucrose and acetic acid) to 3.03 T₆- coconut water from COD added with sucrose and bilimbi juice.

4.1.9 Moisture (%)

There was no significant difference in the moisture content (%) of *Nata* formed in various treatments which is clear from the Table 1. The mean value for moisture content of *Nata* varied from 70.76 (T₂- coconut water from WCT added with sucrose and bilimbi juice) to 72.34 (T₅- coconut water from COD added with sucrose and acetic acid). However, T₁-coconut water from WCT added with sucrose and acetic acid recorded moisture content of 71.94 per cent.

4.2. EFFECT OF SOURCES OF COCONUT WATER AND ACIDULANTS ON PHYSICO-CHEMICAL CHARACTERS OF *NATA*

From the Table 2. , it was clear that various physico- chemical parameters of *Nata* like days to *nata* formation (15.75), thickness of *nata* (7.62mm), weight of *Nata* (362.38g), TSS (7.76° Brix)), Total sugars (1.00g/100g) and protein (0.12%) were found affected by sources of coconut water. These parameters recorded highest value when water from COD was used. The lowest mean value for all these parameters except protein was recorded in *Nata* formed from water of WCT. However, protein content was found to be significantly higher when water from WCT was used for *Nata*

Table 2: Effect of sources of coconut water and acidulants on physico-chemical characters of *Nata-de-coco*.

	Days to <i>Nata</i> formation	Thickness of <i>Nata</i> (mm)	Weight (g)	TSS(° brix)	Acidity (%)	Total sugar (g 100g ⁻¹)	Protein (%)	Moisture (%)	Fibre (%)
Mean (Tall)	15.75	7.62	362.38	7.76	0.60	1.00	0.12	71.35	2.90
Mean (Dwarf)	17.25	8.62	378.12	8.68	0.60	1.26	0.05	72.31	2.89
CD (Source of <i>Nata</i>)	0.78	0.68	10.90	0.62	NS	0.04	0.008	NS	NS
Mean (Acetic acid)	16.25	8.38	375.25	8.45	0.60	1.15	0.08	72.15	2.80
Mean (bilimbi juice)	16.75	7.88	365.25	7.99	0.60	1.12	0.09	71.52	2.99
CD (Source of acid)	NS	NS	NS	NS	NS	NS	NS	NS	NS
CD (Interaction)	NS	NS	NS	NS	NS	NS	0.011	NS	NS

formation. Acidity, moisture and fibre content of *Nata* were not influenced by the sources of coconut water.

With respect to the sources of acidulants, it was found that the various physico-chemical parameters of *Nata* were not affected by the sources of acid. So also the interaction effect of sources of coconut water × sources of acidulants was found to be non-significant for all the physico-chemical parameters except protein.

4.2.0 EFFECT OF SUBSTRATES ON THE YIELD AND QUALITY OF *NATA-DE-COCO*

In the present study, coconut water from tall cultivar (WCT) were blended with fruit/vegetable juices as follows.

T₁= Coconut water from WCT alone

T₂= T₁ + jackfruit juice

T₃= T₁+ beetroot juice

T₄= T₁+ mango juice

T₅= T₁+ pineapple juice

T₆= T₁+ papaya juice

T₇= T₁+lovi-lovi juice

T₈= T₁+ watermelon juice.

Various substrates were added with starter culture, mother liquor and kept for growth of the bacteria. The results of the study on screening substrates for enhancing yield and quality of *Nata de-coco* is presented in the Table.3.

4.2.1. Days to *Nata* formation

From the Table.3, it is clear that various treatments significantly affected the days to *Nata* formation. The lowest number of days (15.33) was recorded when coconut water alone was used as a substrate (T₁), followed by coconut water mixed with pineapple juice (T₅) (16.33), coconut water blended with beetroot juice (T₃) (16.66). The highest number of days (19.66) to *Nata* formation was recorded in T₂-coconut

Table. 3 Physico-chemical characters of *Nata* affected by various substrates

Treatment	Days to <i>Nata-de-coco</i> formation	Thickness of <i>Nata</i> (mm)	Weight (g)	TSS (°Brix)	Acidity (%)	Total sugar (g 100g ⁻¹)	Protein (%)	Fibre (%)	Moisture (%)
T ₁	15.33	8.00	365.66	8.24	0.60	1.02	0.11	2.37	72.00
T ₂	19.66	8.33	373.32	9.15	1.22	1.97	0.26	3.63	71.57
T ₃	16.66	8.66	379.67	9.27	1.20	1.66	0.16	3.00	70.30
T ₄	18.00	11.00	401.67	9.58	1.22	2.18	0.19	3.35	73.04
T ₅	16.33	8.33	376.00	9.25	1.23	1.82	0.15	3.31	70.28
T ₆	17.00	9.33	387.65	9.35	1.22	1.96	0.21	3.82	72.27
T ₇	17.33	8.33	374.00	9.19	1.22	1.85	0.17	2.78	71.90
T ₈	17.00	11.33	416.33	9.59	1.21	2.16	0.17	2.61	72.43
C.D (0.05)	1.45	1.49	14.86	0.02	0.01	0.15	0.01	0.22	NS

T₁: Coconut water alone

T₂: T₁ + Jack fruit juice

T₃: T₁+ Beetroot juice

T₄: T₁+ Mango juice

T₅: T₁+ Pineapple juice

T₆: T₁+ Papaya juice

T₇: T₁+ Lovi-lovi juice

T₈: T₁+ Water melon juice

water from WCT blended with jackfruit juice, followed by T₄- coconut water blended with mango juice (18.00) and T₇- coconut water blended with lovi-lovi juice (17.33). The treatments T₄, T₆, T₇ and T₈ (coconut water mixed with mango juice, papaya juice, lovi-lovi juice and watermelon juice) respectively recorded 18.00, 17.00, 17.33, and 17.00 number of days to *Nata* formation and were on par with each other.

4.2.2 Thickness of *Nata* (mm)

Various substrates mixed with coconut water significantly affected the thickness of *Nata* formed as it is clear from Table.3. The highest thickness of *Nata* (11.33) was recorded when coconut water blended with watermelon juice (T₈), followed by 11.00 in T₄ (coconut water mixed with mango juice) which were on par with each other. The lowest thickness (8.00) was obtained when coconut water alone was used as substrate (T₁), followed by T₂- coconut water mixed with jack fruit juice (8.33), T₅- coconut water mixed with pineapple juice (8.33) and T₇-coconut water mixed with lovi-lovi juice (8.33), T₃-coconut water mixed with beetroot juice (8.66), T₆-coconut water mixed with papaya juice (9.33) which were on par with each other (Plate 4).

4.2.3 Weight of *Nata* (g)

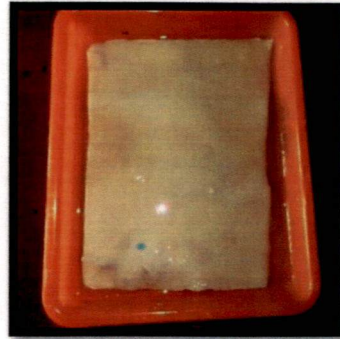
Highest weight of *Nata* (416.33g) was obtained when coconut water was mixed with watermelon juice (T₈), followed by T₄-coconut water mixed with mango juice (401.67g) which were on par. However, T₄ was on par with T₆-coconut water mixed with papaya juice (387.65g). All other treatments namely, T₁, T₂, T₃, T₅, and T₇ were on par with each other and recorded respective weights of 365.66, 373.32, 379.67, 376.00 and 374.00, however, treatment T₁-coconut water alone recorded the lowest weight (365.66) of *Nata*.

4.2.4 Total Soluble Solids (TSS) (°Brix)

When various substrates were blended with coconut water, it was observed that the TSS (°Brix) of the *Nata* formed varied significantly as given in the Table.3. The highest TSS (9.59 °Brix) was observed when coconut water was mixed with water



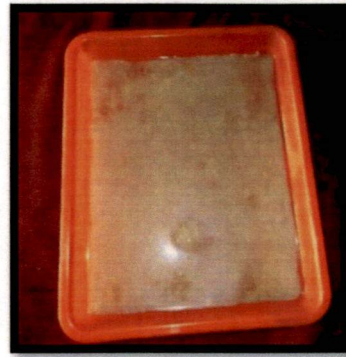
a. Coconut water lone (T₁)



b. T₁+ jackfruit juice (T₂)



c. T₁+ beetroot juice (T₃)



d. T₁+ mango juice (T₄)

Plate. 4 *Nata* formed using various substrates

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melon juice (T₈), for *Nata* production followed by 9.58 °Brix when coconut water mixed with mango juice (T₄) which were on par. The next high mean value for TSS (9.35 °Brix) was recorded when coconut water was mixed with papaya juice (T₆). The treatments T₃-coconut water was mixed with beetroot juice (9.27 °Brix) and T₅-coconut water mixed with pineapple juice (9.25 °Brix) were on par. Upon comparing the CD value, it was observed that TSS of the *nata* formed in T₂-coconut water mixed with jackfruit juice (9.15 °Brix) and T₇-coconut water mixed with lovi-lovi juice (9.19 °Brix) significantly different. *Nata* with lowest TSS (8.24 °Brix) was formed when coconut water alone was used as substrate.

4.2.5 Acidity (%)

It was observed that acidity of the *Nata* formed by using various substrates varied as it is clear from Table.3. *Nata* with highest acidity (1.23) was formed in T₅-coconut water mixed with pineapple juice. Acidity of the *Nata* formed in T₂-coconut water mixed with jackfruit juice (1.22), T₃- coconut water blended with beetroot juice (1.20), T₄-coconut water mixed with mango juice (1.22), T₆-coconut water mixed with papaya juice (1.22), T₇-coconut water mixed with lovi-lovi juice (1.22) and T₈-coconut water mixed with watermelon juice (1.21) were on par with each other. *Nata* with lowest acidity (0.60) was formed when coconut water alone was used as substrate.

4.2.6 Total sugar (g/100g)

The total sugar (g/100g) of *Nata* found to vary significantly among the treatments as shown in the Table.3. *Nata* with highest total sugar (2.18) was formed when coconut water mixed with mango juice (T₄), followed by 2.16 g/100g when coconut water was mixed with water melon juice (T₈) were on par. The next highest mean value of total sugar (1.97) was found when coconut water mixed with jack fruit juice (T₂), followed by 1.96 when coconut water was mixed with papaya juice (T₆), 1.85 when coconut water mixed with lovi-lovi juice (T₇) and 1.82 when coconut water was mixed with pineapple juice (T₅) and all these treatments were on par with each other. However, the treatment T₃-coconut water mixed with beetroot juice showed

marked difference in total sugar of the *Nata* formed (1.66) from other treatments. The lowest mean value for total sugar was found in T₁-coconut water alone (1.02).

4.2.7 Protein (%)

Protein content varied significantly when different substrates were used for *Nata* production as seen in Table.3. *Nata* with highest protein (0.26) was formed when coconut water mixed with jackfruit juice (T₂) followed by T₆-coconut water mixed with papaya juice (0.21) which were significantly different. The next highest mean value for protein content (0.19) was found in *Nata* formed when coconut water was mixed with mango juice (T₄), followed by T₇-coconut water mixed with lovi-lovi juice (0.19) and T₈- coconut water mixed with water melon juice (0.19). The lowest protein content was found when coconut water alone was used as substrate (0.11) followed by T₅-coconut water mixed with pineapple juice (0.15).

