2022-07

Development of plant-based functional yoghurt as an alternative to dairy products

Dusabe, Angelique

NM-AIST

https://doi.org/10.58694/20.500.12479/1497

DEVELOPMENT OF PLANT-BASED FUNCTIONAL YOGHURT AS AN ALTERNATIVE TO DAIRY PRODUCTS

Angélique DUSABE

A Dissertation Submitted in Partial Fulfilment of the Requirements for the Degree of Master in Life Sciences of the Nelson Mandela African Institution of Science and Technology

Arusha, Tanzania

July, 2022

ABSTRACT

Health concerns and risks associated with dairy products such as lactose intolerance, milk allergens, and other considerations like veganism have increased the demand for dairy-free alternatives worldwide. Additionally, the healthy active compounds present in plant source ingredients raise consumers' choices. However, the existing plant-based beverages in the market such as Alpro: coconut, and rice milk not only portray deficient nutritional profiles but are also expensive, making them scanty in limited-resource countries. The main aim of this study was to formulate a convenient plant-based yoghurt (PBY) with a nutritional profile, texture, and flavour comparable to that of cow's milk using plant-based milk from locally available ingredients (coconut, sesame and rice). Objective function (OF), decision variables (DV) and constraints parameters were applied for the optimization of ingredients ratios in the linear programming (LP) model. Fermentation was employed to develop a palatable and functional yoghurt. Laboratory analysis was conducted to validate the nutritional values calculated by LP. Relative differences between the results generated by the LP and the values analyzed in the lab were also calculated. Finally, sensory evaluations were conducted to test consumers acceptability of the PBY. The results showed that LP-optimized PBY can be formulated at a low cost (USD 0.9 per L) which is 60% cheaper than Alpro natural PBY. The formulated PBY were microbiologically stable for 14 days of storage at 4 °C. No significant differences were obtained between the LP and lab results ($P \geq 0.05$). The formulation contained essential nutrients and health-benefiting bioactive compounds, enough to meet the Recommended Daily Intake (RDI) for 2-10-years old. In terms of the overall acceptability, the sensory attributes revealed that PBY was liked very much by consumers. These findings demonstrate that culturally-acceptable functional PBY considered as an alternative to dairy products can be formulated using locally available ingredients.

Keywords: essential nutrients, lactic acid bacteria, linear programming, locally available ingredients, plant-based yoghurt.

DECLARATION

I, **Angélique DUSABE** do hereby declare to the Senate of Nelson Mandela African Institution of Science and Technology that this work is original and has not been submitted elsewhere, nor is it concurrently under consideration for submission for a degree in any other institution.

Angélique DUSABE ________________________ Date: 19/07/2022

Name and signature of candidate

The above declaration is confirmed by

Dr. Musa Chacha Date: 19/07/2022

Supervisor, School of Life Sciences and Bioengineering, NM-AIST, Tanzania

Dr. Sr. John Mary-Vianney Date: 19/07/2022

Supervisor, School of Life Sciences and Bioengineering, NM-AIST, Tanzania

Dr. Jofrey Raymond Date: 19/07/2022

Supervisor, School of Life Sciences and Bioengineering, NM-AIST, Tanzania

COPYRIGHT

CERTIFICATION

The undersigned certify that, they have read and hereby recommend for acceptance by the Nelson Mandela African Institution of Science and Technology (NM-AIST), a dissertation entitled **"Development of plant-based functional yoghurt as an alternative to dairy products"** in partial fulfillment of the requirements for the degree of Master in Life Sciences of the Nelson Mandela African Institution of Science and Technology.

Approval of the Dissertation:

Dr. Musa Chacha Date: 19/07/2022

Supervisor, School of Life Sciences and Bioengineering, NM-AIST, Tanzania

Dr. Sr. John Mary-Vianney Date: 19/07/2022

Supervisor, School of Life Sciences and Bioengineering, NM-AIST, Tanzania

Dr. Jofrey Raymond Date: 19/07/2022

Supervisor, School of Life Sciences and Bioengineering, NM-AIST, Tanzania

ACKNOWLEDGMENTS

I greatly thank the almighty God for his daily grace and blessings that enabled me to undertake my studies successfully. My sincere appreciation also goes to my academic supervisors, Dr. Jofrey Raymond, Dr. Musa Chacha, and Dr. Sr. John Mary Vianney for their continuous guidance and supervision throughout my Master's Research work. I would also like to recognize the hard work of the Arusha Technical College (ATC) lab technicians for their tireless collaboration and contribution while I conducted this study. Furthermore, I would like to thank the Interuniversity Council for East Africa (IUCEA) for funding my studies and research at the Nelson Mandela African Institute of Science and Technology (NM-AIST) through CREATES. Finally, profound gratitude goes to my beloved parents and family members for their encouragement and moral support. I am grateful to my colleagues Steven J. Kitigwa, Frank Sangija, Happyness A. Kisighii, Angela Aluko, Jacinta W. Ndung'u, my fellow Rwandeses in NM-AIST and all my friends for both their academic and moral support.

God bless you all!!!

DEDICATION

This work is dedicated to the Almighty God for His protection and guidance in my academic life. This work is also dedicated to my beloved parents, my relatives, and friends for their unconditional love and care during my studies.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS AND SYMBOLS

ALA	Alpha-Linolenic Acid
AOAC	Association of Official Analytic Chemistry
C.F.U	Coliform Forming Unity
EAC	East African Community
EAS	East African Standard
FAO	Food and Agriculture Organization of the United Nations
FAOSTAT	Food and Agriculture Organization Statistical databases
FSANZ	Food Standards Australia and New Zealand
HPLC	High-Performance Liquid Chromatography
LAB	Lactic Acid Bacteria
LA	Linoleic Acid
LP	Linear Programming
MRS	De Man, Rogasa and Shape
NM-AIST	Nelson Mandela African Institution of Science and Technology
NCDs	Non Communicable Diseases
RDI	Recommended Daily Intake
TFC	Total Flavonoid Content
TFCT	Tanzania Food Composition Tables
TPC	Total Phenolic content
UN	United Nation
UNICEF	United Nations International Children's Emergency Fund
USDA	United States Department of Agriculture
WHO	World Health Organization

CHAPTER ONE

INTRODUCTION

1.1 Background of the problem

Humans have consumed dairy products for centuries (Yiğit, 2020), and Eastern African countries still consume substantial amounts of dairy products to date. Dairy products are considered as good source of calcium, fats, carbohydrates, and proteins, all of which are essential to human nutrition, and comparable balance is hard to find in other food. The world demand for dairy products is connected to a shortage in milk availability, especially in low-income countries (Mäkinen *et al*., 2016). However, the excessive consumption of dairy products also poses some health concerns and risks, including ovarian and prostate cancers (Davoodi *et al*., 2013; Gao *et al.*, 2005).

A more acute risk from milk and dairy products consumption is lactose intolerance that affects approximately 75% of the global population (Mäkinen *et al*., 2016). According to Heaney (2013), about 85% of African people are lactose intolerant; deficient in the lactase enzyme responsible for the digestion of milk sugar lactose. The symptoms of lactose intolerance include gastrointestinal distress, diarrhea, and flatulence. Cases (5-20%) of lactose intolerance were prevalent in agriculturalist communities in Africa. About 50% of nomadic Fulani Nigerian pastoralists population in West Africa have reported to be lactose intolerants (Deng *et al*., 2015). Similar trends have been reported in East Africa. For instance, a study in Morogoro and Njombe districts of Tanzania indicated that 10% of individuals were lactose intolerant with fermented goat milk, and 20% of the intolerant persons in Morogoro reportedly developed ulcers after milk consumption (Mushi, 2014). About 25.5% of severely malnutrished children with diarrhea in Uganda have been reported to be lactose intolerant (Nyeko *et al*., 2010). Contrarily, the lactase persistence cases have previously been reported in cattle-raising nomadic community, particularly the Kenyan and Tanzanian Maasai pastoralists people (Tishkoff *et al*., 2007).

Moreover, food allergies frequently appear to be a common effect of cow's milk consumption. The prevalence of cow's milk allergy (CMA) ranges between 0.5% and 7.5% globally, with children portraying more effects than adults (Bahna, 2002). According to Fiocchi *et al.* (2010) more than 84% of infants lack immunoglobulin E (IgE) which helps individuals to tolerate cow milk protein allergens. Presently, food allergies from animal milk products are increasingly becoming an emerging disease condition in Africa (Atiim & Elliott, 2016; Leung *et al.*, 2018). For instance, a study in Tanzania showed that 16.7 % of the studied individuals experienced allergic reactions to milk (Abla *et al*., 2008). Also, 18% of children under the age of five years in Kenya are allergic to milk products (Kung *et al*., 2014); and 20 % of patients had reactivity to milk allergens in South Africa (Els *et al*., 2010). Additionally, cow's milk consumption has also been linked to chronic constipation in children. All these challenges have caused people to switch to delicious dairy-free alternatives to curb the drawbacks associated with the consumption of animal milk.

Plant-based types of milk are a rising alternative for curbing the health concerns posed by dairy products. Their global market is expected to reach USD 2.89 billion by 2026, and this can aid as an inexpensive alternative in areas with a shortfall in animal milk accessibility (Aydar *et al*., 2020). Plant materials such as coconut, rice, almond, peanuts, quinoa, and sesame are naturally free from lactose and have been used as essential ingredients to produce plant-based beverages present in the market for lactose-intolerant individuals and those allergic to cow's milk (Sethi *et al*., 2016). However, the existing alternatives to milk and dairy products have shown to be highly-priced (Yiğit, 2020) and face many technical issues, either related to processing or preservation, that result in products with poor texture and/or flavor (Sethi *et al*., 2016; Gorlov *et al*., 2019). Further, most of these replacements are unbalanced in specific nutritional values relative to animal-derived products. However, they contain functionally-active components with health-promoting properties that continue to attract health-conscious consumers (Mousel & Tang, 2016).

Fermentation is normally applied to improve sensory, nutritional, and preservation challenges in food production (Tangyu *et al*., 2019). Today, fermented yoghurt is consumed as a probiotic source that improves digestion, enhances gut microbiota, and modulates the immune system (Mahfudh *et al.*, 2021). However, the poor sensory attributes (particularly the texture and flavor) in plant-based yoghurts (PBY) are due to the absence of lactose and fat that exist in cow's milk. Traditional lactic acid yoghurt strains generally require lactose for fermentation. Further, the enjoyable creamy-like texture in yoghurt is partly due to the fat content of the milk and partly due to the interaction between the lactic acid (produced during fermentation) with the casein and whey proteins in cow's milk (Banerjee & Bhattacharya, 2012). Cognizant of this, the absence of these proteins in plant-based milk, creating the desired "creamy-like" textures in PBY remains a daunting task. Therefore, there is a need to develop a PBY using locally available ingredients rich in essential nutrients and functional compounds and providing good flavor and texture to consumers. The main aim of the present study was to formulate a convenient plant-based functional yoghurt with essential nutrients and bioactive compounds comparable to that of cow's milk using locally available ingredients.

1.2 Statement of the problem

Currently, convenient PBY with the flavor and texture comparable to the cow's milk and that provides substantial nutritional benefits to consumers with milk allergy and lactose intolerance are scanty in the Eastern African region. The common plant-based milk in Eastern Africa is soybean milk. Unfortunately, individuals who are allergic to cow's milk may also be allergic to soybean-based products (Jeske *et al.*, 2017). Additionally, plant-based milk products are highly-priced (Sethi *et al.*, 2016) and a majority of them are not acceptable as face the challenge of sensory acceptability (texture and flavor) or deficiency in some nutritional components when compared to cow’s milk yoghurts (Mäkinen *et al.*, 2016; Palacios *et al*., 2010). All these limit lactose intolerant people and other individuals allergic to animal milk proteins from accessing nutritious and palatable dairy alternatives in East Africa.

1.3 Rationale of the study

Yoghurt with active culture is easier to digest than milk. Many people, who cannot tolerate milk, either because of milk protein allergy or lactose intolerance, prefer yoghurt to milk (Perdigon *et al.*, 2002). Yoghurt and other fermented forms of milk not only aid in digestion but have also been shown to ease diarrhea (Aydar *et al*., 2020), boost immunity (Gibson, 2004; Mahfudh *et al*., 2021), fight infections (Banwo *et al*., 2021), and protect against colon cancer (Perdigon *et al.*, 2002). Maintaining an individual's health through sustenance is essential in managing health stresses, improving productivity at the workplace, reducing medical costs and other social expenses which altogether improve people’s lives. The developed healthy dairy-free PBY will increase a choice space for dairy alternative consumers and contribute to food security where there is shortage of animal milk in Tanzania.

1.4 Objectives

1.4.1 General objective

The main objective of this study was to formulate a convenient plant-based functional yoghurt with a comparable nutritional profile to that of cow’s milk.

I. 4. 2 Specific objectives

i) To identify the suitable ingredients for formulating plant-based functional yoghurt .
ii) To determine the optimal formulation of a PBY rich in essential nutrients and bioactive compounds using linear programming (LP).
iii) To analyze the nutritional content, functional values, and sensory acceptability of the final optimized PBY.

I.5 Research Questions

i) Which locally-available ingredients can be used for formulating PBY?
ii) What proportion of ingredients is suitable for formulating convenient plant-PBY?
iii) What are the nutritional and functional values and consumer acceptance of formulated PBY?

1.6 Significance of the study

The PBY in this research study was made from ingredients other than animal milk and can address the identified drawbacks of the consumption of animal milk in the region and provide options for lactose intolerant people and individuals allergic to animal milk products. The product will significantly contribute to the current global efforts to curb diseases related to nutrition, such as lactose intolerance and animal milk allergens, in children and the general population. In a long-term plan, this product will be commercialized to create direct employment and increase the value of indirect jobs, particularly in Tanzania and in East Africa at large.

I.7 Delineation of the study

This study developed PBY rich in essential nutrients and functional compounds with acceptable sensory attributes. Food composition databases were used to create a checklist of the locally available nutritious ingredients in Tanzania. The ratios utilized to formulate the optimal product were calculated using the LP technique. The fermentation method was applied not only for processing and preservation purposes but also for health-promoting benefits and sensory development in yoghurt. Laboratory analysis and sensory evaluation were performed to validate the developed product's nutritional, functional, and sensory qualities. The findings from this work-study display acceptable and convenient ready-to-drink yoghurt rich in functional and nutrition values formulated from available ingredients in Tanzania.

