

**DEVELOPMENT OF A FERMENTED SOY BEAN FORTIFIED MAIZE
MEAL SNACK (*MKARANGO*) TO ALLEVIATE PROTEIN ENERGY
MALNUTRITION IN SCHOOL GOING CHILDREN**

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DECLARATION

DECLARATION BY THE CANDIDATE

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DEDICATION

I dedicate this work to my husband Charles Prisca, my father George Rapando, my mother Inez Rapando and my siblings.

ABSTRACT

Protein Energy Malnutrition (PEM) is a serious public health problem among school going children in Africa and other developing countries. Fortification of food fed to children with legumes is one of the most sustainable methods of alleviating PEM. The aim of this study was to improve the protein quality and increase the nutrient density of a Kenyan traditional fermented maize meal snack by fortifying with soy bean for use among school-age children to alleviate PEM. Nine variations of the snack were produced by compositing maize meal with soy at ratios of 100;0 70;30 and 50;50. Each variation was fermented for 0, 3 and 5 days at ambient temperature. To establish the nutrient composition, proximate analyses including, moisture, protein, ash, fat, carbohydrate and energy were conducted. Functional properties determined were bulk density, water and oil absorption capacities, titratable acidity and pH. Sensory characteristics were evaluated using a 12-member descriptive panel. Acceptability was evaluated using adults and eight to nine-year-old school children. Satiety was further analyzed from the acceptability study and proximate results. Compared to the 100% maize fermented snack, fortification of maize meal with soy bean at 1:1 ratio increased the protein, fat and ash contents by 256, 78 and 285%, respectively while carbohydrate reduced by 30%. Fortification and fermentation increased the titratable acidity, water and oil holding capacities and reduced the bulk density. Principal component analysis revealed that 45%, 29% and a further 12% of the variation in sensory properties among the samples was due to fermentation, fortification with soy and level of soy fortification, respectively. The fermented and soy fortified samples were associated with sour, fermented maize and soy bean flavour, aroma and aftertaste while the unfermented samples were described as sweet, vanilla and roasted flavour, aroma and aftertaste. Adults and children scored the 100% maize: soy, 3 day fermentation snack highest and its liking by children increased in 4 days. The 50:50 maize: soy snack had the highest drop in liking from the sensory specific satiety test. The glycemic load changed from high to medium in the 50:50 maize: soy snack. Fortification with Soy bean and fermentation of the snacks improves nutrient density, functional properties, imparts some desirable sensory characteristics, and satiating ability. Soy fortified fermented maize meal snack has considerable potential for use as supplementary food for increased protein and energy content in the prevention of PEM among school going children in developing countries.

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LIST OF ACRONYMS

AMDR	Adequate Macronutrient Dietary Intake
AOAC	Association of Official Analytical Chemists
CIMMYT	International Maize and Wheat Improvement Center
CRD	Completely Randomized Design
CSB	Corn Soy Blend
FAO	Food and Agriculture Organization of the United Nations
GI	Glycemic Index
GL	Glycemic Load
HGSFP	Home Grown School Feeding Program
HMF	Hydroxymethylfurfural Fusion
LNS	Lipid-based Nutrient Supplement
PDCAAS	Protein Digestibility Corrected Amino Acid Score
PEM	Protein Energy Malnutrition
RUTF	Ready to Use Therapeutic Food
RCBD	Randomized Complete Block Design
SDGs	Sustainable Development Goals
SFP	Supplementary Feeding Program
SPI	Soy Protein Isolate
SSS	Sensory Specific Satiety
UNESCO	United Nations Educational, Scientific and Cultural Organization
UNICEF	United Nations International Children's Fund
USAID	United States Agency for International Development
USDA	United States Department for Agriculture
VLIR – UOS	Vlaamse Interuniversitaire Raad-University Development Cooperation

WFP World Food Programme
WHO World Health Organization

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CHAPTER ONE

INTRODUCTION

1.1 Background Information

Protein-energy malnutrition (PEM) continues to be a major health burden causing illness and death of young children in the developing world (Müller and Krawinkel, 2005). The most recent estimates show that one third and a quarter, of the 156 million stunted and 50 million wasted children under five years, respectively reside in Africa (UNICEF/WHO/World Bank Group, 2018). School going children are a special group that is nutritionally at risk because the consequences of PEM are impaired physical and cognitive development and increased susceptibility to metabolic and infectious disease (Stipanuk and Caudill, 2013) which affect educational achievement and school attendance (World Food Programme, 2013).

The problem of hunger and poor health among school-age children has been recognized and one means of solving it is through programs (Del Rosso, 1999) in which an estimated 368 million children, 66 million of whom are undernourished are fed in school (World Food Programme, 2018). School feeding programs (SFPs) worldwide are designed to alleviate hunger, improve school enrollment and attendance and food security status of poor rural households (Langinger, 2011). Such programs may provide meals at breakfast or lunch, or high energy biscuits or snacks (World Food Programme, 2018). However, school feeding programs in developing countries face challenges. These may include lack of basic infrastructure such as kitchens, water, cooking equipment, manpower and fuel to produce nutritious food on time (UNESCO, 2004).

