

**PROBIOTIC VIABILITY AND STORAGE STABILITY
OF YOGHURT ENRICHED WITH BAOBAB PULP
(*Adansonia digitata*)**

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**Probiotic Viability and Storage Stability of Yoghurt Enriched with
Baobab Pulp (*Adansonia digitata*)**

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Science in Food Science and Nutrition in the Jomo Kenyatta
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DECLARATION

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

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DEDICATION

This work is dedicated to my parents, my late beloved father, Mr. Ezra Andy Aluko, and my mother, Esther Mwaikambo who made the foundation of my education.

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ACRONYMS AND ABBREVIATIONS

DMSO	: Dimethyl Sulfoxide
EU	: European Union
FAO	: Food and Agriculture Organization
FOSHU	: Food for Specified Health Use
FUFOSE	: Functional Food Science in Europe
GR	: Glycemic Response
MIC	: Minimal Inhibitory Concentration
NTFP	: Non-Timber Forest Products
RDA	: Recommended Dietary Allowance
SFA	: Saturated Fatty Acid
USDA	: United States Department of Agriculture
WHO	: World Health Organization

ABSTRACT

Baobab fruit is important as non-timber forest product having a good source of Vitamin C, fiber and minerals although it is underutilized. Incorporation of its pulp to the probiotic yoghurt have significance to the health of human being. Therefore current study was conducted to analyze the physicochemical, microbiological and sensory properties of baobab blended yoghurt and able to cover the objectives which were i) To analyse the nutritional and functional properties of baobab pulp from Makuyuni, Tanzania ii) To determine the optimum levels and viability of baobab enriched probiotic yoghurt formulated from different combinations of starter and probiotic during fermentation and storage. iii) To monitor shelf life and acceptability of the baobab enriched probiotic yoghurt. Parameters analyzed included proximate analysis, vitamin C, minerals (calcium, magnesium, Zinc), beta carotene, fatty acid, sugars and functional properties (foaming, emulsification and gelling capacities) of baobab fruit pulp which was obtained from Tanzania. Yoghurts were prepared by using fresh milk inoculated with starter cultures containing *Streptococcus salivarius subsp. Thermophilus* and probiotics *Bifidobacterium spp* and *Lactobacillus acidophilus*. These were then blended with pasteurized baobab pulp at 0%, 10%, 20%, 30% and 40% to obtain baobab blended yoghurts, and analyzed for physicochemical (pH, viscosity, titratable acidity, total solids) and microbial for 1,7,14 ,21 and 28 days of storage. In addition proximate, minerals (calcium, magnesium, zinc) and vitamin C were analyzed for the prepared yoghurts. Results showed significant difference ($p \leq 0.05$) between locations was on fat, crude fiber,

carbohydrates and fructose. Vitamin C, beta-carotene, protein, ash, moisture, sucrose, glucose, calcium, magnesium and zinc showed no significant difference ($p \leq 0.05$) among locations which are Kwa Muhindi, Oldonyo Orng'ina and Naitolia camp, Makuyuni area in Tanzania. Emulsification, foaming and gelling properties ranged between 37.9-45.15%, 1.85-6.57% and 11-12% respectively and were significantly different ($p \leq 0.05$) among locations. Yoghurt showed significant difference ($p \leq 0.05$) for moisture, crude fiber, carbohydrate, Vitamin C and magnesium while crude ash, fat, protein, calcium and zinc were not significantly different ($p \leq 0.05$). After 28 days of storage, all yoghurt samples revealed a significant difference ($p < 0.05$), decrease in pH value and an increase in titratable acidity, viscosity and total soluble solids. Microbiologically, yoghurts were stable and with satisfactory sanitary conditions for consumption. Survival rate of probiotic on simulated gastrointestinal tract was 22%-52%. Based on sensory evaluation result, the yoghurts enriched with 10% baobab pulp was the most preferred. Results from this study revealed that the composition and nutritional potential of the baobab pulp may be of high interest to health of consumers hence promoting the greater use of wild fruits.

Keywords: Baobab pulp, Functional property, microbiological quality, Probiotic Yoghurt, Physico-chemical properties, sensorial properties.

CHAPTER ONE

INTRODUCTION

1.1. Background of the Study

The Baobab (*Adansonia digitata*) belongs to the Bombacaceae family (Baidoo *et al.*, 2013; Gadanya, Atiku & Otaigbe, 2014; Mulani & Kharate, 2015) which consists of around 20 genera and around 180 species including closely interrelated species such as *Adansonia gregori* and *Adansonia madagascariensi* (Kamatou, Vermaak & Viljoen, 2011). Also known as the “upside down tree”, on pollination by fruit bats, it produces large green or brownish fruits which are capsules and characteristically indehiscent. The capsules contain a soft whitish powdery pulp, kidney shaped seeds, (Sidibe & Williams, 2002).

The baobab is an important indigenous fruit tree throughout the drylands of Africa, in Malaysia, China, Jamaica and Australia (Jama *et al.*, 2005). Several studies in different African countries such as Benin, Burkina Faso, Malawi, Mali, Nigeria, Tanzania and South Africa have highlighted this deciduous stem-succulent taxon as priority species for domestication and enhanced utilization (Sidibe & Williams, 2002; Lamien-Meda *et al.*, 2008).

The baobab fruit is also used daily in the diet of rural communities in West Africa (Sidibe & Williams, 2002; Assogbadjo *et al.*, 2008a; Ibrahima *et al.*, 2013). The species contribute to rural incomes (Kamatou *et al.*, 2011) and

has various important medicinal and food uses (Kaboré *et al.*, 2011). Traditionally the pulp is consumed in different forms. It is also used in the formulation and preparation of cereals and beverage.

Although, baobabs are widely known, the current scientific knowledge on the biochemistry and significance of its fruit in human nutrition is scarce. Most studies have focused on *A. digitata* in particular in relation to its botanical, agronomical and biochemical characteristics (Gebauer, El-siddeing & Ebert, 2002; Ibrahima *et al.*, 2013). It is reported that baobab pulps have many nutrients including vitamin C, riboflavin, niacin, pectin and citric, malic and succinic acids, while the oil also contains the vitamins A, D and E (Besco *et al.*, 2007; Donkor *et al.*, 2014).

Biochemical studies also showed that the pulp of *A. digitata* is rich in dietary fibers and carbohydrates (Ibrahima *et al.*, 2011). The soluble fibers of baobab fruit pulp are prebiotics: non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of beneficial microflora hence supporting probiotics, (Gruenwald & Galizia, 2005).

The interest in prebiotic and probiotic as functional foods has grown enormously (Gruenwald & Galizia, 2005). Probiotics are the bacteria which make up the natural flora of our gastro-intestinal system. These trillions of good bacteria benefit the body by keeping the growth of the harmful bacteria regulated, synthesizing vitamin K,

producing certain hormones and promoting digestion, absorption and elimination (Naik *et al.*, 2012).

Dairy products such as yoghurt are among the key probiotic products (Siró *et al.*, 2008, Lollo *et al.*, 2013). Yoghurt is a coagulated milk product that results from the fermentation of lactic acid in milk, by Lactic Acid Bacteria (LAB), *Streptococcus thermophilus* and *Lactobacillus bulgaricus* (Mazahreh, Omer & Mamdosh, 2009; Chipurura, Pswarayi & Muchuweti, 2014).

The most studied and widely employed probiotic bacteria are Lactic acid Bacteria (LAB) and *bifidobacteria*, as are normal components of the intestinal microbiota and have a long tradition of safe application within the food industry (Siró *et al.*, 2008). Yoghurt particularly probiotic yoghurts contribute to human health by providing natural nutrients and by enrichment of the intestinal microbiota with lactic acid bacteria and probiotic cultures. They provide several benefits for humans, among them are better resistance to infections, stimulation of the immune system and better absorption of minerals and lactose, (Fadaei, 2013).

Interest for probiotics has arisen in recent years especially in relation to the addition of *Bifidobacterium*, *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, *Lactobacillus casei*, *Lactobacillus reuteri* to the fermented dairy products such as yoghurt (Fadaei, 2013). This is because probiotic cultures are believed to play a significant role in the intestinal system against some of the pathogenic

microorganisms such as *Helicobacter pylori*, *Salmonella typhi* and *Yersinia enterocolitica* (Fadaei,2013).

Other human research has shown that inflammation markers and obesity are linked to the state of our gut flora. The studies show that the lower the probiotic diversity of the gut, the higher the incidence of obesity, insulin resistance and other inflammatory conditions (Soccol *et al.*,2010).

1.2. Problem Statement and Justification

1.2.1. Problem Statement

In recent years, there has been a tremendous increase in demand for functional foods among the consumer due to their potential in providing health benefits. Fruit such as baobab has received attention due to its contribution to human health(Arjamand, 2011). Research on baobab as a medicinal and nutritional food has grown. Baobab has recently become more popular because of the attribute of health benefits. Baobab and its derivatives such as juice, pulp and seeds are rich source of several high value compounds with beneficial physiological activities (Arjamand, 2011).

To date, enhancing health and nutrition by provision of probiotics to less-affluent communities in a locally sustainable way is still a major challenge. The use of indigenous foods as potential vehicles for transmission of probiotics has been given little attention even though it is an option with great potential in developing countries.

To deliver health benefits, probiotic products should contain an adequate amount of live bacteria (at least 10^6 to 10^7 cfu/g), (Lourens-hattingh and Viljoen,2001,Larsson,2009 and Swidan,2009) able to survive the acidic conditions of the upper GI tract and proliferate in the intestine, a requirement that is not always fulfilled.

Monitoring the survival of the probiotic cultures in foods is hampered by the lack of accurate, reliable, convenient, and sensitive methods of identification, with the ability to distinguish the strains of interest among other closely related microorganisms present in the products. Therefore to facilitate the consumer's access to health benefits of baobab, a dairy based functional food such as yoghurt can be developed. This can achieved by enriching yoghurt using baobab pulp.

1.2.2. Justification

Interest in non-timber forest products (NTFPs) is increasing rapidly and the use of these products constitute a source of income for many rural people in Africa. Baobab pulp can be beneficial not only to rural communities but also because baobab pulp is an ideal candidate in the functional food market as it is very high in vitamin C and the powder has high pectin and fiber content.

This is quite important in promoting well-being, providing nutritional value and aid in a preventative health management. Therefore; this study will be of great impact in revising ongoing health benefit of functional food, through development of yoghurt enriched with baobab pulp. Despite the advantageous characteristics of baobab as NTFP, the tree

species is currently underutilized. Underutilized crops are characterized by the gap between their economic potential and their actual economic contribution and a lack of research.

The enhancement of the production of cost effective probiotic products made from locally available resources has the potential to lift rural communities which depend on it out of poverty by integrating them into sustainable markets. In addition to that, the scientifically proven yoghurt product can be adopted for commercialization by young entrepreneurs to improve the live-hoods of people living in areas where there is abundance.

1.3. Objectives

1.3.1. Main Objective

To develop and evaluate probiotic yoghurt product enriched with baobab pulp.

1.3.2. Specific Objectives

- i. To analyse the nutritional and functional properties of baobab pulp from Makuyuni, Tanzania.
- ii. To determine the optimum levels and viability of baobab enriched probiotic yoghurt formulated from different combinations of starter and probiotic during fermentation and storage.
- iii. To monitor shelf life and acceptability of the baobab enriched probiotic yoghurt.

1.3.3. Hypotheses

- i. There is no significance difference in the nutritional quality and functional property of baobab pulp within the location and among the trees.
- ii. There is no significance difference in optimum levels and viability of baobab enriched probiotic yoghurt formulated from different combinations of starter and probiotic during fermentation and storage days.
- iii. There is no significance difference in Shelf-life and acceptability of the baobab enriched probiotic yoghurt products.

1.3.4. Expected outcomes

- i. The nutritional quality and functional properties of baobab pulp were determined.
- ii. Microbiological, nutritional and physical properties and sensory preferences probiotic yoghurts were developed.
- iii. Shelf-life and acceptability, viability of different combinations of starter and probiotic during fermentation, storage of enriched probiotic yoghurt products were established.

CHAPTER TWO

LITERATURE REVIEW

2.1. Introduction

The origin of the vernacular name “baobab” is uncertain. However, most scientists believe it is derived from the Arabic name “buhibab” meaning fruit with many seeds. The genus name *Adansonia* is used in honour of Michel Adanson (1727–1806) who brought seeds to Paris in 1754 and who was the first person to provide a comprehensive description accompanied by a drawing of the plant (Kamatou *et al.*,2011 ;Zahra'u, Mohammedi,Ghazali&Karim,2014; Namratha & Sahithi,2015), after a trip to Senegal. The species name *digitata* (hand-like) was selected in reference to the shape of the leaves. Several names are used to describe the baobab depending on its geographical location. This include “magic tree”, “chemist tree”, “symbol of the earth”, “upside-down tree” and “monkey bread of Africa” amongst numerous others (Kamatou *et al.*,2011; Zahra'u,*et al.*,2014; Namratha & Sahithi,2015;Gebauer *et al.*,2016).

2.2. Botanical description, habitat and distribution of baobab in Africa

The baobab is found in many African countries. Eight baobab species have been identified globally and six species found on the island of Madagascar are endemic to that region. It is postulated that the centre of evolutionary origin of the genus *Adansonia* is Madagascar .The African species *A. digitata* is indigenous and widely distributed throughout the savannas and savanna woodlands of sub-Saharan Africa(Sanchez,

2010;Kamatou *et al.*, 2011;Ibrahima *et al.*, 2013). Some baobab trees bear leaves only for three months per year. During the leafless period physiological processes such as photosynthesis take place in the trunk and branches, utilizing water stored in the trunk (Gebauer, *et al.*,2002).

It is estimated that it takes between eight and twenty-three years before the baobab produces seeds and the mature plant (over 60 years) can produce more than 160–250 fruits per year (Kamatou *et al.*, 2011). The baobab produces large pendulous white flowers from October to December and showed correlation between soil minerals and/or soil type and fruit production. For instance, the higher the pH– KCl, the percentage of total nitrogen, organic carbon and organic matter, the higher the number of seeds produced by an individual baobab tree (Kamatou *et al.*,2011).

It was also noted that the higher the clay and crude silt content of the soil, the better the fruit production. Baobab is restricted to hot, semi-arid regions, dry woodland and stony places with low rainfall (less than 1500 mm annually) (Gebauer *et al.*,2002) and grows on a wide range of well-drained soils, from clays to sands, but not on deep unconsolidated sands, where it is unable to obtain sufficient moisture (Kamatou *et al.*, 2011).

2.3. Traditional and modern uses of baobab

Throughout Africa the baobab is regarded with awe by most indigenous people; some even consider it bewitched (Kamatou *et al.*, 2011). Almost all parts of the tree are used in traditional medicine in Africa although this varies from one country to another. The leaves and fruit pulp are used as febrifuge as well as an immuno stimulant (Kaboré *et al.*, 2011; Namratha & Sahithi, 2015). In India, it is reported that baobab pulp is used externally with buttermilk for the relief of diarrhoea and dysentery, while the young leaves are crushed and used to treat painful swellings (Sidibe & Williams, 2002).

