

**STUDY ON DEVELOPMENT AND PHYSICO-CHEMICAL
ANALYSIS OF PROBIOTIC KEFIR YOGHURT USING
ELEUSINE CORACANA, COCOS NUCIFERA AND
MANILKARA ZAPOTA**

*Dissertation submitted to Mahatma Gandhi University in partial
fulfilment of the requirements for the award of degree of*

Bachelor of Vocational Studies

B.Voc Food Processing Technology

By

Anju TR (VB21FPT003)

J. Disha (VB21FPT009)

Vishnupriya KA (VB21FPT022)

Under the Guidance of

Ms. Elizabeth Zarina Jacob

Assistant Professor



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COLLEGE WITH POTENTIAL FOR EXCELLENCE

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Kottayam -686560

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DECLARATION

We **Anju TR (VB21FPT003), J. Disha (VB21FPT009) and Vishnupriya KA (VB21FPT022)** hereby declare that this project entitled "**Development and Physico-Chemical Analysis of Probiotic Kefir Yoghurt using *Eleusine coracana*, *Cocos Nucifera* and *Manilkara zapota***" is a bonafide record of the project work done by us during the course of study and that the report has not previously formed the basis for the award to us for any degree, diploma, fellowship or other title of any other university or society.

Date

Place: Ernakulam

Anju TR

J. Disha

Vishnupriya KA

CERTIFICATE

This is to certify that the project report entitled " **Development and Physico-Chemical Analysis of Probiotic Kefir Yoghurt using Eleusine coracana, Cocos Nucifera and Manilkara zapota** " submitted in partial fulfilment of the requirements for the award of the degree of B.Voc Food processing Technology to St. Teresa's College, Ernakulam is a record of bonafide research work carried out by Ms. Anju TR, Ms. J. Disha and Ms. Vishnupriya KA under my guidance and supervision and that no part of the project has been submitted for the award of any other degree diploma fellowship or other similar titles or prize and that the work has not been published in part or full in any scientific or popular journal or magazine

Signature of HOD

Signature of the Guide

Ms. Elizabeth Zarina Jacob
Assistant Professor,
B.Voc. Food Processing Technology
St. Teresa's College (Autonomous)
Ernakulam

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Anju TR

J. Disha

Vishnupriya KA

ABSTRACT

Yogurt, originating from Western Asia and the Middle East, is a nutritious staple in various cultures due to its probiotics, supporting gut health and immunity. This study aimed to create yogurt from eleusine coracana and cocos nucifera milk infused with Sapota flavor. Ragi and coconut milk mixed with Sapota pulp are heated with agar-agar, followed by the addition of kefir culture. After fermentation and refrigeration, a well-set fermented yogurt is obtained. Ragi and coconut milk serve as vegan substitutes, thus it is suitable for individuals experiencing lactose intolerance and dairy allergies and they are rich in fiber, calcium, iron, Vitamin C, E, selenium, and zinc. Sapota was chosen for its nutrient richness and ability to mask the nutty flavor of ragi milk. The use of agar agar aids in the gelling process. The newly developed yogurt was well-received, with a nutrient analysis showing 120 Kcal energy, 5.66% fat, 1.13% protein, 29.32mg calcium, 0.96mg iron, and 0.27% total-ash per 100g.

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CHAPTER 1

INTRODUCTION

The demand for food is projected to rise by 50% within the next three decades, and current estimates suggest that global food production accounts for 26% of all yearly greenhouse gas emissions. Dairy products are a significant contributor to these emissions in the food production sector. For centuries, humans have included dairy products in their diets and it is valued for its nutritional content, providing essential elements like calcium, fats, carbohydrates, and proteins that are vital for human health. Although milk offers various benefits, its consumption can lead to elevated levels of saturated fats in diets, consequently increasing the risk of heart diseases, as well as prostate and breast cancers. Additionally, health considerations such as cow milk allergy, lactose intolerance, concerns related to cholesterol, and adherence to vegan diets have prompted many consumers to seek dairy-free alternatives. Reports indicate that approximately 75% of the world's population is lactose intolerant (Dusabe et al., 2021). As consumers become increasingly conscious of sustainability, healthier living, and animal welfare, the plant-based food industry has experienced rapid growth over the past decade. This surge in demand has led to an expanding array of plant-based products entering the market, providing consumers with alternatives to traditional dairy products.

Probiotics are defined as “live microorganisms that confer health benefits to the host when administered in sufficient amounts.” Probiotics, on the other hand, have been characterised as live microbial feed supplements that benefit the host animal by improving the microbial balance in its gut (Nagpal et al., 2012). Probiotics can help prevent and treat gastrointestinal infections, irritable bowel syndrome, lactose intolerance, allergies, urogenital tract infections, cystic fibrosis, and cancer, they are also used to treat dental caries, periodontal disease, and bad breath, as well as minimise the negative effects of some antibodies (Bodke and Jogdand, 2022).

Yogurt, a fermented dairy product that has been known for centuries, enjoys worldwide acceptance for its nutritional and health benefits. It is made by fermenting milk (whole, reduced-fat, or low-fat) with lactic acid-producing bacteria, primarily *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. Additional bacteria such as *Acidophilus* and other strains may also be incorporated into the culture. Yogurt serves as a carrier for probiotics and can be classified into two main groups: standard culture yogurt and bio- or probiotic yogurt. Standard

yogurt is produced using *Lactobacillus bulgaricus* and *Streptococcus thermophilus* cultures. On the other hand, bio yogurts, also known as probiotic yogurts, are made by introducing additional beneficial microorganisms, typically probiotic strains such as *Bifidobacteria* and *Lactobacillus acidophilus*, into the culture. (Hadjimbei et al., 2022).

Yoghurt is a highly nutritious and easily digestible dairy product that contains over ten vital components, including minerals and vitamins. It is a good source of calcium, riboflavin, B6 and B12 and important amino acids (Banerjee et al., 2017). Yogurt's nutritional composition varies depending on the strains of starter culture used in the fermentation, the type of milk used (whole, semi, or skimmed milk), the type of milk solids, solid non-fat, sweeteners, and fruits added before fermentation, and the length of the fermentation process. Yoghurt is considered a healthy food because of its high digestibility as well as bioavailability of nutrients. It can also be recommended to people with, gastrointestinal disorders such as inflammatory bowel disease and irritable bowel disease, and helps in immune function and weight control (Weerathilake et al., 2014).

