

**FORMULATION OF PROBIOTIC MANGO JUICE AND ASSESSMENT OF
PHYSICOCHEMICAL CHARACTERISTICS, NUTRITIVE QUALITY, CULTURE
VIABILITY AND STORAGE STABILITY**

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DECLARATION

This thesis is my original work, and it has not been presented for a degree in any other University.

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DEDICATION

This work is dedicated to my loving family, husband, Mr Stephen Wanyi, our children, Vincent, Bramwell, Daniel Mutisya and my parents, Mr. and Mrs. Mwanzia Muasya.

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LIST OF ABBREVIATION

ANOVA	: Analysis of Variance
AOAC	: Association of Official Analytical Chemists
CFU	: Colony Forming Units
DMRT	: Duncan's Multiple Range Test
FAO	: Food and Agriculture Organization
HCDA	: Horticultural Crop Development Authority
HPLC	: High-Performance Liquid Chromatography
ICMSF	: International Commission on Microbiological Specifications for Foods
JKUAT	: Jomo Kenyatta University of Agriculture and Technology
KEBS	: Kenya Bureau of Standard
LAB	: Lactic Acid Bacteria
MRS	: Man Rogosa Sharpe
MT	: Metric Tones
NACOSTI	: National Commission for Science, Technology, and Innovation
PCA	: Plate Count Agar
PJ	: Pasteurized Juice
SDA	: Sabouraud Dextrose Agar
TPC	: Total Plate Count
TSS	: Total Soluble Solid
TTA	: Total Titratable Acidity
USDA	: United States Department of Agriculture
VRBGA	: Violet Red Bile Glucose Agar

OPERATIONAL DEFINITION OF TERMS

Acceptability: Ability of a product to be tolerated or allowed by consumers.

Aseptic: Free from contamination caused by pathogenic microorganisms.

Culture viability: Probiotics' ability to survive or proliferate in fermented mango juice.

Formulation: Mixing ingredients appropriately to have a product of desired characteristics.

Functional foods: Foods containing substances that have positive benefits on health beyond basic nutrition by promoting optimal health and reducing risk of disease.

Inoculate: Introducing fermentative culture to mango fruit juice aseptically.

Probiotics: Live microorganisms added to food that provides health benefits when consumed in an appropriate amount.

Product development: Complete process of developing a new product or a product with different characteristics that offer new or additional benefits to the consumer and widen the product range.

Process stability: Viability of the probiotic culture in the pasteurized juice during fermentation.

Sensory: Physical or perceived senses as assessed using sensory scores of appearance, consistency, color, flavor and taste.

Shelf stability: Storage of food product at ambient temperature or refrigeration to retain its quality (taste, color, culture viability and nutrient content).

Value addition: Changing the original form of the fruit to diverse as well as more valuable and healthy products.

ABSTRACT

Probiotics are live microorganisms added to food products to confer health benefits such as gut health, cancer protection and improved immunity when consumed. Fruits juices, particularly mango (*Mangifera indica l.*) fruit juice, are considered as potential channels for delivering probiotics as they contain essential nutrients suitable for the growth of probiotics and are free from allergen as opposed to milk based products. There is limited information on the processing and stability of probiotic fermented mango juice in Kenya. The study was undertaken to formulate, evaluate the nutritive value, physicochemical, sensory acceptability and storage stability of a fermented probiotic mango fruit juice for the Kenyan market. Mature ripe apple mango variety were sourced from Masii in Machakos county, transported to Jomo Kenyatta University of Agriculture and Technology (JKUAT) Food Science Department and stored in a cold room (6-10⁰C). Three different mixed probiotic cultures were used in this study (Abt-5[®] culture *Streptococcus thermophilus*, *Bifidobacterium* and *Lactobacillus acidophilus*); Fiti[®] culture (*Lactobacillus rhamnosus*) and Yo-mix[®] culture (*Streptococcus thermophilus* and *Lactobacillus delbruikii ssp bulgaricus*). The viability of the cultures was also assessed. The mango juice was formulated using mango juice to water ratios of 100:0 (control), 25:75, 50:50 and 75:25 (v/v) and adjusted to 17⁰brix using cane sugar. The formulated mango juice was pasteurized at 90⁰C for 1 minute, cooled using ice water and refrigerated at 10⁰C. Sensory evaluation was carried out using a 9-point hedonic scale, a questionnaire was administered to a sample of 35 untrained panelists between 18-50 years of age randomly selected from the Food Science Department. The preferred mango juice samples based on overall acceptability were incubated at 42⁰C for 0, 12, 16, 24, 36, 48 and 72 hours. The final product was refrigerated at 4⁰C for analysis. There was a general increase in cell count in all cultures with mango juice fermented with Fiti[®] culture (*Lactobacillus rhamnosus* GR-1) for 48 hours recording the highest viable cell count of log₁₀ 9.14 CFU/ml. Fermentation significantly changed the final product's physicochemical, nutritional, and sensory acceptability (P<0.05). The highest pH decrease was observed in 50:50 formulation with a significant (p<0.05) increase in total titratable acidity from 0.15% to 0.69% using Fiti[®] culture (*Lactobacillus rhamnosus* GR-1) within 72 hours. Subsequently, the total soluble solids decreased from 17⁰ Brix to 14.47⁰ Brix. The decrease in pH and increase in viable count indicated that probiotic bacteria utilized the available sugars in the juice for cell synthesis. β-carotene levels increased by 3-8%, whereas vitamin C retention was >68%. The culture viability, pH and total soluble solids decreased during the four weeks of storage at 4⁰C. The fermented mango juice was microbially stable during the four weeks of storage at 4⁰C. The recommended storage period for the probiotic mango juice is four weeks and one week for the juice fermented using Fiti[®] culture (*Lactobacillus rhamnosus*) and Yo-mix[®] culture (*Streptococcus thermophilus* and *Lactobacillus delbruikii ssp bulgaricus*) respectively. On the overall, mango juice fermented using Fiti bacterial culture for 48 hours was superior in quality based on sensory and culture viability. Therefore, the results of this study are beneficial to food industries, small and medium scale enterprises. The study recommends the popularization of probiotic fermented mango juice using Fiti culture[®] as a healthy drink.

CHAPTER ONE: INTRODUCTION

1.1 Background to the Study

Probiotics are defined as healthful bacteria which offer a beneficial health effect to the body if consumed in the right amount (I et al., 2016). Probiotic foods are among the most commonly known functional foods which promote human health (Nasef et al., 2020). They contain bioactive compounds such as vitamins, live bacteria (probiotics) and minerals that balances gut health (Lebaka, Wee, Narala and Joshi, 2016). The beneficial health effect of probiotics depends on the amount of useful bacteria in the food at consumption. It has been reported that ingestion of 10^6 CFU/ml of viable probiotic cells in a day can significantly promote human health (Bansal, Mangal, Sarma and Gupta, 2016).

Probiotic foods represent 60%–70% of functional foods (Perricone, Bevilacqua, Altieri and Sinigaglia, 2015). The most commonly used probiotics species are *Lactobacilli*, *Bifidobacteria* and *Streptococcus*. They are used in both dairy and non-dairy products (Perricone et al., 2015). Their action and health benefits depend on the strain used. The use of these probiotic, particularly *Lactobacillus rhamnosus* have proven to improve gut health, especially in the prevention and treatment of diarrhea and after antibiotic medication attributed to bacterial imbalance in the digestive system (Westerik et al., 2018). The use of probiotic therapy can restore gut health. The health benefits of *Streptococcus thermophilus*, *Bifidobacterium* and *Lactobacillus acidophilus* includes; boosting body immunity, reducing the risk of developing colon cancer, lowering blood pressure, counteracting the effects of food-borne pathogens and vitamin production (M. Mustafa et al., 2016; Rafiq, Sharma, Nazir, Rashid and Sofi, 2016).

Milk-based products such as yogurts and beverages are traditionally considered the best carriers of probiotic bacteria to the gut. The products are available in a wide range in the market (Khatoon and Gupta, 2015). Demand for functional beverages has been rising worldwide due to the increased number of vegetarian consumers, lactose intolerance associated with dairy products, milk protein allergies, and milk fats contributing to high cholesterol content in a product (Mustafa et al., 2016). This has led to research on several fruit-based probiotic foods which proved that various non-dairy media such as fruit juices could be used to develop functional foods (Khatoon and Gupta, 2015). A healthy probiotic fermented fruit juice with acceptable sensory characteristics and good shelf life serves as an alternative to milk-related disorders. In addition, mango fruit contains essential nutrients, vitamins and minerals necessary for the growth of probiotics. However, fermentation of mango juice using mixed probiotic starter culture is expected to change the Physico-chemical characteristics of the juice and its nutritional content. Starter culture utilizes sugars and other nutrients during fermentation to produce lactic acid which acidifies the media hence affecting the growth of probiotic bacteria (Kumar et al., 2015).

Mango is one of the tropical fruits consumed in many parts of the world. It is currently grown in more than 90 countries; the Asian continent is considered the leading producer of mangoes accounting for 77% of the total mango production worldwide (Reddy et al., 2015). In the recent past, mango production in Kenya has increased, with limited value addition and high postharvest losses experienced during harvesting (Mutua et al., 2016). Mango is widely consumed mainly due to its sensory attributes like color and sweet taste as well as increased awareness of its health benefits to most of the population. It is also the second most-produced fruit traded domestically by small-scale farmers in Kenya and

an important source of income (Guan et al., 2016; Mutonyi, Beukel, Gyau and Hjorts, 2016).

Fresh mangoes contain essential nutrients such as vitamins C and A, which are antioxidants and cancer-preventing agents, sugars that are important for the growth of probiotics (Guiamba, 2016). β -carotene is a biologically active compound and a precursor for vitamin A. Mangoes also contain high organic acid levels and low pH, making them suitable carriers of probiotic bacteria that can survive in an acidic environment (Mustafa et al., 2016). During processing, survival and the activity of probiotics in the final product are crucial as they can be affected by pH, incubation and storage temperature (Reddy et al., 2015). Fermentation of fruit juices with probiotics improves flavor and taste and extends shelf life, contributing to the product's better sensory characteristics. The development of probiotic fruit juice without compromising sensory traits is an innovative idea in developing a functional food. In addition, the use of probiotics could change the original fruit flavor and aroma (Nematollahi et al., 2016).

There is a scarcity of information on the potential of utilizing mango fruit juice as a probiotic product in Kenya. Therefore, the study seeks to formulate and characterize fermented probiotic mango juice and assess the sensory acceptability, nutritional and storage stability of the product. Formulation of fermented mango fruit juice containing bioactive components that positively affect the gut may be one method that can be adapted to develop a new functional food.

1.2 Problem Statement

Mango is a tropical crop widely grown in East Africa. It is seasonal, easily available and highly perishable fruit; a glut is experienced during harvesting season, where postharvest losses of 40-50% are registered due to poor postharvest handling practices and minimal value addition. Mango value addition in Kenya is low at approximately 10% (Kumar et al., 2015). Adoption of value addition methods such as developing new products would otherwise reduce the high postharvest losses experienced. Value addition also prolongs mango shelf life through processing, offers a healthy product and alleviates poverty through enhanced food and nutrition security (Mutonyi et al., 2016). In addition, the diversity of various mango-based products is enhanced.

Currently, 78% of probiotic products worldwide are delivered through dairy products (Kandylis, Pissaridi, Bekatorou and Kanellaki, 2016). The allergies may restrict the consumption of dairy products. Global estimate indicates that 70% of the population is affected by lactose intolerance, cholesterol diseases and vegetarianism (Perricone et al., 2015). Fruits are potential channels for delivering probiotics. They are rich in vitamins and do not contain allergens, lactose and cholesterol that might limit their consumption by a certain part of the population (Hossain, Hoque, Kabir and Yasin, 2019). Research conducted using several tropical fruits such as pineapple, mango and orange blends has proven that fruits can be used as a suitable base to produce probiotic drinks (Perricone et al., 2015). Fermented mango juice has both the nutritional effects of the fruit and the added health promoting benefits from the bacteria. There is a scarcity of information on the formulation and use of probiotics in fermented mango fruit juice. The study focused on formulating fermented probiotic mango fruit juice and assessing its nutritional qualities, stability and sensory acceptability.

1.3 Purpose of the study

The study aimed to formulate fermented probiotic mango fruit juice and establish the physicochemical characteristics, nutritional quality, culture viability, and storage stability.

1.3.1 Specific objectives

1. To formulate a fermented probiotic mango juice product.
2. To determine the physicochemical characteristics, sugars and vitamins A and C content of the control and fermented probiotic mango juice.
3. To assess the sensory acceptability of the control and probiotic fermented mango juices.
4. To establish the probiotic culture viability during processing and storage.
5. To assess the storage stability of probiotic fermented mango juice and the control.

1.4 Study Hypothesis

1. H_0 : There is no significant difference between sugars, vitamin A & C content of the probiotic fermented juice and pasteurized mango juice.
2. H_0 : There is no significant difference between probiotic fermented mango juice and pasteurized mango juice on sensory acceptability.
3. H_0 : There is no significant difference between probiotic fermented mango juice and the pasteurized mango juice on shelf stability.
4. H_0 : There is no significant effect of processing and storage of fermented mango juice on probiotic viability

1.5 Significance of the study

The study may provide a formulated probiotic fermented product to Kenyan industries. Consumers may use information emanating from the study for home-based value addition and managing various health conditions.

1.6 Conceptual framework

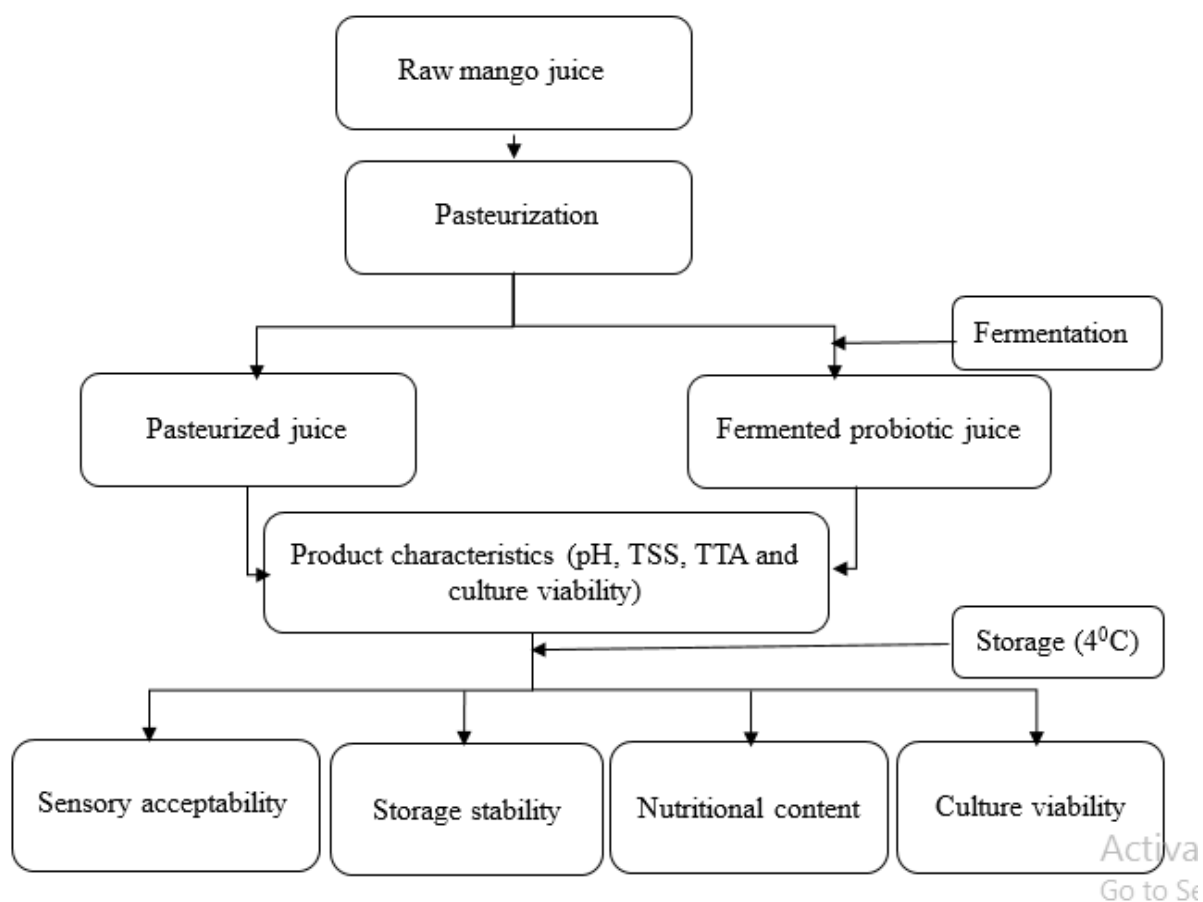


Figure 1.1: Conceptual framework for probiotic fermented mango juice processing

Adapted and modified from (Ranadheera et al., 2017)

The conceptual framework of the study was based on the formulation of fermented probiotic mango juice as one aspect among many in value addition. The process involves the addition of water to mango pulp to get a product of desirable characteristics.

Pasteurization destroys pathogenic microorganisms; minimizes the undesirable enzymatic reactions while enhancing and maintaining the quality of starting fruit (Reddy et al., 2015). Fermentation and inoculation temperatures, pH and total soluble solids of the juice determines the product's culture viability (Khosravi et al., 2015).

Probiotics ferment sugars present in the mango fruit juice, thus affecting the pH, TSS, TTA. Therefore, these parameters are monitored during processing and storage (4⁰C) as they affect the viability and activity of the probiotics during product development (Nguyen et al., 2019). The probiotic mango juices taste, flavor, color and texture may change during processing compared to the pasteurized mango juice; hence sensory evaluation is vital in determining the product's acceptability by consumers.

CHAPTER TWO: LITERATURE REVIEW

2.1 Probiotic foods and their health benefits

Probiotics are live bacteria in a product that on consumption provide beneficial effects to the consumer (Sánchez et al., 2017). Probiofortification is one method of developing new functional food containing biologically active substances that improves the gut microbiome (Francino, 2017). Functional foods have several health benefits for the body which include boosting body immunity, reducing the risk of heart disease, lower blood pressure and reducing the risk of colon cancer. These benefits depend on the number of viable cells present in the product at consumption (10^6 CFU/ml).

Fermented milk products are the most commonly used probiotic foods. However, there is rising demand for fruit-based probiotic products. This has contributed to various studies on several food matrices that include fruits (Corbo et al., 2014; Reddy et al., 2015). Research has been conducted on several fruits to determine their suitability for the production of probiotic drinks (Perricone et al., 2015). However, it has been reported that fruits can be used as suitable media for the growth of probiotics (Rafiq et al., 2016). The study seeks to develop a novel probiotic mango fruit juice with superior quality characteristics and health promoting effects on consumers.