The baobab is extremely important to humans and animals in the dry areas of Africa because it offers shelter, a source of nutrition, clothing as well as raw material for many useful items. In Benin, the bark has been used for making ropes (Kaboré *et al.*, 2011). Baobab is used for several purposes and these include: fruit for food; oil from the seeds; rope, cordage and cloth from the bark fiber; tannin for curing leather from the tree bark; glue from the pollen grain of the flowers; pulp for making paper from the harvested tree (although of low quality), seasoning and as an appetizer (Sidibe & Williams, 2002).

In some countries in West Africa, the leaves, fruit pulp and seeds are the main ingredient in sauces, porridges and beverage (Ibrahima *et al.*, 2013). The major interest in baobab products is as a result of its ascorbic acid and dietary fiber content. It was noted that baobab fruit pulp has very high vitamin C content (280–300 mg/100 g), almost six times that of oranges (51 mg/100 g), (Caluwé & Damme, 2010).

One study demonstrated that the consumption of 40 g of baobab pulp provides 100% of the recommended daily intake of vitamin C in pregnant women (19–30 years), (Kamatou *et al.*, 2011). The ascorbic acid content was evaluated in the fruit of *A. digitata* and it was found to contain 337 mg/100 g of ascorbic acid (Gebauer,*et al.*,2002).The calcium content found in the fruit pulp varies according to authors and the origin of the samples tested. Kamatou *et al.*,(2011), reported a calcium content of 344.2 mg/100 g sample which differs from the value of 295.0 mg/100 g reported by Osman (2004).

Similarly, the level of potassium in the fruit pulp was found to be 1578.5 mg/100 g sample (Kamatou *et al.*, 2011) and 1240.0 mg/100 g (Osman, 2004).The leaves are particularly rich in calcium (307 to 2640 mg/100 g dw), they are known to contain good quality proteins and it is estimated that it contains three to five times more calcium than milk. The seeds have a relatively high lipid content of 11.6 to 33.3 g/100 g dw, (Kamatou *et al.*, 2011).Several studies have revealed that baobab is rich in fiber (approximately 44%) which can balance the intestinal flora. However, the fiber content varied considerably between studies which could be due to several factors such as the origin of the plant material and the extraction procedure used.

Several studies have demonstrated a significant correlation between the intake of fruits and vegetables and the occurrence of inflammation and diseases such as cardiovascular disease and cancer (Adejuyitan,Abioye,Otunola & Oyewole,2012).Natural anti-oxidants, including polyphenolic compounds from plants, vitamins E and C, and carotenoids, are

believed to be effective nutrients in the prevention of these oxidative stress-related diseases (Besco *et al.*,2007).

Baobab oil or fruit pulp contains several vitamins that are essential for skin care. These include vitamins A and E (anti-oxidant and anti-ageing effects) and vitamin D3 which increases calcium absorption and decreases blood pressure among the elderly (Kamatou *et al.*, 2011). Linoleic acid (found in baobab seed oil) is the most frequently used fatty acid in cosmetic products as it moisturizes the skin, aids in the healing process of dermatoses and sun burns and is used for the treatment of *Acne vulgaris* (Kamatou *et al.*,2011).

2.4. Phytochemistry of baobab

Several classes of compounds have been identified from various parts of baobab (fruit pulp, seed oil, leaves, roots) including terpenoids, flavonoids, sterols, vitamins, amino acids, carbohydrates and lipids (Kamatou *et al.*, 2011). Ten aromatic compounds including isopropyl myristate and nonanal were identified in the fruit pulp using GC–MS. Several compounds have been isolated from the pericarp using column chromatography and include: (–)-epicatechin, epicatechin-(4β.8)-epicatechin (B2), epicatechin-(4β.6)-epicatechin (B5), epicatechin- (2β.O.7, 4β.8)-epicatechin (A2), and epicatechin-(4β.8)- epicatechin-(4β.8)-epicatechinC1 (Kamatou*etal.*, 2011).Epicatechin is known to exhibit strong anti-oxidant activity and can also promote survival in diabetic mice (Kamatou *et al.*, 2011).

Compounds such as campesterol, cholesterol, isofucosterol, β -sitosterol, stigma sterol and tocopherols (a, β , and d) have been detected in the seed oil. Fatty acids present in the seed oil include linoleic and oleic acids in high concentration as well as lesser amounts of palmitic, linolenic, stearic and arachidic acids (Osman, 2004). The presence of organic acids such as citric, tartaric, malic, succinic and ascorbic acid in the fruit pulp was first highlighted in the early fifties. The pulp represents 14 to 28% of the total fruit weight and the pulp water content is low (less than 15%) (Kamatou *et al.*, 2011).

The baobab dry fruit pulp was reported Manfredini, *et al.*, (2002) to contain 2.3% protein, 0.27% lipids, 5.2% soluble and insoluble fiber, 75.6% carbohydrates. Baobab Fruit Company (2002) reported a high Ca, Mg and P contents (4310, 2090 and 733 mg/100g, respectively) and moderate Fe, Zn and Na contents. Several amino acids such as alanine, arginine, glycine, lysine, methionine, proline, serine, valine (from fruit pulp), vitamins (B1, B2, B3, A, C) (from fruit pulp and/or leaves) (Kamatou *et al.*, 2011).

2.5. Biological activity

2.5.1. Antibacterial activity

Antimicrobial activity of baobab plant parts (stem and root barks) against gram-negative bacteria and yeast. The aqueous and ethanolic root and stem bark extracts inhibited the growth of various micro-organisms with the Minimal Inhibitory Concentration (MIC) values ranging from 1.5 to 6 mg/ml. The antibacterial activity of the plant could be

attributed to the presence of tannins, phlobatannins, terpenoids and saponins in the stem bark (Kamatou *et al.*, 2011).

2.5.2. Anti-inflammatory activity, Phenolic, flavonoid content and anti-oxidant activity

Fruit pulp extract and aqueous leaf extract showed significant inhibition against cytokine IL-8 (Kamatou *et al.*, 2011). Phenolic and flavonoid compounds are well known for their good anti-oxidant activity. The total phenolic levels in fresh ripe fruits were significantly higher in the aqueous methanol than in the aqueous acetone extracts (4057.5 and 3518.3 mg GAE (gallic acid equivalents)/100 g of fruit for total phenolics, respectively).

Similarly, the flavonoid level was higher in the aqueous methanol extract of the fresh ripe fruit than in the aqueous. Acetone extracts (42.7 and 31.7 mg QE (quercetin equivalents)/ 100 g of fruit for total flavonoids, respectively). The antioxidant activity of fresh ripe fruit of *Adansonia. digitata* was 1000 mg AEAC/100 g (ascorbic acid equivalent anti-oxidant content) (Lamien-Meda *et al.*,2008).

2.5.3. Anti-insecticidal activity and repellency

Smoke from pellets of *A. digitata* leaves was assayed for toxicity and repellency against adult (0–7 days old) *Anopheles gambiae* (African malaria mosquito), *Musca domestica* (housefly) and *Periplaneta americana* (American cockroach) insects. Results indicated that the smoke caused the death of *A. gambiae* and *M. domestica*, but not *P. americana*. The median lethal dose for *A. gambiae* was 0.47, 0.50 and 0.20 g for the pellets

containing 0, 0.01 and 0.05 g of d-allethrin, respectively, while it was 0.46, 0.52 and 0.46 g for *M. domestica* (Denloye, Teslim & Fasasi, 2006).

2.5.4. Antiviral activity

Several *in vitro* and *in vivo* studies have been carried out to determine the antiviral activity of various baobab plant parts, (Kamatou *et al.*, 2011), investigated the antiviral activity of several plants against the herpes simplex, sindbis and polio viruses. The baobab extract from leaves was found to have the most potent effect (Kaboré *et al.*, 2011) investigated the antiviral activity of *A. digitata* leaves, fruit-pulp and seed extracted with water, DMSO and methanol.

2.6. Nutrient composition of Baobab fruit pulp

About 100 g of Baobab fruit pulp contain 75.6 % of total carbohydrates, 2.3 % of proteins and a very low content of lipids (0.27% of total lipids), (Stapleton, 2015). Baobab fruit is known for its high content of ascorbic acid (Vitamin C) (Ndabikunze, Masambu & Tiisekwa, 2010); in particular, 100 grams of pulp contain up to 300 mg of vitamin C, approximately five times more than the ascorbic acid content of one orange (Stapleton, 2015).

The fruit also contains other essential vitamins, such are riboflavin (vitamin B2), necessary for growth and maintenance of the integrity of nervous fibers, skin and eyes, and niacin (vitamin B3), which is important for the regulation of several metabolic processes (Magaia, *et al.*, 2012). The fruit can contribute to the supply of

other important dietary nutrients such as minerals and essential fatty acids. 100 grams of pulp contains 36 mg of calcium, 1221.0 mg of potassium, 199 mg of phosphorus, and α -linolenic acid (27 μ g of acid per gram of product expressed in dry weight).The summary of nutrients present in baobab fruit pulp is shown in Table 2-1.

Table 2-1 Nutritional content of baobab pulp

Nutrients	Baobab fruit	Nutrients	Baobab fruit
	pulp/100g		pulp/100g
Energy	305	Potassium	1221.0
(Kcal)		(mg)	
Protein content	4.2	Sodium	3.0
(g)		(mg)	
Total fat content	1.3	Zinc	0.3
(g)		(mg)	
Fiber	5.1	Vitamin C (mg)	201
(g)			
Calcium	36	Vitamin B1	0.0
(mg)		(mg)	
Iron	1.9	Vitamin B2	0.6
(mg)		(mg)	
Magnesium	46	Vitamin B3	3.4
(mg)		(mg)	
Phosphorus	199.0	Vitamin B6	0.1
(Mg)		(mg)	

Source: Tanzania Food Composition Tables (2008)

2.7. Probiotic Concepts

2.7.1. Definition

The word “probiotic” (origins: Latin pro meaning “for” and Greek bios meaning “life”) was first used in 1954 to indicate substances that were required for a healthy life. Out of a number of definitions, the most widely used and accepted definition is that proposed by a joint FAO/ WHO panel (FAO, 2001). “Live micro-organisms which, when administered in adequate amounts, confer a health benefit on the host”. The most commonly used probiotics in foods are species from the genera *Lactobacillus* and *Bifidobacterium*, but yeasts such as *Saccharomyces spp.* have also been used (Binns,2013).

2.7.2. Probiotics and human health

Nowadays, consumers are aware of the link among lifestyle, diet and good health, which explains the emerging demand for products that are able to enhance health beyond providing basic nutrition. The list of health benefits accredited to functional foods continues to increase and the probiotics are one of fastest growing categories for which scientific researchers have demonstrated therapeutic evidence (Soccol *et al.*,2010).

2.7.3. Application of probiotics in food

Probiotic organisms are used in a variety of foods, the main category being dairy products, but they are also present as food supplements in capsule or tablet form. Since viability is an essential property of a probiotic, the final product must contain an adequate amount of living probiotic(s) until the end of its shelf life, (Binns,2013).

2.8. Yoghurt as a probiotic product

Yoghurt is a cultured milk product that is produced through a fermentation process (Amanze, 2011).It is a fermented milk product that evolved empirically some centuries ago by allowing naturally contaminated milk to sour at a warm temperature, in the range of 40-50^oC. The micro-organisms which are used conventionally in this process are referred to as starter culture.

Since the renewed interest in probiotics, different types of products were proposed as carrier foods for probiotics by which consumers can ingest large numbers of their cells for the therapeutic effect. More than 90 probiotic products containing one or more groups of probiotic organisms are available worldwide (Arjmand, 2011).

A number of probiotic organisms including *Lactobacillus acidophilus*, *Bifidobacterium spp.*, *Lactobacillus casei*, *Lactobacillus rhamnosus*, and propionic bacteria are incorporated in dairy foods. These organisms grow slowly in milk during product manufacture (Kamatou *et al.*,2011).Yoghurt is typical fermented milk consumed all

around the world. As a major dairy product it has long been recognized as having desirable health effects, and it is not surprising that most consumers consider yoghurt to be 'healthy'.(Lourens-hattingh & Viljoen, 2001).

2.9. Functional food: defining the concept

Functional food is a food that beneficially affects one or more target functions in the body beyond adequate nutritional effects in a way that is relevant to either an improved state of health and well-being and/or reduction of risk of disease. It is consumed as part of a normal food pattern. It is not a pill, a capsule or any form of dietary supplement (European Commission, 2010).

According to Ashwell,(2002), functional foods cannot be a single, well defined or well characterized entity, a wide variety of food products are(or in the future will be characterized as functional foods). These include a variety of components, nutrients and no-nutrients, affecting a range of body functions relevant either to state of wellbeing and health and /or to the reduction of risk of a disease. Although many functional food products are in the market, it is easier to explain the scientific rationale behind these foods as a function-driven concept. In this way, the concept can be universal and not influenced by the local characteristics or cultural traditions that determine the products in specific food market (Ashwell, 2002).

Japan is the birthplace of the term functional food. A variety of terms, more or less related to the Japanese Food for Specified Health Use FOSHU (Grajek,Olejniak&Slip,2005) and has appeared World-Wide. These include more terms, such as nutraceuticals, designer foods, medical food and the more traditional dietary supplements and fortified foods. All foods or food products marketed with the message of benefit to health (Ashwell,2002).According to Mortazavian,Mohammadi&Sohrabvandi,(2012) probiotic food products are classified in the category of functional foods and represent a significant part of this market that probiotic foods comprise between 60 and 70% of the total functional food market.

2.9.1. Functional Food: European Consensus

The European Consensus Concerted Action on Functional Food Science in Europe (FUFOSE) actively involved a large number of the most prominent European experts in nutrition and related sciences and was coordinated by International Life Science Institute (ILSI Europe).It reached a consensus on “Scientific Concepts of Functional Foods in Europe” in 1999.To reach that final objectives, three major steps were taken (Ashwell,2002).

- i. A critical assessment of the science base required to provide evidence that specific nutrients and food components positively affect target functions (biological responses) in the body
- ii. An examination of the available science from a function-driven rather than a product-driven perspective

- iii. An elaboration of a consensus on targeted modifications of food and food constituents and on options for their applications.

Because functional foods are a concept rather than a well-defined group of food products, the FUFUSE Consensus Document proposed a working definition. A food can be regarded as “functional” if it is satisfactorily demonstrated to affect beneficially one or more target functions in the body, beyond adequate nutritional effects, in a way that is relevant to either an improved state of health and well-being and/or reduction of risk of disease. Functional foods must remain foods, and they must demonstrate their effects in amounts that can normally be expected to be consumed in the diet,(Shahidi,2012) , they are not pills or capsules, but part of a normal food pattern.