The word 'kefir' comes from the Turkish word 'keif,' which meaning 'good feeling'. Kefir can be made by inoculating milk with kefir grains, which are bacteria and yeasts in a symbiotic matrix. Most microorganisms found in kefir are non-pathogenic bacteria, particularly *Lactobacillus* sp. And yeasts. Kefir contains vitamins, amino acids, carbon dioxide, acetoin, alcohol, and essential oils, all of which have been combined to improved health. (John and Deeseenthum, 2015).

Consuming kefir on a regular basis can help to ease intestinal issues, promote bowel movement, reduce gas, and develop a healthy digestive system. It effectively cleanses the entire body, promoting a balanced inner ecosystem for optimum health and longevity. It is also easily digestible, contains beneficial bacteria and yeast, vitamins and minerals, and complete proteins, and is a nourishing food that contributes to a healthy immune system. It has been used to help patients suffering from AIDS, chronic fatigue syndrome, herpes, and cancer (Otlles and Çağındı, 2003).

Traditional yogurt is created through the fermentation of milk. Bacteria ferment the sugars present in milk, producing acid, which in turn affects the milk proteins and contributes to the formation of the characteristic texture of yogurt. However, with a growing number of individuals experiencing lactose intolerance and dairy allergies, plant-based yogurt serves as a suitable alternative for them (Baskar et al., 2022). People with lactose intolerance, high

cholesterol intake in diet or malabsorption needs proteins derived from alternative sources to satisfy their health and ethical needs. Though these milk alternatives lack the nutritional value compared to cows or other dairy milk, they contain functionally active components with health promoting properties that appeal to the health-conscious consumers (Sethi et al., 2016). It also helps to overcome milk shortage in certain areas. Plant based milks can be derived from cereals, pseudo cereals, legumes and are used in different combination to achieve optimal textural and nutritional quality of products (Montemurro et al., 2021).

Finger millet (*Eleusine coracana*) belongs to the Poaceae family and is generally known as Mandua or Ragi in India. (Jagati et al., 2021). Ragi grains have a high calcium content of 300-400 mg, which is approximately 10 times higher than that of conventional grains like wheat and rice. It comprises several micronutrients such as thiamine, iron, magnesium, manganese, zinc and iodine (Patil et al., 2023).

Ragi has the ability to grow in most fertile soils without the use of chemical fertilizers. Ragi is considered to be one of the most nutritious, easily digestible and least allergenic grains among the grains. Due to its health benefits for humans, it is considered a model plant for nutraceuticals. Numerous epidemiological studies have shown that regular consumption of whole ragi grains and its derivatives can help to reduce type II diabetes, cardiovascular disease, gastrointestinal cancer, and a number of other diseases. Whole ragi grains are rich in minerals, fiber, vitamins, and phenols that help fight high blood sugar and oxidative stress (Akhtar et al., 2020). The seed coat, high in dietary fibre and phenolic compounds, has been shown to reduce blood glucose and cholesterol levels, protect the kidneys, and prevent cataracts and finger millet extracts have been shown to have free radical scavenging, anti-protein glycation, anti-cataractogenic, and antibacterial activities (Gupta, 2014).

Coconut milk is a white, milky substance extracted from the flesh of matured coconuts that incorporates the form of medium-chain triglycerides fatty acids that are easily digested without additional processing, serving as the quickest re-assets of power for the mind in comparison to other milk analogues containing long-chain fatty acids. Coconut milk contains lauric acid, a lipid that induces apoptosis, or cell death, in breast and endometrial cancer cells, therefore boosting the immune system and maintaining blood vessel pliability (Tulashie et al., 2022).

Sapodilla (*Manilkara zapota*) is a nutritious fruit of the Sapotaceae family that is often grown in tropical wet to subtropical chilly and dry climates. It has a rough brown skin and a soft, delicious flesh that ranges from light brown to reddish brown. Sapota fruits are astringent when

raw, but delicious when mature. The flesh is frequently grainy, similar to a pear, and contains three to four flat, smooth black seeds (Jadhav et al., 2018).Sapodilla fruit is mostly composed of water (73%), followed by bioactive components (antioxidants and polyphenols), vitamins (A, C, folate, niacin, and pantothenic acid), minerals (Cu, K, and Fe), sugars, ascorbic acid, and fat, making it a nutritious fruit (Bangar et al., 2022).

Stabilizers and gelling agents are used to improve plant-based yogurt texture and creaminess. Commonly used substances include pectin, starch, gelatin, and various gums. However, in this particular research, agar is utilized as a gelling agent because it is capable of forming very strong gels at very small concentration as 1%.

1.2 RELEVANCE OF THE STUDY:

The demand for plant-based yogurt continues to surge as consumers increasingly prioritize sustainability, health, and ethical considerations in their food choices. With growing awareness of the environmental impact of animal agriculture and concerns about animal welfare, many individuals are seeking alternatives to traditional dairy products. Additionally, the rise in lactose intolerance, dairy allergies, and concerns about saturated fats and cholesterol in dairy have propelled the popularity of plant-based yogurt. Plant-based yogurts offer a diverse range of options, including almond, soy, coconut, oat, and other bases, catering to various dietary preferences and restrictions. Furthermore, these yogurts often contain probiotics, promoting gut health and digestion, while also providing essential nutrients such as calcium, vitamins, and proteins. Through this study, our goal is to develop a probiotic vegan yogurt that effectively substitutes conventional dairy-based yogurt, utilizing ragi, coconut milk and sapota.

1.3 OBJECTIVES:

- To develop Probiotic Kefir Yoghurt using Eleusine coracana, Cocos Nucifera and Manilkara zapota
- To ascertain the best composition from 3 different formulations in terms of their sensory acceptability.
- To do a comparative study of the nutrient profile of the selected formulation of yoghurt with a control.

CHAPTER 2

REVIEW OF LITERATURE

2.1. PROBIOTICS AND ITS HEALTH BENEFITS:

Lilly and Stilwell proposed the word ‘probiotic’, which means ‘for life’ in Greek. The name was coined in contrast to the word ‘antibiotic,’ which refers to a chemical produced by one microorganism to kill another. Probiotics are a type of microorganism that is connected with food to improve its nutritional content and digestive health. They are heavily promoted for their capacity to improve gastrointestinal health and boost the immune system. Currently, the ingestion of probiotic cells through food products is in high demand, and they are also termed functional foods. Numerous scientific studies demonstrate the beneficial effects of probiotics on human health. “Live microorganisms, when administered in sufficient amounts provide a health benefit to the host” is how the FAO and WHO defined probiotics. Fruit juices and dairy products both use lactic acid bacteria (LAB) as food additives in a commercial basis. By balancing the amount of beneficial and harmful bacteria, they change the dynamics of the microbial community in the host’s digestive tract. They also assist in the treatment of urogenital infections, pouchitis, and gastrointestinal disorders like Crohn’s disease (Kumar et al., 2022)

The consumption of probiotics offers numerous benefits, including the regulation of intestinal health by balancing the microbiota, bolstering and training the immune system, enhancing the synthesis and absorption of nutrients, alleviating symptoms of lactose intolerance, and reducing the risk of various diseases. Historically, probiotics have been primarily utilized in medicine to manage and prevent gastrointestinal infections and ailments. (Nagpal et al., 2012).