CHAPTER TWO

LITERATURE REVIEW

2. 1 Opportunity of future research on functional foods in East Africa

The term "functional foods" was invented as a marketing slogan in Japan in the 1980s but their description is frequently misconstrued because they are not legally recognized in most nations. Serafini *et al*. (2012) define a functional food as one that is taken as a normal diet and contains many bioactive compounds that can provide additional health benefits beyond the basic nourishment. It influences one or more body functions in a significant way to cause either an improved state of health and well-being or to lessen the risk of disease (Roberfroid, 2002).

According to Banwo *et al*. (2021), functional foods are not single, well-defined, or well-characterized individual foods but rather a diverse range of food products. These comprise various components, nutrients, and non-nutrients that affect various physiological functions relevant to one's overall well-being and health and disease prevention. A functional food ingredient has to be non-toxic and contains bioactive compounds that improve health. It can be sourced from animals, plants, microbes, or herbs and spices. It should include probiotics, prebiotics, functional fatty acids, functional peptides, antioxidants, vitamins, and minerals, which are commonly found in natural foods (e. g: broccoli and tomatoes), processed foods (e. g: fermented foods and yoghurt), medical foods (gluten-free and sugar-free foods), babies' foods, energy bar, and sports drinks.

Probiotics, yoghurt, and other fermented foods have well-documented health and nutritional benefits with good scientific evidence to verify their usefulness in diets. The most documented probiotic bacteria with proven human efficiency are the *Bifidobacterium* and *Lactobacillus* genera, which are widely employed in the fermentation of commercial products. Probiotics can colonize the intestine after passing through the upper gastrointestinal tract and present the following health-promoting benefits:

i) Providing the host with the end product of anaerobic carbohydrate fermentation, such as org anic acids.
ii) Successfully competing with the pathogenic bacteria in terms of foods and space.
iii) Producing particular polysaccharides to stimulate the immunological responses of the host.

Prebiotics are the non-digestible food ingredients that favor the host by encouraging the growth activities of one or a few bacteria in the colon, hence boosting the gut flora. Prebiotics are fermentable ingredients that induce modifications either in composition or gut microbiota activities, resulting in positive effects on the host's wellness. Dietary prebiotics must be resistant to digestion to reach the bowel with their chemical structures and be fermented by large intestine microbiota, boosting probiotic longevity, metabolism, growth, and beneficial activities in the digestive system. The common examples of prebiotics examples in diets are inulin, β-glucan, pyrodextrins, xylans, and oligosaccharides. Synbiotics are a mix of probiotics and prebiotics that benefit the host by increasing the survival of live bacteria in the digestive tract more effectively than either prebiotics or probiotics alone (Gorlov *et al*., 2019).

As long as functional foods are widely accepted, functional beverages, foods, and ingredients will play an important part in human nutrition. The extraction and quantification of biological compounds is a crucial step in developing functional foods. Since most individuals do not include fruits and vegetables in their meals as sources of bioactive compounds, the use of isolated chemical components known as nutraceuticals added in capsules and tablets is becoming popular in the market. However, consumers prefer foods grown and processed sustainably, considered safe, natural, and fresh with nutritional contents. This shows that plant-based foods will soon be a better source of essential nutrients worldwide, which calls for the development of new functional foods by researchers, and food industries that focus on the use of plant-based foods such as fruits and vegetables, whole grains, and beans.

According to the World Health Organization (WHO) (2021), non-communicable diseases (NCDs) kill about 41 million (71%) each year worldwide, and 77% of all NCDs occur in low-and-middle-income countries. Children, adults, and the elderly are vulnerable to the risk factors of NCDs. Unhealthy diets, changes in lifestyle, and less physical activities are linked to the metabolic risk factors of these diseases. Plant-based foods have been an essential source of vitamins, fibers, and natural substances known as phytochemicals that help to maintain good health by exhibiting anti-tumor, antioxidant, anti-viral, and anti-proliferation activities that protect the human body against NCDs like diabetes, cancers, cardiovascular diseases, stroke, and diseases of the digestive, endocrine, and immune systems. However, the relationships among food and health maintenance and disease prevention have only been known in recent times. The functional food and health trends presented new opportunities and perspectives on food ingredients over metabolism and consumers' physical and health function.

2.2 Plant-based products acceptance and their shortfalls

The survey by Palacios *et al*. (2010), in the US showed how cow's milk is more consumable and preferred than plant-based milk despite cases of lactose intolerance. This is because most of the sensory attributes of plant-based milk products do not exhibit animal milk's preferred flavor and texture. Some plant-based milk products that consumers like have been shown to have similar attributes to animal-based milk. For example, a study by Akoma *et al.* (2000) in Nigeria showed that skimmed cow's milk does not differ organoleptically with pure coconut milk and coconut with cow milk composites. The acceptability of newly developed food can depend on various criteria, for instance, nutritional information, health benefits, good or familiar taste, and environmental features. All these criterion can influence the increase of the community's willingness to try a new formulated food. According to market analysis, the value of non-dairy-based milk alternatives has steadily been increasing and will reach 26 billion by 2025 (Tangyu *et al*., 2019), with the key aspects of plant substitutes are improving sensory, nutritional quality, and awareness.

Research findings reported in 2020 by the US National Medicine Library showed that lactose-free diets are available for patients with lactose intolerance. Non-dairy substitutes are irreplaceable in vegan food industries as raw critical ingredients for plant-derived apple cheese, butter, yoghurt, kefir, and ice cream (Banerjee & Bhattacharya, 2012). Biologically important compounds like antioxidants, probiotics, unsaturated fatty acids, fiber, and functional fatty acids reduce cardiovascular disease risks, cancer, type II diabetes, and atherosclerosis (Manasa *et al*., 2020). Also, milk from plants is free from cholesterol (Aydar *et al*., 2020).

Despite their nutritional advantages, dairy alternatives occasionally contain poor texture and flavor linked to phase separation due to the lack of an appropriate process to develop stable products that affect consumers' acceptance. Generally, most of the dairy alternatives contain low contents of saturated fat, proteins, vitamins (Vitamin D, vitamin B 12), and minerals (Ca) when compared to cow's milk (Jeske *et al*., 2017). To date, fortification and nutritional values of raw ingredients are key points for improving dairy alternatives' nutritional and functional values. The study by Sethi *et al.* (2016) suggested that combining ingredients rich in protein, fats, iron, folate, calcium, vitamin D, and other essential nutrients vital for children's growth should be considered in formulating plant-based milk alternatives.

2.3 Potential sources of plant-based yoghurt ingredients

Plant-based yoghurt is obtained by semi-fermenting water-based extracts (plant-based milk) from plant source materials (cereals, nuts, legumes, or fruits pulp). Primary crops that can produce vegetable milk include coconut, rice, sesame, etc. Lactose-intolerant people and those that present milk allergies can consume vegetable milk which is generally lactose-free. Coconut (*Coconut nucifera*) is a native crop of Malaysia distributed across continents and all over Africa, especially in the coastal countries from Sahara to South Africa in Zambia, Tanzania, and Kenya. Fresh coconut milk is among the traditional food ingredients rich in nutrients and bioactive compounds with the potential to improve consumers' healths. Some of the nutrients in coconut fruit are calcium (144.4 mg/L), Iron (24.56 mg/L), zinc (7.06 mg/L), Vitamin E (0.14 mg/L) and vitamin C (0.46 mg/L). Additionally, coconut contains phenolic compounds and phytosterols, antioxidants linked to the prevention of NCDs (Ngampeerapong *et al*., 2018).

Rice belongs to the grasses family; *Poaceae* and subfamily; *oryzoideae*. It originated from India, Southern Asia and spread all across the world. The Netherlands and Portugal colonies brought rice into West Africa, from where its journey continued to all African countries (Verma & Shukla, 2011). Rice is a common staple food crop with > 60% consumers worldwide and various ready-to-use products such as instant and rice flakes, puffed and popped rice. Canned and fermented rice products are also produced. Tanzania, which is the largest rice producer in EAC countries, produces about 3.2 million metric tons (MT) of rice and contributes to approximately 65% of all rice produced in the EAC (Kilimo-Trust, 2014). Rice contains carbohydrates (starch), an important component for energy provision in the human body. Rice is also rich in vitamins (vitamin B and vitamin E) and minerals. Rice milk is a great alternative to conventional milk, free from cholesterol and lactose. The bioactive compounds of rice-based products include tocopherols, dietary fibers, phenolic acids, gamma oryzanol, β-sitosterol, and vitamin E with health-linked benefits (Ryan, 2011). However, rice is poor in protein and fat components.

Sesame (*sesamum Indicum*) is an oilseed crop that originated in Asia and belongs to the Pedaliaceae family. Africa's sesame seed trade has doubled, accounting for more than 40% of global production (FAOSTAT, 2015). This increase is due to the presence of natural antioxidants like sesamin, sesamolin, and sesamol, with important health benefits which have raised the consumer demand for the crop. Sesame seeds are high in phytosterols which are linked to lower blood cholesterol levels. According to United States Department of Agriculture (USDA, 2019), sesame seeds contain proteins (20.25 g), fats (61.21 g), dietary fibers (11.6 g), and carbohydrates (11.73 g). Methionine, tryptophan, cysteine, leucine, and arginine are also found in sufficient amounts in sesame seeds, with a recommended daily intake (RDI) of around 100%. Combining rice, coconut, and sesame seed milk in producing PBY- Like appears to be innovative, given that these crops are well-known for the bioavailability of critical nutrients and biological compounds.

2.4 Plant-based yoghurt alternatives to dairy products

Yoghurt is a Turkish-derived name for a fermented milk product. Yoghurt must be packed with live active probiotics, commonly known as starter cultures which are acid-forming bacteria that reduce pH of milk and contribute to the sour taste, and thus increasing the viscosity of the yoghurt curd. These probiotic microorganisms comprise Lactic acid bacteria (LAB) such as *Lactobacillus acidophilus, L. bulgaricus, L. casei, L. Plantarum, L. rhamnosus,* and *Streptococcus thermophilus* (Ani *et al*., 2018). Hydrolysis of milk proteins occurs during yoghurt fermentation, producing bacterial metabolites that contribute to the taste, develop health-promoting properties, and inhibit the growth of antagonistic food poisoning bacteria (Gorlov *et al*., 2019).

Active LAB in yoghurt operate as an immunomodulator, assisting in preventing and treating gastrointestinal disorders and the digestion and absorption of more complex nutrients. Thus, they play a vital role in the healthiness of the gut microbiota due to their content in probiotics (Tangyu *et al*., 2019). The proteins in yoghurt are more digestible than those in milk. They can be a staple diet for children who cannot tolerate milk and consumers with allergies to milk protein or intolerant to milk lactose. In some countries where dairy products are a dietary stable, milk and yoghurt are nutritious foods that have several health benefits to humans. Still, some consumers may not tolerate lactose, the main sugar in animal milk, making it necessary to replace its use. The suggested treatment for lactose intolerance and milk allergies is avoidance of dairy products and use their alternatives (Facioni *et al.,* 2020).

Plant-based yoghurt alternatives are the best substitutes as a rich source of health-promoting probiotics to the gastrointestinal tract of consumers. Most of the existing PBY are reportedly low in calcium levels and other essential nutrients like vitamin D and B-12 that are present in animal milk (Oak & Jha, 2019) Therefore, plant-based substitutes can be safe for intestine and bone health if well formulated by focusing on the combination of essential nutrients in raw materials or fortification following Food Standard Australia and New Zealand (FSANZ) guidelines on plant-based alternatives and other international standards (Facioni *et al.*, 2020).

2.5 Prevalence of lactose intolerance and milk allergens

A predictable 70-75% of the global population are lactase enzyme-deficient (Heine *et al*., 2017; Mäkinen *et al*., 2016). According to the World Gastroenterology Organization (2019) lactose intolerance is prevalent in South America, Asia, and Africa. Previous reports show that about 65-85% of Africans are unable to digest lactose appropriately due to the inability to produce the lactase enzyme (Heaney, 2013). Similarly, 10-20% of Tanzanians were found to be lactose intolerant after consuming fermented goat and cow milk (Mushi, 2014). Lactose intolerance is a form of disaccharidase deficiency disorder linked to the incapacity to digest lactose into its galactose and glucose constituents (Heine *et al*., 2017).

The cause of lactose intolerance is the low levels of lactase enzyme in brush edge duodenum. Although lactase enzyme levels are high after birth, continue to decline with age. The symptoms of such deficiency are pain and abdominal bloating, flatulence, diarrhea, and nausea (Bailey *et al*., 2013). The measures to treat lactose intolerance are avoiding milk derivatives and having special diets aid in digestion and health care costs. Allergens caused by cow's milk are an immune response to milk proteins (casein and whey) that regularly follow ingestion. It is the most famous form of initiation of food allergy with an estimation of 0.5 - 3% alternating from the age of one year in a developed country (Flom & Sicherer, 2019). Approximately 18% of milk allergies in five-year-old children have also been reported in Kenya (Kung *et al*., 2014) and 20% of asthmatic children had reactivity to milk allergens in South Africa (Els *et al.*, 2010). In Tanzania, 16.7% of food allergies have also been reported (Abla *et al*., 2008).

2.6 Usage of locally sourced ingredients in plant-based yoghurt production

Nowadays, consumers are pursuing products with minimal or non-added sugar made from simple and natural ingredients to have a so-called clean label. In developed countries, manufacturers present aromatic and plant-derived single and blended yoghurts such as Lava PBY available in seven flavors and Provamel plant-based alternatives to yoghurt. Most industries use their locally available ingredients, for example, fiber-rich plantains, magnesium-rich pili-nuts, cassava roots, almonds, coconut, cashews, oat, squash, and carrot with advanced homogenization processing technologies (Gorlov *et al*., 2019). However, these substitutes are highly-priced compared to lactose-encompassing foods and are only famous in high-income countries (Bernat *et al.*, 2014). In developing countries, certain researchers have used locally available commodities such as *Moringa oleifera* seeds, Bambaranut, corn, rice, and soybeans to produce novel blended PBY (Ani *et al*., 2018; Belewu *et al.*, 2013).