From a practical point of view, a functional food can be:

- i. A natural food in which one of the components has been naturally enhanced through special growing conditions
- ii. A food to which a component has been added to provide benefit (e.g. the addition of selected probiotic bacteria with proven health beneficial characteristics to improve gut health
- iii. A food from which a component has been removed so that the food has less adverse health effects (e.g. the reduction of Saturated Fatty Acids-SFA)
- iv. A food in which the nature of one or more components has been chemically modified to improve health (e.g. The hydrolyzed protein in infant formulas the likelihood of allergenicity)

- v. A food in which the bioavailability of one or more components has been increased to provide greater absorption of a beneficial component
- vi. Any combination of the preceding possibilities (Ashwell,2002) and (Howlett, 2008).

2.9.2. Functional Foods and Health

Functional food science is based on the way in which specific nutrients and food components positively affect target functions (biological responses) in the body. Infact, several important areas of human physiology that are relevant to functional food science can be used to illustrate the concept:

- i. Early development and growth
 - ii. Regulation of basic metabolic processes
 - iii. Defence against oxidative stress
 - iv. Cardiovascular physiology
 - v. Gastrointestinal physiology.
 - vi. Cognitive and mental performance, including mood and alertness
- Ashwell,(2002)and Howlett,(2008).

2.10. Yogurt as a Functional food

Dairy products have the largest probiotics food market share. A total of 78% of current probiotic sales in the world are delivered through yoghurt (Mortazavian,*et al.*,2012).Yogurt products have been commonly supplemented with probiotics such as *Lactobacillus acidophilus*, *Lactobacillus casei* and *Bifidobacteria* to enhance their therapeutic value and to establish a market as a functional food (Han, Lee, Zhang & Guo *et al.*, 2012; Irvine & Hekmat, 2011; Toma & Pokrotnieks, 2006).

The incorporation of probiotic bacteria in various dairy products including yogurt, has become an increasing trend. Today, yogurt has moved from being a “health food” to being a mainstream “healthy food” that people of all ages enjoy (Han, *et al.*, 2012). The conventional yoghurt starter, *Lactobacillus.bulgaricus* and *Streptococcus thermophilus*, do not have ability to survive passage through intestinal tract and consequently so, they are not considered as probiotics. But the addition of *L.acidophilus* and *B.bifidum* into yoghurt adds extra nutritional and physiological values (Homayouni *et al.* , 2012).

This “biotechnological” food is considered as having high nutritional value (namely low lactose and high calcium levels) and positive bioactive effects (containing prebiotic ingredients and probiotic bacteria). The natural plain yoghurt is produced by adding lactic acid bacteria that induce the lactic fermentation (Coïsson *et al.*, 2005) and according to Codex Standard (243-2003) yoghurt is classified as fermented milks and could contain a maximum of 50% (m/m) of non- dairy ingredients (such as fruits and vegetables as well as juices, purees, pulps) and its commonly used starter cultures are *Streptococcus salivarius ssp. thermophilus* and *actobacillus delbrueckii ssp. bulgaricus*.

A number of health benefits have been claimed for probiotic bacteria and consequently, they are increasingly incorporated into dairy foods (Kamatou,*et al.*,2011). In recent years some yoghurt products (bio-yoghurt) have been reformulated to include live strains of *L. acidophilus* and species of *Bifidobacterium* known as AB-cultures(Lourens-hattingh & Viljoen,2001). According to Codex Standard (243-2003) to provide health benefits, the concentration of probiotic bacteria should be no less than 10^6 CFU/g of the product.

CHAPTER THREE

METHODOLOGY

3.1. Materials and Methods

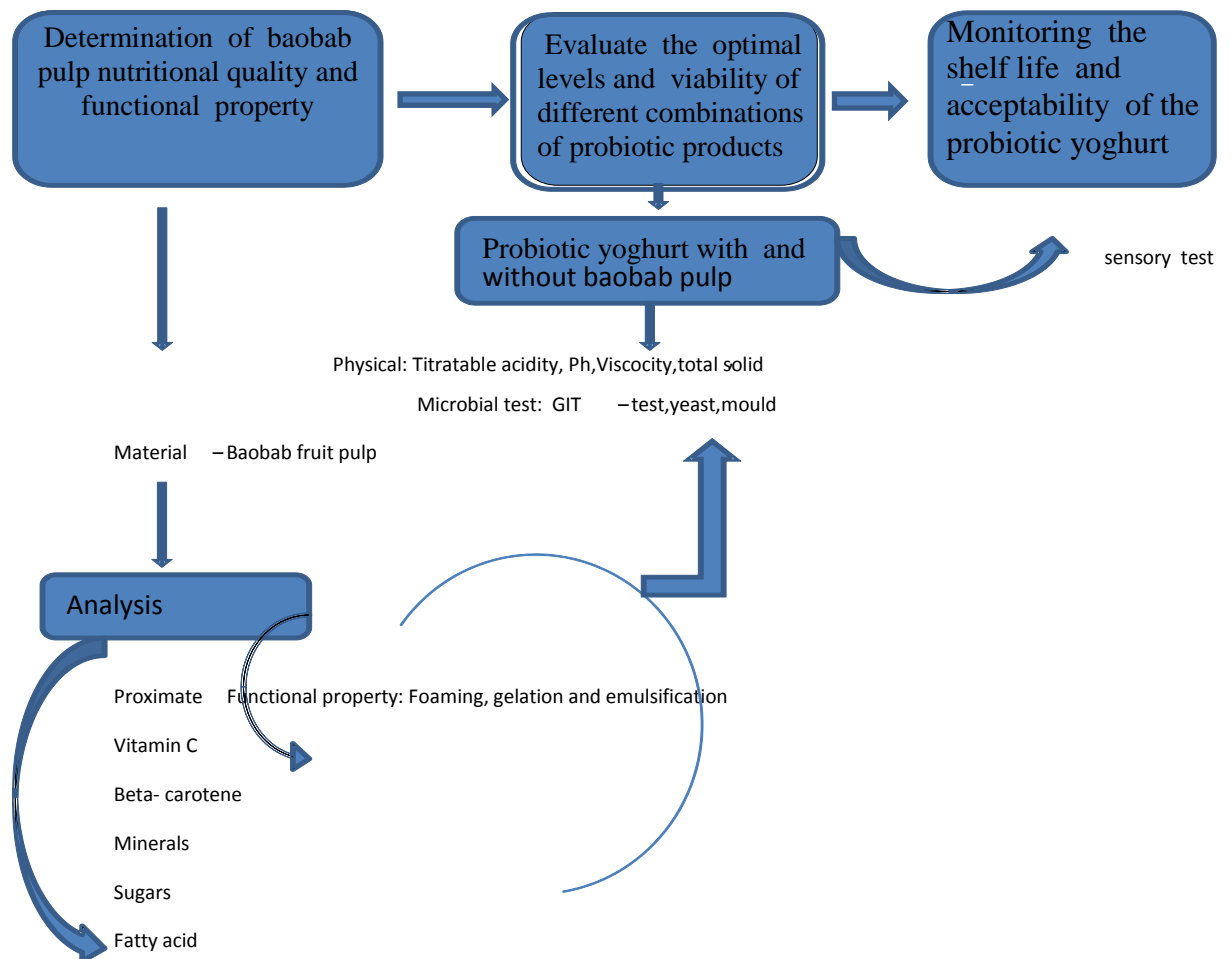


Figure 0-1 Project flow chart

3.2. Materials

Figure 3-1 shows the summary of the work done and explained in detail in heading 3.2.1 to 3.11

3.2.1. Baobab fruits

The baobab fruit samples were collected from Arusha, Makuyuni areas from three locations, including Naitolia camp, Kwa Muhindi and Oldonyo Orng'ina. Baobab fruit was harvested from three trees per location. Criteria used for selection was baobab fruit from trees whose trunks were cylindrical and with a tapering shape .The fruits were collected in September, 2015, at which time the trees had shed the leaves and hence only the trunk shape was used as the harvesting criteria.

3.2.2. Description of the area where baobab samples collected

Samples were collected from Naitolia camp, Kwa Muhindi and Oldonyo Orng'ina in Makuyuni, Arusha. Makuyuni is 77km from Arusha Town as in Figure 3-2 and Table 3-1.



Figure 0-2 Tanzania and Makuyuni

Table 0-1 GPS for Makuyuni area

Location	South (S)	East(E)	Elevation(m)
Naitolia Camp (Station one) –A			
1	03°41.413’	036°06.609’	1119
2	03°41.450’	036°06.595’	1122
3	03°41.417’	036°06.562’	1120
Kwa Muhindi(Station two)- B			
1	03°42.386’	036°08.784’	1175
2	03°41.777’	036°08.680’	1148
3	03°41.888’	036°08.603’	1150
Oldonyo Orng’ina (Station three)- C			
1	03°39.819’	036°37.558’	1099
2	03°39.973’	036°07.709’	1102
3	03°40.001’	036°07.846’	1104

3.2.3. Milk

Fresh cow milk for preparation of yoghurt was obtained from local suppliers in Juja, Kenya.

3.2.4. Starter Cultures and Probiotics

The starter culture contained *Streptococcus salivarius subsp. thermophilus*), Probiotics included *Bifidobacterium spp* and *Lactobacillus acidophilus*. Starter and probiotic cultures were bought from Chris-Hansen company through Promaco Ltd, in Kenya.

3.3. Sample preparation

3.3.1. Preparation of Baobab pulp

Whole baobab fruits were weighed, and their hard woody shells carefully crushed to expose the white flesh powder surrounding the seeds. The pulp was separated from the seeds by grinding using pestle and mortar. The mixture was sieved using a 0.09 micron sieve to obtain a fine powder. The powder was weighed and immediately packed in polyethylene bags sealed and stored in a dark cool place. The procedure is presented in Figure 3-3.

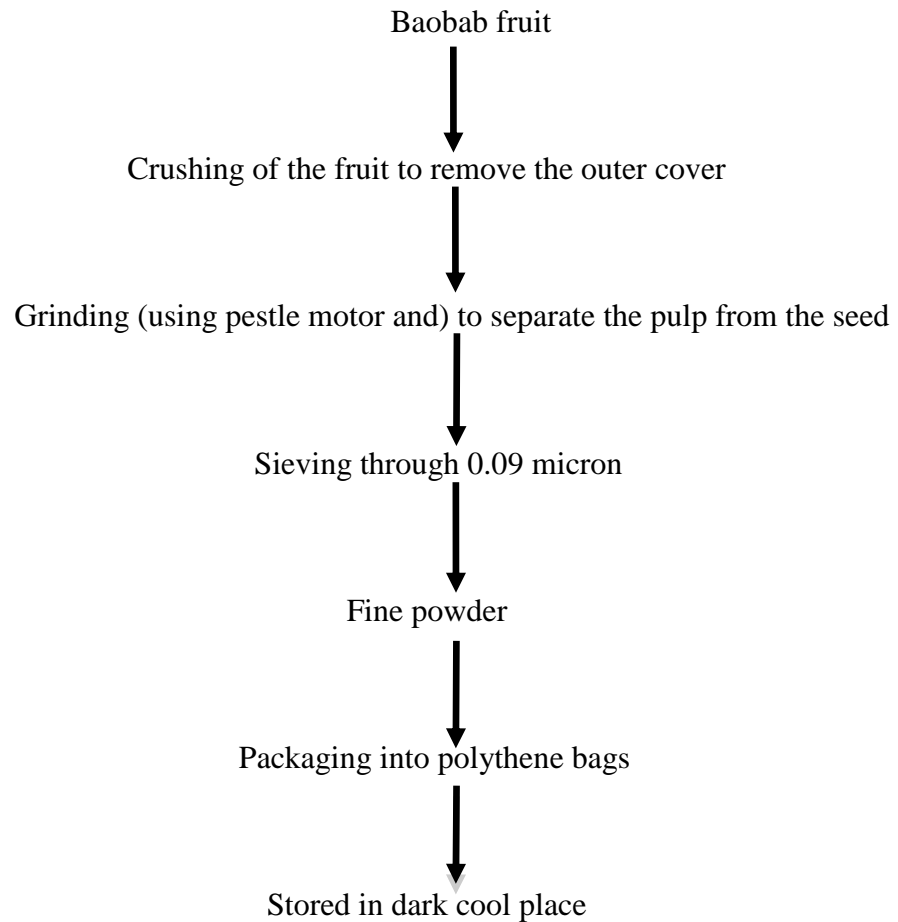


Figure 0-3 Flow chart of baobab pulp production

(Ndabikunze, Masambu, Tiisekwa & Issa-Zacharia, 2011).

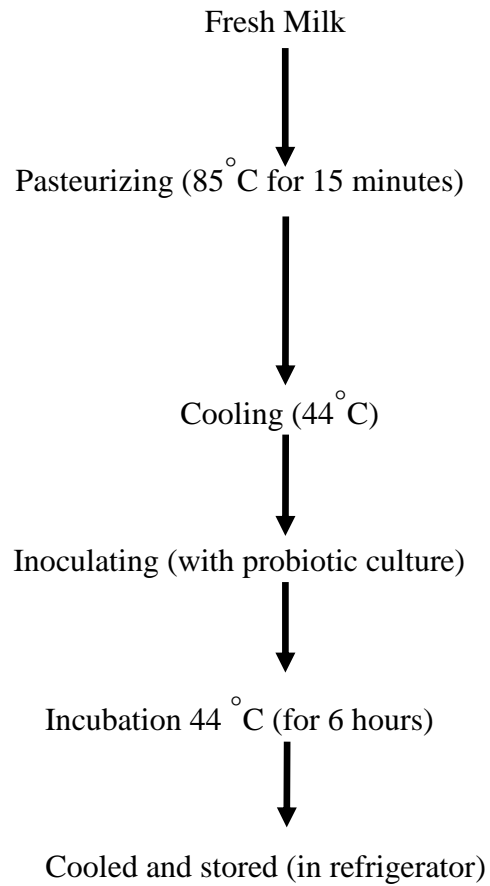


Figure 0-4 Flow chart of probiotic yoghurt formulation (Ndife *et al.*, 2014).

The milk was heated to about 85 °C to kill undesirable bacteria and to partially break down the milk proteins. The samples were then cooled to about 4 °C. Commercial probiotic culture of *Bifidobacterium spp*, *Lactobacillus acidophilus* and *S. thermophilus* was added. This active culture was used to inoculate each of the 1litre (1000 ml) milk ,at the same temperature of 45 °C which was maintained for 6 hours to allow for fermentation and the rapid production of lactic acid by the inoculated bacteria, which was led to the coagulation of the milk(figure 3-4),and baobab pulp was added (figure3-5).Then, pH, the total count and intestinal survival test was done on a weekly basis for 28 days (1, 7, 14, 21 and 28).