Poor roads in the wet season preventing the delivery of perishable foods and unsafe water may also be a hindrance (Aliyar, Gelli, and Hamdani, 2015). This calls for ready to eat products like snacks which are shelf stable, and do not need long preparation time. African governments including Kenya endorsed, the Home Grown School Feeding model (HGSF) as an entry point for interventions designed to reduce malnutrition, food insecurity and poverty in targeted communities (World Food Programme, 2018). The model encourages the use of staples that are commonly produced in rural households by small holder farmers. In Kenya, maize is the major staple food and is commonly purchased for school feeding programs through HGSFP (World Food Programme, 2017a). However, maize is deficient in lysine, an indispensable amino acid, required for growth and development in young children (Shiriki, Igyor, and Gernah, 2015). Overdependence on this cereal therefore leads to PEM (Tumwine, Atukwase, Tumuhimbise, Tucungwirwe, and Linnemann, 2018). Fortification of cereal staples with legumes is a strategy recommended by FAO (2000) for delivering proteins to vulnerable populations and therefore one of the suitable ways of combating PEM in growing children. Cereal-legume blends are relatively high in protein quality and quantity because legumes supply lysine which is deficient in cereals, while the cereals provide cysteine and methionine which are low in legumes (Serrem, De Kock, and Taylor, 2011).

Soy bean (*Glycine max* (L) Merrill) among legumes is recognized for its high nutritional value as it contains about 40 % protein, 23 % carbohydrate, 20 % oil and 4 % minerals (Shiriki *et al.*, 2015). It has a protein content twice that of pulses, groundnuts, meat and fish. Soy protein contains eight essential amino acids, and is superior to other plant proteins (Singh, Kumar, Sabapathy, and Bawa, 2008).

Soy bean is an excellent source of mineral elements such as potassium, and vitamins such as riboflavin, choline, thiamine and pantothenic acid and high in energy due to its oil content (Swick, 2007).

Mkarango is a traditional Kenyan fermented cereal snack that is very popular among children of school-going age in rural farming communities. The snack is commonly made using 100% maize, but millet or sorghum flours can also be used. *Mkarango* has very low moisture content, is shelf stable and only requires the addition of a liquid before consumption. However, it has poor protein quality and low content because it is made of pure cereal. There is no documented evidence of this ready-to-eat fermented snack, fortified with soy bean to improve its nutrient composition. Therefore, the aim of the study was to improve the protein quality and increase the nutrient density of the fermented maize meal snack through soy fortification for use among school-age children, to alleviate PEM.

1.2 Statement of the problem

School going children are vulnerable to malnutrition because they are commonly fed on starchy staples that are inadequate in protein quality and quantity with foods such as porridge or fermented maize products. Furthermore, the government commonly uses staples such as maize and sorghum in school feeding programs. Maize is a poor source of high quality protein. For example, it lacks lysine which is an indispensable amino acid for growth and development in children. Overdependence on this which is poor in protein quantity leads to PEM. Fortification with Soy bean flour which is rich in protein will improve the protein quality and quantity and nutrient density, hence alleviate PEM. Therefore, this study addressed the problem of PEM in this age group through development of a Soy bean fortified fermented maize meal snack.

1.3 Objectives

1.3.1 Broad objective

To formulate, develop and determine the nutrient composition, sensory quality and satiating ability of a Soy bean fortified fermented maize meal snack.

1.3.2 Specific objectives

1. To formulate and develop a Soy bean fortified fermented maize meal snack
2. To determine the effect of fortification with Soy bean on the proximate composition of the fermented maize meal snack.
3. To determine the effect of fortification with Soy bean on the functional properties of the fermented maize meal snack.
4. To evaluate the sensory quality of the fermented soy bean fortified maize meal snack.
5. To evaluate the satiating ability of the fermented soy bean fortified maize meal snack

1.4 Hypotheses

H_A: Fortification with Soy bean significantly improves the physico-chemical properties of the fermented maize meal snack

H_A: Fortification with Soy bean significantly improves the sensory properties of the fermented maize meal snack

H_A: Fortification with Soy bean significantly influences the satiating power of fermented maize meal snack

1.5 Justification of the study

Maize is the most important cereal staple in Kenya and the most commonly used in the Home grown school feeding programs. However, its protein content is low and of

poor quality because it is limiting in the essential amino acid, lysine so fortification with Soy bean will improve the protein quality. This predisposes children, including those who are of school going age to Protein Energy Malnutrition. *Mkarango* is a maize based fermented snack, which is popular among school children in rural Kenya. It also has a long shelf life because of the low moisture content and it is ready to eat after the addition of warm or cold liquid. In this study the product was improved using the principle of complementation by compositing maize meal with soy flour before fermentation. This product will benefit school going children, the Ministry of Education, Ministry of Health, Non-Governmental Organizations dealing with children's health. Because *mkarango* is popular in school going children and it is normally made from maize, and maize is limiting in lysine, therefore fortification with soy will improve the nutrient density and thus alleviate Protein Energy Malnutrition.

CHAPTER TWO

LITERATURE REVIEW

2.1 Malnutrition

Malnutrition refers to deficiencies or imbalances in a person's intake of energy and/or nutrients (UNICEF/WHO/World Bank Group, 2018). The term covers two broad groups of conditions, under-nutrition and over-nutrition. Under-nutrition includes stunting (low height-for-age), wasting (low weight-for-height), underweight (low weight-for-age) and micronutrient deficiencies (insufficient supply of critical minerals and vitamins). Over-nutrition encompasses obesity and diet-related non-communicable diseases (UNICEF/WHO/World Bank Group, 2018).