Microbial activity in the gut, particularly from beneficial cultures, has been shown to enhance the availability, quantity, and digestibility of certain nutrients. Consumption of probiotics has been associated with increased synthesis of riboflavin, niacin, thiamine, vitamin B6, vitamin B12, and folate. Probiotics also improve the absorption of calcium, iron, manganese, copper, and phosphorus, as well as the breakdown of protein and fat present in yogurt. This enzymatic breakdown leads to an increase in free amino acids and short-chain fatty acids. Through LAB fermentation, organic acids like acetate and lactate are produced, lowering the pH of intestinal contents and creating unfavorable conditions for pathogenic bacteria. (Thantsha et al., 2012)

Years of research have demonstrated the potential of probiotics in preventing, treating, and slowing the progression of cancer cells. Extensive studies using human cancer cells and cell lines have revealed that probiotics exhibit anti-proliferative or pro-apoptotic effects across a broad spectrum of cancer types, including colon, stomach, breast, cervix, and myeloid leukemia cells. Another significant application of probiotics in human health is their ability to reduce serum cholesterol levels in the blood. Elevated levels of low-density lipoprotein cholesterol (LDL-C) are a major risk factor for conditions like hypertension, hyperlipidemia, coronary heart disease, and the formation of atherosclerotic plaque in the arteries. Maintaining serum LDL cholesterol levels within normal ranges may significantly decrease the risk of developing these disorders. (Nazir et al., 2018).

2.2. YOGHURT AND ITS HEALTH BENEFITS:

Yoghurt is a milk-based dairy product fermented by lactic acid bacteria. According to the Code of Federal Regulations of the United States Food and Drug Administration (FDA), yoghurt can be defined as a food produced by culturing one or more of the optional dairy ingredients, namely, cream, milk, partially skimmed milk, and skim milk, used alone or in combination with a characteristic bacterial culture that contains lactic acid producing bacteria (Weerathilake et al., 2014).

Yogurt is a fantastic provider of easily absorbed calcium. It's a nourishing option for a meal and offers valuable probiotics. Including yogurt in your diet is linked to better overall dietary habits, including increased consumption of potassium, vitamins B12 and B2, calcium, magnesium, and zinc. Additionally, yogurt provides high-quality proteins and essential fatty acids. These components contribute to reducing levels of circulating lipids, glucose, systolic blood pressure, and insulin resistance. (Hadjimbei et al., 2022).

Early research suggests that adding yogurt to one's diet significantly decreases serum cholesterol levels, thus reducing the risk of cardiovascular diseases, given that high cholesterol is a major risk factor. Consumption of yogurt is associated with lower body weight, a reduced waist-to-hip ratio, smaller overall circumference, and generally lower BMI. Moreover, those who consume yogurt tend to have lower fasting total cholesterol and insulin levels. Notably, overweight or obese individuals who consume yogurt exhibit a more favorable cardio-metabolic profile compared to non-consumers within the same BMI range, demonstrated by

lower plasma levels of triglycerides and insulin (Cormier et al., 2016). Babio et al in 2015 indicated that participants who consumed the most whole-fat yoghurt were less likely to experience abdominal obesity, hypertriglyceridemia, low HDL cholesterol, high blood pressure, and high fasting plasma glucose, which are all components of metabolic syndrome.

Dysbiosis in colonic microflora, characterized by reduced levels of coliforms, lactobacilli, and bifidobacteria, is associated with a higher risk of Irritable Bowel Syndrome (IBS) compared to individuals with a healthy gut microbiota. Consumption of yogurt is beneficial in alleviating symptoms of IBS. The microflora present in yogurt acts as a protective barrier against foreign infectious microorganisms. It helps prevent both pathogenic microorganisms and pathogenic antigens from colonizing the gut, as highlighted by Banerjee et al. in 2017

2.3. PLANT BASED YOGHURT:

There's a growing demand for non-dairy milk substitutes, largely driven by the increasing number of people with dietary restrictions. The market for these substitutes was expected to grow significantly, with a projected compound annual growth rate of 11.7% from 2017, reaching an estimated value of 14.36 billion USD by 2022. Previous research has extensively highlighted the important roles of these plant-based beverages in enhancing or managing the immune system. They offer potential antimicrobial effects, reduce the risk of cardiovascular and gastrointestinal diseases while improving physiological functions, and decrease the risk of low bone mass. Additionally, they provide high levels of antioxidants with free radical scavenging properties (Anuyahong et al., 2020).

Plant-based yoghurts are primarily made from fermented aqueous extracts derived from a variety of raw materials, including cereals, pseudo-cereals, oil seeds, and certain legumes. Because of adequate material breakdown and homogenization, these extracts resemble cow milk or other animal-based milk in both look and consistency. The activity of bioactive substances is stimulated by spontaneous or controlled fermentation. Moreover, the product's overall quality is enhanced by the addition of certain useful and nutritionally significant components, which primarily improve the product's protein quality and mineral bioavailability along with a few other vital elements (Singh and Rathi, 2020).

Previous researches has thoroughly established the benefits of plant-based beverages in enhancing or managing the immune system. These benefits encompass viable antimicrobial

effects, a reduced risk of cardiovascular and gastrointestinal diseases coupled with improved physiological functions, a decreased risk of low bone mass, and elevated levels of antioxidants with free radical scavenging properties. (Huang et al., 2022).

2.4. KEFIR CULTURE:

Kefir, an acidic-alcoholic fermented milk with a creamy consistency can be made by fermenting milk with commercial freeze-dried starter cultures, traditional grains, or the remaining product after removing the grains. Kefir grains are a gelatinous yoghurt starter that range in size from 0.3-3.5 cm in diameter (Prado et al., 2015).