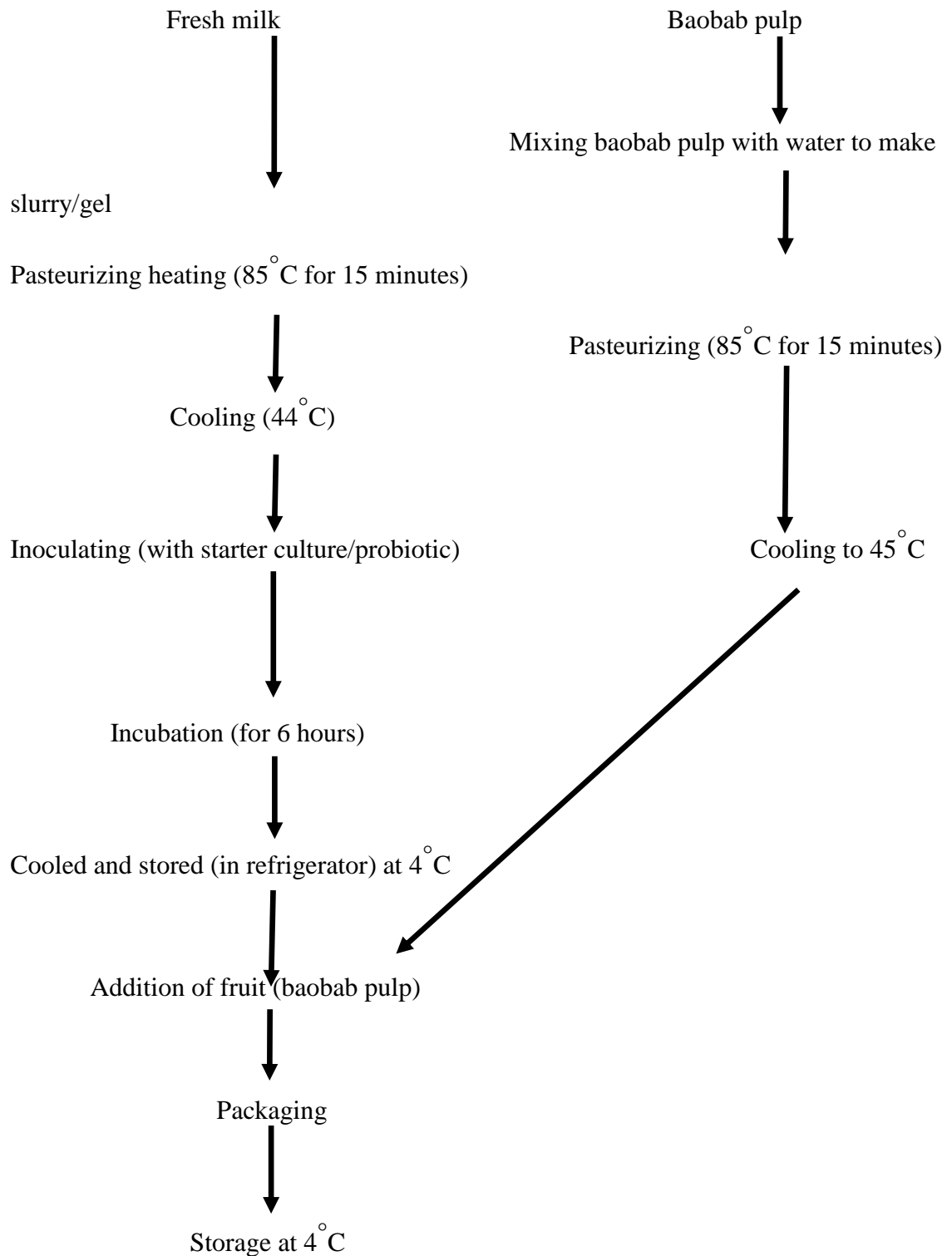


Figure 0-5 Flow chart of enriched baobab yoghurt (Ndife, Idoko & Garba,2014)

The baobab pulp was blended with yoghurt at 0%, 10% 20% 30% and 40% as stipulated on Table 3-2.

Table 0-2 Formulation of enriched yoghurt with baobab pulp

Samples	Ingredients	
	Yoghurt (%)	Baobab pulp (%)
A	100	0
B	90	10
C	80	20
D	70	30
E	60	40

3.4. Analysis for determination of baobab quality and probiotic yoghurt

3.4.1. Functional properties of Baobab pulp powder

3.4.1.1. Foaming

Two grams of baobab powder were dissolved in 100 mL of distilled water and blended at high speed for 1 min. The volume of the mixture was measured. The foaming capacity was calculated as the volume of the mixture after blending compared to the original volume (Santana,Huda&Yang,2012). The formula used for calculation was:

$$\text{Foaming capacity} = \frac{v_2 - v_1}{v_1} * 100$$

Where: v_1 = Initial volume

v_2 = Final volume

3.4.1.2. Gelation

Baobab powder sample suspensions of 10-15% were prepared in distilled water and vortexed for 5 minutes. Ten milliliter of each prepared dispersion was transferred into a test tube. The tubes were heated at 90°C for 30 minutes in water bath and then placed in a cold room at 4°C for 30 minutes. The gelation concentration is determined as the lowest concentration at which the sample does not fall down or slip from an inverted test tube (Santana *et al.*, 2012).

3.4.1.3. Emulsification

A 5 g baobab powder sample was dissolved in 20 mL of distilled water, mixed with 20 mL of corn oil, blended for 1 minute and centrifuged at 7500 rpm for 5 minutes (Santana *et al.*, 2012). The formula used for calculation was:

Emulsification capacity =

$$\frac{\text{Volume after homogenization} - \text{Volume after centrifugation}}{\text{Volume after homogenization}} \times 100$$

3.5. Proximate composition

The samples (baobab pulp and probiotic yoghurt) were analyzed for moisture by drying method, protein by semi kjeldah method, fat by Soxhlet extraction method, ash by ashing in a muffle furnace at 550°C, fiber and carbohydrates by difference. These procedures were adopted from Association of Official Analytical Chemists (AOAC) 2003 and conducted in Jomo Kenyatta University of Agriculture and Technology, Food Science and Technology laboratory.

3.5.1. Determination of moisture content

Two grams of each of the sample was weighed into dried weighed moisture dish. The samples were put into a moisture extraction oven at 105°C, and heated for 3hours. The dried samples were put into desiccators, allowed to cool and reweighed. The process was repeated until constant weight was obtained. The difference in weight was calculated as a percentage of the original sample (AOAC,2003).

$$\% \text{ Moisture} = \frac{w_1 - w_2}{w_1} * 100$$

Where

W_1 =Weight of the sample before

drying

W_2 = Weight of sample after

drying

3.5.2. Determination of ash

Clean empty crucibles were placed in a muffle furnace at 600 °C for an hour, cooled in desiccators and then weight of empty crucible was noted (W_1). Two gram of each of the samples was taken into crucibles (W_2). The sample was ignited over a burner with the help of blowpipe, until it is was charred. The crucibles were placed in muffle furnace at 550 °C for 6 hours. The appearances of gray white ash indicate complete oxidation of all organic matter in the sample. The crucible was cooled and weighed (W_3).The percentage ash was calculated as follows: (AOAC, 2003).

$$\% \text{ Ash} = \text{Difference in weight of ash} * \frac{100}{\text{Weight}} \text{ of the sample}$$

$$\text{Difference in weight of ash} = W_3 - W_1$$

3.5.3. Determination of Protein

Protein in the sample was determined by Kjeldahl method. 1 g of samples was introduced in digestion flask. 10 ml of concentrated H_2SO_4 and 8 g of digestion mixture of $\text{K}_2\text{SO}_4:\text{CuSO}_4$ (8: 1) was added. The flask was swirled in order to mix the contents thoroughly placed on heater to start digestion till the mixture become clear (blue green in color). It took 2 hours to complete the process.

The digest was cooled and transferred to 100 ml volumetric flask. The volume was topped up to mark using distilled water. Ten milliliters of the digest was introduced in the distillation tube where 10 ml of 0.5 N NaOH was gradually added. Distillation continued for 10 minutes and NH_3 produced was collected as $\text{NH}_4 \text{OH}$ in a conical flask containing 20 ml of 4% boric acid solution with a drop of modified methyl red indicator. During distillation yellowish colour appeared due to $\text{NH}_4 \text{OH}$. The distillate was then titrated against standard 0.1 N HCL solutions till a pink colour observed. A blank was also run as previously.

The percentage of protein was calculated using the following formula (AOAC, 2003).

$$\text{Nitrogen\%} = (V_1 - V_2) \times N \times f \times 0.014 \times 100 / V \times 100 / S$$

Where:

V_1 = Titer for the sample (ml) V_2 = Titer for blank (ml)

S = Weight of sample taken (g)

F = Factor of standard HCL solution

N = Normality of HCL solution (0.002)

D = Dilution of sample after digestion

V = Volume of diluted digest taken for distillation (10ml)

0.014 = Milli equivalent weight of Nitrogen

Protein % = Nitrogen \times protein factor

3.5.4. Fat Analysis by Soxhlet's method

Five grams (5 g) of sample was extracted in the thimble using Petroleum Spirit (boiling point of 40-60°C) for 16 hours. Solvent was removed by Vacuum rotary evaporator at 40°C and the fat dried in the oven at 70°C for 30 minutes. The weight of the oil was obtained by subtracting the weight of the empty flask and expressed as a percentage of the sample weight (AOAC,2003).

Calculations were done using the formula below;

$$Fat(\%) = \left(w1 - \frac{w2}{w1} \right) * 100$$

Where

W1= Weight of sample before extraction

W2 = Weight of sample after extraction

3.5.5. Garber fat method for fat analysis on yoghurt

Sulphuric acid, 10ml was added to the butyrometer followed by 11 mls of well mixed yoghurt. Next 1 ml of amyl alcohol was then added, the stopper was inserted and the butyrometer was shaken carefully until the curd dissolved and no white particles were seen. The butyrometer was placed in the centrifuge with the stem (scale) pointing towards the centre of the centrifuge. It was spinned for 5min at 100 rpm. Then butyrometer was removed from the centrifuge, and the butterfat percentage was read.

3.5.6. Fiber Determination

Two grams (2 g) samples were put into 200 ml of 1.25% of H₂SO₄ and boiled for 30 minutes. The solution and the content were then poured into Buchner funnel equipped with muslin cloth and secured with elastic band. This was allowed to filter and residue was then put into 200ml boiled NaOH and boiling was continued for 30 minutes, then transferred to the Buchner funnel and filtered. It was washed twice with alcohol; the material obtained was again washed thrice with petroleum ether. The residue

obtained was put in a clean dry crucible and dried in the moisture extraction oven to a constant weight. The dried crucible was removed, cooled and weighed. Then, difference in weight (i.e. loss in ignition) was recorded as crucible fiber and expressed in percentage crude fiber (AOAC, 2003).

$$= W_1 - \frac{W_2}{W_3} * 100/1$$

Where

W₁ = weight of sample before incineration

W₂ = weight of sample after incineration

W₃ = weight of original sample.

3.5.7. Carbohydrate Content Determination

The content of total carbohydrates were calculated by subtracting the sum of moisture, protein, fat, ash and crude fiber from 100 (AOAC, 2003).

3.6. Post Acidification and Titratable Acidity

Four pots of each treatment were homogenized before analysis. The yoghurt post acidification was determined as pH after 1, 7, 14, 21 and 28 days using pH meter. pH values of all samples were measured using a pH-meter calibrated with pH 7.0 and 4.0 standard buffers. Titratable acidity of samples was determined by using 0.1M NaOH to the end point of pH 8.1 according to the Association of Official Analytical Chemists

(AOAC) method 942.15 (AOAC 2000) and was reported as % citric acid (w/v) for baobab pulp and %Lactic acid(w/v) for yoghurt . pH and TA samples were determined according to (Arjamand, 2011).

3.7. Microbiological Analyses

The starter and probiotic cultures were tested for growth in MRS (De Man Rogosa and Sharpe) medium for *Bifidobacteria spp*, and *Lactobacillus acidophilus* (was incubated at 37° C for 5 days).

3.7.1. de Man, Rogosa and Sharpe Agar (MRS agar)

MRS agar was prepared as recommended by the manufacturer by suspending 62 g of media in 1 L of distilled water. The suspension was warmed gently to boiling point to dissolve the agar, and then autoclaved at 121 °C for 15 minutes followed by immediate cooling.

3.7.2. Coliforms, Yeast and Molds Count

Coliforms, yeast and moulds was tested as follows:

i. Total yeast and moulds

Serial dilutions in ratios of 1:10 were prepared using peptone water. The dilutions were shaken uniformly by rotation and tilting. 0.1ml of the inoculum from each of the dilutions was picked and spread gently on already sterilized Subouroud Dextrose Agar

(SDA) plates. The plates were incubated at 20⁰c for 48hrs. The plates were examined for colonies appearing on the medium, which were then counted.

ii. Coliform / E coli

Serial dilutions in ratios of 1:10 were prepared using peptone water. The dilutions were shaken uniformly by rotation and tilting. 0.1ml of the inoculum from each of the dilutions was picked and spread gently on already sterilized Violet Red Bile Agar (VRBA) plates for E-coli test. The plates were incubated at 37⁰ C for 24hrs. The plates were examined for colonies appearing on the medium, which were then counted. Lactose broth was used for coliform test, after sterilization durhum tubes were put in the test tubes for gas detection and incubation was done for 24hrs at 30⁰c.

3.7.3. Enumeration of yeast/moulds, coliform and probiotic

Ten gram of each cultured yoghurt samples was diluted with 90 ml of 0.15% sterile peptone water Ten-fold serial dilutions (10⁻² - 10⁻⁸) was prepared in 9 ml of 0.15% sterile peptone water (Arjmand, 2011). The bacterial counts of each treatment were carried out in quadruplicate after intervals of 1, 7, 14, 21 and 28 days. Enumeration was done using the pour plate technique. Plates were gently mixed clockwise and anticlockwise to disturb the samples uniformly and allowed to set. Plates were then incubated under anaerobic condition (using Gas-pack system, AnaeroGen - 1.3) at 37 °C for 72 h, according to (Arjmand, 2011). The numbers of Colony Forming Units (CFU) on plates containing 15 to 300 colonies (Arjmond , 2011) were calculated per gram of samples as shown below:

$$\text{CFUg} - 1 = \frac{(\text{Number of colonies} * \text{Volume of dilute suspension})}{\text{Dilution factor}}$$

3.8. Survival in Gastral Intestinal Tract (GIT) Test for Selected Probiotic bacteria

3.8.1. Preparations of artificial fluids

3.8.1.1. Artificial gastric fluid

Artificial gastric fluid was prepared by supplementing basic gastric fluid with pepsin. The basic gastric fluid was prepared according to Malgorzata and Zareba,(2010). It contained 4.8 g of NaCl, Poland), 1.56 g of NaHCO₃ 2.2 g of KCl and 0.22 g of CaCl₂ dissolved in 1 L of distilled water. After autoclaving at 121°C/15 min, the pH of the basic gastric fluid was adjusted to 2.4 ±0.2 using 1 M HCl and 2 mg of pepsin added per 50 mL of the artificial gastric fluid.