Under-nutrition among children in developing countries is commonly caused by hunger (World Food Programme, 2013). Hunger can be temporary, such as not having enough to eat for a meal or a day, or can be long lasting when the person does not get enough to eat to maintain physical needs over many days, weeks, months or years (World Hunger Education Service, 2019). Hunger for a sustained period of time causes mild or severe under-nutrition depending on physiological needs and food intake (World Food Programme, 2013).

According to FAO/IFAD/UNICEF/WFP and WHO (2017), it is estimated that in 2016 the number of chronically under-nourished people worldwide increased to 815 million, up from 777 million in 2015. Furthermore, it is estimated that 155 million children were stunted and 52 million wasted in 2016, most of whom live in developing countries (FAO *et al.*, 2017). Malnutrition due to under nutrition is a major cause of PEM particularly in developing countries, and Sub-Saharan Africa is the most affected (FAO, 2015). Protein Energy Malnutrition is a major consequence of under nutrition and is classified into three forms:

2.1.1 Kwashiorkor

Kwashiorkor, also called wet PEM, is characterized primarily by protein deficiency. This condition usually appears at the age of about 12 months when breastfeeding is discontinued, but can develop at any time during a child's formative years (Manary, Broadhead, and Yarasheski, 1998). Kwashiorkor usually manifests with fluid retention (oedema) starting in the legs and feet and spreading, in more advanced cases, to the hands and face making children look “fat” so that their parents mistake them for well fed (Manary *et al.*, 1998).

2.1.2 Marasmus

Early marasmus occurs usually in the first year of life in children who have been weaned from breast milk or who suffer from weakening conditions like chronic diarrhoea. It is frequently associated with contaminated bottle-feeding in urban areas (Pinstrip-Andersen, Burger, Habicht, and Peterson, 1993). Primarily marasmus is caused by energy deficiency from prolonged starvation. It may also result from chronic or recurring infections with marginal food intake (De Onis, Wijnhoven, and Onyango, 2004). Marasmus is characterized by stunted growth and wasting of muscle and tissue. Wasting indicates recent weight loss, whereas stunting results from chronic weight loss.

2.1.3 Marasmic kwashiorkor

This is a severe wasting in the presence of oedema. It is a mixed form of PEM, and manifests as oedema occurring in children who may or may not have other signs of Kwashiorkor (Manary *et al.*, 1998).

2.2 School going children and their nutrition requirements

School children from 5 years of age grow very rapidly and can be very active and therefore providing adequate energy and nutrients are essential (World Food Programme, 2013). Furthermore, appetite and stomach capacity for food among 5 year olds is sometimes small, so it is particularly important for such children to have nutrient-dense diets that include healthy snacks to ensure nutrient requirements are met (World Food Programme, 2013). The nutrient requirements of the children are shown in Table 2.1.

Adequate energy intake is essential for growing children as they expend more of this during the growth process and that their bodies are developing. Further, for school going children from developing countries, most energy is expended during play and also walking for long distances to school. Therefore provision of adequate energy in meals provided at home and also for school feeding programs is necessary for children at this critical stage of growth (Aliyar *et al.*, 2015).

2.2.1 Protein in school children's diet

Proteins are essential for human body cells as they form the basic parts within the cells are responsible for providing the amino acids and nitrogen required for non-essential amino acids and nitrogen balance in the body (World Food Program, 2013).

The amino acids are important in linear growth, repair and maintenance of body tissues, formation of antibodies to defend the body against infections, control of body electrolytes and fluid balance, regulation of acid balance, transportation of nutrients and provision of energy (Rolfes, Pinna, and Whitney, 2014). Proteins from animals are complete as they contain all the essential amino acids, while majority of the plant proteins are incomplete. It is recommended that the protein intake of the total energy intake should be between 5 to 20% of the total energy intake (Rolfes *et al.*, 2014).

Inadequate protein intake among school going children predisposes them to PEM and can further lead to poor growth, poor cognitive functioning affecting school performance and can lead to absenteeism from school (World Food Programme, 2018).

Table 2.1 Nutrient requirements for young school going children

Nutrients	Units	1-3 years	4-6 years	7-9 years	10-13 years
Energy ¹	Kcal	1117	1561	1814	2316
Protein	G	13	19	23	24
Vitamins²					
Thiamin	Mg	0.5	0.6	0.9	1.0
Riboflavin	Mg	0.5	0.6	0.9	1.0
Niacin	Niacin equivalent	6	8	12	16
Vitamin C	mg/day	30	30	35	40
Folate	µ/day	120	160	250	300
Biotin	µ/day	8	12	20	25
Panθοthenate	mg/day	2.0	3.0	4.0	5.0
Vitamin B12	µ/day	0.7	1.0	1.5	2.0
Vitamin D	µ/day	5	5	5	5
Vitamin A	µRE/day	190	200	250	330-400
Vitamin K	Mg/day	15	20	25	35-55
Minerals²					
Calcium	mg/day	500	600	700	1300
Iodine	µ/day	90	90	90	120
Iron	mg/day	11.6	12.6	17.8	37.6
Magnesium	mg/day	60	76	100	220
Zinc	mg/day	3	5	5	5

¹(FAO, 2004) ; ²(FAO and WHO, 2004)

2.2.2 Protein and satiety

Protein has been shown to be the most satisfying macronutrient (Halton & Hu, 2004). High protein food results in higher sensory specific satiety and decreases the feeling of hunger more than similar low protein food (Vandewater & Vickers, 1996). This is attributed to the low Glycemic Index (G.I) of protein rich foods. Several short term studies have been conducted to explain the connection between GI and feelings of satiety and hunger.