Kefir is traditionally made by fermenting milk with kefir grains, which are composed up of many microbial species. Milk kefir grains are mostly inhabited by lactic acid bacteria (LAB), but can include yeasts and acetic acid bacteria (AAB). Kefir drinks vary in acidity, flavour, viscosity, and fluffiness based on age.

LAB cultures are employed in the food business to improve food safety and features such as taste, technology, nutrition, and health. Many bacterial strains in LAB exhibit multifunctional properties, including high fermentative activity and positive effects on humans. LAB produce antimicrobial compounds, such as organic acids and bacteriocins and bioactive substances like enzymes, vitamins, conjugated linoleic acid, exopolysaccharides, and gamma-aminobutyric acid (Kondrotiene et al., 2023).

Streptococcus thermophilus is a non-pathogenic, facultative anaerobic lactic acid (LAB) bacterium commonly used in the production of fermented dairy products, particularly yoghurt, both at home and in industrial settings. As a milk starter, *S. thermophilus* converts lactose into lactic acid, reducing pH and causing casein coagulation. This bacteria improves yoghurt processing qualities, including flavour, acidity, viscosity, and water holding capacity (Linares et al., 2016).

L. lactis is a well-known and characterised species of LAB that may be used as a model organism for research. *L. Lactis* is a mesophilic species from the Streptococcaceae family. *Lactococcus lactis* is a crucial microorganism in the production of dairy products such as cheese and fermented butter. It plays a role in acidification and flavour development, for which selected strains of this species are used as essential starters. *L. lactis* thrives in harsh gastrointestinal circumstances, producing lactic acid to maintain an acidic environment and

inhibit pathogenic bacteria development. *L. Lactis* has been shown to improve gut barrier integrity by strengthening tight junctions between intestinal epithelial cells. This feature reduces intestinal permeability and prevents hazardous substances from entering the blood circulation (Kondrotiene et al., 2023).

Lactobacillus acidophilus is a Gram-positive, rod-shaped bacteria that may be found in a variety of bodily regions, including the stomach and the female genital tract (Molina et al., 2023). *L. acidophilus* may ferment carbohydrates to produce lactic acid, resulting in an acidic environment that limits the growth of pathogenic bacteria while promoting the growth of helpful bacteria. It has been found to reduce symptoms of irritable bowel syndrome in adults and relieve dysentery in children. *L. acidophilus* is regarded to play an important role in preserving healthy microbiomes and promoting human health. It mostly resides in the small intestine. It can produce and release lactic acid and acetic acid, as well as secrete a variety of anti-biotin substances such as acidolin, acidophilin, lactic acid bacteria, and so on, all of which have antibacterial effects on pathogenic bacteria in the intestinal tract, reduce the production of various toxins, reduce the burden of liver detoxification, and promote the establishment of the host's own Beneficial intestinal microflora (Wen et al., 2023).

2.5. RAGI:

Finger millet, one of India's ancient crops, is referred to as "nritha-kondaka" in ancient Indian Sanskrit literature, meaning "dancing grain," and was also known as "rajika" or "markataka." The key botanical components of the millet kernel include the seed coat, embryo (germ), and endosperm. Various color varieties such as yellow, white, tan, red, brown, or violet exist, but globally, red-colored varieties are the most commonly cultivated. The pericarp, which is the outermost layer of the millet, provides minimal nutritional value. Unlike other millets like sorghum, pearl millet, and proso millet, the seed coat, or testa, of finger millet is multilayered, consisting of five layers. (Shobana et al., 2013).

India holds a significant share, approximately 41%, of the global finger millet output, with Africa following closely. Finger millet ranks as the fourth most important millet crop worldwide, trailing behind sorghum, pearl millet, and foxtail millet. In India, finger millets, also known as ragi, are primarily cultivated in regions such as Karnataka, Tamil Nadu, Andhra Pradesh, Odisha, Bihar, and Gujarat. Rich in fiber, minerals, micronutrients, macronutrients,

amino acids, and fatty acids, Indian finger millets are recognized as functional millets. Ragi, as it's commonly called in India, holds a significant position as a staple food in the country and is often consumed without peeling. Due to its easy digestibility and nutrient-rich composition, ragi is frequently used as a baby food in India. (Somarajan and Morya, 2022).

Ragi indeed stands out with its high calcium content among cereals, playing a vital role in bone growth and preventing osteoporosis, a condition characterized by brittle bones, Regular consumption of ragi can potentially reduce the risk of diabetes, thanks to its significant levels of fiber and polyphenols, which contribute to stabilizing and lowering blood sugar levels. Moreover, ragi serves as a natural anti-aging ingredient and skincare product, containing amino acids like methionine and lysine that protect the skin against rashes and wrinkles. Its antioxidants help combat stress and slow the aging process by rejuvenating skin cells, promoting a youthful and healthy appearance. Additionally, ragi is rich in Vitamin E, which supports skin health by promoting spontaneous wound healing and providing lubrication and protection for the skin, fostering its development (Vagdevi et al., 2022).

2.6. COCONUT MILK:

The coconut tree is a versatile plant that brings great benefits to humanity. The seeds or fruit of the coconut tree are called coconuts. The coconut tree's scientific name is *Cocos nucifera* L., belongs to the Arecaceae (Palmae) family, and is a monocotyledonous tree. Botanically, the coconut is a drupe, not a true nut, it has three layers: outer exocarp, middle mesocarp and inner endocarp. Exocarp and mesocarp make up the “shell” of the coconut. The mesocarp is made from fiber, called coir, which has many common and commercial uses. The shell has three germination holes (stoma) or “eyes” that are visible on its outer surface after the shell is removed (Devi and Ghatani, 2022).

Coconut milk is the word used to denote the liquid obtained by manual or mechanical force from coconut pulp. It is a white oil-in-water emulsion extracted from fresh coconut pulp with or without added water. It is made from finely grated coconut pulp, soaked in hot water and then filtered. Coconut milk is increasingly becoming an important ingredient in home cooking as well as in the food industry. It is estimated that 25% of global coconut production is consumed as coconut milk. It is the main and essential ingredient in the preparation of a variety of food products such as curries, desserts, coconut spreads, coconut syrup, coconut cheese,

baked goods and beverages. It can also be used as a milk substitute in certain desserts such as chocolate and other confections with an exotic coconut milk flavor (Alyaqoubi et al., 2015).

Coconut milk stands as a highly sought-after plant-based alternative to animal-origin milk. Its increasing global demand stems from several factors, including the surge in vegetarian diets, the growing preference for lactose-free milk, and the recognition of health benefits associated with coconut milk's fats and vitamins. Pure coconut milk comprises over 30% saturated fats and lauric acid. (Abdullah et al., 2022).