2.2 Probiotic viability in fruit juices

There are several strains of LAB, such as *Lb. plantarum*, *Lb. acidophilus*, *Lb. casei*, *Lb. rhamnosus* and *Bifidobacteria* that are capable of growing in fruit juice as they can survive in an acidic environment (Patel, 2017). It has been reported that probiotic bacteria culture takes 24 fermentation hours to achieve optimal growth (Lebaka et al., 2018). Probiotics provide antimicrobial, antiviral and antioxidant activities (Mustafa et al., 2016). The health effects of probiotics are determined by their concentration and

viability during storage (Tamang et al., 2016). Maintaining the viability and activity of probiotics in food products is a great achievement in product development as it guarantees the consumer its health benefits. Most *Lactobacilli* can survive in juices with low pH (2.9 to 4.5) (Khosravi et al., 2015; Perricone et al., 2015). However, there are factors found to affect probiotic survival in fruit juices they include: pH, titratable acidity, water activity, heat treatment, incubation temperature, storage methods, strains and proportion of inoculation (Tripathi and Giri, 2014). The study established the viability of probiotics in fermented mango juice stored at 4⁰C for one month.

2.3 Production and nutritional value of mango

Mango (*Mangifera indica l.*) is a commercial crop grown in many countries. It is mainly grown in Australia, Florida, Israel, the Middle East, East Africa and South Africa. In Kenya, the area under mango production by 2014 was 47,620 hectares with an estimated production of 744 million tons (MT) valued at ~\$US 101 Million (Mujuka et al., 2020). Mango has an attractive fragrance, flavor, high concentration of sugars and antioxidants like carotene (Hossain et al., 2019). The location of mango fruit production and the season in which fruits are grown determine their nutritional composition. Rainfall and excess water supply reduce soluble solids content while soil mineral composition influences the nutritional quality of the fruit (Reddy et al., 2015). The apple mango variety was used in the study due to its stability during processing, high total soluble solid and nutritional content (M & A, 2013).

Mangoes provide up to 64-86 calories of energy per 100g which balances the human diet. Mango fruit contains phytochemicals and other nutritional compounds essential for human body. Carotenoids and other antioxidants like vitamin C protect humans from certain cancers, enhance the immunity system and protect against stroke (Kandyliis et al.,

2016; Mkandawire et al., 2016). Therefore, mango juice has health-promoting benefits if consumed regularly.

Mango fruit being seasonal and highly perishable during the harvesting period leads to a glut in the market. They are scarce when offseason hence uneven supply throughout the year. Despite high-level mango fruit production in Kenya, there is food insecurity at the household level. There is little fruit processing on a small scale and farmers experience losses as they often sell fresh fruits within weeks of harvesting at low prices (Geburu et al., 2015). Probiotification is an alternative and profitable method of using surplus/excess mango if it becomes accepted.

2.4 Formulation and processing of probiotic fermented mango juice

Proper product formulation gives a quality product with different characteristics from the original fruit or product with added health benefits (Santhirasegaram et al., 2015). Thermal processing helps conserve the juice while guaranteeing the quality and safety of the final product, although it may change sensory characteristics or lower nutrient bioavailability (Gallina, Barbosa, Ormenese and Garcia, 2019). Development and characterization of probiotic fermented smoothie beverage by Gallina et al., (2019) showed that processing conditions such as temperatures, pH and Brix are monitored during fermentation as they affect culture viability.

Fermentation is a food processing method that uses microorganisms and their enzymes to modify the biochemical components of the primary foodstuff (Lee and Hong, 2016). It involves inoculation of culture into fruit juice which contains fermentable sugars (fructose, glucose and sucrose) and incubating it at an appropriate temperature (40-45⁰C) for the bacteria to reproduce (Kumar et al., 2015).

During fermentation citric acid and other organic acids such as succinic acid, malic acid are produced, contributing to improved organoleptic properties of the product (Di Cagno et al., 2016). Fermentation of fruit-based probiotics requires; proper selection of microbial strain, physicochemical properties of the fruit and conditions for optimal growth (Mustafa et al., 2016). There is scarce information on the processing requirements of fermented probiotic mango fruit juice for product quality.

2.5 Physico-chemical characteristics of fermented probiotic mango juice

The mango juice's intrinsic characteristics, such as sugars, pH, Brix, and total titratable acidity, are essential when developing a new product. They determine the sensory acceptability and the quality of the final product. They also help predict microbial growth hence the product storage period (Olayinka et al., 2018; Tripathi and Giri, 2014). The study seeks to characterize the developed new product.

2.6 Sensory acceptability of fermented probiotic mango juice

Tropical fruit juices have desirable color, flavor and taste. The main challenge of using probiotics in developing fruit juices is consumer acceptability. Probiotics may improve flavor, texture and mouthfeel, contributing to better sensory characteristics (Lee and Hong, 2016). Several studies show that some probiotics do not affect the overall acceptance of juices such as pineapple juice containing (*Lb. reuteri*). On the contrary, others alter the original flavor and aroma of the fruit (Perricone et al., 2015). Fruit such as pineapple, mango, or passion fruit may impart the aroma or flavor of the fruit to the finished product in a positive way but also avoids off-flavors associated with the addition of probiotics (Kaur et al., 2016). The sensory traits of juice depend on the bacteria species used and the condition under which the product is stored (Perricone et al., 2015).

There is a scarcity of information on consumer acceptability of probiotic fermented mango juice. The study, therefore, seeks to establish the product's sensory acceptability to Kenyan consumers.

2.7 Process and shelf stability of fermented probiotic mango

Product shelf stability is the period under specified storage conditions, after processing or packaging, during which a product remains sound and safe for consumers (Mkandawire et al., 2016). Currently, consumers prefer food with extended shelf life. Food fermentation affects the shelf stability, nutritional and sensory properties of food. Lactic acid bacteria (LAB) convert fermentable sugars to lactic acid, ensuring product safety by producing bacteriocins and organic acid, which act against pathogenic and spoilage microorganisms, prolonging the product's shelf life (Khosravi et al., 2015).

Fermentation helps stabilize and transform food material especially perishable foods, whereby desirable and improved nutritional, organoleptic and functional attributes of probiotic food are attained, contributing to health-promoting benefits (Marsh et al., 2014). Chemical, physical, sensory and microbiological changes are monitored during storage to determine product shelf stability (Mkandawire et al., 2016). The current study investigates the suitability of mango juice for probiotification and evaluates probiotic viability during processing and cold storage.

2.8 Summary of Literature Review

Recently, the consumption of probiotic foods has been on the rise due to health benefits associated with the prevention and management of various health conditions being one of the key reasons. The health benefits of probiotic foods include; boosting body

immunity, reducing the risk of developing some cancers, lower high blood pressure and reducing heart disease.

Mango fruit is seasonal, perishable and highly produced in Kenya. Postharvest losses are experienced during harvesting and transportation. Product development/value addition may help reduce postharvest losses while providing a healthy product throughout the year. Mango fruit juice has been an excellent carrier of probiotics to the gut. It has favorable intrinsic characteristics for bacterial growth (nutrient and pH) and appealing organoleptic qualities for consumers. Therefore, probiotic fruit juice, especially mango fruit, can be used as an alternative for lactose-intolerant consumers and as a product with a low cholesterol content. There is limited information on the formulation, nutrient content, culture viability and sensory acceptability of fermented probiotic mango fruit juice.

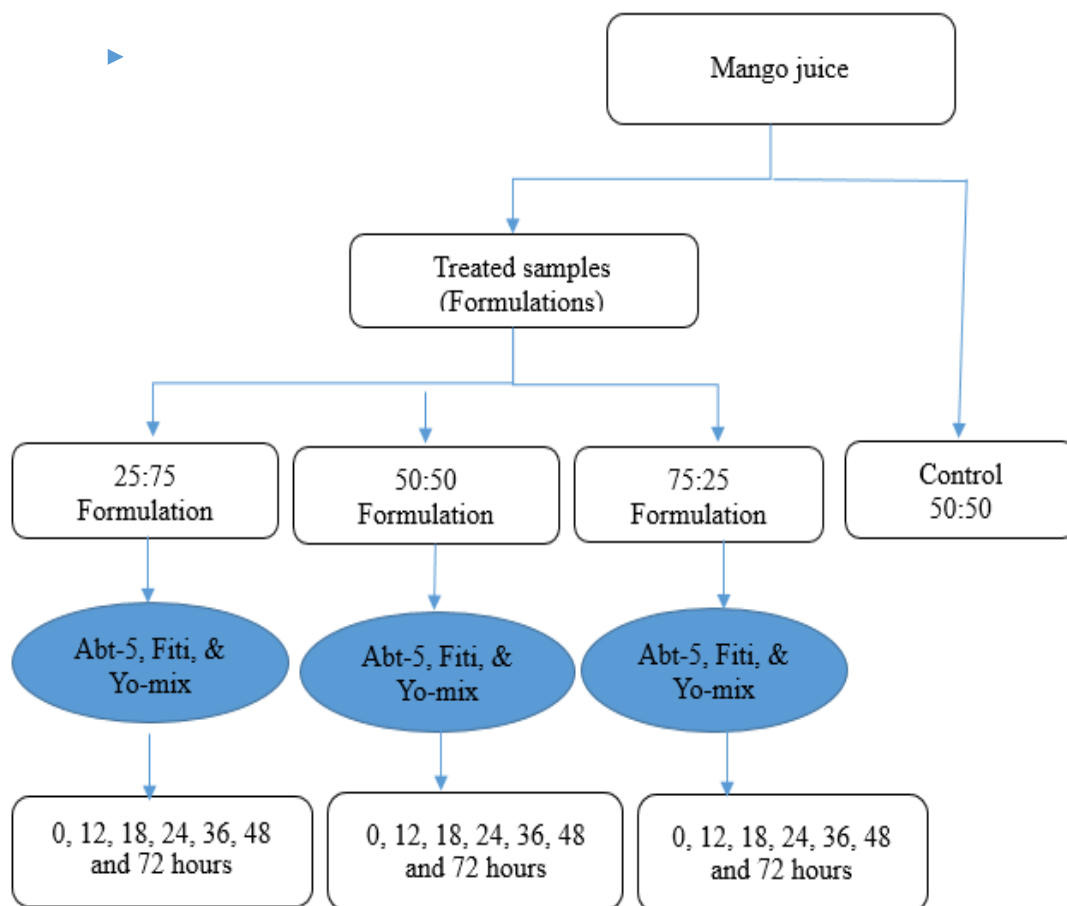
The product formulation is a process of producing quality products with added health benefits. It involves changing the original form of the fruit to add value or developing a product with beneficial health effects. The intrinsic characteristics of a fruit (total soluble solids and pH) are important during the development of probiotic foods as they determine culture viability and product shelf life. Probiotics should be viable and in the required amount during consumption to confer health benefits to the body. Fermentation could affect the fruit's original taste and flavor, which determines the sensory acceptability of the fruit juice. There is limited information on the optimal process for producing fermented probiotic mango juice to achieve quality products.

CHAPTER THREE: MATERIALS AND METHODS

3.1 Study design

The study adopted an experimental laboratory design using a fractional factorial design to formulate probiotic fermented mango juice. Mature, well ripe Apple mango (*mangifera indica l.*) fruits were sourced from Masii market in Machakos County of Kenya during February- march harvesting season. They were packed on lined crates and transported to the Department of Food Science and Technology (JKUAT), stored in a chiller room at 6-10⁰C before processing. All the equipment's used during processing were cleaned with boiling water before use and good manufacturing practices including wearing hand gloves and hair nets were observed during the study to minimize contamination. The mango fruits were sorted, washed with portable water and food-grade detergent and rinsed thoroughly. They were peeled with a kitchen knife and was flesh sliced into pieces and homogenized using a commercial blender. The mango juice obtained was diluted into different ratios with water to obtain different samples of juice as described in section 3.3.

The formulated mango juice was subjected to sensory evaluation to evaluate consumer acceptability. The preferred formulation based on sensory acceptability was fermented with three different cultures (Abt-5[®] (*Streptococcus thermophilus*, *Bifidobacterium* and *Lactobacillus acidophilus*), Yoba-Fiti[®] (*Lactobacillus rhamnosus*) and Yo-Mix[®] (*Streptococcus thermophilus* and *Lactobacillus delbruikii ssp bulgaricus*) (Appendix I). The samples were incubated at different times (0, 12, 18, 24, 36, 48 and 72 hours). The control sample was pasteurized mango juice (Fig 3.1).



Activ.

Figure 3.1: Fractional factorial design for probiotic fermented mango fruit juice formulation

3.2 Study variables

The independent variables in the study included product formulation, type of cultures used, fermentation and storage time of the fermented and unfermented mango fruit juice. On the other hand, the dependent variables were sensory acceptability, shelf stability and nutrient content of the product as well as culture viability of the product during processing and storage.

3.3 Mango fruit juice formulation and processing

The first step in the formulation of probiotic fermented mango juice was to dilute mango pulp with distilled water. Formulations were made based on KEBS standards

specifications for fruit juice in terms of the amount of fruit puree for ready to drink fruit juice, total soluble solids and pH (©KEBS, 2016). The KEBS standard requires a minimum of 25%v/v fruit puree, 13⁰Brix and a minimum pH of 2.5. Formulations containing slightly higher mango juice ratios were also chosen to assess consumer preferences. The 100.0 ratio was the control sample. Three mango fruit juice samples were prepared with fruit juice to water ratios as follows: 25 parts of mango juice and 75 parts of water; 50 parts of mango juice and 50 parts of water; 75 parts of mango juice and 25 parts of water. (25:75, 50:50 and 75:25). The fermentable sugars (TSS) were standardized to 17⁰brix using cane sugar. The samples were pasteurized at 90⁰C for 1 minute using steam jacketed pan (de Oliveira et al., 2021). The pasteurization temperature was based on juice safety, mango fruit pH and quality of fruit juice composition (Moreira et al., 2017). The pasteurized mango juice samples were cooled in an ice water bath and refrigerated at 4⁰C prior to sensory evaluation. These samples were fermented using the selected bacteria cultures.

3.4 Fermentation of formulated mango fruit juice

The second step of the formulation was done based on the physicochemical characteristics to obtain the best formulation and also to optimize fermentation time. A liter of the three formulations (25:75; 50:50; 75:25) of the juice was transferred to sterile opaque cans to minimize exposure to light during processing and storage which can lead to loss of some vitamins. The juice was immediately cooled to 42⁰C with ice water. Preparation and addition of probiotic culture to mango juice were conducted as described by Moreira et al., (2017). A pre-inoculum (mother culture) was prepared using the three different cultures (Abt-5, Fiti and Yo-mix) where one gram of each of the bacteria culture (Abt-5, Fiti and Yo-mix) were aseptically added to a liter of separate juice sample and stirred manually. They were incubated at 42⁰C for 24 hours (Appendix J). After 24

hours, 100ml of inoculum containing probiotic bacteria was added to 900ml of the pasteurized juice (totaling nine treatments) and were incubated at 42⁰C for 0, 12, 18, 24, 36, 48, and 72 hours (Appendix K). The fermented products were cooled to 4⁰C and stored for further analysis (Appendix L).

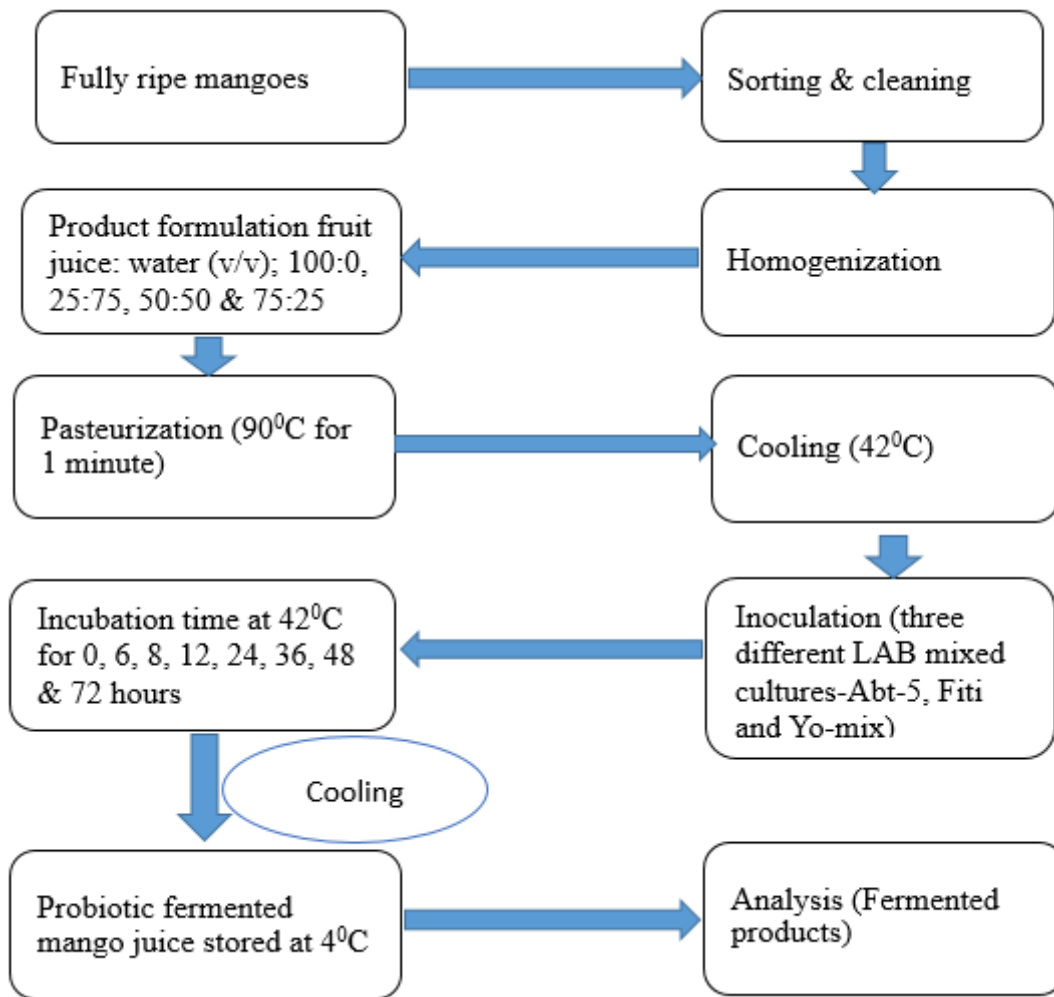


Figure 3.2: Flow chart on formulation and processing of probiotic fermented mango juice

3.5 Physico-chemical properties of the control (pasteurized) and probiotic fermented mango juice.

3.5.1 Determination of pH

pH of the control and fermented mango juice was determined using a calibrated pH Meter (Model HI 2211, Hanna) as per Soon & Hendershot, (2006). The meter electrode probe was standardized using acidic buffer (4.0), rinsed with distilled water before standardizing with alkaline buffer (6.0) solution. The mango juice pH was measured with the electrode probe.