3.8.1.2. Artificial duodenal fluid

It was prepared by supplementing the basic duodenal fluid with the enzyme complex. The basic duodenal fluid was prepared according to Malgorzata and Zareba.,(2010). It contained 5.0 g of NaCl 0.6 g of KCl 0.03 g of CaCl₂ and 17 g of bile salts was dissolved in 1 L of 1 mol/L NaHCO₃ After autoclaving at 121°C/15 min, the pH of the basic juice was 7.0 ±0.2 using 1 M NaOH and the enzyme complex was added (two capsules per 50 mL of fluid). The pharmaceutical prepared source of enzyme complex (pancreatic enzyme) was used.

3.8.1.3. Survival of the microorganisms in the simulated gastric and duodenal fluids

Ten grams of each product was suspended in 50 mL simulated gastric fluid for 3 h at 37°C. Next the mixture was transferred into 50 mL simulated duodenal fluid for 5 h at 37°C. Immediately after bacteria inoculums addition and at the end of experiment, the number of selected probiotic was measured. The number of *Bifidobacteria spp*, *Lactobacillus acidophilus* was measured using classical plate counting method MRS agar.

3.9. Nutrition composition determination

3.9.1. Sugars

Ten grams of ground homogenous sample was mixed with 50ml of 96% ethyl alcohol. The mixture was refluxed at 100°C for 1 hour and the slurry filtered. The conical flask was rinsed 3 times with 5ml of 80% Ethyl alcohol. The filtrate was evaporated to dryness at 60°C and dissolved in 10ml distilled water. Two ml of the solution was mixed with 2 ml of acetonitrile and filtered through 0.45µm. Then 20ul was injected into Higher Performance Liquid Chromatography (HPLC) (Shimadzu 20A Series, with column -NH₂ -LUNA-100A (250X4.6mm), Diameter -5ul, Refractive Index Detector and the peaks quantified against standards.

3.9.2. Determination of β -carotene

Five gram (5 g) of sample was put in the separating funnel, then about 40 ml of petroleum ether and acetone were added. Distilled water was added slowly along the neck without shaking to avoid emulsion formation. The two phases were then left to separate and the lower aqueous layer discarded. The sample was washed 3 - 4 times with 200ml distilled water each time to remove residual acetone. In the last phase, washing was done ensuring that no amount of the upper phase was discarded. Then, the upper layer was collected into 50 ml flask using anhydrous sodium sulphate filter arrangement to remove residual water. The absorbance was determined at 450 nm using UV-visible spectrophotometer (Shimadzu –UV 1800).The concentration of beta carotene was calculated using the equation of the standard curve.

3.9.3. Determination of vitamin C

Vitamin C determination was done according to the method described by Vikram,Ramesh&Prapulla,(2005), 1g of the sample was mixed with 30 ml of metaphosphoric acid and centrifuged at 10,000 rpm for 10 minutes at 4⁰C in a refrigerated centrifuge (Model H-2000C). The supernatant was filtered through whatman No 4 filter paper. The filtrate was then diluted with 1ml of 0.8% metaphosphoric acid and filtered with 0.450 Millipore filter and 20ul of the sample injected into the HPLC(Shimadzu 20 A Series, with column –NH₂ –LUNA-100A (250X4.6mm), Diameter -5ul,Refractive Index Detector and the peaks quantified against standards. The concentration was calculated from the standard graph in mg/100g.

3.9.4. Determination of Calcium, Magnesium and Zinc

Five grams of sample were weighed in crucibles and transferred to hot plates in the fume hood chamber where they were charred to clear all the smoke from the carbonations material before transferring them to the muffle furnace. The charred materials were then incinerated at 550°C until they were reduced to white ashes. The ashes were cooled, 15 ml of 6N HCl was added to each of them in the crucibles before transferring them to 100 ml volumetric flasks. Distilled water was used to top them up to the mark before mineral analysis (AOAC, 2003). Atomic Absorption Flame Emission Spectrophotometer was used for the samples analysis (Model A A-6200, Shimadzu, Corp., and Kyoto, Japan). The various mineral standards were also prepared to make the calibration curve.

3.9.5. Fatty Acid Profile

About 40mg of the sample was weighed into a conical flask. Then 10ml of methanolic HCL solution was added, heated under reflux for 1 hour, and then cooled under tap water. Methyl esters were extracted and transferred into a funnel and 10ml of hexane added, the mixture was shaken vigorously and left to stand.

Hexane layer was collected and aqueous layer was returned and extraction was repeated. The hexane fractions was combined and washed with 3 portions of distilled water to remove acid. This was filtered by deffated cotton wool and anhydrous sodium sulphate. The filtrate was then concentrated using rotary evaporator at 40⁰C. The sample was ready for injection to the Gas Chromatography (GC) -Shimadzu 8A Series, with column –

Diethyl Glycol Succination(DEGS) glass packed (15%), Diameter -5ul, mobile gas nitrogen, detector which was Flame Ionization detector(FID) contained Hydrogen and Oxygen, running time 45 minutes, ATT/range 3 and speed 10.

3.9.6. Total Soluble Solids

The total solid was determined according to Association of Official Analytical Chemists (AOAC) method 932.12 (AOAC, 2002) with a calibrated hand-held refractometer.

3.9.7. Viscosity

The method of (Arukwe, *et al* .,2012) was adopted. The sample was suspended in distilled water and mechanically stirred for 2h at room temperature (25°C). Viscometer was used to measure the viscosity.

3.10. Sensory evaluation

Cooled samples that had been stored overnight, were subjected to sensory evaluation on a 9-point hedonic scale. The panel consisted of twenty Food science student panelists from Jomo Kenyatta University of Agriculture and Technology (JKUAT). Samples were served at 4°C immediately after removing from the refrigerator. Panelists were requested to fill in the sensory evaluation form as shown in the appendix 1.

3.11. Data Analysis

All physicochemical tests were conducted in triplicate while microbial experiments were done in quadruplicate and the mean values \pm standard deviation (SD) was reported. Statistical analyses were performed by applying Analysis of Variance (ANOVA) to determine the significance of the 95% confidence interval and correlation coefficient using SPSS version 20 as statistical software. The mean differences were analyzed by Least Significance Difference (LSD) test.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1. Baobab pulp yield

The yield for baobab fruit pulp from different locations is depicted in Table 4-2. Both the pulp yield and percentage loss was observed higher in samples obtained from Oldonyo Orng'ina compared to those from Naitolia camp and Kwa Muhindi.

Table 0-1 Baobab pulp yield

Location	Weight of baobab fruits(g)	Baobab pulp yield(g)	% yield of baobab pulp
Kwa Muhindi	2635	200	7.6
Naitolia camp	4465	285	6.0
Oldonyo Orng'ina	4020	320	7.9

Yield percentage was obtained by baobab pulp yield (g) divided by weight of baobab fruits (g)

4.2. Chemical Composition

The chemical composition of raw baobab fruit pulp obtained from the three selected sites was as shown below.

4.2.1. Moisture content

Moisture content for baobab pulp from the three locations are shown in Table 4-2. The value obtained from Naitolia camp was higher than that of 7.87%-8.59% as reported by Abdalla, Mohammed & Mudawi, (2010), and higher than those reported by Osman (2004). There were no significant differences at ($p \leq 0.05$) in moisture contents between samples from all three locations.

Table 0-2 Proximate for baobab pulp

Proximate (%)	Location			p-value	LSD
	Kwa Mhindi	Naitolia camp	Oldonyo Orng'ina		
Moisture	9.94±0.54 ^a	10.30±1.28 ^a	9.16±1.15 ^a	0.08	0.36
Fat	1.98±0.69 ^a	1.11±0.35 ^b	0.46±0.13 ^c	0.00	0.64
Ash	4.87±0.51 ^a	5.21±0.39 ^a	4.75±0.51 ^a	0.11	0.11
Crude fiber	6.29±2.57 ^a	9.65±0.36 ^b	5.91±1.42 ^c	0.00	0.38
Protein	3.23 ± 0.23 ^a	3.53±0.29 ^a	3.52±0.27 ^a	0.38	0.29
Carbohydrate	83.58±2.77 ^a	80.49±0.48 ^b	80.49±0.48 ^b	0.00	1.61

Values are means ± SD. Means sharing a common superscript letter in a row are not significantly different ($p < 0.05$)

4.2.2. Fat content

The crude fat values of baobab pulp were as shown on Table 4-2. Significant differences exist among the three locations at ($p \leq 0.05$). Present findings were noticeably higher compared to the value of 0.3% reported by Osman, (2004), 0.2% Zahra'u, *et al.*, (2014)

and for 1.43%. Abdalla *et al.*,(2010). The low level of fat in the fruits means that these fruits are not good source of energy and are therefore recommended for loss or maintaining of weight, supply of nutrients and lowering of blood pressure (Othman ,Fabian&Lugwisha .,2014).

4.2.3. Ash content

Results showed that there is no significant difference ($p \leq 0.05$) in ash content as shown in Table 4-2. Present findings for the locations were within the range as reported value of 4.5% stated Osman,(2004) and 5.5% Magaia,Uamusse,Sjoholm&Skong,(2013).,but lower for both location as reported value of 5.8% Sidibe and Williams, (2002).The results in this study suggest that the fruits has high deposit of minerals.

4.2.4. Fiber content

As shown in Table 4-2 pulp collected from baobab from Kwa Muhindi, Naitolia camp and Oldonyo Orng'ina was found to contain fiber in a range of $5.91 \pm 1.42\%$ to $9.65 \pm 0.36\%$. There was significance difference among location at ($p \leq 0.05$). Results obtained were comparable to the results reported by Sidibe and Williams, (2002);Osman,(2004), Caluwé and Damme,(2010;) which ranged between 5.4 and 11.5%. Fiber is essential to the human body as it helps to maintain the health of the gastrointestinal tract and in weight regulation (Othman *et al.*,2014).

4.2.5. Protein content

Protein content of baobab fruit pulp was not significantly different ($p \leq 0.05$) among the three locations as stipulated on Table 4-2. Results reported were within the range of 2.5-17% presented by Ibrahima *et al.*, (2013) and Sidibe and Williams,(2002), but higher than the 2.1-2.4% concentration reported by Gebauer *et al.*, (2002) and Magaia *et al.*, (2012) respectively.

4.2.6. Total carbohydrates

The carbohydrate content of the baobab pulp was in a range of $80.49 \pm 0.48\%$ to $85.19 \pm 1.53\%$ for both locations. Samples from Oldonyo Orng'ina showed significantly higher carbohydrate content ($P \leq 0.05$) as shown on Table 4-2. Data obtained were within the range of 73.7 to 81% which was at per values previously reported by Osman, (2004), Abdalla *et al.*,(2010), and Rahul *et al.*,(2015).

4.3. Ascorbic acid (vitamin C) content

As presented in Table 4-3, pulp obtained from baobab fruit from Kwa Muhindi location had the highest ascorbic acid content followed by Oldonyo Oring'ina, while that from Naitolia camp showed the lowest vitamin C level and the results showed no significant difference ($p \leq 0.05$).

Table 0-3 Vitamin C, beta-carotene and mineral content for baobab fruit pulp

Nutrients(mg/100g)	Location			p-value	LSD
	Kwa Mhindi	Naitolia camp	Oldonyo Orng'ina		
Vitamin C	231.57±140.41 ^a	169.74±85.43 ^a	211.99±84.82 ^a	0.47	19.57
Beta-carotene	2.16±1.77 ^a	3.03±1.79 ^a	3.19±1.68 ^a	0.42	0.16
Calcium	274.65±69.71 ^a	279.38±149.15 ^a	322.93±100.93 ^a	0.61	4.73
Magnesium	184.60±41.44 ^a	174.13±64.36 ^a	175.27±17.98 ^a	0.87	1.13
Zinc	3.11±1.05 ^a	2.67±1.41 ^a	2.89±1.05 ^a	0.73	0.22

Values are means ± SD. Means sharing a common superscript letter in a row are not significantly different ($p < 0.05$)

The vitamin C content range was higher compared to 34-200 mg/100g reported by Sidibe and Williams ,(2002), and lower in comparison with content of 300 mg/100g and 355.7 mg/100g as reported by Gebauer *et al.*,(2002) and Almustafa ,(2003) respectively. The high values of ascorbic acid in baobab pulp signify the potential use of the fruit as an antioxidant. The recommended daily intake (RDI) of ascorbic acid is about 30 mg/day for adults and 17 mg/day for children Othman *et al.*,(2014). Therefore, these fruits could be considered as good sources of ascorbic acid for purposes of human nutrition.

4.4. Beta carotene

Beta carotene was found to range between 2.16-3.19mg/100g for both locations as shown in Table 4-3. Oldonyo Orng'ina sample showed higher beta carotene content compared with those from Naitolia camp and Kwa Muhindi. In this research, the results showed that there was no significant difference ($p \leq 0.05$) in beta-carotene content among three locations.

4.5. Minerals

Mineral composition of juices and fruits are among the criteria that guide consumers' choice. The minerals analyzed (Table 4-3) in this study are calcium, magnesium and zinc. Calcium content was higher, than other minerals analyzed, and had no significant difference between the three source areas at $p < 0.05$. The high calcium contents of the fruit pulp makes the baobab fruit of interest as a natural source of calcium supplementation for pregnant and lactating women, as well as for children and the elderly (Osman,2004).The high variability in mineral contents in the baobab pulp has been largely highlighted by Ibrahima *et al.*, (2013). It may be associated, at least in part, with the soil type and origin of samples.

Calcium and Magnesium are major minerals in human nutrition; therefore, the baobab pulp might be an important source of calcium, higher than that of milk. Caluwé and Damme,(2010) reported the contribution of baobab pulp to the recommended daily intake (RDI) for zinc and calcium for children and pregnant women. The coverage of these minerals is possible only when the highest reported values are considered for the pulp. Thus, considering the highest reported values; the consumption of 40 g of baobab pulp for children is enough to cover 25.4% of the RDI for zinc and 35% for the RDI for calcium. For pregnant women, the consumption of 60 g and 100 g would cover 17.3% and 28.7% of the RDI for zinc and 42.1% and 70.1% of the RDI for calcium, respectively.

4.6. Sugars

Sugars tested were glucose, fructose and sucrose and the results are stipulated in table 4-4. Fructose was high in pulp from baobab tree collected from Naitolia camp while glucose and sucrose were high in pulp from baobab tree collected from Kwa Muhindi location. Ibrahima *et al.*,(2013) reported 7.9 g/100g and 7.0 g/100g for glucose and fructose respectively which were higher and 1.7 g/100 for sucrose which lower in comparison with the result obtained. The results show that there was significant difference at ($p \leq 0.05$) for fructose while no significant difference was observed for sucrose and lactose among the locations.