According to the Food and Agriculture Organization of the United Nations (FAO) (2019), food production has remained at the subsistence level in East Africa, and a majority of income to farmers comes from selling their produce to local restaurants or whole-food shops. Consumers with milk lactose intolerance and milk allergies are not conscious of alternatives to dairy products because of the lack of these substitutes in the local markets. Conversely, there are limited innovations and research on the appropriate processes for fostering products derived from primary commodities. Ingredients must be preserved to avoid cross-contamination, which is the critical factor of consumer acceptability (flavor, color, and texture) to add nutritional values (Bernat *et al.*, 2014; Grasso *et al*., 2020). Therefore, community-based food processing industries can keep the local economy healthy by providing job opportunities, and purchasing produce from nearby areas; as a result, the choice of food commodities varieties in urban areas will also increase (FAO, 2019) . The development of such natural products can strengthen rural and urban economies by increasing traditional food demand and reducing urban reliance on food imports, particularly urban consumers' preferences, attitudes, behaviors, and perceptions about food (Brixi, 2018; Chileshe *et al*., 2020). This demonstrates the need to use primary ingredients to enhance individuals' healths while minimizing and preventing the problems associated with malnutrition to increase a choice space for consumers with lactose intolerance and allergic to milk proteins, and all consumers in the East African Community (EAC).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study design and sample collection

The food samples such as whole coconut, broken rice, sesame seeds (LINDI 02 variety); oyster mushroom (*Pleurotus ostreatus*) powder, and date palm fruits (*Phoenix dactylifera*, Medjool variety) were conveniently collected from farmers' local markets in Arusha, Tanzania. The commercial plain yoghurt was purchased from local supermarket at Tengeru, Tanzania. The guar gum (Bob's Red Mill Natural Foods Inc., USA), α-amylase (K-CERA, 96156 Ceralpha), and thermophilic freeze-dried lactic culture (YF-L811, YoFlex®, Denmark) were obtained from suppliers of food additives in Mbezi, Dar es Salaam. For prototype formulation, all ingredients were transported to the Nelson Mandela African Institution of Science and Technology (NM-AIST) food kitchen.

3.2 Formulation and prototype tasting

The general procedure for formulating and testing the model involved three key stages: The first stage involved determining the formulation's desired composition and potential ingredients to use. Local and global food composition databases and published reports were surveyed to get nutrient composition data of each ingredient. The optimal formulation was relied on the accuracy of the food composition database that was chosen. The second stage created a definite LP model key elements: the decision variables, objective function, and constraints. As described by Dibari *et al.* (2012), these parameters were utilized to set up and solve the LP model using Microsoft Excel Office 2010 (version 14.0.7268.5000) and the Solver add-in. The third stage involved the formulation (preparation) and sensory evaluation of the prototypes formulated by the model. The formulated prototypes were evaluated for mixing feasibility and predicted full-scale production feasibility at the NM-AIST laboratory.

3.2.1 Food composition database choices

The selection of the food composition database was based on the following features:

i) The most comprehensive collection of commodity composition data from around the world,

ii) The highest amount of nutrient data values per food ingredient,

iii) Food descriptors that correspond to the ingredients chosen,

iv) Internationally recognized datasets with methods that have been cited in peer-reviewed journals (Charles *et al.*, 2020; Dibari *et al*., 2012).

3.2.2 The selection of potential ingredients

In modern world, consumers no longer consider beverages as simple thirst-quenchers only but are also looking for beneficial functionalities in these drinks. Therefore, the bioavailability of active compounds in these brews can address users' different lifestyles and needs. The great impact on prototype quality is the choice of raw materials and technological processes to apply (Mäkinen *et al.*, 2016). During the selection of ingredients, all potential ingredients farmed in the EAC were shortlisted (Appendix 1). The selection was made based on the local availability, nutrient composition, ingredients prices, and cultural acceptability. In particular, the criteria of choosing the ingredients highlighted on commodities with healthy bio-active compounds, rich in essential nutrients like vitamin B9, vitamin C, iron, zinc, and vitamin E; and producing acceptable milk equivalent cow's milk in terms of flavor and texture. The ingredients proposed in the selection process were coconut, rice, cashew nut, peanut, and sesame. Food composition databases have nutritional sufficiency values and are locally found in Tanzania.

The inclusion of oyster mushroom powder in the formulation was due to its high fibers known as prebiotics such as β-glucans, chitin, and xylans, which have been shown to promote microbial activity in the colon and improve the host's health (Gibson, 2004). A combination of prebiotic and probiotic in yoghurt forms a feasible synbiotic linkage that can improve the gut's well-being as described by Tupamahu and Budiarso (2017). Date palm syrup is used as a natural sweetener and impacts the nutritional and functional values of yoghurt as propounded by Gad *et al*. (2010). The nutrition information for each ingredient was collected from the following databases: the US National Library of Medicine (2020), USDA Nutrient Database, Tanzania and Kenyan Food Composition Database, SELF Nutrition Database, FAO/IN FOODS Food Composition Databases, and SCI peer-reviewed journals. The price information for each ingredient, local and international, is paramount and was collected from the Tanzania consumer price index (CPI) and FAOSTAT, respectively (Charles *et al*., 2020).

3.2.3 Developing a Linear Programming model for plant-based yoghurt

Linear programming is an appropriate mathematical model in formulating novel optimized food products. It is a tool that aids in the usage of possible cheapest food ingredients to set and meet nutritional necessities while respecting multiple linear constraints (Sheibani *et al*., 2018). In this study, LP was used to minimize the objective function *Z*, which is the cost of the formulation. The decisions variables (DVs) were values of ingredients weights which can be changed to minimize the cost of formulating *Z*. The LP model is expressed in Equation (1);

$$Z = A_1X_1 + A_2X_2 + ... + A_nX_n \quad (1)$$

Where Z is the total cost for ingredients, A_1, $A_2...An$ are objective function coefficients which were constant equivalents to cost per unit weight for food ingredients and X_1, $X_2...X_n$ are the values of DVs in formulation Z.

The linear constraints are the optimization process limitations as described by Dibari *et al*. (2012). The main purpose was to minimize the cost of formulation *Z* while meeting a range of different constraints. The limitations of constraints such as equality, greater, or less than are imposed on the DVs to ensure that the product's nutrient content met the requirements designed and exceeded the upper thresholds. When all of its constraints were achievable, the solution was feasible upon solving the LP. In particular; when the highest possible nutrient concentration was less than a minimum constraint or the lowest concentration of a nutrient was higher than a maximum constraint, an equality constraint could not be respected, the model can be considered unfeasible (Brixi, 2018). Constraints in prototype formulation problems may be non-linear or linear; however, LP can be applied when all the constraints are linear. A constraint is considered linear when it can be expressed as shown in Equation 2;

$$\begin{Bmatrix} c1X1 + c2X2 + ... + cnXn \geq \mathrm{d1} \\ c1X1 + c2X2 + ... + cnXn \leq d2 \\ c1X1 + c2X2 + \ldots + cnXn = d3 \end{Bmatrix} \quad (2)$$

Where, c_i for (i = 1, , ...n) represents quantity of constraint coefficients per 100g,

d_i for (i = 1, 2, 3) constraint ranges and X_i for (i = 1, , ...n) DVs for ingredients.

Due to the lack of PBY standards in East Africa, FSANZ (2019) for plant-based substitutes and the East African Standards (EAS 33:2006) were used to set the constraints. Also, the peer review journals of related similar products were surveyed to design the optimal formula. The constraints for the LP model were as follows: nutrients concentration and energy, texture, palatability, anti-nutrients, total food ingredients, and ratios to fats, carbohydrates, and proteins to energy. In the prototype development, a LP was used to reduce the traditional trial-and-error method and minimize the production cost.

i) Nutrient concentration and energy constraints

Nutrient concentration and energy constraints: LP constraints were set to ensure that the optimized formula met the FSANZ (2019) specifications for energy proportion and energy ratios to fats, carbohydrates, and proteins. For instance, care was taken to obtain the energy quantity between 67-272 KJ/100 g and caloric distribution between 20-33% from fat and 5 to 6% from protein. According to Dibari *et al*. (2012), the energy constraint is a ratio with lower and upper limits on the energy proportion delivered from the nutrients; this makes the constraints non-linear. Therefore, the reformulated linear equations are expressed in Equation (3), where the first equations represent the minimum ratios and the second equations represent the maximum ratios as follows;

$$\begin{Bmatrix} X1(C1 - b\,(min)\,E1) + X2(C2 - b\,(min)\,E2) \ldots Xn\,(Cn - b\,(min)\,En) \geq 0 \\ X1(C1 - b\,(max)\,E1) + X2(C2 - b\,(max)\,E2) \ldots Xn\,(Cn - b\,(max)\,En) \geq 0 \end{Bmatrix} \quad (3)$$

Where X_i are ingredients weights with (i = 1, 2, n), C_n represents the energy content values of nutrients (fats, proteins...), E_i is the total energy values of ingredients with (i=1, 2...n), b (min), and b (max) are the lower and upper limits for energy proportion coming from the nutrients, respectively.

Protein digestibility corrected amino acid score (PDCAAS) was constrained in Equation (4) and (5) given by FAO (2013) to ensure the quality of protein. The quantity of each ingredient was multiplied by the true protein digestibility factor of each ingredient and the quantity of essential amino acid within the ingredient following the USDA database to calculate PDCAAS in the formulated product. On the other hand, adding the protein digestibility adjusted the total of each amino acid in the formula and dividing by the total protein quantity. The resulting values were divided by the respective amino acid quantity per gram of reference protein for ingredient, as determined by FAO, which were constrained to be not less than the desired PDCAAS score. This is expressed as follows;

$$Qa = \sum_{i=1}^{n} Ci\alpha i\beta i \quad (4)$$

$$\frac{Qa}{R} \geq 0.95(\text{ga}) \quad (5)$$

Where Q_a represents the total quantity of amino acid a, C_i is the quantity of ingredient i, α_i is the quantity of amino acid (a) per 1 g of ingredient i, β_i is the protein digestibility factor ingredient i. R the total quantity of protein in the formula, and g_a the goal quantity of amino acid as per 1 *g* reference protein.

ii) Palatability and anti-nutrient constraints

Palatability constraint is a preference influenced by sugar content and fermentation result in yoghurt. Dibari *et al*. (2012) and Brixi (2018) explained the use of LP in designing a consistent, palatable prototype. In computing the values to be used in the formulation of the food prototype using LP, the palatability constraint was introduced to obtain the acceptable taste. The dried date (7-10 g/100g) was included to enhance the sweetness of the formulation. Therefore, based on the study by Dibari *et al.* (2012), the low sugar content of 15-25g was constrained in LP. The present work presents a plausible and sustainable approach with non-added sugar products free from lactose and gluten, acceptable by adults and infants. Lower sugar content as the best predictor of likeability, the PBY was expected to be comparable with cow's milk yoghurt in terms of acceptability and nutrients for suitable consistency.

According to FSANZ (2019), anti-nutrient factors in food are substances that reduce nutrient intake, digestibility, absorption, and utilization. However, they may cause other adverse effects. Phylate, oxalate, and polyphenols (tannin) are among the anti-nutrient factors potentially in nuts, seeds, and cereals that can affect micro-nutrient absorption. Anti-nutrients factors were inserted in the LP model to ensure that the optimized formula met the FSANZ (2019) specifications for anti-nutrient factors in food. As stipulated in the LP delimited and constrained the phytic acid content must less or equal to 22.8 mg per 100 g.

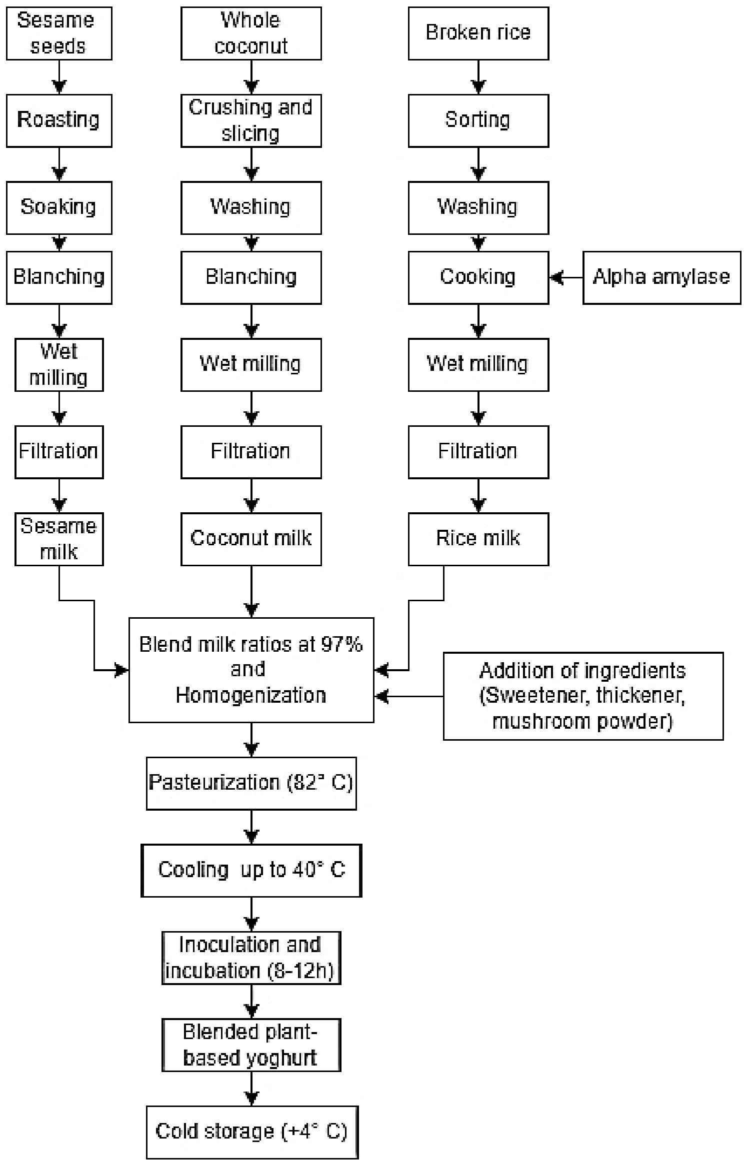
iii) Texture and total weight constraints

Food texture in prototype formulation is paramount, as is the specific consistency in the food mix that determines the uniformity in composition and stability (Awasthi & Singh, 2020). The texture-related constraint, the solid contents of yoghurt expected to be 8.25g and fat content with a range from 0.8-6.8g to provide a better body and texture that is smooth and firm enough to be spooned. Since the fat composition makes the texture of the product softer, squeezable, and more easily swallowable by consumers, fat compositions and yoghurt total solid content was constrained as they can affect the texture and consistency of the prototype.