In about half of the 31 studies analyzed by Raben (2002), low GI products decreased the feeling of hunger or increased feeling of satiety more than high GI products. Ingestion of high glycemic index food increases hunger and lowers satiety, and this has been attributed to the rapid decline in blood glucose level following a hyperinsulinemic response caused by a sharp increase in glucose level to the high G.I food (Niwano et al., 2009). Increasing the protein content in school children's diet has potential to alleviate short term hunger.

2.3 School feeding programs

In both developing and developed countries, an estimated 368 million children receive a meal at school every day (World Food Programme, 2018). For millions of school going children today, hunger is one of the most pervasive and damaging dilemmas, and it has far reaching effects on the development of individuals and nations (World Food Programme, 2013). Hunger negatively affects the development of children, impeding their chances of educational success where both acute and chronic hunger affects children's access to school, attention span, behavior in class and educational outcomes (Lawson, 2012). For example, children suffering from short term hunger, due to skipping breakfast have difficulty concentrating in class and performing complex tasks (World Food Programme, 2013). Thus, the need to reduce hunger while increasing school enrollment in these children is evident, and school feeding programs have been developed to target this multifaceted problem.

There are two main ways to distribute food through school feeding programs in developing countries: on-site meals and take-home rations (Aliyar *et al.*, 2015). On-site meals are foods that are distributed to children while at school during morning and afternoon meal and snack times, which may include a bowl of porridge or nutrient-fortified crackers.

Take-home rations are a collection of basic food items, such as a bag of rice and a bottle of cooking oil, which may be sent home and transferred to the families of children that regularly attend school (World Food Programme, 2013).

Most of the meals provided in school feeding programs do not adequately meet the nutritional needs of children (Kearney, 2010). In Kenya, most of the programs feed children on porridge made of staples such as maize, sorghum and millet, and in some instances, *githeri*, a boiled mixture of maize and beans (Kenya Kids Can Organization, 2019). This shows that the protein quality and quantity of these foods is low. According to Kearney (2010) foods used for school feeding programs should have a high nutritional value, be rich in protein, energy, micronutrients and moderate in fats and sugar. Maize, a grain commonly used in school feeding programs is lacking in lysine which is an essential amino acid for growth in young children.

According to a review by World Food Programme (2017b), school feeding programs directly contribute to Sustainable Development goal (SDG) 2 (Achieve zero hunger), 4 (Ensure quality education) and 5 (Achieve gender equality). School feeding programs help end hunger and all forms of malnutrition and ensure access to safe, nutritious and sufficient food particularly among vulnerable populations such as infants and young children. With regards to SDG 4, when a school meals programme is part of a package of investments in education, it can help maximize the return of these investments, because school meals facilitate access to school, increase enrolment and attendance rates and improve the nutritional status, health and cognitive development of children (WFP, 2017). School feeding programs contribute to SDG 5 through narrowing the gender gaps between boys and girls and because girls are more exposed to hunger and malnutrition than boys.

2.4 Homegrown school feeding program

In Kenya, in an effort to transition from WFP assistance and create a more sustainable and locally integrated program, the Ministry of Education began implementing a Homegrown School Feeding Program (HGSFP) in 2009 (Espejo, 2009). The HGSFP Program targets about 600,000 Kenyan children (World Food Programme, 2017a). The aim of the programme is to encourage and facilitate increased consumption of locally produced food items, including the promotion of innovative school feeding programs that use food items sourced from the local farming community (USDA, 2009). Increasingly, HGSF is being adopted to not only improve education outcomes but also farmers' livelihoods through increased production, market access and improved nutrition (World Food Programme, 2017a).

The HGSFP currently encourages the purchase of key “orphan crops” like sorghum, millet, and cowpeas due to their drought-resistance and Arid and Semi-Arid Land (ASAL) suitability (World Food Programme, 2017a). However, maize constitutes a majority of the food purchased for school meal programs (USDA, 2009). In these ways, the Kenyan government hopes to integrate schools more fully into rural communities, provide an economic stimulus for impoverished villages, boost local agricultural productivity, and establish a sustainable school meals program independent from heavy foreign subsidization.

2.5 Maize

Maize (*Zeamays*) is a large grain plant, first domesticated by the indigenous people of Southern Mexico 10,000 years ago (FAO, 2007). Though maize is the third most important staple food globally, after wheat and rice it is the leading cereal grain worldwide as measured by production (Gwirtz and Garcia-Casal, 2014).

Maize is not only used as food for humans but also animal feed, and industrially to produce ethanol, corn starch and corn syrup. In Kenya, according to (World Food Programme, 2017a), maize remains the main staple food crop and its consumption is expected to increase despite diversification of the Kenyan diet. It is commonly consumed as stiff porridge (*ugali*), drinking porridge (*uji*) or cooked mixed with beans (*githeri*). Kenya produced an estimated 4,023,000 Million tons of maize by mid 2017 (FAO, 2007).