Table 0-4 Sugar content for baobab pulp

Sugar(g/100g)	Location			p-value	LSD
	Kwa Mhindi	Naitolia camp	Oldonyo Orng'ina		
Fructose	0.62±0.17 ^a	0.81±0.17 ^b	0.56±0.15 ^c	0.01	0.05
Glucose	0.87±0.31 ^a	0.81±0.24 ^a	0.77±0.26 ^a	0.76	0.04
Sucrose	0.84±0.29 ^a	0.79±0.23 ^a	0.75±0.25 ^a	0.76	0.04

Values are means \pm SD. Means sharing a common superscript letter in a row are not significantly different ($p < 0.05$)

Fructose, sucrose and glucose contribute to the pulp sweetness (Sidibe & Williams, 2002;Caluwé & Damme, 2010;Namratha & Sahithi, 2015).

4.7. Fatty acid

Fatty acid profile results for baobab pulp are presented in Table 4-5 .Saturated fatty acids constituted the range of 41.22-43.78% .The higher percentage was on Oldonyo Orng'ina, and the dominant saturated fatty acid in all location was palmitic acid.

Table 0-5 Fatty acid profile of baobab pulp

	Naitolia camp	Kwa Muhindi	Oldonyo Orng'ina
Fatty acid %			
		Saturated	
Butyric (C4:0)	0.73	0.00	4.02
Caprylic (C8:0)	1.27	0.38	0.36
Capric (C10:0)	0.56	0.14	0.20
Lauric (C12:0)	2.75	5.60	9.17
Myristic (C14:0)	1.69	2.22	2.50
Palmitic (C16:0)	29.85	30.43	24.68
Stearic (C18:0)	4.37	3.82	2.85
Saturated %	41.22	42.59	43.78
		Mono unsaturated	
Palmitoleic (C16:1)	1.33	0.56	0.76
Oleic (C18:1)	9.38	8.58	8.38
Mono unsaturated%	10.71	9.14	9.14
		Poly unsaturated	
Linoleic (C18:2)	10.39	8.00	5.47
Linolenic (C18:3)	26.43	31.61	27.76
Poly-unsaturated%	36.82	39.61	32.23

Mono unsaturated fatty acid profile ranged between 9.14-10.71%, the highest being from Naitolia camp location, and oleic acid was the leading fatty acid all locations. Linolenic acid was the dominant with a percentage range of 32.23-39.61%, and Kwa Muhindi recording the higher percentage fatty acid profile. Therefore, according to these results the major fatty acid in baobab pulp is linolenic acid followed by palmitic and oleic acid respectively. This qualifies baobab as an excellent source of mono and polyunsaturated fatty acid (Osman,2004).

The quality of the fat in the diet is also important, especially the content of unsaturated fatty acids. The presence of polyunsaturated fatty acids in baobab pulp are important for the maintenance of the immune system and cell membranes, and for functioning of the brain and skin and also in the reduction of the risk of heart diseases (Magaia *et al.*,2013 ;Zahra'u, *et al.*,2014).

4.8. pH

Values of pH for baobab fruit pulp from different locations are presented in Table 4-6. Pulp obtained from Kwa Muhindi and Naitolia camp had a pH value of 3.3, which was higher compared to the value obtained from samples from Oldonyo Orngina.

Table 0-6 pH for baobab pulp

Location	pH
Naitolia camp	3.3
Kwa Muhindi	3.3
Oldonyo Orng'ina	3.2

Values are means triplicates determination

4.9. Titratable Acidity

Values of % TTA for baobab fruit pulp from different locations are depicted in Table 4-7. There is a significant difference among location ($p \leq 0.05$). Pulp obtained from Naitolia camp had % TTA value of $1.13 \pm 0.16\%$, which was higher compared to the value demonstrated by both Kwa Muhindi and Oldonyo Orng'ina.

Table 0-7 Baobab pulp titratable acidity

Location	Mean \pm Sd Titratable Acidity
Naitolia Camp	1.13 ± 0.16
Kwa Muhindi	1.01 ± 0.33
Oldonyo Orng'ina	1.08 ± 0
p-value	0.024

Each value in average of three replicates with Mean \pm Standard deviation

4.10. Colour

Hue describes a visual sensation according to which an area appears to one or two proportions of the perceived colours, red, yellow, green and blue(Yumbya,Ambuko&Owino,2014). Hue angle is therefore the actual colour. Chroma (C) is the intensity of fundamental colour with respect to amount of white light on the background. L* value is therefore an indication of lightness (León *etal.*, 2006).As shown in Table 4-8 ,L* values ranged from 91.0 to 94.7,Chroma 16.4 to 24 and Hue 86.51 to 93.9. L* values are approximately to the maximum value which is 100 (Hunterlab) ,indicating the lightness (0=black and maximum 100).

Table 0-8 Baobab pulp colour

Tree	L*	A	B	Chroma	Hue Angle
1	91.5	-1.4	24	24.04	93.34
2	93.6	0	16.4	16.4	86.51
3	94.6	0	17.3	17.3	86.69
4	92.9	0.5	17.3	17.31	88.34
5	92.1	0.2	18.6	18.60	89.38
6	92.1	0.1	17.4	17.40	89.67
7	91.5	0	21.8	21.80	87.37
8	94.7	-1.3	18.6	18.65	93.99
9	91.1	0.2	18.2	18.20	89.37

L*indication of light,A and B indicating maximum value of lightness(0=back and maximum 100)

4.11. Functional properties

Rapid increase in world's population and the lack of animal protein, especially in the developing countries, has led to an increase in the demand of both conventional and nonconventional plant protein. These alternative plant proteins must be of high nutritional value and should have good functional properties for them to be used as food ingredients (Atta & El-Shenawi, 2013).

4.11.1. Gelation

The lowest concentration at which the sample does not fall down/slip from an inverted test tube was used as index of gelation capacity. The lower the Lowest Gelation Concentration (LGC), the better the gelation ability of the protein ingredient (Atta & El-Shenawi, 2013). As shown in Figure 4-1 a good gel was formed at 11% (w/v) which is within the range similar to 8% -18% attained by Eltayeb, Abou-arab & Abu-salem, (2011).

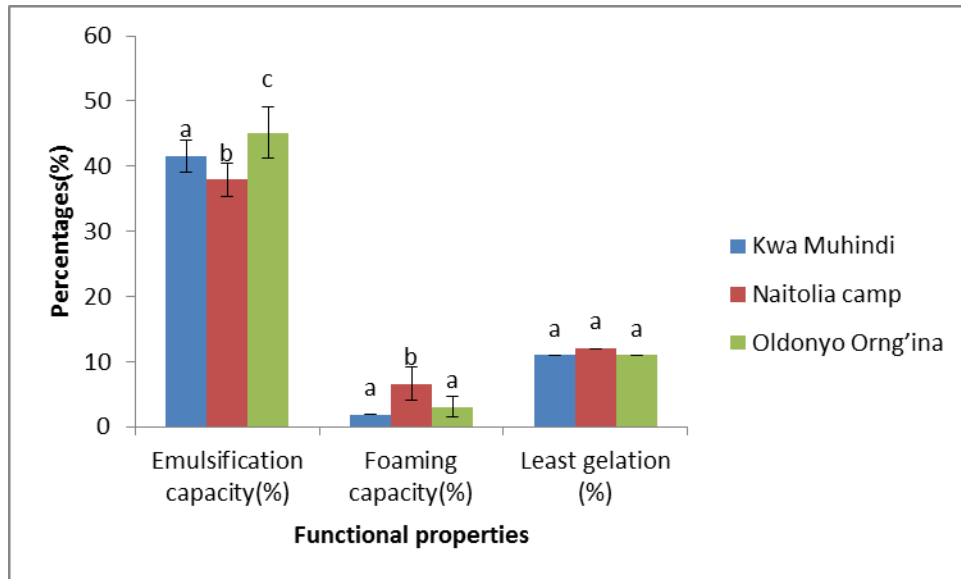


Figure 0-1 Functional properties of baobab pulp

Letter a, b, c are showing standard error, and those sharing common letter are not significantly different ($p < 0.05$)

The low LGC observed in the baobab pulp may be an advantage in respect to the production of products such as curd as their production requires materials with high gelation capacity like milk protein(casein) Kisambira,*et al.*,(2015),and in jam making due to higher content of pectin which contribute to the excellent gelling capacity (Ndabikunze *et al.*,2011).

4.11.2. Foaming Capacity

Low foaming capacity was reported on baobab pulp powder from Kwa Muhindi location whereas the highest was from Naitolia camp (Figure 4-1). In comparison with baobab pulp mixed with ogi flour (Adejuyitan, *et al.*,2012) ranged at 3.2-11.7 %, which

is within for Naitolia camp. Baobab pulp powder reported lower foaming capacity in comparison with egg yolk powder which is widely used for its excellent foaming capacity value of 38.50% (Ndife,Ejimeke&Amaechi,2010).

In comparison to other fruits like watermelon seed flour the value are lower as reported by (Oyeleke *et al.* 2012) with 23.5% and higher compared to the dehulled and cooked jackbean flour which is 0.02%,(Obiageli, 2008). In regard with this result, use of baobab pulp powder for foaming application may therefore, require modification.

4.11.3. Emulsification capacity

As shown in Figure 4-1, the highest emulsion of baobab pulp powder was reported on Oldonyo Orng'ina whereas the lowest was on Naitolia camp. This results showed that baobab pulp powder had almost half emulsification capacity(74%) Ndife *et al.*,2010 as that emulsification of egg yolk powder which is one of the excellent emulsifiers. In comparison with other food products ,baobab pulp collected from Naitolia camp has slightly similar value to yam bean flour 35.70% (Kisambira, *et al.*,2015) .This is however higher compared to pulp obtained from Kwa Muhindi and Oldonyo Orng'ina. On the other hand reported higher emulsification capacity compared to jack fruit flour 2.53-3.16% reported by Obiageli ,(2005) for both locations. According to this result, the uses of baobab pulp powder for emulsification application may therefore, require modification.

Variations

The variation for the proximate results may be due to, the provenance of the sample, the treatment before analysis, storage conditions, processing methods, a probable genetic variation, ripening age difference (Fagbohun *et al.*,2012) and physical chemical characteristics of the soil (Assogbadjo *et al.*, 2012b;Fagbohun *et al.*,2012).Apart from the variability in the material, the analytical methods and inherent variability may also be a cause of variability. Moreover, micronutrients, such as vitamins and minerals, are biologically active. They can interact with other nutrients and change in their bioavailability, hence it may be also a contributing factor for variation (Caluwé & Damme,2010).

4.12. Chemical Properties of the Yoghurt during storage

4.12.1. Total soluble solids

The percentage soluble solids content in yoghurt (control) and those enriched with baobab decreased throughout the storage period as shown in Figure 4-2.

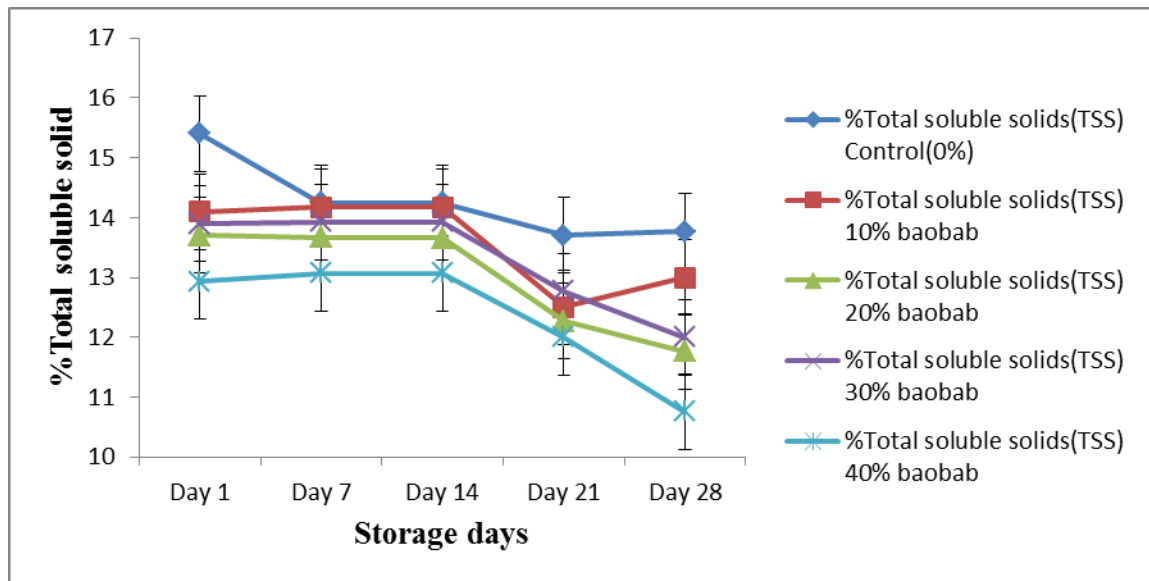


Figure 0-2 Changes in total soluble solids of yoghurt during storage

The graph above shows the total soluble solids percentages at different storage days for yoghurt as control with no baobab added and with 10%, 20%, 30% and 40% baobab pulp added, and statistically significant difference at $p < .005$. On day 28, TSS in yoghurt with 40%, 30%, 20% and 10% baobab pulp had decreased by 16.78%, 13.67%, 14.08% and 7.80% respectively. The control sample decreased by 10.71%, therefore the highest decrease was on the yoghurt enriched with 40% baobab pulp.

The percentage TSS is reasonably lower compared to the findings of (Igbabul *et al.*,2014) who reported a TSS range of 18.4% to 21.41% ,however results are within the range reported by Ndife *et al.*,(2014) having 14.77% to 19.90%. The significant decrease in total solids could be due to syneresis i.e. oozing out of whey which contains whey proteins, lactose and minerals (Selvamuthukumran &Farhath,2014).

4.12.2. pH

The results of the pH of the different yoghurt samples as presented in Figure 4-3 which indicate a decrease of pH as days increases.