The benefits of coconut milk extend to aiding digestion, supporting skin health, and regulating body temperature. It is linked to various health advantages, including potential anticancer, antibacterial, and antiviral properties. Coconut milk shares similarities with breast milk, containing saturated fat and lauric acid. Moreover, it is a plentiful source of essential nutrients and minerals like iron, calcium, potassium, magnesium, and zinc. (Tulashie et al., 2022).

2.7. SAPOTA:

Sapota (Manilkara Zapota L.) is one of the major tropical fruit trees in India and belongs to the Sapotaceae family. This cultivation originated in tropical South America and also inhabited tropical coastal India, which is sometimes considered the indigenous culture of that country. It is also known as Naseberry, Mud Apple and Sapodilla Plum. Chikoo grows in warm climates and takes about 5 to 8 years to mature (Singh et al., 2019).

Sapota is a significant small fruit with various health benefits. It is a delicious fruit known for its soft, sweet pulp, granular texture, and pleasant aroma. Its pulp is red or light brown in color and the thin peel is yellowish with a dull brown tint. It includes minerals such as iron, potassium, copper and calcium, as well as proteins, amino acids and phenolic substances such as carotenoids, catechins, chlorogenic acid, gallic acid, ascorbic acid, leucodelphinidin, leucopelargonidin and leucocyanidin. With abundance of phytochemicals, sapota is a rich source of antioxidants and free radical scavengers (Chaudhary et al., 2023).

Several researchers have highlighted the multitude of phytochemicals responsible for various biological effects, including anti-inflammatory, anti-arthritic, antibacterial, antifungal, antioxidant, anti-tumor, and antidiabetic properties. Despite its significant economic and medical importance, this substance remains relatively unpopular due to its perishable nature, which leads to quick spoilage. (Bano and Ahmed, 2017).

CHAPTER 3

METHOD AND METHODOLOGIES

INTRODUCTION

This chapter deals with materials and method used for the development and analysis of the Yoghurt. This study was carried out at the Department of B.VOC Food Processing Technology, St Teresa's College Ernakulam during the year 2023-2024.

3.1 DEVELOPMENT OF NUTRIENT RICH AND FLAVOURED YOGHURT USING RAGI AND COCONUT MILK:

MATERIALS REQUIRED:

- 1. Ragi : Purchased from market**
- 2. Coconut : Purchased from local market**
- 3. Chikku : Purchased from local market**
- 4. Agar agar : Purchased from Stanes bake world**
- 5. Kefir culture : Purchased online from Amazon**

Yogurt was made by blending ingredients in three different ratios, labeled T1, T2, and T3, with each set of ingredients placed in separate plates. Ragi grains were soaked in drinkable water for 18 to 20 hours, then ground, strained, and milk was extracted. Fresh coconut flesh from recently cracked coconuts was collected, ground, and strained to obtain fresh coconut milk. Fresh chikku were bought and their flesh was scooped off leaving the peels, seeds and the central white part which may impart off taste. The flesh was then beaten in mixer followed by straining to get smooth chikku pulp. The required amounts of ragi milk, coconut milk, and chikku pulp were carefully weighed and combined in a milk pan. After warming the mixture to the appropriate temperature, agar agar was added as a setting agent. The mixture was boiled until reaching the desired thickness, then taken off the heat and allowed to cool until slightly warm. Next, the measured kefir culture was added to the mixture and thoroughly mixed. The product was then sealed and left to ferment for 6 to 8 hours before being refrigerated for chilling. Once set and chilled, the yogurt was ready to be enjoyed.

SAMPLE	RAGI MILK	COCONUT MILK	CHIKKU JUICE	AGAR-AGAR	KEFIR CULTURE	COW MILK
T1	200ml	50ml	100ml	6gm	0.25gm	0
T2	125ml	125ml	100ml	6gm	0.25gm	0
T3	150ml	100ml	100ml	6gm	0.25gm	0
T0	0	0	0	0	0.25gm	250ml

TABLE 1: FORMULATIONS OF THREE DIFFERENT YOGHURT

3.2 SELECTION IF THE BEST FORMULATION OF YOGHURT:

The field of sensory evaluation plays a crucial role in determining the acceptability of food products, and its importance is expected to grow significantly in the coming years. Sensory evaluation involves the scientific assessment of food attributes such as taste, aroma, texture, and appearance by human senses, primarily through methods like tasting panels and consumer testing. (Murray et al., 2003). Sensory evaluation of the three different samples namely T1, T2 and T3 after the preparation was carried out by a panel of 5 judges with the help of a scorecard. A model of the score card used is given in Appendix.

3.3 NUTRIENT PROFILE OF THE DEVELOPED SAMPLE:

After the selection of the best sample by the sensory panel they were further subjected to chemical analysis to study their energy, protein, fat, iron and calcium and total ash content.

3.3.1 DETERMINATION OF ENERGY

The total energy content of the yogurt sample was determined using the methodology outlined in Pearson's Composition and Analysis of Foods: 9th edition. This involved listing out all the ingredients used in the yogurt and obtaining their respective nutritive values from published data sources. These values, such as calories, protein, carbohydrates, fats, etc., were then aggregated to calculate the total energy content of the sample.

3.3.2 DETERMINATION OF TOTAL FAT

The determination of fat content in yoghurt was done using IS: 12711:1989 method. In order to determine the fat content, 5g of material was weighed accurately in a suitable thimble and dried for 2 hours at $100 \pm 2^\circ \text{C}$. Then the material was placed in a Soxhlet extraction apparatus and it was extracted with petroleum ether ($40-60^\circ \text{C}$) for 8 hours. The Soxhlet flask which contained the extract were dried, the empty mass of which has been previously determined by taring at $95-100^\circ \text{C}$ for 1 hour. After this step, it was cooled in a desiccator and weighed. The process of drying, cooling, and weighing were repeatedly conducted for almost half hour intervals until the difference in mass between two successive weighing was less than 2 milligrams. Finally, the lowest mass obtained was recorded.

Calculation:

$$\text{Fat percent by mass} = 100(M1 - M2)/M$$

Where,

M1 = mass, in g of Soxhlet flask with the extracted fat

M2 = mass in g of empty Soxhlet flask, and

M = mass in g of the material taken for test

3.3.3 DETERMINATION OF PROTEIN CONTENT

The determination of protein content in yoghurt was done using AOAC method. In order to conduct this test, 0.70 to 2.20g sample was weighed into a digestion flask. Then 0.7g HgO and 15g Na₂SO₄ were added. To this 25 ml of sulphuric acid was poured. The flask was placed in an inclined position on a heater and heated gently until frothing ceases. The sample was boiled until it becomes clear.