2.5.1 Anatomy of the maize kernel

The maize kernel is composed of four primary structures from a processing perspective. These are endosperm, germ, pericarp, and tip cap, making up 83%, 11%, 5%, and 1% of the maize kernel, respectively (Fig. 2.1). The endosperm is primarily starch surrounded by a protein matrix (Eckhoff, 2010). The germ of the maize kernel is high in fat, and also contains B complex vitamins and vitamin E (Gwirtz and Garcia-Casal, 2014). Maize germ oil is rich in polyunsaturated fatty acids, while the pericarp is high in fiber (Eckhoff, 2010).

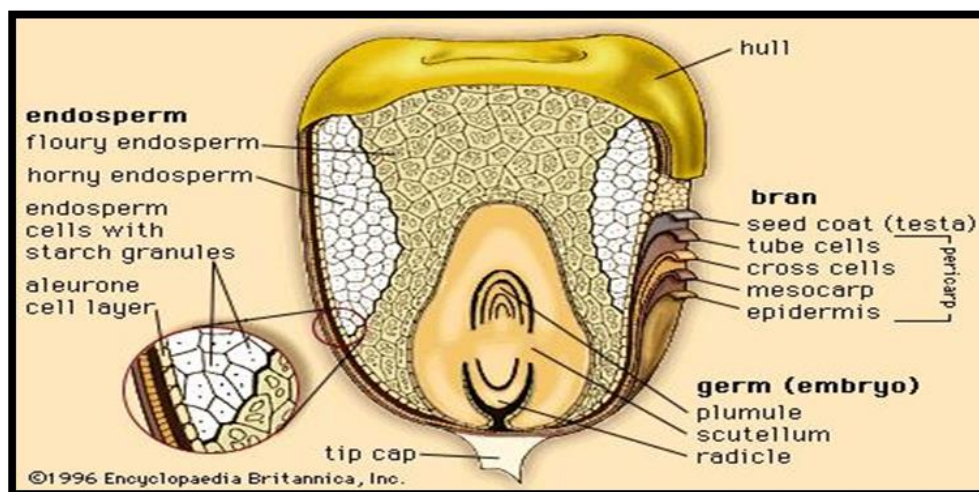


Figure 2.1: Cross sectional section of the maize grain (Encyclopaedia Britannica, 1996)

2.5.2 Maize kernel nutrient composition

The major chemical component of the maize kernel is starch, which provides up to 72 to 73 percent of the kernel weight. Other carbohydrates are simple sugars present as glucose, sucrose and fructose in amounts that vary from 1 to 3 percent of the kernel (FAO, 2007). The starch in maize is made up of two glucose polymers: amylose, an essentially linear molecule, and amylopectin, a branched form. In common maize, with either the dent or flint type of endosperm, amylose makes up 25 to 30 percent of the starch and amylopectin 70 to 75 percent (Shannon, Garwood, and Boyer, 2009).

The protein content in maize varies among common varieties from about 8 to 12 percent of the kernel weight. It is well documented that maize protein compared to wheat and rice, has low biological value and net protein utilization of 59% and 49%, respectively (Dendy and Dobraszczyk, 2001). The poor quality can be attributed to the prolamins of maize grain, called zeins. They consist of one major class, α -zeins and three minor classes β , γ and δ , constituting about 50-70% of maize endosperm which are poor in lysine and tryptophan content (Sofi, Wani, Rather, and Wani, 2009). Additionally, niacin in maize is not bio-available because it exists as niacytin, a bound form (Dendy and Dobraszczyk, 2001). Consequently, dependence on high maize diet as a source of all nutrients by large populations and its common use in school feeding programs has resulted in protein (PEM) and niacin (Pellagra) deficiencies, where no measures are taken to improve its nutritional quality.

2.6 Approaches to improving nutritive value of maize

Maize is a very important staple food for large populations in developing countries and its low nutritional value with respect to protein content has driven efforts in improving the biological utilization of its nutrients (Eckhoff, 2010).

2.6.1 Dietary diversification

This is an approach whose aim is to enhance the availability, access and consumption of foods that are nutrient dense and have high bioavailability of nutrients (FAO, 1997). In terms of protein intake, this approach includes the intake of animal source foods that are rich in lysine such as meat, dairy, fish and poultry (Nair, Augustine, and Konapur, 2016) with maize. Nutrition education helps to increase knowledge on consumption of nutrient dense foods, preparation of food to preserve most nutrients, combination of foods to increase nutrient availability, quality and utilization in the body, and the importance of balanced diets (Vardanjani, Reisi, Javadzade, Pour, and Tavassoli, 2015).

2.6.2 Bio-fortification

Bio-fortification is the use of conventional breeding techniques to increase the nutrient concentration in the desired crops (HarvestPlus, 2017). The advantage of this method is that bio-fortified crops offer a good rural intervention, reaching the most remote populations, which comprise a majority of the under-nourished in many countries, and then penetrates to urban populations as production surpluses are marketed (Bouis and Saltzman, 2017).

The bio-fortification of maize was first conducted by the International Maize and Wheat Improvement Center (CIMMYT) in the 1960s, who developed Quality Protein Maize (QPM) an improved cultivar containing almost twice the amount of the limiting amino acids lysine and tryptophan compared to conventional maize (Sofi *et al.*, 2009). Currently, QPM has been adopted as a maize variety that can be used to address the problem of PEM among children in some maize consuming developing countries.