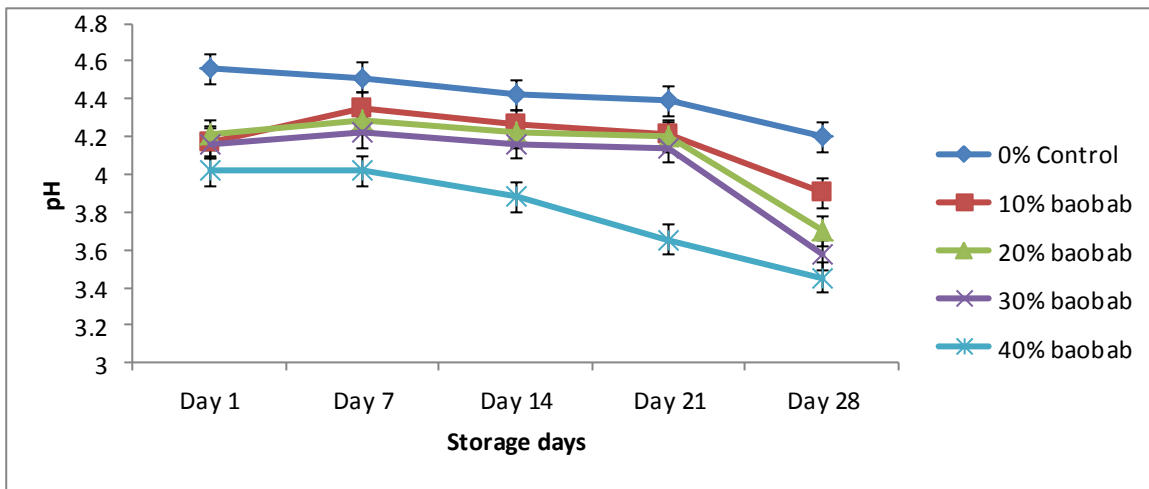


Figure 0-3 Changes in pH of yoghurt during storage

From day one, the yoghurt having 40 % of baobab showed low value of 3.45 ± 0.05 while the control showed high. pH value of 4.57 ± 0.01 . The decrease on day 28 was 4.20 ± 0.2 , 3.90 ± 0.1 , 3.70 ± 0.2 , 3.57 ± 0.03 and 3.45 ± 0.05 for control, 10%, 20%, 30% and 40% baobab enriched yoghurt respectively, and were significant different with ($p\leq 0.05$). Lactic acid Bacteria produce lactic acid during fermentation of milk lactose thus lowering the pH (Ndife *et al.*, 2014).

In addition, the presence of naturally occurring organic acid such as citric, tartaric, malic, succinic and ascorbic acid in baobab pulp (Kamatou *et al.*, 2011) and Yeast could be a contributing factors towards decrease in pH of the baobab enriched yoghurt (Larsson, 2009). pH shows a steady decline throughout the storage time. pH of the yoghurt decreases with longer storage times. Storage time in fermentation is the strongest factor influencing the final acidity of the product (Larsson, 2009).

4.12.3. Viscosity

The viscosity of the samples as shown on Figure 4-5 indicates decrease in viscosity as the day increases. The baobab enriched yoghurt had a unique trend as it shows an increase on day 7 and 14 for 10% and 20%, while 30% and 40% there was decrease on day 14. Yoghurt with 10% baobab was more viscous on day 14 (Fig 4-4). The low viscosity on day 28 was reported on the control yoghurt with a value of 3.68 (cP). Moreover the results showed significant differences ($p\leq 0.05$).

Viscosity is affected by the strength and number of bonds between casein micelles in yoghurt, as well as their structure and spatial distribution (Izadi,Nasirpour,Garousi&Tamjidi,2014).As it is seen in figure 4-4, viscosity of enriched yoghurts with 40% is lower than the control on the first day .This is because yogurt is a gel/matrix of casein micelles with entrapped water. Adding baobab pulp may interrupt the gel structure of the enriched sample. It was reported that the apparent viscosity of yoghurt during storage time decreases and also can increase over time due to the rearrangement of protein and protein-protein contacts (Izadi *et al.*,2014).

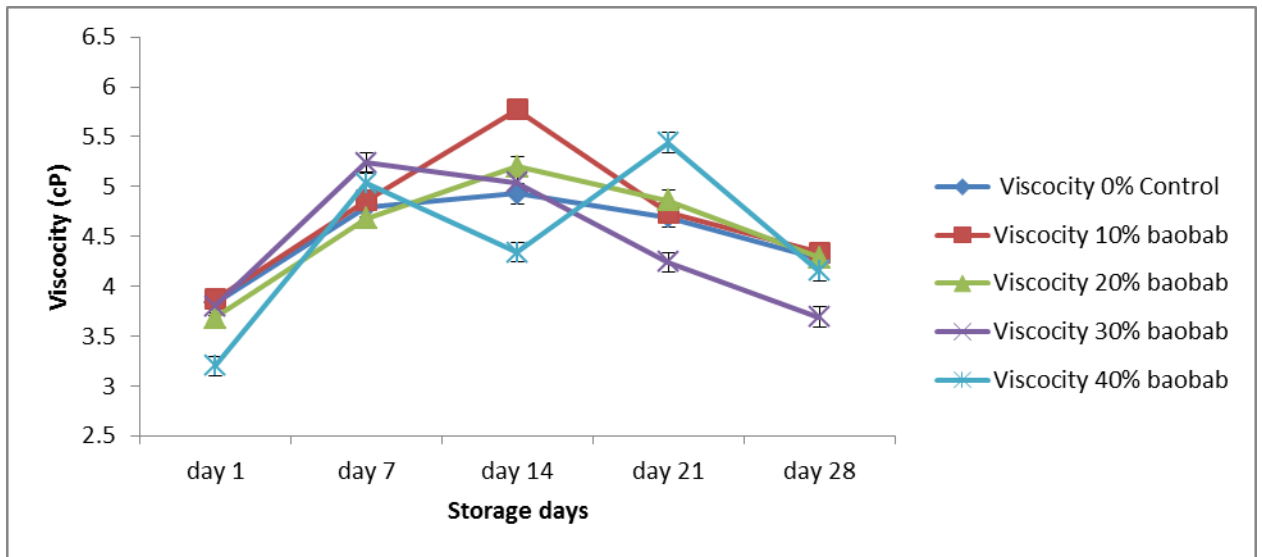


Figure 0-4 Changes in viscosity of yoghurt during storage

4.12.4. Titratable Acidity (TTA)

Figure 4-5 shows % TTA of yoghurt samples during storage. It ranged between 0.52 % and 0.76% which agreed with 0.6% minimum according to Ojotu, Ngozi & Haruna, (2013). Results show slight decrease in TTA for control yoghurt, 10% baobab and 30% baobab. A significant increase in TTA for the 40% baobab on day 28 was observed. Titratable acidity obtained from this study showed significant difference among yoghurt samples ($p \leq 0.05$).

This phenomenon can be explained through the fact that whey separation caused by lactose hydrolysis leads to slow rate of acid production in yogurt (Chalas, 2013). As lactose is hydrolyzed by lactic acid bacteria the amount of lactic acid produced increased. This behavior may be due to the availability of more quantity of easily fermentable sugar (glucose) which is required for the faster growth of starters (Chalas, 2013). Fan *et.al.*, (2008) reported that changes in titratable acidity do not necessarily have an effect on pH values.

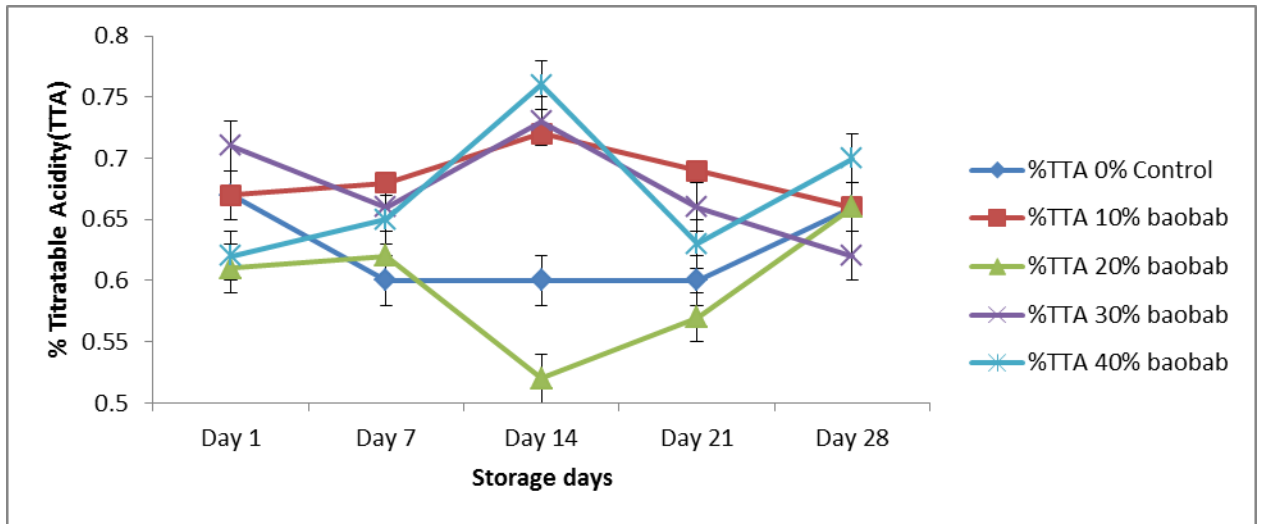


Figure 0-5 Changes in Titratable acidity of yoghurt during storage

4.13. Proximate Composition for yoghurt

Table 4-9 shows the result of the proximate analysis of different yoghurt samples evaluated in this study. The composition of foods is known to exert considerable influence on their physical, nutritional, sensory and shelf characteristics(Zubeir, 2009) .The moisture content of the yoghurt samples ranged between 24.82 ± 1.09 % to 81.87 ± 5.35 % and control had the highest moisture value (81.87%) compared to the enriched yoghurts.

Table 0-9 Proximate for Yoghurt

Proximate (%)	Baobab pulp%					p-value
	0	10	20	30	40	
Moisture	81.87±5.35 ^a	72.78±1.87 ^b	67.72±2.26 ^b	25.92±2.91 ^c	24.82±1.09 ^c	<.001
Crude ash	0.65±0.06 ^a	1.35±1.26 ^a	4.59±3.95 ^a	5.07±4.52 ^a	6.04±5.21 ^a	0.33
Crude fiber	0.14±0.05 ^a	0.40±0.08 ^b	0.54±0.05 ^b	0.68±0.03 ^b	0.93±0.14 ^c	<.001
Fat	6.33±0.58 ^a	6.00±1.00 ^b	6.67±0.58 ^c	7.00±0.33 ^c	7.57±0.58 ^d	0.60
Protein	2.53±0.39 ^a	2.35±0.28 ^a	2.40±0.21 ^a	2.62±0.46 ^a	1.78±0.14 ^b	0.06
Carbohydrate	8.14±6.17 ^a	17.11±2.26 ^b	16.22±5.22 ^b	59.13±4.90 ^c	60.44±4.77 ^c	<.001

Values are means ± SD. Means sharing a common superscript letter in a row are not significantly different ($p < 0.05$)

The fat content ranged between 6.00±1.00 to 7.57± 0.58% in the enriched yoghurts compared to the control (6.33± 0.12%). Fat content has been reported by other researchers to have positive influence on the physical and sensory characteristics and negative impact on the shelf stability of yoghurts (Ndife, *et al.*,2014) and (Weerathilake *et al.*, 2014) reported maximum fat content of 15% for yoghurt.

Ash content increased as the proportion of baobab pulp increased in the yoghurts, as shown on table 4-9, the value ranged from 1.35±1.26% to 6.04±5.21% for baobab enriched yoghurt and 0.65±0.06% for control yoghurt. This can be explained by the fact that baobab powder has a high ash content which implies to high mineral content in the

baobab enriched yoghurts. This findings are similar to results from other plant based enriched yoghurt which have equally reported high ash content (Belewu ,Belewu & Bamidele,2010; Eke,Olaiten & Sule,2013).

Fiber content in the enriched-yoghurts ranged between $0.40\pm 0.08\%$ to $0.93\pm 0.14\%$ compared to the control ($0.14\pm 0.05\%$).The baobab powder contains fiber (soluble and insoluble) which are indigestible polysaccharides that could assist in the viscosity and stabilization of the yoghurts and that could be reason for the increase of crude fiber, in addition to serving as prebiotics. According to Igbabul *et al.*,(2014) fibers contributes to the health of the gastrointestinal system and metabolic system in man.

The protein content of yoghurt decreased with increase in baobab pulp (Table 4-9).Protein content of control yoghurt was $2.53\pm 0.39\%$ while that of enriched with the baobab yoghurt had a range of $1.78\pm 0.14\%$ to $2.62\pm 0.46\%$. According to Madora,Takalani&Mashau,(2016) the protein content decreased with increase in baobab pulp because of the inhibitory effect of baobab pulp on the proteolytic organisms that could harbour breakdown of proteins.

The increase in protein content in yoghurt depends on the proteolytic activity of lactic acid bacteria which hydrolyses proteins (caseins) into peptides and amino acids. The observed carbohydrates content was $8.14\pm 6.17\%$ for the control, $17.11\pm 2.26\%$ for 10% $16.22\pm 5.22\%$ for 20%, $59.13\pm 4.90\%$ for 30% and $60.44\pm 4.77\%$ for 40% had significance different at ($p\leq 0.05$) as shown on Table 4-9.Lactose is a major carbohydrate found in milk, and is converted to lactic acid during yoghurt (fermentation) production process. Therefore fermentation and conversion of lactose to lactic acid

accounts for the low content of carbohydrates of yoghurt as observed in the result (Ihemeje *et al.*,2015).

4.14. Vitamin C

Vitamin C content was low in the control with value of 0.60 ± 0.04 mg/100g, and higher in the baobab enriched yoghurt at 7.07 ± 0.10 mg/100g as shown on Table 4-10. The value is smaller compare to the baobab which contain 300 mg/100g (Stapleton,2015; Ndabikunze *et al.*,2011). Low value of vitamin C in the yoghurt enriched with baobab pulp was due to the processing since the Vitamin C is easily lost during heating. However the content of vitamin C keeps increasing as the concentration of the baobab pulp increases.

Table 0-10 Vitamin C and mineral contents of Yoghurt

Nutrients (mg/100g)	Baobab pulp%					p-value
	0	10	20	30	40	
Vitamin C	0.60 ± 0.04^a	2.75 ± 0.22^b	2.95 ± 0.02^c	3.35 ± 0.03^d	7.07 ± 0.01^e	<.001
Calcium	64.12 ± 6.69^a	65.89 ± 8.18^a	67.86 ± 17.57^a	85.87 ± 26.19^a	88.47 ± 6.65^a	0.21
Magnesium	12.05 ± 2.36^a	21.56 ± 1.84^b	21.87 ± 1.58^b	22.05 ± 6.79^b	31.03 ± 3.98^c	0.002
Zinc	0.83 ± 0.19^a	0.85 ± 0.19^a	0.86 ± 0.17^a	1.01 ± 0.12^a	1.59 ± 1.42^a	0.59

Values are means \pm SD. Means sharing a common superscript letter in a row are not significantly different ($p < 0.05$).

4.15. Minerals analysis

Results for mineral analysis of control (mg/100g) for calcium, magnesium and zinc were as shown on Table 4-10. The results justifies the ascertainment of Ihemeje,Nwachukwu&Ekwe,(2015) that yoghurt is a very good source of essential minerals needed for human metabolism or functionality of cells. Addition of baobab pulp caused an increase in calcium, magnesium and zinc. A similar increase of minerals was also observed by Ihemeje *et al.*,(2015) when carrot,pineapple,ginger and pepper fruit were used. Baobab pulp had earlier been reported to contain minerals, vitamins, protein, fiber, ash and carbohydrate. According to Ibrahima,*etal.*,(2013) baobab pulp is rich in calcium(302 mg/100 g) and magnesium(195 mg/100 g).This may have caused the observed increased in mineral values of the products.