After this step, the sample was cooled and about 200 ml of distilled water was added and again cooled to room temperature. To this, 25 ml of thiosulphate solution (8% in water) was added and mixed to precipitate mercury. To make strongly alkaline solution, hydroxide solution was carefully added through the sides of the flask. The apparatus was assembled taking care that the tip of the condenser extends below the surface of a known quantity of standard sulphuric

acid. To this, 5-7 drops of methyl red indicator was added. After all these steps, the solution was heated immediately until all ammonia has distilled (150 ml).

The receiver was lowered before stopping distillation and the tip of the condenser was washed with distilled water. It is then titrated against standard hydroxide solution. Finally, it was corrected for blank determination of reagents.

Calculation:

Nitrogen content (N) in % = $[(M_{acid})(ml_{acid}) - (ml_{NaOH})(M_{NaOH})] \times 1400.67$

mg test portion wt

Where,

M_{acid} - molarity of standard acid

ml_{acid} - volume in ml of acid used as trapping solution M_{NaOH} - molarity of standard base

ml_{NaOH} = volume in ml of standard base used for titrating

3.3.4 DETERMINATION OF CALCIUM CONTENT

Determination of calcium content in yoghurt was done using IS: 9497:1980(RA 2015). For this test, 2g of sample was weighed accurately in porcelain dish. In order to obtain a carbon free ash, the sample was ignited in a furnace. This residue was boiled in 40 ml HCl (1+3) and a few drops of HNO₃ was added. This was transferred to a 250 ml standard flask, and diluted to a perfect volume and mixed. 25 ml of clear liquid was pipetted into a beaker, diluted to 100 ml and 2 drops of methyl red was added.

To obtain pH 5.6 which is a brownish orange color, NH₄OH (1+1) was added in a dropwise manner. Two or more drops of HCl was also added to obtain pink color that has a pH ranging from 2.5-3.0. It was diluted to 150 ml and boiled. To this boiled diluted solution 10 ml of hot saturated solution of ammonium oxalate were added slowly with constant stirring. If the color changes from red to orange or yellow, HCl was added in dropwise until it turns pink.

The solution was kept overnight for the precipitate to settle. The supernatant was filtered through Whatman no 40 and the precipitate was washed thoroughly with NH₄OH (1+50). Paper was placed on an original beaker and a mixture of 125 ml water and 5 ml H₂SO₄ were

added. Heat to 75° C and titrate against 0.02M KMnO₄ (0.1N) to slight pink color. It was then corrected for blank determination test.

Calculation :

$$\text{Calcium (as Ca)} = \frac{\text{titre volumes} \times \text{normality of KMnO}_4 \times 100 \times 28 \times 40}{\text{Sample weight} \times 1000 \times 56}$$

$$\text{Sample weight} \times 1000 \times 56$$

3.3.5 DETERMINATION OF IRON CONTENT

The iron content was determined using AOAC Method. Sample is prepared by Microwave digestion Method. Weigh accurately about 25 g of well homogenized sample into a clean silica dish. Add 25 ml of 20% sulphuric acid. Mix thoroughly with a glass stirring rod ensuring all sample material is wetted by the acid. Rinse stirring rod with water into silica dish. Dry the contents of the dish thoroughly on a steam bath or in an oven around 110°C. When the sample is thoroughly dry, heat the contents of the dish with a soft flame (such as that of a Bunsen burner) until all volatile or readily combustible matter has been removed. Transfer the dish to a furnace set at 250°C. Slowly raise temperature to 500 °C. Ash at this temperature for about 6 to 8 hours. Remove the dish and cool. Ash should now be white or brownish red and essentially be carbon free. If ash contains carbon particles, wash down sides of dish with water and add 2 ml of HNO₃ and mix well. Dry thoroughly on hot plate. Return dish to furnace at 500°C and ash for 30 minutes. Repeat nitric acid treatment using 1 ml increments of HNO₃ until white/brownish red, carbon free ash is obtained. When clean ash is obtained, remove the dish from furnace, cool and add 1ml HNO₃ and 10 ml of water. Heat on hot plate till sample ash is dissolved. Quantitatively transfer the contents of the dish to a 50 ml volumetric flask, heat the dish with 10 ml of HCl (1+1) and transfer the solution again to the same volumetric flask to volume with water. Prepare sample blank solution by following the same procedure as described for sample. Use same quantities of reagents including water for both sample and blank. Subject both sample and sample blank to identical treatment (even the length of time kept in furnace etc..).

Calculation:

$$\text{Calcium (Ca) (mg/L)} = \frac{\text{Concentration (mg)} \times \text{Volume (V)} \times \text{Dilution Factor (DF)}}{\text{Weight of sample}}$$

3.3.6 DETERMINATION OF TOTAL ASH

Take fresh sample for the determination, rather than left over after determination of moisture. Ignite the dried material in the dish left after the determination of moisture with the flame of a burner till charred. Transfer to a muffle furnace maintained at 550-600°C and continue ignition till grey ash is obtained. Cool in a desiccator and weigh. Repeat the process of heating, cooling and weighing at half hour intervals till the difference in weight in two consecutive weighings is less than 1 mg. Note the lowest weight. If ash still contains black particles add 2-3 drops of pre-heated water at 60°C. Break the ash and evaporate to dryness at 100-110°C. Re-Ash at 550°C until ash is white or slightly grey.

The percentage ash was calculated as follows,

$$\% \text{ of ash} = (B - C) / A \times 100$$

Where, A = sample weight prior to drying

B = weight of dish and contents after ashing

C = weight of empty dish

CHAPTER 4

RESULT AND DISCUSSION

The present investigation was undertaken to develop probiotic vegan yogurt using ragi milk, coconut milk, and sapota. In total 3 different formulations were prepared during the study. They were T1, T2, and T3.