2.6.3 Fortification

Food fortification is the addition of nutrients to food that were not previously present in the food, or they were present in minute amounts (Lockyer, White, and Buttriss, 2018). A complementary approach to improve maize quality is the direct fortification with micronutrients, including synthetic amino acids or protein sources rich in amino acids (Young and Pellett, 1990). Related maize fortification studies include a study by Waliszewski, Estrada, and Pardo (2000) who established that nixtamalized maize flour fortified with FAO recommended concentrations of lysine and tryptophan could supply 83% of requirement. Another study by Scrimshaw, Bressani, Béhar, and Viteri (1958) found that addition of lysine and tryptophan at 3 g protein per kilogram of body weight per day and 100 Kcal per kilogram of body weight per day to corn *masa* in the diet of children increased nitrogen retention.

Fortification through supplementation with food sources of high biological value or food-to food fortification has also been used to improve the protein quality of maize. Studies have developed foods from corn fortified with animal source foods. Shaviklo, Thorkelsson, Rafipour, and Sigurgisladottir (2011) developed three types of corn-fish snacks and while Kinyuru *et al.*, (2015) developed maize based complementary foods fortified with fish and termites. Fernandes, Madeira, Carvalho, and Pereira (2016) made pellets using corn grits and whey protein.

For developing countries, animal source foods are too expensive, due to poverty; therefore fortification with legumes such as Soy bean could improve the amino acid content of the maize products. Complementation of maize with legumes improves the amino acid profile of the cereal-legume blends because legumes supply the lysine which maize lacks, while maize provides cysteine and methionine which are low in legumes (Serrem, De Kock, and Taylor, 2011).

2.7 Soy bean

Soy bean (*Glycine max*) belonging to the family *leguminosae* is one of the oldest cultivated crops in the tropics and sub-tropical regions, and one of the world's most important sources of protein and oil Ding, Zhao, and Gai (2008). Soy bean as a domesticated crop has its origin in Eastern Asia, mainly China (Dugje *et al.*, 2009).

2.7.1 Nutrient composition of Soy bean

The protein content of Soy bean is almost twice that of milk, beef and meat (Swick, 2007). Soy bean is an important vegetable for vegetarians and vegans due to its high nutritional benefits (Hassan, 2013). Protein isolates from Soy beans have been shown to be important in human nutrition, where their digestibility is approximated to be 92-100%, which is a measure of the protein quality (Michaelsen *et al.*, 2009). Soy bean protein has also been used as a standard against which other proteins are measured. The average protein content in Soy bean is around 40% even though there could be variations depending on the varieties (Agengo, Serrem, and Wakhu-Wamunga, 2015). It is also a rich source of amino acids, particularly lysine (Kamau, Serrem, and Wakhu-Wamunga, 2015; Serrem, de Kock, and Taylor, 2011) which is an indispensable amino acid required for child growth (Michaelsen *et al.*, 2009).

The carbohydrate content of Soy bean is approximately 30%, out of which 10-13% is soluble carbohydrates (Sato, Van Schoote, Wagentristl, and Vollmann, 2014). The principle soluble carbohydrates of mature Soy beans are disaccharide sucrose, stachyose and raffinose. Soy beans are a good source of several dietary fibres, micronutrients, phytochemicals and isoflavones (Messina, 1999). The maximum moisture content in Soy bean should be 12% as higher moisture content tends to dilute

the nutritional value of Soy bean meal (Britzman, 1994). Oil from Soy bean contains about 22% of monounsaturated oleic acid (Ding *et al.*, 2008).

The oil is healthy as it is free from cholesterol and saturated fats. The high energy content of Soy bean fortified foods is attributed to the oil content (Kamau *et al.*, 2015).

Soy beans also contain anti-nutrients such as trypsin inhibitors which inhibit protein absorption from the beans (Sato *et al.*, 2014). Furthermore, the presence of low molecular weight oligosaccharides, primarily raffinose, stachyose and verbascose are linked to flatulence characterized by stomach cramps, nausea, diarrhea, intestinal and gastric disorders caused by production of gas in the intestinal tract. Ways of reducing effects of the anti-nutrients include fermentation, removal of seed coat prior to cooking, heat treatment such as boiling and roasting, germination and soaking (Bursens *et al.*, 2011).

2.7.2 Soy bean fortified food products

Soy bean has been utilized as a fortificant for commonly consumed cereals used in relief programs because of its high protein content as well as high energy attributed to its high oil content. Currently, the World Food program and its partners such as United States Agency for International Development (USAID) are using a wide range of specialized foods made from Soy beans for food aid and relief programs around the world (World Food Programme, 2017a). These foods range from Fortified Blended Foods (FBF's), Ready-to-use Therapeutic Foods (RUTF) and High Energy Biscuits (HEB). These products have been used to meet the nutritional needs of young children and vulnerable groups of people when food is unavailable during drought and emergency situations. Corn Soy Blend (CSB) has also been used in school feeding programs and has been shown to improve the nutritional status of school going

children (Were *et al.*, 2010). A study by Ronoh, Were, Waku-Wamunga, and Wamunga (2017) showed that feeding school going children with Soy bean fortified porridges significantly improved their nutritional status.

Studies have shown that fortification of maize based foods with Soy bean improves the amino acid profile. A study by Kamau *et al.*, (2015), showed that fortification of banana, millet, cassava and maize complementary flours with added Soy bean resulted in increased growth rate in rats indicating improved quality of the indispensable amino acids. Similar results were reported by Serrem, De Kock, Oelofse, and Taylor (2011) who fed soy fortified biscuits developed for school feeding to rats. Kure and Wiyasu (2013) also found that fortifying sorghum with Soy beans increased the levels of lysine, methionine and tryptophan.