3.5.2 Determination of Total Soluble Solids (TSS) content

The TSS was determined using digital Atago Hand Refractometer (Model RX 5000, Atago, Tokyo, Japan). A drop of mango juice sample was placed on the prism of calibrated digital hand refractometer and the lid closed, total soluble solids were read at $20^{\circ}\text{C} \pm 1$ from the scale. The results obtained were expressed in $^{\circ}\text{brix}$.

3.5.3 Determination of Total Titratable Acidity (TTA) content

TTA was conducted as described by Sadler and Murphy, (2010). Approximately 10ml of the sample was pipetted into a 100ml volumetric flask and 2-3 drops of 1% phenolphthalein were used as an indicator to mark the endpoint. Titrated using 0.1N sodium hydroxide until the persistent pink color appeared. Titer volume was noted and TTA was calculated as % citric acid, the main organic acid in mango fruit (Ueda et al., 2000).

3.6 Sensory evaluation of mango juice

Sensory evaluation of the control and fermented probiotic mango juice was conducted at day one of processing and during the four weeks of storage where the samples were stored at a refrigeration temperature of 4⁰C. The sensory panelist (35 untrained staff (20) and (15) students) were recruited using simple random sampling from JKUAT, Food Science and Technology Department. The room was lit with normal room temperature and cubicles with sinks. Each panelist was served with a 20ml of juice in a clear plastic cup at 10⁰C placed on a table. The samples were coded and randomly presented to the panelist to minimize carryover and first order effect. They were also provided with cold water to rinse their mouth after each test. Preference testing was done using a hedonic scale rating (9-point) where 1 denoted dislike extremely and 9 denoted like extremely. The parameters evaluated were; juice color, appearance, taste, aroma/flavor, consistency and overall acceptability of the final product (Appendix B) according to (Larmond, 1977).

3.7 Microbiological analysis for culture viability testing and quality control

3.7.1 Determination of the viability of lactic acid bacteria

Culture viability was assessed using the standard method (Hoben and Somasegaran, 1982). The growth and viability of the three cultures Abt-5 (*Streptococcus thermophilus*, *Bifidobacterium* and *Lactobacillus acidophilus*), Fiti (*Lactobacillus rhamnosus*) and Yo-Mix (*Streptococcus thermophilus* and *Lactobacillus delbruiikii ssp bulgaricus*) in 50:50 formulated probiotic fermented mango juice were determined using DeMan Rogosa Sharpe (MRS) agar. Agar was dissolved by adding distilled water and adjusted to the final volume of 500ml. It was sterilized by autoclaving at 121⁰C for 15 minutes, then cooled to 50⁰C before dispensing aseptically to a sterile petri dish in triplicate.

Quarter strength of ringers' salt solution was prepared and dispensed in test tubes each 9mls, sterilized and cooled for serial dilution of the sample. Sterile pipette tips were used and 1ml was drawn and dispensed in the first test tube of the prepared diluent making 10^1 dilution. Serial dilution was done up to 10^6 for probiotic counts. The spread plate technique was used for the serial diluted samples. Plates were incubated in an upside-down position at 37°C for 48 hours, after which colonies were enumerated and the results obtained indicated as colony-forming units per milliliter (CFU/ml).

$\text{CFU/ml} = \text{No of colonies} \times \text{Dilution factor} / \text{Volume of Sample}$

3.7.2 Determination of the microbiological quality of the product

Total coliform were assessed using violet red bile glucose agar (VRBGA), plate count agar (PCA) for Total Plate Count (TPC) and yeasts and molds using Sabouraud Dextrose Agar (SDA) as described by Buchanan et al., (1998). Media was prepared, sterilized, and aseptically poured on a sterile plate placed on a clean bench; sample caps were wiped with 75% ethanol before opening to avoid contamination. The spread plate method was used followed by incubation at 37°C for TPC (24 hours), coliform (24 hours) and yeast and molds were incubated for five days at 25°C . The number of CFU/ml was calculated considering the dilutions. The analysis was carried out in triplicate.

3.8 Nutritional quality analysis of the probiotic fermented mango juice

3.8.1 Determination of glucose, fructose and sucrose content

Selected sugars were analyzed for their content in the juice. Sugars were determined using High-Performance Liquid Chromatography (UFLC 20A series Shimadzu) HPLC method as described by Gancedo and Luh, (1986). The mango juice (5ml) was refluxed in 20ml ethanol for one hour. The extract was strained through a filter paper,

concentrated using a rotary evaporator and diluted with 5ml of 50% acetonitrile. The extract and the standard solution were introduced into HPLC equipped with a Refractive Index detector under the following conditions: 350⁰C (oven temperature), 0.8ml/min (flow rate), 20uL (sample volume injected) and column-NH₂P-50E (250mm × 4.6mm × 0.5μl) was used to detect fructose, glucose and sucrose. The mobile phase used in the current study was 75% acetonitrile. Standard curve of peak area (y-axis) against concentration (x-axis) were drawn using standard sugar solution and the amount of sugar present in the sample was quantified.

3.8.2 Determination of β-carotene content

β-carotene content was determined using a modified UV-Vis spectrophotometer (Shimadzu 1800) as described by Heinonen, (2002). β-carotene is light liable, thus the experiment was carried out in a dark room. A spatula full of hyflosuperel (celite) to aid in grinding (frictional grist) was added to 5ml of the sample and extraction was done using 25ml of acetone until the residue becomes colorless. Partitioned with 25ml petroleum ether in a separating funnel of 250ml capacity.

The acetone extract was added to the separating funnel. Acetone was removed by slowly adding distilled water to the wall of the separating funnel. The two phases were allowed to separate (2-3 minutes). The lower phase was removed, discarded, and then washed with distilled water (three times) until acetone was completely removed. The carotenoids were now in the petroleum spirit fraction. A conical flask was used to collect the petroleum spirit layer containing the carotenoids through a funnel with Anhydrous Na₂SO₄ then topped up to 50ml using pet-ether. At this point, β-carotene content was measured at 440 nm using a UV-Vis spectrophotometer and the absorbance obtained was used to calculate β-carotene content. Stock solution of β-carotene at 100ug/ml was used

to make a working standard solutions with different concentrations (0.5, 1.0, 2.0, 4.0, 5.0 µg/ml β- carotene). Absorbance values of the solutions were read at 440 nm and a standard curve of absorbance against concentration was plotted.

3.8.3 Determination of Ascorbic Acid content

HPLC method was used to determine ascorbic acid as described by Vikram et al., (2005). Approximately 10mls of the sample were weighed and extracted with 20mls metaphosphoric acid (0.8%), centrifuged (10000 rpm), passed through a filter (0.45 µL) and 20 µL administered into the HPLC machine. Ascorbic acid standards of different concentrations were prepared to plot a calibration curve. The ascorbic acid was determined using a Shimadzu UV-VIS detector (C₁₈ 250mm × 4.6mm× 0.5µl). The HPLC machine used a mobile phase (0.8% metaphosphoric acid), flow rate (1.2 mL/min) and wavelength (266 nm).

3.9 Determination of shelf-life stability

Physicochemical, nutritional, probiotic viability and microbial quality tests were conducted weekly for four weeks (28 days at 4⁰C) during storage to determine the shelf stability of the three most preferred formulated products. The analysis was done only for the final products selected based on sensory acceptability trials.

3.10 Quality control, validity and reliability

All the experiments were conducted in triplicates. Portable distilled water was used during processing, good manufacturing practices were followed to minimize contamination during processing. Calibrated machines were used for weighing and measuring samples; all the apparatus were cleaned using a detergent and distilled water,

rinsed severally and then oven-dried at 80⁰C to minimize contamination. Analytical grade reagents were used for nutrient analysis. Standards for vitamin A (Appendix D), vitamin C (Appendix C) and sugars (Appendix E) were run before the sample for reproducible and reliable results. For microbial analysis, aseptic techniques were assured. Validated questionnaires for sensory analysis were used (Singh-Ackbarali and Maharaj, 2014).

3.11 Statistical analysis

The means and standard deviation were calculated using Genstat programme, 14th Edition by comparing the means for control and fermented mango juice at a 5% level of significance using Analysis of Variance (ANOVA) and Duncan's Multiple Range Test (DMRT). Data presented using tables and graphs.

3.12 Logistical considerations and ethical clearance

Authority to conduct the research was given by Graduate School of Kenyatta University (Appendix H). Ethical clearance was obtained from Kenyatta University Ethical Review Committee (KUERC) (Appendix G) while a research permit was obtained from the National Council for Science, Technology and Innovation (NACOSTI/P/21/9778) (Appendix F). Informed consent was sought from research participants (Appendix A). Participation was voluntary and confidentiality of the information obtained from the participants was ensured.

CHAPTER FOUR: RESULTS

4.1 Formulation of probiotic fermented mango juice

The formulated mango juice was subjected to sensory evaluation as presented in Table 4.1. Sensory acceptability scores of the four formulations exhibited a significant difference ($P < 0.05$) in the consumer liking of most sensory attributes; color, consistency, flavor and overall acceptability in 25:75, 50:50, 75:25, and 100:0 ratios. The 50:50 formulation had the highest overall mean acceptability score of 7.44 ± 0.03 (like moderately) according to the hedonic scale ranking. The least accepted formulation was the 25:75 formulation with an overall mean acceptability score of 2.44 ± 0.08 (dislike very much). Mango juice 25:75 formulation was significantly different ($p < 0.05$) from the other formulations in color, flavor and consistency. The consistency of the 50:50 formulation was ranked the highest (7.79) while the 25:75 formulation was ranked lowest (5.11). The results showed no significant difference in consistency and flavor ($P > 0.05$) between 100:0 and 75:25 formulations. The panelist evaluation further indicated that 100:0 and 75:25 formulations were too viscous while the 25:72 formulation was too watery.

Table 4.1 Sensory evaluation of the four formulations of unfermented mango juice

Mango pulp to water ratio	Consistency	Color	Taste	Flavor	Overall Acceptability
25:75	5.11 ± 0.05^c	$6.83 \pm 0.04_b$	7.33 ± 0.03^a	6.44 ± 0.1^b	2.44 ± 0.08^c
50:50	7.79 ± 0.02^a	$7.72 \pm 0.08_a$	7.66 ± 0.02^a	7.66 ± 0.01^a	7.44 ± 0.03^a
75:25	6.33 ± 0.02^b	$7.56 \pm 0.04_a$	7.42 ± 0.03^a	7.75 ± 0.02^a	6.22 ± 0.08^b
100:0	6.44 ± 0.01^b	$7.78 \pm 0.03_a$	7.44 ± 0.03^a	7.74 ± 0.01^a	6.44 ± 0.07^b

Values are means of three replicates (\pm standard deviation). Means with different superscript letters in the same column were significantly different ($P < 0.05$) according to Duncans Multiple Range Test (DMRT)

N/B: 25:75-25 parts of pulp 75 parts of water; 50:50-50 parts of pulp 50 parts of water; 75:25-75 parts of pulp 25 parts of water.

4.2 Physicochemical characteristics of the formulated probiotic fermented mango juice

The changes in Physicochemical properties for the four formulations (25:75, 50:50, 75:25 and 100:0) after fermentation of the pasteurized mango juice with the different cultures Abt-5[®] culture (*Streptococcus thermophilus*, *Bifidobacterium*, and *Lactobacillus acidophilus*), Fiti[®] (*Lactobacillus rhamnosus*) and Yo-mix[®] (*Streptococcus thermophilus* and *Lactobacillus delbruiikii ssp bulgaricus*) at different time intervals (0, 12, 18, 24, 36, 48 and 36 hours) are presented in Figures 4.1 to 4.9. This was conducted to establish the best-fermented formulation.

4.2.1 Changes in Total Titratable acidity (TTA) during fermentation

The change in TTA during fermentation for the three formulations was monitored in Abt-5[®], Fiti[®] and Yo-mix[®] cultures. The results findings are presented in Figures 4.1 to 4.3. The three mango juice formulations (25:75, 50:50 and 75:25) fermented with Abt-5[®], Fiti[®] and Yo-mix[®] cultures showed an increase in TTA with fermentation time. The TTA (%) increased from 0.15 to 0.32%; 0.31 to 0.69, and 0.5 to 0.65(%) for 25:75, 50:50 and 75:25 mango juice formulations respectively. The highest acidity was observed in the 50:50 formulation (Figure 4.2) fermented with Fiti[®] culture after fermentation for 72 hours.

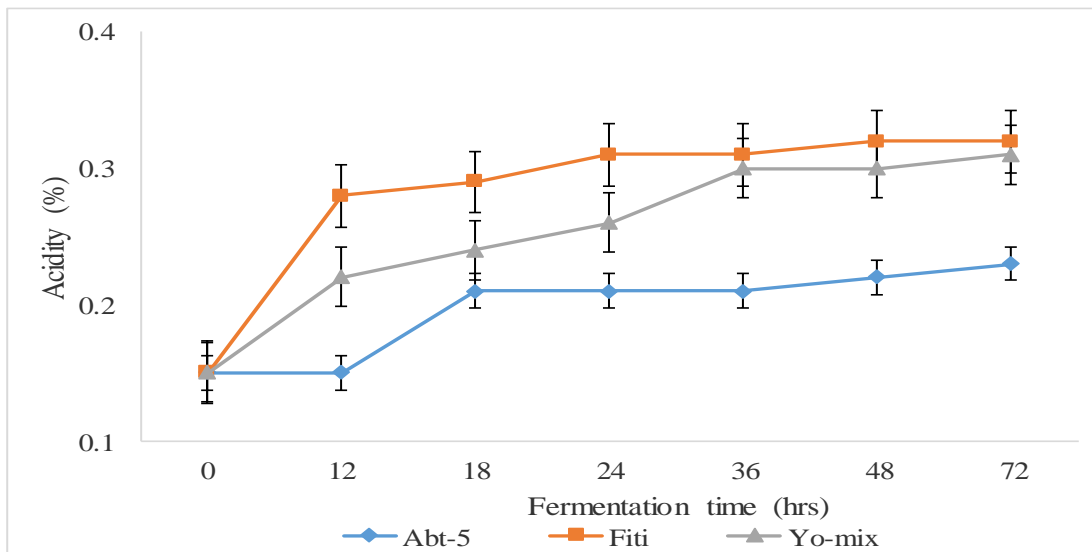


Figure 4.1: Change in total titratable acidity with fermentation in 25:75 mango juice formulation

Abt-5[®] (*Streptococcus thermophilus*, *Bifidobacterium*, and *Lactobacillus acidophilus*), *Fiti*[®] (*Lactobacillus rhamnosus*) and *Yo-mix*[®] culture (*Streptococcus thermophilus* and *Lactobacillus delbruiikii ssp bulgaricus*)

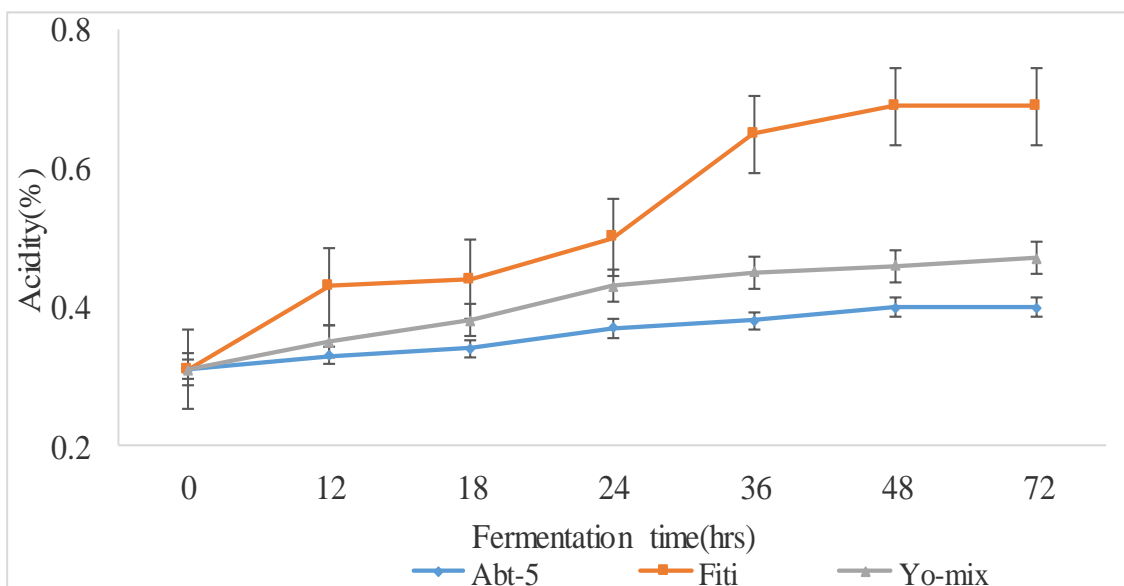


Figure 4.2: Change in total titratable acidity with fermentation in 50:50 mango juice formulation

Abt-5[®] (*Streptococcus thermophilus*, *Bifidobacterium*, and *Lactobacillus acidophilus*), *Fiti*[®] (*Lactobacillus rhamnosus*) and *Yo-mix*[®] culture (*Streptococcus thermophilus* and *Lactobacillus delbruiikii ssp bulgaricus*)

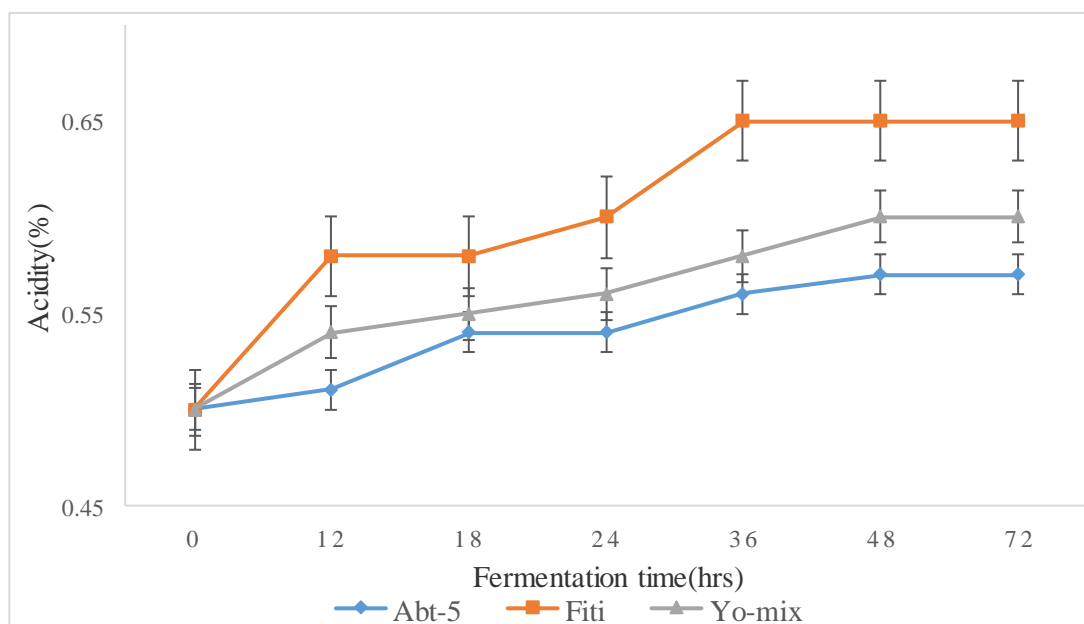


Figure 4.3: Change in total titratable acidity with fermentation in 75:25 mango juice formulation

Abt-5[®] (*Streptococcus thermophilus*, *Bifidobacterium*, and *Lactobacillus acidophilus*), *Fiti*[®] (*Lactobacillus rhamnosus*) and *Yo-mix*[®] culture (*Streptococcus thermophilus* and *Lactobacillus delbruiikii ssp bulgaricus*)

4.2.2 Changes in pH levels during fermentation

The pH levels change in mango juice fermented by *Abt-5*[®], *Fiti*[®] and *Yo-mix*[®] starter cultures at 42⁰C for 72 hours are presented in Figures 4.4 to 4.6. Overall, the pH decreased for all formulations during fermentation. Generally, the rate of pH decrease in *Fiti*[®] and *Yo-mix*[®] culture was highest within 0-12 hours, after which the rate was gradual. pH reduction was lowest in *Abt-5*[®] culture for all formulations. The pH decreased from 4.4 to 4.4 in 25:75 formulation; 4.4 to 3.2 in 50:50 formulation, and 4.4 to 3.8 in 75:25 formulation. The highest decrease was observed in 50:50 mango juice formulation fermented with *Fiti*[®] culture (3.2) compared to control (4.4). However, the least pH change was observed in sample 25:75 formulation fermented with *Abt-5*[®] culture (4.3) with slight pH change after 72 hours.