The total food ingredient weights were constrained to allow sufficient space for inclusion of premix addition during finalizing the product. The inclusion of equality constraint was considered to weigh the food ingredients at 97 g. This setting was based on calculations that showed that up to 3% of the final product's weight was required for premixes (natural sweetener, thickener, and yoghurt starter culture). This formulation was done when the prototype was shown to be accepted according to Dibari *et al*. (2012). To obtain the optimized values as shown in mathematical computation using LP and software involved five steps as applied by Dibari *et al*. (2012) and Charles *et al*. (2020): (a) Creating the data layout in Microsoft Excel spreadsheet, data layout was created, (b) activation of add-in Solver Function with Excel installation standard, (c) assignment of decision variables, constraints, and objective function constraints, (d) Resolution of the objective function (OF) by running LP procedure, and (e) Sensitivity analysis (Appendix 2).

3.2.4 Prototype preparation and formulation

The preparation underwent various steps; the first step was the traditional processing of ingredients such as soaking, roasting, blanching, and cooking. This was done to reduce anti-nutrients compounds (tannins, oxalates, saponins, and phytic acids) and improve raw ingredients' nutritional and sensory profile (Aydar *et al*., 2020). For instance, qualitative questions were focused on "Does a food processor mix the formulated prototype? "Which blending equipment type might be used? The second phase was to process milk from raw materials. The last phase was the fermentation process using mixed starter culture, the prototype preparation presented in Fig. 1.



Source: (Aydar *et al*., 2020; Montemurro *et al*., 2021)

Figure 1: Flowchart for the production of the blended plant-based yoghurt

i) Preparation of plant-based milk

Coconut milk was prepared following the method Akoma *et al.* (2000) described with slight modifications. By shelling the nut using a dull knife, the meat was separated. The brown skin of the meat was removed using a sharp knife and then washed with clean water. Later, coconut meat was chopped into small pieces. In a bowl of warm water, the chopped meat pieces were soaked for 30 minutes to allow the extracted oil and aromatic compounds. The coconut meat was homogenized with water in a blender at 18 000 revoluation per minute (rpm/minute) and filtered through cheesecloth. The obtained milk (supernatant) was stood and fat and water separated to form a float coconut cream.

The method described by Belewu *et al*. (2013) was used to prepare rice milk. Rice was manually sorted and washed with clean potable water. The rice was cooked with 1:3 parts of water at medium temperature for 30 minutes, and α-amylase (0. 22%) was added to fasten the cooking rate. The soupy gelatinized filtered through cheesecloth and extracted milk was obtained. Sesame milk was prepared following the method reviewed by Ahmadian-Kouchaksaraei (2014). The sesame seeds were sorted, roasted in an oven (145° C for 20 minutes), and soaked overnight for 16 hours at room temperature (25 ± 2°C). This was done to reduce chalkiness and bitterness thus improving the flavor and acceptability of milk. The seeds were drained, rinsed in tap water, and blanched for 15 minutes in boiling water. After draining, the blanched sesame seeds were wet milled in a blender with water (5:1) for 20 minutes. The resulting slurry was kept at room temperature (25 ± 2°C) for about an hour and was later filtered through a double-layered cotton cloth to get sesame milk.

ii) Preparation of date syrup

A natural sweetener was extracted from dried date fruits. The fruits (500 g) were soaked in hot water (1000 mL) for 2 hours, mashed, and filtrated through a cheesecloth to get the water extract. The extract was collected and different concentrations used as part of water (0.0, 6.0, 8.0 and 10.0% v/v). The mixture boiled by stirring until a thick consistency like honey was obtained (Gabsi *et al*., 2013).

iii) Plant-based yoghurt production

Based on the above evaluation, the optimized ingredients' ratios were calculated in LP and used to formulate four prototypes with different concentrations of added date's syrups. The milk mixture was triple sieved using the muslin cloth. The four final formulations were prepared and blended until a homogenized smooth solution was achieved (18 000 rpm/minute). The milk samples were pasteurized in 30 minutes at 85°-87 °C, then filtered using gauze filters and cooled at 40 °C. The milk blends of 100 mL were poured in four sterile containers with different portions of date syrup (0.0, 6.0, 8.0, and 10.0% v/v), 0.05% (w/v) of guar gum, and 1% (w/v) of oyster mushroom powder. By stirring, 0.1% (w/v) of thermophilic yoghurt culture YF-L811 (YoFlex®, Denmark) packed with *L. bulgaricus* and *S. thermophilus* (50:50) was added. The milk blends were incubated at 43 °C for 8-12 hours up to the dropped pH of 4.5. The four types of yoghurt (Appendix 3) were cooled rapidly and stored at + 4 °C for 14 days. Experienced panelists carried out the sensory evaluation and the formulation which ranked the best was selected and evaluated for proximate analysis, fatty acids profile, nutrients bio-availability, health bioactive compounds, and microbiological stability compared to the commercial cow's milk yoghurt (Control).

3.2.5 The sensory evaluation

The sensory testing was accomplished to assess consumers' satisfactoriness level of the formulated non-dairy yoghurt relative to cow's milk yoghurt. Twenty panellists (25-35 of age range) from NM-AIST, including students and staff members (twelves females and eight males), were involved in the sensory test. The panelists were chosen based on their socioeconomic statuses, such as education, willingness, capacity, and experience in conducting the sensory evaluation. The sensory attributes acceptability of produced yoghurt was determined based on a 9-hedonic scale ranging from like extremely (9) to dislike strongly (1) concerning taste, texture, odour, colour, and overall acceptability between optimized PBY prototypes and the control (Charles *et al*., 2020).

3.3 Laboratory analysis of the optimized prototype

3.3.1 Analysis of proximate composition

Prototype proximate analyses determined the ash, moisture, crude fat, crude protein, carbohydrate, and crude fiber content of the optimized prototype

i) Determination of moisture content

The moisture content was determined according to the methods developed by the Association of Official Analytical Chemists (AOAC) (2012). By drying 5 g of a sample in a weighed crucible at 105 °C for 3 hour, the moisture content was computed as the percent weight of the initial weight of the sample as expressed in Equation 6.

$$\% \text{ Moisture} = \frac{(w1-w2)\times 100}{(W1)} \quad (6)$$

Where W1= crucible and sample weight before drying (g), W2 = crucible, and sample weight after drying (g)

ii) Determination of ash content

The ash content was determined by burning 5g of sample in a muffle furnace for 3 hours at 500 ºC following the AOAC (2012) method. The crucible with the sample was reweighed after cooling in a desiccator. The ash content was determined as the total percentage weight of inorganic residue remained as shown in Equation 7:

$$\% \text{ ash} = \frac{W2-W1}{W1} \times 100 \quad (7)$$

Where $W_{1=}$ the initial weight of crucible and sample, W_2 = the weight of crucible and sample after ashing

iii) Fat content determination

The fat content was analyzed using the Gerber fat method (AOAC, 2012). About 10 g of sample was taken, 1 mL of amyl alcohol and 10 mL of sulfuric acid (H_2SO_4) were mixed in the butyrometer and closed with rubber cork. The mixture was shaken carefully and centrifuged at 1000 rpm for 15 minutes. The tube was transferred into a water bath at 65°C for 5 minutes, and the fat percentage was read out from the fat column.

iv) Crude protein content analysis

The Kjeldahl method was used to quantify the crude protein content in the PBY as described by Ibrahim (2017). In a digestion flask, about 2 g of sample, a catalyst (copper sulfate and sodium sulfate in the ratio of 5:1) and concentrated H_2SO_4 were added for the digestion process. Then, 40% of NaOH/thiosulphate solution, 2% of boric acid, and two drops of bromocresol green and methyl red with indicator added where distillation continued to turn the boric acid mixture from pink to yellowish-green. Titration against 0.1M HCL followed till the endpoint. The nitrogen and crude protein contents were calculated as shown in Equations 8 and 9, respectively.

$$\% \text{ nitrogen} = \frac{(\text{mL standard acid-mL blank}) \times \text{N of acid} \times 0.0014 \times 100}{\text{Weight of sample in grams}} \quad (8)$$

crude protein (g/100 g) = 6.38×% total nitrogen (9)

v) Crude fiber content

The fibre content was determined by first boiling 2 g of defatted PBY sample in 1.25% of H_2SO_4 for 30 minutes (AOAC, 2012). The mixture was then filtered by washing the residues using hot distilled water until the washings were no longer acidic. Secondly, the solution boiled with 1.25% NaOH for another 30 minutes. The mixture was filtered through a linen cloth, and the NaOH in the digested sample was neutralized by washing with hot distilled water until no alkaline remained. Lastly, the residues were transferred by a spatula into a crucible. The remaining residues were washed off by 15 mL of ethanol into the crucible, followed by drying in the oven for 2 hours at 100°C and cooling in the oven with a desiccator before weighing. The contents were incinerated at 600 °C in a furnace until the carbonaceous matter was burnt, cooled, and reweighed. The loss in weight was used to determine the fibre content, as shown in Equation 10.

$$\% \text{ Crude fibre} = \frac{W1 - W2}{W} \times 100 \quad (10)$$

Where W1=the mass of the crucible and dried sample, W2= the mass of crucible and ash, and W= the mass of sample used.

vi) Carbohydrate content determination

The carbohydrate value was expressed as the difference of the protein, fat, crude fibre, total ash, and moisture content of the sample from 100 using Equation 11.

$$Carbohydrate\,\% = 100 - \%\,(ash + protein + fat + crude\,fibre + moisture) \quad (11)$$

3.3.2 Minerals analysis

The analysis of minerals was performed as described by Ibrahim (2017). About 5 g of samples were weighed into porcelain crucibles and ashed in a muffle furnace at 500 °C for 6 hours to get white ashes. The ashes were cooled in a desiccator, weighed, dissolved in 2 mL of hydrogen peroxide (H_2O_2) and 3 mL of concentrated nitric acid (HNO_3), before diluting with distilled water in a 25 mL calibrated flask to the mark with vigorous shaking. A Flame Atomic Absorption Spectrophotometer (WFX-210) was used to determine the zinc (Zn), iron (Fe), copper (Cu), manganese (Mn), and magnesium (Mg) contents. All analyses were performed in triplicates. Standards solutions were made by appropriate dilution of the stock solutions of graded chemicals. A flame photometer (FP-6440) was used to determine calcium, potassium, and sodium according to AOAC (2012), and a DR 2700 spectrophotometer was used to quantify the phosphorus content.

3.3.3 Water-soluble vitamins

i) Vitamins B group

For vitamins B_1 and B_9 determination, the sample was incubated in 0.1N of H_2SO_4 for 30 minutes at 121°C. The extract was adjusted to the pH of 4.5 with 2.5M of sodium acetate followed by the Takadiastase enzyme and stored overnight at 35°C. The mixture was filtered through a Whatman No.4 filter, diluted with 50 mL of pure water, and re-filtered through a micropore (0.45 µm) filter. Working solutions of vitamin B_1 and B_9 were prepared following the standard methods described by Sami *et al*. (2014). Twenty microliters of the filtrate were injected, and chromatographic separation was completed on a reverse-phase (RP)-High-Performance Liquid Chromatography (HPLC) column through the isocratic delivery mobile phase at a flow rate of 0.5 mL/minute UV-absorbance was recorded at 270 nm at room temperature.

ii) Vitamin C

Vitamin C was determined using acetic acid (1.4 M) and metaphosphoric acid (0.3M) extracting solution, as reported by Charles *et al*. (2020). Ten grams of the sample was blended and homogenized within 50 % of an extracting solution containing acetic acid (1.4M) and metaphosphoric acid (0.3M). Then into a conical flask, the mixture was placed and agitated at 10,000 rpm for 15 minutes. Then, through a Whatman No.4 filter, the mixture was brought to 100 mL within the same solution of metaphosphoric acid/acetic acid. Finally, the solution was filtered, and samples were extracted in triplicate. Preparation of the ascorbic acid stock standard was done by dissolving 100 mg of L-ascorbic acid in 50% of a metaphosphoric acid (0.3M)/acetic acid (1.4M) solution at a final concentration of 100 mL of the same solution (0.1 mg/mL) and based on four measured concentration levels. The calibration line was converted to a linear range, and an Agilent HPLC system was used to quantify the content of ascorbic acid. The separation was accomplished on an RP-HPLC column through isocratic delivery of a mobile phase at a 1 mL/minute flow rate. UV-absorbance was recorded at 254 nm at room temperature.

3.3.4 Fat-soluble vitamins

Fat-soluble vitamins (vitamin A, E, and β-Carotene) were quantified using the methods described by Sami *et al.* (2014). The 70 % of alcoholic KOH, 1 g of pyrogallic acid, and 10 g of sample were stirred and refluxed for 40 minutes in a 50°C water bath. The extracts were prepared using 50, 30, and 20 mL of ether concentration three times, then neutralized with distilled water to make a concentration of 5 mL. The extract was diluted with 10 mL, filtered using a 0.45 μm membrane, and finally, 20 µl of sample oil injected into the HPLC. For β-carotene quantification, the extracts were chromatographed using a reverse-phase TC-C18 column with acetonitrile-methyl alcohol-ethyl acetate solvent (88:10:2) UV- absorbance was read at 453 nm. Fat-soluble vitamins were quantified using the XDB-C18 column with methanol as solvent, and the UV absorbance read at 325 and 290 nm for vitamins A (Retinol) and E, respectively. Standards solutions were prepared daily by serial dilution of stock solution to a concentration of 1, 5, and 10 mg per litre for vitamins E, A, and β-carotene, respectively.