Fortification of cereals with Soy bean flour improves the Protein Digestibility Corrected Amino Acids Score (PDCAAS), a measure of protein quality, and an estimate of the true value of dietary protein for the human body. A study by Serrem, De Kock, and Taylor, (2011) found that complementation of Soy bean and sorghum in the ratio 50:50 could yield a PDCAAS that meets the threshold for children aged 1-10 years. Improvement in the protein digestibility of cereals fortified with Soy bean have also been reported by Kamau *et al.*, (2015) where fortification of maize flour with 30% Soy bean improved the PDCAAS value of the diet from 53% to 70%, meeting the requirement for infant and young children food (Michaelsen *et al.*, 2009). The amino acid requirements for 2 and 3-10-year-old children are shown in table 2.2.

Table 2.2: Comparison of essential amino acids (mg/g protein) content of maize and Soy bean to FAO/WHO suggested amino acids (mg/g crude protein) requirements for 2-10 year old children

Amino acid	Maize ^a	Soy bean ^b	Amino acid requirements ^c	
			2 years	3-10 years
Lysine	29	63	58	48
Isoleucine	40	47	28	31
Leucine	125	85	66	61
Threonine	38	38	34	25
Tryptophan	7	11	11	6.6
Valine	50	49	35	40
Histidine	26	25	19	19
Phenylalanine+ Tyrosine	86	96	63	22
Methionine+Cysteine	40	68	25	22

^aValues from Koziol (1992)

^bValues from Shewry (2006)

^cAmino acid requirements for selected age groups male and female combined (WHO, 2007)

Soy bean proteins have a low glycemic index and have been recommended as appropriate part of diet intended to improve blood glucose and insulin levels (Blair, Henley, and Tabor, 2006). For instance, Sugiyama, Tang, Wakaki, and Koyama (2003) demonstrated that adding Soy bean products (miso, natto and ground Soy bean flour) lowered the G.I of white rice by 20-40%. Fujiwara (2014) while working with bread fortified with Soy Protein Isolates (SPI) showed that the bread had a lower G.I due to increased protein content. This therefore is a pointer that fortification of maize, which is of high G.I, with soy increases the satiation power of the snack by lowering the G.I and load. Therefore, soy protein has the potential to keep the children full for a long time, which is one of the main goals of school feeding programs in developing countries.

2.8 Processing of maize-based food products

The most common processing method for maize is milling, which grinds the maize into coarse whole-grain pieces or fine flour and removes much of the bran and germ. Milled maize can be processed by heat into foods such as porridge, grits, baked goods, and other locally named dishes. Traditional preparation methods often include further processing, such as soaking, fermentation, and nixtamalization.

2.8.1 Fermentation

Fermentation is a common processing method in many developing countries. Fermentation is a process in which microorganisms cause chemical changes in organic substrates through the action of enzymes produced by these microorganisms (Li *et al.*, 2004). Developing countries rely on fermentation to process and preserve its food at a cost within the means of the average consumer making it one of the most important food processing technologies indigenous to African cultures and has been used for centuries (Ross, Morgan, and Hill, 2002). Lactic fermented food products constitute the bulk of foods given to children and generally form part of the daily main dishes of the average individual in Africa. For example, *ogi*, a lactic fermented maize based gruel is the major indigenous traditional weaning food common in the whole of West Africa (Oyewole, 1997). In the Northern parts of Nigeria, *Kunun-zaki*, a cereal-based fermented beverage is commonly consumed by children and adults as a breakfast drink (Ndulaka, Obasi, and Omeire, 2014) and sometimes as a weaning drink for infants (Adebayo, Otunola, and Ajao, 2010). In Benin, *gowe*, a traditional product made from malted and non-malted maize or sorghum flours which are fermented and then cooked to give sweet dough. It is consumed as is or after diluting in water often with the addition of sugar. It is consumed by children and adults as an energy drink (Adinsi *et al.*, 2014).

Fermentation makes food palatable by enhancing its aroma and flavour (Chelule, Mokoena, and Gqaleni, 2010). These sensory properties are responsible for increasing consumer acceptability of fermented foods compared to unfermented foods. Therefore fermentation is a unique way in which foods can be modified in diverse ways and that in effect result in new sensory properties in the fermented products (Leroy and De Vuyst, 2004).

The ability of the Lactic Acid Bacteria on preservative activity has been documented in cereal products. This is due to lowering of pH to below 4 through acid production, which inhibits growth of pathogenic micro-organisms which cause food spoilage and also food poisoning disease (Chelule *et al.*, 2010). Lactic acid bacteria inhibit the growth of fungus (Rouse, Harnett, Vaughan, and Sinderen, 2008). Therefore, fermentation increases the shelf life of the food product.

2.8.2 Effects of fermentation on the nutrient content of cereal based foods

Lactic acid bacteria have been shown to improve the nutritional value and digestibility of fermented foods (Chelule *et al.*, 2010). Acids that are produced in the fermentation process enhance the activity of microbial enzymes at temperature ranging 22-25⁰C. These enzymes include amylases, proteases and lipases that modify the primary food products through hydrolysis of polysaccharides, proteins, phytates and lipids, respectively (Rouse *et al.*, 2008).

Studies have shown that fermentation reduces the energy content of food. For example, a study by Adebowale and Maliki (2011) demonstrated that energy content of fermented pigeon pea seed flour significantly decreased after a fermentation period of 5 days.