4.2.8 Fibre (%)

The fibre content (%) of *Nata* formed varied significantly when different substrates used varied significantly as shown in table.3. *Nata* with highest fibre content (3.82) was formed when coconut water was mixed with papaya juice (T₆), followed by 3.63% when coconut water was mixed with jackfruit juice (T₂), which were on par. The next highest mean value for fibre content (3.35) was noted in *nata* formed when coconut water was mixed with mango juice (T₄) followed by 3.31 in T₅-coconut water mixed with pineapple juice which were on par. The treatment T₃-coconut water mixed with beetroot juice (3.00) showed significantly different mean value for fibre content when compared with others. T₇-coconut water mixed with lovi-lovi juice (2.78) and T₈-coconut water mixed with watermelon juice (2.61) were on par with each other. The lowest mean value for fibre content (2.37) was found in *Nata* formed when coconut water alone used as substrate.

4.2.9 Moisture (%)

Various substrates recorded no significant effect in the moisture content (%) of the *Nata* formed as it is clear from table.3. However, the mean value for moisture

content of *nata* ranged from 70.28 to 73.04 which was found in T₃-coconut water mixed with beetroot juice and coconut water mixed with mango juice (T₄) respectively.

4.2.2.0. Microbial population (cfu/g)

Microbial population in *Nata* was enumerated by plate count method immediately after harvest (table 4). Bacterial and yeast population were observed, whereas fungi population were not observed. (Plate. 6)

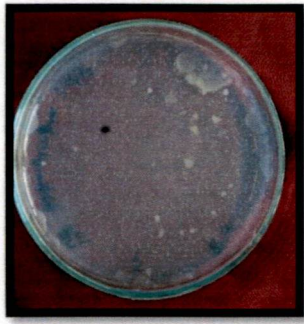
Highest bacterial population were recorded in T₈- coconut water blended with water melon juice (1.89), followed by T₄- coconut water blended with mango juice (1.76), T₇- coconut water blended with lovi-lovi juice (1.48) and T₆- coconut water blended with papaya juice (1.44). However, lowest bacterial count was observed in T₁- coconut water alone used as substrate (1.24). (Plate. 5)

Highest yeast count was observed in T₈- coconut water blended with water melon juice (1.71) followed by T₄- coconut water blended with mango juice (1.45 cfu/g), T₆- coconut water blended with papaya juice (1.39) and T₃- coconut water blended with beet root juice (1.31). Lowest yeast count was observed in T₁- coconut water alone used as substrate (1.18), followed by T₂-coconut water blended with jackfruit juice (1.26). (Plate. 7)

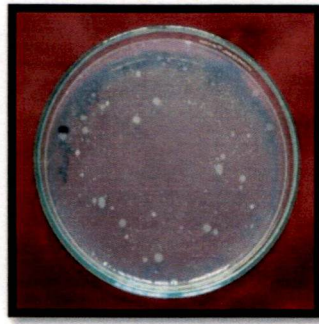
4.2.2.0 ORGANOLEPTIC SCORING OF *NATA-DE-COCO* FORMED FROM VARIOUS SUBSTRATES

The mean score obtained for various sensory attributes like appearance, colour, flavour, taste, after taste, texture, chewiness and overall acceptability of *Nata* are presented in Table.5. Scores obtained for various quality attributes of *Nata* was found to be statistically significant among treatments.

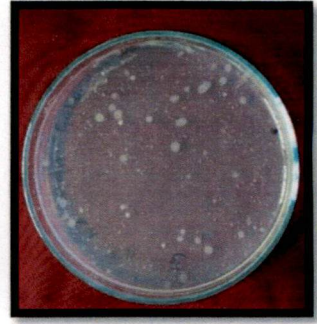
As evident from the Table.5, T₅ - coconut water blended with pineapple juice recorded highest mean score of 8.06 (7.43) followed by (T₇) – coconut water blended with lovi-lovi juice 7.81(7.14) for appearance and were on par with other. T₁- coconut



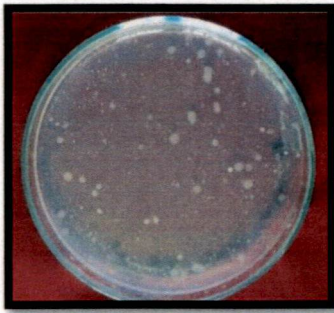
Coconut water alone (T₁)



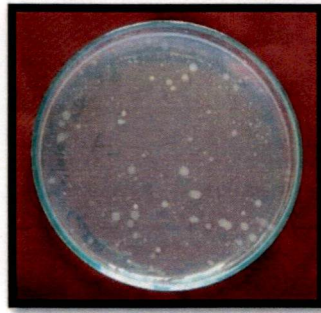
T₁+ jackfruit juice (T₂)



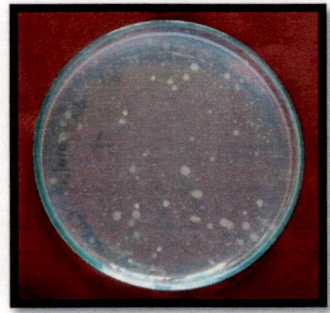
T₁+ beetroot juice (T₃)



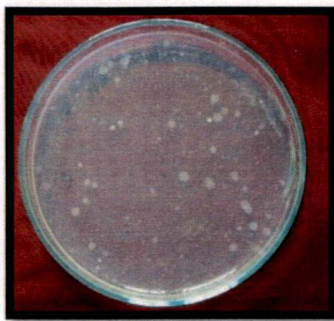
T₁+ mango juice (T₄)



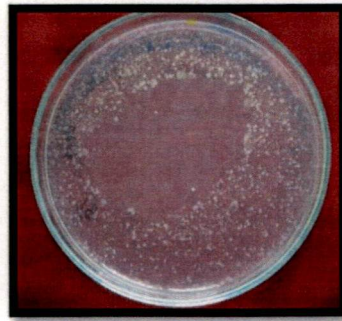
T₁+ pineapple juice (T₅)



T₁+ papaya juice (T₆)

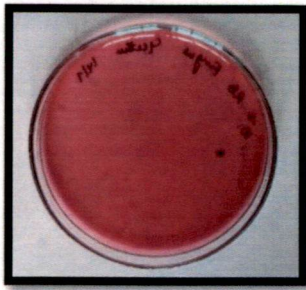


T₁+ lovi-lovi juice (T₇)



T₁+ water melon juice (T₈)

Plate 5 Bacterial population in Nata formed using various substrates



Coconut water alone (T₁)



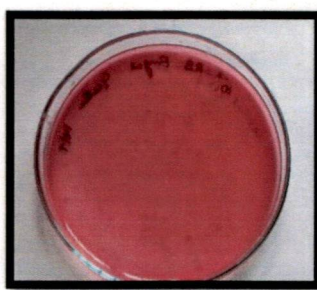
T₁+ jackfruit juice



T₁+ beetroot juice



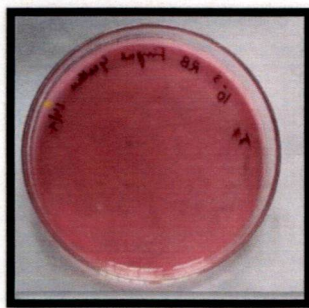
T₁+ mango juice



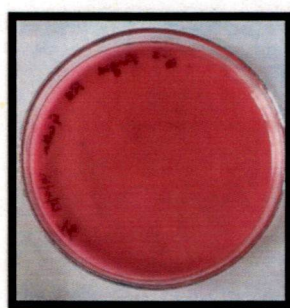
T₁+ pineapple juice



T₁+ papaya juice



T₁+ lovi-lovi juice



T₁+ water melon juice

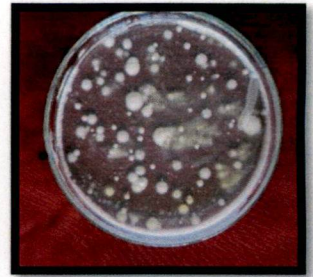
Plate. 6 Fungal population in *Nata* from various substrates



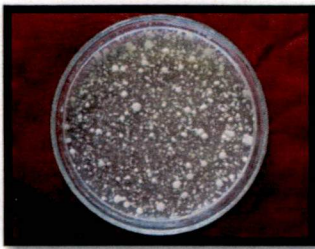
Coconut water alone (T₁)



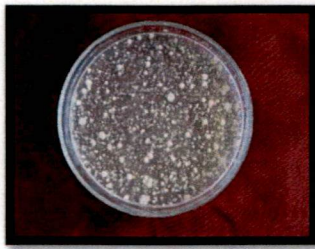
T₁+ jackfruit juice



T₁+ beetroot juice



T₁+ mango juice



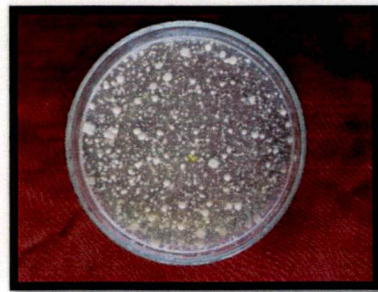
T₁+ pineapple juice



T₁+ papaya juice



T₁+ lovi-lovi juice



T₁+ water melon juice

Plate. 7 Yeast population in *Nata* from various substrates

Table. 4 Effect of different substrates on microbial population in *Nata*

Microbial population (cfu/g)			
Treatments	Bacteria x10 ⁵	Fungus x10 ³	Yeast x10 ²
T ₁	1.24	Absent	1.18
T ₂	1.26	Absent	1.26
T ₃	1.35	Absent	1.31
T ₄	1.76	Absent	1.45
T ₅	1.42	Absent	1.28
T ₆	1.44	Absent	1.39
T ₇	1.48	Absent	1.32
T ₈	1.89	Absent	1.71

T₁: Coconut water alone

T₂: T₁ + Jack fruit juice

T₃: T₁+ Beetroot juice

T₄: T₁+ Mango juice

T₅: T₁+ Pineapple juice

T₆: T₁+ Papaya juice

T₇: T₁+ Lovi-lovi juice

T₈: T₁+ Water melon juice

Table. 5 Sensory qualities of *Nata* produced from various substrates

Treatment	Appearance	Colour	Flavour	Taste	After taste	Texture	Chewiness	Overall acceptability	Total score
T ₁	6.26 (3.07)	6.06 (2.47)	5.8 (1.90)	6.06 (2.23)	5.86 (2.64)	6.13 (2.23)	6.24 (2.87)	6.23 (2.76)	48.64
T ₂	6.66 (4.04)	6.66 (4.10)	6.33 (3.13)	6.93 (4.07)	6.26 (3.43)	6.73 (4.60)	6.53 (3.63)	6.86 (4.05)	52.96
T ₃	6.80 (4.96)	6.62 (3.87)	6.60 (3.87)	6.46 (3.43)	6.46 (4.21)	6.66 (3.30)	6.61 (3.83)	6.66 (3.29)	52.87
T ₄	6.73 (4.93)	6.81 (4.33)	6.61 (3.80)	6.80 (3.67)	6.46 (4.25)	6.60 (4.27)	6.46 (3.57)	6.73 (4.87)	53.20
T ₅	8.06 (7.43)	7.46 (6.30)	8.40 (7.53)	8.20 (7.20)	7.26 (6.46)	7.43 (7.37)	6.44 (3.37)	8.40 (7.62)	61.65
T ₆	6.66 (4.93)	6.46 (3.23)	6.73 (4.23)	6.46 (3.50)	6.41 (4.21)	6.93 (3.33)	6.61 (3.93)	6.66 (4.91)	52.92
T ₇	7.81 (7.14)	7.40 (5.93)	7.93 (7.00)	7.60 (7.40)	7.06 (6.18)	8.53 (6.33)	6.35 (3.07)	8.26 (6.21)	60.94
T ₈	7.00 (5.36)	7.26 (5.77)	6.86 (4.53)	6.93 (4.50)	6.62 (4.61)	7.00 (4.57)	6.80 (4.50)	7.00 (4.58)	55.47
Kendall's W test	0.39	0.42	0.69	0.70	0.34	0.70	0.47	0.65	

T₁: Coconut water alone

T₂: T₁ + Jack fruit juice

T₃: T₁+ Beetroot juice

T₄: T₁+ Mango juice

T₅: T₁+ Pineapple juice

T₆: T₁+ Papaya juice

T₇: T₁+ Lovi-lovi juice

T₈: T₁+ Water melon juice

water alone recorded lowest mean score 6.26 (3.07). All other treatments T₂, T₆, T₄ and T₃ were on par.