3.3.5 Determination of phytoconstituents

The phytate content was determined as described by Hailu and Addis (2016). The phytic acid concentration was determined using wade reagents of 0.03% $FeCl_3.6H_2O$ and 0.3% sulfosalicylic acid. A standard phytic acid curve was constructed under the same conditions and the results were expressed as phytic acid mg/100 g of fresh weight of the sample. A UV-VIS spectrophotometer was used to measure the absorbance at 500 nm. The total phenolic contents (TPC) were determined using 10% of Folin-Ciocalteu's reagent (FCR) and 7% Na_2CO_3, according to Chandra *et al.* (2014). Several concentrations of Gallic acid solution in methanol (5-500 mg/L) were prepared from the standard curve. TPC was calculated as mg Gallic acid equivalents per gram of fresh weight of the sample (GAE/fw). The extract's Total Flavonoid Content (TFC) was investigated using the aluminium chloride colorimetric method described by Chandra *et al.* (2014). The standard calibration curve was prepared for Quercetin; the TFC was expressed as milligram quercetin equivalent per gram of extracted sample based on a standard curve of Quercetin (mg QCE/g sample).

3.3.6 Fatty acid profile

The quantification of fatty acids profile was assessed using a Gas Chromatography Hewlett-Packard 5890:5971A system (Hewlett-Packard, Walbronn, German) with an SP 2331 column (0.25 mm of diameter, 60 m of length, and 0.25 μm of film thickness) following a previously published method by Dreiucker and Vetter (2011). The injection volume of the prepared sample was 1.0 μL, and the carrier gas was Helium. The normalized peak area and equations generated from the calibration curves were used to compute the concentration of methylated fatty acids in the sample.

3.3.7 Microbiological analysis

The methods described by Ani *et al.* (2018) were used to determine the lactic acid bacteria (LAB) probiotic viability and storage stability. The enumeration of viable LAB colonies was tested following the method applied previously by Fatima and Hekmat (2020). The sample was subjected to a 10-fold serial dilution. Aliquots portions (0.1 mL) were picked and transferred by spread-plating on MRS agar (HiMedia, M641-500G, India) plates per dilution factor. The incubation occurred at 37 °C for 24-48 hours. The same quantity of aliquot was inoculated on Potato dextrose agar (PDA) (HiMedia, M096-500G, India) to enumerate yeasts and moulds. Plates were incubated at 25°C for 3-5 days. Coliform bacteria were determined on MacConkey agar (HiMedia, MM081-500G, India), incubated at 37°C for 24 hours (Moh *et al*., 2017). For *salmonella* counting, dilution was spread plated on *Salmonella Shigella* agar (SSA) and incubated for 24 - 48h at 37° C. For all enumerations, the plates with between 30 and 300 bacteria colonies were counted. The total microbial counts were expressed as log Colony Forming Units per mL of yoghurt (CFU/mL). The microbiological properties were evaluated at the 1st, 7th, and 14th days of refrigerated storage (4°C).

3.4 Relative difference between the results generated by the LP and the values analyzed in the lab

The relative difference between the LP computed values, and the lab analyzed values were calculated as described by Dibari (2012). An absolute difference (AD) for each nutrient was calculated by subtracting the LP values from the lab analyzed results. The relative difference (RD) was computed by dividing the absolute difference values with the calculated LP values (C), as shown in Equation 12.

$$\text{RD} = \frac{(B)x100}{C} \qquad (12)$$

Where C = the calculated values from LP, E= the analyzed value from the lab, and B is the absolute value (AD or E-C)

3.5 Statistical analysis

Means of the LP-calculated values' nutritional values were compared to the lab-analyzed values using a paired t-test. The main goal was to check if there were significant differences between the LP-designed product and those from the lab. A Kruskal-Wallis H test was used to assess statistically significant variations across the sensory qualities (odour, taste, colour, texture, and overall acceptability) of the optimized product on a 9-point hedonic Likert scale at P = 0.05. IBM SPSS 23 (IBM®SPSS®Statistics, USA) and R (library version 3.6.1) software were used to analyze the data.

CHAPTER FOUR

RESULTS AND DISCUSSIONS

4.1 Results

4.1.1 Sensory evaluation of the optimized prototypes

The statistical significant differences (p-value < 0.05) between the sensory attributes of the formulated PBY was shown by the Kruskal-Wallis H test. About 85%, 79, 78, 84, and 80% of descriptive analysis showed that the respondents liked the product (PBY-10%) very much for odor, texture, colour, taste, and overall acceptability, respectively. The likely mean scale ratings are presented in Table 1. The plain yoghurt derived from cow's milk (control) was more acceptable (liked extremely) than the formulated PBY-10% date syrup (liked very much toward moderately) in terms of overall acceptability.

Table 1: Average results of sensory evaluation

Sample	Odour	Texture	Colour	Taste	Overall acceptability
PBY-0%	$5.0{\pm}0.14^{b}$	$5.2{\pm}0.26^{e}$	$6.1{\pm}0.21^{f}$	$7.9{\pm}0.19^{a}$	$6.3{\pm}0.25^{c}$
PBY-6%	$6.2{\pm}0.12^{a}$	$6.0{\pm}0.15^{b}$	$6.9{\pm}0.18^{a}$	$8.0{\pm}0.15^{e}$	$7.5{\pm}0.27^{a}$
PBY-8%	$7.7{\pm}0.11^{d}$	$7.0{\pm}0.18^{f}$	$7.7{\pm}0.14^{c}$	$8.2{\pm}0.12^{e}$	$7.7{\pm}0.15^{a}$
PBY-10%	$8.5{\pm}0.19^{c}$	$7.9{\pm}0.25^{d}$	$7.8{\pm}0.280^{c}$	$8.45{\pm}0.52^{b}$	$8.0{\pm}0.48^{d}$
Plain Yoghurt	$8.85{\pm}0.10^{e}$	$8.88{\pm}0.13^{a}$	$9.0{\pm}0.10^{d}$	$8.9{\pm}0.09^{f}$	$8.93{\pm}0.13^{b}$

Values expressed in form of mean±sd (standard deviation, n=3). Means which differ on superscripts within columns are significantly different from each other (p<0.05). PBY-0%: Plant-based yoghurt without date palm syrup; PBY-6%: Plant-based yoghurt with 6% of syrup; PBY-8%: Plant-based yoghurt with 8% of syrup; and PBY-10%: Plant-based yoghurt with 10% of syrup.

4.1.2 Attributes of the final optimized product

The mathematical LP method was used to create the functional PBY using locally grown commodities by small-scale farmers in East Africa like coconut, broken rice, roasted sesame seeds, date palm syrup, and mushroom powder. The replacement of animal milk and the low price of local ingredients where the key cost savings obtained in the optimized PBY. The prices per kilogram of the main ingredients were about 0.4 USD for ground rice and coconut, USD 0.6 for sesame, USD 0.7 for mushroom powder, and USD 0.5 for dried date fruit. In this pilot study, the LP tool was valuable to certify that the cost was minimized while the nutritional value requirements and product palatability were met. The LP analysis result of the formulated LP model given from Equation 1 indicated a low cost of 0.9 USD/kg (2078.10 TZS/L), which is 60% cheaper than Alpro (Soya-coconut blend) PBY (2.50 Euro/L) and have a comparable price to the commercial plain yoghurt available in Tanzania. About 97-100 g of blended PBY contained significant amounts of essential nutrients (Tables 2 and 3) and bioactive compounds (Tables 4 and 5) important to human nutrition.

The protein quality of the optimized formula achieved > 95% of the PDCAAS. In addition, the optimized formulation met the standard ratios of minerals (Ca, Fe, and Zn) to phytic acid (Table 2), which are the determinants of minerals absorption and are within the ranges that favors micronutrients bioavailability in the body. Moreover, the plant-based milk and yoghurt encompassed enough Polyunsaturated Fatty Acids (PUFAS) of 15.52 - 18.88% (Table 4), which increased after fermentation. The formulae contained natural cofactors required for long-chain fatty acids metabolism (carbon atom of 20-22) in the body and contained a balanced omega-6 to the omega-3 fatty acid ratio (3:1), both of which are rare in other common dairy free-alternatives.

The number of LAB identified were within the accepted quantitative standard of a minimum of 10^6 to 10^7 CFU/mL which is corresponding to 6 -7 log CFU/mL (Table 5). Yeast and moulds were not detected at 10^{-1} using spread plate, thus less than 100 CFU/mL was reported at the end of storage. *Salmonella* spp, and coliforms were not detected in yoghurts within storage time.

Table 2: Nutrition composition of optimized PBY formulation (per serving)

Analysed nutrients	Optimized PBY-10% (100 g)	Cow's milk yoghurt (100 g)	FSANZ (100 g)
Moisture, g	85±0.66[a]	84.90±0.46[a]	50-100
Proteins, g	3.1± 0.25[a]	3.95±0.53[b]	not less than 3%
Fibers, g	0.88±0.06[a]	0.00±0[b]	0-1.9
Ash, g	1.93±0.02[a]	0.59±0.18[b]	NS
Carbohydrate, g	6.38±0.41[b]	7.60±0.40[b]	NS
Fats,g	2.90±0.22[a]	3.00±0.30[a]	no more than 2.5%
Total saturated FA, %TFA	43.25±4.25[d]	NS	NS
Monounsaturated FA, %TFA	37.87±2.98[a]	NS	NS
Polyunsaturated FAs, %TFA	18.88±1.78[b]	NS	NS
Calcium, mg	117±2.08[b]	120±0.0[c]	120
Zinc, mg	1.59±0.02[e]	0.90±0.05[b]	0.8
Potassium, mg	362±1.53[f]	231.00±2.53[d]	200
Phosphorus, mg	95±1.14[e]	112±6.66[b]	100
Iron, mg	9.6±0.38[b]	0.07±0.02[c]	5-7
Sodium, mg	14.00±1.52[a]	50.00±2.52[e]	NS
Magnesium	10.81±0.21[c]	16.86±0.07[f]	11
Vitamin A, mcg RE	54.00±0.61[a]	27.50±1.04[b]	55-62.5
Vitamin B9, mcg	8.08±0.12[d]	10.90±0.15[a]	6
Vitamin C, mg	35±0.01[f]	0.009±0.05[d]	NS
β- carotene, mcg	86.50±1.11[a]	21.00±0.06[f]	NS
Vitamin E, mcg	29.01±1.06[b]	50.00±0.23[e]	NS
Thiamine, mcg	50.00±1.52[f]	59.01±0.57[c]	50
TPC, (mg GAE/100g)	120.10±0.61[a]	0.00[b]	NS
TFC, (mg QCE/100g)	69.01±1.06[a]	0.00[b]	NS
Phytic acid, mg	0.23±0.01[a]	0.00[b]	<22.8
Molar ratio:			
Phytic acid: Fe	0.0020	NS	<2.5
Phytic acid: Zn	0.0145	NS	<15
Phytic acid: Ca	0.0001	NS	<0.24
Ascorbic acid: Fe	1.162	NS	<3.8
Energy, kJ	265.23±14.54[a]	305.87±6.04[b]	67-272

Values expressed in form of mean±sd (standard deviation, n=3). Means which differ on superscripts within rows are significantly different from each other ($p<0.05$). PBY: Plant-based Yoghurt; FSANZ: Food Standards Australia and New Zealand; NS: Not specified.

4.1.3 Relative difference of nutritional values between lab analyzed values and LP calculated values

The calculated RD (Table 3) confirmed that the LP-computed nutritional values of developed PBY were in line with the laboratory analysed values. The paired sample t-test showed no significant differences between the calculated values from the lab and the LP.

Table 3: Relative difference between the LP calculated values and lab analyzed values

Component	C (LP)	E (Lab)	[1]A.D E-C=B	[2]R.D (B×100)/C	P-Value
Moisture, g	84.7	85	0.3	0.34	0.20
Proteins, g	3.06	3.1	0.04	1.30	0.83
Fiber, g	0.90	0.88	0.02	2.2	0.66
Ash,g	1.87	1.93	0.06	3.20	0.35
Carbohydrate,g	6.55	6.38	-0.17	-2.59	0.11
Fats,g	2.92	2.90	-0.002	-0.68	0.30
Energy, KJ	271.41	265.23	-6.18	-2.27	0.04
Protein Energy/Total Energy, KJ	52.02	52.7	0.68	1.30	0.92
Fat energy/Total energy, KJ	108.04	107.3	-0.74	-0.68	0.17
Calcium, mg	117	120	2	1.73	0.10
Zinc, mg	1.55	1.59	0.04	2.58	0.77
Potassium, mg	362	362	0.00	0.00	0.05
Phosphorus, mg	94	95	1	1.06	0.19
Iron, mg	9.4	9.6	0.2	2.17	0.63
Sodium, mg	13.8	14	0.2	1.44	0.50
Magnesium	10.5	10.81	0.31	2.95	0.08
Vitamin A, mcg	53	54	1	1.88	0.42
Vitamin B9, mcg	8	8.08	0.08	1	0.05
Vitamin C, mg	34	35	1	2.94	0.81
β-carotene, mcg	85.8	86	0.2	0.23	0.34
Vitamin E, mcg	29	29.01	0.01	0.03	0.05
Thiamine, mcg	51.5	50	-1.5	-2.91	0.86
TPC, mg GAE/g	119	120.10	1.1	0.92	0.09
TFC, mg QCE/g	70	69.01	-0.04	-0.57	0.65
Phytic acid, mg	0.22	0.23	0.01	4.54	0.50
Molar ratio:					
Phytic acid: Fe	0.0020	0.0020	0.00	0.00	0.00
Phytic acid: Zn	0.0145	0.0145	0.00	0.00	0.00
Phytic acid: Ca	0.0001	0.0001	0.00	0.00	0.00
Ascorbic acid: Fe	1.162	1.162	0.00	0.00	0.00
PDCAAS,%	100	-	-	-	-

[1]AD: Absolute Difference, [2]RD: Relative Difference, PDCAAS: Protein Digestibility Corrected Amino Acid Score

Table 4: Fats acids profile of optimized prototype before and after fermentation

Fatty acids	Non-fermented blended milk (% TFA)	Fermented blended yoghurt (% TFA)
Butyric acid, C4:0	0.00±0.00^{a}	0.00±0.00^{a}
Caprylic acid, C8:0	0.00±0.00^{a}	0.00±0.00^{a}
Capric acid, C10:0	0.00±0.00^{a}	0.00±0.00^{a}
Lauric acid, C12:0	2.25±0.19^{a}	3.01±0.26^{b}
Tridecanoic acid, C13:0	0.00±0.00^{a}	0.00±0.00^{a}
Myristic acid, C14:0	5.65±0.46^{c}	4.00±0.30^{b}
Myristoleic acid, C14:1	0.00±0.00^{a}	0.00±0.00^{a}
Pentadecanoic acid, C15:0	0.00±0.00^{a}	0.00±0.00^{a}
Palmitic acid, C16:0	38.86±3.90^{c}	34.44±2.49^{d}
Palmitoleic acid, C16:1	1.44±0.12^{c}	2.07±0.20^{c}
Heptadecanoic acid, C17:0	0.12±0.01^{d}	0.00±0.00^{a}
Stearic acid, C18:0	3.23±0.30e	1.11±0.12^{f}
Oleic acid, C18:1	31.18±2.81^{f}	34.85±2.49^{e}
Elaidic, C18:1 (Trans)	0.00±0.00^{a}	0.00±0.00^{a}
Linoleic acid, C18:2	15.03±1.05^{e}	18.05±1.70^{b}
Linoelaidic, C18:2 (Trans)	0.00±0.00^{a}	0.00±0.00^{a}
α-Linolenic acid, C18:3	0.49±0.038^{c}	0.83±0.07^{d}
Arachidic, C:20:0	1.17±0.1^{d}	0.69±0.05^{b}
Eicosenoic acid, C20:1	0.58±0.04^{b}	0.95±0.08^{b}
Total saturated Fatty acid	51.28±4.90^{e}	43.25±4.25^{d}
Total monounsaturated Fatty acid	33.2±2.78^{c}	37.87±2.98^{a}
Total polyunsaturated Fatty acid	15.52±1.43^{f}	18.88±1.78^{b}

Means which differ on superscripts within rows are significantly different from each other ($p<0.05$).