4.1 DEVELOPMENT AND PHYSICO-CHEMICAL ANALYSIS OF PROBIOTIC KEFIR YOGHURT USING ELEUSINE CORACANA, COCOS NUCIFERA AND MANILKARA ZAPOTA

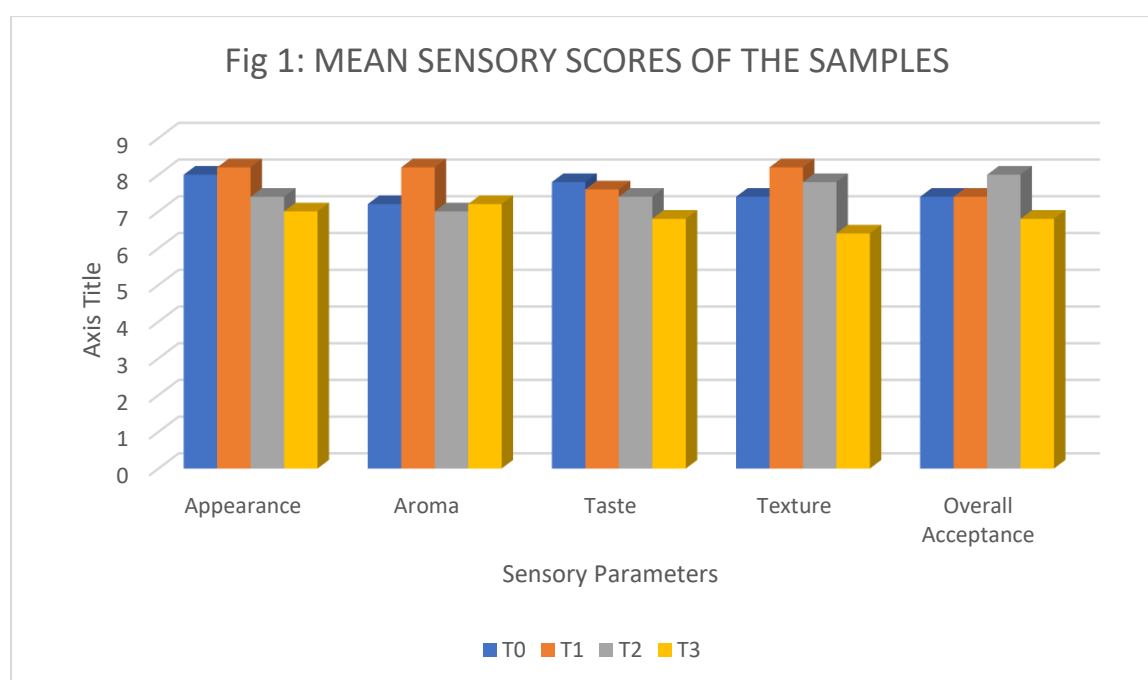
In the present study 3 different formulations of yoghurt were made by making small alterations in the composition of ragi milk and coconut milk and were subjected to sensory evaluation for selecting the best formulation along with the control.

4.2 SELECTION OF THE BEST FORMULATION OF YOGHURT

The acceptability of the three samples were found by a panel of 5 semi trained members using a nine-point hedonic scale. It was found that sample T2 with 50% ragi milk and 50% coconut milk has got more acceptance whereas the sample T3 with 60% ragi milk and 40% coconut milk got the least acceptance. It was noted that the yogurt sample, prepared using the described method and ingredients, received the same level of acceptance in terms of sensory parameters as the control sample made from milk. The yogurt sample exhibits a subtle brown hue attributed to the inclusion of ragi milk and sapota juice, while the addition of coconut milk contributes to its creamy texture. This suggests that despite the variations in ingredients and ratios used in the yogurt sample, it was able to match the sensory appeal of the traditional milk-based yogurt. Image of the final product is attached in appendix number 3.

**TABLE 2: MEAN SENSORY SCORES OF THREE DIFFERENT FORMULATIONS
OF YOGHURT ALONG WITH CONTROL**

SL NO	QUALITY PARAMETERS	T0	T1	T2	T3
1	APPEARANCE	8	8.2	7.4	7
2	AROMA	7.2	8.2	7	7.2
3	TASTE	7.8	7.6	7.4	6.8
4	TEXTURE	7.4	8.2	7.8	6.4
5	OVERALL ACCEPTANCE	7.4	7.4	8	6.8

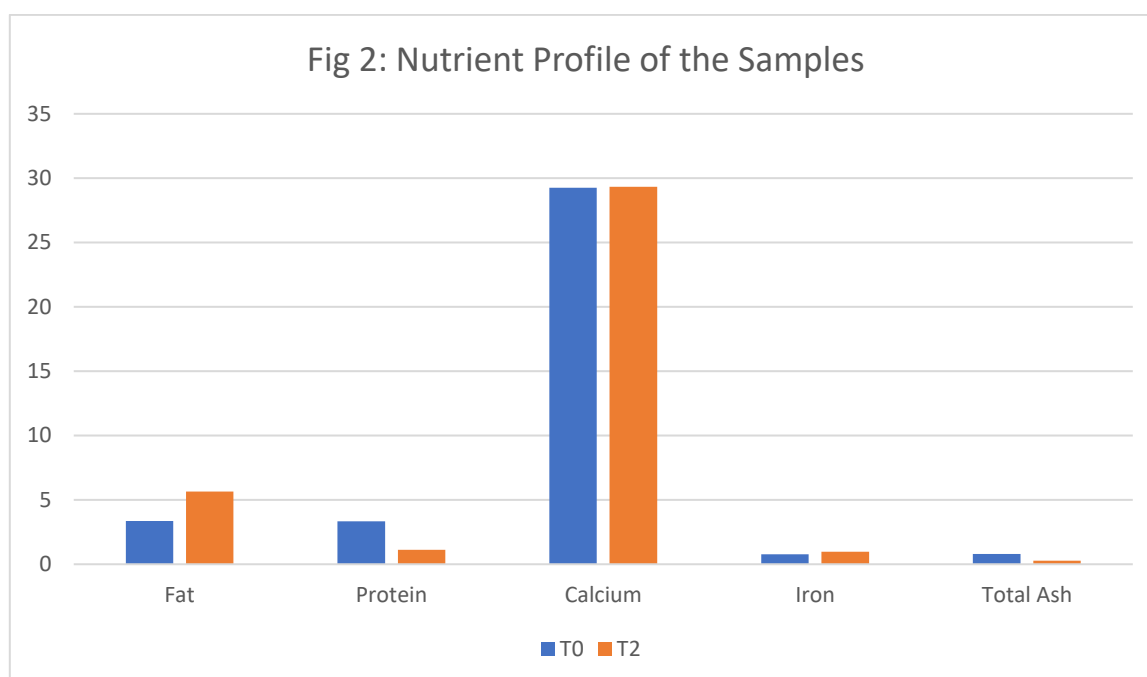


4.3 NUTRIENT PROFILE OF THE DEVELOPED SAMPLE

The yoghurt which is rich in ragi and coconut has got much nutritious properties and have a remarkable health benefit so, it is attracted to every age group. Iron's main role is to help transport oxygen around the body. It's also important for brain function, and a healthy immune system. Calcium is vital for healthy bones and teeth and it also helps normal blood clotting. So, It is important to impart in our daily life.