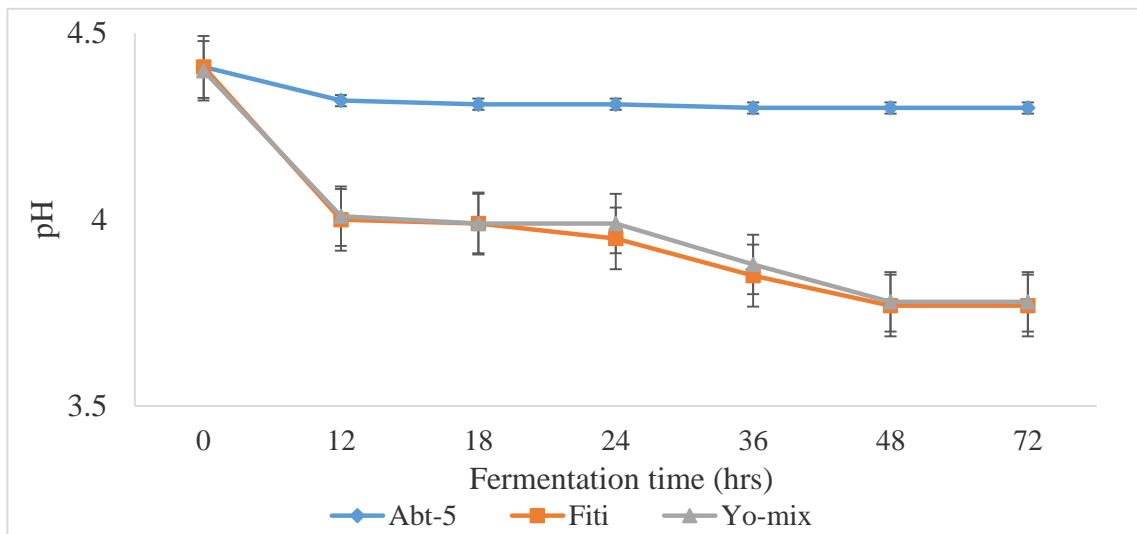
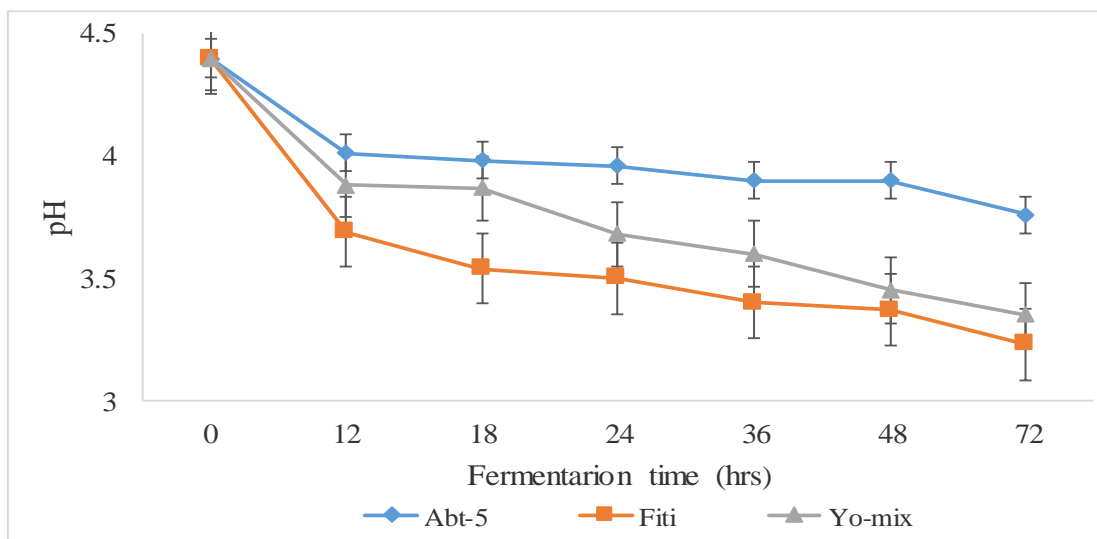


Figure 4.4 : Change in pH levels with fermentation in 25:75 mango juice formulation

Abt-5[®] (*Streptococcus thermophilus*, *Bifidobacterium*, and *Lactobacillus acidophilus*), *Fiti*[®] (*Lactobacillus rhamnosus*) and *Yo-mix*[®] culture (*Streptococcus thermophilus* and *Lactobacillus delbruiikii ssp bulgaricus*)



4.5 Change in pH levels with fermentation in 50:50 mango juice formulation

Abt-5[®] (*Streptococcus thermophilus*, *Bifidobacterium*, and *Lactobacillus acidophilus*), *Fiti*[®] (*Lactobacillus rhamnosus*) and *Yo-mix*[®] culture (*Streptococcus thermophilus* and *Lactobacillus delbruiikii ssp bulgaricus*)

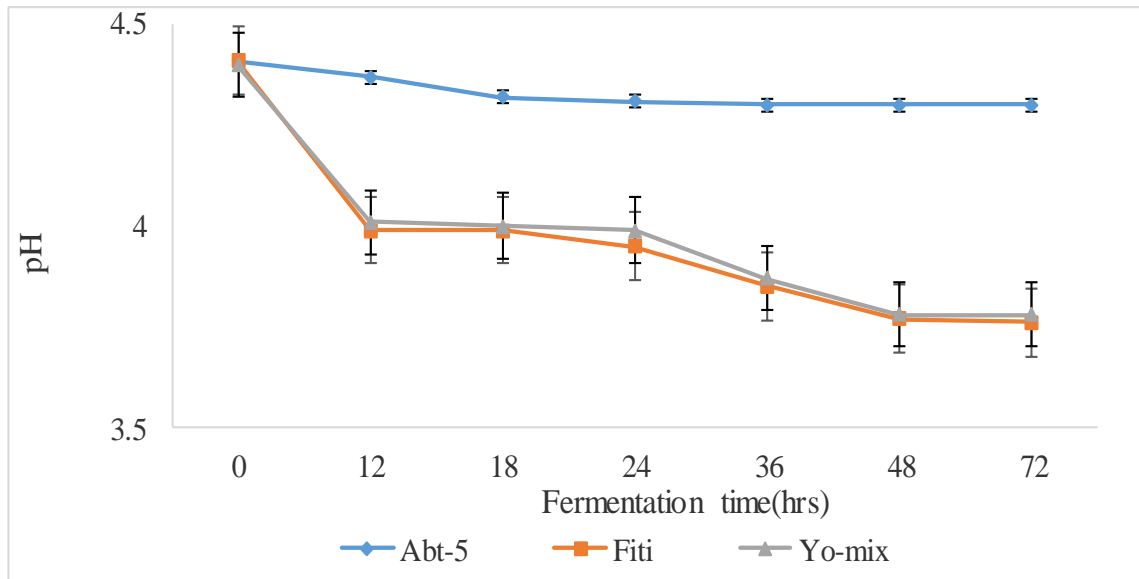


Figure 4.6: Change in pH levels with fermentation in 75:25 mango juice formulation

Abt-5[®] (*Streptococcus thermophilus*, *Bifidobacterium*, and *Lactobacillus acidophilus*), *Fiti*[®] (*Lactobacillus rhamnosus*) and *Yo-mix*[®] culture (*Streptococcus thermophilus* and *Lactobacillus delbruiikii ssp bulgaricus*)

4.2.3 Change in Total Soluble Solids (TSS)

The trends of TSS reduction was similar to decrease in pH. However, in the current study, the rate of substrate utilization and production of metabolites was not investigated. The decrease in TSS was observed in the three formulations fermented by *Abt-5*[®], *Fiti*[®] and *Yo-mix*[®] cultures and the results are as presented in Figures 4.7 to 4.9. Total soluble solids decreased from 17 to 14.47 °brix within 72 hours with a rapid drop observed in 50:50 mango juice fermented by *Fiti*[®] culture. However, a slight decrease occurred in 75:25 formulation (17 to 16.33 °brix) fermented using *Abt-5*[®] culture after 72 hours of fermentation.

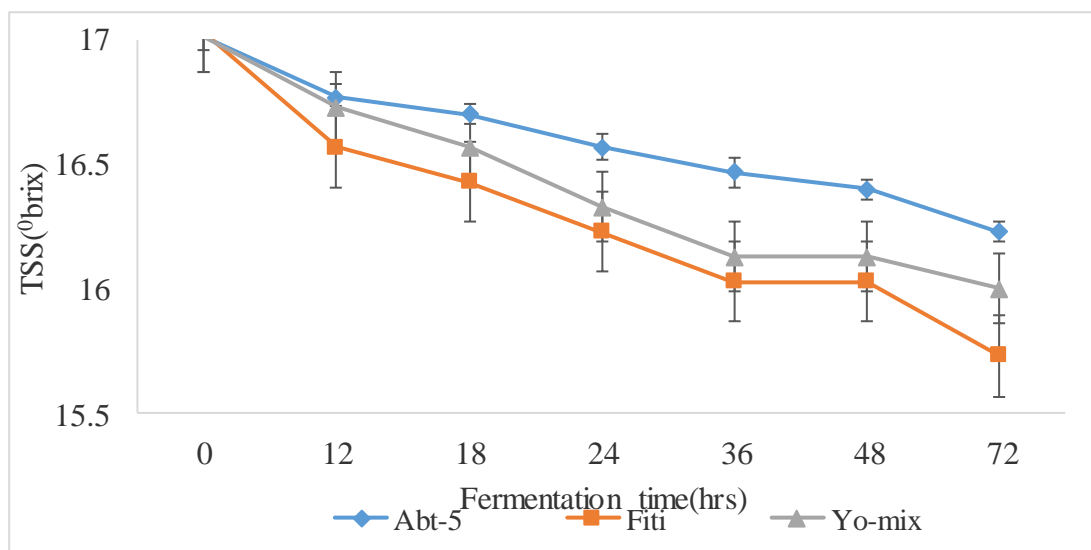


Figure 4.7: Change in TSS with fermentation in 25:75 mango juice formulation

Abt-5[®] (*Streptococcus thermophilus*, *Bifidobacterium*, and *Lactobacillus acidophilus*), *Fiti*[®] (*Lactobacillus rhamnosus*) and *Yo-mix*[®] culture (*Streptococcus thermophilus* and *Lactobacillus delbruiikii ssp bulgaricus*)

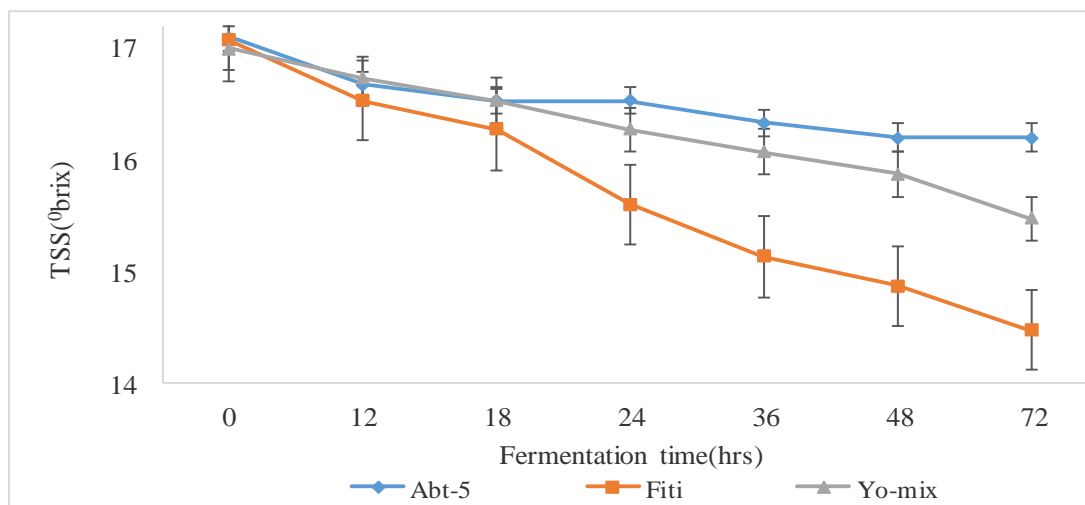


Figure 4.8: Change in TSS with fermentation in 50:50 mango juice formulation

Abt-5[®] (*Streptococcus thermophilus*, *Bifidobacterium*, and *Lactobacillus acidophilus*), *Fiti*[®] (*Lactobacillus rhamnosus*) and *Yo-mix*[®] culture (*Streptococcus thermophilus* and *Lactobacillus delbruiikii ssp bulgaricus*)

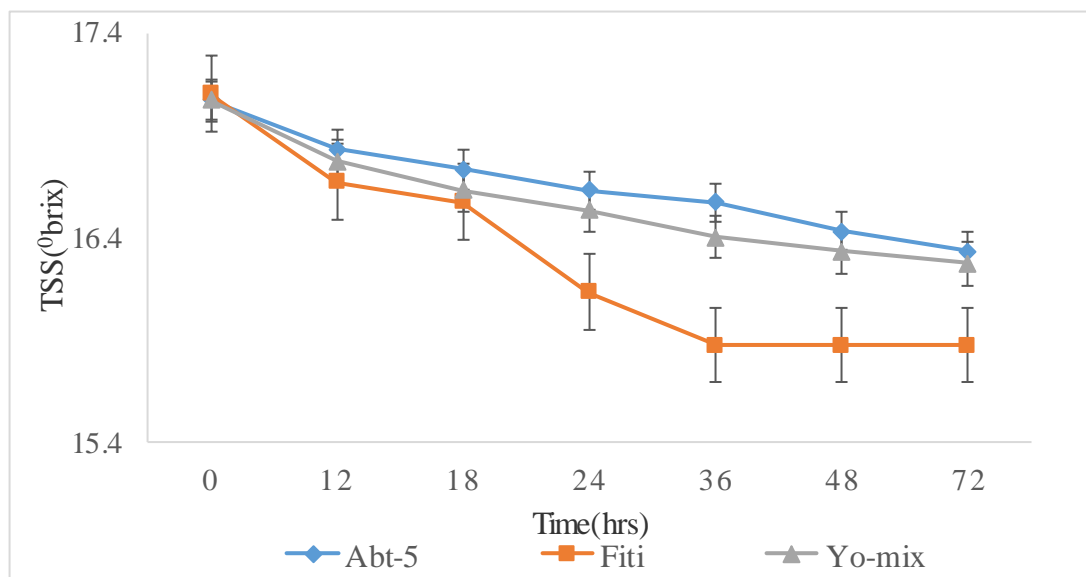


Figure 4.9: Change in TSS with fermentation in 75:25 mango juice formulation

Abt-5[®] (*Streptococcus thermophilus*, *Bifidobacterium*, and *Lactobacillus acidophilus*), *Fiti*[®] (*Lactobacillus rhamnosus*) and *Yo-mix*[®] culture (*Streptococcus thermophilus* and *Lactobacillus delbruiikii ssp bulgaricus*)

The 50:50 formulation was finally selected for further testing (nutrient content, culture viability and microbial quality) due to its superior qualities in overall sensory acceptability and physicochemical characteristics.

4.3 Starter culture activity in fermented mango juice

4.3.1 Pre-inoculum (mother culture) activity for *Abt-5*[®], *Fiti*[®] and *Yo-mix*[®] cultures

The starter culture activity of the 50:50 sample was determined after 24 hours to confirm their presence and activity behavior before inoculating them in the preceding samples (Figure 4.10). The cell population in all cultures increased within 24 hours with the highest count being observed in *Fiti*[®] culture (\log_{10} 8.9 CFU/ml) followed by *Yo-mix*[®] culture (\log_{10} 7.7 CFU/ml) and the lowest count recorded in *Abt-5*[®] culture (\log_{10} 4.5 CFU/ml).

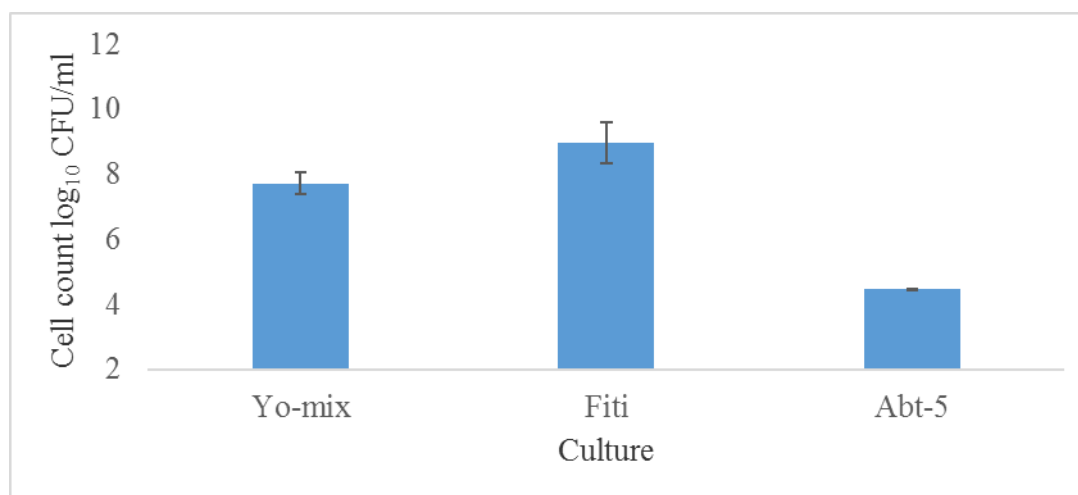


Figure 4.10: Activity of the probiotic cultures in mango juice

Abt-5[®] (*Streptococcus thermophilus*, *Bifidobacterium*, and *Lactobacillus acidophilus*), *Fiti*[®] (*Lactobacillus rhamnosus*) and *Yo-mix*[®] culture (*Streptococcus thermophilus* and *Lactobacillus delbruiikii ssp bulgaricus*)

4.3.2 Cell viability of the final product (*Abt-5*[®], *Fiti*[®], and *Yo-mix*[®]) during fermentation

The cell viability of the three probiotic cultures was determined for the 50:50 mango juice formulation and results were recorded as indicated in Figure 4.11. Immediately after inoculation the cell count for the three mixed cultures ranged from log₁₀ 3.32 CFU/ml to log₁₀ 6.73 CFU/ml despite using the same volume of inoculant. *Fiti*[®] culture showed the highest viable cell count. Slow multiplication was observed within the first 18 hours of the fermentation process. During fermentation, a gradual increase in cell count was observed after 18 hours for the *Fiti*[®] culture (log₁₀ 6.73 CFU/ml to log₁₀ 9.14 CFU/ml) with optimal growth attained within 48 hours of the fermentation process. The same trend was observed in *Yo-mix*[®] culture (log₁₀ 5.25 CFU/ml to log₁₀ 8.2 CFU/ml) with optimal growth attained within 48 hours.

The lowest cell growth was observed in *Abt-5*[®] starter culture with a maximum count

(log₁₀ 5.4 CFU/ml) attained within 48 hours of fermentation lower than the recommended cell count, followed by a sharp decline. Fiti[®] was the only culture that achieved the minimum allowable limit after 12 hours. In addition, mango juice fermented using Fiti and Yo-mix starter cultures attained high viable cell count and were the best-fermented samples. Consequently, the samples were subjected to further nutritional quality analysis, microbial quality, and sensory evaluation tests.

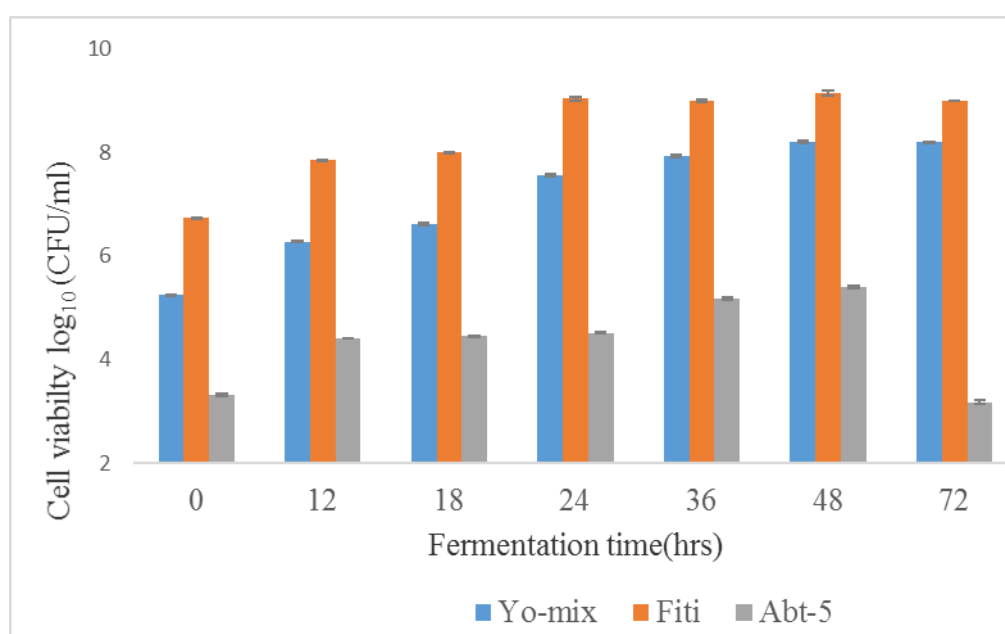


Figure 4.11: Viability of the probiotic cultures

Abt-5[®] (*Streptococcus thermophilus*, *Bifidobacterium*, and *Lactobacillus acidophilus*), *Fiti[®]* (*Lactobacillus rhamnosus*) and *Yo-mix[®]* culture (*Streptococcus thermophilus* and *Lactobacillus delbruiikii ssp bulgaricus*)

4.4 Probiotic viability of the fermented mango juice during storage at 4⁰C

Cell viability of the fermented mango juice by Fiti[®] and Yo-mix[®] culture during storage at 4⁰C was explored and the results are shown in Table 4.2. The results revealed a decreasing trend in cell viability for both cultures during the four-week storage. The viable cell count for fermented mango juice using Fiti[®] starter culture was above the

recommended values during storage (10^6 CFU/ml). The levels gradually decreased with storage in mango juice fermented for 24 and 48 hours to $6.8\pm 0.1\times 10^8$ and 5.5×10^7 CFU/ml, respectively. However, cell viability for Fiti[®] starter culture remained high (above 10^6 CFU/ml) within the four weeks of storage. The Yo-mix[®] starter culture viability decreased to unacceptable levels after one week and attained a minimum of 1.2×10^4 (Y48) and 2.2×10^3 (Y24) CFU/ml within four weeks of storage.

Table 4.2 Culture viability of fermented mango juice during storage

Storage Time (weeks)	Fiti [®] (F24) (CFU/ml)	Fiti [®] (F48) (CFU/ml)	Yo-mix [®] (Y24) (CFU/ml)	Yo-mix [®] (Y48) (CFU/ml)
0	$1.1\pm 0.05\times 10^9$	$1.2\pm 0.08\times 10^9$	$5.7\pm 0.12\times 10^7$	$1.6\pm 0.05\times 10^8$
1	$1.2\pm 0.01\times 10^9$	$1.2\pm 0.05\times 10^9$	$3.1\pm 0.02\times 10^5$	$1.2\pm 0.12\times 10^7$
2	$1.2\pm 0.08\times 10^9$	$7.8\pm 0.05\times 10^8$	$9.0\pm 0.07\times 10^4$	$4.2\pm 0.17\times 10^6$
3	$9.3\pm 0.08\times 10^8$	$9.6\pm 0.09\times 10^7$	$3.1\pm 0.12\times 10^4$	$1.7\pm 0.12\times 10^5$
4	$6.8\pm 0.1\times 10^8$	$5.5\pm 0.12\times 10^7$	$2.2\pm 0.03\times 10^3$	$1.2\pm 0.08\times 10^4$

F24 - Fiti culture fermented for 24 hours; *F48* - Fiti culture fermented for 48 hours; *Y24* - Yo-mix culture fermented for 24 hours; *Y48* - Yo-mix culture fermented for 48 hours

4.4.1 Product microbial quality during processing

The microbial analysis of the pasteurized juice and fermented probiotic mango juice was done to ascertain the quality of the fermented product to ensure final product safety for the consumers (Table 4.3). The control's total plate count (TPC) was 1.5×10^2 CFU/ml, lower than the KEBS recommended levels of 10^3 CFU/ml. The yeast and molds present in both pasteurized (control) and probiotic fermented mango samples were 1.0×10^1 CFU/ml which is also less than 3.0×10^1 CFU/ml for fresh fruit juices thus, the product microbial quality was within the acceptable range (©KEBS, 2016). There were no coliforms, detected in the control and formulated products.

Table 4.3 Microbial quality of the formulated fermented mango juice

Sample	Total Plate Count (CFU/ml)	Yeast and molds (CFU/ml)	Total coliforms (CFU/ml)
Control (PJ)	1.5×10^2	1.0×10^1	ND
Fiti [®] (F24)	-	1.0×10^1	ND
Fiti [®] (F48)	-	1.0×10^1	ND
Yo-mix [®] (F24)	-	1.0×10^1	ND
Yo-mix [®] (Y48)	-	1.0×10^1	ND
KEBS allowed limits for fruit juices	1.0×10^3	3.0×10^1	NIL

PJ-PJ; Pasteurized Juice; F24- Fiti[®] culture for 24 hours; F48- Fiti[®] culture for 48 hours; Y24 - Yo-mix[®] culture fermented for 24 hours; Y48- Yo-mix[®] culture for 48 hours; ND-Not Detected; CFU/ml-Colony forming units per milliliter.

4.5 Sensory acceptability of the control and fermented probiotic mango juice

Sensory evaluation was performed to examine the three fermented probiotic mango juice acceptability to consumers against the control (pasteurized juice); appearance, color, mouthfeel, taste, flavor and overall acceptability (Table 4.4). This test is key in showing us how the product will fare on in the market. The sensory results revealed that the appearance and color of the fermented samples did not change significantly ($P > 0.05$); however, mouthfeel, taste, flavor and general acceptability varied significantly compared to the control. Mango juice fermented using Fiti[®] culture for 24 hours had the highest sensory mean score in taste (8.37 ± 0.14), followed by the juice sample fermented using Fiti[®] culture for 48 hours which was not significantly different ($P > 0.05$) from each other. However, the most preferred juice in overall acceptability was Fiti[®] culture fermented for 48 hours, followed by mango juice fermented with Fiti[®] culture for 24 hours.

Table 4.4 Sensory acceptability of probiotic fermented mango juice during processing

Sample	Appearance	Color	Mouth feel	Taste	Flavor	Overall acceptability
Control(PJ)	8.10±0.01 ^a	8.05±0.05 ^a	6.00±0.05 ^b	7.56±0.12 ^b	7.60±0.01 ^f	7.33±0.24 ^b
Fiti [®] (F24)	8.05±0.05 ^a	8.03±0.05 ^a	7.85±0.09 ^a	8.37±0.14 ^a	8.10±0.12 ^{cd}	8.17±0.09 ^a
Yo-mix [®] (Y24)	8.03±0.03 ^a	8.03±0.01 ^a	7.76±0.02 ^a	8.30±0.06 ^a	8.09±0.04 ^{cd}	7.98±0.03 ^a
Fiti [®] (F48)	8.10±0.14 ^a	8.03±0.05 ^a	7.97±0.2 ^a	8.27±0.33 ^a	8.57±0.05 ^a	8.23±0.20 ^a
Yo-mix [®] (Y48)	8.05±0.01 ^a	8.00±0.01 ^a	7.75±0.22 ^a	8.20±0.12 ^a	8.10±0.09 ^{cd}	8.00±0.01 ^a

Means with different superscript letters in the same column are significantly different at $P < 0.05$; (n=3); F24: mango juice fermented by Fiti[®] culture for 24 hours; F48: mango juice fermented by Fiti[®] culture for 48 hours; Y48: mango juice fermented by Yo-mix[®] culture for 48 hours;

4.6 Changes in vitamin C and β -carotene content during the processing of fermented mango juice

Vitamins present in a food product are important as they indicate product nutritional quality. They are essential nutrients for the probiotic bacteria proliferation. The vitamin C and β -carotene content in pasteurized and probiotic fermented mango juice are as presented in Table 4.5. Vitamin C content decreased with an increase in fermentation time. There was a significant difference at $P < 0.05$ in vitamin C content between control and fermented samples. Control juice had the highest vitamin C retention of 80.24%. The lowest vitamin C retention (68.09%) was recorded in Yo-mix[®] culture fermented for 48 hours. Vitamin C content in mango juice fermented using Fiti[®] and Yo-mix[®] cultures for 48 hours was not significantly different. β -carotene content slightly decreased after pasteurization but increased significantly on fermenting the juice, with the highest β -carotene increase observed in mango juice fermented using Fiti[®] culture for 48 hours

(108.0%). Overall, β -carotene was found to differ significantly in all the various samples studied (F24, F48 and Y48) at $P < 0.05$.

Table 4.5 Change in vitamin C and β -carotene content in fermented mango juice

Sample	Vitamin C content (mg/100ml)	β -carotene content (mg/100ml)
Fresh juice (FJ)	56.01 \pm 0.01 ^a (100%)	1.13 \pm 0.06 ^c (100%)
Control (PJ)	45.04 \pm 0.05 ^b (80.24%)	1.12 \pm 0.01 ^c (99.1%)
Fiti [®] (F24)	40.32 \pm 0.5 ^c (72%)	1.17 \pm 0.01 ^b (103.5%)
Fiti [®] (F48)	38.20 \pm 0.43 ^d (68.21%)	1.23 \pm 0.01 ^a (108.1%)
Yo-mix [®] (Y48)	38.13 \pm 0.44 ^c (68.09%)	1.17 \pm 0.01 ^b (103.5%)

Values are means of three replicates (\pm standard deviation). Means with different superscript letters across the column are significantly different ($P < 0.05$) according to Duncans multiple Range Test (DMRT); FJ: Fresh juice; PJ-Pasteurized Juice; F24-Fiti[®] culture for 24 hours; F48- Fiti[®] culture for 48 hours; Y48- Yo-mix[®] culture for 48 hours.

4.7 Change in selected sugars during the processing of mango juice

Mango fruit contains sugars such as glucose, fructose and sucrose. Changes in sugars during fermentation are presented in Table 4.6. There was a decreasing trend in sugar content in the studied samples, each varied significantly ($P < 0.05$). Sucrose content was the highest among the three sugars, followed by fructose and glucose. The highest percentage decrease was observed for glucose in Fiti[®] fermented mango juice for 48 hours, followed by fructose and sucrose, 40.9%, 40.5% and 26.9%, respectively. Fructose content ranged from 5.29.35 \pm 0.29 to 3.15 \pm 0.06 mg/ml. The highest glucose content was recorded in the pasteurized juice (1.17 \pm 0.08mg/ml), while the lowest glucose content of 1.01 \pm 0.03mg/ml was recorded in the juice fermented using Fiti[®] culture for 48 hours. A significant decrease ($P < 0.05$) in sucrose content was observed after fermentation (control and fermented juice) from 25.34 \pm 0.57 to 18.53 \pm 0.31mg/ml.

Table 4.6 Change in selected sugars during processing of fermented mango juice

Sample	Fructose (Mg/ml)	Glucose (Mg/ml)	Sucrose (Mg/ml)
Control (PJ)	5.29±0.29 ^a	1.71±0.02 ^a	25.34±0.57 ^a
Fiti [®] (F24)	3.40±0.05 ^b	1.27±0.02 ^b	21.60±0.18 ^b
Fiti [®] (F48)	3.15±0.06 ^d	1.01±0.03 ^d	18.53±0.31 ^d
Yo-mix [®] (Y48)	3.25±0.09 ^c	1.15±0.31 ^c	20.51±0.31 ^c

Values are means of three replicates (± standard deviation). Means with different superscript letters across the column are significantly different (P<0.05) according to Duncans multiple Range Test (DMRT); FJ: Fresh juice; PJ-Pasteurized Juice; F24-Fiti[®] culture for 24 hours; F48- Fiti[®] culture for 48 hours; Y48- Yo-mix[®] culture for 48 hours

4.8 Shelf stability of the control and probiotic fermented mango juice

4.8.1 Change in Physicochemical characteristics and nutrient content of the control and probiotic fermented mango juice during storage

Physicochemical characteristics (pH, TTA and TSS) and changes in nutrient content (Beta carotene, vitamin C, and sugars) of the probiotic fermented mango juice, were investigated and the results are presented in Table 4.7. The results manifested a significant decrease in pH in all juice samples studied except for the control juice, which was not significantly different (P>0.05) during the four weeks of storage. Control recorded the highest pH (4.4), significantly different from the fermented samples. The least pH (3.30) was recorded in mango juice fermented using Fiti[®] culture for 48 hours during the fourth week of the study period. A slight increase in total titratable acidity was observed in fermented samples as opposed to the control with the highest TTA (0.69%) observed in mango juice fermented using Fiti[®] culture for 48 hours. The increase in acidity was not significantly different in the first two weeks of storage within the same samples. The highest TSS (17⁰brix) was recorded in the control sample and the least TSS (14.67⁰ Brix) in mango juice fermented using Fiti[®] culture for 48 hours. There

was no significant difference in TSS in the pasteurized sample during storage, whereas the TSS of the fermented samples further decreased significantly after the third week of storage.

Table 4.7 Change of physicochemical characteristics of the fermented mango juice during storage.

Sample	Storage period (Weeks)	pH	TTA (%)	TSS (⁰ brix)
Control (PJ)	W0	4.4±0.01 ^a	0.31±0.04 ^j	17.03±0.08 ^a
	W1	4.4±0.01 ^a	0.31±0.01 ^j	17.03±0.05 ^a
	W2	4.4±0.16 ^a	0.31±0.01 ^j	17.00±0.16 ^a
	W3	4.4±0.01 ^a	0.32±0.01 ^j	16.93±0.01 ^a
	W4	4.4±0.01 ^a	0.32±0.01 ^j	17.00±0.12 ^a
Fiti[®] (F24)	W0	3.54±0.01 ^b	0.50±0.01 ^f	16.10±0.08 ^b
	W1	3.54±0.01 ^b	0.50±0.01 ^f	16.07±0.09 ^b
	W2	3.45±0.01 ^d	0.50±0.01 ^f	16.05±0.01 ^b
	W3	3.43±0.01 ^d	0.52±0.01 ^e	15.80±0.01 ^d
	W4	3.42±0.01 ^{de}	0.53±0.01 ^d	15.60±0.09 ^e
Fiti[®] (F48)	W0	3.37±0.01 ^f	0.69±0.01 ^c	14.87±0.09 ^f
	W1	3.37±0.01 ^f	0.69±0.01 ^c	14.87±0.01 ^f
	W2	3.32±0.08 ^{gh}	0.69±0.01 ^c	14.85±0.08 ^f
	W3	3.31±0.01 ^b	0.70±0.01 ^b	14.67±0.09 ^g
	W4	3.30±0.01 ^h	0.71±0.01 ^a	14.67±0.09 ^g
Yo-mix[®] (Y48)	W0	3.49±0.08 ^c	0.46±0.08 ^h	15.85±0.09 ^d
	W1	3.49±0.09 ^c	0.46±0.01 ^h	15.81±0.09 ^d
	W2	3.38±0.01 ^{ef}	0.46±0.01 ^h	15.80±0.01 ^d
	W3	3.37±0.01 ^f	0.47±0.01 ^g	15.60±0.01 ^e
	W4	3.36±0.01 ^f	0.48±0.01 ^g	15.60±0.01 ^e

Means with different superscript letters in the same column are significantly different at $P < 0.05$; (n=3); PJ: Pasteurized juice; F24: mango juice fermented by Fiti[®] culture for 24 hours; F48: mango juice fermented by Fiti[®] culture for 48 hours; Y48: mango juice fermented by Yo-mix[®] culture for 48 hours; W0: processing date; W1, W2, W3, W4: first, second, third and fourth week of storage.

4.8.2 Change in nutrient content of the control and probiotic fermented mango juice during storage

The three fermented mango fruit juices revealed a gradual increase in beta carotene content during processing and storage. The beta carotene content in control and fermented mango juice ranged between 1.13mg/100m and 1.23mg/100ml during storage. A slight increase was observed during storage, with the highest increase recorded in mango juice fermented using Fiti[®] culture for 48 hours. There was a significant difference ($P<0.05$) in beta carotene content of fermented mango juice stored for different weeks. The four juices demonstrated a loss in vitamin C with storage. They lost 19.58%, 28.00%, 31.79% and 31.99% corresponding to control, mango juice fermented by Fiti[®] culture for 24 and 48 hours and mango fruit juices fermented by Yo-mix[®] culture for 48 hours respectively.

Table 4.8 Change in the nutrient content of the control and probiotic fermented mango juice during storage.

Sample	Storage period (Weeks)	β -Carotene (mg/100ml)	Vitamin C (mg/100ml)
Control (PJ)	W0	1.13±0.01 ^e	45.04±0.05 ^a
	W1	1.13±0.03 ^e	43.59±0.04 ^a
	W2	1.13±0.01 ^e	40.25±0.14 ^b
	W3	1.13±0.06 ^e	38.54±0.09 ^{bc}
	W4	1.13±0.02 ^e	36.38±0.25 ^{de}
Fiti[®] (F24)	W0	1.17±0.01 ^d	40.32±0.4 ^b
	W1	1.21±0.04 ^{ab}	34.95±0.85 ^e
	W2	1.21±0.01 ^{ab}	29.36±1.10 ^{fg}
	W3	1.22±0.03 ^{bc}	28.73±0.37 ^g
	W4	1.22±0.01 ^{bc}	24.32±0.45 ^h
Fiti[®] (F48)	W0	1.23±0.01 ^a	38.20±0.4 ^{cd}
	W1	1.23±0.06 ^a	32.15±0.56 ^f
	W2	1.23±0.01 ^a	30.82±0.57 ^f
	W3	1.23±0.04 ^a	23.69±2.25 ^{hi}
	W4	1.20±0.01 ^{bc}	22.11±1.86 ^{ij}
Yo-mix[®] (Y48)	W0	1.17±0.01 ^d	38.13±0.04 ^{cd}
	W1	1.19±0.08 ^c	31.10±0.69 ^f
	W2	1.20±0.03 ^{bc}	28.86±0.27 ^g
	W3	1.20±0.01 ^{bc}	24.53±1.52 ^h
	W4	1.20±0.02 ^{bc}	24.42±0.24 ^j

PJ: Pasteurized juice; F24: mango juice fermented by Fiti[®] culture for 24 hours; F48: mango juice fermented by Fiti[®] culture for 48 hours; Y48: mango juice fermented by Yo-mix[®] culture for 48 hours; W0: processing date; W1, W2, W3, W4: first, second, third and fourth week of storage.

4.8.3 Change in sugar content of the control and fermented mango juice during storage

Sugar content (glucose, fructose and sucrose) in control and fermented probiotic mango juice samples refrigerated at 4°C were monitored for four weeks of storage and the results are presented in Table 4.9. The three sugars analyzed showed a decreasing trend during the juice fermentation process and storage, where they varied significantly at $P < 0.05$. Sucrose occurred most abundantly of the three sugars, followed by fructose and

lastly, glucose. There was a significant difference in sucrose content between the samples but no significant change was observed with storage. The extent of sucrose reduction was dependent on the culture used. The highest sucrose content of 25.34mg/ml was recorded in control which did not significantly vary ($P>0.05$) during storage. The least sucrose content of 18.30mg/ml was exhibited by mango juice fermented using Fiti culture for 48 hours.

The fructose content of the analyzed samples ranged from 5.29mg/ml to 3.15mg/ml as exhibited by control and mango juice fermented using Fiti[®] culture for 48 hours. There was a significant decrease in fructose content in mango juice fermented using Fiti[®] and Yo-mix[®] culture for 48 hours after the third week of storage. The highest glucose content was recorded in the control sample (1.71mg/ml), while the least occurrence was observed in mango juice fermented with Fiti[®] culture for 48 hours. The glucose content was significantly different in the analyzed samples. It was observed that there was no significant change in glucose and fructose content within the samples after the third week of storage.

Table 4.9 Change of sugars in probiotic fermented mango juice during storage.

Sample	Storage period (Weeks)	Fructose (mg/ml)	Glucose (mg/ml)	Sucrose (mg/ml)
Control (PJ)	W0	5.29±0.29 ^a	1.71±0.02 ^a	25.34±0.57 ^a
	W1	5.26±0.56 ^a	1.70±0.02 ^a	25.33±0.53 ^a
	W2	5.25±0.39 ^a	1.70±0.12 ^a	25.33±0.4 ^a
	W3	5.23±0.16 ^a	1.70±0.49 ^a	25.30±0.47 ^a
	W4	5.23±0.13 ^a	1.70±0.29 ^a	25.29±0.29 ^a
Fiti [®] (F24)	W0	3.40±0.05 ^b	1.27±0.02 ^b	21.60±0.18 ^b
	W1	3.36±0.03 ^b	1.17±0.05 ^c	21.48±0.17 ^b
	W2	3.18±0.34 ^b	1.16±0.24 ^{cd}	21.32±0.50 ^b
	W3	3.18±0.13 ^b	1.16±0.15 ^{cd}	21.32±0.27 ^b
	W4	3.11±0.06 ^{bcd}	1.16±0.05 ^{cd}	21.31±0.19 ^b
Fiti [®] (F48)	W0	3.15±0.06 ^c	1.01±0.03 ^{gh}	18.53±0.31 ^d
	W1	3.15±0.08 ^c	0.97±0.01 ^h	18.36±0.51 ^d
	W2	3.13±0.12 ^d	0.89±0.43 ⁱ	18.31±0.10 ^d
	W3	3.13±0.03 ^d	0.88±0.06 ⁱ	18.30±0.32 ^d
	W4	3.13±0.08 ^d	0.88±0.45 ⁱ	18.30±0.26 ^d
Yo-mix [®] (Y48)	W0	3.25±0.09 ^d	1.15±0.31 ^{de}	20.51±0.31 ^c
	W1	3.23±0.12 ^d	1.12±0.10 ^{cdef}	20.31±0.09 ^c
	W2	3.11±0.22 ^{bcd}	1.08±0.05 ^{defg}	20.30±0.19 ^c
	W3	3.11±0.07 ^{bcd}	1.07±0.10 ^{efg}	20.20±0.16 ^c
	W4	3.10±0.05 ^{bcd}	1.04±0.006 ^{fgh}	20.19±0.28 ^c

Means different superscript letters in the same column are significantly different at $P < 0.05$; ($n=3$); PJ: pasteurized juice; F24: mango juice fermented by Fiti[®] culture for 24 hours; F48: mango juice fermented by Fiti[®] culture for 48 hours; Y48: mango juice fermented by Yo-mix[®] culture for 48 hours; W0: processing date; W1, W2, W3, W4: first, second, third and fourth week of storage.

4.9 Microbial quality of the fermented probiotic mango juice during storage

The microbial quality analysis of the juices was assessed to determine the keeping quality during four weeks of storage at 4⁰C. The pasteurized juice (control) was analyzed for total plate count, yeast and molds and total coliform, while probiotic fermented juices were analyzed for yeast and molds and total coliform. The microbial quality of the fermented probiotic mango juice during storage is shown in Table 4.10. It was observed that the control and probiotic fermented mango juice had no coliform counts after pasteurization and fermentation as well as during the storage period. The total plate

count in pasteurized juice (control) increased slightly during storage. However, the general microbial quality of control juice was within the KEBS set limits. In the fermented mango juice, yeast and molds growth was observed during the third week of storage but were also within the KEBS, (2016) allowable limits of 3.0×10^1 CFU/ml.

Table 4.10 Microbial quality of probiotic fermented mango juice during storage

Time (Weeks)	TPC (CFU/ml)	Yeast & molds (CFU/ml)			Total coliform (CFU/ml)			
		Control (PJ)	F24	F48	Y48	F24	F48	Y48
0	1.5×10^2		1.0×10^1	1.0×10^1	1.0×10^1	ND	ND	ND
1	2.0×10^2		1.0×10^1	1.0×10^1	1.0×10^1	ND	ND	ND
2	2.0×10^2		1.0×10^1	1.0×10^1	2.0×10^1	ND	ND	ND
3	2.5×10^2		1.0×10^1	2.0×10^1	2.0×10^1	ND	ND	ND
4	3.0×10^2		2.0×10^1	2.0×10^1	2.0×10^1	ND	ND	ND
KEBS Standards	1.0×10^3			3.0×10^1			NIL	

PJ-pasteurized Juice; F24- Fiti[®] culture for 24 hours; F48- Fiti[®] culture for 48 hours; Y48- Yo-mix[®] culture for 48 hours; ND-Not Detected

4.10 Sensory evaluation of probiotic fermented mango juice

The sensory evaluation of the control and fermented mango juice was carried out using a 9-point hedonic scale rating weekly for four weeks to establish sensory changes during storage (4°C). The samples were randomly presented to the panelist and the sensory attributes rated were; appearance, color, mouthfeel, taste, flavor and overall acceptability. The results obtained were recorded as shown in Table 4.11. The three fermented mango juices had a superior appearance compared to the control with the highest mean score of 8.10 and the lowest mean score of 7.80 in control. All the mango juices were not significantly different ($P < 0.05$) from the control in appearance. The color of the fermented and unfermented juices (control) changed significantly ($P < 0.05$) after

the first three weeks of storage. The mouthfeel of the control sample was significantly different ($P < 0.05$) from the fermented probiotic mango juices with higher scores observed in fermented mango juice. The mouthfeel scores of probiotic fermented mango juice by the two cultures were not significantly different ($P > 0.05$).

Generally, the taste and flavor of fermented mango juice were superior to the control. The taste of the control sample was not significantly different during storage. According to the organoleptic scores, the taste of fermented mango juice using Fiti® culture for 24 hours changed significantly ($P < 0.05$) after the third week of the study period. A significant change ($P < 0.05$) in taste was observed after the first week of storage in Fiti® and Yo-mix® culture fermented for 48 hours, as shown in the results. Mango juice fermented using Fiti® culture for 24 hours was ranked the best during the first week of the storage with a taste mean score of 8.37.

The flavor of the control did not change significantly ($P > 0.05$) during storage. Among the juice, mango juice fermented using Fiti® culture for 48 hours was ranked the best in flavor with a score of 8.57, whereas the control sample was the least scored (7.50). However, the flavor of fermented probiotic mango juices changed significantly ($P < 0.05$) after the third week of the storage period. The overall acceptability during storage for the fermented juice was higher than the control. However, it decreased significantly ($P < 0.05$) after three weeks of storage. There was no significant difference in the overall acceptability of the fermented mango juice during the first three weeks of storage. Based on the overall acceptability, the most preferred juice by the panelist was the probiotic mango juice fermented using Fiti® culture (F48) whose mean score was 8.23 and the least preferred was control (6.97) at the fourth week of storage. It also showed that the

fermentation influences the product's overall acceptability since in all the parameters tested, the unfermented juice (control) scored the least.

Table 4.11 Sensory characteristics of fermented mango juice during storage at 4⁰C.

Sample	Storage (Weeks)	Appearance	Color	Mouthfeel	Taste	Flavor	Overall acceptability
Control (PJ)	W0	8.1±0.02 ^a	8.0±0.04 ^a	6.0±0.02 ^b	7.5±0.07 ^b	7.6±1.17 ^f	7.3±0.04 ^b
	W1	8.0±0.01 ^a	8.0±0.02 ^a	6.0±0.04 ^b	7.5±0.43 ^b	7.5±0.08 ^f	7.2±0.02 ^b
	W2	8.0±0.03 ^a	8.0±0.08 ^a	5.9±0.12 ^b	7.4±0.21 ^b	7.5±0.06 ^f	7.1±0.12 ^b
	W3	8.0±0.01 ^a	7.9±0.04 ^a	5.9±0.06 ^b	7.4±0.05 ^b	7.5±0.03 ^f	7.0±0.90 ^b
	W4	8.0±0.27 ^a	7.5±0.03 ^b	5.9±0.03 ^b	7.4±0.11 ^b	7.5±0.05 ^f	6.9±0.10 ^b
Fiti [®] (F24)	W0	8.0±0.01 ^a	8.0±0.1 ^a	7.8±0.02 ^a	8.3±0.04 ^a	8.1±0.04 ^{cd}	8.1±0.6 ^a
	W1	8.0±0.06 ^a	8.0±0.01 ^a	7.6±0.11 ^a	8.3±0.32 ^a	8.0±0.01 ^{cd}	8.0±0.02 ^a
	W2	7.9±0.02 ^a	7.9±0.10 ^a	7.4±0.22 ^a	8.2±0.42 ^a	8.0±0.04 ^{cd}	7.9±0.32 ^a
	W3	7.9±0.04 ^a	7.9±0.07 ^a	7.2±0.46 ^a	8.2±0.25 ^a	8.0±0.03 ^{cd}	7.9±0.2 ^a
	W4	7.90±0.12 ^a	7.4±0.02 ^d	7.1±0.36 ^a	8.0±0.43 ^{ab}	7.7±0.02 ^{def}	7.3±0.34 ^b
Fiti [®] (F48)	W0	8.0±0.36 ^a	8.0±0.02 ^a	7.9±0.06 ^a	8.2±0.01 ^a	8.5±0.90 ^a	8.2±0.03 ^a
	W1	8.0±0.50 ^a	8.0±0.02 ^a	7.7±0.09 ^a	8.2±0.12 ^a	8.5±0.12 ^a	8.0±0.06 ^a
	W2	7.9±0.10 ^a	7.9±0.08 ^a	7.4±0.10 ^a	8.0±0.08 ^{ab}	8.5±0.82 ^a	7.9±0.04 ^a
	W3	7.8±0.40 ^a	7.9±0.02 ^a	7.3±0.14 ^a	7.9±0.25 ^{ab}	8.5±0.10 ^a	7.9±0.08 ^a
	W4	7.8±0.10 ^a	7.5±0.50 ^b	7.3±0.25 ^a	7.5±0.48 ^b	8.4±0.54 ^{ab}	7.1±0.90 ^b
Yo mix [®] (Y48)	W0	8.0±0.37 ^a	8.00±0.01 ^a	7.7±0.05 ^a	8.2±0.02 ^a	8.1±0.05 ^{cd}	8.0±0.11 ^a
	W1	8.0±0.25 ^a	8.0±0.03 ^a	7.6±0.01 ^a	8.1±0.07 ^a	8.0±0.03 ^{cd}	8.0±0.05 ^a
	W2	7.9±0.36 ^a	7.9±0.06 ^a	7.5±0.04 ^a	7.9±0.04 ^{ab}	8.0±0.02 ^{cd}	7.9±0.03 ^a
	W3	7.9±0.05 ^a	7.9±0.04 ^a	7.3±0.9 ^a	7.7±0.05 ^{ab}	8.0±0.04 ^c	7.9±0.07 ^a
	W4	7.8±0.10 ^a	7.6±0.03 ^b	7.2±0.14 ^a	7.5±0.03 ^b	7.9±0.03 ^{cde}	7.1±0.09 ^b

Means with different superscript letters in a column are significantly different ($P<0.05$); ($n=3$); PJ: Pasteurized juice; F24: mango juice fermented by Fiti[®] culture for 24 hours; F48: mango juice fermented by Fiti[®] culture for 48 hours; Y48: mango juice fermented by Yo-mix[®] culture for 48 hours.

CHAPTER FIVE: DISCUSSION

5.1 Formulation of fermented mango juice

Food value addition is important in achieving food and nutrition security. The current study was designed to formulate a new fermented probiotic product based on mango juice by optimizing process conditions for better culture viability and considering consumer acceptability. Four pasteurized mango juice formulations (25 parts of mango pulp and 75 parts of water, 50 parts of mango pulp and 50 parts of water, 75 parts of mango pulp and 25 parts of water and 100 parts of mango juice (control) were rated by sensory panelists based on color, flavor, taste, consistency and general acceptability in the preliminary evaluation (Table 1). The most preferred formulation based on overall acceptability was the 50:50 formulation which had the highest means score (7.44) in overall acceptability as it was more appealing compared to 100:0 and 75:25.

Mango fruit contains sugars and organic acids such as malic acid, citric acid and succinic acid which contributed to the taste and flavor of the probiotic fermented mango juice (Acevedo-Martínez et al., 2018; Rafiq et al., 2016). The current findings imply that water to pulp ratio and consequently the mango juice concentration influences consumer acceptability. The findings of the current study agree with the findings of Ryan et al., (2020) who reported that the pulp to water ratio significantly changed the viscosity, color and overall liking of the juice resulting in poor rating by panelists. This was however, one of the criteria considered during the preliminary steps of the formulation.

5.2 Physicochemical characteristics of the formulated pasteurized and fermented mango juice

Probiotic mango juice is rich in sugars, organic acids mainly lactic acid and vitamins including vitamin C and β -carotene among other vitamins, making it a nutritive product consumed as a snack and a beverage to replace commonly consumed non-nutritious juices. However, the composition of mango juice can vary based on ecological and agronomical conditions. The ecological zone, harvesting season, and soil mineral composition determine the mango fruit's nutritional composition as high rainfall or excess water supply reduces the total soluble content (Reddy et al., 2015).

Physicochemical properties of fruit juices such as TSS, TTA and pH affect the fermentation and, consequently, the final product's quality (Ryan et al., 2020). The current study showed that the pH (4.41) of pasteurized mango juice was within the acceptable range (3.2 to 4.5) for the lactic acid bacteria survival and is in agreement with other scholars (Ryan et al., 2020; Rafiq et al., 2016).

The pH plays a significant role in final product preservation and flavor development (Kumar et al., 2015). The pH changed progressively with an increase in fermentation time and during storage for four weeks at 4⁰C. The Abt-5[®] culture (*Streptococcus thermophilus*, *Bifidobacterium*, and *Lactobacillus acidophilus*), Fiti[®] (*Lactobacillus rhamnosus*) and Yo-mix[®] (*Streptococcus thermophilus* and *Lactobacillus delbruiikii ssp bulgaricus*) cultures fermented mango juice within 72 hours. They lowered the pH values from an initial 4.41 to 3.23 (Figure 4.4 to 4.6). The rapid drop in pH could have been attributed to probiotic bacteria by-products of fermentation namely lactic acid and acetic acid which increased the products acidity hence lowering the pH (Xu et al., 2020). The cultures were able to break down complex sugars to simple sugars and utilize them

during fermentation and storage causing a decrease in glucose, fructose and sucrose with the most utilized sugar being sucrose as observed in Table 4.9.

The difference in pH values among the fermented juices (Abt-5[®], Fiti[®] and Yo-mix[®]) could be attributed to strains of LAB used during fermentation. Different species have been reported to have different abilities to utilize sugars to produce lactic acid (Perricone et al., 2015). The results of the current study were in agreement with those reported by Kumar et al., (2015) who fermented probioticated mango and sapote juice using *Lactobacillus plantarum*. Probiotic mango juice fermentation using LAB by Reddy et al., (2015) also reported a drop in pH during fermentation and storage while Panghal et al., (2018) using non-dairy probiotic products reported similar results. Low pH in probiotic fermented mango juice could have hindered the growth of pathogenic microorganisms, thus guaranteeing product safety and prolonged shelf life (Panghal et al., 2018). This was demonstrated by the excellent keeping quality of the developed product (Table 4.10).

There was a considerable increase in titratable acidity (Figure 4.1 to 4.3) in all cultures for the three formulations (25:75, 50:50 and 75:25) during fermentation and storage. The highest acid production was observed in 50:50 formulation fermented by Fiti[®] culture with initial acidity of 0.31%, which increased to 0.69% compared to 0.32% and 0.65% by Abt-5[®] and Yo-mix[®] for 25:75 and 75:25 pulp respectively. The increase in acidity could be due to sugar metabolism by probiotic bacteria producing lactic acid (Tayo & Akpeji, 2016). In addition to acid production, acidity may have increased during storage due to the hydrolyzation of sugars induced by release of enzymes present in dead probiotic cells (Zhu et al., 2020). The findings are in agreement with those reported by Reddy et al., (2015) who recorded an increase in titratable acidity in probiotic mango juice by LAB stored for four weeks at 4⁰C; Kumar et al., (2015) reported an increase in

total titratable acidity in mango and sapota juice enriched with *L. plantarum*. The current finding also corroborates with the findings reported by Oliveira et al., (2020) on mixed juice (mango and carrot) fermented using *L. rhamnosus*, *L. plantarum*, and *L. acidophilus* stored for 35 days at 8⁰C.

A similar trend to that of the pH of the product was observed for the TSS of the product. The TSS measures carbohydrates, fats, proteins and minerals in fruit by determining the refractive index (Khatoon & Gupta, 2015). Brix, a widely used parameter, determines sugar concentration in a product. The lactic acid cultures rapidly fermented the sugars in the mango juice, causing a decrease in total soluble solids. The solid matters present in mango juice were utilized as substrates by the cultures during processing and storage days. Similar findings on the decrease in total TSS have been reported in the probiotification of mango juice LAB during processing and storage by Hossain et al., (2019).

5.3 Culture viability of fermented mango juice during processing and storage

The amount of live probiotic cells present in a product at the time of use determines the product's health benefits to the consumer (Fernandes Pereira & Rodrigues, 2018). The minimum recommended level to maintain therapeutic effect is 10⁶CFU/ml viable cells in a product at consumption (Bansal et al., 2016). However, this depends on the fruit's intrinsic characteristic and the culture strain used. Several factors affect culture viability in a product, such as pH, incubation temperature, storage temperature and presence of oxygen (Reddy et al., 2015). In the present study, cell multiplication was noted in all cultures as shown in Figure 4.10. The results in Figure 4.11 indicate that Abt-5[®], Fiti[®] and Yo-mix[®] cultures were able to survive and utilize nutrients, vitamins and minerals in

mango juice for cell synthesis; consequently, a decrease in pH was observed as reported by Ryan et al., (2020).

The slow growth in Fiti culture within 18 hours could have been associated with the adaptation of microorganisms to the new environment which differs due to the strain used, dissolved oxygen and intrinsic characteristics of the product (Khatoon & Gupta, 2015). The growth rates observed in the current study were higher than those reported by White and Hekmat, (2018) log 7 CFU/ml in fruit juices using *Lactobacillus Rhamnosus GR-1* fortified with inulin fiber. Lactic acid bacteria have a wide pH (2.9-6) and temperature (40-46⁰C) range and some can survive in an acidic environment. Yo-mix[®] can also be used in both dairy and non-dairy products as a direct vat. There is scarce information on the use of Yo-mix[®] culture in fruit juices. Previous studies have shown that fortification of yogurt with chicken pea using Yo-mix[®] culture increased the viable cell count to 10⁸ CFU/ml (Chen X, Singh, Bhargava and Ramanathan, 2018) which agrees with our findings. Therefore, mango juice fermented with Fiti and Yo-mix culture maintained higher count levels than the minimum recommended values by Codex standards (10⁶ CFU/ml) for a healthy probiotic product throughout the fermentation period.

The lowest cell count was recorded in the Abt-5[®] starter culture. The optimum pH for Abt-5[®] culture growth is 6.5 according to the manufactures description. The low cell count could be attributed to the pH of the fermented fruit juice being a limiting factor for Abt-5[®] starter culture growth (Elshagabee et al., 2021). In addition, most of the beverages fermented with Abt-5 starter culture are milk based products with log 7.40 CFU/ml (Chen et al., 2018) and milk-fruit based formulations with 3.7×10⁹ CFU/ml (El-Sohaimy et al., 2020) which gives higher viable count than the current study.

Researchers reported lower counts of bifidobacteria in the probiotic dairy beverages fortified with pomegranate juice (Nour-Eldin et al., 2020).

Mango juice fermented using Fiti[®] culture for 24 hours, 48 hours and Yo-mix[®] culture for 48 hours were able to withstand the fermentation conditions (mainly temperature and pH) and proliferate during fermentation. Fiti[®] and Yo-mix[®] cultures maintained higher levels than the minimum recommended values by Codex standards (10^6 CFU/ml viable cells) for probiotic products throughout the fermentation and storage period.

Microorganism growth and change of other variables such as pH, acidity and soluble solids during fermentation and storage indicated that lactic acid bacteria could ferment sugars in the mango juice (Mauro et al., 2016). After 48 hours of fermentation (Figure 4.11), there was a slight decrease in cell count, which may be attributed to acid accumulation in the fermented mango juices. pH plays an essential role in determining probiotic survival in fermented fruit juices during processing and storage (Ashurst, 2016; Reddy et al., 2015). It has been reported that the low pH (2.5-3.7) of fruit juices creates a stressful environment for lactic acid bacteria (Perricone et al., 2015). According to the current study, the Abt-5[®] bacterial culture did not grow to attain the minimum recommended viability levels (10^6 CFU/ml) since the optimum cell count was 10^5 CFU/ml. This could be attributed to low pH as some species of the probiotic culture may not survive in the acidic environment according to Shori, (2016) and Sarao & Arora, (2016) and Abt-5[®] could be one of them.

It has been reported that probiotic bacteria take a long time to reach optimal growth in non-dairy products (Perricone et al., 2015). However, the current study achieved optimal growth after 24 hours of fermentation with Fiti culture and 48 hours with Abt-5[®] and Yo-mix. The high viable cell count (Fiti[®] and Yo-mix[®] cultures) in probiotic mango juice

indicated that the cultures could survive and utilize essential nutrients (sugars and vitamins) present in mango juice for cell reproduction and metabolism as characterized by a drop in pH and total soluble solids achieved after 24 hours of fermentation. The difference in fermentation time could be due to the different bacterial species used during processing. However, Reddy et al., (2015) study on fermentation of mango juice using *Lactobacillus acidophilus* (MTCC10307), *Lactobacillus delbrueckii* (MTCC911), *Lactobacillus plantarum* (MTCC9511) and *Lactobacillus casei*, obtained higher levels of the viable bacterial count of above 10^9 CFU/ml after 40 hours of fermentation.

The probiotic cell population decreased during storage as indicated in Table 4.2, with the lowest viability observed in juice sample fermented by Yo-mix[®] culture for 48 hours after the third week of storage. Several factors could have affected the viability of probiotic bacteria during storage, mainly the production and accumulation of lactic acid creating an unfavorable environment for reproduction. It has been reported that acid production, mainly during post-incubation, affects the viable cell count of probiotic bacteria such as *L. acidophilus* and *Bifidobacterium bifidum* (Reddy et al., 2015). Fermentation of sugars by probiotics continued during storage, causing a decrease in pH (Oliveira et al., 2020).

5.4 Microbial quality of the control and fermented mango juice

The control and probiotic fermented mango juice's microbial quality was assessed to determine the efficiency of pasteurization, hygiene during the processing and the keeping quality over the four weeks of storage at 4⁰C. No coliforms were detected in the processed product and during storage (4⁰C) for the four weeks in all juice samples. Additionally, the total plate count, yeast and mold for both control and fermented mango juice were within the set KEBS, (2016) of 1.0×10^3 CFU/ml. This indicated that hygiene

handling and aseptic packaging were observed during the final product's production and handling.

The heating process applied to mango juice during pasteurization was adequate to destroy the most viable spoilage and pathogenic cells as the impact of thermal destruction is high at low pH (Xu et al., 2018). According to the results pasteurization process (90⁰C for 1 min) effectively destroyed most of the viable microorganisms. Low storage temperature has been reported to enhance storage stability (Perricone et al., 2015). Moreover, most spoilage and pathogenic bacteria do not survive pH levels <4.5 (Ryan et al., 2020). In addition, the low pH in the current study could also have contributed to the good microbial quality of the product during processing and storage. Similar results on the microbial safety of stored fermented beetroot juice were reported by Czyżowska et al., (2020).

5.5 Sensory evaluation of the control and fermented mango juice during processing and storage

Sensory properties such as color, appearance, taste, mouthfeel and flavor are considered essential for consumer preference, these parameters may be affected by raw material, fermentation conditions and the culture (Ayed, Mhir and Hamadi, 2020). The mean flavor scores increased with fermentation hours (Table 4.5). The higher scores in fermented mango juice by Fiti[®] for 48 hours compared to control were due to the sweet and fruity flavor of fresh mango juice masking the sour/acidic taste developed during fermentation which influenced consumer liking.

In sensory evaluation, sweetness and sourness correspond to sugars and organic acid, respectively (Ryan et al., 2020). Glucose, fructose and sucrose were the main sugars in mango fruit juice and source of sweetness. However, after fermentation, glucose and

fructose were utilized by probiotic bacteria producing organic acids and aroma compounds which may have contributed to the taste and flavor of the finished product. The volatile acids, carbonyl compounds and non-volatile acids form aroma/flavor compounds (Ayed et al., 2020; Ozcan et al., 2015) The acidic (sour) nature of mango juice fermented using Fiti[®] culture for 48 hours could be the reason it achieved high consumer acceptability in flavor and taste sensory attributes, although they were not significantly different during processing.

There was no significant change in appearance (syneresis, particles and brightness) of the control and fermented mango juice during processing and storage. The mean scores for the flavor and taste of the fermented mango juice significantly decreased after the third week of storage which negatively influenced the overall consumer acceptability (Table 4.7). There was a slight increase in sourness (acidity) after the third week due to metabolic activities of the cultures and excessive acid production, which could have been attributed to the poor scores obtained.

5.6 Change in selected sugars, vitamin C and β -carotene content in pasteurized and fermented mango juice

It is desirable that processing conditions achieve maximum possible nutrient retention while maintaining food safety and overall quality. The sugars decreased during processing and storage (Table 4.7). Sucrose was more utilized, followed by fructose. However, the observed decrease in sucrose could be due to the breakdown of sucrose into glucose and fructose. Probiotics were able to ferment added sugars (sucrose) for their growth and other metabolic activities to produce lactic acid. This may have influenced the taste and flavor of the final product during processing and storage at 4⁰C for four weeks.

The current findings were in agreement with those obtained by Sivudu et al., (2014) who analyzed fermented watermelon and tomato juice stored for four weeks at 4⁰C. The study is also supported by Rafiq et al., (2016) who reported a decrease in sugars with an increase in fermentation hours, in the development of probiotic carrot juice using LAB. However, sugars, vitamins and minerals present in mango juice supported the growth and survival of probiotic cultures in fermented mango juice during storage.

Vitamin C content decreased with processing and storage of the mango juice, and this was attributed to thermal degradation during pasteurization. It is also unstable when exposed to oxygen and light; this was minimized by hot filling and use of opaque cans during packaging (Kesavanath et al., 2015; Xu et al., 2018). The difference in vitamin C content between the control and the fermented juice could be due to the use of antioxidants (vitamin C) by the probiotic's during fermentation and storage to protect them against oxidative stress (Fernandes Pereira & Rodrigues, 2018). Similar results in the reduction of vitamin C were reported by Guan et al., (2016) on mango juice stored for 60 days. In the current study, it was observed that vitamin C content gradually decreased with storage, indicating that vitamin C degrades with time. Also, the probiotic bacteria could have used vitamin C for their growth. The findings are in line with shelf-life stability research on kiwi fruit beverages stored for 40 days (Chen et al., 2020).

The β -carotene content in probiotic mango juice increased during fermentation and storage for four weeks compared to the control. A significant difference was observed on the fermented mango juice as different probiotic strains synthesize β -carotene differently (Reddy et al., 2015). The observed increase in β -carotene content during fermentation could be due to its synthesis and release by the fermenting cultures during processing

and storage (Xu et al., 2020). Xu et al., (2020) also reported that LAB synthesizes β -carotene as a protective mechanism against oxidative stress.

CHAPTER SIX: SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.0 Summary of the findings

The 50 parts of pulp and 50 parts of water mango fruit juice formulation was the most acceptable pre-fermentation which also yielded the best product after fermentation in terms of physicochemical, organoleptic and culture viability. The pH levels of the mango fruit juice and the presence of fermentable sugars offered a conducive environment for the survival and proliferation of the fermentation cultures. Fermentation significantly changed the physicochemical properties as observed in the decreased pH, total soluble solids and sugar content with increased total titratable acidity. The highest acid increase was observed in mango juice fermented using Fiti bacterial culture for 48 hours. This was followed by mango juice fermented using Fiti bacterial culture for 24 hours which had a ratio of 50:50.

The control mango juice was rich in vitamin C content but the content significantly decreased during processing and storage. This could be attributed to heat processing, exposure to light and oxygen during processing and storage. Fairly high vitamin C retention was observed in the fermented products. On the other hand, β -carotene content increased significantly ($P < 0.05$). β -carotene content increased by approximately 3-8% in the fermented juices which could be associated with its synthesis by probiotic cultures.

Sensory scores indicated that mango fruit juice fermented using Fiti[®] culture for 48 hours had the highest overall sensory rating followed by juice fermented by Fiti[®] culture for 24 hours and Yo-mix[®] culture for 48 hours. The culture viability significantly increased during fermentation with Fiti[®] bacterial culture for both 48 hours and 24 hours and Yo-mix[®] culture fermented for 48 hours attaining a viable count of above 10^6 CFU/ml. The viable cell count of the juice fermented using Yo-mix culture for 24 and 48 hours

decreased with storage attaining levels below the recommended viable cell count of 10^6 CFU/ml after two weeks of storage. Samples fermented using Fiti[®] culture sustained the cell count within the recommended range during the four weeks of storage. Based on culture viability results obtained, the mango juice fermented using Fiti culture for 24 hours is recommended for industrial uptake, not only does it give a count above the recommended daily intake but it's also economically viable product. It is recommended that further investigation be done on probiotic fermented mango juice stored beyond four weeks.

Culture activity during storage resulted in a significant decrease ($P < 0.05$) in sugars, pH and total soluble solids. This was evident after three weeks of storage. The microbial quality for both fermented and control juice during storage was within the set limit by KEBS. The probiotic juice stability during the four weeks of storage was associated with the effectiveness of pasteurization temperature, low pH environment and refrigeration temperatures.

6.1 Conclusion

The two probiotic cultures; Fiti[®] and Yo-mix[®] can be used to ferment mango juice to attain the recommended viable count (10^6 CFU/ml) within 48 hours of the fermentation process. The current study showed that the 50:50 (v/v) formulation was rated the best during sensory evaluation. Upon fermentation of the mango juice, the 50:50 formulation yielded a superior fermented product compared to other formulations.

The probiotic cultures survived low pH during fermentation and storage at 4°C for four weeks. A significant decrease was recorded in vitamin C content ($P < 0.05$) in both control and fermented mango juice, which could have been attributed to both high temperatures and oxidation effects during processing and storage. β -carotene content in the

pasteurized juice (control) remained stable during processing and storage. There was a slight increase in β -carotene content during fermentation and storage. This could have been attributed to β -carotene synthesis by fermentative cultures. Hence, the first null hypothesis that no significant difference in sugars, vitamin A & C content between the probiotic fermented juice and pasteurized mango (control) juice was **rejected**.

Sensory attributes are important in product development. A significant difference was observed in sensory acceptability of pasteurized (control) and fermented juice, with fermented mango fruit juice scoring better in most sensory scores than the control. The acceptability was attributed to the sour/acidic taste and flavor thus the **second null** that there is no significant difference between probiotic fermented mango juice and pasteurized mango juice (control) on sensory acceptability was **rejected**.

The fermented mango juice was microbially stable during storage at 4⁰C and this could be due to low pH and low storage temperature. The recommended storage period for the probiotic mango juice fermented using Fiti culture is three weeks while the storage period for the juice fermented by Yo-mix culture is one week based on sensory and culture viability. There was a significant difference ($P < 0.05$) in the storage stability of pasteurized (control) and fermented mango juice. No significant change was found in nutrient content, physicochemical and sensory attributes of pasteurized mango juice (control) during storage for 4 weeks at 4⁰C. Therefore, the **third null hypothesis** that there is no significant difference between probiotic fermented mango juice and the pasteurized mango juice (control) on shelf stability was **rejected**.

The culture viability for Fiti culture was relatively higher than the minimum recommended allowable limits at the fourth week of storage whereas Yo-mix culture viability declined significantly ($P < 0.05$) to below the acceptable limit after the first week

of storage. Therefore the fourth **null hypothesis** that there is no significant effect of processing and storage of fermented mango on probiotic viability was **rejected**.

In conclusion, fermented mango juice was more preferred to control mango fruit juice; therefore, probiotic fruit juices can be consumed by people with milk allergies and lactose intolerant. Mango fruit juice has the potential for value addition to fruit-based probiotic fermented products which are consumed as healthy functional foods.

6.2 Recommendations

6.2.1 Recommendations for practice

- Industries and small-scale enterprises to be trained on the processing of probiotic mango juice by research institutes using Fiti culture since it gives high quality and healthy probiotic fermented fruit products to upscale the production for their financial gain.
- Consumers to be sensitized on the benefits of consuming fermented probiotic mango juice to embrace it.

6.2.2 Recommendations for policy

- Ministry of Health to create awareness on the presence and consumption of fermented probiotic fermented mango juice and its health benefits to the public.

6.2.3 Recommendations for further research

- The study recommends further investigation on the viability of Fiti culture in fermented mango juice beyond four weeks of storage.
- Further research is conducted to develop fermented probiotic fruit juices using other locally available fruits.

- Research be done to establish the cut-off point for optimum acidity at which the fermented juice is most palatable at optimal probiotic cell counts for a healthy product (10^6 CFU/ml and above).

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APPENDICES

Appendix A: Consent Form

Introduction

Dear panelist,

My name is Margaret Mwanzia. I'm a postgraduate student from Kenyatta University doing research on "Formulation of probiotic mango juice and assessment of physicochemical characteristics, nutritive quality, culture viability and storage stability" The information will be used by the Kenyan industries and farmers in value addition.

Study procedures

You are invited to fill out a questionnaire on sensory evaluation of fermented probiotic mango fruit juice. The aim of the study is to determine the sensory acceptability of probiotic fermented mango juice. You are supposed to taste and cleanse your palate with tap water and then rate the samples for the intensity of each given characteristic. Voluntary involvement in this research is encouraged, and you can ask questions at any time regarding the study.

Discomforts and Risks

Minimal discomforts may be experienced when participating in the study, but if you have experience of allergy when consuming probiotic fruit juices, you are not allowed to part in this study. Also, you are not supposed to continue with the study if you experience allergic reactions.

Benefits

There is no immediate benefit when taking part in the study, but also you can pull out of the research any time, for whatever reasons.

Voluntary participation

Participation in this research is voluntary, no monetary compensation is expected for your involvement.

Confidentiality

Data and information emanating from the study will be confidential, and responses will be coded for confidentiality. Presentation of the results will only include panelist performance. Confidentiality will be ensured by not sharing the information regarding the panelist with any person or organization.

Contact information

In case of any questions contact Dr. Kiio Supervisor 1. On 0725999448 or Dr. Okoth Supervisor 2. On 0710780832 or the Kenyatta University Ethical Review Committee Secretariat on chairman.kuerc@ku.ac.ke, secretary.kuerc@ku.ac.ke, secretariat.kuerc@ku.ac.ke

Participant’s statement

I fully understand the above details concerning my involvement in the study. I will participate voluntary as my confidentiality is well assured.

I agree.....or disagree..... to participate in this study.

Participant’s name

Signature

Date

Interviewer statement

I, the undersigned, have expounded to the panelist in a language they comprehend, the procedure to be observed while undertaking the research and the benefits or risks for their involvement.

Name of interviewer

Signature

Date

Appendix B: Sensory evaluation form for fermented probiotic mango juice

Panelist code

Age

Gender

You have been provided with fermented fruit juice samples labeled A-D as indicated on the cups. You've also been provided with portable drinking water labeled W. Please rinse your mouth in between samples and spit it out for better assessment. Assess them in the order presented and rate them on color, appearance, mouthfeel, taste, consistency, flavor and general acceptability using 9-point hedonic scale.

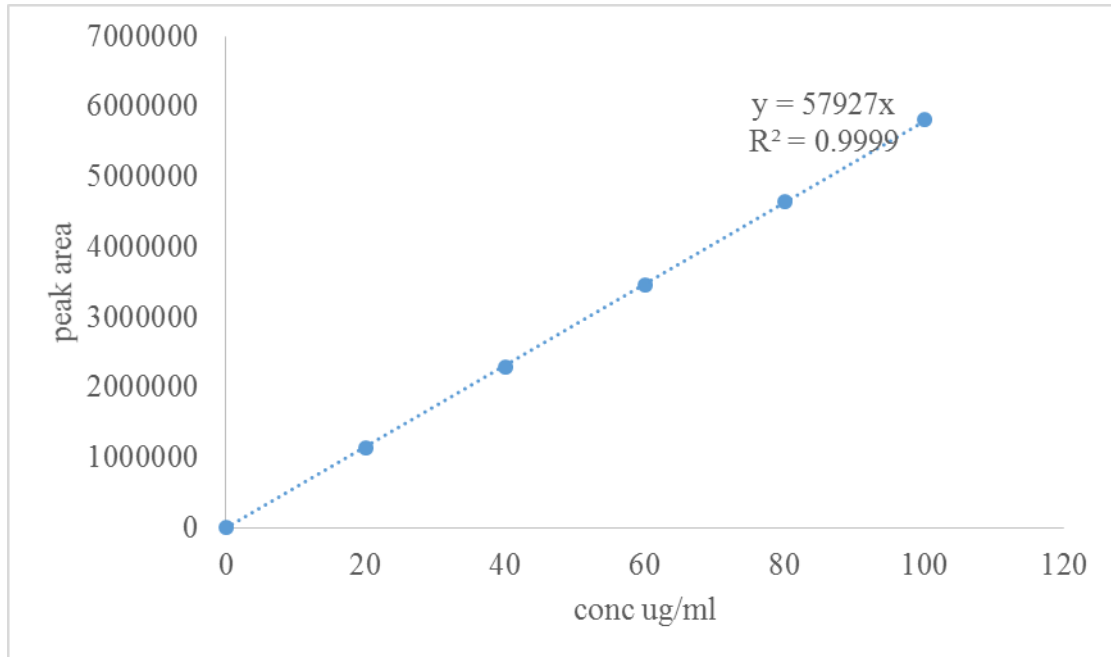
HEDONIC SCORE

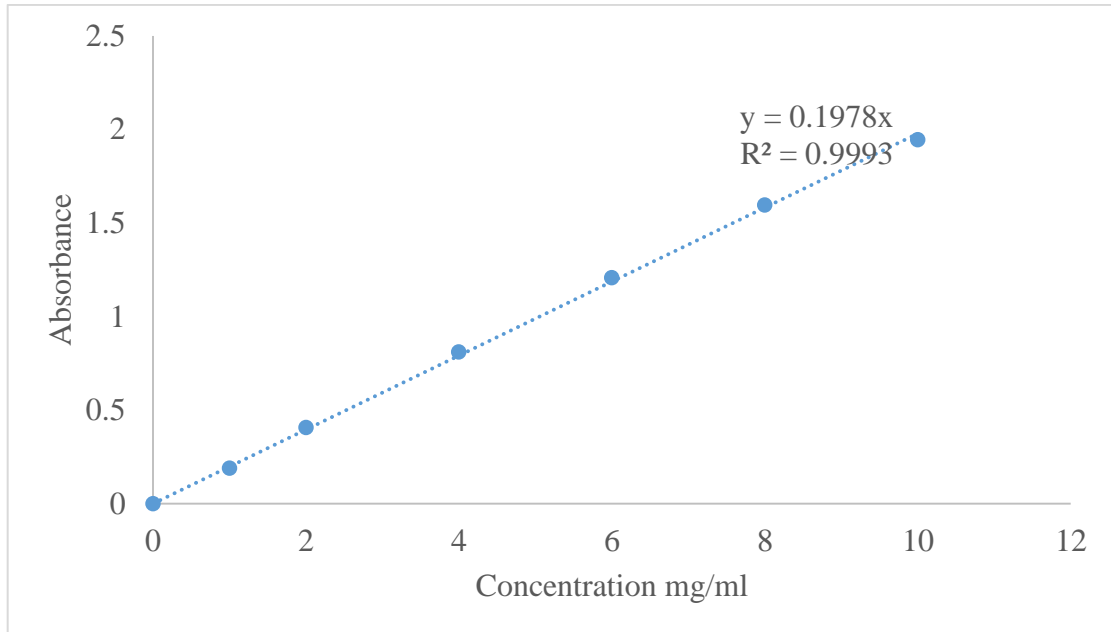
1 denotes dislike extremely, 2 dislike very much, 3 dislike moderately, and 4 dislike it. 5 neither like it nor dislike it, 6 like it and 7 like it moderately, like it very much, 9-like it extremely.

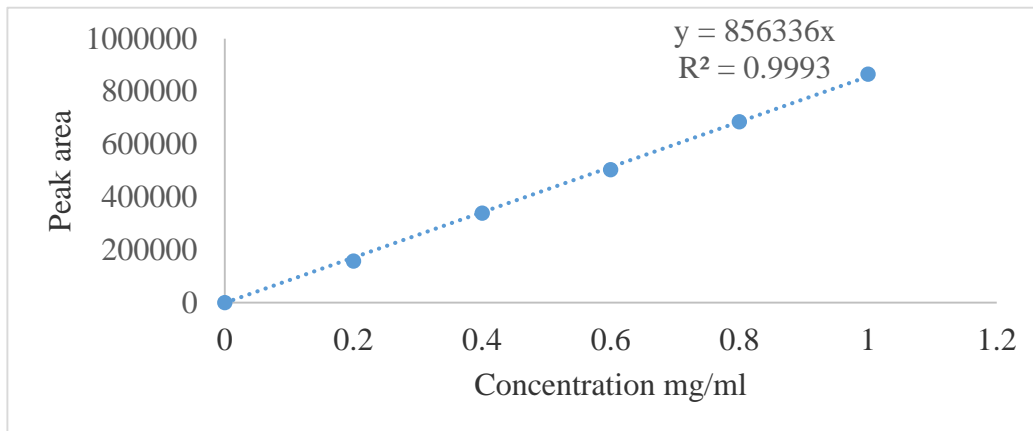
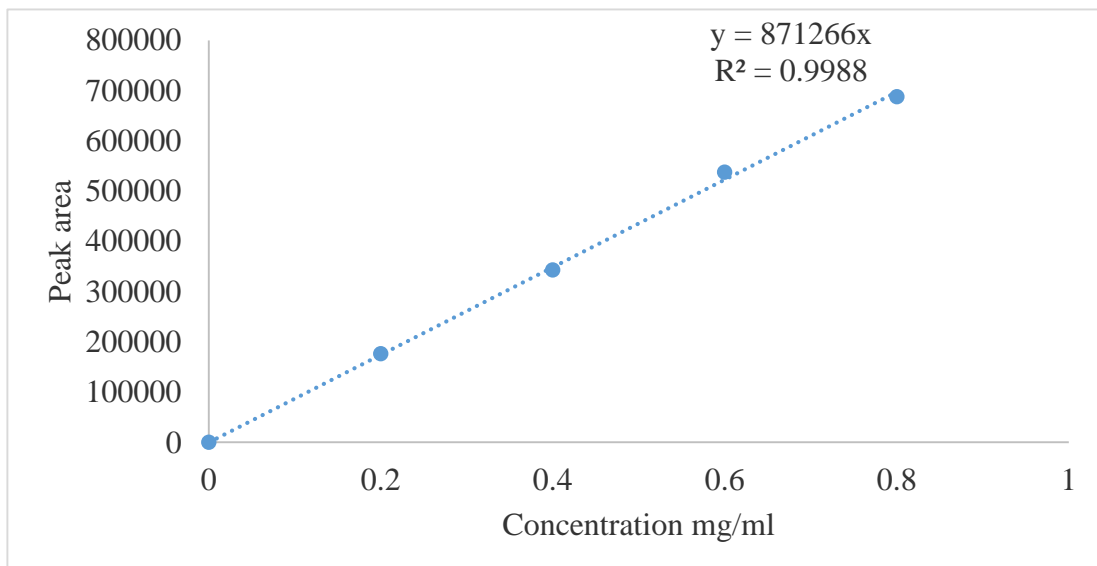
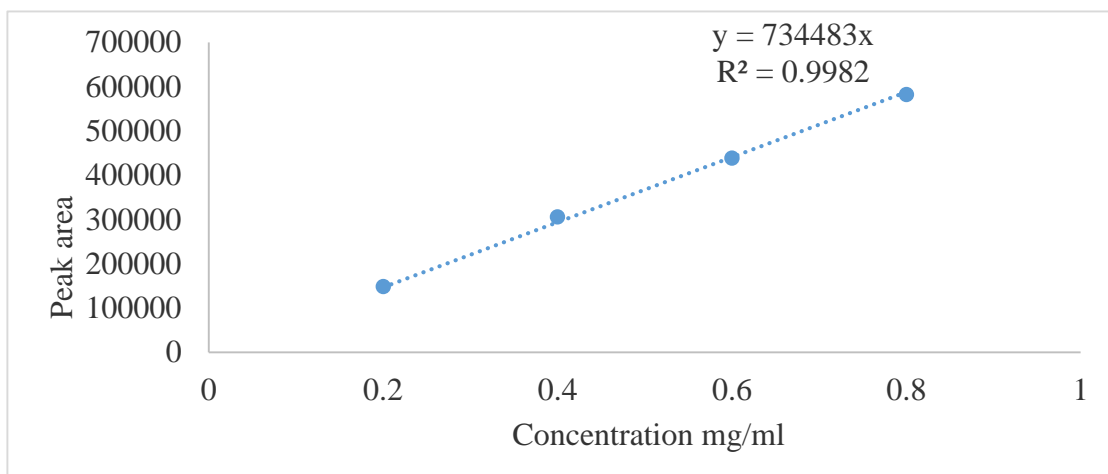
Samples	Color	Flavor	Consistency	Taste	mouthfeel	Appearance	Overall acceptability
A							
B							
C							
D							

General comment






.....

Appendix C: Vitamin C standard curve peak area against concentration

Appendix D: Beta-carotene standard curve absorbance against concentration

Appendix E: sugars standard curves peak area against concentration**Fructose****Glucose****Sucrose**

Appendix F: NACOSTI research authorization letter

 REPUBLIC OF KENYA	 NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY & INNOVATION
Ref No: 277804	Date of Issue: 20/March/2021
RESEARCH LICENSE	
	
<p>This is to Certify that Ms. Margaret Mueni Mwanza of Kenyatta University, has been licensed to conduct research in Kilambu on the topic: FORMULATION OF PROBIOTIC MANGO JUICE AND ASSESSMENT OF NUTRITIVE QUALITY, ACCEPTABILITY AND SHELF LIFE STABILITY DURING PROCESSING AND STORAGE for the period ending : 20/March/2021.</p>	
License No: NACOSTI/21/977E	
277804 Applicant Identification Number	 Director General NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY & INNOVATION
	Verification QR Code 
<p>NOTE: This is a computer generated License. To verify the authenticity of this document, Scan the QR Code using QR scanner application.</p>	

Appendix G: KUERC approval letter



**KENYATTA UNIVERSITY
DIRECTORATE OF ETHICS REVIEW COMMITTEE**

Fax: 8711242/8711575
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 Nairobi, 00100
 Website: www.ku.ac.ke
 Our Ref: KU/ERC/APPROVAL/VOL.1

P. O. Box 43844,
 Tel: 8710901/12
 Date: 20th January, 2021

Margaret B. Mwanzia
 P.O Box 43844-00100
 NAIROBI

Dear Ms. Mwanzia,

RE: APPLICATION NUMBER: PKU/2195/11339 FORMULATION OF PROBIOTIC MANGO JUICE AND ASSESSMENT OF NUTRITIVE QUALITY ,ACCEPTABILITY AND SHELF LIFESTYLE STABILITY DURING PROCESSING AND STORAGE

This is to inform you that **KENYATTA UNIVERSITY DIRECTORATE OF ETHICS REVIEW COMMITTEE** has approved version 4 of the study protocol together with the attached consent forms dated 12.09.2020. Your application approval number is **PKU/2195/11339**. The approval period is **20th January, 2021 TO 20th January, 2022**.

This approval is subject to compliance with the following requirements;

- i. Only approved documents including (informed consents, study instruments, MTA) will be used
- ii. All changes including (amendments, deviations, and violations) are submitted for review and approval by **KENYATTA UNIVERSITY DIRECTORATE OF ETHICS REVIEW COMMITTEE**.
- iii. Death and life threatening problems and serious adverse events or unexpected adverse events whether related or unrelated to the study must be reported to **KENYATTA UNIVERSITY DIRECTORATE OF ETHICS REVIEW COMMITTEE** within 72 hours of notification
- iv. Any changes, anticipated or otherwise that may increase the risks or affected safety or welfare of study participants and others or affect the integrity of the research must be

reported to **KENYATTA UNIVERSITY DIRECTORATE OF ETHICS REVIEW COMMITTEE** within 72 hours

- v. Clearance for export of biological specimens must be obtained from relevant institutions.
- vi. Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. Attach a comprehensive progress report to support the renewal.
- vii. Submission of an executive summary report within 90 days upon completion of the study to **KENYATTA UNIVERSITY DIRECTORATE OF ETHICS REVIEW COMMITTEE**.

Prior to commencing your study, you will be expected to obtain a research license from National Commission for Science, Technology and Innovation (NACOSTI) <https://oris.nacosti.go.ke> and also obtain other clearances needed.

Yours sincerely



Prof. Judith Kimiywe

DIRECTOR- KENYATTA UNIVERSITY ETHICS REVIEW COMMITTEE.



Appendix H: Kenyatta University research approval letter



**KENYATTA UNIVERSITY
GRADUATE SCHOOL**

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 NAIROBI, KENYA
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Internal Memo

TO: Dean, Graduate School

DATE: 2nd June, 2020

Ms. Margaret M. Mwanzia
 C/o Department of Foods, Nutrition & Dietetics
 KENYATTA UNIVERSITY

REF: H60/CTY/33368/14

SUBJECT: APPROVAL OF RESEARCH PROPOSAL

This is to inform you that the Graduate School Board at its meeting 20th May, 2020 approved your Sc. Research Proposal entitled "Formulation of Probiotic Mango Juice and Assessment of Nutritive Quality, Acceptability and Shelf Life Stability during Processing and Storage".

You may now proceed with your Data collection, subject to clearance with the Director General, National Commission for Science, Technology & Innovation.

As you embark on your data collection, please note that you will be required to submit to Graduate School completed supervision Tracking and Progress Report Forms. The Forms are available at the University's Website under Graduate School webpage downloads.

Thank you.

JULIANA GITU
 DEAN, GRADUATE SCHOOL

Registrar (Academics) Att: Mrs Lucy Njenga
 Chairman, Department of Foods, Nutrition & Dietetics

Supervisors

1. Dr. Juliana Kiiro
 Department of Foods, Nutrition & Dietetics
 Kenyatta University
2. Dr. Everlyn Okoth
 JKUAT
 C/o Department of Foods, Nutrition & Dietetics
 Kenyatta University

cc:ao

Appendix I: Mango fermenting bacteria cultures used in the study



1. *Lactobacillus rhamnosus*.



2. *Streptococcus thermophilus*, *Bifidobacterium* and *Lactobacillus acidophilus*



3. *Streptococcus thermophilus* and *Lactobacillus delbruiikii ssp bulgaricus*

Appendix J: Illustration of fermentation chamber.**Fermentation chamber****Appendix K: Illustration of experimental design and test samples.****Appendix L: Fermented Samples using different cultures**