4.16. Microbial Quality

Table 4-11 shows the microbial populations of different yoghurt samples on day one and after seven days of cold storage at 4°C.They were enumerated to evaluate the finished product on the survival of starter organisms as well as the presence of undesirable spoilage and pathogenic organisms.

Table 0-11 Microbial count for yoghurt during storage

	Storage days	Treatment				
		0%	10%	20%	30%	40%
		baobab	baobab	baobab	baobab	baobab
Total count(cfu/ml)	1	0	0	0	0	0
	7	0	0	0	0	0
	14	0	0	0	1x10 ¹	0
	21	1x10 ²	1x10 ²	0	1x10 ²	0
	28	5x10 ²	55x10 ¹	1x10 ²	2x10 ²	40x10 ¹
Yeast/Moulds(cfu/ml)	1	0	0	0	0	0
	7	0	0	0	0	0
	14	17x10 ²	0	0	2x10 ²	2x10 ³
	21	27x10 ²	0	1x10 ³	4x10 ²	4x10 ³
	28	34x10 ²	46x10 ²	22x10 ²	24x10 ²	47x10 ²
Coliform	1	-ve	-ve	-ve	-ve	-ve
	7	-ve	-ve	-ve	-ve	-ve
	14	-ve	-ve	-ve	-ve	-ve
	21	-ve	-ve	-ve	-ve	-ve
	28	-ve	-ve	-ve	-ve	-ve

Values are means triplicates determination

The total viable counts for freshly prepared control, ranged from (1×10^2) CFU.ml⁻¹ to (5×10^2) CFU.ml⁻¹ at 4°C. The total viable counts of control and baobab enriched yoghurts on day 1 to 7th day of storage were zero but on day 14 was 1×10^1 CFU.ml⁻¹ for 30% baobab pulp yoghurt.

Coliform, Escherichia coli and Salmonella spp were not detected in control and baobab pulp enriched yogurts throughout the storage time. The absence of *Escherichia coli* signifies that all yogurt samples were free from faecal contamination. However, yeasts and molds count were detected on 14th day of storage for 0% ,30% and 40% and for 20% and 10%, on day 21 and 28. According to Sengupta, Bhowal, Bhattacharyya, (2013) the count kept increasing with days due to an increase in acidity or reduction in potential oxygen during fermentation.

4.17. Viability in simulated gastric and duodenum

The probiotic used for making the yoghurt were tested under condition of simulated gastric and duodenum fluid for Survival in Gastro Intestinal Tract. All the products showed survival rates of 22% -52% after the 3 hours in the gastric juice and duodenum (Table 4-12). Yoghurt with 30% baobab pulp had higher survival rate of 52% and initial count of 8.95 log₁₀(cfu/ml) for 40% baobab pulp added yoghurt while the rest had survival rates less than 50% and initial count ranging from 7.3 – 8.45 log₁₀(cfu/ml). This suggest that they can tolerate conditions of the gastral intestinal tract and also addition of baobab pulp to the product support its growth as the product enriched baobab pulp has higher survival rate and initial count.

The reason for higher survival rates and number for initial count is that the baobab pulp has soluble fiber (25%) which stimulates the growth and metabolic activities of beneficial organism-probiotic (Abdalla *et al.*, 2010). Moreover, products observed to contain the recommended number of bacteria 10^6 cfu (6 log₁₀ cfu) required in a standard probiotic food (Granato, Branco, Cruz, Faria & Sha 2010; Cencic & Chingwaru, 2010). This fulfills the aim of compensating the possible reduction in the number of probiotic during passage through the gut.

Table 0-12 Survival rates probiotics (*Lactobacillus* and *Bifidobacteria*) used to make yoghurt in response to stimulated gastric and duodenum fluid

Product(%baobab)	Initial count	Duodenum					
		Gastric log 10CFU/ml			log 10 CFU/ml		
		1 h	2h	3h	4 h	5h	6h
0%	7.3	3(41)	2.78(38)	2.6(36)	2.78(38)	2(27)	0
10%	7.48	3.41(46)	3.20(43)	3(40)	3.68(49)	3.32(44)	2.30(31)
20%	8.38	3.89(46)	3.51(42)	3.44(41)	3.83(46)	3.58(43)	2.30((27)
30%	8.45	4.39(52)	4.2(50)	4(47)	3.90(46)	3.68(44)	2.85(34)
40%	8.95	2.69(30)	3(34)	3.08(34)	2.78(31)	2(22)	2(22)

Figures in brackets represent the survival rate

4.18. Sensory properties

The sensory evaluation results of probiotic yoghurt enriched with baobab pulp are shown in Figure 4-6.

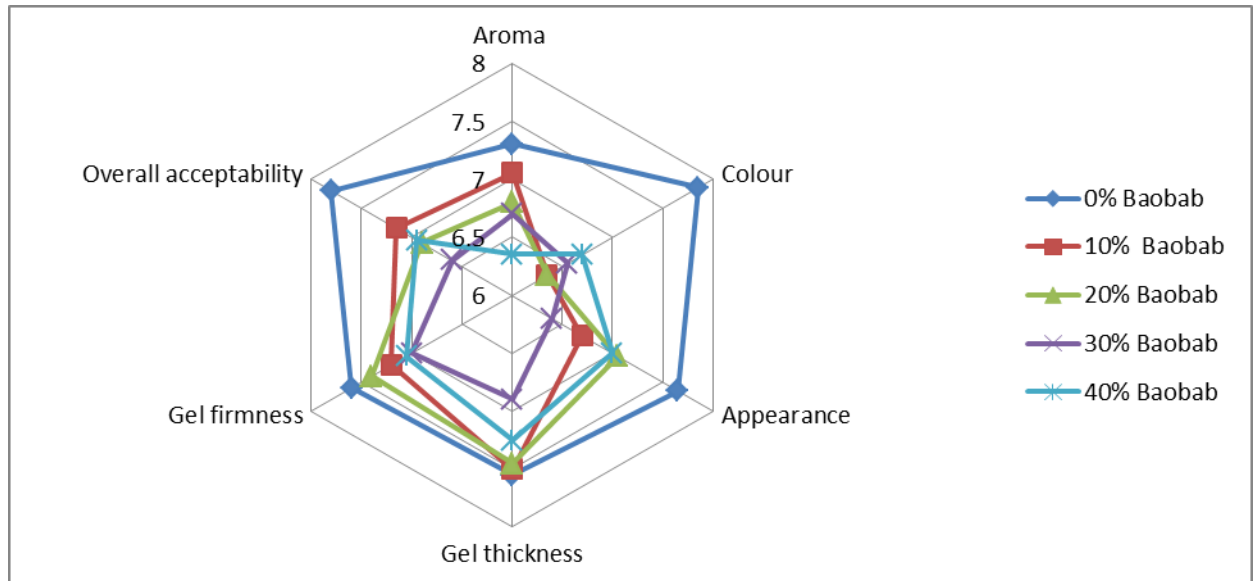


Figure 0-6 Effect of baobab pulp concentration on sensory evaluation of probiotic yoghurt

Results for aroma showed that enriched yoghurt with 10% baobab pulp had higher mean score of 7.15. Yoghurt enriched with 40% baobab had higher mean score of 6.70 while 10% and 20% baobab pulp yoghurt had a similar mean score of 6.35. Appearance for 20% baobab pulp yoghurt had higher mean score of 7.05, and in the case of gel thickness and firmness the results showed 20% baobab pulp had higher mean score of 7.45 and 7.40 respectively. Overall acceptability of 10% baobab enriched yoghurt showed higher mean score of 7.15, indicating good acceptance by consumer, hence most preferred.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5.1. Conclusion

This study aimed at developing probiotic yoghurt enriched with various concentrations of baobab pulp while monitoring its characteristics during 5 weeks of storage. Baobab pulp has nutritional benefit as it has lot minerals, vitamin C and fiber. Beside probiotic contributes to health benefit in the prevention of gastral intestinal tract disorders, immunity enhancement and improvement of lactose intolerance disorders. Considering the above benefits then incorporating baobab pulp to the probiotic yoghurt is beneficial, as it results in additional value.

Incorporation of baobab pulp to the probiotic yoghurt is of advantageous nutritionally, as it contribute to the health benefit to the human body. Generally, 10% and 20% baobab enriched yoghurt was more acceptable as it has the highest score compared to 30% and 40% baobab enriched yoghurt. In addition to that, the physical-chemical characteristics, pH, TSS, and viscosity shows decrease as days increase while titratable acidity increases.

The yeast/mold counts was low and coliforms were absent in all samples, which is indicative of non-post processing contamination hence the product is safe for human consumption. The addition of baobab pulp supports the growth of probiotics as higher initial count and survival rates were higher in the simulation gastral intestinal tract .*A. digitata* pulps can provide large amounts of nutrients, such as minerals and dietary fiber. The pulp also may help to supply fat and protein. The data obtained from this study will be vital in efforts to promote the greater use of wild fruits in supporting Non-Timber Forest Products, for addition of value to the products like yoghurt, and the education of local communities with regard to the nutritional benefits of baobab pulp as free sources of food in their environment. The findings can also contribute to the domestication of wild fruit trees, and the enhancement of cultural heritage and forests.

5.2. Recommendation

This study analyzed proximate, physicochemical, microbiological, and nutritional content of the baobab pulp grown at Makuyuni area in Tanzania but further studies are needed on the baobab varieties of species in the area and east Africa in general. The formulated probiotic yoghurts used combined starter culture (*Acidophilus spp*, *Bifidobacteria* and *Thermophilus* (ABT), hence there is a need for further study on formulation yoghurt enriched with baobab pulp using single strain starter culture. Further research is also needed on the consumer acceptability for the products formulated, and involvement of animals or human feed trials to substantiate the health

benefits of the probiotic yoghurt developed in the research. In addition to that marketing of the baobab enriched yoghurt as a products is needed.

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APPENDICES

Appendix 1: Questionnaire for sensory evaluation of yoghurt samples using 9 point hedonic scale.

Instruction: You are given different yoghurt samples coded, please evaluate them on the, nine points hedonic scale as below:

Remove the lid of the cups and evaluate aroma first and then the colour and appearance by visual observation. For textural properties break down the yoghurt gel with spoon and gently mix the samples to evaluate the yoghurt thickness. After placing product in your mouth evaluate the gel firmness, flavour and overall acceptability.

Then tick on the table below:

Attribute to be tested	Like extremely	Like very much	like moderately	Like slightly	neither like nor dislike	Dislike slightly	Dislike moderately	Dislike very much	Dislike extremely
Aroma									
Colour									
Appearance									
Gel thickness									
Gel firmness									
Overall acceptability									

Additional comments on aroma flavour or texture of samples:

Appendix 3: Recommended Dietary Allowance (RDA)

Weight		Height		Recommended Dietary Allowance (RDA)																				
kg	lb	cm	inch	ENERGY	PROTEIN	VITAMIN A	VITAMIN D	VITAMIN E	VITAMIN K	VITAMIN C	THIAMIN	RIBOFLAVIN	NIACIN	VITAMIN B6	FOLATE	VITAMIN B12	CALCIUM	PHOSPHOROUS	MAGNESIUM	IRON	ZINC	IODINE	SELENIUM	
				(kcal)	(g)	(µg)	(µg)	(mg)	(µg)	(mg)	(mg)	(mg)	(mg)	(µg)	(µg)	(mg)	(mg)	(mg)	(mg)	(mg)	(mg)	(µg)	(µg)	(µg)
Males																								
11-14	45	99	157	62	2500	45	1000	10	10	45	50	1.3	1.5	17	1.7	150	2.0	1200	1200	270	12	15	150	40
15-18	66	145	176	69	3000	59	1000	10	10	65	60	1.5	1.8	20	2.0	200	2.0	1200	1200	400	12	15	150	50
19-24	72	160	177	70	2900	58	1000	10	10	70	60	1.5	1.7	19	2.0	200	2.0	1200	1200	350	10	15	150	70
25-50	79	174	176	70	2900	63	1000	5	10	80	60	1.5	1.7	19	2.0	200	2.0	800	800	350	10	15	150	70
51+	77	170	173	68	2300	63	1000	5	10	80	60	1.2	1.4	15	2.0	200	2.0	800	800	350	10	15	150	70
Females																								
11-14	46	101	157	62	2200	46	800	10	8	45	50	1.1	1.3	15	1.4	150	2.0	1200	1200	280	15	12	150	45
15-18	55	120	163	64	2200	44	800	10	8	55	60	1.1	1.3	15	1.5	180	2.0	1200	1200	300	15	12	150	50
19-24	58	128	164	65	2200	46	800	10	8	60	60	1.1	1.3	15	1.6	180	2.0	1200	1200	280	15	12	150	55
25-50	63	138	163	64	2200	50	800	5	8	65	60	1.1	1.3	15	1.6	180	2.0	800	800	280	15	12	150	55
51+	65	143	160	63	1900	50	800	5	8	65	60	1.0	1.2	13	1.6	180	2.0	800	800	280	10	12	150	55
Pregnant				+300	60	800	10	10	65	70	1.5	1.6	17	2.2	400	2.2	1200	1200	320	30	15	175	65	
Lactating																								
1st 6 mo.				+500	65	1300	10	12	65	95	1.6	1.8	20	2.1	280	2.6	1200	1200	355	15	19	200	75	
2nd 6 mo.				+500	62	1200	10	11	65	90	1.6	1.7	20	2.1	260	2.6	1200	1200	340	15	16	200	75	

Source: International drug mart.com

Appendix 4: Cow's Milk (whole), Nutritional value per 100 g (3.5 oz)

Energy	252 kJ (60 kcal)
Carbohydrates	5.26 g
Sugars	5.26 g
Lactose	5.26 g
Fat	3.25 g
• saturated	1.865 g
• monounsaturated	0.812 g
• polyunsaturated	0.195 g
Protein	3.22 g
Tryptophan	0.075 g
Threonine	0.143 g
Isoleucine	0.165 g
Leucine	0.265 g
Lysine	0.140 g
Methionine	0.075 g
Cystine	0.017 g
Phenylalanine	0.147 g
Tyrosine	0.152 g
Valine	0.192 g
Arginine	0.075 g
Histidine	0.075 g
Alanine	0.103 g
Aspartic acid	0.237 g
Glutamic acid	0.648 g
Glycine	0.075 g
Proline	0.342 g
Serine	0.107 g
Water	88.32 g
Vitamin A	equiv. 28 µg (3%)
Thiamine (Vit. B1)	0.044 mg (3%)
Riboflavin (Vit. B2)	0.183 mg (12%)
Vitamin B12	0.44 µg (18%)
Vitamin D	40 IU (10%)
Calcium	113 mg (11%)
Magnesium	10 mg (3%)
Potassium	143 mg (3%)

Source: USDA Nutrient database

Appendix 5: Standards

Vitamin C Standard for Baobab

pulp