Among the eight treatments, T₅- coconut water blended with pineapple juice showed maximum mean score for colour 7.46 (6.30) followed by T₇-coconut water blended with lovi-lovi juice with mean score of 7.40 (5.93) and T₈- coconut water blended with water melon juice with a mean score of 7.26 (5.77) were on par. T₁- recorded lowest mean score of 6.06 (2.47) for colour of *nata*. All other treatments T₄, T₂ and T₃ were on par.

Superior score for flavour was recorded in (T₅) - coconut water blended with pineapple juice 8.40 (7.53) followed by T₇- coconut water blended with lovi-lovi juice with a mean score of 7.93 (7.00). The treatment T₈- coconut water blended with water melon 6.8 (4.53) followed by T₆, T₄ and T₃ were on par. T₁- coconut water alone used as a substrate recorded lowest mean score of 5.80 (1.90), followed by T₂ with a score 6.33 (3.13) and were on par.

Highest score for taste was recorded in T₅ - coconut water blended with pineapple juice with a mean score of 8.20 (7.20) followed by T₇- coconut water blended with lovi-lovi juice 7.60 (7.40) were on par with other. T₁ recorded lowest mean score of 6.06 (2.23) followed by T₃- coconut water blended with beetroot juice with mean score of 6.46 (3.43) and T₆- coconut water blended with papaya juice 6.46 (3.50).

After taste was recorded highest in T₅ – coconut water blended with pineapple juice with a score of 7.26 (6.46), followed by T₇ coconut water blended with lovi-lovi juice with a mean score of 7.06 (6.18). Treatments T₂ 6.26 (3.43), T₃ 6.46 (4.21), T₄ 6.46 (4.25), T₆ 6.41 (4.21) were on par. Treatment T₈.coconut water blended with water melon juice recorded a mean score of 6.62 (4.61) for after taste. Lowest after taste was recorded in T₁- coconut water alone used as substrate with a mean score of 5.86 (2.64).

Superior score for texture was recorded in T₇- coconut water blended with lovi-lovi juice with a highest mean score of 8.53 (6.33) followed by T₅- coconut water blended with pineapple juice with mean score of 7.43 (7.37). Lowest mean score of

6.13 (2.23) was observed in T₁ – coconut water alone used as a substrate. The treatments T₈, T₆, T₂, T₃, and T₄ were on par with other.

Nata prepared by blending coconut water with watermelon juice (T₈) recorded highest score for chewiness 6.80 (4.50) followed by T₆- coconut water blended with papaya juice with mean score of 6.61 (3.93) and T₃ –coconut water blended with beetroot juice with mean score of 6.61 (3.83) were on par. Coconut water alone used as a substrate recorded lowest chewiness with a mean score of 6.24 (2.87). However it was on par with other treatments.

The overall acceptability was highest for T₅ - coconut water blended with pineapple juice with mean score of 8.40 (7.62) followed by T₇- coconut water blended with lovi-lovi juice 8.26 (6.21). There were no significant difference between the treatments T₈, T₂, T₄, T₃ and T₆. Lowest overall acceptability was recorded in coconut water alone used as substrate (T₁) with mean score of 6.23 and mean rank of 2.76.

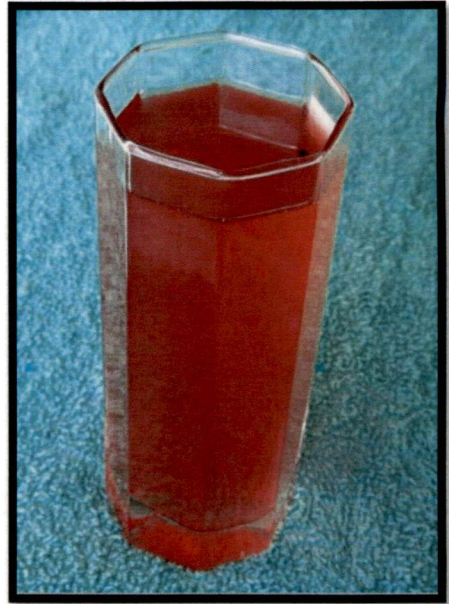
Coconut water blended with pineapple juice (T₅) recorded highest total score (61.65) followed by (T₇) coconut water blended with lovi-lovi juice (60.94) followed by T₈- coconut water blended with water melon juice (55.47). Lowest total score was observed in (T₁) – coconut water alone used as substrate (48.64). The treatments T₂, T₃ and T₄ recorded total scores of 52.96, 52.87 and 53.20 respectively.

4.3.0. IMPROVING COLOUR AND FALVOUR OF *NATA-DE-COCO* BY USING ADDITIVES.

The present experiment was conducted using coconut water from tall (WCT) and dwarf (COD) cultivars added with sucrose, acetic acid, 0.5 per cent Ammonium sulphate and inoculated with *Acetobactor xylinium* culture, mother liquor and kept for growth of bacteria and production of *Nata*. *Nata* was harvested, washed several times in water to remove sourness. Then cut into pieces, packed in three syrups (° Brix) namely, Sugar syrup, rose apple syrup, and west Indian cherry syrup (Plate 8) and such packed *Nata* were flavoured with two additives namely, ginger extract and vanilla extract. The present experiment consisted of the following treatments namely,



Sugar syrup



Roseapple syrup



West Indian Cherry syrup

Plate 8. Syrups used as additives for *Nata-de-coco* production

GH

- T₁= *Nata* formed from coconut water from WCT packed in Sugar syrup, flavoured with Ginger extract.
- T₂= *Nata* formed from coconut water from WCT packed in Sugar syrup, flavoured with Vanilla extract.
- T₃= *Nata* formed from coconut water from WCT packed in Rose apple syrup, flavoured with Ginger extract
- T₄= *Nata* formed from coconut water from WCT packed in Rose apple syrup flavoured with Vanilla extract
- T₅= *Nata* formed from coconut water from WCT packed in West Indian cherry syrup flavoured with Ginger extract
- T₆= *Nata* formed from coconut water from WCT packed in West Indian cherry syrup flavoured with Vanilla extract
- T₇= *Nata* formed from coconut water from COD packed in Sugar syrup flavoured with Ginger extract
- T₈= *Nata* formed from coconut water from COD packed in sugar syrup flavoured with vanilla extract
- T₉= *Nata* formed from coconut water from COD packed in Rose apple syrup flavoured with Ginger extract
- T₁₀= *Nata* formed from coconut water from COD packed in Rose apple syrup flavoured with Vanilla extract
- T₁₁= *Nata* formed from coconut water from COD packed in West Indian cherry syrup flavoured with Ginger extract
- T₁₂= *Nata* formed from coconut water from COD packed in West Indian cherry syrup flavoured with Vanilla extract.

The *Nata* formed from WCT and COD cultivars packed in three syrups and flavoured with two flavours (Plate 9) were analysed for various sensory qualities such as appearance, colour, flavour, taste, texture, overall acceptability were evaluated by a panel of selected 15 judges on the nine point hedonic scale (Plate. 10). The results of the experiment are presented in the Table.6.



Nata packed with sugar syrup



Nata packed with roseapple syrup



Nata packed with West Indian cherry syrup



Plate 10. Organoleptic scoring of *Nata* added with additives and flavours

Table.6 Effect of additives and flavours on sensory qualities of *Nata-de-coco*

Treatment	Appearance	Colour	Flavour	Taste	After taste	Texture	Chewiness	Overall acceptability	Total score
T ₁ (TC, S,G)	7.00 (4.79)	6.15 (3.15)	6.28 (2.25)	6.35 (3.10)	7.00 (3.28)	6.75 (5.15)	6.81 (3.88)	6.95 (3.83)	53.30
T ₂ (TC, S,V)	7.13 (5.20)	6.60 (3.70)	6.46 (3.61)	6.51 (3.14)	7.13 (3.31)	6.54 (3.25)	6.91 (4.61)	7.01 (5.01)	54.30
T ₃ (TC, R, G)	8.20 (9.03)	8.06 (9.37)	6.86 (4.30)	6.73 (4.10)	6.53 (5.13)	7.26 (5.53)	6.86 (3.97)	7.40 (5.13)	57.90
T ₄ (TC, R, V)	8.26 (9.37)	8.73 (9.54)	8.40 (9.40)	8.53 (9.56)	7.46 (8.93)	7.80 (7.83)	7.73 (8.30)	8.13 (8.40)	65.04
T ₅ (TC, W,G)	7.33 (5.93)	7.20 (6.00)	7.06 (4.83)	7.13 (5.50)	6.73 (6.13)	7.67 (6.13)	7.60 (7.73)	7.66 (6.27)	58.38
T ₆ (TC,W,V)	7.33 (5.77)	7.06 (5.43)	7.93 (7.77)	7.73 (7.80)	6.86 (6.30)	7.66 (7.50)	7.26 (6.03)	7.46 (5.20)	59.29
T ₇ (DC,S,G)	6.42 (2.33)	6.40 (3.07)	6.26 (2.23)	7.00 (3.27)	6.20 (3.20)	7.40 (5.90)	7.26 (5.97)	7.06 (3.37)	54.00
T ₈ (DC,S,V)	6.81 (3.63)	6.46 (3.23)	6.53 (3.13)	6.73 (3.90)	6.33 (4.50)	7.40 (6.40)	7.33 (6.40)	7.13 (6.16)	54.72
T ₉ (DC,,R, G)	8.21 (9.07)	8.26 (9.23)	6.73 (3.77)	6.60 (3.30)	6.40 (4.67)	7.40 (6.37)	6.93 (4.70)	7.46 (5.47)	57.99
T ₁₀ (DC,R,V)	8.46 (9.41)	8.60 (9.51)	8.46 (9.57)	7.73 (7.67)	7.66 (9.47)	7.86 (8.47)	7.35 (8.03)	8.53 (9.67)	65.30
T ₁₁ (DC,W,G)	6.73 (7.03)	7.33 (6.53)	7.13 (5.10)	7.00 (4.87)	6.74 (6.15)	7.46 (6.70)	7.53 (7.17)	7.66 (6.37)	57.58
T ₁₂ (DC,W,V)	7.32 (5.75)	7.13 (5.70)	8.33 (9.13)	7.60 (7.33)	7.06 (7.43)	7.66 (7.47)	7.06 (7.03)	7.73 (7.20)	60.69
Kendall's W test	0.542	0.78	0.69	0.64	0.30	0.14	0.31	0.40	

TC: *Nata* formed using coconut water from tall cultivar (WCT)

S: Sugar syrup, R: Roseapple syrup, W: West Indian cherry syrup

DC: *Nata* formed using coconut water from dwarf cultivar (COD)

G: Ginger extract V: Vanilla extract

The scores obtained for various quality attributes of *Nata* were found to vary significantly among treatments.