Table 5: Microbial count (log CFU/mL) during storage (4 °C)

Storage days	Parameters	PBY[1]	Plain Yoghurt[2]	EAS and Codex Alimentarius specifications (log CFU/mL or g)
Day 1	LAB	8.24 ± 0.28[a]	8.50±0.36[e]	Minimum of 6-7log cfu/mL
	YMC	ND	ND	2 log cfu/mL
	Coliforms	ND	ND	Absent
	Salmonella	ND	ND	Negative in 25mL
Day 7	LAB	8.19± 0.33[a]	8.46± 0.26[e]	Minimum of 6-7log cfu/mL
	YMC	ND	ND	2 log cfu/mL
	Coliforms	ND	ND	Absent
	Salmonella	ND	ND	Negative in 25mL
Day 14	LAB	8.14 ± 0.19[a]	7.85±0.36[d]	Minimum of 6-7log cfu/mL
	YMC	2.00±0.0[b]	ND	2 log cfu/mL
	Coliforms	ND	ND	Absent
	Salmonella	ND	ND	Negative in 25mL

[1, 2] Formulated plant-based yoghurt with 10% of date palm syrup and cow's milk yoghurt, respectively. LAB (Lactic Acid Bacteria), YMC (Yeast and Mould Count), ND (Not Detected). Means which differ on superscripts within columns are significantly different from each other ($p<0.05$).

4.2 Discussions

The analyses of the present study have shown that it is technically possible to design and develop suitable culturally acceptable nutritious and functional PBY rich in health-promoting bioactive compounds from locally available ingredients (other than animal milk) in Tanzania. Despite the scarcity of scientific literature on blending plant-based milk, Sethi *et al*. (2016) established that blending two or more plant-based milk varieties to produce a product with a higher nutritional value comparable to cow's milk is crucial in food production. The blending of ingredients to improve the nutritional balance and sensory acceptability of PBY has also been studied by Awasthi and Singh (2020). The best ranked formulated prototype (PBY-10%) satisfactorily met consumers' sensory preferences regarding odour, taste, and overall acceptability (liked very much), whereas the texture and colour were moderately liked. The odour acceptability was increased with increases of roasted sesame milk concentration in formulation. The pleasant taste obtained was enhanced by the increases of date syrup concentration and fermentation result of natural sugar present in blended milk by use of mixed culture microbial species: *S. thermophilus* and *L. bulgaricus*, which have been shown to improve the sensory attributes, organoleptic quality, bioavailability of mineral and vitamins, and the shelf life of the fermented plant-based products (Tangyu *et al*., 2019). The Plain yoghurt (control) was liked extremely in all parameters compared to the PBY formulated. This acceptance was due to the creaminess, thickness, and pleasant aroma of cow's milk yoghurt linked to milk proteins (casein and whey).

The present study discourages the use of highly-priced imported raw materials to formulate ready-to-use therapeutic foods as a means of addressing the cost constraints in developing countries, as per UNICEF (2009). A combination of local ingredients acted as sources of monounsaturated and polyunsaturated functional fatty acids such as palmitoleic acids, linoleic acid (LA), and oleic, which augmented after fermentation, and their quality was maintained because of the high content of vitamin E and C antioxidants in the formulation. Additionally, coconut acted as a source of lauric acid and monolaurin-functional compounds against harmful pathogens such as bacteria, viruses, and fungi (Ngampeerapong *et al*., 2018). The formulated PBY can replace dairy products in terms of bioactive components accessibility from non-animal sources needed to reduce risk of chronic diseases and brain development.

According to FSANZ (2019), at least 20–33% of the total energy must be provided from fats and the rest from proteins and carbohydrates, and a total energy density of 67-272 kJ/100 mL was suggested. The total energy density content of 265.23 kJ/100g was obtained with a protein-to-energy ratio (0.198), fat-to-energy ratio (0.404), and carbohydrate-to-energy ratio (0.408). The optimized formula meets the protein content recommendation of approximately 3% and 0.5 PDCAAS as per international standards (FAO, 2013). The ingredients were then rationed to favour the bioavailability of the most limited necessary nutrients and biological health-promoting compounds. Consumers should get access to omega-6 fatty acids, represented by linoleic acid (LA)

(18:2), and omega-3 fatty acids like alpha-linolenic acid (ALA, 18:3) in a ratio that does not compromise the bioavailability of omega-3 fatty acids upon consumption. Both are essential fatty acids that act as cofactors to be metabolized into long-chain fatty acids in the body. During metabolism, LA converts into arachnoid acid (C20:4), whereas ALA converts into eicosapentaenoic acid (EPA, C20: 5) and docosahexaenoic acid (DHA, 20:6). The PBY was designed to help consumers to access other micronutrients such as vitamins A, C, B_9, and $B_{1,}$ Fe, Zn, and Mg which are also coenzymes of ALA conversion to docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), and docosapentaenoic acid (DPA) in the human body (Charles *et al.*, 2020). At a single-serving, the analysis of this study showed that PBY could provide the recommended amount of linoleic acid (10 g) and ALA (0.9 g) for children between 4-8 years of age, which are negligible in many dairy alternatives. There is no current dietary omega-6: omega-3 guideline ratio, but the recommended intake of omega-6 and omega-3 can be used to access the amount of dietary intake a consumer would have if they followed them. Sheppard *et al.* (2018) reported that omega-6 to omega-3 lower ratios (< 10:1) were linked to a healthy diet and adequate intake of a variety of other nutrients.

According to FAO & WHO (2004), the optimized formula meets 100 and 50% RDI of Fe and vitamin A respectively in children under-3 years old per serving. In addition, the RDI requirements for women of reproductive age were met in 70% of cases (Fe) and 30% of cases (Vitamin A). Likewise, one serving of formulated yoghurt can contribute to the RDI of 15% for Mg, Na, vitamin E and B1; 30% for Ca and B9, 60-70% for Zn and P, and ≥ 100% RDI for K, Fe, and vitamin C and B9 for the infants under three years. There was a most-significant difference ($P < 0.001$) in micronutrients such as vitamins (β-carotene, Vit A, E, C, B1 and B9 and minerals (Na, K, Na, Fe, and Mg) between PBY-10% and the control. The micronutrients of PBY were higher than the cow's milk yoghurt. The sourced raw materials from plants rich in micronutrients are attributed to enough amounts of minerals and vitamins, which are negligible in cow's milk. Formulated PBY-10% experienced a little high amount of fats beyond the FSANZ specifications (> 2.5%). The slight high amounts were recognized to be due to high-fat content in the raw materials like coconut and sesame seeds (Appendix 1).

In addition, the presence of sesame in the formulation can enable consumers to access sesame proteins that contain adequate essential amino acids to meet 100% RDI for methionine, tryptophan, and cysteine, which are the most limiting amino acids among children < 3 years old in developing countries (Aydar *et al.*, 2020). Likewise, access to functional biological compounds was raised by the inclusion of probiotics in the formulation, which intervene in the absorption of plant source phytoconstituents such as polyphenols, polyunsaturated fatty acids, flavonoids, α-Tocopherol, and short-chain fatty acids (Banwo *et al*., 2021). The study demonstrated that the formulated product contained potentials TPC and TFC profile which are absent in dairy products (control). A similar range of TPC (49.60-74.75 mg GAE/100g) was obtained by Aydar *et al.* (2021). According to the approved requirements for ready-to-use foods and beverages, the current formulation does not contain artificial antioxidants or flavorings. Additionally, the PBY-10% was also sugar-free, relying on dried fruits sugar used as a natural sweetener.

The ratios of minerals (Ca, Fe, and Zn) to phytic acid of the optimized formulation were within the standard ranges, which in turn favors micronutrients bioavailability in the body. This shows that there is no impairment of nutrient absorption due to interactions between minerals and phytate. The low amount of anti-nutrients phytic acids in the formulation was caused by the processing techniques of ingredients. For instance, roasting and soaking raw sesame seeds has been established to minimize phytic acids and tannins while increasing the extraction yield of milk (Aydar *et al*., 2020). The fermentation process can also have beneficial effects on minerals absorption. Abd El-Gawad *et al.* (2014) demonstrated that the minerals in raw materials such as calcium showed partial digestion due to the suppression of *L. acidophilus* on calcium metabolism, which increased calcium concentrations. The present PBY-10% formulation is a future promising product for all consumers, including those with diabetes and vegetarians in developing countries, due to the high content of biological and functional compounds with essential nutrients to human nutrition. Additionally, the present innovative product is free from lactose and milk proteins, which may be the basic source of lactose intolerance and milk allergency health concerns to some people.

The microbiological quality of developed PBY was assessed to expel doubts of the product's microbiological deterioration during its anticipated shelf-life and ensure consumer protection against exposure to any health hazard. Yoghurt and alternative yoghurt must contain at least 10^6 CFU/mL (g) LAB colonies during consumption time to provide a therapeutic advantage to the host (Fatima & Hekmat, 2020). The LAB count was almost constant during 14 days of refrigerated storage (+4 °C), with minor decreases especially for the control which had a slight drop in LAB towards the end of storage due to the type of strain used (Table 5). Similarly, the slight constant of LAB during storage time of 15 days agrees with the results obtained by Fatima & Hekmat, (2020).

Yeast and mould (YMC) concentrations of no more than 2 log cfu/mL are allowed in yoghurt as because yoghurts with YMC more than 2 log cfu/mL deteriorate quickly even before being refrigerated (Moh *et al*., 2017). In current study, YMC were not detected at 10^{-1} using spread plate, thus less than 100 CFU/mL was reported at the end of storage (Table 5). This can be attributed to the presence of LAB, which prevents the proliferation of fungus in yoghurt during storage. A previous study by Ani *et al.* (2018) also obtained least amount of yeasts and moulds during storage time of 14 days (+4° C). Differently, a previous study by Falade *et al*. (2015) established that YMC increased in yoghurt with increased storage due to increased acidity associated with decreased oxygen potential during the fermentation process, which provides the yeasts and moulds with suitable growing conditions. *Salmonella* spp, and coliforms were not detected in PBY during storage times. This absence indicates Good Manufacturing Practices (GMP), such as effective cleaning and pasteurization employed during the production. For instance, blanching occurred for coconut inactivated natural enzymes linked to odour loss, texture, lipid oxidation, and decreased microbial load (Tangyu *et al*., 2019). Moreover, organic acids and sensory metabolites such as bacteriocins produced by starter culture acted against pathogenic and spoilage bacteria during fermentation. Therefore, the lack of Enterobacteria indicates the safety level of optimized PBY.

The drawback of the present LP analysis is the discrepancies among ingredients' nutritional values from various nutrient data sources. The local food composition data like the Tanzanian Food Composition Tables lacks all nutritional information for the selected ingredients thus other publicly available sources such as SELF Nutrition Data (2018), USDA (2019) nutritional databases, and peer-review papers were used to obtain the nutritional composition data. The ingredients' nutritional composition may differ according to the geographical locations across the world, and these deviations may affect the final product composition (Merchant & Dehghan, 2006). Hence, the developed prototype was analyzed for nutritional values in the laboratory to validate the quality of the LP formulated prototype.

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusion

The proximate and nutritional values analyzed clearly show that, soon, plant-based foods will be a better source of nutrients worldwide. The findings demonstrated that the proposed yoghurt based on plants was comparable to the cow's milk product. This study successfully developed a product that is microbiologically stable for 14 days of storage (+4 °C), rich in bioavailable essential nutrients and bioactive compounds, and meets acceptable standards specified by FSANZ and the EAS for yoghurt (EAS 33:2006).

With the aid of LP, the present study showed that the use of primary ingredients (broken rice, coconuts, and dates palm fruit and sesame oilseeds) is one of the possible cost-effective ways to reduce the high cost of importing raw materials from foreign countries, which is always incurred by food industries that manufacture nutritious and complementary foods and beverages. The results indicated that at a serving size of 100 mL, 1% of oyster mushroom powder could be a good prebiotic source to produce culturally acceptable synbiotic yoghurt; moreover, 10% of date syrup had a significant smooth texture and sweetness associated with its nutritional values. The present study can be used commercially in the future development of PBY alternatives to dairy. It can expand the choice space for consumers with lactose intolerance, allergenic to milk proteins, and in general to all consumers in the EAC. This is the first study in East Africa to generate PBY with considerable omega-6 and omega-3 content. It is also the first study to highlight the need to develop dietary guidelines of plant-based substitutes in Africa, which is currently lacking.

5.2 Recommendations

Despite the achievement of this study, clinical trials are needed to validate the efficacy and acceptability of the developed ready-to-serve PBY among individuals who are lactose intolerant or have milk allergies. Furthermore, additional researches are needed to evaluate the optimized product on nutrients and bioactive compounds lacking in this study. Willingness to pay studies and consumer acceptance with relatively large sample size are needed to substantiate all potential factors of consumers acceptability in our community.

The study also recommends that researchers and food industries switch to the use of locally available commodities to produce products that can substitute imported foods, thus reducing production and distribution costs and improving the region's overall prosperity through the export of finished goods.

REFERENCES

Abd El-Gawad, I. A., Abou Elsamh, M. M., Saleh, F. A., & Rayan, E. A. (2014). Bioavailability of Ca, P and Zn and bone mineralization in rats fed yoghurt and soy-yoghurt containing bifidobacteria. *European Journal of Nutrition & Food Safety*, *4*(2), 110–126.

Abla, O., Justin-Temu, M., Massawe, A., & Risha, P. (2008). Incidence; Knowledge and Healh Seeking Behaviour for Perceived allergies at Household Level: A Case Study in Ilala District Dar Es Salaam Tanzania. *East African Journal of Public Health*, *5*(2), 90–93.

Ahmadian-Kouchaksaraei, Z., Varidi, M., Varidi, M. J., & Pourazarang, H. (2014). Influence of processing conditions on the physicochemical and sensory properties of sesame milk: A novel nutritional beverage. *LWT-Food Science and Technology*, *57*(1), 299–305.

Akoma, O., Elekwa, U. O., Afodunrinbi, A. T., & Onyeukwu, G. C. (2000). Yogurt from coconut and tigernuts. *Journal of Food Technology in Africa*, *5*(4), 132–134.

Ani, E., Amove, J., & Igbabul, B. (2018). Physicochemical, microbiological, sensory properties and storage stability of plant-based yoghurt produced from bambaranut, soybean and Moringa oleifera seed milks. *American Journal of Food and Nutrition*, *6*(4), 115–125.

AOAC. (2012). *Official Methods of Analysis* (20^{th} Ed.) Association of Official Analytical Chemists.

Atiim, G. A., & Elliott, S. J. (2016). The global epidemiologic transition: Noncommunicable diseases and emerging health risk of allergic disease in sub-Saharan Africa. *Health Education & Behavior*, *43*(1), 37S-55S.

Awasthi, Y., & Singh, N. (2020). A comparative study on assessment of physiochemical properties of blended plant based yoghurt alongside commercial dairy yoghurt. *Gujarat Agricultural Universities Research Journal*, *45*(4), 229–232.

Aydar, A. Y., Mataracı, C. E., & Sağlam, T. B. (2021). Development and modeling of a novel plant-based yoghurt produced by Jerusalem artichoke and almond milk using l-optimal mixture design. *Journal of Food Measurement and Characterization*, *15*(4), 3079-3087.

Aydar, E. F., Tutuncu, S., & Ozcelik, B. (2020). Plant-based milk substitutes: Bioactive compounds, conventional and novel processes, bioavailability studies, and health effects. *Journal of Functional Foods*, *70*, 103975.

Bahna, S. L. (2002). Cow's milk allergy versus cow milk intolerance. *Annals of Allergy, Asthma & Immunology*, *89*(6), 56–60.

Bailey, R. K., Fileti, C. P., Keith, J., Tropez-Sims, S., Price, W., & Allison-Ottey, S. D. (2013). Lactose intolerance and health disparities among African Americans and Hispanic Americans: An updated consensus statement. *Journal of the National Medical Association*, *105*(2), 112–127.

Banerjee, S., & Bhattacharya, S. (2012). Food gels: Gelling process and new applications. *Critical Reviews in Food Science and Nutrition*, *52*(4), 334–346.

Banwo, K., Olojede, A. O., Adesulu-Dahunsi, A. T., Verma, D. K., Thakur, M., Tripathy, S., Singh, S., Patel, A. R., Gupta, A. K., & Aguilar, C. N. (2021). Functional importance of bioactive compounds of foods with Potential Health Benefits: A review on recent trends. *Food Bioscience*, *43*, 101-320.

Belewu, M. A., Abdulsalam, K. O., Belewu, K., & Belewu, N. (2013). Rice-Coconut Yoghurt: Preparation, Nutritional and Sensory Qualities. *Asian Journal of Agriculture and Rural Development*, *3*(393-2016–23802), 924–928.

Bernat, N., Cháfer, M., Chiralt, A., & González-Martínez, C. (2014). Vegetable milks and their fermented derivative products. *International Journal of Food Studies*, *3*(1), 93–124.

Brixi, G. (2018). Innovative optimization of ready to use food for treatment of acute malnutrition. *Maternal & Child Nutrition*, *14*(4), e12599.

Chandra, S., Khan, S., Avula, B., Lata, H., Yang, M. H., ElSohly, M. A., & Khan, I. A. (2014). Assessment of total phenolic and flavonoid content, antioxidant properties, and yield of aeroponically and conventionally grown leafy vegetables and fruit crops: A comparative study. *Journal of Evidence-Based Complementary and Alternative Medicine*, *2014*, 253875.

Charles, C. N., Swai, H., Msagati, T., & Chacha, M. (2020). Development of a Natural Product Rich in Bioavailable Omega-3 DHA from Locally Available Ingredients for Prevention of

Nutrition Related Mental Illnesses. *Journal of the American College of Nutrition*, *39*(8), 720–732.

Chileshe, J., Talsma, E. F., Schoustra, S. E., Borgonjen-Van den Berg, K. J., Handema, R., Zwaan, B. J., & Brouwer, I. D. (2020). Potential contribution of cereal and milk based fermented foods to dietary nutrient intake of 1-5 years old children in Central province in Zambia. *PloS One*, *15*(5), e0232824.

Davoodi, H., Esmaeili, S., & Mortazavian, A. (2013). Effects of milk and milk products consumption on cancer: A review. *Comprehensive Reviews in Food Science and Food Safety*, *12*(3), 249–264.

Deng, Y., Misselwitz, B., Dai, N., & Fox, M. (2015). Lactose intolerance in adults: biological mechanism and dietary management. *Nutrients*, *7*(9), 8020-8035.

Dibari, F., Diop, E. H. I., Collins, S., & Seal, A. (2012). Low-cost, ready-to-use therapeutic foods can be designed using locally available commodities with the aid of linear programming. *The Journal of Nutrition*, *142*(5), 955–961.

Dreiucker, J., & Vetter, W. (2011). Fatty acids patterns in camel, moose, cow and human milk as determined with GC/MS after silver ion solid phase extraction. *Food Chemistry*, *126*(2), 762–771.

Els, C., Boonzaaier, L., & Green, R. J. (2010). Atopy in asthmatic children attending a tertiary hospital in Pretoria. *Current Allergy & Clinical Immunology*, *23*(4), 180–182.

Facioni, M. S., Raspini, B., Pivari, F., Dogliotti, E., & Cena, H. (2020). Nutritional management of lactose intolerance: The importance of diet and food labelling. *Journal of Translational Medicine*, *18*(1), 260. https://doi.org/10.1186/s12967-020-02429-2

Falade, K. O., Ogundele, O. M., Ogunshe, A. O., Fayemi, O. E., & Ocloo, F. C. (2015). Physico-chemical, sensory and microbiological characteristics of plain yoghurt from bambara groundnut (Vigna subterranea) and soybeans (Glycine max). *Journal of Food Science and Technology*, *52*(9), 5858–5865.

FAO. (2013). *Dietary protein quality evaluation in human nutrition*. *Food and Agriculture Organization of the United Nations*. http://www.fao.org/documents/card/en/c/ab5c9fca-dd15-58e0-93a8-d71e028c8282/

FAO. (2019). *Agriculture food and nutrition for Africa—A resource book for teachers of agriculture. Agriculture Food and Nutrition for Africa*. from http://www.fao.org/3/w0078e/w0078e05.htm

FAO, & WHO. (2004). *Vitamin and mineral requirements in human nutrition* (2nd ed.). World Health Organization. https://books.google.co.ke/books?

FAOSTAT. (2015). *Food and Agriculture Organization statistical databases*. http://faostat.fao.org/

Fatima, S. M., & Hekmat, S. (2020). Microbial and sensory analysis of soy and cow milk-based yogurt as a probiotic matrix for Lactobacillus rhamnosus GR-1. *Fermentation*, *6*(3), 74.

Fiocchi, A., Brozek, J., Schünemann, H., Bahna, S. L., Von Berg, A., Beyer, K., Bozzola, M., Bradsher, J., Compalati, E., & Ebisawa, M. (2010). World Allergy Organization (WAO) diagnosis and rationale for action against cow's milk allergy (DRACMA) guidelines. *World Allergy Organization Journal*, *3*(4), 57–161.

Flom, J. D., & Sicherer, S. H. (2019). Epidemiology of cow's milk allergy. *Nutrients*, *11*(5), 1051.

FSANZ. (2019). *Approval Report: Application A1104 Voluntary Addition of Vitamins and Minerals to Nut- and Seed-based Beverages* (33(1)(b) of the FSANZ Act). https://www.foodstandards.gov.au/consumer/nutrition/milkaltern/Pages/default.aspx

Gabsi, K., Trigui, M., Barrington, S., Helal, A. N., & Taherian, A. R. (2013). Evaluation of rheological properties of date syrup. *Journal of Food Engineering*, *117*(1), 165–172.

Gad, A., Kholif, A., & Sayed, A. (2010). Evaluation of the nutritional value of functional yogurt resulting from combination of date palm syrup and skim milk. *American Journal of Food Technology*, *5*(4), 250–259.

Gao, X., LaValley, M. P., & Tucker, K. L. (2005). Prospective studies of dairy product and calcium intakes and prostate cancer risk: A meta-analysis. *Journal of the National Cancer Institute*, *97*(23), 1768–1777.

Gibson, G. (2004). From probiotics to prebiotics and a healthy digestive system. *Journal of Food Science*, *69*(5), M141–M143.

Gorlov, I. F., Shishova, V. V., Slozhenkina, M. I., Serova, O. P., Mosolova, N. I., Zlobina, E. Y., & Barmina, T. N. (2019). Synbiotic yoghurt with walnut and cereal brittle added as a next-generation bioactive compound: Development and characteristics. *Food Science & Nutrition*, *7*(8), 2731–2739.

Grasso, N., Alonso-Miravalles, L., & O'Mahony, J. A. (2020). Composition, physicochemical and sensorial properties of commercial plant-based yogurts. *Foods*, *9*(3), 252.

Hailu, A. A., & Addis, G. (2016). The content and bioavailability of mineral nutrients of selected wild and traditional edible plants as affected by household preparation methods practiced by local community in Benishangul Gumuz Regional State, Ethiopia. *International Journal of Food Science*, *2016*, 761-853.

Heaney, R. P. (2013). Dairy intake, dietary adequacy, and lactose intolerance. *Advances in Nutrition*, *4*(2), 151–156.

Heine, R. G., AlRefaee, F., Bachina, P., De Leon, J. C., Geng, L., Gong, S., Madrazo, J. A., Ngamphaiboon, J., Ong, C., & Rogacion, J. M. (2017). Lactose intolerance and gastrointestinal cow's milk allergy in infants and children–common misconceptions revisited. *World Allergy Organization Journal*, *10*(1), 1–8.

Ibrahim, A. I. E. (2017). *Physicochemical, Microbiological and Sensory Properties of Yoghurt Processed by Addition of Rice Flour* [Doctoral Dissertation, University of Gezira]. http://repo.uofg.edu.sd/bitstream/handle/123456789/978/Alya%20Ismat%20Elyas%20Ibrahim.pdf?sequence=1

Jeske, S., Zannini, E., & Arendt, E. K. (2017). Evaluation of physicochemical and glycaemic properties of commercial plant-based milk substitutes. *Plant Foods for Human Nutrition*, *72*(1), 26–33.

Kilimo Trust. (2014). *Expanding rice markets in the East African Community (EAC) Region* (No. 64; pp. 1–94). https://www.kilimotrust.org/documents/2018/

Kung, S.-J., Steenhoff, A. P., & Gray, C. (2014). Food allergy in Africa: Myth or reality? *Clinical eviews in Allergy & Immunology*, *46*(3), 241–249.

Leung, A. S., Wong, G. W., & Tang, M. L. (2018). Food allergy in the developing world. *Journal of Allergy and Clinical Immunology*, *141*(1), 76–78.

Mahfudh, N., Hadi, A., & Solechan, R. (2021). Immunomodulatory activity of yogurt fortified with roselle (Hibiscus sabdariffa L.) extract. *International Food Research Journal*, *28*(2), 255–261.

Mäkinen, O. E., Wanhalinna, V., Zannini, E., & Arendt, E. K. (2016). Foods for special dietary needs: Non-dairy plant-based milk substitutes and fermented dairy-type products. *Critical Reviews in Food Science and Nutrition*, *56*(3), 339–349.

Manasa, R., Harshita, M., Prakruthi, M., & Shekahana, N. R. (2020). Non-dairy plant based beverages: A comprehensive review. *The Pharma Innovation Journal*, *9*(10), 258–271.

Merchant, A. T., & Dehghan, M. (2006). Food composition database development for between country comparisons. *Nutrition Journal*, *5*(1), 1–8.

Moh, L. G., Keilah, L. P., Etienne, P. T., & Jules-Roger, K. (2017). Seasonal microbial conditions of locally made yoghurt (shalom) marketed in some regions of Cameroon. *International Journal of Food Science, 2017*.

Montemurro, M., Pontonio, E., Coda, R., & Rizzello, C. G. (2021). Plant-Based Alternatives to Yogurt: State-of-the-Art and Perspectives of New Biotechnological Challenges. *Journal of Foods*, *10*(2), 316.

Mousel, T., & Tang, X. (2016). *Analysis of consumer behavior towards plant-based meat and dairy alternatives market in Sweden* [Masters Thesis, Uppsala University]. http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-297183.

Mushi, P. M. (2014). *A study on lactose intolerance and milk intake among people in Mgeta and Njombe areas, Tanzania* [MSc. Thesis, Norwegian University of Life Sciences]. http://hdl.handle.net/11250/217128.

Ngampeerapong, C., Chavasit, V., & Durst, R. W. (2018). Bioactive and nutritional compounds in virgin coconut oils. *Malaysian Journal of Nutrition*, *24*(2), 257–267.

Nyeko, R., Kalyesubula, I., Mworozi, E., & Bachou, H. (2010). Lactose intolerance among severely malnourished children with diarrhoea admitted to the nutrition unit, Mulago hospital, Uganda. *BMC pediatrics*, *10*(1), 1-9.

Oak, S. J., & Jha, R. (2019). The effects of probiotics in lactose intolerance: A systematic review. *Critical Reviews in Food Science and Nutrition*, *59*(11), 1675–1683. https://doi.org/10.1080/10408398.2018.1425977

Palacios, O. M., Badran, J., Spence, L., Drake, M. A., Reisner, M., & Moskowitz, H. R. (2010). Measuring acceptance of milk and milk substitutes among younger and older children. *Journal of Food Science*, *75*(9), S522–S526.

Perdigon, G., de LeBlanc, A. d. M., Valdez, J., & Rachid, M. (2002). Role of yoghurt in the prevention of colon cancer. *European Journal of Clinical Nutrition*, *56*(3), S65–S68.

Rasika, D. M., Vidanarachchi, J. K., Rocha, R. S., Balthazar, C. F., Cruz, A. G., Sant'Ana, A. S., & Ranadheera, C. S. (2021). Plant-based milk substitutes as emerging probiotic carriers. *Current Opinion in Food Science*, *38*, 8–20.

Roberfroid, M. B. (2002). Functional foods: Concepts and application to inulin and oligofructose. *British Journal of Nutrition*, *87*(S2), S139–S143. Cambridge Core. https://doi.org/10.1079/BJN/2002529

Ryan, E. P. (2011). Bioactive food components and health properties of rice bran. *Journal of the American Veterinary Medical Association*, *238*(5), 593–600. https://doi.org/10.2460/javma.238.5.593

Sami, R., Li, Y., Qi, B., Wang, S., Zhang, Q., Han, F., Ma, Y., Jing, J., & Jiang, L. (2014). HPLC analysis of water-soluble vitamins (B2, B3, B6, B12, and C) and fat-soluble vitamins (E, K, D, A, and β-carotene) of okra (Abelmoschus esculentus). *Journal of Chemistry*, *2014*, 831357.

SELF Nutrition Data. (2018). *Food facts, information and calories: Cereals, Grains and Pasta*. https://nutritiondata.self.com/

Serafini, M., Stanzione, A., & Foddai, S. (2012). Functional foods: Traditional use and European legislation. *International Journal of Food Sciences and Nutrition*, *63*(sup1), 7–9. https://doi.org/10.3109/09637486.2011.637488

Sethi, S., Tyagi, S. K., & Anurag, R. K. (2016). Plant-based milk alternatives an emerging segment of functional beverages: A review. *Journal of Food Science and Technology*, *53*(9), 3408–3423.

Sheibani, E., Dabbagh Moghaddam, A., Sharifan, A., & Afshari, Z. (2018). Linear programming: An alternative approach for developing formulations for emergency food products. *Journal of the Science of Food and Agriculture*, *98*(4), 1444–1452.

Sheppard, K. W., & Cheatham, C. L. (2018). Omega-6/omega-3 fatty acid intake of children and older adults in the US: dietary intake in comparison to current dietary recommendations and the Healthy Eating Index. *Lipids in Health and Disease*, *17*(1), 1–12.

Tangyu, M., Muller, J., Bolten, C. J., & Wittmann, C. (2019). Fermentation of plant-based milk alternatives for improved flavour and nutritional value. *Applied Microbiology and Biotechnology*, *103*(23), 9263–9275.

Tishkoff, S. A., Reed, F. A., Ranciaro, A., Voight, B. F., Babbitt, C. C., Silverman, J. S., & Deloukas, P. (2007). Convergent adaptation of human lactase persistence in Africa and Europe. *Nature Genetics*, *39*(1), 31-40.

Tupamahu, I. P. C., & Budiarso, T. Y. (2017). *The effect of oyster mushroom (Pleurotus ostreatus) powder as prebiotic agent on yoghurt quality*. https://doi.org/10.1063/1.4983433

UNICEF. (2009). *A supply chain analysis of redy-to-use theurapeutic foods for the horn of Africa. Report. 2009; (May):1–136* (pp. 1–136). http://public.kenan-flagler.unc.edu/Faculty/swaminaj/Global-Health/RUTFSupplyChainProject

U.S. National Library of Medicine. (2020). Lactose intolerance. *Genetics Home Reference*. https://ghr.nlm.nih.gov/condition/lactose-intolerance

USDA. (2019). *Data from: Fooddata central*. fdc.nal.usda.gov

Verma, D. K., & Shukla, K. (2011). Nutritional value of rice and their importance. *Indian Farmers Digest*, *44*, 21–35.

WHO. (2021). Noncommunicable diseases. *Who*, https://www.who.int/news-room/fact-sheets/detail/noncommunicable-diseases

World Gastroenterology Organization. (2019). Lactose Intolerance scientIfIc revIew paper. *Grobal Garduian of Digestive Health*, *1*(1), 35–176.

Yiğit, A. A. (2020). Animal and plant-based milk and their antioxidant properties. *Veterinary Journal of Mehmet Akif Ersoy University*, *4*(2), 113–122.

APPENDICES

Appendix 1: Checklist of raw materials screenings

Brand	Names	Energy (kcal)	Protein (g)	Fats(g)	Carbs.(g)	Fiber(g)	Ca (mg)	B_9(μ_g)	Fe (mg)
FDC ID: 1100522	Coconut	354	3.33	33.49	15.23	9	14	26	2.43
FDC ID: 1100608	Sesame	631	20.45	61.21	11.73	11.6	60	11.5	6.36
FDC ID: 1578329	Quinoa	357	14.29	7.14	64.44	6.7	44	ns	4
FDC ID: 170162	Cashew	553	18.22	43.85	30.19	3.3	37	6.68	25
FDC ID: 170556	Pumpkin seeds	559	30.23	49.05	10.71	6	46	8.82	58
FDC ID: 1097552	Rice	47	0.28	0.97	9.17	0.3	118	0.2	2
FDC ID: 1581178	Pea	48	3.2	0.4	8	2.4	16	ns	1.15
FDC ID: 169702	Millet	378	11.02	4.22	72.85	8.5	8	85	3.01
FDC ID: 170178	Macadamia	718	7.91	75.77	13.82	8.6	85	11	3.69
FDC ID: 1517737	Sunflower seeds	283	11.67	25	10	5	67	ns	3
FDC ID: 175034	Mushroom powder	33	2.9	0.2	6.9	3.48	2.5	63	0.7
E 017	Dates fruits	310	2.38	0.35	72.67	9.10	ns	ns	ns

Data values were determined from USDA (2019); SELF Nutrition Data (2018), and expressed per serving 100g, ns: not specific

Appendix 2: Sensitivity analysis of the optimized formulation (PBY)

Constraits		**Allowable increase**	**Allowable decrease**
Total energy, kJ/100g	>67	2.90	1.15
	<272	46.50	3.10
Protein energy/Total energy	>0.02	12.67	1.90
	<0.025	<0.001	5.52
Fat/energy/Total energy	0.08	40.6	<0.001
	<0.12	<0.001	3.00
Constraints: Palatability			
Date palm syrup, g	>7	0.02	0.9
Sugar, g	<10	5	0.003
	>15	0.50	<0.001
Constraint: Texture			
Fat content, g	<6.9	0.89	<0.001
Solid content, g	8.25	0.001	0.97
Constraints: Anti-nutrient			
phytic acid, mg	≤ 22.8	5.19	<0.001
PDCAAS,%	≥ 95	<0.001	31.9
Total ingredients weight, g	97	0.08	0.001
Minerals and vitamins			
Calcium, mg	120	0.001	0.003
Zinc, mg	0.8	0.33	0.001
Potassium,mg	200	0.006	0.001
Phosphorus,mg	100	0.001	0.003
Thiamine,mg	5	0.001	0.004
Vitamin B9, mg	0.6	0.023	0.002
Vitamin A,mgRet	≤0.6	0.001	0.004

Appendix 3: Plant-based yoghurt formulations from coconut, rice, and sesame milks

Ingredients	Control	PBY-0%	PBY-6%	PBY-8%	PBY-10%
Cow's milk yoghurt	100	-	-	-	-
Coconut milk	-	-	85	40.7	38.5
Rice milk	-	92	5	39.3	38.5
Sesame milk	-	4	-	8	9
Mushroom powder	-	1	1	1	1
Guar gum	-	0.05	0.05	0.05	0.05
Date palm syrup	-	0	6	8	10
Total (%)	**100**	**97.00**	**97.00**	**97.00**	**97.00**

RESEARCH OUTPUTS

i) Publication

Dusabe, A., Chacha, M., Vianney, J. M., & Raymond, J. (2022). Development of Plant-Based Yoghurt Rich in Bioavailable Essential Nutrients and Bioactive Compounds from Ingredients Available in East Africa. *Current Research in Nutrition and Food Science Journal*, *10*(1), 250-266.

ii) Poster Presentation

Development of plant-based functional yoghurt as an alternative to dairy products

Angelique Dusabe: angeliqued@nm-aist.ac.tz Dr. Jofrey Raymond: jofrey.raymond@nm-aist.ac.tz
Dr. Musa Chacha: musa.chacha@nm-aist.ac.tz Dr. Sr. John-Mary Vianney: john-mary.vianney@nm-aist.ac.tz

Introduction

Humans have consumed dairy products for centuries, and today, Eastern African countries still consume substantial amounts of dairy products. They are considered as good source of calcium, fats, carbohydrate, and proteins essential to human nutrition, and comparable balance is hard to find in other foods. This advantage is connected to a shortage of milk availability compared to the global demand, especially in low-income countries. Despite the benefits of milk, its excessive consumption may significantly elevate the amounts of saturated fats in diets, increasing the risk of heart diseases, prostate and breast cancers. Moreover, health concerns and risks such as cow milk allergy, lactose intolerance, veganism, and cholesterol concerns have compelled some consumers to switch to dairy-free beverages as alternatives to cow milk. Example of these alternative are plant-based yoghurt (PBY) which are obtained by fermenting water-based extracts (plant-based milk), suspensions from plant source materials like cereals, nuts, legumes, oilseeds that resemble to cow milk in appearance. Plant materials such as coconut, rice, and sesame seeds are naturally free from lactose and locally available in Tanzania. They can be used as essential ingredients in plant-based beverages for consumers who are intolerant to lactose or allergic to cow's milk. Several food industries have tried to explore on these alternatives but are very expensive and some of these substitutes on the market are deficient in nutrients, texture and flavours. Therefore, the present research aimed to formulate a palatable plant-based functional yoghurt rich in the bioavailability of essential nutrients and biological active compounds from locally available ingredients apart from dairy sources with aid of Linear Programming Model. The cost effective developed PBY can expand a choice space for consumers with lactose intolerance, allergies to milk proteins and all consumers in general in the East African Community (EAC).

Problem statement

Currently, convenient and acceptable PBY that provides substantial nutritional benefits to consumers with milk allergy and lactose intolerance are scanty in the EAC. The common plant-based milk in Eastern Africa is soybean milk. Unfortunately, individuals who are allergic to cow's milk may also be allergic to soybean-based products. Additionally, plant-based milk products are highly-priced and a majority of them are not acceptable as face the challenge of sensory acceptability (texture and flavor) or deficiency in some nutritional components when compared to cow's milk yoghurts. All these limit lactose intolerant people and other individuals allergic to animal milk proteins from accessing nutritious and palatable dairy alternatives on East African market.

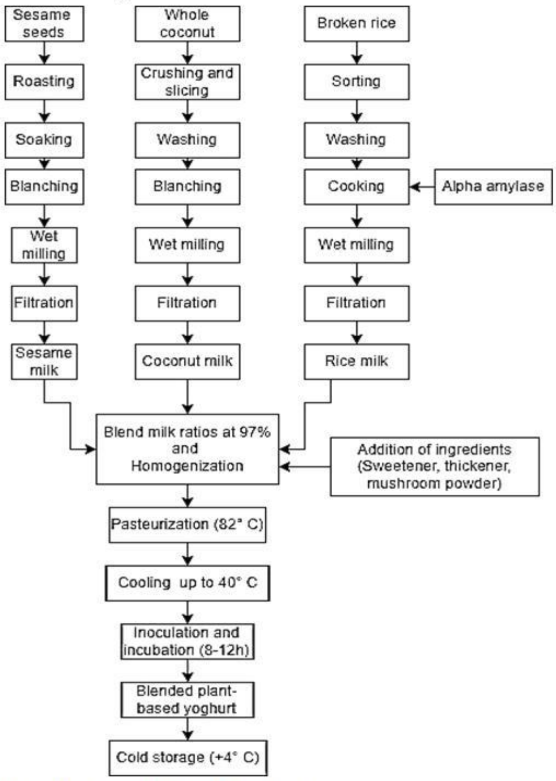
Objectives

The general objective of this study was to formulate a convenient plant-based functional yoghurt with comparable nutritional profile to that of cow's milk.

Specific objectives

i. To identify the suitable ingredients for formulating PBY
ii. To determine the optimal formulation of a PBY using LP Model
iii. To analyze the nutritional content, functional values, and sensory acceptability of the final optimized PBY.

Flow Diagram of PBY Production



Results and Conclusions

The formulated prototype considered as dairy-free alternative satisfactorily met consumers' sensory preferences (liked very much). The LP analysis result indicated a low cost of 0.9 USD/kg, which is 60% cheaper than Alpro (Soya-coconut blend). The findings showed that the cost-effective PBY comparable to the cow's milk in terms of nutritional values can be formulated from locally available ingredients. This study successfully developed a product that is microbiologically stable in 14 days of storage (+4 °C), rich in bioavailable essential nutrients and bioactive compounds, and meets acceptable standards for yoghurt specified by Food Standard Australia New Zealand, Codex Alimentarius specifications and the East Africa Standards (EAS 33:2006).