TABLE 3: NUTRIENT PROFILE OF THE SAMPLE

SL NO.	PARAMETERS	UNIT	T0	T2
1	ENERGY	KCAL	60.0	120.0
2	TOTAL FAT	%	3.36	5.66
3	PROTEIN	%	3.33	1.13
4	CALCIUM	mg/100g	29.26	29.32
5	IRON	mg/100g	0.78	0.96
6	TOTAL ASH	%	0.79	0.27



4.3.1 ENERGY

Energy content is typically measured in calories or kilocalories (kcal) and represents the amount of energy released when a food is metabolized by the body. Understanding the energy content of foods is essential for maintaining a balanced diet and managing calorie intake. It helps individuals make informed choices about their dietary habits, especially when it comes to weight management and overall health. From the above results it is noted that the energy content of T2 is 120Kcal whereas that of T0 is 60kcal. This difference in energy content could

be due to several factors, including variations in ingredient ratios, processing methods, or the addition of certain ingredients with higher energy densities.

4.3.2 FAT

The fat content in yogurt was analyzed using the IS: 12711:1989 method. Fat is essential for providing energy and essential fatty acids necessary for maintaining healthy skin. Moreover, a higher fat content assists in the absorption of fat-soluble vitamins like A, D, E, and K. Additionally, fats play a role in enhancing the absorption and retention of flavors. (Adel et.al. 2017). An average healthy person should consume 58 grams of fat per day, according to RDA guidelines. It was found out that T2 contains 5.66g/100g of fat content that means an average of 24.39 % of daily requirement can be met by consumption of 1 cup (250gms) of yogurt. A higher fat content was found in T2 compared to T0 this may be due to the addition of coconut milk in the sample.

4.3.3 PROTEIN

The protein content in yogurt was assessed using the AOAC method. Proteins are nitrogen-containing compounds composed of amino acids. When consumed, the body breaks down proteins into their constituent amino acids. These amino acids are linked together by α -peptide bonds. Proteins serve as structural components of muscles and various bodily tissues. They also contribute to the production of essential molecules such as hemoglobin, enzymes, and hormones. On average, an adult requires approximately 54 grams of protein daily for maintaining overall health and bodily functions. (Hoffman and Falvo, 2004). It was found that T0 and T2 contains 3.33 and 1.13% respectively.

4.3.4 CALCIUM

Calcium plays an important role in muscle contraction, building strong bones and teeth, nerve impulse, blood clotting, oocyte activation, regulating heart beat and fluid balance within the cells etc. The human body contains of about 2% of Ca and 98% of this occupies in the bones. The daily requirement of calcium for normal adult is 600 mg/day. (Beto, 2015). From the present investigation it was found that T0 and T2 contains 29.26 and 29.32 mg calcium per 100 g of the sample. This indicates that the developed plant-based yogurt is capable of providing a

comparable amount of calcium to that of yogurt made from milk. The slightly increased calcium levels in the plant-based yogurt sample T2 is likely due to the inclusion of ragi as an ingredient. Ragi, or finger millet, is renowned for its comparatively elevated calcium content compared to other grains.

4.3.5 IRON

The iron content in yogurt was analysed using the AOAC method. Iron deficiency and anaemia are prevalent health concerns globally and are frequently encountered in clinical settings. Iron deficiency occurs when the body's iron needs are not adequately met, often due to factors like blood loss or insufficient dietary intake. Iron is essential for various physiological functions in the human body. It plays critical roles in oxygen transport, supporting immune function, facilitating cell division and differentiation, and contributing to energy metabolism. Therefore, ensuring an adequate intake of iron is crucial for maintaining overall health and preventing conditions related to iron deficiency, such as anemia (Piskin et al., 2022). The nutrient analysis of the yoghurt revealed that T0 and T2 contains about 0.78 and 0.96 mg iron per 100g of the sample. The increase in iron content of T2 may be due to the addition of ragi and sapota.

4.3.6 TOTAL ASH

The mineral content in a product is assessed through its ash content, which is closely linked to the material's purity and cleanliness. Through the oxidation of organic substances present in yogurt, the ash content serves as a measure to determine the overall mineral concentration (Fox, 1998). The ash content of T0 and T2 was found to be 0.79 and 0.27 respectively.

CHAPTER 5

SUMMARY AND CONCLUSION

The study aimed to develop and analyze probiotic kefir yogurt using a combination of *Eleusine coracana*, *Cocos nucifera*, and *Manilkara zapota*. Different formulations were prepared and evaluated for organoleptic qualities, with sample T2, containing a 50:50 ratio of ragi milk to coconut milk, showing the highest mean score. Chemical and nutritional analyses revealed high energy, fat, calcium, and iron content, suggesting superior nutritional benefits compared to commercial dairy yogurt. Shelf life testing was not conducted due to time constraints, but further studies could explore this aspect. Overall, the study suggests that ragi milk and coconut milk can serve as viable substitutes for dairy milk in producing vegan probiotic yogurt with enhanced nutritional value.

CHAPTER 6

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CHAPTER 7

APPENDICES

APPENDIX 1: INGREDIENTS USED IN THE PREPARATION OF YOGHURT



RAGI



COCONUT



SAPOTA



AGAR-AGAR



KEFIR-CULTURE

APPENDIX 2: DIFFERENT STAGES OF PROCESSING



RAGI MILK



COCONUT MILK



SAPOTA PULP

APPENDIX 3 FINAL PRODUCT & CONTROL



T2



T0

APPENDIX 4

SCORE CARD

HEDONIC RATING SCALE

Name:

Product:

Date:

You are provided with 4 samples. Please evaluate the sample for acceptability and allot a score from the Hedonic scale as below:

	Appearance	Aroma	Taste	Texture	Overall acceptability
T ₀					
T ₁					
T ₂					
T ₃					

9 – Point Hedonic Scale	
9	Like Extremely
8	Like Very Much
7	Like Moderately
6	Like Slightly
5	Neither Like or Dislike
4	Dislike Slightly
3	Dislike Moderately
2	Dislike Very Much
1	Dislike Extremely

Comments:

Signature: