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Optimized processing method for producing Avocado and Mango seed-based composite flour for functional Foods

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OPTIMIZED PROCESSING METHOD FOR PRODUCING AVOCADO AND MANGO SEED-BASED COMPOSITE FLOUR FOR FUNCTIONAL FOODS

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A Dissertation Submitted in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Life Sciences of the Nelson Mandela African Institution of Science and Technology

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ABSTRACT

Avocado and mango seed kernels are considered as wastes although they are rich in essential nutrients and bioactive compounds for human health. Lack of commercial application, presence of anti-nutrients, difficulty in processing and little information on nutritional and functional values contribute significantly to their underutilization. These factors underscore the need for processing these seeds into consumable products to utilize them as functional foods. The aim of this study was to optimize a processing technique capable of reducing antinutrients to acceptable levels (above 50%), retain nutrients and functional values, and improve sensory qualities of avocado and mango seeds-based composite flour for functional foods. Different processing conditions for probiotic fermentation, boiling and soaking techniques were studied to optimize processing conditions for the seeds. The antinutrients, antioxidant activity, total phenolics and selected nutrients of the seeds were analyzed using standard analytical methods. Furthermore, avocado and mango seeds-based composite flour was formulated using Nutrasheets integrated with the USDA National Nutrient Database for Standard Reference. The formulated composite flour was assessed for safety and quality parameters for a period of 84 days under different storage temperatures. The results showed that all processing techniques significantly ($p < 0.05$) reduced the antinutrients by over 50% for processed avocado and mango seed kernels. The highest total phenolics and antioxidant activity (IC_{50}) for both seeds were observed at a fermentation temperature of 37^{o}C with *Lactobacillus plantarum*. Soaking and boiling reduced the analyzed minerals in both avocado and mango seed kernel except for potassium and zinc on soaked mango seeds, whereas probiotic fermentation retained 100 % or increased the concentration of the analyzed minerals, ascorbic acid and α-tocopherol. In contrast, boiling and soaking reduced the contents of ascorbic acid and antioxidant activity of the analyzed samples. The Nutrasheets analysis showed that the formulated composite flour can achieve the nutritional and functional values for the functional foods. The shelf life analysis showed that 115 days (4 months) is the maximum shelf life for the flour where all the assessed safety and quality parameters can still be within the maximum recommended tolerable limits for human consumption. Therefore, the results of this study indicate that unlike boiling and soaking methods, the probiotic fermentation is an optimized processing method which can significantly reduce the antinutrients to acceptable levels, retain nutrients and bioactive compounds, and improved sensory attributes of avocado and mango seeds-based composite flour for functional foods.

DECLARATION

I, Joseph Runyogote, do hereby declare to the Senate of the Nelson Mandela African Institution of Science and Technology that this dissertation is my own original work and that it has neither been submitted nor being concurrently submitted for degree award in any other institution.

Joseph Runyogote

Name and Signature of Candidate DATE

The above declaration is confirmed

Dr. Jofrey Raymond

Name and Signature of Main Supervisor DATE

Dr. Musa Chacha

Name and Signature of Co-Supervisor DATE

Prof. Bernadette Ndabikunze

Name and Signature of Co supervisor DATE

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CERTIFICATION

The undersigned certify that they have read and found it to be acceptable for examination; hereby recommends for examination of the dissertation entitled: **"***Optimized processing method for producing avocado and mango seed-based composite flour for functional foods",* as a fulfillment of the Award of Doctor of Philosophy in Life Sciences at The Nelson Mandela African Institution of Science and Technology.

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DEDICATION

This work is dedicated to my parents; Kashunju Runyogote and Mary Nzige who laid down the foundation of my education and my lovely wife Irene Temu and my children (Jennah Joseph Runyogote, Jaydan Joseph Runyogote and Junior Joseph Runyogote) for their tireless support, prayers and encouragement.

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LIST OF ABBREVIATIONS AND SYMBOLS

AAE	Ascorbic Acid Equivalents
AAS	Atomic Absorption Spectrophotometer
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemistry
BHA	Butylated HydroxyAnisole
BHT	Butylated HydroxyToluene
Ca	Calcium
CF	Crude fiber content
CFU	Colony Forming Unit
CL	Crude Lipid Content
CREATES	Centre for Research, Agricultural Advancement, Teaching Excellent and Sustainability
DPPH	1, 1-diphenyl-2-picrylhydrazyl
EE	Ether Extract
FAO	Food and Agriculture Organization
FDA	Food and Drug Administration
Fe	Iron
GAE	Gallic Acid Equivalents
GC	Gas Chromatography
HPLC	high performance liquid chromatography
ISO	International Standard Organization
ITC	International Trade Centre
LAB	Lactic Acid Bacterial
Na	Sodium
NAD	Nicotinamide Adenine Dinucleotide
NM-AIST	Nelson Mandela African Institution of Science and Technology
NRRL	Agricultural Research Service Culture Collection
^{o}C	Degree Centigrade
ODS	Octadecyl-Silica
PB	Policy Brief
PCA	Principal Component Analysis
PDA	Potato Dextrose Agar
QDA	Quantitative Descriptive Analysis

ROS	Reactive Oxygen Species
TAHA	Tanzania Horticultural Association
TPC	Total Phenolic Content
URT	United Republic of Tanzania
WFP	World Food Program
WHO	World Health Organization
XLD	Xylose Lysine Deoxycholate
Zn	Zinc

CHAPTER ONE

INTRODUCTION

1.1 Background of the Problem

Malnutrition and other chronic diseases affect more than half of the world's population, especially in developing countries where plants are the major source of nutrients and functional values (Hendek & Bektaş, 2018; Lasano *et al.*, 2019). The cause of this problem is partly due to anti-nutrients binding micronutrients such as minerals and vitamins forming insoluble complexes hence reducing the bioavailability of these nutrients. Anti-nutritional factors combine with nutrients and reduce their availability upon intake, digestion, absorption, and utilization (Yacout, 2016). The negative effects of these anti-nutrients rely on their concentration, chemical structure, time of exposure and interaction with other dietary components (Popova & Mihaylova, 2019). Improving the nutritional and functional values of foods by inactivating or reducing the anti-nutrients can improve the nutritional and health status of the population (Gupta *et al.*, 2015). This can be achieved by developing a processing technique that can reduce anti-nutritional compounds to an acceptable level such as reduction of tannin content to above 50**%**, and yet retain the nutrients, functional values and improve sensory attributes in the intended final products (Ojha, 2020).

Recent studies have shown that avocado and mango seed kernels are potential sources of essential nutrients that can improve nutrition in areas where malnutrition is pervasive. These seed kernels are also good sources of bioactive compounds which have a wide range of physiological functions including anti-tumoral, antiviral, anti-bacterial and cardio-protective (Dabas *et al.*, 2013; Kittiphoom, 2012; Mahawan *et al*., 2015; Tremocoldi *et al.*, 2018). Despite their usefulness, these seeds are always regarded as wastes and discarded away into the environment (Dabas *et al.*,2013; Yatnatti *et al.*, 2014). Processing these seeds into a consumable product could potentially minimize this problem. However, so far, there is no appropriate processing method for turning these seeds into consumable products. Common processing methods have a number of technological weaknesses such as reduction of only the anti-nutrients without considering their negative effect on other nutrients (soluble and heat sensitive vitamins) (Hendek & Bektaş, 2018; Ojha, 2020). However, the strength of an optimized method addresses the shortcomings of the common methods in way that it reduces or removes the antinutrients yet retains the nutrients and functional properties, and improves the sensory attributes of the intended final product (Alexey, 2021). This underscores the need for developing proper

processing method that could help practitioners to tap health opportunities that are available in these seeds.

Through proper processing method, these seeds can be developed into composite flour, which can then be incorporated into various dried products like biscuits, bread, and other related products. Therefore, developing composite flour incorporating functional foods from these seeds could provide nutrients and functional properties to consumers. It is also the best way of adding value to these seeds and generating income for all stakeholders who are engaged in this subsector. Furthermore, the products from these seeds could eventually add variety and offerings to benefit consumers. Therefore, this study aimed at optimizing a processing technique that can reduce the antinutritional compounds, retain nutrients, improve sensory attributes and extend the shelf life of the intended final products from avocado and mango seed extracts.

1.2 Statement of the Problem

Tanzania has a diverse number of edible fruits. However, for most fruits, a relatively small portion of the entire fruit is consumed. The other parts of the fruit are discarded due to their poor taste, difficulty in processing, lack of commercial applications (unlike the oil seeds) and lack or little information about their nutritional and functional values (Henly*et al.*, 2015). Avocado and mango are among the fruits in which their utilized portion is less than 50% compared with wastes such as peels and seeds despite their relatively higher content of health-promoting bioactive compounds and essential nutrients. Among the four seeds (mango, tamarind, avocado, and jackfruit) mango and avocado seed kernels exhibited the highest (95%) total antioxidant activity and phenolic content (Soong & Barlow, 2004).

Nevertheless, optimized processing method that can improve sensory attributes, retain nutrients and reduce antinutritional compounds in mango and avocado seed kernels is not in place (Ojha, 2020). This suggests a need to optimize a processing method for proper utilization of these beneficial seeds. This method can be used to produce flour from these seeds for utilization in functional foods for human consumption. This, in turn, would contribute to food security, generate income and reduce poverty among stakeholders who are involved in the avocado or mango industry. On the other hand, utilization of these seeds would reduce environmental pollution emanating from their disposal.

1.3 Rationale of the Study

Recent studies have shown that avocado and mango seed kernels are potential sources of essential nutrients that can improve nutrition in areas where malnutrition is pervasive. These seed kernels are also good sources of bioactive compounds which have a wide range of physiological functions including anti-tumoral, antiviral, anti-bacterial and cardio-protective (Dabas *et al.*, 2013; Kittiphoom, 2012; Mahawan *et al.*, 2015; Tremocoldi *et al.*, 2018). Despite their usefulness, these seeds are always regarded as wastes and discarded away into the environment (Dabas *et al.*,2013; Yatnatti *et al.*, 2014). Processing these seeds into a consumable product could potentially minimize this problem. However, so far, there is no appropriate processing method for turning these seeds into consumable products. Common processing methods have a number of technological weaknesses such as reduction of only the anti-nutrients without considering their negative effect on other nutrients (soluble and heat sensitive vitamins) (Hendek & Bektaş, 2018; Ojha, 2020). However, the strength of an optimized method addresses the shortcomings of the common methods in way that it reduces or removes the antinutrients yet retains the nutrients and functional properties, and improves the sensory attributes of the intended final product (Alexey, 2021). This underscores the need for developing proper processing method that could help practitioners to tap health opportunities that are available in these seeds.

1.4 Research Objectives

1.4.1 General Objective

The main objective of this study was to optimize a processing method for producing avocado and mango seeds-based composite flour which is rich in nutrients and functional properties, improved sensory attributes and shelf life stable.

1.4.2 Specific Objectives

(i) To optimize a processing method for reducing antinutrional compounds, retaining nutrients and functional values, and improving sensory attributes of avocado and mango seed kernels.

(ii) To formulate avocado and mango seeds-based composite flour from optimized method and determine nutritional values, safety and consumer acceptability.

(iii) To determine the shelf life of the developed avocado and mango seeds-based composite flour using a predictive modeling approach.

1.5 Research Hypothesis

Properly processed avocado and mango seed-based products can be utilized as food, provided that anti-nutritional factors are reduced, and consumers are convinced of their sensory attributes, safety, nutritional and health benefits.

1.6 The significance of the Study

This study intended to contribute maximum utilization of avocado and mango seed kernels which are always regarded as wastes in various places of the world. The study also meant at enhancing value addition of these seeds and opening of the business opportunities for stakeholders by generating income in Tanzania and other countries which produce avocado and mango fruits. Thus, the significance of this study in general focused on increasing food security by utilizing the avocado and mango seed kernels, increasing product variety for consumers to choose. Generate income and reduce poverty among stakeholders who are involved in this sub-sector and decreasing wastes in the environment as a result of these seeds.

1.7 Delineation of the Study

This study was conducted with the scope of optimizing a processing method suitable for processing avocado and mango seed kernels as well as determining nutritional, functional and sensory attributes of the processed product. However, the big challenge encountered in this study was the outsourcing of some laboratory equipment and the strains (lactic acid bacteria) that were used in this study. Additionally, issuing ethical clearence and covid-19 pandemic outbreak affected timeline of the planned schedule.

CHAPTER TWO

LITERATURE REVIEW

2.1 Avocado and Mango Fruits in Tanzania

2.1.1 Avocado

Avocado (*Persea americana*) is an important plant from the family Lauraceae bearing fruits that are highly appreciated for their nutrition and health benefits (Dreher *et al*., 2013). The fruits of avocado can be 7 to 20 cm long and up to 15 cm wide, weighing 0.01 kg to 1kg (Juma *et al.*, 2020). In Tanzania, avocado cultivation was first reported in Zanzibar in 1892, and then increased in other areas during the 1900s (Juma *et al.*, 2020). Avocado was grown for diverse purposes; fruits were used as food by humans, leaf and seed for feeding animals, and stems for timber and firewood (Geleta *et al.*, 2020). Avocados are becoming Tanzania's green gold. Production of avocados in Tanzania is highly concentrated around small scale growers who are spaced across the growing regions (Policy Brief [PB], 2018). Currently, the prominent avocado producing areas are in the regions of Mbeya, Njombe, Songwe and Iringa in the southwest, as well as in Kilimanjaro, Arusha and Tanga in the northeast of the country (United Republic of Tanzania [URT], 2019). The other regions are Kigoma and Kagera in the northwest and Morogoro in the east of Tanzania**.**

Two common types (cultivars) of avocado are grown in Tanzania, the Hass and Fuerte cultivars (Juma *et al.,* 2020). The Fuerte has a medium size, pear-shaped fruit with a green, leathery and easy to peel skin, the skin ripens green and this is the most preferred by families in Tanzania while the Hass cultivar is a fruit with black pebbled skin and can withstand low temperatures up -1 °C upon storage time (URT, 2019).

Report of Tanzania Horticultural Association (TAHA) figures show that the country produces about 39 000 tons annually. This is due to an increase in domestic awareness about the global opportunities (Juma *et al.,* 2019). The level of awareness among the farmers regarding the commercial avocado cultivars and their value on external markets determined the type of avocado to be grown. During the year 2018, avocados earned the country $12.6 million which is equivalent to 27.6 billion Tshs (UTR, 2019). However, export of most produced avocados in the country is dominated by Rungwe Avocado Company Limited and Africado Limited, which is based in Mbeya and Kilimanjaro respectively. The markets for avocados from Tanzania are the Netherlands, Germany, United kingdom, South Africa, Kenya, Dubai and Gulf states (PB,

2018). Despite of the avocado being exported in various countries in the world, the most of the avocados farmers need to adhere to good agricultural practices in order to meet market requirements; that is the, quality and standards.

2.1.2 Mangoes

Mangoes are edible fruits produced by the tropical tree (*Mangifera indica*) which is believed to have originated from the region between northwestern Myanmar, Bangladesh, and northeastern India (Das *et al.*, 2019). The fruits vary in shape (nearly round, oval, ovoid-oblong), size (from 100 grams to more than 2.5 Kg), and colour (greenish, greenish-yellow, yellow, red, orange, or purple) upon maturity depending on the variety (Lo *et al.*, 2005). Mangoes are harvested in three stages: immature (no shoulder development), half-mature (the shoulder has developed) and full mature fruits (the shoulder has filled out and extends to beyond the stem insertion point) all the three stages depended on the ultimate goal for use (Majeed & Jeffery, 2002). For example, the green fruit (immature) can be used to make curries, pickles, jellies and dehydrated slices whereas, the ripe (full mature) mangoes are used to make ice creams, juice, nectar and jam (Amagro, 2011).

In Tanzania, the regions that are highly growing mangoes include Tabora, Shinyanga, Tanga, Morogoro, Lindi, Dar es Salaam, Mwanza and Mtwara (ITC, 2014). There are two kinds of varieties of mangoes grown in Tanzania; traditional or local varieties and improved or exotic varieties (International Trade Centre [ITC], 2014). The traditional varieties that existed in the country for a long period include varieties like Dodo, Bolibo, Muyuni, Viringe and Bonyoa grown mainly by small scale farmers (URT, 2019). The improved cultivars were introduced in the country ten years ago and include; Apple, Keitt, Kent and Alfonso. This type account for around 1% of the total production of mangoes in Tanzania and are mostly grown by medium and large scale farmers (ITC, 2014). The harvest is often achieved in two seasons in the months of October to November and May to July (URT, 2018).

Over 99% of mangoes produced in Tanzania are consumed by the local market and essentially as a fresh fruit or in juice form and only a very small percentage (less than 0.05%) of the total mango produced are being exported mainly to the Middle East (Mma, 2008). The slow pace observed in moving to commercial production of export is being attributed by key constraints such as lack of clean planting material, inadequate technology transfer support, the length of the production cycle, pests and disease infestation and inadequate postharvest handling (AMAGRO, 2011). However, the government of Tanzania has started initiatives of removing these

constraints through trainings, researches and encouraging public private partnership so as to meet the required standards of the final produce for both consumption and exportation (Mgaiwa & Poncian, 2016).

2.2 An Overview of Avocado and Mango Seed Kernels and their Potential Use

Plants naturally contain a mixture of compounds that may act independently or in combined form to improve the health of humans (Lasano *et al.,* 2019). Recent studies have found higher levels of potential nutrients and bioactive compounds in waste food materials than in the actual products, comprising a wide category of fruits and seeds (Olawoye & Gbadamosi, 2017). Avocado and mango seeds kernels are among the part of the fruits which have a great potential to improve nutrition and wellbeing of people. In most cases, these seeds are discarded (Soong & Barlow, 2004; Dabas *et al.,* 2013). However, avocado and mango seeds are good source of protein, fat starch, vitamins amino acids, polyphenols and phytosterols (Akho, 2011; Kittiphoom, 2012). The compositions of avocado and mango seed kernels vary between cultivars and sometimes vary between the same cultivar. This phenomenon is normal because each cultivar is different and there are many factors that influence the composition of the fruit during its development, including the region of avocado and mango production, climate, altitude, precipitation as well as genetics (Torres-León *et al*., 2016; Araújo *et al*., 2018)

Avocado and mango seeds kernels are the rich source of nutrients and phytochemical compounds such as vitamins, minerals, dietary fiber, complex mixture of polyphenolic compounds and healthy sugars which have a wide range of physiological functions including; cholesterol lowering, anti-tumor, anti-viral, anti-bacterial activities, cardio-protective, dermatological use, anti-inflammatory and inhibition of lipid and protein oxidation (Kittiphoom, 2012; Dabas *et al.*, 2013; Mahawan *et al*., 2015; Tremocoldi *et al.,* 2018). Because of these health benefits, people are increasingly processing these seeds into various products for human consumption in various places of the world (Henly *et al*., 2015).

In some societies of Tanzania, these seeds are locally prepared into flour and used as flavor ingredient in porridge or drinking tea. They also use the flour as a relief drink in treating diarrhea, teeth aches as well as skin diseases (Henry *et al.,* 2015). However, one of the challenges these people face is the absence of a proper processing method that would ensure the quality and safety of the intended end products. So, given the curative evidence of these seeds, there is a need to explore optimized processing technique that will enhance the utilization of these seeds that are usually regarded as wastes in many countries to be in cooperated in various

functional foods as natural ingredients rather than relying on synthetic additives which may exhibit toxicity, manufactured at high costs and show lower efficiency (Song & Barrow, 2004).

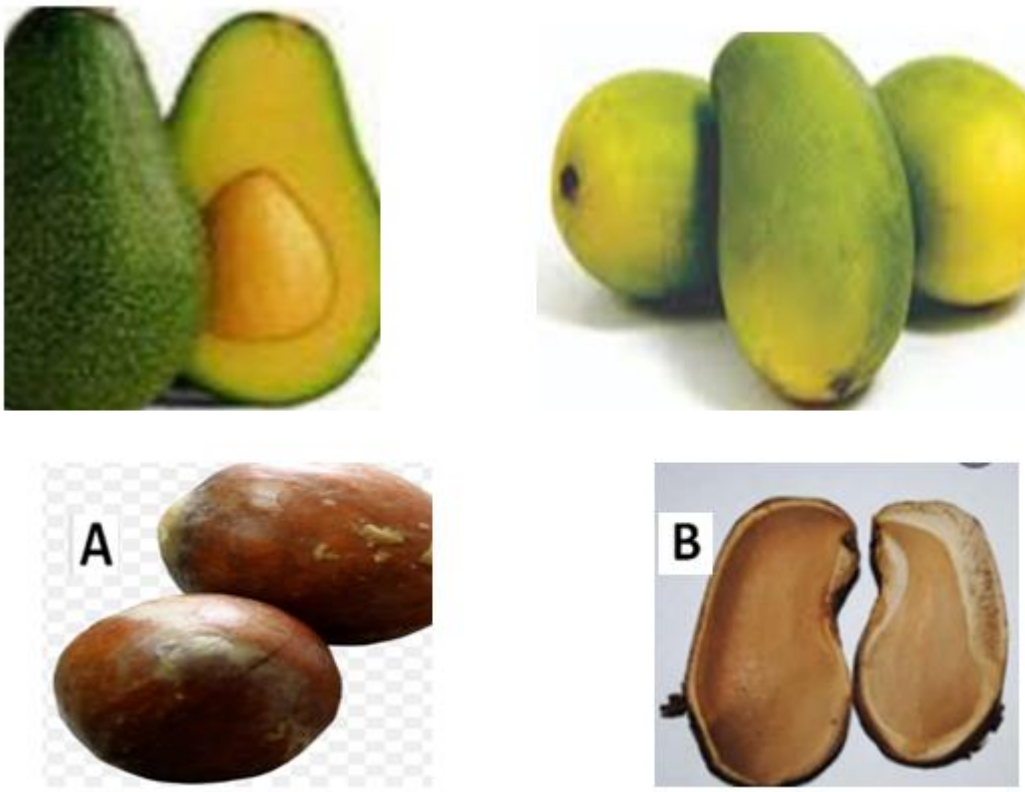


Figure 1: Avocado seeds (A); (B) Mango seed kernels

2.3 Factors Hindering Utilization of Avocado and Mango Seeds

A number of factors that limit optimal utilization of avocado and mango seeds in the food industry have been reported (Henly *et al*., 2015). Some of these factors include high levels of anti-nutritional factors such as tannin, phytates, oxalates and saponin which contribute to the astringent taste of seed kernels; lack of commercial application (unlike the oil seeds) and popularity; the absence of optimal processing method for ensuring safety and nutritional qualities of the intended end product; and limited or lack of informations on the nutritional and health benefits of the seeds. These factors include but not limited to what described below.

2.3.1 The high Content of anti-nutritional Factors in the Seeds

Anti-nutritional factors are substances when present in any food tend to complex the potential nutrients through their metabolic process or product, hence reduce the availability of one or more nutrients and bioactive compounds upon intake, digestion, absorption and utilization of foods in the human digestive system (Yacout, 2016). For examples, tannin contents bind protein

to form insoluble complexes (Henly *et al.,* 2015). The negative effects of these anti-nutrients rely on their concentration, chemical structure, time of exposure and interaction with another dietary component (Popova & Mihaylova, 2019). The anti-nutritional factors are regarded such due to their negative effect or non-nutritive effect on human health. On the contrary, these anti-nutritional compounds are useful in plants and seeds as they are used to protect them against molds, bacteria, birds and other predators (Akho, 2011).

High anti-nutritional factors in avocado and mongo seeds signify potential threat in the use of these seeds in the human diet, despite their high nutritional and functional values. These compounds are present in higher concentration in the seeds and thus results in complexes of essential nutrients and bioactive compounds which may eventually lead to allergic reactions after consumption. For example, the bitterness of the seeds of avocado and mango is attributed to the presence of tannins which bind proteins and form insoluble complexes. These complexes render proteins indigestible by intestinal enzymes thereby interfering with their bioavailability and in some cases cause allergic reactions (Talabi *et al.,* 2016; Henry *et al*., 2015; Torres-León *et al.,* 2016).

Furthermore, phytic acid (phytates) which is abundantly found in these seeds is a strong chelator of many divalent minerals such as iron, magnesium, and calcium and thus renders them biologically unavailable (Lopez *et al*., 2002). However, the antinutritional factors have biological beneficial responses, for example some are widely applied in pharmacology as active ingredients. Saponins for example, if present in acceptable levels bind cholesterol and form insoluble complexes, which prevent cholesterol reabsorption and hence reduce the level of serum cholesterol in the body (Henry *et al.,* 2015; Tadele, 2015). Additionally, epidemiological and controlled-case studies reported by McCranie *et al*. (2011) showed that many anti-nutritional compounds when present in a low level which is acceptable may offer beneficial effects, especially in the prevention of non-communicable diseases like cancer and coronary diseases. This implies that anti-nutrients when reduced to acceptable levels may not be harmful (McCranie *et al*., 2011). Therefore, there is a need to develop a processing technique that will reduce them to acceptable levels so as to allow their beneficial functions in the human body.

2.3.2 Lack of Commercial Application and Popularity

Unlike the oil seeds such as sunflower, sesame, castor, chia, mustard, cotton and palm seeds, mango and avocado seed kernels have not generally received much attention despite being packed with essential nutrients and bioactive compounds. This popularity may be contributed by

less commercial applications (Song & Barrow, 2004). There are high amount of these seeds arising upon consumption and avocado or mango processing plants in various places of the world. This has led to disposal of these seeds which has eventually caused accumulation of waste in places where these fruits are being cultivated especially during the peak seasons. For example, there is about 3×10^5 tons of dry mango seed kernels available annually in India after consumption or industrial processing of mango fruits (Song & Barrow, 2004). Therefore, it would be beneficial to utilize the complete fruit instead of only consuming or processing the edible parts. This can be achieved by processing the thrown seeds into consumable products using optimal method so as to benefit the packed essential and bioactive compounds from them.

2.3.3 Limited Information on the Nutritional and Health Benefits of Avocado and Mango Seed Kernels

Underutilization of avocado and mango seeds is partly contributed by limited informations regarding their nutritional and health benefits to humans. Thus regarded as waste in most of the countries including Tanzania despite the fact that they are a rich source of most essential nutrients and health-promoting bioactive compounds (Henry *et al.,* 2015). The higher content of phytonutrients in these seeds can be harnessed for nutritional and functional purposes or as an alternative to most commonly used synthetic antioxidants like *butylated hydroxyanisole* (BHA) or butylated hydroxytoluene (BHT) which in high doses may become toxic in the body (Raihana *et al., 2015;* Moure *et al.,* 2001). Lack of scientific information on effectiveness of the processing techniques to reduce toxic substances has led sensitive consumers not to consume the seeds even though there are some people who use these seeds locally as a relief drink in treating diarrhea, teeth aches and skin diseases (Henry *et al.,* 2015). This means that if these seeds are properly processed and consumers are well informed on their importance of contributing to human health, they may be utilized to the maximum benefit.

2.3.4 The Absence of Optimized Method for Reducing Anti-nutritional Compounds

The absence of optimized method that can reduce anti-nutritional compounds and at the same time retain nutrients and improve sensory attributes is another limiting factor for the reported underutilization of avocado and mango seeds (De Lange *et al.,* 2000; Diarra, 2014). Various techniques employed in the reduction of anti-nutritive substances in the seeds of these fruits include simple treatments such as thermal, non-thermal, autoclaving and natural fermentation (Hendek & Bektaş, 2018). Natural fermentation is a process in which microorganisms are already parts of the natural micro flora present in the foodstuff and they only require creation of

the necessary conditions (e.g., the creation of anaerobic conditions) for their development (Voidarou *et al.*, 2021). These methods often aim only at reducing or removing the anti-nutritional compounds without regarding their effects on other potential nutrients and bioactive compounds such as water soluble and heat sensitive nutrients. In this regard an optimized method for reducing anti-nutritional compounds in the seeds of avocado and mango to an acceptable level (e.g., reduction of tannin content to above 50%**)**, yet retaining nutrients, improving sensory attributes and increasing the shelf life of the intended final product is needed (Ojha, 2020). This method may help practitioners to optimally harness the potential of avocado and mango seeds in the food industries.

2.4 Methods for Processing Avocado and Mango Seeds

2.4.1 Common Methods

There are various existing methods that have been employed to improve taste by reducing anti-nutritional compounds in the seeds of avocado and mango. Some of these methods include thermal and non-thermal techniques, anaerobic fermentation and addition of chemicals that have high affinity to anti-nutritional compounds (Akho, 2011). None of these methods have been proven to be effective on nutrients and functional value retention in the intended final products (De Lange *et al.,* 2000). Also, those methods which have been deployed have produced mixed results. For example, a study by Diarra (2014) showed that soaking method reduced up to 95.8% of tannis in mango seed kernels. On the contrary, a similar study by Ojha (2020) reported relatively smaller proportions (39%) of tannins were removed by soaking in the final product despite being soaked at similar conditions.

In fact, there is no proper justification to indicate that a specific technique has been proven to reduce the anti-nutritional compounds to an acceptable level (e.g., reduction of tannin content to above 50%) and at the same time retaining essential nutrients and improving sensory attributes. This calls for the development of an optimal processing method that can reduce anti-nutritional compounds in these seeds and yet retain essential nutrients and functional values such as water soluble and heat sensitive nutrients.

2.4.2 Optimized Method

Optimized method is a method which often shows best performance or results in comparison to other methods and usually is the method of choice if it can solve a target problem (Alexey, 2021). For optimization, the method that will reach the highest degree of reducing or removing

antinutrients to acceptable levels, yet retaining nutrients and functional values and improving sensory attributes is considered as an optimized method for processing avocado and mango seed kernels. In other hand common methods have a number of technological weaknesses which need to be addressed (Ojha, 2020). However, the strength of an optimized method addresses the shortcomings of the common methods. Therefore, based on the original assumptions of the common methods and the suggested optimization, an optimized method defines for the evaluation in the remediation of the best represents (Braun *et al.*, 2019).

2.4.3 Fermentation

Fermentation is a process that helps break down large organic molecules via the action of microorganisms into simpler ones (Sharma *et al.*, 2020). For example, yeast enzymes convert sugars and starches into alcohol, while proteins are converted to amino acids. The microbial or enzymatic actions on food ingredients tend to ferment food, leading to desirable biochemical changes responsible for the significant modification to the food. In this regard, fermentation is a natural way of improving vitamins, essential amino acids, anti-nutrients, proteins, food appearance, flavors and enhanced aroma (Rollan *et al.*, 2019). Therefore, the activity of microorganisms involved in the fermentation process play a significant role in the fermented foods by showing changes in both chemical and physical properties. Fermented foods have several advantages such as; longer shelf life than the original foods, increase or retaining the bioavailability of essential nutrients and enhancement of organoleptic properties for example, cheese has more enhanced organoleptic properties in terms of taste than its raw milk (Sanlier, 2019). Mechanism of action of food fermentation essentially takes place in anaerobic conditions when there is no oxidative phosphorylation to maintain the production of ATP (Adenosine triphosphate) by glycolysis (Nkhata, 2018). During fermentation pyruvate is metabolized to diverse compounds such as alcohol, carbon dioxide, acetic acid, ethanol and hydrogen depending on the type of fermentation. However, conversion of pyruvate to fermentation end-products does not produce any energy since it rejuvenates nicotinamide adenine dinucleotide (NAD), which is required for the glycolysis process (Sharma *et al.*, 2020).

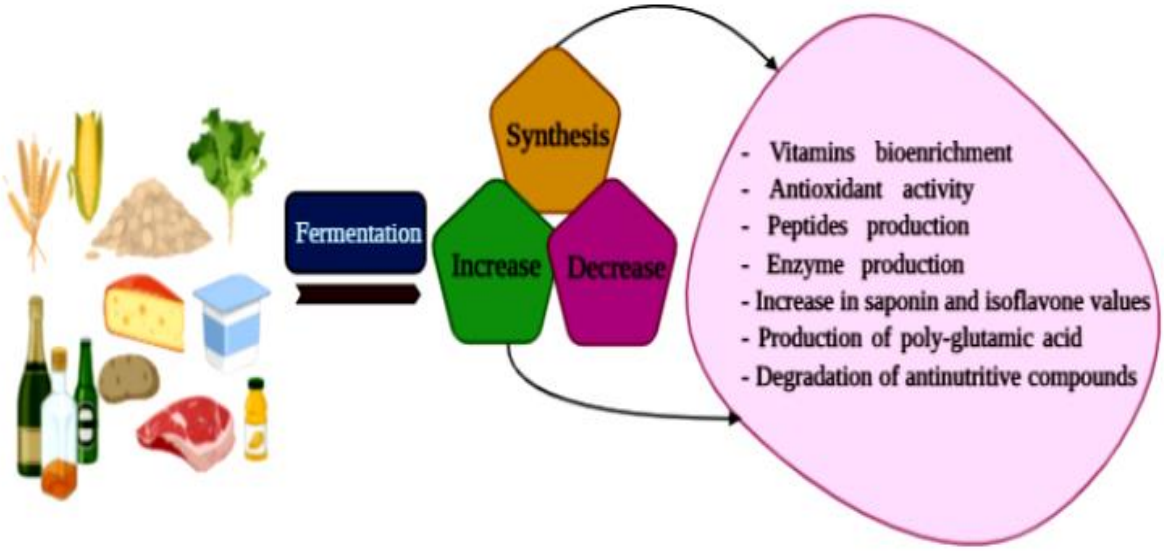


Figure 2: Nutritional enhancement of fermented foods (Sharma, 2020)

2.5 Environmental Pollution by Mango and Avocado See Kernels

Solid waste management is both an urban and rural problem and it has been noted to be a global universal issue, which affects every individual, families, communities and governments (Nyampundu *et al.,* 2020). Pollution in the world is attributed to different actions and the base is found in the excessive use of natural resources and lack of environmental education which all result into health problems (Lorenzo-Santiago *et al.,* 2018). Tanzania like other developing countries faces a serious concern and challenges on solid waste management, which is more pronounced in the commercial and market places where most people visit to sell or buy goods without necessary infrastructures and quality social services (Nyampundu *et al.,* 2020). The most common market solid wastes in the country include among others; food/animal product remains, bottles, metals, garbage, papers, plastics and glasses, However, the status has caused environmental problems especially in market places that endanger public health. Moreover, the limited sorting process of solid wastes at the source that leads to improper collection, storage, transportation, treatment, and final disposal at the dumping areas is experienced in the country (Nyampundu *et al.,* 2020).

This indicator might be attributed by either lack of enforcement of environmental laws, regulations, awareness levels, unwillingness of the urban dwellers and vendors to participate or pay for the waste management services (Nyampundu *et al.,* 2020). Based on that solid waste management in cities and towns might not be as effective to manage ever increasing volume and variety of waste, solid waste collection, storage transportation and disposal system as it is required. The disposal of wastes generated from avocado and mango fruits into the environment

pose a problem as they decompose hence emitting harmful greenhouse gases and objectionable odors. In Tanzania solid wastes are generated at the market account for 86% where fruits residues are inclusive (Nyampundu *et al.,* 2020). The wastes from theses fruits are extensively disposed as wastes by consumers and fruit processors during the eating of fresh fruits, making of juice, jams and snacks. For example, there is about 3×10^5 tons of dry mango seed kernels not utilized annually in India and thrown into the environment after consumption or industrial processing of mango fruits (Soong & Barlow, 2004). Thus, it would be useful by preventing the disposal of these wastes from mango and avocado fruits as animal feeds or turning them into utilizable foods so as to prevent them being thrown into the environment (Henly *et al*., 2015)

2.6 Safety of Avocado and Mango Seed Kernel Extracts

Reports have revealed that many parts of the plants used as foods or traditional medicine have cytotoxic, mutagenic and genotoxic properties (Padilla-Camberos *et al.*, 2013; Adegbehingbe *et al.,* 2017). This shows that there is a need to understand the toxicological profile of substances that are in direct or indirect contact with humans. Therefore, to be sure of the toxicological profile of the avocado and mango seed extracts, it is also necessary to test or get scientific proofs conducted in areas of potential damage, such as those related to the immune system and those that alter endocrine function.

Based on earlier studies, literature has shown that there have been various studies conducted on avocado and mango seed extracts using animal models. For instance, results obtained in the study of Padilla-Camberos (2013) and Mas'Ud *et al.* (2017) using extracts of avocado and mango seed kernels in animal models were recommended safe to be incorporated into various foods. Other studies have reported that acute and sub-chronic safety of orally administered seeds extract of avocado evaluated in the rat was found to be safe even at high doses and this was observed when an aqueous extract of avocado seed (2-10 g/kg) administered as a single dose to rats with no signs of toxicity in the two subsequent weeks observed (Cárdenas, 2012).

It was also found that toxicological evaluation of rat which was fed by mango seed extract revealed that the liver serum, total cholesterol, triglyeerides and total lipids of the administered animal was within the normal levels and the studied various organs showed no abnormalities (Rukmini & Vijayaraghavan, 1984). Thus, the study conducted recommended that mango kernel extract can be substituted for any foods without adverse effects. On the other hand, study conducted by Abdalla *et al*. (2007) and Araújo *et al.* (2018) on avocado and mango seed kernel extracts reduced total bacterial count, inhibited coliforms growth, showed remarkable

antimicrobial activity against *Escherichia coli* strain and extended the shelf-life of fermented extracts of avocado and mango seed kernels. With these evidences it is true to incorporate the developed extracts of avocado and mango seeds kernels into various functional foods so as to tap their potential benefits without compromising their composition as reported by Maisuthisakul and Gordon (2009) and Olaleye *et al.* (2020).

2.7 Food Security, Nutritional and Functional Foods

2.7.1 Food Security

Food security is defined as a situation that exists when all people, at all times, have physical, social and economic access to sufficient, safe and nutritious food that meets their dietary needs and food preferences for an active and healthy life (Food and Agriculture Organization [FAO], 2006). Food security is a linked pathway from production to consumption and through distribution to processing (Bilali, 2019). The absence of food security brings the stresses of food insecurity. In Tanzania the main source of food to the majority is through agricultural production (USDA, 2015). Despite the fact that the country is not drought prone, but food insecurity is temporary in nature (Saruni *et al.,* 2018). Temporary food insecurity arises from instability of food production, food prices, or household's income commonly experienced in marginal areas of the central and northern regions of Dodoma, Singida, Shinyanga, Tabora, Tanga, Arusha, Kilimanjaro and Manyara (Saruni *et al.,* 2018). It has been observed that even in areas with surplus production of food, farm households tend to sell their surplus produce immediately after harvest; as a result six to nine months later in the year many do not have their own crop or the cash to purchase food from the market hence suffer from food insecurity (Babantunde *et al.,* 2007). Other factors contributing to seasonal food insecurity include, overselling due to competing needs for cash including health, education and clothing. Inadequate postharvest management knowledge also contributes to food insecurity (URT, 2019).

Achieving food security presents a profound challenge to the government to implement social and economic policies to meet households' dietary requirements. For example statistics indicates that 30% of children were underweight at the age of 5, and 30% of Tanzanians live below the poverty line (Saruni *et al.,* 2018). Since food insecurity incidence increases with increase in household size, efforts should be made at improving programs: (a) through crop and livelihood diversification by decision makers (b) provision of input such as herbicides, fertilizer and improved seeds will motivate farming households and also increase their productivity (c) through innovative ways by products of emanating from fruits' wastes like the seeds of avocado

and mango should be utilized into consumable products this will increase the volume of food produced hence stabilize food security in Tanzania (Henly *et al.,* 2015). Therefore, processing avocado and mango seed kernels into consumable products will increase the foods and product variety for consumers to choose.

2.7.2 Nutritional Versus Functional Foods

Functional foods are considered to be those whole, fortified, enriched or enhanced foods that provide health benefits beyond the provision of essential nutrients (for example, vitamins and minerals), when they are consumed at efficient levels as part of diet on a regular basis (Hasler, 2018). The effect of functional foods in human body, lays on their physiological benefits which can reduce the risk of chronic diseases such as cancer, diabetes and cardiovascular illnesses (Cencic & Chingwaru, 2010). However, any health benefits attributed to functional foods should be based on sound and accurate scientific proofs on their efficacy (Hasler, 2018). On the other side the nutritional foods are foods that are packed with substances such as proteins, vitamins, fats and carbohydrates which provide nourishment essential for the growth of the human body (FAO, 2019). Consuming nutritional and functional foods in the daily diet may contribute to immune competence and lack of these foods impairs the immune system and suppresses immune functions that are fundamental to the protection of human body (Calder & Kew, 2002). Thus, avocado and mango seed kernels are the rich source of proteins, starch, phenolic contents, antioxidants and minerals which play a great role in the body growth and immune competence (Henly *et al.,* 2015).

2.8 Future Prospects

Based on the reported potential health benefits and business opportunities around avocado and mango seeds, researchers have been urged to develop a solution for maximum utilization of these seeds. The most fascinating thing in this aspect is the applying of an optimized processing method which will help processors and consumers benefit from both economic and health potential of the seeds. This has led many researchers to put their thoughts on the science of developing this processing method. One of the things scientists have noted so far is that existing processing methods have a number of technological weaknesses which need to be addressed (Ojha, 2020). It is under this ground that optimized processing method should be developed for proper utilization of avocado and mango seeds. This method would help processors to produce the extract from these seeds which can then be incorporated into production of various functional and nutritious products such as biscuits, bread and other related products for human

consumption. This approach is expected to add value to these seeds, open business opportunities for stakeholders to generate income and reduce environmental pollution emanating from their disposal.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

Samples were collected from two districts in Tanzania namely Muheza (Tanga) and Rungwe (Mbeya) whereby sample preparations and all laboratory works were carried out at Sokoine University of Agriculture (SUA) and Jomo Kenyatta University of Agriculture and Technology (JKUAT).

3.2 Methods

3.2.1 Optimizing a Processing Method Suitable for Reducing Anti-nutritional Compounds, Retaining Nutrients and Functional Values, and Improving Sensory Attributes of Avocado and Mango Seeds

(i) Research Design

Purposive sampling procedure was used to collect a variety of a chosen avocado (Fuertes) and mango (Dodo). The 'Fuertes' and Dodo fruits were chosen in this study because they are highly cultivated varieties and most preferred by families of Tanzania (URT, 2017). Randomized Complete Block Design (RCBD) was used in this study to assess the effect of the selected processing techniques (probiotic fermentation, boiling and soaking) on the analyzed nutritional and functional properties of avocado and mango seed kernel extracts. Laboratory analysis was conducted to validate the analyzed nutrients, antinutrients, minerals, total phenol and antioxidant activity using analytical standard methods and obtained data were statistically analyzed using R statistical package (R Development Core Team, Version 3.6.2 Vienna, Austria).

(ii) Sample Collection

Selected matured and ripen fruits (280 *Fuertes* avocado and 340 *Dodo* mangoes) were collected directly from farmers in Muheza and Rungwe districts to ensure freshness of the produce and to ascertain that they were the exact cultivars of the study districts. The fruits were packed in clean containers and then transported to Sokoine University of Agriculture. The stated quantities of fruits above delivered 4 kg of flour from each sample enough for laboratory analysis and product development (formulations, sensory evaluation and shelf life determination) requirements. The indicated quantities of avocado or mango seed kernels had been

predetermined (where 70 dried avocado seeds gave 1 kg flour and 85 dried mango seed kernel gave 1 kg of flour) on dry basis after being dried using a solar tunnel dryer.

(iii) Sample Preparation

Collected fruits were first washed with clean water to remove debris on the upper layer (skin) followed by separating seeds from the edible parts using a kitchen knife. The separated seeds were immediately sliced cross-sectionally at 0.2 cm thickness for each slice. The seeds were cut using a food grade machine slicer equipped with the cutting discs which were flexible to be adjusted depending on the size required (Henan, China). This thickness was within the range as reported by other scholars in the processing and drying of fruit seeds. For example, the study conducted by Hau *et al.* (2019) on the optimization for extraction of polyphenols from avocado seeds used the thickness of 0.25 cm. Also Dorta *et al.* (2012) used the thickness of 0.5 cm in drying treatments for stabilizing mango seed kernel on the effect of antioxidant activity. The chopped pieces were then placed into open containers ready for the probiotic fermentation, boiling and soaking.

Figure 3: Sample preparation of avocado and mango seed kernels before being subjected to different processing techniques

(iv) Processing Methods

In order to learn or examine the effects of different processing methods on the reduction of anti-nutritional factors, retaining of nutrients, functional values and improving of sensory attributes sliced avocado and mango seed kernels were fermented, boiled and soaked as discussed below.

- **Probiotic Fermentation**

The overall process of probiotic fermentation involved three key steps. The first step was the preparation of the starter cultures and samples. Lactic acid bacterial (LAB) namely *Lactobacillus plantarum* (B-3058), *Lactobacillus johnsonii* (B-2178) and *Lactobacillus rhammnosus* (B-58149) were obtained from the Agricultural Research Service Culture Collection (NRRL) United States, Department of Agriculture, Peoria, Illinois, USA. The culture were collected from NRRL, USA because this is a chief supplier of all the starter culture for research purpose on free basis and they only require you to give them feedback and acknowledgement. The selection of these probiotics was due to their superior properties of working better in reducing of antinutrients, retaining or improving of the essential nutrients and

bioactive compounds of various foods during fermentation (Filannino *et al.,* 2020; Moroni *et al.,* 2015). Preferably, the selected probiotics are of food grade with all components appropriate for use in the preparation of food ingredients (Moroni *et al.,* 2015). All the strains received were in a lyophilized state, thus activation process was needed in order for them to work as required. Activation of the lyophilized strains were achieved following the procedures as reported by Nyamete *et al*. (2016). The sliced pieces of the avocado or mango seed kernels were firstly dipped in ethanol followed by several times of washing with deionized water prior to inoculation to kill any indigenous microorganism that could interfere with the controlled fermentation.

The second step encompassed inoculation of the samples, which was done by mixing the prepared sample with portable water in the ratio of 1:1 in the sterile covered containers followed by inoculation with 1 ml of lactic acid bacteria culture. The inoculated samples were mixed well and covered aseptically, then put into the incubators at temperatures of 30 ^{o}C, 37 ^{o}C and 42 ^{o}C following the manufacturer's instructions (NRRL).

The third step involved monitoring of probiotic fermentation by measuring parameters which included the pH and lactic acid prior to fermentation at an interval of 6 hours to completion of fermentation within 24 hours. The pH was measured using the digital pH meter while lactic acid was measured by calorimetric method according to Taylor (1996). After fermentation, all samples were removed from the incubators and dried into the solar tunnel dryer till the required moisture content was attained (8%).

- **Boiling**

Approximately 2 liters of portable water was poured into the water bath and then switched on till boiling was attained. The sliced pieces of avocado or mango seed kernels were put separately for each seed into the boiled water in the water bath set at 100 ^{o}C. The samples put in the water bath were monitored at a constant boiling temperature with different time interval of 5 minutes, 10 minutes, 15 minutes and 20 minutes as reported by Talabi *et al.* (2016). Finally the samples were removed from the water bath and dried with the solar dryer tunnel until the required moisture content was obtained.

- **Soaking**

Small seized pieces of fresh avocado or mango seed kernels were soaked in the portable water with varying time of 6 hours, 12 hours, 18 hours and 24 hours according to the methods

portrayed by Adeleke *et al.* (2017) with some modifications. The seeds were soaked in water at the ratio 200 g:12 L; seed kernels:water. This ratio was adapted from previous published work by Oboh *et al.* (2016) with some modifications. The modification in this experiment based on amount of water to sample weight ratio used. After soaking, all the samples were removed from the water and dried into the solar dryer tunnel until the required moisture content was obtained.

- **Experimental Set-up**

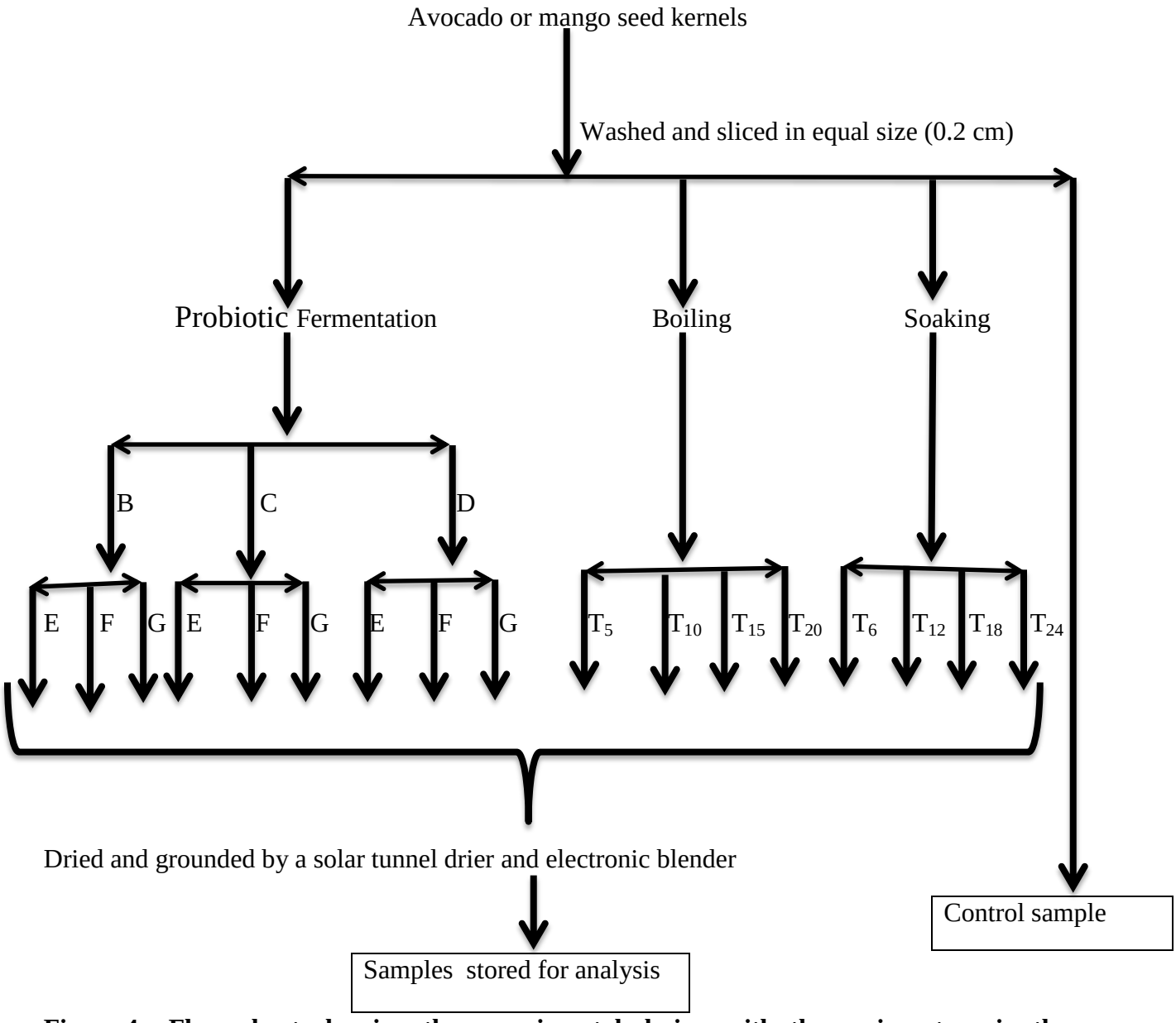


Figure 4: Flow chart showing the experimental design with the major steps in the processing avocado and mango seed kernels. B= *Lactobacillus plantarum*, C= *Lactobacillus johnsonii*, D= *Lactobacillus rhammnosus*, E=30^{o}C, F=37^{o}C, G=42^{o}C, T= Time, T5=Five minutes, T10=ten minutes, T_{15} =fifteen minutes, T_{20}=Twenty minutes, T_6=six hours, T_{12}=Twelve hours, T_{18}=Eighteen hours and T_{24}= Twenty four hours

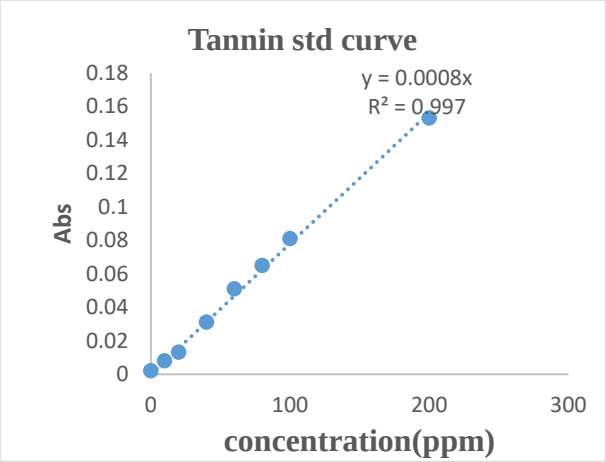
(v) Drying Procedures and Pulverization

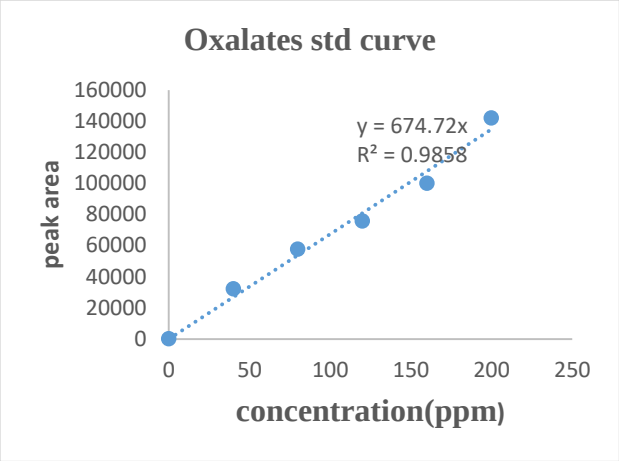
All samples after being treated to processing methods (probiotic fermentation, boiling and soaking) were dried into a solar drier tunnel while monitoring moisture content until it reached the steady state which is 8% (Mongi, 2013). The dried samples were grounded using an electric blender into fine powder. The grounded powder was packed in airtight polyethylene bags and stored at room temperature until analysis.

(vi) Determination of Antinutritional Compounds, Nutritional and Functional Values of the Selected Avocado and Mango Seed Kernels

- **Antinutrients Analysis**

In this study, the anti-nutrients analyzed included oxalates, phytates, tannins and saponin. Oxalates and phytates were analyzed by using the HPLC as described by Vũ *et al.* (2013). Tannin content was determined by using UV-Vis spectrophotometer (Shimadzu model UV – 1601 PC, Kyoto, Japan) as described by Arslan *et al*. (2016), while Saponin was determined using the screening method as reported by Ejikeme *et al*. (2014) (Fig. 5).





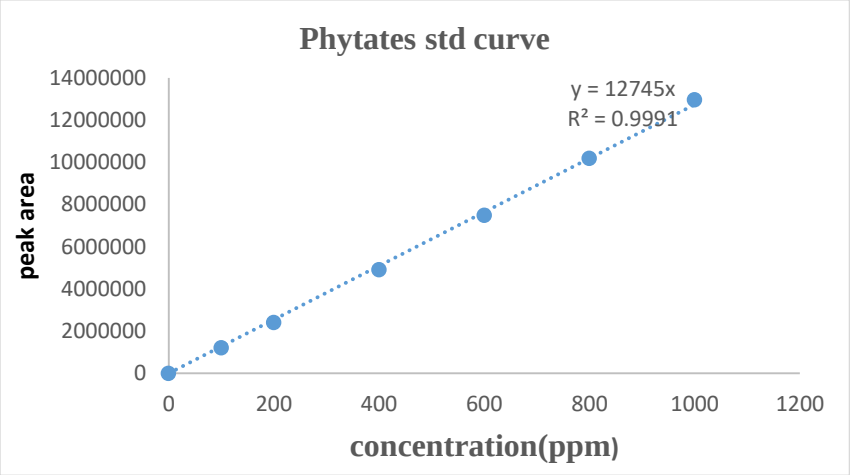


Figure 5: Stand curves for absorbance/peak area against concentrations

- **Total phenolic Compounds**

Total phenolic compounds were determined by using the method described by Molyneux (2004) and Arslan *et al.* (2016) with some modifications. Briefly, 10 milligrams of the sample was extracted with 20 ml of 50% aqueous methanol at 80 ºC for 1 hour, followed by filtration and volume made to 50 ml. Further, 1 ml of the solution was put into 50 ml volumetric flask and 20 ml of distilled water added followed by 2.5 ml of folin-ciocalteu reagent and 10 ml of 17% sodium carbonate. The mixture was homogenized and made to 50 ml with distilled water and after 20 minutes, absorbance was read on UV spectrophotometer at 760 nm using gallic acid as a standard. The results of total phenolic contents were calculated using the standard calibration curve of gallic acid and expressed as gallic acid equivalents (GAE) per 100 g (Fig. 6).

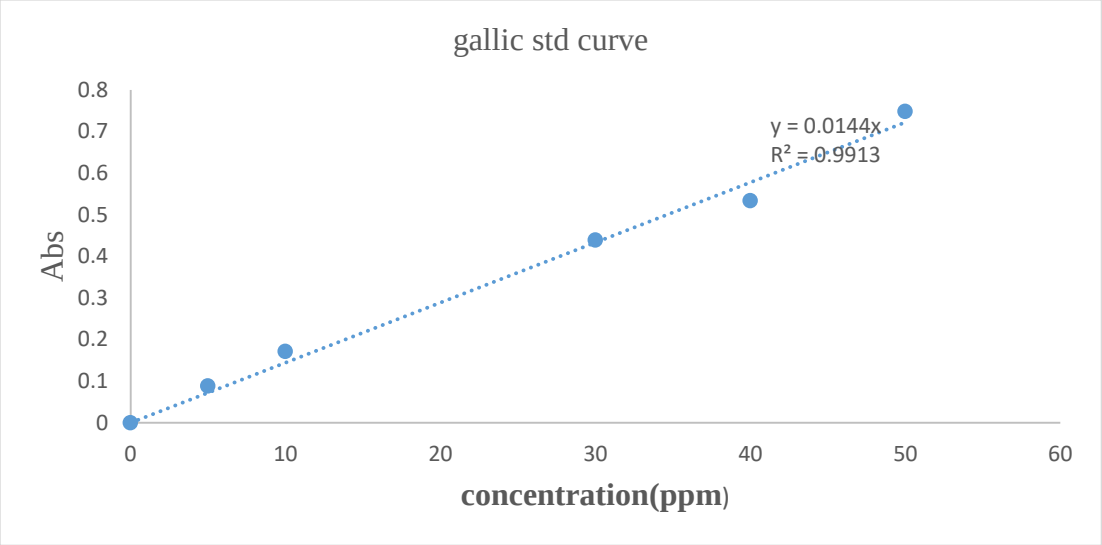


Figure 6: The graph of Gallic standard curve

- **Free Radical Scavenging Activity**

The radical scavenging activities of the extracts was determined in accordance with the Molyneux (2004) method with slight modifications, which is based on the principle of scavenging the DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical. Briefly, 0.5 ml DPPH was added to the solutions prepared with 2.5 ml extracts of avocado or mango seed kernel and 3 ml of methanol. The obtained mixture was vigorously shaken for 20 minutes and left to stand in the dark room for 1 hour. The same procedure was performed for the control sample containing 3 ml of methanol and 0.5 ml of DPPH. The UV-Vis spectrophotometer (Shimadzu model UV – 1601 PC, Kyoto, Japan) was used to read the absorbance at 517 nm. Vitamin C was used as the antioxidant standard. The radical scavenging activity was calculated using the following formula:

$$\text{Percentage inhibition of DPPH} = \{(A_B - A_A)/A_B\} \times 100 \quad (1)$$

Where A_B is the absorption of blank sample and A_A is the absorption of tested extract solution. The results were expressed as percentage inhibition of DPPH and mean inhibitory concentrations (IC_{50}) was determined from a plot of percentage inhibition of DPPH versus concentration of the of avocado or mango seed kernel extract.

- **Mineral Determination**

The minerals that were determined in this study were potassium, sodium, calcium, iron and zinc. Minerals were analyzed using atomic absorption spectrophotometer (AAS) according to the method of Nanda *et al.* (2003). A 5.0 g of weighed sample was placed into a clean dry crucible. The weighed crucible containing the sample was placed into the muffle furnace whose temperature was set at 550 ^{o}C. After complete ashing the crucible was removed from the muffle furnace and cooled at room temperature. The ash was transferred to 100 mL beaker using 20 mL of 1N HCL, then heated at 80-90 ^{o}C on a hot plate for 5 minutes. This was then transferred to 100 mL volumetric flask and filled to the mark using 1N HCL. Insoluble matters were filtered and the filtrate kept in a labeled prepared containers. The absorbance of the extract was read by atomic absorption Spectrophotometer. Mineral standards were prepared to make the calibration curve (Fig. 7).

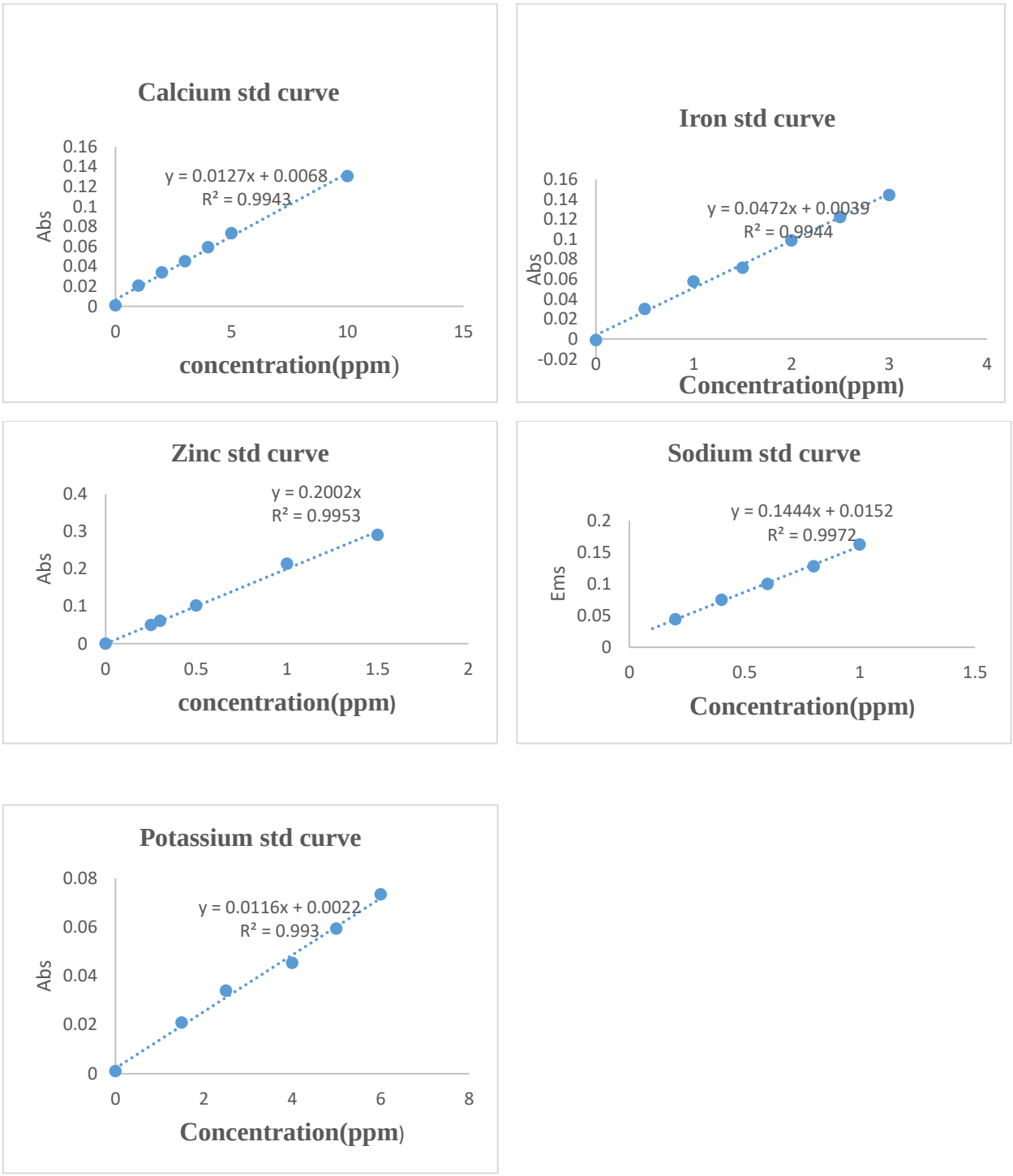


Figure 7: Standard curves of absorbance versus concentration of different analyzed minerals

- **Ascorbic Acid**

Analysis of vitamin C (ascorbic acid) content was performed by high performance liquid chromatography (HPLC) using a Shimadzu UV-VIS detector of 254 nm as reported by Vikram *et al*. (2005) with slight modifications. Triplicate samples of ≥2.0 g were weighed and extracted with 0.8% metaphosphoric acid as a mobile phase. The mixture was made to 20 mL followed by

agitation at 10000 rpm for 15 min. The supernatant was filtered and diluted with 10 mL of 0.8% metaphosphoric acid. This was passed through 0.45 µ filter and the isolate supernatant was injected into the HPLC. Various concentrations of ascorbic acid standards were made and used to draw the calibration curve (Fig. 8).

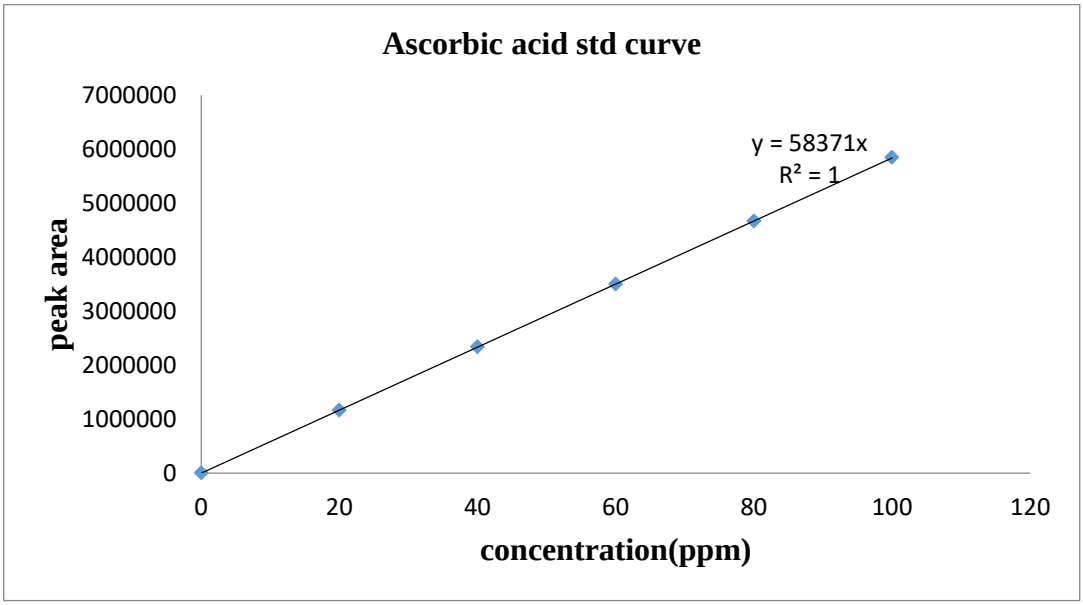


Figure 8: Graph of ascorbic acid standard curve

- **Determination of α-tocopherol Content**

Vitamin E (α-tocopherol) was analyzed using a modification of the method of Barker *et al.* (1998) using the HPLC. About 2.0 g of the homogenized sample was mixed with 4 ml of 95% ethanol and 1ml of 50% KOH. The mixtures were saponified by heating at 70°C in the water bath for 15 minutes, followed by cooling in an ice bath container. Fat-soluble vitamins were extracted with 1ml hexane containing 0.2% BHT, and a 1ml aliquot of the hexane layer was evaporated under nitrogen. Saponification, extraction and evaporation procedures were performed under yellow light. There after the samples were reconstituted with 0.25 ml ethanol containing 0.1% BHT. Quantification of α-tocopherol as a measure of vitamin E was done by a Shimadzu 20A Series liquid chromatograph equipped with a 250 x 4.0 mm stainless steel ODS reversed- phase column. The mobile phase used was in the ratio of 96:4 (methanol: water) for α-tocopherol detection. Also the α-tocopherol was monitored at 285 nm (Shimadzu SPD 20A) and the external standard was compared to sample extracts for determination of vitamin concentrations (Fig. 9).

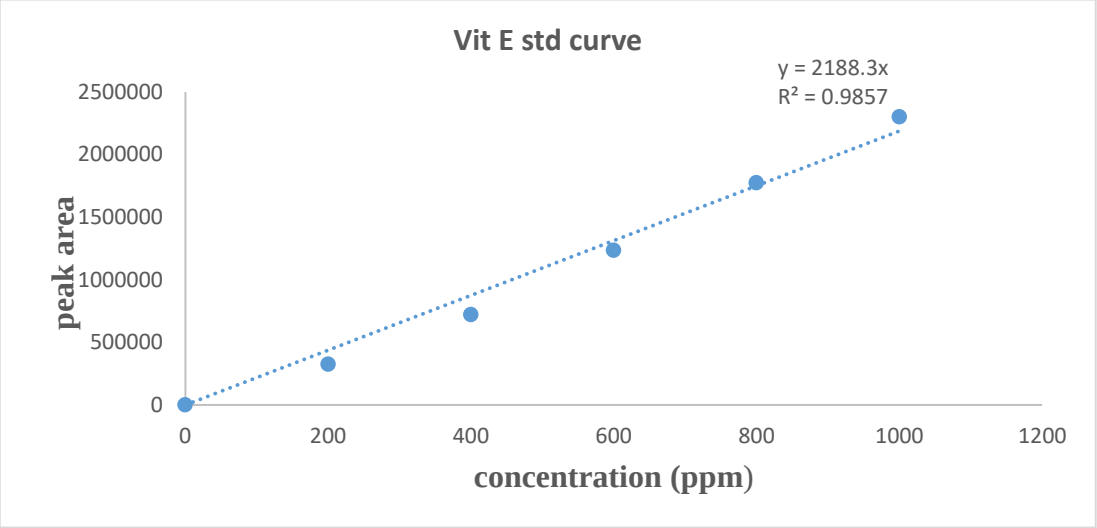


Figure 9: Vitamin E standard curve

- **Profiling of Fatty Acid**

Profiling of fatty acid was determined using a gas chromatographic (GC) method as described in previous studies (Cavonius *et al.,* 2014). About 150 mg of the sample was dissolved in 0.5N methanolic sodium hydroxide and heated on a steam bath. Thereafter, 5ml of methanol was added to the solution and the mixture was boiled. To float the methyl esters up, saturated sodium chloride was added to the solution. The solution was allowed to cool at room temperature and distilled water was added followed by adding n-hexane. Then, the solution was transferred into a separating funnel and left to settle until a clear separation between the organic phase and the aqueous phase was visible. The aqueous layer was drained first into a conical flask. The organic layer was transferred to the second conical flask and anhydrous sodium sulfate was added to remove any trace of water in the organic layer. The methylated fatty acid contained in n-hexane was then transferred to vials and nitrogen gas was blown to remove hexane. The vials were placed on a liquid auto-sampler and a 1.0 µL injection of the prepared sample was introduced into the GC.

(vii) Determination of Consumer Acceptability, Descriptive Sensory Profiling and Preference Mapping of the Products from an Optimized Method

- **Quantitative Descriptive Analysis**

A descriptive sensory profiling was conducted by trained sensory panelists comprising 15 assessors whose age ranged from 23 to 28 years according to the method described in Lawless and Heyman (2010). The assessors were selected and trained according to ISO 586 standard (1993). In a pre-testing session, the assessors were trained in developing sensory descriptors, the

definition of the sensory attributes, test vocabulary in describing differences between the samples and agreed on a total number of attributes to be used. The attributes were color hue, sweetness, texture and aroma as indicated in Table 1 below. An unstructured line scale was used for rating the intensity of an attribute. The left side of the scale corresponded to the lowest intensity of each attribute (value 1) and the right side corresponded to the highest intensity (value 9) as attached in Appendix 2.

Descriptive analysis was carried out in two sessions, in the first session assessors evaluated 3 prepared samples from avocado and the second session involved the analysis of 3 samples from mango seed kernels. The samples were coded with 3-digit random numbers from statistical tables of random numbers and the samples were served to each panelist in a randomized order and instructed to rate the colour hue, sweetness, texture and aroma attributes. Water was served alongside the samples for rinsing the mouth before evaluating another sample during the test session. Thus the average responses were used in the univariate and multivariate analyses.

Table 1: Definitions of sensory attributes used in descriptive sensory analysis

Parameters	Attribute	Definitions
Colour	Colour hue	Yellow/ any colour of egg york of indigenous chicken
Aroma	Fruity (mango/avocado)	Aromatics associated with fresh ripen fruit (mango/avocado)
Taste	Sweetness	The taste associated with sucrose solution (Reference 0.1% sucrose)
Texture	Hardness	Resistance of the sample during pressing

Lawless and Heyman (2010)

- **Consumer Test (Hedonic)**

The test was carried out in the Department of Food Technology, Nutrition and Consumer Sciences, Sokoine University of Agriculture, Tanzania by untrained consumers using a 9-point hedonic scale (where 9- Like extremely, 8-Like very much, 7-Like moderately, 6-Like slightly, 5-Neither like nor dislike 4-Dislike slightly, 3- Dislike moderately, 2-Dislike very much, 1-Dislike extremely) as described by Lawless and Heyman (2010). The prepared samples were coded with three digit random numbers using statistical random tables and served to the panelists at 11:00 am with clean drinking water in a randomized manner. The assessors were instructed to rate the taste, color, aroma, texture and general acceptability attributes indicating

the degree of liking or disliking by putting a number as provided in the hedonic scale according to their preference. The test was done in 2 sessions and each judge evaluated 3 samples per session. This test was completely voluntary and it involved participants of 23 to 28 years of age. The age group of the assessors observed was the turn up of people after publically announced two weeks before the sensory evaluation test. Consent information to conduct the study is shown in Appendix 2. Also the study was approved by the Tanzania National Institute for Medical Research and was given an ethical clearance certificate with a reference number KNCHREC00034 (Appendix 4).

- **Relationship Between Sensory and Consumer Data (Preference Mapping)**

The preference mapping was evaluated by combining the descriptive data and hedonic data obtained from all assessors and the direction of the degree of liking of the products were assessed based on the attributes which drove the panelists to like the products. Preference mapping was done to study the relationship between descriptive data and hedonic liking from the consumers.

3.2.2 Formulation of Avocado and Mango Seeds-based Composite Flour from Optimized Method and Determination of Nutritional Values, Safety and Consumer Acceptability

(i) Formulation of Avocado and Mango Seeds-based Composite Flour

A Recipe and Nutrition Management Workspace integrated with the USDA National Nutrient Database for Standard Reference were used in formulating the composite flour. Avocado and mango seed extracts were used as the main ingredients, green banana as the filler that provided baking characteristics, chia seeds used as a binder that provided stability and thickness and the potato starch was used for adding taste and palatability of the formulated composite flour. Nutrasheets^{TM}, was used to custom batches, add ingredients such as avocado and mango seed kernels which were missing in the USDA database and compute nutrition facts and formulation costs. Finally all the ingredients were blended together using a food grade grinder to obtain the intended composite flour that met the nutrients and functional values as computed by the Recipe and Nutrition Management Workspace.

- **Protocol for Formulation of Avocado and Mango Seeds-based Composite Flour**

Fermented avocado and mango seed kernels by *Lactobacillus plantarum* at 37 ^{o}C, green banana flour, chia seeds and potato starch were blended together using a food grade grinder to obtain the avocado and mango seeds-composite flour. The percentage ratio of the blend was; fermented avocado seeds (28%): fermented mango seed kernels (25.5%): green banana flour (32%): chia seeds (0.8%): potato starch (13.7%). These ratios were computed by a Recipe and Nutrition Management Workspace integrated with the USDA National Nutrient Database for Standard Reference with regard to the set requirements of nutrients and functional values of avocado and mango seeds-based composite flour.

(ii) Determination of Nutrients of the Formulated Composite Flour

- **Proximate Analyses of the Developed Composite Flour**

Determination of moisture, crude ash, crude proteins, crude lipids and crude fiber of the formulated composite flour were carried out in triplicate according to the AOAC (1990) standard methods. Dry matter was determined by drying 2 g of triplicate samples to constant weight in an oven at 105 ºC overnight. Crude ash was determined by incineration at 550 ℃ for 4 hours in a combustion oven (ISO, 2002). Protein content was determined by the standard Kjeldahl nitrogen method following Dumas principle (ISO, 2008). Crude lipid (CL) content (ether extract, EE) was quantitatively determined after extraction with diethyl ether with a Soxhlet system (ST 243 SoxtecTM, Hilleroed, Denmark) (ISO, 1999). Crude fiber (CF) content was determined in triplicate according to the AOAC (1990) standard method 962.09. Finally, carbohydrate was obtained by difference from moisture, crude ash, crude proteins, crude lipids and crude fiber.

- **Minerals, Fatty Acid, Ascorbic Acid and Alpha-tocopherol**

Minerals were analyzed using atomic absorption spectrophotometer (AAS) according to the method of Nanda *et al.* (2003) while profiling of fatty acids were determined by a gas chromatographic (GC) method as described in previous studies of Cavonius *et al.* (2014). Finally, ascorbic acid content was performed by high performance liquid chromatography (HPLC) using a Shimadzu UV-VIS detector of 254 nm as reported by Vikram *et al*. (2005) and alpha tocopherol was analyzed using a modification of the method of Barker *et al.* (1998).

(iii) Determination of Safety Parameters (Salmonella, Molds and Yeast) and Consumer Acceptability of the Developed Avocado and Mango Seeds-based Composite Flour

- **Salmonella**

Determination of Salmonella was done using EN ISO 6579:2002/Amd.1:2007 procedures (Mainar-Jaime *et al*., 2013). This involved four stages which are: (a) Preparation of the initial suspension by adding each test portion to the quantity of non-selective broth (buffered peptone water) to yield a ten-fold dilution, (b) pre-enrichment, (c) planting out the suspected culture on semi solid Rappaport vassaliadis agar and Xylose Lysine Deoxycholate (XLD) and (d) Confirmation of the suspected colony by biochemical test. The counted number of colony forming units (CFU) per milliliter were calculated using the following formula:

$$CFU/ml= (\sum C)/ (n_1+n_2)\ d$$

Where; $\sum C$ is the sum of colonies counted on the retained dishes, n_1 is the number of colonies in the dishes retained in the first dilution, n_2 is the number of colonies in the dishes retained in the second dilution, d is the dilution factor corresponding to the first dilution.

- **Molds and Yeast**

Molds and yeast were enumerated on Potato Dextrose Agar (PDA) with Chloramphenicol (2%) as described by Victor *et al.* (2013). About 15 ml of the medium containing rose bengal chloramphenicol previously melted and maintained at 45 °C in the water bath into each petri dish was carefully mixed with the inoculum. The petridishes were rotated and allowed to mix and solidify. The prepared dishes were inverted and placed in the incubator set at 25 °C for 5 days.

Immediately after the removal of the petridishes from the incubator colonies were counted. The counted number of colony forming units (CFU) per milliliter were calculated using the following formula:

$$CFU/ml= (\sum C)/ (n_1+n_2)\ d$$

Where; $\sum C$ is the sum of colonies counted on the retained dishes, n_1 is the number of colonies in the dishes retained in the first dilution, n_2 is the number of colonies in the dishes retained in the second dilution, d is the dilution factor corresponding to the first dilution.

Consumer Acceptability

The test was carried out in the Department of Food Technology, Nutrition and Consumer Sciences, Sokoine University of Agriculture, Tanzania by untrained consumers using a 9-point hedonic scale (where 9- Like extremely, 8-Like very much, 7-Like moderately, 6-Like slightly, 5-Neither like nor dislike 4-Dislike slightly, 3- Dislike moderately, 2-Dislike very much, 1-Dislike extremely) as described by Lawless and Heyman (2010). The assessors were instructed to rate the taste, color, aroma, texture and general acceptability attributes indicating the degree of liking or disliking by putting a number as provided in the hedonic scale according to their preference. This test was completely voluntary and it involved participants of 23 to 28 years of age.

3.2.3 Determination of Shelf Life of Developed Avocado and Mango Seeds-based Composite Flour using a Predictive Modeling Approach

(i) Shelf Life Determination

Shelf life of the developed avocado and mango seeds-based composite flour was determined using a predictive modeling approach to evaluate how the deterioration process behaves as a function of time as described by Phimolsiripol *et al.* (2016). The microbial safety (Salmonella, yeast and molds), peroxide value and water activity, pH was first defined and determined as the first priority for shelf life of the developed product. Then the samples were exposed to the set extreme conditions (maximum and low) of temperatures and then the peroxide value was used to predict the shelf life of the developed product. The following kinetic equation was used in the prediction of shelf life:

$$r_A = \frac{d[A]}{dt} = K[A]^n$$

Where; k = kinetic constant, r = rate of reaction, t= time, A= net rate of reaction and n= order of reaction

(ii) Microbiological Analysis

- **Salmonella**

Determination of Salmonella was done using EN ISO 6579: 2002/Amd.1: 2007 procedures (Mainar-Jaime *et al*., 2013). This involved four stages which are: (a) Preparation of the initial

suspension by adding each test portion to the quantity of non-selective broth (buffered peptone water) to yield a ten-fold dilution, (b) pre-enrichment, (c) planting out the suspected culture on semi solid Rappaport vassaliadis agar and Xylose Lysine Deoxycholate (XLD) and (d) Confirmation of the suspected colony by biochemical test. The counted number of colony forming units (CFU) per milliliter were calculated using the following formula:

$$CFU/ml= (\sum C)/ (n_1+n_2)\ d$$

Where; $\sum C$ is the sum of colonies counted on the retained dishes, n_1 is the number of colonies in the dishes retained in the first dilution, n_2 is the number of colonies in the dishes retained in the second dilution, d is the dilution factor corresponding to the first dilution.

- **Molds and Yeast**

Molds and yeast were enumerated on Potato Dextrose Agar (PDA) with Chloramphenicol (2%) as described by Victor *et al.* (2013). About 15 ml of the medium containing rose bengal chloramphenicol previously melted and maintained at 45 °C in the water bath into each petri dish was carefully mixed with the inoculum. The petridishes were rotated and allowed to mix and solidify. The prepared dishes were inverted and placed in the incubator set at 25 °C for 5 days.

Immediately after the removal of the petridishes from the incubator colonies were counted. The counted number of colony forming units (CFU) per milliliter were calculated using the following formula:

$$CFU/ml= (\sum C)/ (n_1+n_2)\ d$$

Where; $\sum C$ is the sum of colonies counted on the retained dishes, n_1 is the number of colonies in the dishes retained in the first dilution, n_2 is the number of colonies in the dishes retained in the second dilution, d is the dilution factor corresponding to the first dilution.

(iii) Determination of Physio-chemical Properties

- **Water Activity**

Water activity was determined using the Lab swift-water activity analyzer as described by Novasina (2010). The samples was filled into the sample dish (three-quarters of its volume) then

put into the measurement chamber and closed. The analyzer button was pressed. Thereafter, the results were read when the stable value is displayed.

- **pH Measurement**

pH was measured using the AOAC (1999) standard procedures. A 20 g of the homogenized food sample was dissolved in 100 ml of distilled water for a period of 2hrs and then filtered. The obtained extract was used to measure the pH using a glass electrode pH meter.

- **Peroxide Value**

Determination of peroxide value was done using the standard method of ISO 3976 | IDF 74: 2006. The test method was based on the co-oxidation of Fe (II) to Fe (III) by hydro peroxides from sample and the formation of the reddish Fe (III)-thiocyanate complex which was read at 500 nm using spectrophotometer to obtain the absorbance. The peroxide value was calculated using the following formula:

$$PV = \frac{Abs}{55.84 * w} * \frac{1}{b} \text{ [mEqo}_2\text{/kg fat]}$$

Where; w= fat weight (g); Abs= absorbance; 55.84= atomic weight of Fe3+; b= the slope of the Fe (III) calibration curve and mEqo$_2$/kg= milliequivalents of active oxygen per kilogram of fat

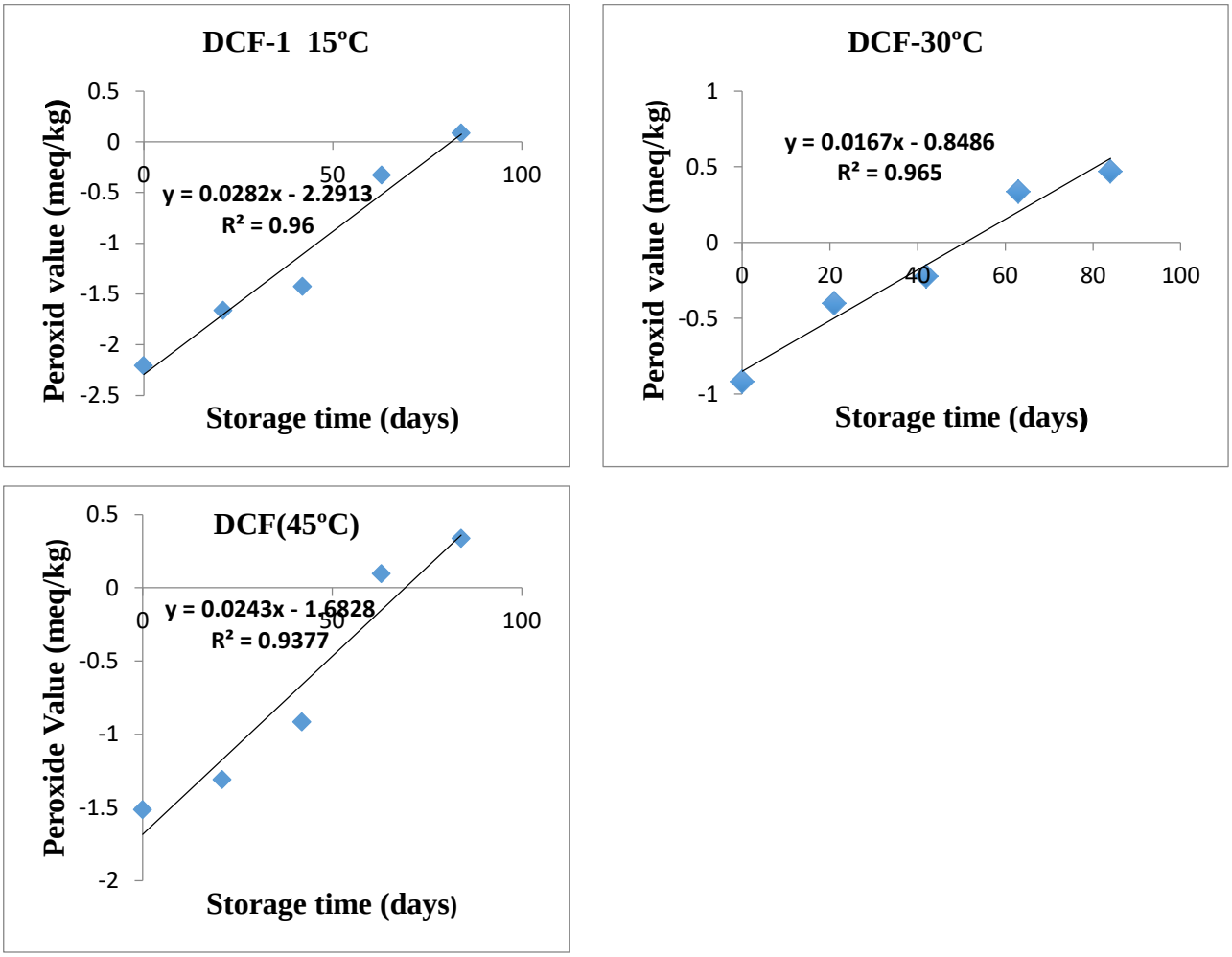


Figure 10: Peroxide value standard curves

3.3 Statistical Data Analysis

The data were analyzed using R statistical package (R Development Core Team, Version 3.0.6, and Vienna, Austria) in a two way analysis of variance in order to determine the significant differences and interactions between the processing methods. Means were separated by post hoc pair wise test (Turkeys Honest Significant Difference) at $p<0.05$. The results were presented in tables, charts, spider plots and bi plots as mean ± SD. Principal Component Analysis (PCA) was used to study the main sources of systematic variations in the average sensory descriptive data. The sample difference in relation to the attributes was analyzed using panel check software while the relationship between descriptive data and hedonic data were analyzed by consumer check software whereby the direction of liking of the consumers was evaluated. Correlation loading plots were applied with cycles indicating 50 and 100% explained variance respectively.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Results

4.1.1 Optimized Processing Method for Producing Suitable Avocado and Mango Seed Flour

(i) Effects on Nutrients, Bioactive Compounds, and Antinutritional Factors in Avocado and Mango Seed Kernels before and after Treatments

- **Antinutritional Compounds**

In this study, the effect of probiotic fermentation, boiling and soaking on the antinutrients reduction in avocado and mango seed kernels is shown in Tables 1 and 2. All processing methods had a significant reduction ($P<0.05$) of the antinutritional compounds in the samples at various conditions. In the raw avocado seed kernel the lowest content of the antinutrients were represented by oxalates (0.44 mg/100 g) and the highest contents by phytates (0.272 mg/100 g) (Table 2) while mango seed kernel, among the analyzed antinutritional factors tannin was observed to have the lowest content (0.49 mg/100 g) and phytates had the highest content (2.91 mg/100 g). This means with the raw avocado, it had the highest concentration of oxalates and highest content of phytates. On the other hand, the highest content and lowest concentration of the antinutrients in mango seed kernel were phytates and tannin respectively.

Reduction of phytates, oxalates and tannins increased as time increase for boiling and soaking in both processed avocado and mango seed kernels. No saponin content was detected at all set conditions after boiling, probiotic fermentation and soaking of the mango seed kernels. In fermented mango seed kernels the reduction of tannins and oxalates was more than 55% at both 37 ^{o}C and 42 ^{o}C. The maximum reduction (> 60%) of tannins and oxalates in boiled mango seed kernels was achieved at 20 minutes while soaking reduced the contents of tannins and oxalates in mango seed kernels for more than 86% from 6 hours to 24 hours (Table 3).

It was also observed that boiling avocado seeds for 10 minutes or less, the effect of reducing tannins, phytates and oxalates was less than 50% and increase of time above 10 minutes the total reduction was more than 55% while saponin was not detected at all and the highest reduction of anti-nutrients with boiled avocado seeds were noted at 20 minutes (Table 2). Soaked avocado seeds for more than 12 hours no oxalate content was observed and similarly at above 18 hours

saponin contents were not detected. Tannin content in soaked avocado seeds for 24 hours significantly reduced to more than 90%. As observed above, probiotic fermentation also reduced the tannins, phytates and oxalates contents of avocado seeds above 50%. Upon screening of saponin, no detection was observed in avocado seeds fermented by *Lactobacillus plantarum* and *Lactobacillus johnsonii* in all set temperatures. Similarly avocado seeds fermented by *Lactobacillus plantarum* also showed no detection of tannin upon analysis in all set temperatures (Table 2). This observation of completely removal of the tannin and saponin was good as the aim was to reduce their concentration to above 50% (harmless) or completely removed.

Table 2: Effect of boiling, soaking and probiotic fermentation on the antinutritional reduction in avocado seeds on dry weight basis

Methods	Treatment	Tannins (mg/100 g)	Oxalates (mg/100 g)	Phytates (mg/100 g)	Saponin
Soaking	Raw	0.45±0.03^{a}	0.44±0.02^{a}	2.72±0.14^{a}	+
	T_6	0.20±0.02^{b}	0.37±0.07^{b}	2.67±0.07^{a}	+
	T_{12}	0.19±0.02^{b}	0.14±0.01^{c}	1.97±0.01^{b}	+
	T_{18}	0.17±0.02^{b}	Nd	0.75±0.07^{c}	+
	T_{24}	0.14±0.01^{c}	Nd	0.25±0.00^{d}	nd
Boiling	Raw	0.45±0.03^{a}	0.44±0.02^{a}	2.72±0.14^{a}	+
	T_5	0.33±0.02^{b}	0.42±0.04^{a}	1.51±0.04^{b}	+
	T_{10}	0.30±0.02^{b}	0.40±0.04^{a}	1.01±0.02^{c}	nd
	T_{15}	0.20±0.01^{c}	0.11±0.00^{b}	0.54±0.01^{d}	nd
	T_{20}	0.06±0.00^{d}	0.074±0.00^{b}	0.50±0.04^{d}	nd
Fermentation (30 ^{o}C)	Raw	0.45±0.03^{a}	0.44±0.02^{a}	2.72±0.14^{a}	+
	B-3058	nd	Nd	0.17±0.07^{b}	nd
	B-2178	0.09±0.01^{c}	0.04±0.00^{c}	0.20±0.00^{b}	nd
	B-59149	0.22±0.00^{b}	0.12±0.01^{b}	0.26±0.01^{b}	+
Fermentation (37 ^{o}C)	Raw	0.45±0.03^{a}	0.44±0.02^{a}	2.72±0.14^{a}	+
	B-3058	nd	0.04±0.00^{c}	0.22±0.00^{b}	nd
	B-2178	0.07±0.01c	0.07±0.00^{b}	0.09±0.00^{b}	nd
	B-59149	0.13±0.01^{b}	0.04±0.00^{c}	0.21±0.01^{b}	nd
Fermentation (42 ^{o}C)	Raw	0.45±0.03^{a}	0.44±0.02^{a}	2.71±0.14^{a}	+
	B-3058	nd	0.19±0.00^{b}	0.65±0.12^{b}	nd
	B-2178	0.01±0.00^{b}	0.03±0.00^{c}	0.35±0.27^{c}	nd
	B-59149	nd	0.41±0.04^{a}	0.50±0.02^{b}	+

Data presented as means ±SD (n=3). The means in columns with different superscript letters are significantly different ($p<0.05$) (a=) while means in columns with same superscript letters are not significant ($p<0.05$) (relative to what?); nd= not detected, B-3058= *Lactobacillus plantarum*, B-2178= *Lactobacillus johnsonii*, 59149= *Lactobacillus rhammnosus*, T=Time, T_5 =five minutes, T_{10} =ten minutes, T_{15} = fifteen minutes, T_{20} =twenty minutes, T_6 =six hours, T_{12} =twelve hours, T_{18} =eighteen hours and T_{24} =twenty four hours

Table 3: Effect of soaking, boiling and probiotic fermentation on the antinutrients reduction in mango seed kernels on dry weight basis

Methods	Treatment	Tannins (mg/100 g)	Oxalates (mg/100 g)	Phytates (mg/100 g)	Saponin
Boiling	Raw	0.49±0.01^{a}	0.67±0.01^{a}	2.91±0.49^{a}	+
	5 minutes	0.30±0.02^{b}	0.33±0.06^{b}	1.41±0.19^{b}	nd
	10 minutes	0.29±0.01^{b}	0.18±0.01^{c}	1.35±0.05^{b}	nd
	15 minutes	0.23±0.02^{c}	0.17±0.00^{c}	0.10±0.01^{c}	nd
	20 minutes	0.17±0.02^{d}	Nd	0.06±0.00^{c}	nd
Soaking	Raw	0.49±0.01^{a}	0.67±0.01^{a}	2.91±0.29^{a}	+
	6 hours	0.06±0.00^{b}	0.05±0.01^{b}	1.58±0.08^{b}	nd
	12 hours	0.05±0.00^{b}	0.04±0.00^{b}	1.47±0.06^{b}	nd
	18 hours	Nd	0.04±0.00^{b}	0.69±0.04^{c}	nd
	24 hours	nd	0.04±0.00^{b}	0.51±0.03^{c}	nd
Fermen't (30^{o}C)	Raw	0.49±0.01^{a}	0.67±0.01^{a}	2.91±0.49^{a}	+
	B-3058	0.29±0.00^{b}	0.36±0.04^{b}	0.47±0.02^{b}	nd
	B-2178	0.21±0.02^{c}	0.25±0.04^{c}	0.93±0.02^{bc}	nd
	B-59149	0.31±0.01^{b}	0.18±0.02^{d}	0.28±0.01^{c}	nd
Fermen't (37^{o}C)	Raw	0.49±0.01^{a}	0.67±0.01^{a}	2.91±0.45^{a}	+
	B-3058	0.20±0.07^{c}	0.18±0.01^{c}	0.20±0.02^{c}	nd
	B-2178	0.24±0.02^{b}	0.21±0.01^{b}	1.11±0.21^{b}	nd
	B-59149	0.03±0.00^{d}	0.07±0.01^{d}	0.16±0.00^{c}	nd
Fermen't (42^{o}C)	Raw	0.49±0.01^{a}	0.67±0.01^{a}	2.91±0.49^{a}	+
	B-3058	0.28±0.01^{b}	0.03±0.00^{c}	1.75±0.09^{b}	nd
	B-2178	0.03±0.00^{c}	0.05±0.00^{b}	1.71±0.07^{b}	nd
	B-59149	0.03±0.00^{c}	0.03±0.00^{c}	1.67±0.05^{b}	nd

Data presented as means ±SD (n=3). The means in columns with different superscript letters are significantly different ($p<0.05$) (relative to processing method) while means in columns with same superscript letters are not significant ($p<0.05$) (relative to processing method); nd= not detected, T=Time, B-3058= *Lactobacillus plantarum*, B-2178= *Lactobacillus johnsonii*, 59149= *Lactobacillus rhammnosus* and Fermen't = fermentation

- **Vitamins (Ascorbic Acid and α-tocopherol), Total Phenol and Antioxidant Activity**

The effect of probiotic fermentation, boiling and soaking on the selected vitamins, total phenol and antioxidant activity in avocado and mango seed kernels is presented in Tables 4 and 5. For vitamin C (ascorbic acid), it was noted that boiling and soaking had a decrease for all the treatments in both avocado and mango seed kernels. Ascorbic acid contents of the raw avocado and mango seed kernels were 9.18 mgAAE/100 g and 0.68 mgAAE/100 g respectively. The decrease in ascorbic acid contents of mango seed kernels ranged from 0.68 to 0.06 mgAAE/100 g (soaked) and 0.68 to 0.05 mgAAE/100 g (boiled) while that of avocado seeds ranged from 9.18 to 0.51 mgAAE/100 g (soaked) and 9.18 to 1.70 mgAAE/100 g (boiled) (Tables 4 and 5).

Reduction of ascorbic acid contents with boiled and soaked avocado and mango seed kernels increased with processing time. In avocado seeds the decrease was observed to be above 51% in all treatments while the reduction of ascorbic acid in all conditions for boiled and soaked mango seed kernels was higher than 66% (Tables 4 and 5). On the other hand, probiotic fermentation showed contrary results of ascorbic acid in both avocado and mango seed kernels. For example, the results of probiotic fermentation showed a significant ($p < 0.05$) increase of ascorbic acid contents in all fermented avocado seeds. Similar increase of ascorbic acid contents were observed in fermented mango seeds by *Lactobacillus plantarum* at temperatures of 30 ^{o}C (0.70 mgAAE/100g) and 37 ^{o}C (0.95 mgAAE/100 g). However, the highest contents of ascorbic acid (13.5 mgAAE/100 g) in avocado seeds and (0.95 mgAAE/100 g) in mango seed kernels were both observed at an optimized temperature of 37 ^{o}C (Tables 4 and 5).

In this experiment, no significant differences were observed in vitamin E (α-tocopherol) contents of the analyzed avocado and mango seed kernels in all the treatments. Vitamin E (α-tocopherol) contents of raw avocado and mango seed kernels were 0.63 mg/100 g and 1.28 mg /100 g respectively. However the results of avocado and mango seed kernels ranged from 0.63-0.89 mg/100 g (avocado seeds) and 1.28-1.41 mg/100 g (mango seed kernels) (Tables 4 and 5)

For the total phenolics, soaked avocado seeds revealed a significant decrease from 18.22 to 15.84 mgGAE/g and the highest loss was at 24 hours which accounted for 13.1%. Boiled and fermented avocado seeds retained the total phenolic contents in a sense that boiling did not show any significant differences while fermentation had a significant increase. The increase of total phenolic contents in fermented avocado seeds ranged from 18.22 to 33.3 mgGAE/g and the highest increment (45.3%) was observed at a temperature of 37 ^{o}C. Total phenolic contents of raw mango seed kernels was found to be 70.68 mgGAE/g. Soaked mango seed kernels showed a

significant reduction ($p<0.05$) in total phenol while probiotic fermented and boiled mango seed kernels presented a significant increase of total phenol. The maximum reduction of total phenol in the soaked mango seed kernel was 11.2% and it was observed at 24 hours. The increase of total phenol in probiotic fermented and boiled mango seed kernels ranged from 70.68 to 94.84 mgGAE/g and 70.68 to 90.78 mgGAE/g respectively where the highest (24.16%) increase in total phenol content was observed at a fermentation temperature of 37 ^{o}C in mango seed kernels fermented by *Lactobacillus plantarum* (Tables 4 and 5).

The DPPH (1, 1-diphenyl-2-picrylhydrazyl) of the radical scavenging activity (IC_{50}) of avocado and mango seed kernels ranged from 0.8 to 2 mg/ml and 1.25 to 2.5 mg/ml, respectively. A lower IC_{50} value reflects a greater antioxidant activity of the samples. Moderate reduction of DPPH radical scavenging activity in avocado seeds was observed after boiling and soaking compared with the analyzed raw avocado seeds whereas probiotic fermentation had a significant increase of the antioxidant activity. Avocado seeds fermented by *Lactobacillus plantarum* had the highest antioxidant activity. The loss of antioxidant activity was observed in both boiled and soaked mango seed kernels. The highest reduction of antioxidant activity in both boiled and soaked mango seed kernels were at 20 minutes and 24 hours, respectively. In mango seed kernels also probiotic fermentation presented a significant increase in the antioxidant activity and the highest percentage (41.5%) was noted with mango seeds kernels fermented by *Lactobacillus plantarum*. In addition, the extent of increased antioxidant activity of fermented mango seed kernel varied with the microorganisms used (Tables 5).

Table 4: Effect of boiling, soaking and probiotic fermentation on the analyzed vitamins, total phenol and antioxidant activity in avocado seeds on dry weight basis

Methods	Treatment	Ascorbic acid (mgAAE/100 g)	α-tocopherol (mg/100 g)	Total phenol (mgGAE/g)	DPPH radical IC_{50} (mg/ml)
Soaking	Raw	9.18±0.54^{a}	0.78±0.03^{a}	18.22±0.10^{a}	1.5
	T_6	3.85±0.04^{b}	0.76±0.05^{a}	17.96±0.06^{a}	1.7
	T_{12}	2.34±0.02^{c}	0.74±0.04^{a}	16.80±0.08^{b}	1.61
	T_{18}	1.70±0.01^{c}	0.73±0.02^{a}	16.07±0.09^{c}	1.75
	T_{24}	0.51±0.01^{d}	0.71±0.02^{a}	15.84±0.04^{c}	1.8
Boiling	Raw	9.18±0.54^{a}	0.78±0.03^{a}	18.22±0.10^{c}	1.5
	T_5	4.42±0.03^{b}	0.66±0.07^{a}	19..68±0.13^{bc}	1.8
	T_{10}	3.91±0.09^{b}	0.63±0.02^{a}	21.34±0.20^{ab}	1.75
	T_{15}	2.08±0.02^{c}	0.70±0.02^{a}	21.56±1.60^{a}	1.85
	T_{20}	1.70±0.04^{c}	0.77±0.07^{a}	22.07±0.90^{a}	2
Fermentation (30 ^{o}C)	Raw	9.18±0.54^{bc}	0.78±0.03^{a}	18.22±0.10^{d}	1.5
	B-3058	12.81±0.74^{a}	0.80±0.06^{a}	23.21±0.14^{a}	1.2
	B-2178	9.93±0.17^{b}	0.67±0.04^{a}	21.10±0.24^{b}	1.4
	B-59149	9.45±0.17^{c}	0.89±0.05^{a}	20.32±1.5^{c}	1.1
Fermentation (37 ^{o}C)	Raw	9.18±0.56^{c}	0.78±0.03^{a}	18.22±0.10^{d}	1.5
	B-3058	13.50±0.12^{a}	0.93±0.09^{a}	33.30±0.71^{a}	0.8
	B-2178	12.19±0.03^{ab}	0.89±0.06^{a}	28.65±0.56^{b}	0.9
	B-59149	9.28±0.68^{c}	0.83±0.02^{a}	24.73±0.46^{c}	1.0
Fermentation (42 ^{o}C)	Raw	9.18±0.56^{a}	0.78±0.03^{a}	18.22±0.10^{b}	1.5
	B-3058	9.95±0.54^{b}	0.82±0.06^{a}	18.38±0.71^{ab}	0.9
	B-2178	9.80±0.32^{b}	0.75±0.00^{a}	18.48±1.30^{a}	1.4
	B-59149	9.61±0.41^{b}	0.75±0.05^{a}	17.11±0.20^{c}	1..3

Data presented as means ±SD (n=3). The means in columns with different superscript letters are significantly different ($p<0.05$) (relative to processing method) while means in columns with same superscript letters are not significant ($p<0.05$) (relative to processing method); B-3058= *Lactobacillus plantarum*, B-2178= *Lactobacillus johnsonii*, 59149= *Lactobacillus rhammnosus*, GAE= Gallic acid equivalents, AAE= Ascorbic acid equivalents, T= Time, T_5 =five minutes, T_{10} =ten minutes, T_{15} = fifteen minutes, T_{20} =twenty minutes, T_6 =six hours, T_{12} = twelve hours, T_{18} =eighteen hours and T_{24} =twenty four hours

Table 5: Effect of soaking, boiling and lactic acid fermentation on the selected vitamins, total phenol and antioxidant activity in mango seed kernels on dry weight basis

Methods	Treatment	Ascorbic acid (mgAAE/100 g)	α-tocopherol (mg/100 g)	Total phenol (mgGAE/g)	DPPH radical IC_{50} (mg/ml)
Boiling	Raw	0.68 ± 0.02^{a}	1.28 ± 0.05^{a}	70.68 ± 1.23^{c}	2
	5 minutes	0.19 ± 0.04^{b}	1.13 ± 0.02^{a}	87.21 ± 6.95^{b}	2
	10 minutes	0.14 ± 0.01^{bc}	1.14 ± 0.05^{a}	88.15 ± 1.27^{ab}	2.05
	15 minutes	0.11 ± 0.01^{cd}	1.02 ± 0.00^{a}	90.09 ± 0.23^{a}	2.15
	20 minutes	0.05 ± 0.00^{d}	1.04 ± 0.02^{a}	90.78 ± 0.84^{a}	2.25
Soaking	Raw	0.68 ± 0.02^{a}	1.28 ± 0.05^{a}	70.68 ± 1.23^{a}	2
	6 hours	0.23 ± 0.02^{b}	1.17 ± 0.15^{a}	69.66 ± 2.30^{a}	2.01
	12 hours	0.16 ± 0.01^{b}	1.23 ± 0.04^{a}	68.98 ± 1.07^{ab}	2.25
	18 hours	0.07 ± 0.01^{c}	1.20 ± 0.01^{a}	68.34 ± 0.28^{ab}	2.3
	24 hours	0.06 ± 0.00^{c}	1.10 ± 0.06^{a}	62.75 ± 3.41^{b}	2.4
Fermen't (30 ^{o}C)	Raw	0.68 ± 0.02^{a}	1.28 ± 0.05^{a}	70.68 ± 1.23^{b}	2
	B-3058	0.70 ± 0.03^{a}	1.34 ± 0.02^{a}	82.86 ± 1.01^{a}	1.55
	B-2178	0.60 ± 0.04^{b}	1.40 ± 0.02^{a}	80.71 ± 7.27^{a}	1.55
	B-59149	0.54 ± 0.01^{c}	1.31 ± 0.01^{a}	80.16 ± 1.40^{a}	1.8
Fermen't (37 ^{o}C)	Raw	0.68 ± 0.02^{ab}	1.28 ± 0.05^{a}	70.68 ± 1.23^{c}	2
	B-3058	0.95 ± 0.02^{a}	1.33 ± 0.03^{a}	94.84 ± 6.73^{a}	1.25
	B-2178	0.64 ± 0.01^{ab}	1.29 ± 0.07^{a}	92.82 ± 1.41^{a}	1.5
	B-59149	0.49 ± 0.09^{b}	1.15 ± 0.07^{a}	86.49 ± 0.23^{b}	1.3
Fermen't (42 ^{o}C)	Raw	0.68 ± 0.02^{a}	1.28 ± 0.05^{a}	70.68 ± 1.23^{b}	2
	B-3058	0.60 ± 0.03^{ab}	1.41 ± 0.04^{a}	87.69 ± 3.90^{a}	1.8
	B-2178	0.57 ± 0.05^{b}	1.24 ± 0.02^{a}	85.54 ± 3.68^{a}	1.55
	B-59149	0.55 ± 0.0^{b}	1.34 ± 0.01^{a}	75.68 ± 6.95^{b}	1.61

Data presented as means ±SD (n=3). The means in columns with different superscript letters are significantly different ($p<0.05$) (Relative to processing method) while means in columns with same superscript letters are not significant ($p<0.05$) (Relative to processing method); T= Time, B-3058= *Lactobacillus plantarum*, B-2178= *Lactobacillus johnsonii*, 59149= *Lactobacillus rhammnosus* and Fermen't=fermentation

- **Minerals**

Tables 6 and 7 present the mineral contents of raw, fermented, boiled and soaked avocado and mango seed kernels. Three major minerals and two trace minerals were analyzed in both avocado and mango seed kernels. From the results, it was observed that all samples analyzed had moderately higher values of calcium, sodium and potassium, but low values of iron and zinc Probiotic fermented avocado seeds showed no significance differences of all mineral analyzed in this experiment among the treatments while boiled avocado seeds had a significant ($p < 0.05$) lower values than the raw samples for calcium, sodium, potassium and iron except for zinc which was insignificant at $p < 0.05$. Soaked avocado seeds showed a significant lower variations among the treatments for calcium, sodium and iron than unsoaked avocado seeds except for potassium and zinc which were also insignificant at $p < 0.05$ (Table 6).

However, the maximum significant decrease in percentage for minerals in soaked avocado seeds were Ca (9%), Na (19.8%) and Fe (23.6%) while for boiled avocado seeds were Ca (26.6%), Na (21.3%), K (14.6%) and Fe (27.7%). For mango seed kernels, probiotic fermentation had no significant differences in the analyzed minerals (calcium, potassium, iron, and zinc) though there were some apparent differences detected among the samples in the treatments. Boiling significantly reduced the minerals contents of mango seed kernels (calcium 15.7%, sodium 11.6%, potassium 17.2%, iron 29.8% and zinc 21.9%) while soaking reduced the components of some analyzed minerals of mango seed kernels (calcium 18.9%, sodium 14.9% and iron 36%). Of all the minerals analyzed in mango seed kernels, highest reduction was observed in soaked mango seed kernels at 24 hours in iron whereas the lowest reduction was noted in boiled mango seed kernels at 20 minutes in calcium (Table 7).

Table 6: Effect of soaking, boiling and probiotic fermentation on the analyzed minerals in avocado seeds (mg/100 g) on dry weight basis

Methods	Treatment	Calcium	Sodium	Potassium	Iron	Zinc
Soaking	Raw	77.73±1.87^{a}	212.70±0.36^{a}	232.90±0.40^{a}	3.61±0.08^{a}	0.18±0.01^{a}
	T_6	77.29±1.59^{a}	191.61±1.17^{b}	229.41±8.81^{a}	3.36±0.02^{ad}	0.19±0.02^{a}
	T_{12}	73.47±1.48^{ab}	187.32±3.85^{b}	227.43±2.26^{a}	3.14±0.02^{bc}	0.18±0.01^{a}
	T_{18}	70.73±2.49^{b}	182.15±0.20^{c}	225.80±1.16^{a}	2.81±0.27^{cd}	0.18±0.01^{a}
	T_{24}	71.13±1.79^{b}	170.05±0.93^{d}	225.22±3.10^{a}	2.75±0.14^{d}	0.18±0.01^{a}
Boiling	Raw	77.73±1.87^{a}	212.70±0.36^{a}	232.90±0.40^{a}	3.61±0.08^{a}	0.18±0.01^{a}
	T_5	76.28±3.56^{a}	201.70±4.76^{b}	222.28±2.11^{b}	3.36±0.01^{a}	0.20±0.02^{a}
	T_{10}	74.66±2.43^{ab}	183.42±2.24^{c}	218.87±0.21^{c}	3.19±0.05^{ab}	0.18±0.01^{a}
	T_{15}	68.68±1.19^{b}	178.81±1.33^{c}	210.41±2.95^{c}	2.73±0.39^{bc}	0.17±0.01^{a}
	T_{20}	57.08±3.56^{c}	167.32±2.13^{d}	198.97±1.11^{d}	2.61±0.04^{c}	0.17±0.01^{a}
Fermentation (30 ^{o}C)	Raw	77.73±1.87^{a}	212.70±0.36^{a}	232.90±0.40^{a}	3.61±0.08^{a}	0.18±0.01^{a}
	B-3058	78.61±7.91^{a}	235.76±4.44^{a}	237.21±4.63^{a}	3.69±0.40^{a}	0.19±0.08^{a}
	B-2178	89.73±3.25^{a}	214.05±1.24^{a}	235.44±4.51^{a}	3.74±0.08^{a}	0.19±0.01^{a}
	B-59149	84.11±1.27^{a}	222.40±4.63^{a}	233.25±3.44^{a}	3.65±0.14^{a}	0.19±0.05^{a}
Fermentation (37 ^{o}C)	Raw	77.73±1.87^{a}	212.70±0.36^{a}	232.90±0.40^{a}	3.61±0.08^{a}	0.18±0.01^{a}
	B-3058	77.10±1.34^{a}	210.72±10.9^{a}	229.82±6.91^{a}	3.72±0.37^{a}	0.18±0.01^{a}
	B-2178	78.79±5.34^{a}	212.58±8.60^{a}	229.82±6.91^{a}	3.91±0.01^{a}	0.21±0.01^{a}
	B-59149	80.38±1.10^{a}	212.99±4.80^{a}	233.73±9.25^{a}	3.67±0.01^{a}	0.18±0.01^{a}
Fermentation (42 ^{o}C)	Raw	77.73±1.87^{a}	212.70±0.36^{a}	232.90±0.40^{a}	3.61±0.08^{a}	0.18±0.01^{a}
	B-3058	77.73±0.15^{a}	213.71±1.07^{a}	233.55±6.91^{a}	3.66±0.08^{a}	0.18±0.01^{a}`
	B-2178	78.97±4.36^{a}	212.34±3.19^{a}	230.10±2.38^{a}	3.68±0.04^{a}	0.18±0.02^{a}
	B-59149	77.45±5.66^{a}	213.76±0.73^{a}	233.17±9.28^{a}	4.01±0.28^{a}	0.18±0.01^{a}

Data presented as means ±SD (n=3). The means in columns with different superscript letters are significantly different ($p<0.05$) (Relative to processing method) while means in columns with same superscript letters are not significant ($p<0.05$) (Relative to processing method); B-3058= *Lactobacillus plantarum*, B-2178= *Lactobacillus johnsonii*, 59149= *Lactobacillus rhammnosus*, T= Time, T_5 =five minutes, T_{10} =ten minutes, T_{15} = fifteen minutes, T_{20} =twenty minutes, T_6 =six hours, T_{12} =twelve hours, T_{18} =eighteen hours and T_{24} =twenty four hours

Table 7: Effect of soaking, boiling and probiotic fermentation on the analyzed minerals in mango seed kernels (mg/100 g) on dry weight basis

Methods	Treatment	Calcium	Sodium	Potassium	Iron	Zinc
Boiling	Raw	120.77±0.87^{a}	275.28±2.34^{a}	301.45±2.57^{a}	4.70±0.12^{a}	0.64.±0.05^{a}
	5 minutes	118.02±1.91^{a}	258.21±0.19^{b}	289.54±3.14^{a}	4.13±0.06^{b}	0.51±0.01^{b}
	10 minutes	117.40±1.38^{a}	254.74±6.18^{bc}	285.83±10.84^{ab}	4.02±0.08^{b}	0.51±0.02^{b}
	15 minutes	103.63±4.28^{b}	247.96±1.48^{cd}	272.54±7.28^{b}	3.57±0.09^{c}	0.50±0.01^{b}
	20 minutes	101.77±2.17^{b}	243.45±0.28^{d}	249.70±2.70^{c}	3.25±0.10^{d}	0.48±0.03^{b}
Soaking	Raw	120.77±0.87^{a}	275.28±2.34^{a}	301.45±2.57^{a}	4.70±0.12^{a}	0.64.±0.05^{a}
	6 hours	120.01±3.61^{a}	273.36±1.90^{b}	299.53±2.09^{a}	4.27±0.07^{b}	0.60±0.10^{a}
	12 hours	117.51±2.42^{ab}	235.07±1.37^{b}	297.23±3.25^{a}	3.68±0.10^{c}	0.57±0.02^{a}
	18 hours	111.97±1.44^{b}	234.83±1.49^{b}	296.69±2.32^{a}	3.36±0.16^{d}	0.54±0.08^{a}
	24 hours	98.08±0.76^{c}	234.39±1.16^{c}	287.39±0.65^{a}	2.99±0.08^{e}	0.50±0.02^{a}
Fermen't (30 ^{o}C)	Raw	120.77±0.87^{a}	275.28±2.34^{a}	301.45±2.57^{a}	4.70±0.12^{a}	0.64.±0.05^{a}
	B-3058	119.96±1.28^{a}	286.92±3.80^{a}	296.87±4.96^{a}	4.63±0.06^{a}	0.60±0.07^{a}
	B-2178	122.34±0.69^{a}	288.70±8.46^{a}	299.29±7.50^{a}	4.63±0.06^{a}	0.69±0.02^{a}
	B-59149	121.77±0.66^{a}	287.95±10.28^{a}	292.44±10.40^{a}	4.54±0.01^{a}	0.64±0.06^{a}
Fermen't (37 ^{o}C)	Raw	120.77±0.87^{a}	275.28±2.34^{a}	301.45±2.57^{a}	4.70±0.12^{a}	0.64.±0.05^{a}
	B-3058	121.86±3.22^{a}	277.92±6.96^{a}	301.44±1.14^{a}	4.76±0.05^{a}	0.57±0.04^{a}
	B-2178	120.87±3.87^{a}	279.26±7.77^{a}	303.95±2.12^{a}	4.66±0.11^{a}	0.60±0.01^{a}
	B-59149	122.51±8.93^{a}	280.45±6.72^{a}	304.78±0.59^{a}	4.62±0.01^{a}	0.60±0.01^{a}
Fermen't (42 ^{o}C)	Raw	120.77±0.87^{a}	275.28±2.34^{a}	301.45±2.57^{a}	4.70±0.12^{a}	0.64.±0.05^{a}
	B-3058	122.25±2.53^{a}	276.33±1.96^{a}	301.29±6.19^{a}	4.70±0.10^{a}	0.62±0.04^{a}
	B-2178	120.08±0.73^{a}	276.79±7.82^{a}	303.10±2.95^{a}	4.53±0.05^{a}	0.64±0.03^{a}
	B-59149	121.66±0.74^{a}	272.02±6.46^{a}	303.65±3.88^{a}	4.51±0.14^{a}	0.64±0.01^{a}

Data presented as means ±SD (n=3). The means in columns with different superscript letters are significantly different ($p<0.05$) (Relative to processing method) while means in columns with same superscript letters are not significant ($p<0.05$); T= time, B-3058= *Lactobacillus plantarum*, B-2178= *Lactobacillus johnsonii*, 59149= *Lactobacillus rhammnosus* and Fermen't=fermentation

- **Fatty Acid Profile**

The mean values of the profiled fatty acid compositions of the raw, boiled, soaked and probiotic fermented avocado and mango seed kernels are shown in Tables 8 and 9. The palmitic acid, oleic acid, linoleic acid and linolenic acid are the major fatty acids detected in the seeds of avocado while in mango seed kernels palmitic acid, stearic acid and oleic acid were observed as the major fatty acids (Tables 8 and 9). Among the profiled fatty acids of the avocado and mango seed kernels, myristic acid, palmitic acid and stearic acid were saturated fatty acids while linoleic acid, linolenic acid and oleic acid were unsaturated fatty acids. Most of the results in this study for both avocado and mango seed kernels showed insignificant differences in the

involved processing techniques, although some significant differences in the profiled fatty acid were observed among the processing techniques at $p < 0.05$. In the present study, mango seed kernels had 44% saturated fatty acid and 56% unsaturated fatty acid whereas avocado seeds contained 33% saturated fatty acid and the remaining 67% were unsaturated fatty acid. In all the treatments for avocado and mango seed kernels, the highest composition of the fatty acids was found in probiotic fermented samples, followed by boiled samples. However, soaking was the only processing method that did not show any significant variations in the processed avocado and mango seed kernels.

Table 8: Effect of boiling soaking and probiotic fermentation on the profiled Fatty acid of avocado seed (mg/100 g) on dry weight basis

Methods	Treatment	Saturated fatty acid	Unsaturated fatty acid	Palmitic acid (16:0)	Stearic acid (18:0)	Myristic acid (14:0)	Linoleic acid (18:2)	Linolenic acid (18:3)	Oleic acid 18:1)
Boiling	Raw	476±3.7^{a}	976.±1.0^{a}	244±1.7^{a}	55±0.6^{a}	25±0.1^{a}	570±0.5^{c}	254±0.3^{a}	146±0.1^{a}
	5 minutes	478±6.0^{a}	964±5.7^{a}	253±2.3^{a}	55±2.0^{a}	24±2.6^{a}	577±2.1^{c}	249±7.0^{a}	157±1.4^{a}
	10 minutes	474±2.8^{a}	960±7.8^{a}	260±4.2^{a}	55±4.2^{a}	25±1.4^{a}	649±6.4^{b}	246±2.1^{a}	155±8.3^{a}
	15 minutes	478±2.1^{a}	979±10.6^{a}	267±1.3^{a}	57±4.9^{a}	24±4.2^{a}	741±4.2^{a}	241±5.0^{a}	164±2.5^{a}
	20 minutes	480±0.0^{a}	978±12.7^{a}	263±7.8^{a}	52±2.1^{a}	27±9.0^{a}	770±10.0^{a}	261±8.5^{a}	171±2.8^{a}
Soaking	Raw	476±3.7^{a}	976±1.0^{a}	244±1.7^{a}	55±0.4^{a}	25±0.1^{a}	570±0.5^{a}	253±0.3^{a}	146±0.0^{a}
	6 hours	462±1.1^{a}	960±6.3^{a}	247±1.7^{a}	54±0.6^{a}	25±2.8^{a}	573±3.8^{a}	254±11.3^{a}	144±7.8^{a}
	12 hours	465±8.5^{a}	968±4.95^{a}	244±6.4^{a}	54±3.0^{a}	27±0.7^{a}	552±2.6^{a}	251±5.5^{a}	143±2.1^{a}
	18 hours	459±2.4^{a}	972±14.0^{a}	239±9.2^{a}	54±0.0^{a}	26±2.1^{a}	564±0.0^{a}	252±4.9^{a}	146±5.0^{a}
	24 hours	456±0.1^{a}	971±2.2^{a}	245±3.0^{a}	55±0.7^{a}	25±4.5^{a}	573±8.5^{a}	250±0.2^{a}	144±1.6^{a}
Fermen't (30 ^{o}C)	Raw	476±3.7^{a}	976±1.0^{a}	244±1.7^{a}	55±0.1^{b}	25±0.1^{a}	570±0.6^{b}	255±0.3^{b}	146±0.0^{a}
	B-3058	487±4.2^{a}	961±7.1^{a}	241±11.3^{a}	71±0.7^{ab}	20±0.1^{c}	587±4.2^{b}	552±12.7^{a}	143±0.7^{a}
	B-2178	474±6.7^{a}	936±12.7^{a}	242±0.7^{a}	73±0.1^{a}	22±0.3^{b}	631±0.4^{b}	548±7.1^{a}	146±0.3^{a}
	B-59149	472±11.9^{a}	940±1.4^{a}	243±0.0^{a}	63±2.1^{ab}	17±0.0^{d}	790±1.4^{a}	532±15.6^{a}	145±9.1^{a}
Fermen't (37 ^{o}C)	Raw	476±3.7^{a}	976±1.0^{a}	244±1.7^{a}	55±0.1^{c}	25±0.1^{a}	570±0.5^{a}	253±0.3^{b}	146±0.1^{a}
	B-3058	493±4.2^{a}	983±4.24^{a}	246±5.0^{a}	98±1.5^{a}	22±0.0^{a}	884±1.4^{a}	556±10.6^{a}	154±3.0^{a}
	B-2178	492±7.1^{a}	970±1.97^{a}	238±12.0^{a}	74±6.3^{b}	23±0.1^{a}	791±1.5^{a}	512±3.4^{a}	148±1.2^{a}
	B-59149	478±12.7^{a}	967±1.55^{a}	243±2.1^{a}	73±4.9^{b}	22±0.1^{a}	836±0.8^{a}	526±4.3^{a}	146±9.3^{a}
Fermen't (42 ^{o}C)	Raw	476±3.7^{a}	976±1.0^{a}	244±1.7^{a}	55±0.1^{b}	25±0.1^{a}	570±0.5^{b}	254±0.3^{b}	146±0.1^{a}
	B-3058	477±3.5^{a}	971±2.2^{a}	237±6.4^{a}	69±2.1^{ab}	22±0.1^{a}	754.5±5^{ab}	485±1.8^{a}	151±6.4^{a}
	B-2178	481±13.3^{a}	938±6.3^{a}	238±8.5^{a}	75±5.0^{a}	22±0.0^{a}	649±0.6^{ab}	514±6.6^{a}	140±4.9^{a}
	B-59149	458±1.4^{a}	955.±3.1^{a}	240±11.6^{a}	70±0.0^{ab}	23±0.1^{a}	820±0.7^{a}	528±2.1^{a}	137±7.7^{a}

Data presented as means ±SD (n=3). The means in columns with different superscript letters are significantly different ($p<0.05$) (Relative to processing method) while means in columns with same superscript letters are not significant ($p<0.05$) (Relative to processing method); B-3058= *Lactobacillus plantarum*, B-2178= *Lactobacillus johnsonii*, 59149= *Lactobacillus rhammnosus* and Fermen't=fermentation

Table 9: Effect of boiling soaking and probiotic fermentation on the profiled Fatty acid of mango seed kernels (g/100 g) on dry weight basis

Methods	Treatment	Saturated fatty acid	Unsaturated fatty acid	Palmitic acid (16:0)	Stearic acid (18:0)	Myristic acid (14:0)	Linoleic acid (18:2)	Linolenic acid (18:3)	Oleic acid 18:1)
Boiling	Raw	43±2.0^{a}	55±1.2^{a}	13±0.1^{a}	29±2.0^{a}	0.53±0.01^{a}	3.9±0.02^{c}	0.26±0.01^{a}	49±0.0^{a}
	5 minutes	42±0.1^{a}	53±0.1^{a}	11±0.0^{a}	28±1.1^{a}	0.59±0.02^{a}	4.3±0.00^{bc}	0.25±0.00^{a}	48±0.1^{a}
	10 minutes	44±0.0^{a}	52±1.0^{a}	10±0.1^{a}	26±0.9^{a}	0.55±0.03^{a}	4.6±0.04^{b}	0.25±0.02^{a}	47±0.2^{a}
	15 minutes	41±0.1^{a}	53±05^{a}	9±0.0^{a}	27±1.7^{a}	0.56±0.00^{a}	5.8±0.00^{a}	0.32±0.03^{a}	45±0.1^{a}
	20 minutes	42±3.0^{a}	54±1.7^{a}	12±0.2^{a}	28±1.0^{a}	0.67±0.01^{a}	4.9±0.01^{b}	0.27±0.00^{a}	46±0.3^{a}
Soaking	Raw	43±2.0^{a}	55±1.2^{a}	13±0.1^{a}	29±2.0a	0.53±0.01^{a}	3.9±0.02^{a}	0.26±0.01^{a}	49±0.0^{a}
	6 hours	43±2.7^{a}	51±0.2^{a}	10±0.6^{a}	27±1.4^{a}	0.59±0.00^{a}	4.0±0.10^{a}	0.22±0.00^{a}	49±1.0^{a}
	12 hours	43±0.2^{a}	51±1.0^{a}	11±0.5^{a}	27±1.3^{a}	0.57±0.10^{a}	4.2±0.20^{a}	0.21±0.00^{a}	50±0.4^{a}
	18 hours	42±1.4^{a}	53±2.9^{a}	12±0.4^{a}	27±0.0^{a}	0.56±0.00^{a}	4.3±0.10^{a}	0.22±0.00^{a}	53±2.1^{a}
	24 hours	40±0.2^{a}	52±0.5^{a}	11±0.2^{a}	26±0.2^{a}	0.51±0.20^{a}	4.4±0.30^{a}	0.20±0.00^{b}	53±0.3^{a}
Fermen't (30 ^{o}C)	Raw	43±2.0^{a}	55±1.2^{a}	13±0.1^{a}	29±2.0^{a}	0.53±0.01^{a}	3.9±0.02^{a}	0.26±0.01^{a}	49±0.0^{a}
	B-3058	43±1.4^{a}	56±2.1^{a}	8±0.1^{b}	37±0.7^{a}	0.53±0.04^{a}	3.2±0.02^{a}	0.33±0.01^{a}	32±2.8^{b}
	B-2178	41±0.7^{a}	51±2.8^{a}	5±0.0^{c}	33±1.4^{ab}	0.51±0.01^{a}	3.0±0.00^{a}	0.34±0.02^{a}	34±2.1^{b}
	B-59149	41±2.8^{a}	55±0.7^{a}	7±0.2^{bc}	31±2.3^{ab}	0.51±0.02^{a}	3.6±0.05^{a}	0.35±0.04^{a}	35±2.3^{b}
Fermen't (37 ^{o}C)	Raw	43±2.0^{a}	55±1.2^{a}	13±0.1^{a}	29±2.0^{b}	0.53±0.01^{a}	3.9±0.02^{a}	0.26±0.01^{b}	49±0.0^{a}
	B-3058	47±0.0^{a}	59±2.1^{a}	9±0.6^{ab}	44±0.3^{a}	0.51±00^{a}	3.8±0.07^{a}	0.40±0.01^{a}	28±2.0^{b}
	B-2178	43±0.7^{a}	54±0.2^{a}	5±0.14^{b}	35±1.1^{b}	0.53±0.02^{a}	3.8±0.21^{a}	0.3±0.01^{b}	34±1.06^{b}
	B-59149	43±3.5^{a}	56±0.6^{a}	5±0.02^{b}	35±2.8^{b}	0.45±0.00^{a}	3.7±0.42^{a}	0.33±0.04^{ab}	33±0.07^{b}
Fermen't (42 C)	Raw	43±2.0^{a}	55±1.2^{a}	13±0.1^{a}	29±2.0^{a}	0.53±0.01^{a}	3.9±0.02^{c}	0.26±0.01^{b}	49±0.0^{a}
	B-3058	42±0.7^{a}	53±0.21^{a}	4.5±0.07^{b}	31±1.4^{a}	0.50±0.00^{a}	3.6±0.14^{a}	0.32±0.0^{ab}	29±0.7^{b}
	B-2178	41±0.5^{a}	52±2.8^{a}	4.5±0.3^{b}	34±0.71^{a}	0.53±0.02^{a}	3.6±0.07^{a}	0.38±0.0^{a}	35±2.8^{b}
	B-59149	39±2.0^{a}	51±2.1^{a}	3.0±0.14^{b}	30±2.4^{a}	0.51±0.04^{a}	3.7±0.35^{a}	0.32±0.04^{ab}	29±0.3^{b}

Data presented as means ±SD (n=3). The means in columns with different superscript letters are significantly different ($p<0.05$) (Relative to processing method) while means in columns with same superscript letters are not significant ($p<0.05$) (Relative to processing method); B-3058= *Lactobacillus plantarum*, B-2178= *Lactobacillus johnsonii*, 59149= *Lactobacillus rhammnosus* and Fermen't=Fermentation

(ii) Consumer Acceptability, Descriptive Sensory Profiling and Preference Mapping of the Avocado and Mango seeds Flour from an Optimized Method

- **Consumer Characteristics**

Table 10 indicates characteristics of the assessors (consumers). The results revealed that, out of 72 assessors, 33 (45.8 %) were female while 39 (54.2%) were male with age group ranging from 19-36 years. The results also indicated that 44 (61.1%) were bachelor degree students, 16 (22.2%) were diploma students and 12 (16.7%) were master degree students. Out of these, 2 (0.03%) were frequent consumers of the samples, while 70 (99.07%) were not users of the samples. This implies that the panel was dominated by not users of the samples female, young people and bachelor degree students. However, most of the consumers who were involved in the testing of the provided products were not frequent user of the samples.

Table 10: Consumer characteristics

Characteristics	Category	Frequency	Percentage (%)
Age group	18-36	71	98.6
	37-45	1	1.4
	Total	**72**	**100**
Gender	Female	33	45.8
	Male	39	54.2
	Total	**72**	**100**
Education level	Diploma	16	22.2
	Bachelor degree	44	61.1
	Master degree	12	16.7
	Total	**72**	**100**
Frequent user	Yes	2	0.03
	No	70	99.07
	Total	**72**	**100**

- **Hedonic Test**

Only fermented samples (mangoes or avocado seed kernels) by *lactobacillus plantarum* were tested, this was due to the fact that the method employed was capable of removing or reducing anti-nutrients to above 50%, maximum retaining or increasing of the analyzed nutrients and bioactive compounds hence adopted as an optimized method. Mean hedonic scores for the analyzed samples of fermented avocado and mango seed kernels are shown in Tables 11 and 12. With the exception of the attribute taste for mango seed kernels which had a significant

difference at ($p<0.05$), 72 consumers showed insignificant difference in all the remaining attributes assessed. For the fermented avocado seeds colour, taste and aroma attributes were statistically significant while texture was not statistically significant at $p<0.05$.

Table 11: Mean hedonic scores for the fermented mango seed kernel samples

Sample	Aroma	Colour	Texture	Taste
B-2178	7.07 ± 0.39^{a}	5.33 ± 0.33^{a}	6.53 ± 0.13^{a}	4.0 ± 0.09^{b}
B-3058	6.53 ± 0.92^{a}	6.40 ± 0.11^{a}	7.20 ± 0.32^{a}	4.93 ± 0.30^{a}
B-59149	7.20 ± 0.47^{a}	6.66 ± 0.64^{a}	6.93 ± 0.00^{a}	3.50 ± 0.12^{b}

Data presented as mean ± SD (n=72). The means in columns with different superscript letters are significantly different ($p<0.05$) (Relative to processing method) while means in columns with same superscript letters are not significant ($p<0.05$) (Relative to processing method); B-3058= *Lactobacillus plantarum*, B-2178= *Lactobacillus johnsonii* and 59149= *Lactobacillus rhammnosus*

Table 12: Mean hedonic scores for the fermented avocado seed samples

Sample	Aroma	Colour	Texture	Taste
B-2178	7.27 ± 0.01^{b}	7.73 ± 0.33^{a}	6.23 ± 0.34^{a}	4.27 ± 0.28^{a}
B-3058	9.47 ± 0.60^{a}	7.33 ± 0.11^{ab}	6.43 ± 0.00^{a}	4.53 ± 0.42^{a}
B-59149	6.67 ± 0.72^{b}	6.60 ± 0.30^{b}	6.00 ± 0.31^{a}	3.30 ± 0.02^{b}

Data presented as mean ± SD (n=72). The means in columns with different superscript letters are significantly different ($p<0.05$) (Relative to processing method) while means in columns with same superscript letters are not significant ($p<0.05$); (Relative to processing method) B-3058= *Lactobacillus plantarum*, B-2178= *Lactobacillus johnsonii* and 59149= *Lactobacillus rhammnosus*

- **Hedonic Liking**

The mean scores for the hedonic liking of avocado and mango seed kernels are shown in Tables 13 and 14 respectively. The results show that consumers did not express any significant difference ($p<0.05$) for both mango and avocado seeds, however mango seed kernels fermented by *Lactobacillus plantarum* scored high mean values followed by *Lactobacillus johnsonii* whereas the sample fermented by *Lactobacillus rhammnosus* had the least mean score. Avocado seeds fermented by *Lactobacillus plantarum* and *Lactobacillus johnsonii* scored similar mean values while *Lactobacillus rhammnosus* also had the lowest mean score.

Table 13: Hedonic liking mean scores for the fermented mango seed kernels

Samples	Overall acceptability
B-2178	6.53 ± 0.02^{a}
B-3058	6.65 ± 0.04^{a}
B-59149	6.41 ± 0.30^{a}

Data presented as mean ± SD (n=72). The means in columns with different superscript letters are significantly different ($p<0.05$) (Relative to processing method) while means in columns with same superscript letters are not significant ($p<0.05$) (Relative to processing method); B-3058= *Lactobacillus plantarum*, B-2178= *Lactobacillus johnsonii* and 59149= *Lactobacillus rhammnosus*

Table 14: Hedonic liking mean scores for the fermented avocado seeds

Samples	Overall acceptability
B-2178	6.47±0.59[a]
B-3058	6.49±0.06[a]
B-59149	6.30±0.31[a]

Data presented as mean ± SD (n=72). The means in columns with different superscript letters are significantly different ($p<0.05$) (Relative to processing method) while means in columns with same superscript letters are not significant ($p<0.05$) (Relative to processing method); B-3058= *Lactobacillus plantarum*, B-2178= *Lactobacillus johnsonii* and 59149= *Lactobacillus rhammnosus.*

- **Descriptive Sensory Profiling**

Quantitative descriptive analysis of the fermented avocado and mango seed kernels samples is shown in Fig. 10 and 11. The Figures indicate the bi-plot with two first significant principal components from the principal component analysis (PCA) on the average sensory attributes. In Fig. 10, principal component 1 accounted for 78.5% of the variation while principal component 2 accounted for 21.5% of the variations. Mango seed kernel fermented by *Lactobacillus johnsonii* and *Lactobacillus rhammnosus* were separated from the mango seed kernels fermented by *Lactobacillus plantarum*. Mango seed kernel fermented by *Lactobacillus johnsonii* and *Lactobacillus rhammnosus* are positively correlated with colour hue. However, in terms of other attributes such as texture, aroma and sweetness they correlated negatively. The samples fermented by *Lactobacillus plantarum* correlated negatively with texture, aroma and sweetness but correlated positively with colour hue attribute. Also the results show that the variation between fermented mango seed kernels were explained by colour hue attribute on the right hand side of principal component one (PC1) while texture, aroma and sweetness explained the variation between samples on the left hand side. In principal component two (PC2) the variation between fermented mango seeds was explained by attributes texture, aroma and sweetness on one side while colour hue explained the variation of the sample on the other side.

In Fig. 11, principal component 1 accounted for 88.1% of the variation while principal component 2 accounted for 11.9 % of the variations. Avocado seed fermented by *Lactobacillus plantarum and Lactobacillus johnsonii* were separated from the avocado seeds fermented by *Lactobacillus rhammnosus.* Avocado seed fermented by *Lactobacillus plantarum and Lactobacillus johnsonii* are positively correlated with texture and colour attributes and correlated negatively with aroma and sweetness. The samples fermented by *Lactobacillus rhammnosus* correlated positively with texture and colour hue and negatively with aroma and sweetness. The findings also indicated that the variation between fermented avocado seeds were explained by colour hue and texture attributes on the right hand side of PC1 while aroma and sweetness explained the

variation between samples on the left hand side. In PC2 the variation between fermented avocado seeds was explained by texture on one side while aroma and sweetness explained the variation of the sample on the other side of the bi-plot.

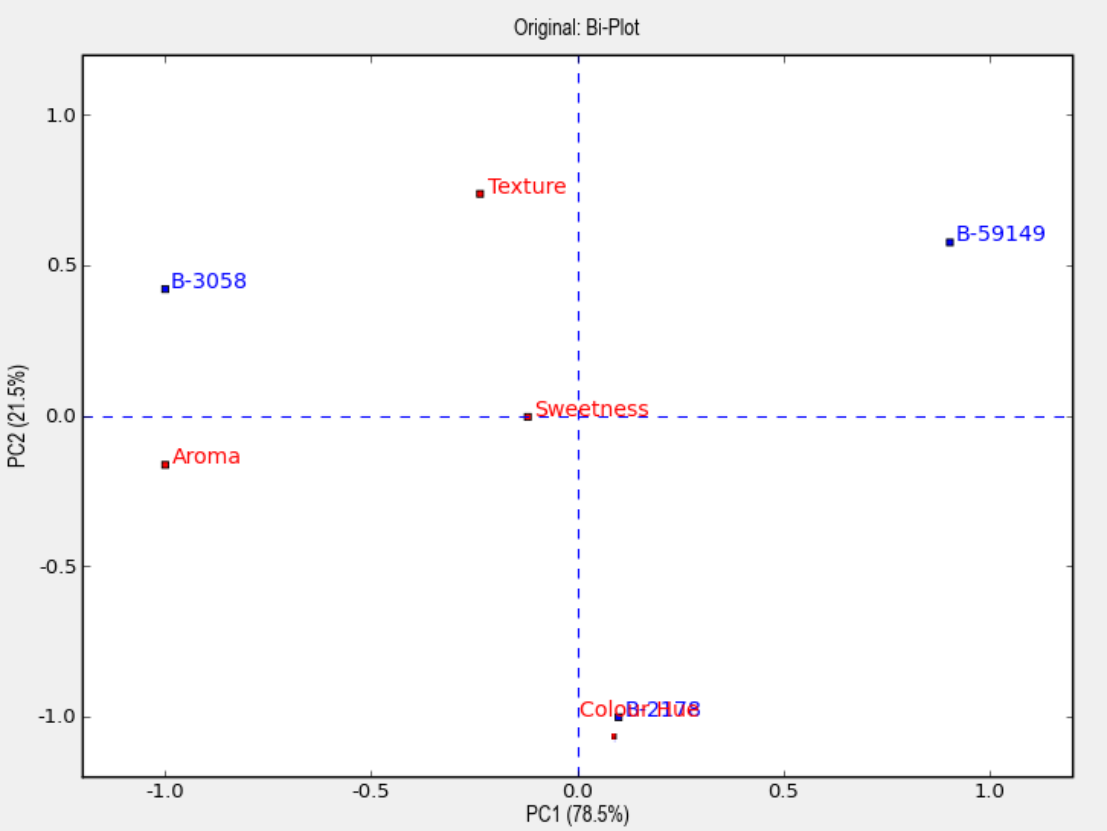


Figure 11: Bi – plot from PCA of descriptive sensory data of the fermented mango seed kernel samples; B-3058= *Lactobacillus plantarum*, B-2178= *Lactobacillus johnsonii* and 59149= *Lactobacillus rhammnosus*

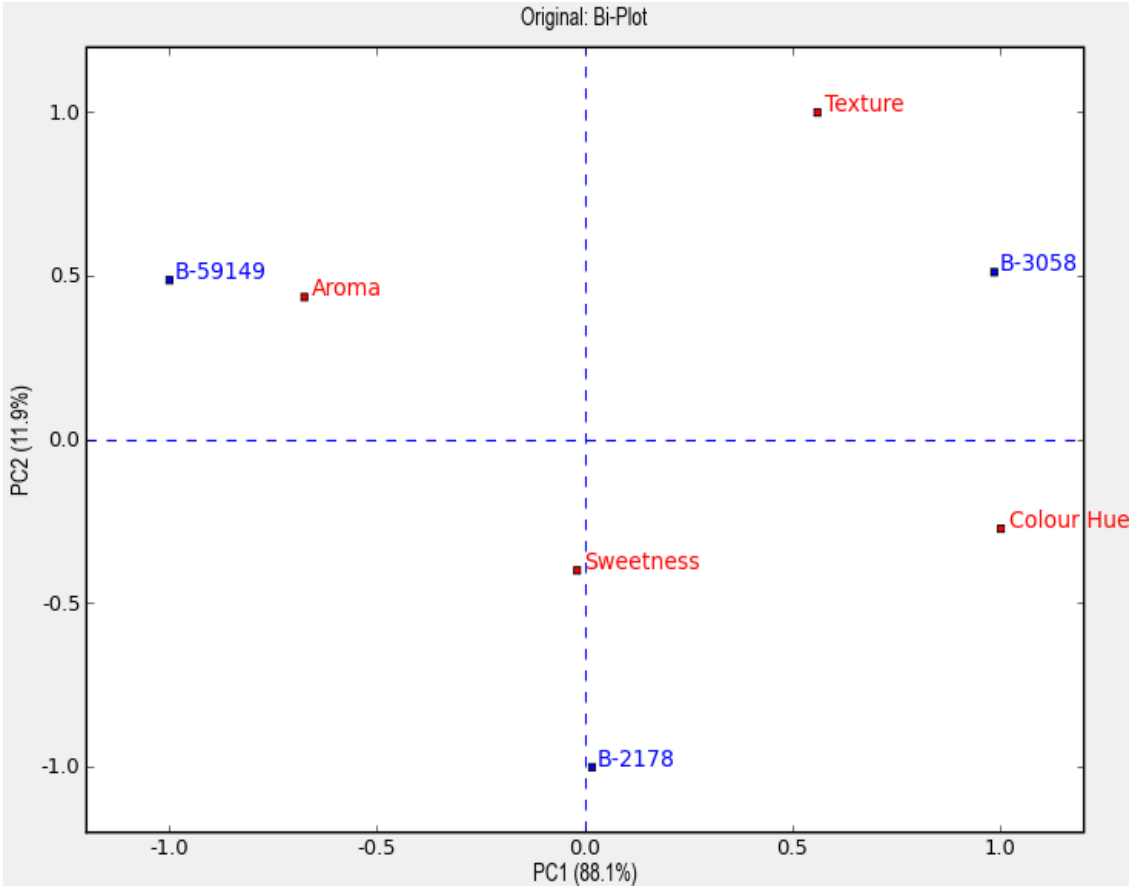


Figure 12: Bi-plot from PCA of descriptive sensory data of the fermented avocado seed samples; B-3058= *Lactobacillus plantarum*, B-2178= *Lactobacillus johnsonii* and 59149= *Lactobacillus rhammnosus*

- **Sample Differences**

The mean intensity ratings of descriptive attributes of fermented avocado and mango seed samples are shown by Fig. 13 and 14. The findings indicated that there was a significant difference at ($p<0.05$) in mean score of aroma, colour hue and texture attributes assessed for both fermented avocado and mango seed kernel samples except for sweetness attribute which showed insignificant results. This implies that fermentation had effect on aroma, colour hue and texture on fermented avocado and mango seed kernel samples.

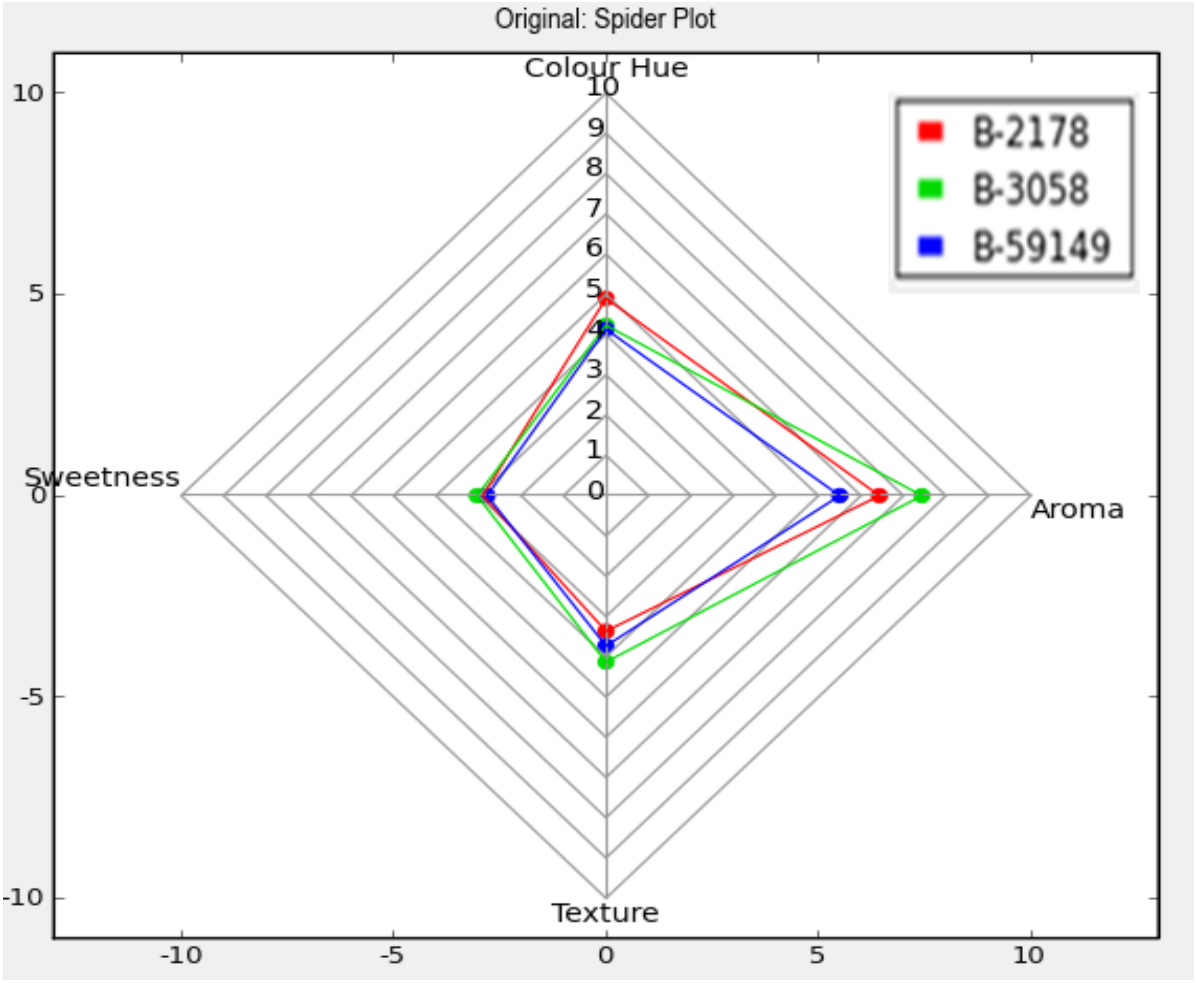


Figure 13: Spider plot showing the mean score of attributes between mango seed kernel samples; B-3058= *Lactobacillus plantarum*, B-2178= *Lactobacillus johnsonii* and 59149= *Lactobacillus rhammnosus*

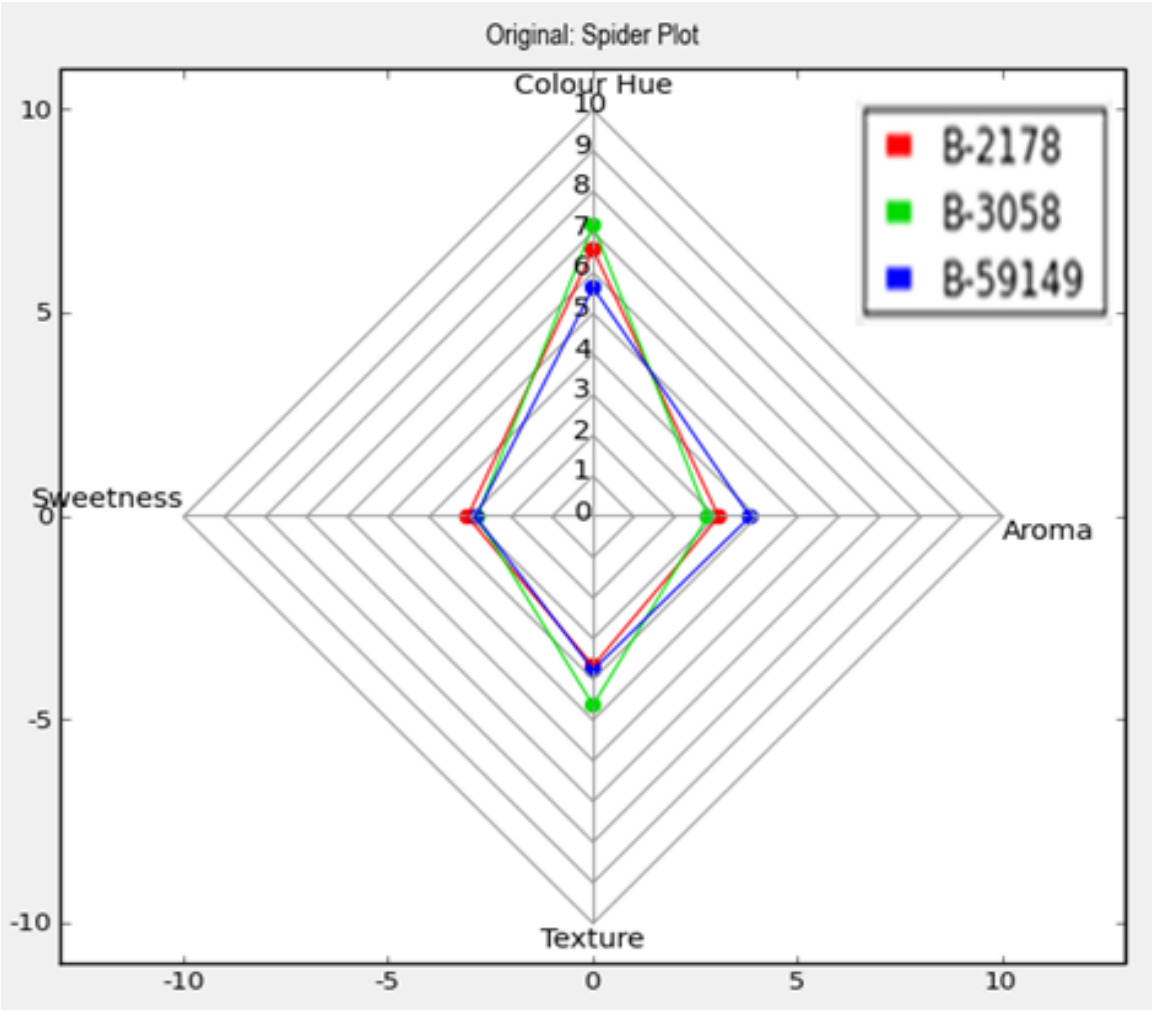


Figure 14: Spider plot showing the mean score of attributes for fermented avocado seed samples

- **Relationship Between Quantitative Descriptive and Hedonic Data (Preference Mapping)**

Figures 15 and 16 present the relationship between descriptive and hedonic data from a consumer check using descriptive data as X- variables and liking rated by the consumers as Y-variables. Results show that high density of consumers fall in the right hand side of the vertical-axis and this means that, the acceptance values of these consumers go in the direction of the samples fermented with *Lactobacillus plantarum* due to texture, aroma and color hue attributes for the fermented mango seed kernels and texture, color hue and sweetness for the fermented avocado seeds.

On the other hand, the samples fermented by *Lactobacillus johnsonii* and *Lactobacillus rhammnosus* were less preferred as they were found in the left hand side of the y- axis as a result

of sweetness attribute for fermented mango seed kernels and aroma for fermented avocado seed samples. Moreover, mango and avocado seeds fermented using *Lactobacillus johnsonii* and *Lactobacillus rhammnosus* are in the left direction from the overall linking indicating that they were not liked by consumers as compared with mango and avocado seed samples fermented using *Lactobacillus plantarum* which was in the right direction of the overall liking. Therefore, colour hue, aroma and texture are the key attributes which drove the consumer to prefer fermented mango seed kernels by *Lactobacillus plantarum* while texture and colour hue were the attributes that influenced the assessors like fermented avocado seed samples using *Lactobacillus plantarum.*

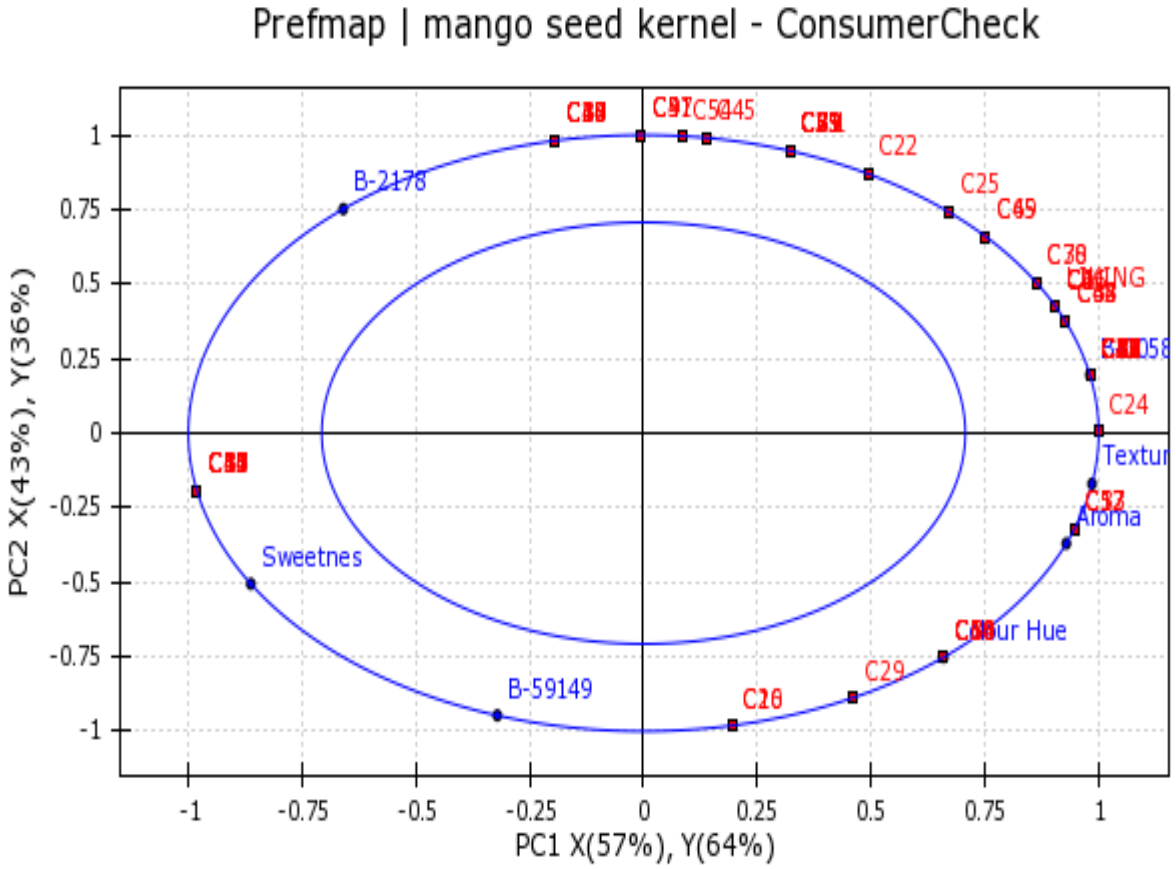


Figure 15: Correlation loadings from a partial least squares regression of three fermented mango seed kernels samples with descriptive data as X variables and hedonic rating as Y variables; B-3058= *Lactobacillus plantarum*, B-2178= *Lactobacillus johnsonii* and 59149= *Lactobacillus rhammnosus*

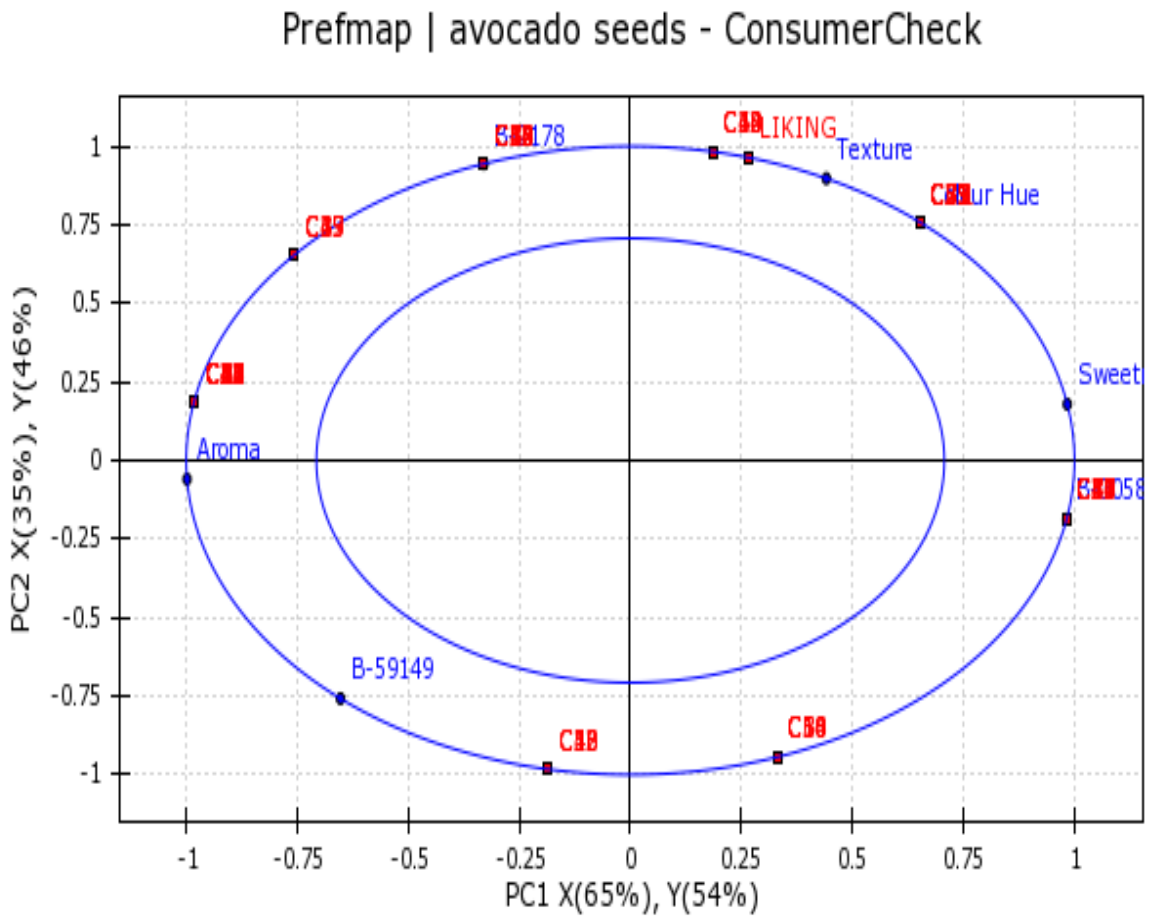


Figure 16: Correlation loadings from a partial least squares regression of three fermented avocado seed samples with descriptive data as X variables and hedonic rating as Y variables; B-3058= *Lactobacillus plantarum*, B-2178= *Lactobacillus johnsonii* and 59149= Lactobacillus *rhammnosus*

4.1.2 Formulation of Avocado and Mango Seeds-based Composite Flour

A Recipe and Nutrition Management Workspace integrated with the USDA National Nutrient Database for Standard Reference were used in formulating the composite flour. Avocado and mango seed extracts (53.5%) were used as the main ingredients. Other additional ingredients of the final included green banana (32%) which acted as the filler that provided baking characteristics, used to lower the content of fat in the final product and the cost of production, chia seeds (0.8%) as a binding material that provided stability and thickness, and the potato starch (13.7%) for taste and palatability of the formulated composite (Table 16). The developed composite flour had all necessary quality characteristics (baking properties, inexpensively cost and binding capacity), nutritional and functional values and improved sensory attributes as seen in Tables 18 and 21. The percentage ratios in each ingredient indicated in Table 16 considered adequate amounts of fatty acids, protein, lipid, fibre and carbohydrates (Table 17). Avocado and

mango seeds were the excellent source of functional values and essential nutrients as indicated in Table 16. Green banana had relatively high levels of carbohydrates and lower contents of lipids (Table 15), and were primarily included for their protein sparing function, filler material which provides the baking properties and its ability of reducing the higher fat contents (which is being restricted by FDA standards in flour based products) (Table 16). Potato starch was added so as to improve the taste and increase palatability of the composite flour while chia seeds were used as a binder material, but also aids in thickening as well as stabilization in food products. Chia seeds also have higher content of dietary fibre which helps in water holding during processing like baking, cooking and extrusion.

Results of crude protein for the developed composite flour from a Recipe and Nutrition Management Workspace were higher (7.23%) than the value from the actual lab analysis (6.71%). The Recipe and Nutrition Management Workspace output also indicated that the composite flour had high amount of carbohydrates (74.96%), which was different from the obtained analytical results (73.49%) (Table 1). However, the energy content of the developed composite flour for the laboratory analysis was higher than that obtained from the Recipe and Nutrition Management Workspace. These differences show the importance of having all nutritional and functional informations in the Nutrasheets database especially for some information of the avocado and mango seed kernels that are not found in the *USDA* National *Nutrient Database* for Standard Reference. However, the developed composite flour was assessed for the safety parameters and sensory quality and it was found safe and accepted by the assessors (Tables 18 and 19). Furthermore, pH and water activity was within the range that could not support the growth of spoilage microorganisms (Table 18).

Table 15: Fatty acid profile and proximate composition of the ingredients to be used in formulating the composite flour

Fatty acid	[1]Mango seed flour	[1]Avocado seed flour	[2]Green banana flour	[2]Chia seeds	[2]Potato starch
	g/100 g	mg/100 g	g/100 g	g/100 g	g/100 g
Saturated	43	476	49.8	16.6	23
Unsaturated	55	976	47.1	74	64
Palmitic acid (16:0)	13	247	41	8.2	11.8
Stearic acid (18:0)	29	55	4	4.05	6.5
Myristic acid (14:0)	0.53	25	3.8	nd	nd
Linoleic acid (18:2)	3.9	570	20.3	17.6	13.5
Linolenic acid (18:3)	0.26	253	20	39.5	9.5
Oleic acid (18.1)	49	146	4.1	14.6	39
Proximate composition	%	%	%	%	%
Moisture	8.1	8.68	9.23	8.6	5.7
Lipid	5.02	8.45	0.78	23.4	2.6
Protein	3.2	8.01	5.14	14.3	2.04
Carbohydrates	75.37	69.34	75.03	21.5	89.5
Ash	3.91	2.62	1.96	3.0	0.16
Fibre	4.4	2.9	7.86	29.2	0.00

[1] Analyzed parameters in the laboratory
[2] *USDA* National *Nutrient Database* for Standard Reference

Table 16: Formulation obtained from the Recipe and Nutrition Management Workspace for the developed composite flour

Ingredients	[1]Composite flour			Functions
	Percentage (%)	Quantity (g)	Cost in ($)	
Avocado seeds, four	28.0	280	1.1	Excellent source of functional values and essential nutrients (antioxidant activity, total phenols, fatty acids, vitamins and minerals such as iron, calcium, potassium and sodium)
Mango seed kernels, flour	25.5	255	1	
Green bananas, flour	32	320	0.4	Used as a filler material which provides the baking properties, has higher amount of carbohydrates which is inexpensive source of energy that spare protein from being used as a source of energy. They are rich in dietary fibre specifically resistance. Also it has been considered due to its ability of reducing the higher fat contents which is being restricted by FDA in flour based products.
Chia seeds	0.8	8	0.04	Binder material which is used to provide thickening as well as stabilization in food products. It has higher content of crude fibre which helps in holding more water during processing like baking. Cooking and extrusion.
Potato starch	13.7	137	0.04	Adds taste and increases palatability. It rich in high carbohydrates, ascorbic acid, and vitamin E predominantly represented by α-tocopherol which is believed to play a key role in the body's defense system against reactive oxygen species (ROS) that is involved in the pathogenesis of aging and many degenerative diseases such as cardiovascular diseases and cancers.
Total	100	1000	2.54	

[1]Quantities weighed as is in Recipe and Nutrition Management Workspace which uses individual moisture content of each ingredient in computing best practical output based on evaporation/absorption desired

Table 17: Recipe and Nutrition Management Workspace output and laboratory analysis values of the developed composite flour

Parameters	[1]Composite flour	[2]Composite flour
Lipid (%)	2.1	3.4
Protein (%)	7.23	6.71
Carbohydrates (%)	74.96	73.49
Ash (%)	1.51	2.3
Crude fibre (%)	5.35	6.3
Moisture content (%)	8	7.8
Energy (kcal/g)	338.18	351.44
Cost ($/kg)	2.54	-
Fatty acids, total saturated (g/100 g)	0.09	0.088
Fatty acids, total trans (g/100 g)	0.00%	0.00
Fatty acids, total polyunsaturated (g/100 g)	0.08	-
Fatty acids, total monounsaturated (g/100 g)	0.42	-
Cholesterol (mg/100 g)	0.00	-
Sodium, Na (mg/100 g)	0.06	0.054
Sugars, total (mg/100 g)	0.06	-
Calcium, Ca (mg/100 g)	5.08	5.09
Iron, Fe (mg/100 g)	0.22	0.23
Potassium, K (mg/100 g)	208	204
Vitamin A, IU	0.43	-
Vitamin C, total ascorbic acid (mg/100 g)	9.22	9.00
Vitamin E ,alpha-tocopherol (mg/100 g)	0.07	0.065
Vitamin K, phylloquinone (µg/100 g)	0.29	-
Thiamin (mg/100 g)	0.02	-
Riboflavin (mg/100 g)	0.04	-
Folate, total (µg/100 g)	11.88	-
Niacin (mg/100 g)	0.45	-
Phosphorus, P(mg/100 g)	19.52	18.6
Magnesium, Mg (mg/100 g)	18.19	18.3
Zinc, Zn (mg/100 g)	0.13	0.14

[1]Recipe and Nutrition Management Workspace output values; [2] laboratory analysis values (n =3); - means not examined; n means the mean was obtained by the sum of three results divide by three

Table 18: Microbiological parameters, water activity and pH of the developed avocado and mango seeds-based composite flour stored at different temperatures

Product	Microbiological test (log_{10} cfu/g)		Water activity(a_w)	pH
	Yeast and molds	Salmonella		
DCF (15^{o}C)	1.41	nd	5.3	5.4
DCF (30^{o}C)	1.35	nd	5.2	5.3
DCF (45^{o}C)	1.36	nd	5.1	5.2

DCF=Developed composite flour; nd= not detected

Table 19: Acceptability scores of the formulated composite flour stored at different temperatures

Product	Acceptability scores				Overall acceptability
	Aroma	Colour	Texture	Taste	
DCF (15^{o}C)	6.45±0.24^a	7.16±0.44^a	6.40±0.03^a	6.45±0.18^b	6.8±0.27^a
DCF (30^{o}C)	6.69±0.67^a	7.29±0.12^a	6.38±0.10^a	7.06±0.22^a	7.2±0.43^a
DCF (45^{o}C)	6.34±0.20^b	6.52±0.40^{ab}	6.33±0.42^a	6.58±0.01^b	6.7±0.56^a

Data presented as mean ± SD (n=72). Means in the column with different superscript small letters indicate significant difference at ($p<0.05$) (Relative to processing method); DCF= Developed composite flour

4.1.3 Shelf Life of the Developed Avocado and Mango Seeds-based Composite Flour

Shelf life prediction of the developed composite flour was determined by using the amount of peroxide values produced during storage time (days) at different temperatures. The peroxide value was used in shelf life prediction because is an indicator for determining the quality and stability of mostly dried foods having fat content since it deteriorates most rapidly in time as results of oxidation. The composite flour was stored at temperatures of 15 ^{o}C, 30 ^{o}C and 45 ^{o}C following the monitoring of the trend of peroxide production for 84 days with an interval of three weeks (Table 21). Other parameters that were analyzed were the microbiological test (salmonella and fungi), water activity and pH within the set time (84 days for the interval of 3 weeks) aiming at determining stability of the developed composite flour as shown in Table 20. The highest fungi count was 1.5 6cfu/g at a temperature of 15 ^{o}C while the lowest fungi count was 1.31 cfu/g which was at a temperature of 42 ^{o}C stored for 42 days and no salmonella was detected at all set Table 20. In the tested microbiological parameters, it was observed that the highest fungi (yeast temperatures for 84 days (Table 20). The analyzed water activity at all set temperature for 84 days ranged from 4.2 to 5.6. The result of the pH in this research also ranged from 4.5 (highest) to 5.4 (lowest). Findings indicate that pH of the developed composite flour increased with time as temperature increased.

The peroxide values at the highest storage time (84 days) were 1.09 meq/kg sample (DCF-1), 1.6 meq/kg sample (CDF-2) and 1.42 meq/kg sample (CDF-3). These were further applied to estimate the shelf life of the developed composite flour (Table 21) using the equations on each product obtained from the graphs and the 10 meq/kg sample as a critical standard reference of the peroxide value for the shelf life stability of foods having fat contents. Therefore, results of this study estimated shelf life at various set temperatures ranged between 79 - 115 days as indicated in Table 22. The maximum estimated shelf life was 4 months. The observed maximum shelf life of the developed composite flour was noted at a room temperature (30 ^{o}C) which is the target storage temperature of the developed product in this study.

Table 20: Analyzed microbiological parameters, water activity and pH of the developed composite flour stored at different temperatures and days

Product	Microbiological test (log_{10} cfu/g)										Water activity(a_w)					pH				
	Yeast and molds					Salmonella														
	0	**21**	**42**	**63**	**84**	**0**	**21**	**42**	**63**	**84**	**0**	**21**	**42**	**63**	**84**	**0**	**21**	**42**	**63**	**84**
DCF (15^oC)	1.41	1.43	1.47	1.52	1.56	nd	nd	nd	nd	nd	5.3	5.4	5.5	5.5	5.6	5.4	5.2	5.1	5.0	4.8
DCF (30^oC)	1.35	1.35	1.37	1.42	1.49	nd	nd	nd	nd	nd	5.2	5.0	5.0	4.8	4.7	5.3	5.0	4.9	4.8	4.6
DCF (45^oC)	1.36	1.33	1.31	1.32	1.34	nd	nd	nd	nd	nd	5.1	4.4	4.3	4.3	4.2	5.2	4.99	4.8	4.7	4.5

DCF=Developed composite flour; nd= not detected; 0, 21, 42, 63 and 84= storage days

Table 21: Peroxide value produced at different storage temperatures (15°C, 30°C and 45°C) and days

Product	Peroxide (meq/kg sample) v/s Temperature variations				
	0	21	42	63	84
DCF (15°C)	0.11±0.01	0.19±0.00	0.24±0.02	0.72±0.04	1.09±0.00
DCF (30°C)	0.4±0.02	0.67±0.03	0.8±0.00	1.4±0.01	1.6±0.02
DCF (45°C)	0.22±0.00	0.27±0.00	0.41±0.01	1.12±0.02	1.42±0.11

Values are expressed as mean±SD (n=2); DCF = Developed composite flour

Table 22: Shelf life prediction by use of peroxide values of the developed composite flour stored at different temperatures and days

Product	Regression equation	R^2	Estimated Shelf life (days)
DCF (15°C)	Y=0.028x--2.2913	0.960	79
DCF (30°C)	Y=0.016x--0.8486	0.965	115
DCF (45°C)	Y=0.024x--1.6828	0.938	81

DCF = Developed composite flour

4.2 Discussion

4.2.1 Optimized Processing Method used to Make Composite Flour from Avocado and Mango Seed Kernels

There has been significant progress in applying different food processing techniques in the food industries such as thermal, non-thermal and fermentation for process optimization (Abakarov & Nuñez, 2012). Optimized processing method aims at coming up with the best solutions for improving food quality parameters of a given food. This can be achieved by setting suitable conditions in order to maximize nutrients, functional values, and sensory attributes and minimize antinutrients to acceptable levels (Banga *et al.*, 2008). In this study, the applications of different processing techniques (probiotic fermentation, boiling and soaking) on the avocado and mango seed kernels were evaluated.

(i) Nutritional, Total Phenols, Antinutritional Compounds and Antioxidant Property of the Selected Avocado and Mango Seed Kernels

- **Antinutritional Compounds**

Antinutritional factors present in the raw seeds of avocado or mango limit the use of these seeds in animal and human nutrition, in spite of their nutritional composition (Fowomola, 2010;

Talabi *et al.,* 2016). The negative effects of these antinutrients can be attributed to their concentration, chemical structure, time of exposure and interaction with another dietary component thus decreasing their bioavailability (Popova & Mihaylova, 2019). Reducing or eliminating these antinutrients to acceptable levels is needed so as to improve the utilization of avocado and mango seed kernels in the food industry.

In this study, all employed processing techniques removed or reduced these natural toxicants found in mango and avocado seeds. The reduction of the antinutrients in mango and avocado seeds upon soaking, boiling and probiotic fermentation had been reported in other previous studies (Torres-León *et al.,* 2016; Adegbehingbe *et al.,* 2017). However, the reduction of the phytates oxalates, tannins and saponin increased as time increased for boiling and soaking in both avocado and mango seeds. Similar results for such reduction of antinutrients as time increased were reported by Sotelo *et al.* (2010). For example, the highest reduction of anti-nutrients with maximum boiling time was observed in a study conducted by Talabi *et al.* (2016). Also it was reported by Handa *et al*. (2017) that there were a decrease in tannins and oxalates upon soaking of underutilized pulse (horsegram) as soaking time increased. The reasons for increased reduction of antinutrients with increase time could be due to more leaching and diffusion of the antinutrients into water as time increased and upon removal of soaked or boiled samples in water the leached or diffused antinutrients remained in water. This was in agreement with results published by Moroni *et al*. (2015) and Adeleke *et al.* (2017).

As observed above, probiotic fermentation also reduced the tannins, phytates and oxalates to the significant levels statistically. Despite the reduction of the antinutrients by probiotic fermentation there were significant variations in the reduction of oxalate, phytates, tannins and saponin by probiotic fermentation due to the action and capacity of lactic acid bacteria in producing different levels of metabolites which could remove or reduce antinutrients at different temperatures. Similar results were described in other studies by Moroni *et al.* (2015) in fermented grains. Thus, this observation helps to come up with an optimized method with the best results in relation to the reduction of the antinutrients. Additionally, the contents of the antinutrients obtained in this study were a bit lower as compared with the findings published by Fowomola (2010) and Adegbehingbe *et al.* (2017). The obtained differences in the contents of antinutrients may be attributed to the different cultivars (as a result of the genetic makeup of the specific plant), climatic conditions, harvesting time and soil properties.

Vitamins (Ascorbic acid and α-tocopherol), Total Phenol and Antioxidant Activity

Among the three processing techniques employed in this study, boiling and soaking reduced the contents of ascorbic acid in both avocado and mango seed kernels. Similar results were reported by other scholars. For example, Han *et al.* (2004) reported the loss of ascorbic acid in boiled potatoes. Also a decline in ascorbic acid content during soaking was observed previously in soaked soy bean as reported by Kaushik and Satya (2010). The loss of ascorbic acid increased as time increased in boiled and soaked avocado and mango seed kernels. This loss of ascorbic acid contents during boiling and soaking was due to the fact that ascorbic acid is a water soluble and heat sensitive vitamin (Okmen & Bayindirli, 1999; Shintani, 2013). Ascorbic acid is an antioxidant that plays a potential role in human health such as neutralizing free radicals in the body, converting proline and lysine to hydroxyproline and hydroxysine both important to the collagen structure it also helps in production of thyroid hormones for the treatment goiter (Iqbal *et al*., 2004). Therefore, loss of ascorbic acid during processing has a disadvantage of reducing the quality of food (Reddy & Love, 1999; Hailemariam & Wudineh, 2020).

On the other hand, fermented avocado and mango seed kernels showed some contrary results of ascorbic acid as compared with boiled and soaked avocado and mango seed kernels. The observed increase in ascorbic acid content upon probiotic fermented avocado and mango seeds might be due to increased activities and ability of fermenting microorganisms that led to the disruption of ester bond linkages thus releasing the ascorbic acid that resulted into its increase as observed by other investigators. It has been observed that vitamin C content of red beans, citrus peels and white cabbage increased with fermentation (Adetuyi & Ibrahim, 2014; Jhan *et al.*, 2015). The observed retention or increase of ascorbic acid as a result of fermented samples is the evidence that final product (avocado and mango seed-based composite flour) is rich in ascorbic acid content.

The non-significant differences observed in vitamin E (α-tocopherol) contents in the processed avocado and mango seeds in all the treatments may be attributed to the inactivation of lipoxygenase enzyme responsible for lipid peroxidation upon boiling and the nature of vitamin E being fat soluble vitamin hence not leached or dissolved in water during fermentation, boiling and soaking (Sistrunk, 1977; Kansson & Jagerstad, 1990).

Soaked avocado and mango seed kernels revealed a significant decrease in the total phenolic contents. The decline in bioavailability of total phenol in soaked mango and avocado seeds

possibly, may have been attributed by leaching and diffusion of phenolics in cell liquids and such decrease was also seen in soaked green gram as reported by Afify *et al.* (2012) and Oghbaei and Prakash (2017). Contrary results were shown by boiled and fermented avocado and mango seed kernels which increased or retained the total phenolic contents. In normal form, phenolic compounds are mutual or bound with sugar which decreases their bio-availability (Adetuyi & Ibrahim, 2014). During probiotic fermentation, usually proteolytic enzymes from the starter organisms hydrolyse complexes of these phenolics into soluble free phenols (Adetuyi & Ibrahim, 2014). Therefore, the increased or retained total phenolic contents observed during boiling and probiotic fermentation of avocado and mango seed kernels were attributed to the opening of the cell matrix during boiling which facilitated the extractability and bio-availability of total phytochemicals and cleavage of the ester linkages by synthesizing enzymes during probiotic fermentation (Acosta-Estrada *et al.,* 2014; Tian *et al.,* 2016). Additionally, in probiotic fermented avocado and mango seed kernels there was a remarkable increase in variations of total phenol at different fermentation conditions which were influenced by the ability of a specific microorganisms at different temperatures for enzymatic processes as explained by Moroni *et al.* (2015).

With regard to 1,1-diphenyl-2-picryl-hydrazyl (DPPH), it is a stable free radical that has an unpaired valence electron on one atom of the nitrogen bridge and has been often used to evaluate antioxidant activity of foodstuff (Oghbaei & Prakash, 2017). The IC_{50} value is defined as the concentration of the sample extracts causing 50 percent inhibition of absorbance and therefore a lower IC_{50} value reflects a greater antioxidant activity of the sample (Ananas *et al.*, 2010; Olawoye & Gbadamosi, 2017).

In this study, the loss of DPPH radical scavenging activity was observed after boiling and soaking of avocado and mango seed kernels. Similar results were reported by other previous scholars. For example, during boiling and soaking there was a significant degradation of the phytochemicals and leaching of the essential antioxidants which all contributes to loss of antioxidant activity (Perla *et al.,* 2012). However, in this study, probiotic fermented avocado and mango seed kernels showed a significant increase in the antioxidant activity. The observed increase in the antioxidant activity as a result of probiotic fermentation reported in this study is in agreement with the data given by Virtanen *et al.* (2007) who also found that fermentation of milk whey when using lactic acid bacteria resulted in an increase of its antioxidant activity. In addition, the extent of increased antioxidant activity of fermented avocado and mango seed kernel varied with the microorganisms were used. Similar variations of antioxidant activities

were observed when different starter microorganisms used during fermentation of soy milk (Wang *et al.,* 2006). Additionally, the high antioxidant activity observed as a result of probiotic fermented avocado and mango seed kernels could be due to the increase in hydroxyl groups or amino groups in the antioxidant compounds and synthesis of the phenolics (Olawoye & Gbadamosi, 2017).

However, fermented avocado and mango seed kernels with *Lactobacillus plantarum* had the highest antioxidant activity. The observed high antioxidant activity in avocado and mango seed kernels fermented with *Lactobacillus plantarum* could be contributed to its exceptional characteristics (genetic makeup and ability to produce varieties of enzymes) in comparison with *Lactobacillus johnsonii* and *Lactobacillus rhammnosus* as reported by other researchers. For example, a study conducted by Filannino *et al.* (2020) on the capability of selected lactic acid bacteria to enrich the portfolio of bioactive compounds of avocado fruit, *Lactobacillus plantarum* showed the highest antioxidant activity compared with other used lactic acid bacteria. Furthermore, a study conducted by Adeleke *et al*. (2017) using various lactic acid bacteria on fermented cassava peels, *Lactobacillus plantarum* demonstrated the best results in the production of linamarase enzyme. In this study it was also observed that fermented avocado or mango seed kernels by *Lactobacillus plantarum* gave the best results (highest antioxidant activity) in comparisons to other used lactobacillus strains (johnsonii and rhammnosus*).* The increase in the antioxidant activity as a result of probiotic fermentation observed in this study is in agreement with the report of Jamro and Starzyn (2008) who found that fermentation of grass pea seeds by starter culture resulted in an increase of its antiradical effect against DPPH.

- **Minerals**

Minerals can be categorized into major and trace elements subject to their concentration present and amount required by human body (Lasano *et al.,* 2019). In the current study, three major minerals and two trace minerals were analyzed in avocado and mango seed kernels. From the results obtained, it was observed that boiled and soaked avocado and mango seed kernels significantly reduced the contents of some minerals. The decline in mineral contents in soaked and boiled avocado and mango seed kernels was in agreement with other scholars. For example, the study conducted by Ojha *et al.* (2020) showed a significant reduction in mineral contents (iron, calcium, and phosphorous) when mango kernel powder was soaked and boiled. Similarly, a significant decrease of calcium in undercorticated castor oil seeds on boiling and soaking was reported by Nsa *et al.* (2011). Also Lestienne *et al.* (2005) observed a significant reduction of

iron in millet, rice and soybean on the effects of soaking whole cereals and legume seeds. This significant reduction of some minerals in boiled and soaked avocado and mango seed kernels was due to leaching in water during processing (Hefnawy, 2011). Processing foods makes it healthier, safer, tastier and shelf-stable and on the other hand processing can also be detrimental, affecting the nutritional quality of foods (Reddy & Love, 1999) However, reduction of nutrients including mineral contents upon processing in this study reflects loss of quality of the final product.

Probiotic fermented avocado and mango seed kernels had no significance differences of all mineral analyzed in this experiment among the treatments. Similar results were reported by Afoakwa *et al.* (2013) who noted insignificant change in sodium content of cocoa beans upon fermentation. Also Bilgiçli *et al.* (2006) observed no significant difference in calcium, potassium and Magnesium on fermented tarhana dough. Nevertheless, the values of some analyzed minerals in avocado and mango seed kernels were found to be lower compared to the data published by Talabi *et al*. (2016) and Yatnatti *et al.* (2014) respectively. These differences may be due to a number of factors including type of the soil, environmental conditions and type of cultivars used.

- **Fatty Acids**

Plant based products are claimed to have low saturated fat, low or no cholesterol content and balanced amount of unsaturated fat which attract most of the consumers trying to reduce fat intake (Tavella *et al.,* 2000). Regulations on the mandatory product labeling vary from country to country, but rarely include details of fatty acid composition. In those cases where there is information about fatty acids, trans-fatty acids are usually not named for the products that contain less than 0.5 gram of total fat in a serving and if declared shall be expressed as zero (Tavella *et al*., 2000; Food and Drug Administration [FDA], 2006). In this study, both avocado and mango seed kernels had a remarkably higher level of unsaturated fatty acids which is good for consumption as it has beneficial effects in human over the saturated fatty acids. The proportions of the major fatty acid obtained in the current findings for both fermented avocado and mango seed kernels were in accordance with the previous studies by Banerjee (2016) and Ge *et al.* (2018) that showed linoleic, palmitic, and oleic acids were the predominant fatty acids in avocado seeds while palmitic acid, stearic acid and oleic acid were observed as the major fatty acids in mango seed kernels. There was a noticeable significance increase of fatty acid in fermented and boiled samples while all soaked samples had no significant change. This

significant increase may be as a result of the synthesis or breakage of the fatty acid bonds during boiling and fermentation which release and accumulate extra fatty acid (Chukwu *et al.,* 2019). However, the insignificant change in fatty acid content during soaking in this study was also reported by Sarkar *et al.* (1996) who noticed no change of fatty acid in soaked soya beans.

(ii) Consumer Acceptability, Descriptive Sensory Profiling and Preference Mapping of the Product from an Optimized Method

- **Consumer Studies**

Development of new products involves sensory and consumers understanding as those are critical for commercialization success (De Andrade *et al*., 2018). The present study involved the demographic and consumption characteristics of consumers, hedonic testing and consumer liking of the innovative products (fermented avocado and mango seed kernels) by lactic acid bacteria. Females dominated the panel used, followed by young people and bachelor degree holders. However, most of the consumers who were involved in the testing of the provided products were not user of the samples. Mean hedonic scores for the sweet taste attribute in both fermented avocado and mango seed kernels showed a statistical difference indicating that different people involved in this test can be classified as either sweet likers or sweet dislikers. This result is in agreement with the report published in a case study on the effect of hedonic claims on consumer hedonic and sensory perception of sugar reduction in orange/passion fruit nectars (Oliveira *et al.,* 2018). Also the insignificant differences witnessed by consumers in some attributes such as aroma, colour and texture for fermented avocado seed samples and texture for fermented mango seed kernels indicated that their sensory properties were close and hence hedonic liking (overall acceptance) in all consumers did not express any significant difference. Hence, it is clear that the attributes colour, aroma and texture in this need no any improvements for the final product.

- **Descriptive Sensory Profiling**

Descriptive sensory profiling is an important tool for any food industry as it guides product development and re-formulation of products so as to identify key sensory drivers essential for consumer acceptance and marketing of products (Reinbach *et al.,* 2014). Based on the obtained findings in this study, it has been explained for each tested product what has made it being preferred by consumers. For example, in Fig. 12 fermented avocado sample by *Lactobacillus plantarum* was more preferred due to its colour hue intensity (highest) compared with fermented

avocado seed samples by *Lactobacillus johnsonii* and *Lactobacillus rhammnosus* consequently it was less preferred as a result of aroma attribute.

Furthermore, sample fermented by *Lactobacillus rhammnosus* correlated positively with texture and colour hue while aroma and sweetness correlated negatively to the same sample. These findings confirm that quantitative descriptive analysis is suitable for capturing differences among products and thus it can be a useful tool for understanding consumer perceptions on the developed sensory descriptors (Reinbach *et al.,* 2014). Similarly, the results are in agreement with earlier studies which indicate that trained panelists can sensorially describe and discriminate between samples (Fonseca *et al.,* 2016).

- **Preference Mapping**

Preference mapping is a perceptual map that is used in understanding the relationship between descriptive sensory and consumer data for product developers to identify design and improvement opportunities relative to the competition within the sample product category (Lovely & Meullenet, 2009; Mongi *et al*., 2013). It can help food designers in their product optimization efforts to meet consumer expectations, especially overall liking and acceptance (Yenket *et al*., 2011; Mongi *et al.,* 2013; Ng'ong'ola-Manani *et al.,* 2014). In this study, three lactic acid bacterial namely *Lactobacillus plantarum*, *Lactobacillus johnsonii* and *Lactobacillus rhammnosus* were used to ferment avocado and mango seed kernels. The used strains were aimed at improving the acceptance of the fermented avocado and mango seed kernels by developing desirable sensory properties as recommended by other scholars (Ng'ong'ola-Manani *et al.,* 2014). During preference mapping, external preference mapping showed that most consumers preferred both fermented avocado and mango seed kernels by *Lactobacillus plantarum*. Drivers of liking for these samples fermented using *Lactobacillus plantarum* included colour, texture and aroma attributes. This is in conformity with the literature showing that colour is one of the initial quality attribute which attract people to food product and thus considered as an index of the inherent good quality of foods associated with the acceptability (Mongi *et al*., 2013). On the contrary, attributes that showed highly on the opposite direction of attributes driving liking of the majority of the consumers can be considered as drivers of dislike of these products (Ng'ong'ola-Manani *et al.,* 2014). Therefore, sweetness as one of the attributes had the lowest intensity in the used scale (1-9).

There were no significant differences in overall acceptance of the products by consumers, even though avocado or mango seed kernels by *Lactobacillus plantarum*. This implies that all avocado and mango seed kernels fermented with *Lactobacillus plantarum* directionally liked. This implies that all avocado or mango seed kernels fermented by *Lactobacillus plantarum*, *Lactobacillus johnsonii* and *Lactobacillus rhammnosus* had the potential of being used by the consumers. However, to increase utilization and acceptance of the fermented avocado and mango seed kernels, it would be useful to optimize drivers of liking which will influence the acceptance of the samples by improving desirable property which had the lowest intensity (sweet taste) so as to increase acceptability and utilization of fermented avocado and mango seed kernels.

4.2.2 Formulation of the Avocado Mango Seeds-based Composite Flour

To the best of my knowledge this was the first study to use the Recipe and Nutrition Management Workspace to formulate the composite flour from avocado and mango seeds kernels as the main ingredients (53.5%) targeting functional foods. Other ingredients used in this study were green banana with the intention of being used as the filler and providing the baking characteristics, chia seeds as the binder while potato starch was aimed at adding taste and increase palatability of the developed composite flour. The use of the locally available ingredients was intended to replace wheat flour. This is the current advocacy of the Food and Agriculture Organization (FAO). The idea is to utilize available inexpensive local foods which are rich source of essential nutrients and functional values (Ituen, 2011; Noorfarahzilah *et al.*, 2014). With exception of avocado and mango seed kernel extracts, the rest of the ingredients used were sourced from the supermarket stores and flour mills which are all reported as the major blends for composite flour used by other scholars with the aim of improving baking characteristics, sensory attributes of the composite flour (Ituen, 2011).

The Recipe and Nutrition Management Workspace used was aimed at reducing the fat content so as to follow to the Food and Drug Administration (FDA) restrictions which allows lower fat contents of the flour related products. This was also meant to increase the amount of carbohydrate to about 75% and availing to other essential nutrients and functional values of the developed composite flour. Thus, Recipe and Nutrition Management Workspace enables intended formulations that take into account all essential nutrients and functional values in balanced proportions. Such balanced proportions of the nutrients in the blends of the composite flour was observed by other researchers as reported by Shafi et *al.* (2017). However, in this

study the final moisture content of the formulated composite flour by the Recipe and Nutrition Management Workspace was 8%. This is within the recommended moisture content suitable for the extended shelf life (Nasir *et al.*, 2003).

Furthermore, developed avocado and mango seed-based composite flour was accepted by the assessors, all safety parameters analyzed were within the recommended tolerable limits and the pH and water activity obtained do not allow the growth of spoilage microorganisms. Generally, the results of this study indicated that the formulated composite flour is suitable to be incorporated into various functional foods since it had all the required essential nutrients and improved sensory attributes.

4.2.3 Shelf Life of the Developed Avocado and Mango Seed-based Composite Flour

Shelf life is an important factor to consider for food products and it must accommodate several requirements such as safety, quality, organoleptic and appearance (sensory quality) of any food to assure customer acceptance (Phimolsiripol *et al.*, 2016). Microbial safety and quality parameters are key factors to consider when determining shelf-life of any food product. Thereafter, key deteriorative reactions that cause quality loss and consumer unacceptability are selected (Phimolsiripol *et al.*, 2016). This study used the peroxide value as the indexes of estimating the shelf life of the developed composite flour since it deteriorates most rapidly in time as results of oxidation. Therefore, it is used as an indicator for determining the quality and stability of mostly dried foods such as flour, cookies and snakes containing fats (Fu, 2012; Alemayhu *et al.*, 2019).

As shown in this study, accelerated shelf-life determination process can be used to quickly assess the safety and stability of food for human consumption. For example, storing food at a higher or lower temperatures has adverse effect on its storage behavior and thus shelf life may become apparent in a shorter time and assist to estimate normal storage conditions by extrapolated data obtained (Phimolsiripol *et al.*, 2016). Results of the tested parameters (microbial, water activity, peroxide value and pH) in the developed composite flour for all specified number of days were within the tolerable range of safety. Flour or flour based products are known to be safe commodities due to their low water activity and moisture content. Additionally, determination of contamination of yeasts and molds in flour or flour based products is of paramount importance when considering the quality and safety of food (Victor *et al.*, 2013). The maximum legal limit for fungi in flour is 5 cfu/g (World Food Programs [WFP],

2012). Yeasts and molds from results (Table 20) were within the recommended limits. However, higher level of fungi more than the legal limits deteriorates the quality of food and causes food borne diseases due to the presence of molds which can be attributed to poor handling practices along the food chain (Victor *et al.,* 2013). Water activity and pH are considered important parameters in food preservation and processing. The range of water activity < 6 a_w obtained in the developed composite flour in this study could not support the growth of spoilage microorganisms especially bacteria (salmonella), yeast and molds in the period of 84 days.

Similar range of water activity was reported by Abdullah *et al.* (2000) who noticed no visible appearance of fungi in stored flours at water activity of 0.65 until 6 months of storage. The pH range on this study also played a great role in stabilizing the shelf life of the developed composite flour as it relied in the acidic side (<5.5) In this regard the observed pH in combination with water activity <6 suggests that even if the microorganisms survive they are unable to grow as reported by Gould (1996). Additionally, the obtained peroxide value (1.6 meq/kg sample) of the developed composite flour stored at a room temperature (30 ^{o}C) used to predict the maximum shelf life (4 months) of the developed composite flour did not exceed the limit for the safety of the food which is 10 meq/kg as stated in standard value of SNI 01-2347-1991 (Rahman *et al.*, 2019).

Generally, with these findings probiotic fermentation technique (by *Lactobacillus plantarum* at 37 ^{o}C) presented better results in reducing or removing antinutritional compounds, while retaining nutrients and functional values of processed avocado or mango seed kernels compared to boiling and soaking methods. This was justified by the highest antioxidant activity, total phenolics, ascorbic acid and minerals. These facts could be explained by the ability of the used microorganism to synthesize and break the ester linkages bonds, availability of suitable substrate and utilization of the substrate by microorganism at a temperature of 37 ^{o}C. Thus, the method was optimized for producing avocado and mango seeds-based composite flour.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

Results of this study showed that probiotic fermentation is the best processing method for reducing antinutritional compounds, while retaining the selected nutrients and bioactive compounds, improving sensory attributes and liking of avocado and mango seed-based composite flour compared with boiling and soaking techniques. Thus, probiotic fermentation technique (using *Lactobacillus plantarum* at a temperature of 37 ^{o}C) in this study was considered as an optimized method for processing avocado and mango seed kernels to be incorporated into various functional foods for human consumption.

Furthermore, optimized processing method used from this study is able to produce safe and nutritious avocado and mango seeds-based composite flour with sensory attributes that are acceptable by consumers. Moreover, the results showed that composite flour from avocado and mango seeds kernels produced by optimized method from this study can have a shelf life stability of a maximum of 4 months when stored at a temperature of 30 ^{o}C . However, further studies are needed to validate the effectiveness of the used optimized processing method on retaining other nutrients and bioactive compounds that were not studied in this study. Thus, for full realization of the health-benefits of avocado and mango seed kernels, exploration of the functional attributes of their fermented flours in different food forms should be intensified, and accordingly, make them part of the daily diet.

5.2 Recommendations

Based on the results obtained in this study, the following recommendations were considered desirable:

(i) Further studies are needed to validate the effect of the optimized processing method on other essential nutrients and bioactive compounds that were not analyzed in avocado and mango seed extracts of this study

(ii) Further studies are needed to identify specific compounds that contributed to the observed antioxidant activity of avocado and mango seed kernels processed from optimized method of this study.

(iii) In vivo and in vitro toxicity studies are needed to further validate other safety parameters of avocado and mango based product developed from optimized method of this study. This will greatly lead to the safe adoption of the developed composite flour.

(iv) Utilization of fermented avocado and mango seed kernel flours as food ingredients to help mitigate nutrient deficiencies and disorders associated with malnutrition.

(v) Cost-benefit analysis of using optimized method in producing avocado and mango seed extracts for commercial purposes.

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APPENDICES

Appendix 1: Consumer test form

Sex…… Age…….

Time…….. Date…….....

Please look and taste each of the 3 coded samples. Indicate how much you like or dislike each sample by checking the appropriate sample attributes and indicate your reference (1-9) in the column against each attribute. Put the appropriate number against each attribute.

9 – Like extremely

8 – Like very much

7- Like moderately

6- Like

5- Neither like nor dislike

4- Dislike

3- Dislike moderately

2- Dislike very much

1- Dislike extremely

	SAMPLE CODE		
Attributes	**476**	**263**	**135**
Appearance/ colour			
Flavor/ taste			
Aroma			
Consistence/texture			
General acceptability			
Would you be interested in buying/ using this product?	Yes No	Yes No	Yes No

Comments

……

……

Appendix 2: Quantitative Descriptive Sensory Evaluation form

Sex……Age……Time……

Please evaluate each sample in the order they are listed. Choose appropriate number in a scale from 1 to 9, where 1 is low intensity and 9 is high intensity. How do you find the following characteristics for different snacks made from fruits? Put the appropriate number against each characteristic.

Colour hue

White _____ yellow

1 2 3 4 5 6 7 8 9

Hardness/texture

Not hard _____ Very hard

1 2 3 4 5 6 7 8 9

Aroma

Not aromatic _____ Very aromatic

1 2 3 4 5 6 7 8 9

Sweetness

Not sweet _____ Very sweet

1 2 3 4 5 6 7 8 9

What is your total liking of the product?

Do not like it _____ like it a lot

1 2 3 4 5 6 7 8 9

Appendix 3: Informed Consent

TITTLE: Optima processing method suitable for producing composite flour using avocado and mango seed kernels for functional foods

This Informed Consent Form has two parts:

Part I: Information sheet, Part II: Certificate of Consent (for signatures if you agree to take part in sensory evaluation)

PART I: INFORMATION SHEET

Introduction: I am Joseph Runyogote, a PhD student from the Nelson Mandela African Institution of Science and Technology (NM-AIST). I am currently involved in conducting a research on "Optimal processing technique suitable for processing of composite flour using mango and avocado seed kernels for functional foods". Please listen carefully and ask any questions you may have before agreeing to take part in the study.

What the study is about: The purpose of this study is to develop composite flour using mango and avocado seed kernels for functional foods.

Participant Selection: The people who will be involved are people of 23 to 28 years of age

Task that you are supposed to do: If you agree to be in this study, I will request you to evaluate the sensory attributes of the developed composite flour made from mango and avocado seed kernels. You will be requested to score the samples using a 9 - point hedonic scale with ranging scale 1 = "dislike extremely" and 9 = "like extremely" of a given attribute for the following characteristics: colour, flavor, aroma, texture and general acceptability.

Risks: The developed composite flour may not be delicious as you expected.

Benefits: The participant who will take part in this study will obtain very useful information on how to enrich the nutritional content of the composite flour made from avocado and mango seeds to be incorporated in functional foods

Compensation: There is no compensation for taking part in this study.

Confidentiality: Your answers will be confidential. The records of this study will be kept private. In any sort of report we make public we will not include any information that will make it possible to identify you. Research records will be kept in a locked file; only the researchers will have access to the records.

Taking part is voluntary: Taking part in this study is completely voluntary. You may skip any questions that you do not want to answer. If you decide not to take part or to skip some of the questions, it will not affect your current or future relationship with Sokoine University of Agriculture. If you decide to take part, you are free to withdraw at any time.

PART II: CERTIFICATE OF CONSENT

I have read/ someone has read for me the fore given informations, and I had the opportunity to ask questions and the questions have been answered to my satisfaction. I consent voluntarily to participate in this research.

Name of participant: _____

Signature of participant: _____ Date: _____

I confirm that the participant was given an opportunity to ask questions about the study, and all the questions asked by the participant have been answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

Name of researcher: Joseph Runyogote

Signature …….Date…….

Thank you very much for your participation in this study.

For any inquiries, please contact Joseph Runyogote 0766430186

Appendix 4: Research ethical clearance certificate

Kibong'oto Infectious Diseases Hospital- Nelson Mandela African Institution of Science and Technology- Centre for Educational Development in Health, Arusha (KIDH-NM-AIST-CEDHA) -KNCHREC

RESEARCH ETHICAL CLEARANCE CERTIFICATE

Research Proposal No: KNCHREC 00034 26TH MAY 2020

Study Title: DEVELOPMENT OF COMPOSITE FLOUR USING MANGO AND AVOCADO SEED KERNELS FOR BREAD AND BISCUITS

Study Area: Muheza (Tanga) and Rungwe (Mbeya)wherebylaboratory work will be carried out at Sokoine University of Agriculture (SUA).

PI Name: JOSEPH RUNYOGOTE

Co-Investigator:

Institutions: THE NELSON MANDELA AFRICAN INSTITUTION OF SCIENCE AND TECHNOLOGY SCHOOLOFLIFE SCIENCE AND BIOENGINEERING

The Proposal has been approved by KNCHREC on 26TH MAY 2020

1. Subject to this approval you will be required to submit your progress report to the KNCHREC, National Institute for Medical Research and Ministry of Health Community Development Gender Elderly and Children
2. Publication of your findings is subject to presentation to the KNCREC and NIMR Approval.
3. Copies of final publication should be made available to KNCHREC, National Institute of Research and Ministry of Health Community Development Gender Elderly and Children

Duration of Study Renewal: Subject to Renewal within ONE YEAR
Span From: 26th MAY 2020 to 25TH MAY 2021.

Simon Njeya

.....
Mr. Simon Njeya
Secretary
KNCHREC

.....
Prof. Raymond Mosha
Chairperson
KNCHREC

RESEARCH OUTPUTS

(i) Publications

Runyogote, J, Chacha, M., Ndabikunze, B., & Jofrey Raymond, J. (2021). Optimized method for processing avocado seeds to improve selected nutrients and functional values. *Food Science and Technology Research*, 27(1), 75-84.

Runyogote, J, Chacha, M., Ndabikunze, B., & Jofrey Raymond, J. (2020). Effect of lactic acid fermentation, boiling and soaking on selected nutrients and health promoting components of mango seed kernels. *International Journal of Biosciences*, 17(6), 26-39.

(ii) Poster Presentation