Appearance of *Nata* packed in syrups and added with additives varied significantly among treatments. It was found that the highest mean score of 8.46 and mean rank of 9.41 for appearance was found in *nata* formed using coconut water from dwarf cultivar (COD) packed in rose apple syrup and flavoured with vanilla extract (T₁₀), followed by T₄- *Nata* formed from coconut water of tall (WCT) cultivar packed in rose apple syrup and flavoured with vanilla extract with a mean score of 8.26 (9.37), T₉- *Nata* formed from coconut water of dwarf (COD) cultivar packed in Rose apple syrup and flavoured with ginger extract with a mean score of 8.21 (9.07), T₃-*Nata* formed using coconut water from tall(WCT) cultivar packed in rose apple syrup and flavoured with ginger extract with a mean score of 8.20 (9.03), which were on par. The lowest mean score of 6.42 (2.33) for appearance of *nata* was recorded when coconut water from COD packed in sugar syrup flavoured with ginger extract (T₇), followed by T₁₁- *Nata* formed from coconut water from COD packed in West Indian cherry syrup flavoured with ginger extract with a mean score of 6.73 (7.03), T₈- *Nata* formed from coconut water from COD packed in sugar syrup flavoured with vanilla extract with a mean score of 6.81 (3.63), which were on par. Overall mean score for appearance of *Nata* in the treatments T₁, T₂, T₅, T₆, and T₁₂ were 7.00 (4.79), 7.13 (5.20), 7.33 (5.93), 7.33 (5.77) and 7.32 (5.75) respectively were on par.

Colour of *Nata* recorded significant variation among treatments. The highest mean score of 8.73(9.54) was recorded in T₄- *Nata* formed using coconut water from WCT packed in rose apple syrup flavoured with vanilla extract, followed by T₁₀- *Nata* formed from coconut water from COD packed in rose apple syrup flavoured with vanilla extract with a mean score 8.60 (9.51), T₉- *Nata* formed from coconut water from COD packed in rose apple syrup flavoured with ginger extract with mean score of 8.26 (9.23) and T₃- *Nata* formed from coconut water from WCT packed in rose apple syrup, flavoured with ginger extract with a mean score of 8.06 (9.37) and were on par. The treatments T₁₁, T₁₂, T₅ and T₆ with mean score of 7.33 (6.53), 7.13 (5.70),

7.20 (6.00), and 7.06 (5.43) respectively were on par. The lowest mean score for colour of *Nata* was recorded in T₁- *Nata* formed from coconut water from WCT packed in sugar syrup, flavoured with ginger extract 6.15 (3.15), followed by T₇-with a mean score of 6.40 (3.07), T₈ with mean score of 6.46 (3.23) and T₂ with a mean score of 6.60 (3.70) and were on par.

Flavour of *Nata* from various treatments varied significantly. The highest mean score 8.46 (9.57) for flavour was recorded in T₁₀- *Nata* formed from coconut water from COD packed in rose apple syrup flavoured with vanilla extract, followed by T₄-with mean score of 8.40 (9.40), T₁₂- with a score of 8.33(9.13) and T₆-with a score of 7.93(7.77) and were on par. The treatments, T₁₁ with mean score of 7.13(5.10), followed by T₅-with a score of 7.06 (4.83), T₃-with a score of 6.86 (4.30) and T₉ with a score of 6.73 (3.77) respectively were on par. The lowest mean score for flavour was obtained in *Nata* formed from T₁- coconut water from WCT packed in sugar syrup flavoured with ginger extract.

The taste of *Nata* packed varied significantly among treatments. The highest mean score 8.53 (9.56) was recorded in T₄- *Nata* formed from coconut water from WCT packed in rose apple syrup flavoured with vanilla extract, followed by T₆ with a score of 7.73 (7.80) and T₁₀ with score of 7.73(7.67) and were on par. The treatments T₅ with a score of 7.13(5.50), T₁₁ with 7.00(4.87), T₃ with 6.73(4.10) respectively were on par. The treatment T₁₂ with 7.60 (7.33) was different from other treatments. The lowest mean score of 6.35(3.10) was recorded in T₁- *Nata* formed from coconut water from WCT packed in sugar syrup, flavoured with ginger extract, followed by T₂ with a score of 6.51(3.14) were on par.

After taste of *Nata* packed and flavoured with various syrups and flavours varied significantly. The highest score of 7.66(9.47) was recorded in T₁₀- *Nata* formed from coconut water from COD packed in rose apple syrup flavoured with vanilla extract, followed by T₄ with a score of 7.46(8.93) were on par. T₂ with a score of 7.13 (3.31) and T₁₂ with a score of 7.06 (7.43) were also on par. The treatments, T₁, T₂ and T₁₂ were on par and T₁₁, T₆ and T₅ were on par respectively recorded the scores of

7.00(3.28), 6.86 (6.30), 6.74 (6.15), 6.73(6.13), 6.53(5.13) and 6.33 (4.50) respectively were on par. The lowest mean score of 6.20 (3.20) was recorded in T₇- *Nata* formed from coconut water from COD packed in sugar syrup flavoured with ginger extract.

The texture of *Nata* packed and flavoured with various syrups and flavours varied significantly. It was found that the highest mean score of 7.86 (8.47) was found in T₁₀- *Nata* formed from coconut water from COD packed in rose apple syrup flavoured with vanilla extract, followed by T₄-with a score of 7.80(7.83), were on par. The treatments T₅-with a score of 7.67(6.13), T₆- with a score of 7.66 (7.50) and T₁₂-with score of 7.66 (7.47) were on par. So also the treatments T₈- with a score of 7.40 (6.40), T₉ with a score of 7.40 (6.37), T₇ -with a score of 7.40 (5.90) and T₃-with a score of 7.26 (5.53) were on par. The lowest score of 6.54 (3.25) was recorded in T₂- *Nata* formed from coconut water from WCT packed in sugar syrup, flavoured with vanilla extract, followed by T-with a score of 6.75(5.15).

The parameter chewiness of *Nata* varied significantly in various treatments. The highest mean score of 7.73 (8.30) was recorded in T₄- *Nata* formed from coconut water from WCT packed in rose apple syrup flavoured with vanilla extract, followed by T₅-with score of 7.60 (7.73), T₁₁ with score of 7.53 (7.17) and were on par. The overall mean for chewiness of *Nata* were in the treatments T₈-with a score of 7.33 (6.40) and T₁₀- with a score of 7.35 (8.03) were on par. The same score for chewiness of *Nata* was recorded in T₆ 7.26 (6.03)) and T₇ 7.26 (5.97), however these treatments recorded ranks differently. Among the treatments, the lowest mean value for chewiness of *Nata* was found in T₁- *Nata* formed using coconut water from WCT packed in sugar syrup, flavoured with ginger extract with a score of 6.81 (3.88), followed by T₃-with a score of 6.86 (3.97), T₂ with a score of 6.91(4.61) and T₉ with a score of 6.93 (4.70).

The overall acceptability of *Nata* packed and flavoured with various syrups and flavours varied significantly. It was observed that the highest mean score of 8.53 (9.67) was in T₁₀- *Nata* formed using coconut water from COD packed in rose apple syrup flavoured with vanilla extract, followed by T₄- *Nata* formed from coconut water from WCT packed in rose apple syrup flavoured with vanilla extract with a score of 8.13

(8.40), which were on par. The mean scores obtained for overall acceptability in the treatments T₁₂-with a score of 7.73 (7.20), T₁₁-with a score of 7.66(6.37), T₅ with a score of 7.66(6.27), T₆ with a score of 7.46 (5.20) and T₃-with a score of 7.40 (5.13) were on par. The lowest mean score of 6.95 (3.83) was found in T₁, followed by T₂ with a score of 7.01(5.01) and T₇ with a score of 7.06(3.37) which were on par.

The total score obtained for various treatments which were packed and flavoured with various syrups and flavours varied. The highest total score of 65.30 was recorded in T₁₀- *Nata* formed from coconut water from COD packed in rose apple syrup flavoured with vanilla extract, followed by T₄ - *Nata* formed from coconut water from WCT packed in rose apple syrup flavoured with vanilla extract with a score of 65.04 and T₁₂ with a score of 60.69 followed by T₆-*Nata* formed using coconut water from tall cultivar packed in West Indian cherry syrup flavoured with vanilla extract (59.29). The lowest score was obtained in the treatment T₁- *Nata* formed from coconut water from WCT packed in sugar syrup, flavoured with ginger extract (53.30).

Discusión

5. DISCUSSION

Coconut, Kalpavriksha-the tree of heaven in which every part of the tree is useful to mankind. Two main products commercially traded are copra and oil. The price of raw coconut in the market is decided by the price of these two main commodities in the international market. To make coconut cultivation more remunerative, it is essential that all the products and byproducts from the palm is fully exploited and commercialised.

Coconut water is an important by-product of copra and oil industry, which is merely, wasted at the copra production centers. Bacterial cellulose produced by the *Acetobacter xylinum* at the air liquid interphase of coconut water is popularly known as *Nata-de-coco*. *Nata* is gaining popularity because of its high dietary fibre content and low calorific value. It is an excellent ingredient for ice creams, cocktails and other food products. Raw *Nata* is transparent without any colour and flavour. In order to enhance consumer acceptability, improving color and flavor of *Nata-de-coco* by using under exploited fruit and vegetables and addition of natural flavours can go a long way in improving palatability and consumer acceptability of the product. Therefore, the present investigation entitled "Screening substrates and additives for enhancing yield and quality of *Nata-de-coco*" was undertaken to study the performance of coconut water from tall, dwarf cultivars to improve yield and quality of *Nata -de-coco*, to evaluate the suitability of substrates for blending with coconut water for *Nata* production and to evaluate the suitability of additives to improve consumer acceptability of *Nata-de-coco*. The data was statistically analyzed and the results of the study are discussed hereunder.

5.1.0. EFFECT OF COCONUT WATER FROM TALL (WCT) AND DWARF (COD) CULTIVARS ON YIELD AND QUALITY ON *NATA-DE-COCO*.

5.1.1: Effect on physico- chemical qualities of *Nata-de-coco*:

The study revealed that coconut water from tall (WCT), dwarf (COD) cultivars showed significant effect on the physical characters of *Nata* formed. It was observed that days to *Nata* formation (17.25) (Fig.1), thickness of *Nata* (8.62) and weight of *Nata* (378.12) were significantly higher when coconut water from COD cultivar was used for the production of *Nata*. These parameters recorded lower mean values when coconut water from WCT was used for *Nata- de-coco* production. This may be due to the fact that coconut water from dwarf cultivars contain more sugars than that present in tall cultivars. Similar results were reported by Thampan (1998), Sabapathy and Kumar (1999). In tall coconut cultivars total sugar content of coconut water increased from 5 months to reach the maximum at seven months. Subsequently, total sugar rapidly decrease at the age of 12 months. This results is in conformity with the results of Pradesh *et al.*, (2012) in which they reported that coconut water from tall cultivars had the lowest total sugar content compare to the dwarf cultivars.

Coconut water from dwarf cultivars possess more Total Soluble Solids (TSS) 5.25° Brix compared to coconut water from tall cultivars (WCT) 4.83°Brix as measured in the present study. Similar results on the quality criteria of tall and dwarf cultivars were reported by Pradesh *et al.*, (2012). Cellulose production by *Acetobacter xylinum* has been known to be affected by concentration of sugar as reported by Embuscado *et al.* (1994). However, days taken for *Nata* formation, was also higher when coconut water from dwarf cultivars used for *Nata* production compared to those from tall cultivars. This result is also in conformity with the result of Jagannath *et al.*, (2008). In which *Nata* sheet was formed 15 to 20 days after inoculation of bacteria in coconut water from dwarf cultivars.

Chemical qualities such as TSS, total sugar, protein were found to be influenced by *nata* formed by sources of coconut water. It was observed that TSS and total sugar were found more in *nata* formed when coconut water from COD were used for *Nata-de-coco* production compared to coconut water from tall cultivars. This result is in line with earlier observation that coconut water from dwarf cultivars recorded more TSS than that from tall cultivars. However protein content (%) of *Nata* was found more when coconut water from tall cultivar (WCT) was used for *Nata* production compared to that from COD (0.008%) (Fig. 2). This may be due to the fact that coconut water from tall cultivars may contain more protein than that found in coconut water from dwarf cultivars. This result is in conformity with the reports of Enonuya, (1988) and Santoso *et al.* (1999).

Qualities such as acidity (%), fibre (%) and moisture (%) were found not affected by sources of coconut water.

When acetic acid, bilimbi juice were used as acidulant sources, it was observed that there were no significant effect for the various acidulant sources on days to *nata* formation, TSS, acidity, total sugar, protein, moisture and fibre content of *nata* formed.

When acetic acid, bilimbi juice were used as acidulant sources it was observed that there were no significant effect for the various acidulant sources on days to *nata* formation, thickness of *Nata* and weight of *Nata*. The acidulant sources brought down the pH of coconut water which favoured the growth of *Acetobacter xylinum* and in turn favoured *nata* formation as reported by Vandamme *et al.* (1998).

5.1.2 Correlation of physico chemical characters of *Nata*

From the table 7, it was clear that thickness of *Nata* is positively correlated with days to *nata* formation (0.18). Weight of *Nata* had significant positive correlation (0.948**) with thickness of *Nata*. Total sugar of *Nata* had significant positive correlation with days to *Nata* formation (0.570**), thickness of *Nata* (0.573**), weight of *nata* (0.635**). TSS of *Nata* had significant positive correlation with days to *Nata*



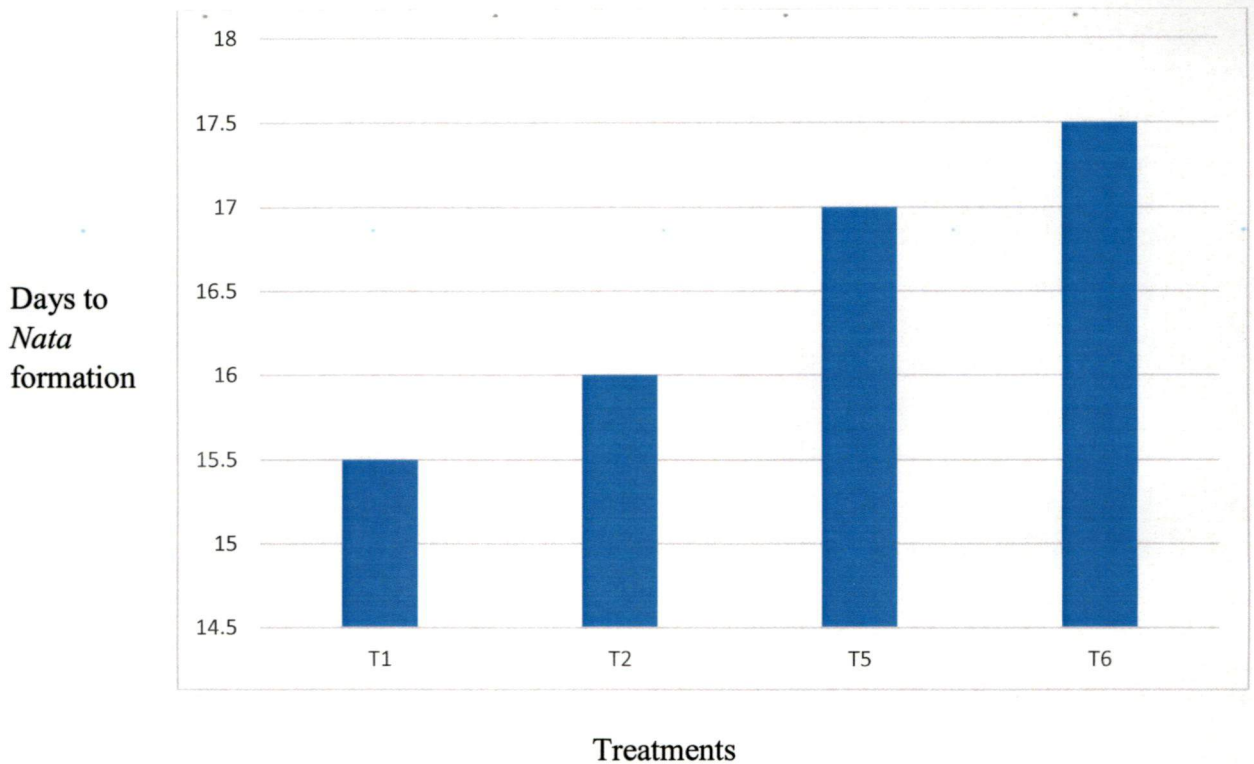
Table. 7. Correlation of physico chemical characters of *Nata*

	Correlations					
	Days to <i>nata</i> formation	Thickness of <i>Nata</i>	Weight	Total sugar	TSS	Fibre
Days to <i>Nata</i> formation	1					
Thickness of <i>Nata</i>	0.180	1				
Weight	0.154	0.948**	1			
Total sugar	0.570**	0.573**	0.635**	1		
TSS	0.407*	0.599**	0.683**	0.935**	1	
Fibre	0.486*	0.007	0.015	0.509*	0.452*	1

** .Correlation is significant at the 0.01 level

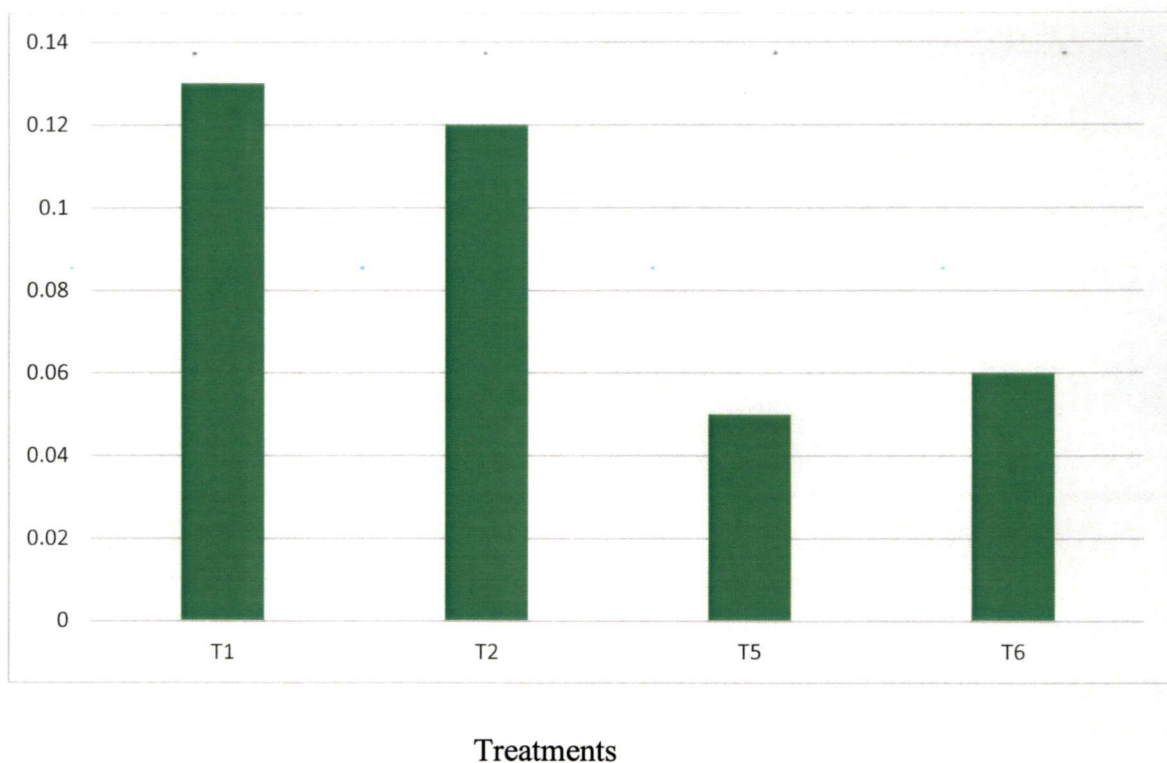
*. Correlation is significant at the 0.05 level

Fig. 1 Days taken for *Nata* formation when coconut water from various cultivars used



- T1: Coconut water from tall cultivar (WCT) added with sucrose and acetic acid
T2: Coconut water from tall cultivar (WCT) added with sucrose and bilimbi juice
T5: Coconut water from dwarf cultivar (COD) added with sucrose and acetic acid
T6: Coconut water from dwarf cultivar (COD) added with sucrose and bilimbi juice

Fig. 2 Protein content in *Nata* formed using coconut water from WCT and COD cultivars



T₁: Coconut water from tall cultivar (WCT) added with sucrose and acetic acid

T₂: Coconut water from tall cultivar (WCT) added with sucrose and bilimbi juice

T₅: Coconut water from dwarf cultivar (COD) added with sucrose and acetic acid

T₆: Coconut water from dwarf cultivar (COD) added with sucrose and bilimbi juice

formation (0.407*), thickness of *Nata* (0.599**), weight of *Nata* (0.683**) and total sugar (0.935**).

Fibre content of *Nata* had significant positive correlation with days to *Nata* formation (0.486*), total sugar (0.509*) and TSS (0.452*).

5.2.0. SCREENING SUITABILITY OF DIFFERENT SUBSTRATES FOR *NATA-DE- COCO* PRODUCTION

5.2.1: Effect on physico chemical characters of *Nata-de-coco*:

From the results it was observed that various substrates used for *Nata* production had significant effect on the physical properties of *Nata* formed. Days to *nata* formation was found highest when coconut water was blended with jack fruit juice followed by coconut water blended with mango juice and lovi-lovi juice. The lowest no. of days for formation of *nata* was recorded when coconut water alone was used as a substrate. However the characters like thickness, weight, TSS (Fig. 3), total sugar of the *nata* formed recorded higher mean values when coconut water blended with water melon juice (11.33mm, 416.33g, 9.59^o Brix, 2.18g) and mango juice (11.00mm, 401.67g, 9.58^o Brix, 2.16g). To assess the superiority of the treatments, a relative ranking was done using physico-chemical characters. According to this combination of characters with lowest ranking score gave the best treatment as evident from fig. 4. It was observed that *Nata* with more thickness recorded higher weight, total sugar and TSS. This might be due to the high TSS of the ripened mango as reported by Anila and Radha (2003).

Chemical qualities such as TSS, acidity, protein and total sugar and fibre content of *Nata* formed from various substrates varied significantly. It was found that higher TSS was found in *Nata* formed from coconut water blended with water melon juice (9.59^o Brix) followed by coconut water blended with mango juice (9.58^o Brix)

were on par with other. It can be inferred that fruit juices with more TSS when blended with coconut water (water melon and mango juice) the *Nata* formed also recorded higher TSS as recorded in the present study.

Lowest TSS was found when coconut water alone used as a substrate. Which recorded low TSS (4.51° Brix) as measured in the present study when compared to the fruit juices. TSS of *Nata* formed from various substrates ranged from 9.15-9.59° Brix. This results are in conformity with the earlier findings of Illiaskutty (2004).

Various substrate used showed varied performance in the acidity content of *Nata*. When pineapple juice mixed with coconut water, the *Nata* formed recorded higher acidity (1.23%). When coconut water blended with jack fruit juice, papaya juice and lovi- lovi juice *Nata* formed recorded same acidity (1.22 %). Beetroot juice blended with coconut water recorded lower acidity (1.20 %) of *Nata* among all the fruit/vegetable juices. The *Nata* with lowest acidity was formed when coconut water alone was used as a substrate (0.60 %).

With respect to the total sugar content of the *Nata* formed it was seen that various fruit juices recorded significant difference. *Nata* with highest total sugar was formed when coconut water was blended with mango juice (2.18 %) followed by water melon juice which were on par. This result is in line with the TSS of *Nata* were formed from fruit juices with more TSS recorded more total sugar content. *Nata* with lowest total sugar content was formed when coconut water alone used as a substrate.

Protein content of *Nata* varied significantly among various fruit juices used as substrates. It was found that the protein content varied from 0.17 to 0.26 % among various substrates. *Nata* with highest protein content (0.26%) was formed when jackfruit juice blended with coconut water followed by papaya juice (0.21%). *Nata* with lowest protein was formed when coconut water alone used as substrate. This might be

Fig. 3 Effect of substrates on TSS of *Nata*

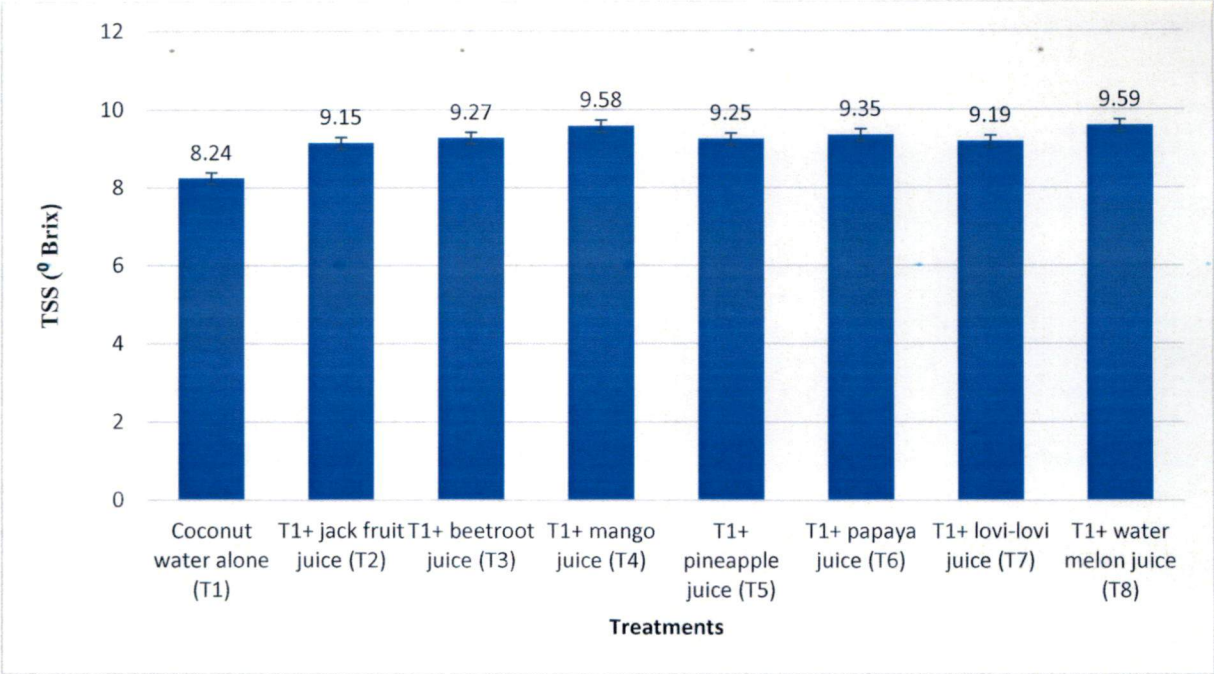
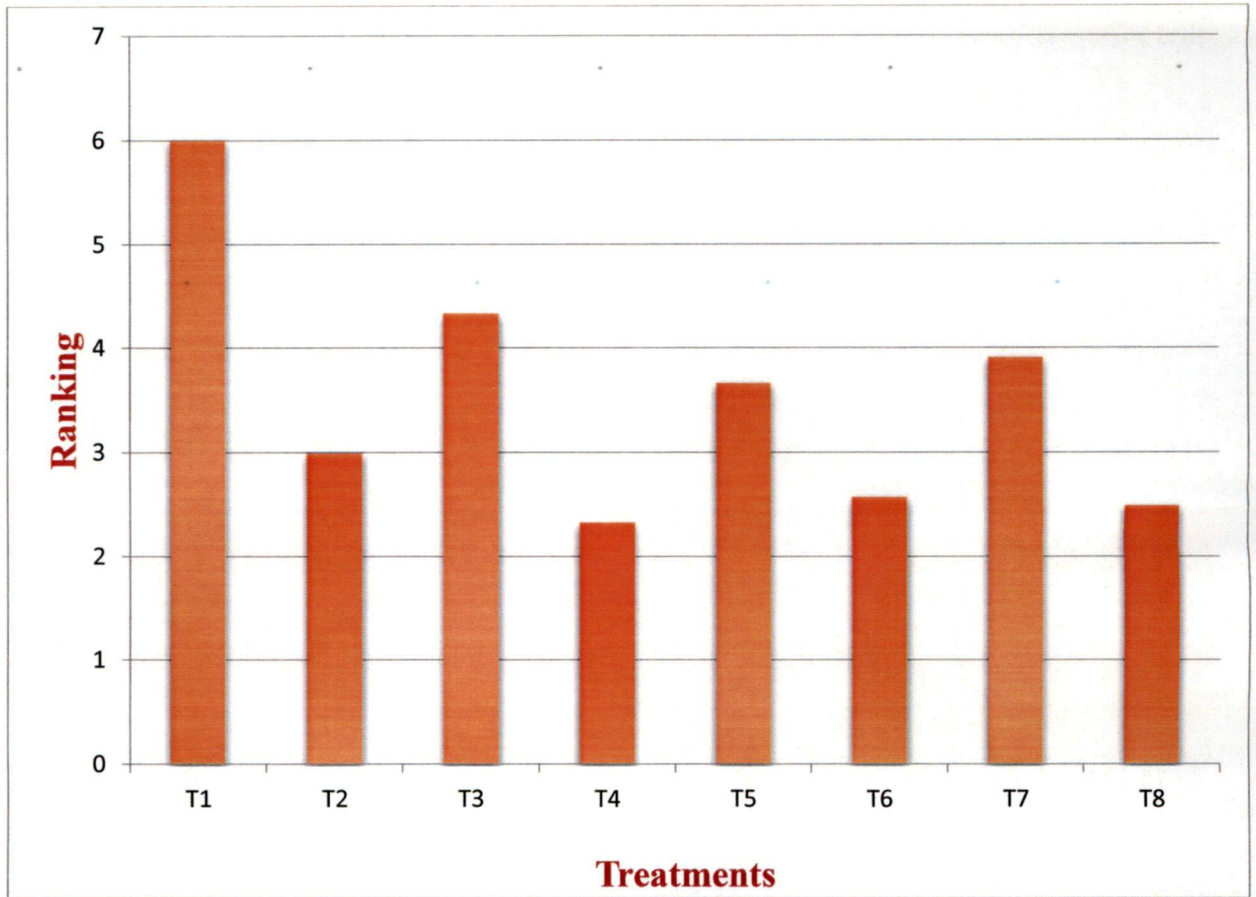


Fig. 4 Ranking scores of physico-chemical characters of *Nata*



- T₁. Coconut water alone
- T₂. T₁+ Jackfruit juice
- T₃. T₁+ Beetroot juice
- T₄. T₁+ Mango juice
- T₅. T₁+ Pineapple juice
- T₆. T₁+ Papaya juice
- T₇. T₁+ Lovi-lovi juice
- T₈. T₁+ Watermelon juice

due to the more protein content in fruit /vegetable juices than coconut water. This results are in conformity with the earlier report of Pradesh *et al.*, (2012).

Fibre content of the *nata* formed when various fruit juices used as substrate from 2.61 % to 3.82%. *Nata* with highest fibre content (3.82 %) was formed when papaya juice was blended with coconut water followed by coconut water blended with jackfruit juice (3.63%). Lowest fibre content of *Nata* was formed when coconut water alone used as substrate. Fibre content of *nata* is an important for which it is valued. So from the study, it was found that blending fruit/ vegetable juices with *Nata* improved the fibre content significantly. Hence it may be inferred that mixing of fruits/vegetable juices may be beneficial on improving the fibre content and overall quality of *Nata*.

Moisture content of *nata* formed from various substrates blended with coconut water did not vary significantly. This results are in conformity with the earlier findings of Illiaskutty (2004) and Afreen and Lokeshappa (2014).

5.2.2 Microbial population (cfu/g)

Highest bacterial population were observed in T₈- coconut water blended with water melon juice (1.87×10^5) followed by T₄-coconut water blended with mango juice (1.76×10^5). The high TSS content of these fruit juices might have favoured the growth of microbes.

Highest yeast population were also observed in T₈- coconut water blended with watermelon juice (1.71×10^2) followed by T₄- coconut water blended with mango juice. This might be due to the higher content of sugar in water melon juice and mango juice and *Acetobacter xylinum* is present along with various types of yeast as reported by Jagannath *et al.* (2008).

5.2.3: Sensory qualities of *Nata-de-coco*

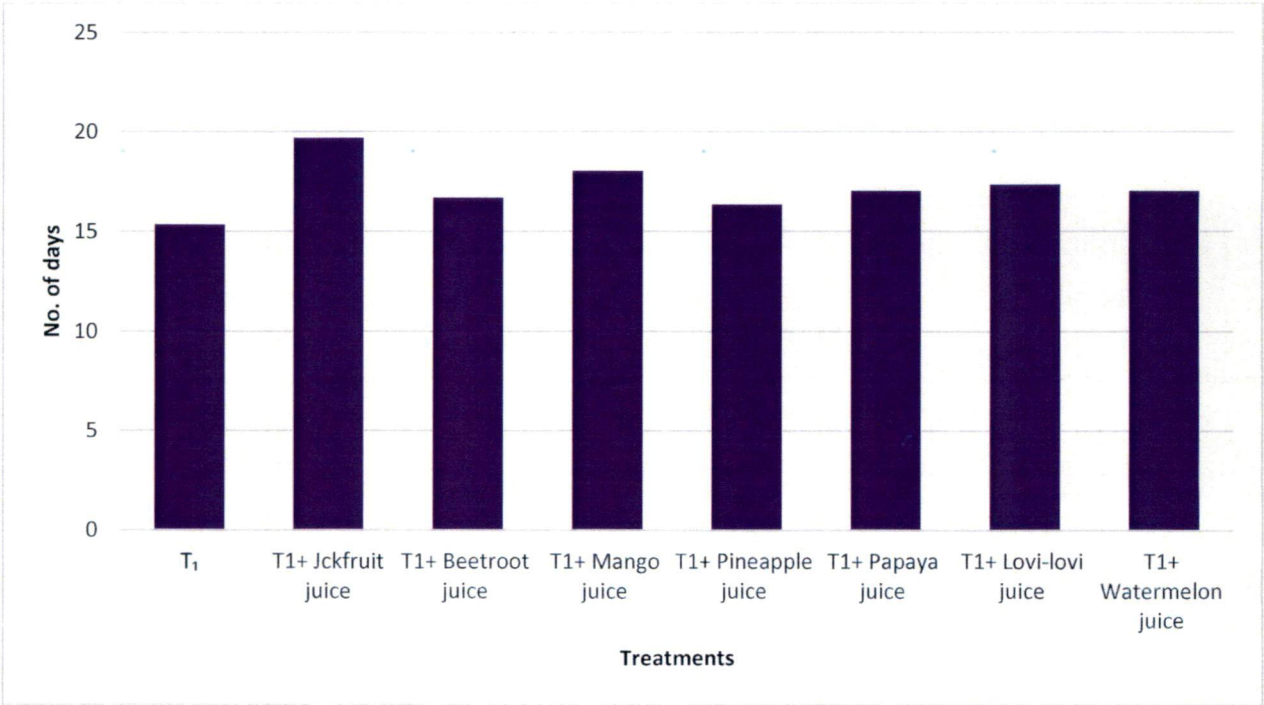
Results of sensory evaluation of *Nata-de-coco* showed that various substrates had influence on various sensory quality parameters such as appearance, colour, flavour, taste, after taste, texture, chewiness, overall acceptability and total score. The highest total score for appearance 8.06 (7.43), colour 7.46 (6.30), flavour 8.4 (7.53), taste 8.2 (7.20), after taste 7.26 (6.46), chewiness 6.44 (3.37), overall acceptability (Fig. 5) 8.4 (7.62) and total score 61.65 was recorded in T₅- coconut water was blended with pineapple juice. Similar results were reported by Illiaskutty (2004).

However highest score of 5.30 (6.33) for texture of *Nata* formed was recorded in T₇- coconut water blended with lovi-lovi juice and the same treatment recorded the next highest values for appearance, colour, flavor, taste, after taste, chewiness, overall acceptability and total score. Similar results has not been reported so far. The lowest score for the above parameters were recorded in *Nata* formed from coconut water alone used as substrate as reported by Illiaskutty, (2004).

5.3.0: Sensory qualities of packed and flavored *Nata-de-coco*

In this experiment *Nata -de -coco* formed from coconut water of WCT and COD cultivars were packed with sugar syrup, roseapple syrup and West Indian cherry syrup. The *Nata* formed from these six treatments were flavored with ginger and vanilla extract. Accordingly *nata* from 12 treatments were given for sensory evaluation. Results of organoleptic scoring of *Nata-de-coco* showed that various substrates additives and flavoures had influence on sensory quality parameters such as appearance, colour, flavour, taste, after taste, texture, chewiness, overall acceptability and total score. The highest score for appearance 8.46 (9.41), flavour 8.46 (9.57), after taste 7.66 (9.47), texture 7.86 (8.47), overall acceptability 8.53 (9.67) and total score (65.30) was recorded higher in T₁₀- *Nata* formed using coconut water from COD packed in roseapple syrup and flavoured with vanilla extract. The next highest score

Fig. 5 Overall acceptability of *Nata-de-coco* as influenced by additives and flavoures



was recorded in T₄- *Nata* formed using coconut water from tall cultivar packed in roseapple syrup and flavoured with vanilla extract were the appearance 8.26 (9.37), colour 8.73 (9.54), flavour 8.40 (9.40), taste 8.50 (9.56), after taste 7.46 (8.93), texture 7.8 (7.83), chewiness 7.73 (8.30), overall acceptability 8.13 (8.40) and total score of 65.04. It was inferred that the consumer acceptability was higher for *Nata-de-coco* formed from water of COD packed with roseapple syrup and flavoured with vanilla extract. From the experiment it was found that the treatments in which *nata* packed with roseapple syrup and flavored with vanilla extract recorded high total score. Therefore it is concluded that roseapple syrup and vanilla extract are excellent additive and flavouring material for *Nata-de-coo*. Similar results has not been reported so far.

The study discloses that, coconut water from WCT and COD cultivars showed significant effect on the physico-chemical characters of *Nata* formed. Various substrates improved the yield and quality of *Nata*. Evaluation of more additives and flavoures can be done to improve the consumer acceptability of *Nata*. For large scale production of starter culture technology standardization is needed.

Summary

6. SUMMARY

The study entitled “Screening substrates and additives for enhancing yield and quality of *Nata-de-coco*” was carried out in the Department of Plantation crops and Spices, College of Horticulture, Vellanikkara during the period 2015-2017. The objective of the study was to evaluate the suitability of substrates and additives to improve yield and consumer acceptability of *Nata-de-coco*. The salient findings are summarized below.

- The study revealed that coconut water from tall (WCT), dwarf (COD) cultivars showed significant effect on the physical characters of *Nata* formed. There were no *Nata* formation in the treatments that were inoculated without sucrose.
- It was observed that days to *Nata* formation, thickness of *Nata* and weight of *Nata* were significantly higher when coconut water from COD cultivar was used for the production of *nata*. These parameters recorded lower mean values when coconut water from WCT was used for *Nata- de-coco* production.
- Coconut water from dwarf cultivars possess more Total Soluble Solids (TSS) 5.25° Brix compared to coconut water from tall cultivars (WCT) 4.83°Brix as measured in the present study.
- Days taken for *Nata* formation, was higher when coconut water from dwarf cultivars used for *Nata* production compared to those from tall cultivars.
- When acetic acid, bilimbi juice were used as acidulant sources it was observed that there were no significant effect for the various acidulant sources on days to *Nata* formation, TSS, acidity, total sugar, protein , moisture and fibre content.
- Correlation studies revealed that thickness of *Nata* is positively correlated with days to *Nata* formation. Total sugar of *Nata* had significant positive correlation with days to *Nata* formation, thickness of *Nata*, weight of *Nata*.

- Fibre content of *Nata* had significant positive correlation with days to *Nata* formation, total sugar and TSS.
- Various substrates used for *Nata* production had significant effect on the physical properties of *Nata* formed. The lowest number of days for formation of *Nata* was recorded when coconut water alone was used as substrate.
- The characters like thickness, weight, TSS, total sugar of the *Nata* formed recorded higher mean values when coconut water blended with water melon juice (11.33mm, 416.33g, 9.59^o Brix, 2.18g) and mango juice (11.00mm, 401.67g, 9.58^o Brix, 2.16g). It was observed that *Nata* with more thickness recorded higher weight, total sugar and TSS.
- Various substrates used showed varied performance in the acidity content of *Nata*. When pineapple juice mixed with coconut water, the *nata* formed recorded higher acidity. The *Nata* with lowest acidity was formed when coconut water alone was used as a substrate.
- *Nata* with highest total sugar was formed when coconut water was blended with mango juice (2.18 %) followed by water melon juice which were on par.
- It was found that the protein content varied from 0.17 to 0.26 % among various substrates. *Nata* with highest protein content (0.26%) was formed when jackfruit juice blended with coconut water and lowest protein was formed when coconut water alone used as substrate.
- *Nata* with highest fibre content was formed when papaya juice was blended with coconut water followed by coconut water blended with jackfruit juice. Lowest fibre content of *Nata* was formed when coconut water alone used as substrate.
- Moisture content of *Nata* formed from various substrates blended with coconut water did not vary significantly.
- Highest bacterial population were observed in T₈- coconut water blended with water melon juice followed by T₄-coconut water blended with mango juice.

- Results of sensory evaluation of *Nata-de-coco* showed that various substrates had influence on various sensory quality parameters such as appearance, colour, flavour, taste, after taste, texture, chewiness, overall acceptability and total score.
- The highest total score for appearance, colour, flavour, taste, after taste, chewiness, overall acceptability and total score was recorded in T₅- coconut water was blended with pineapple juice followed by T₇- coconut water mixed with lovi-lovi juice.
- Various additives and flavoures had influence on sensory quality parameters such as appearance, colour, flavour, taste, after taste, texture, chewiness, overall acceptability and total score.
- The highest score for appearance, flavour, after taste, texture, overall acceptability and total score was recorded higher in T₁₀- *Nata* formed using coconut water from COD packed in roseapple syrup and flavoured with vanilla extract followed by T₄- *Nata* formed using coconut water from WCT packed in roseapple syrup and flavoured with vanilla extract.
- The consumer acceptability was higher for *Nata-de-coco* formed from water of COD packed with roseapple syrup and flavoured with vanilla extract. Roseapple syrup and vanilla extract are excellent additive and flavouring material for *Nata-de-coo*.

References

7. REFERENCES

- Afreen, S. S. and Lokeshappa, B. 2014. Production of bacterial cellulose from *Acetobacter xylinum* using fruits wastes as substrate. *Int. J. Sci. Technol.* 2(8): 57-64.
- Agarwal, G.P. and Hasija, S. K. 1986. *Microorganism in laboratory- A laboratory guide for Microbiology, Mycology and Plant Pathology*. Print House, Lucknow, India, 137 p.
- Alleyne, T., Roache S., Thomas C., and Shirley, A. 2005. The control of hypertension by use of coconut water and mauby: two tropical food drinks. *W. Indian Med. J.* 54 (1): 3-8.
- Alvarez, C., Perez, E., and Larez, M. 2003. Fruit morphology and physical – chemical characteristics of cocoa mucilage from three areas of Argua State. *J. Agron. Trop.* 52(4): 497-506.
- Anila, R. and Radha, T. 2003. Physico-chemical analysis of mango varieties under Kerala conditions. *J. Trop. Agric.* 41: 20-22.
- AOAC. 1984. *Official Methods of Analysis*. Association of Official Analytical Chemist, Washington DC, 896p.
- Bourdeix, R., Santos, G., Labouisse, J.P, and Baudouin, L. 2005. Useful definition of terms and nomenclature. In. Batugal, P., Rao, R.V and Oliver, G (eds), *Coconut genetic resources*. IPGRI, Rome. pp. 9-10.
- Budhiono, A., Rosidi, B., Taher H., and Iguchi, M. 1999. Kinetic aspects of bacterial cellulose formation in *Nata-de-coco* culture system. *Carbohydr. Polym.* 40: 137–143.

- Campbell, D., Thomas, T., Falck, T. M., Tutuo, N., and Clem, K. 2000. The intravenous use of coconut water. *Am. J. Emer. Med.* 18(1): 108-111.
- Chang, C. and Wu, R. 2011. Quantification of (+)- catechin and(-)-epicatechin in coconut water by LC-MS. *Food Chem.* 126: 710–717.
- Chowdhury, M. G. F., Rahman, M. M., Islam, T. A. F. M., Islam, M. S., and Islam, M. S. 2009. Processing and preservation of green coconut water. *J. Innov. Dev. Strategy.* 2(3): 01-05.
- Coconut Development Board. 2016. Coconut Statistics [On-line]. Available: <http://www.coconutdevelopmentboard.nic.in> [16 Jan. 2017].
- Embuscado, M.E., Marks, J. S. and Be Miller, J. N., 1994. Bacterial cellulose. II. Optimization of cellulose production by *Acetobacter xylinum* through response surface methodology. *Food hydrocolloids* 8(5): 419-430.
- Enonuya, D. O. M. 1988. High performance liquid chromatographic analysis of nut water syrup fractions from two varieties of Nigerian coconuts (*Cocos nucifera*, L.). *Nigerian J. Palms and Oil Seeds* 9: 48-58.
- Gallardo, D. E. J., Andress, R. M., and Mango, E.T. 1971. A study on the isolation and screening of microorganisms for production of diverse-textured *nata*. *Philip. J. Sci.* 100: 41-49.
- Gayathry, G. 2015. Production of *Nata-de-coco*- a natural dietary fibre product from mature coconut water using *Gluconacetobacter xylinum* (sju-1). *Int. J. Food. Ferment. Technol.* 5(2): 231-235.

- Huber, G. W., Iborra, S., Corma, A. 2006. Synthesis of transportation fuels from biomass: chemistry, catalysis, and engineering. *Chem. Rev.* 106 (5): 4044-4098.
- Iguchi, M., Yamanaka, S., and Budhiono, A., 2000. Bacterial BC-a masterpiece of nature's arts. *J Master Sci.* 35: 261-270.
- Illiaskutty, N. 2004. Developing value added and diversified products from coconut (*Cocos nucifera L.*). ph.D (Ag) thesis, Kerala Agricultural University, Vellayani, 274p.
- Jackson, J. C., Gordon, A., Wizzard, G., McCook, K., and Rolle, R. 2004. Changes in chemical composition of coconut (*Cocos nucifera*) water during maturation of the fruit. *J. Sci. Food Agric.* 84(9): 1049-1052.
- Jagannath, A., Kalaiselvan, A., Manjunatha, S.S., Raju, P.S., and Bawa, A. S. 2008. The effect of pH, sucrose and ammonium sulphate concentrations on the production of bacterial cellulose (*Nata-de-coco*) by *Acetobacter xylinum*. *W. J. Microbiol. Biotechnol.* 24: 2593-2599.
- Jellinek, G. 1985. *Sensory evaluation of food. Theory and practice.* Ellis Horwood Ltd. New Jersey, 586p.
- Jothi, J. S., Islam, M., Islam, M. S., Rahman, M. R. T., and Akther, S. 2014. Development and Shelf-Life Prediction of Pineapple (*Ananas comosus*) Preserve and Candy. *Int. J. Inno. Sci. Res.* 10(1): 77-82.

- Keshk, S. and Sameshima, K. 2006. The utilization of sugar cane molasses with/without the presence of lingo sulfonate for the production of bacterial cellulose. *Appl. Microbiol. Biotechnol.* 72: 291–296.
- Kongruang, S. 2008. Bacterial cellulose production by *Acetobactor xylinum* strains from agricultural waste products. *Appl. Biochem. Biotechnol.* 148(1-3): 245-256.
- Kurosumi, A., Sasaki, C., Yamashita, Y., and Nakamura, Y. 2009. Utilization of various fruit juice as carbon source for production of bacterial BC by *Acetobactor xylinum* NBRC 13693. *Carbohydr. Polym.* 76: 333-335.
- Lapuz, M. M., Gallardo, E.G., and Palo, M. A. 1967. The *nata* organism cultural requirements, characteristics and identity. *Philipp. J. Sci.* 96(2): 91-108.
- Markose, V. T. and Poduval, S. 2009. Processing and value addition of coconut. *Indian Coconut J.* 56 (9): 19-22.
- Matsui, K. N., Gut, W. J. A., Vitoriano de Oliveira, P., and Tadini, C. C. 2008. Inactivation kinetics of polyphenol oxidase and peroxidase in green coconut water by microwave processing. *J. Food Eng.* 88(2): 169-176.
- Mesomya, W., Pakpeankitvatana, V., Komindr, S., Leelahakul, P., Cuptapun, Y., Hengsawadi, D., Tammarate, P. and Tangkanakul, P. 2006. Effects of health food from cereal and *Nata-de-coco* on serum lipids in human. *J. Sci. Technol.* 28(1): 24-28.
- Nielsen, S. S. 2010. Phenol-sulfuric acid method for total carbohydrates. *Food Anal. Lab. Mannual.* U.S 8(2): 47-53.

- Okiyama, A., Shirae, H., Kano, H., and Yamanaka, S. 1993. Bacterial cellulose I. Two-stage fermentation process for cellulose production by *Acetobacter aceti*. *Food Hydrocolloids* 6(5): 471-477.
- Phisalaphong, M. and Chiaoprakobkij, N., 2012. Applications and products—Nata de Coco. *Bacterial Nano Cellulose: A Sophisticated Multifunctional Material* 27 (1): 143-156.
- Prades, A., Dornier, M., Diop, N., and Pain, J. P. 2012. Coconut water preservation and processing: a review. *Fruits* 67(3): 157-171.
- Ranganna S. 1997. *Handbook of Analysis and Quality Control for Fruit and Vegetable Products* (2nd Ed.). Tata McGraw Hill Pub. Co. Ltd., New Delhi, India, 336p.
- Reddy, E. M and Lakshmi, T. M. 2014. Coconut Water - Properties, Uses, Nutritional Benefits in Health and Wealth and in Health and Disease: A Review. *J. Curr. trends Clin. Med. LaB. Biochem.* 2(2): 6-18.
- Rethinam, P. and Kumar, T. B. N. 2001. Tender coconut –an overview. *Indian Coconut J.* 32: 2–22.
- Sabapathy, S. and Kumar, R. 1999. Physico-chemical constituents of tender coconut (*Cocos nucifera* L.) water. *Indian J. Agric. Sci.* 69(10): 24-29.
- Sadasivam, S., & Manickam, A. 1992. *Biochemical Methods for Agricultural Science*, Wiley Estern Ltd. New Delhi, 256p.

- Sangamithra, A., John, G.S., Prema., S.R, Chandrasekhar, V. and Sasikala, S. 2014. Coconut: An overview on value added products. *Indian J. Food Ind.* 32 (6): 27-36.
- Santoso, U., Meekawa, A., and Kubo, K. 1999. Nutrient composition of *kopyor* coconuts (*Cocos nucifera* L.). *Food Chem.* 57(2): 299-304.
- Setiaji, B., Setyopratiwi, A., and Cahyandaru, N. 2002. Exploiting a benefit of coconut milk skim in coconut oil process as *Nata de coco* substrate. *Indonesian J. Chem.* (3): 167-172.
- Shaw, M. and Srivastava, B.I.S. 1963. Purine like substances from coconut endosperm and their effect on senescence in excised cereal leaves. *Plant Physiol.* 38: 528–531.
- Shi, Z., Zhang, Y., Phillips, G. O., and Yang, G. 2014. Utilization of bacterial cellulose in food. *Food Hydrocolloids* 35: 539-545.
- Singh, H.P. and Gopalakrishnan, R. 2002. Approaches for increasing the farm income through product diversification and product utilization. In: Singh, H. P. and Mathew, M. T. (eds.), *Sustainable Production and Utilization of Coconut*. Coconut Development Board, Kochi, pp.1-11.
- Sutanto, A. 2012. Pineapple liquid waste as *Nata-de-pina* raw material. *Makara J. Technol.* 16(1): 63-67.
- Thampan, P. K. 1998. The strategy of coconut development in root wilt affected coconut tracts in Kerala. *Indian Coconut J.* 23(9): 1-5.

- Ullah, H., Santos, H. A., and Khan, T. 2016. Applications of bacterial cellulose in food, cosmetics and drug delivery. *Cellulose* 23(4): 2291-2314.
- Unagul, P., Assantachai, C., Phadungruengluij, S., Suphantharika, M., Tanticharoen, M., and Verduyn, C. 2007. Coconut water as a medium additive for the production of docosahexaenoic acid. *Bioresource technol.* 98(2): 281-287.
- Vandamme, E. J., De Baets, S., Vanbaelen, A., Joris, K., and De Wulf, P. 1998. Improved production of bacterial cellulose and its application potential. *Polym. Degradation Stability* 59 (1-3): 93-99.
- Wonganu, B., and Kongruang, S. 2010. Red bacterial cellulose production by fermentation of *Monascus purpureus*. *Chem. Eng.* 24(6): 137-141.

Appendices

Score card for sensory evaluation of packed and flavoured *Nata-de-coco*

Name: _____

	SVT	SVD	SGT	SGD	RVT	RVD	RGT	RGD	WVT	WVD	WGT
Appearance											
Colour											
Flavour											
Texture											
Taste											
After taste											
chewiness											
Overall acceptability											

Nine point hedonic scale

- 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

Score card for sensory evaluation of *Nata-de-coco* formed from various substrates

Exp. No.2

Name:

Date:

	T1R 1	T1R 2	T1 R3	T2R 1	T2R 2	T2 R3	T3R 1	T3R 2	T3 R3	T4R 1	T4R 2	T4 R3	T5 R1	T5R 2	T5R 2	T6R 1	T6R2	T6R 3	T7 R1	T7 R2	T7 R3	T8 R1	T8 R2	T8 R3
Appearance																								
Colour																								
Flavour																								
Texture																								
Taste																								
After taste																								
chewiness																								
Overall acceptability																								

9 point hedonic scale:

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

Signature

**SCREENING SUBSTRATES AND ADDITIVES FOR
ENHANCING YIELD AND QUALITY OF *NATA-DE-COCO***

BY

**Geethumol Thankappan
(2015-12-018)**

ABSTRACT OF THE THESIS

**Submitted in partial fulfillment of the
requirement for the degree of**

Master of Science in Horticulture

**Faculty of Agriculture
Kerala Agricultural University**



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2017**

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ABSTRACT

Coconut water is an important by-product of copra and oil industry, which is merely, wasted at the copra production centres. Bacterial cellulose produced by the *Acetobacter xylinum* at the air liquid interphase of coconut water is popularly known as *Nata-de-coco*. *Nata* is gaining popularity because of its high dietary fibre content and low calorific value. It is an excellent ingredient for ice creams, cocktails and other food products. Raw *Nata* is transparent without any colour and flavour. In order to enhance consumer acceptability, improving colour and flavour of *Nata-de-coco* by using under exploited fruit and vegetables and addition of natural flavours can go a long way in improving palatability and consumer acceptability of the product. Therefore, the present investigation entitled "Screening substrates and additives for enhancing yield and quality of *Nata-de-coco*" was undertaken to study the performance of coconut water from tall and dwarf cultivars to improve yield and quality of *Nata -de-coco*, to evaluate the suitability of substrates for blending with coconut water for *nata* production and to evaluate the suitability of additives and flavours to improve consumer acceptability of *Nata-de-coco*.

The study revealed that coconut water from tall (WCT), dwarf (COD) cultivars showed significant effect on the physico-chemical characters of *Nata* formed. It was observed that days to *Nata* formation (17.25), thickness of *Nata* (8.62 mm) and weight of *nata* (378.12g), TSS (8.72° Brix), total sugar (1.20 per cent) were significantly higher when coconut water from COD cultivar was used. These parameters recorded lower mean values when coconut water from WCT was used for *Nata- de-coco* production. Fibre (%) and moisture (%) were found to be not influenced by sources of coconut water, so also sources of acidulants did not influence acidity. However, protein (%) was found significantly higher when water from WCT was used.

When various fruit juices were blended with coconut water, significant effect was observed on physico-chemical qualities of *Nata*. The parameters like thickness, weight, TSS, total sugar of the *Nata* recorded higher mean values when coconut water blended

with water melon juice (11.33mm, 416.33g, 9.59⁰ Brix, 2.18g) and mango juice (11.00mm, 401.67g, 9.58⁰ Brix, 2.16g).The highest mean score for appearance (8.06), colour (7.46), flavour (8.40), taste (8.20), after taste (7.26), chewiness (6.44), overall acceptability (8.40) and total score 61.65 was recorded in T5- coconut water was blended with pineapple juice.

Nata-de-coco from water of WCT and COD packed with sugar syrup, West Indian cherry syrup, and roseapple syrup and flavoured with ginger and vanilla extract. The result showed that highest mean score for appearance, colour, after taste, texture and overall acceptability such as 8.46, 8.60, 7.66, 7.86, and 8.53 respectively when *Nata* obtained from COD was packed in roseapple syrup and flavoured with vanilla extract.

From the present study it can be concluded that *Nata* produced from water of COD gave highest thickness, weight, TSS and total sugar. Acidulant sources such as acetic acid and bilimbi juice are equally effective in maintaining the pH of substrates. Blending coconut water with water melon juice/mango juice can be done to improve yield and quality of *Nata*. *Nata* packed in roseapple syrup and flavoured with vanilla extract improve the consumer acceptability.

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