The decrease in energy value was attributed to the decrease in fat content. Ijarotimi and Keshinro (2012) reported similar results where increase in the fermentation period reduced the fiber and the carbohydrate content of cereals, accounting for the low energy content of the fermented food. The effect of fermentation on the protein content of both legumes and grains has also been studied. Adebowale and Maliki (2011) using pigeon pea flour fermented for five days further showed that there was an increase in the crude protein content of the flour as the fermentation days progressed.

2.8.3 Heating and Maillard reaction

The Maillard reaction was first described in 1912 by Louis-Camille Maillard, who found that upon gently heating sugars and amino acids in water, a yellow-brown color developed (Bastos, Monaro, Siguemoto, and Séfora, 2012). Browning development occurs after an induction period, characterized by the production of fluorescent uncolored intermediates. Fluorophores are considered precursors of brown pigments and allow detecting the progress of the reaction before any visual change occurs. Fluorescence from the Maillard reaction is attributed to molecular structures with complex bonds between carbon and nitrogen, and the contribution of sugar caramelization to global fluorescence is insignificant in amino-acid containing systems (Matiacevich and Buera, 2006). The Maillard reaction is exceptionally widespread in foods (Nass *et al.*, 2007). Maillard reaction can be divided into three major steps depending on colour formation. Briefly, in the first step, sugars and amino acids condense and following condensation Amadori re-arrangement and 1-amino-1-deoxy-2-ketose form (Rozycki, Buera, Piagentini, Costa, and Pauletti, 2010). The Amadori rearrangement is irreversible since the reaction is blocked by methyl group, and therefore re-arrangement becomes impossible (Rozycki *et al.*, 2010).

In the second stage, dehydration and fragmentation occur in the sugar molecules. Amino acids are also degraded in this stage. This leads to the formation of hydroxymethylfurfural fusion (HMF) such as pyruvaldehyde and diacetyl. This stage often results in slight yellow or colourless food products. The third is ascorbic acid oxidation. The last, although it need not involve any enzyme at all, is nearest to enzymic browning, since it often does involve ascorbic acid oxidase, which, however, does not affect the phenols, which are the normal substrate in enzymic browning, but may involve other enzymes, *e.g.*, laccase or peroxidase (Wang, Qian, and Yao, 2011).

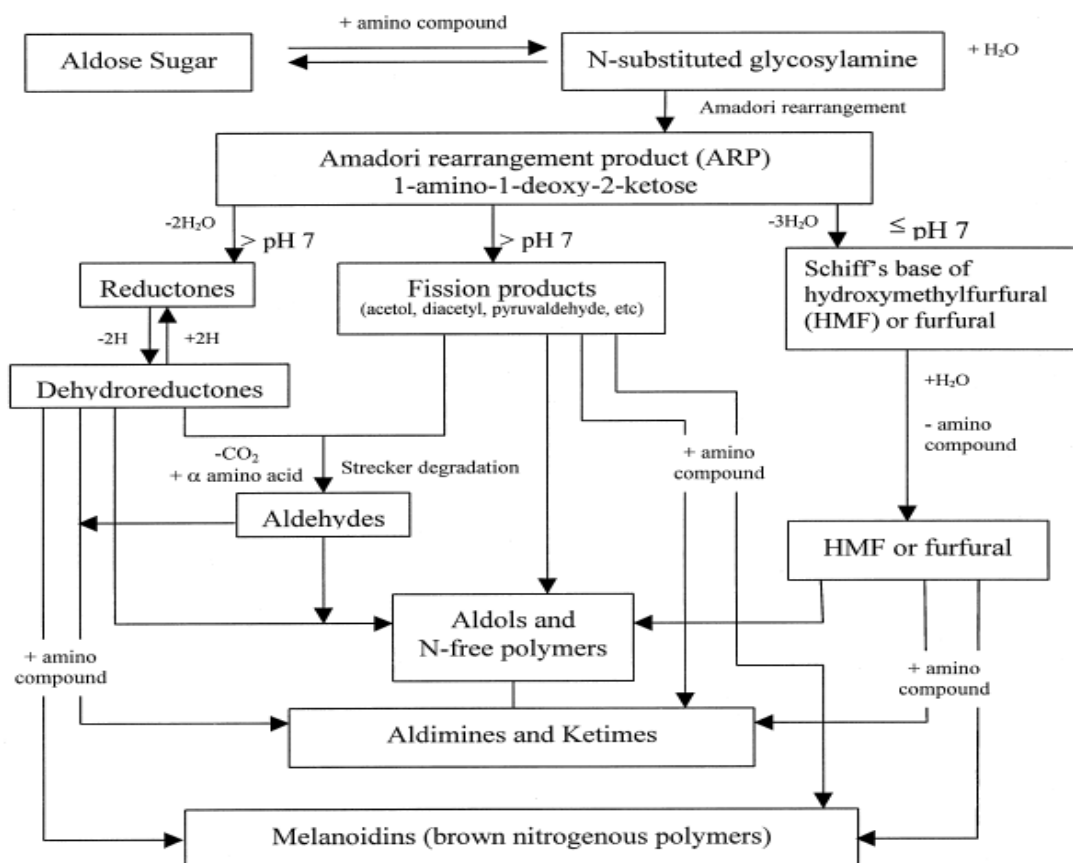


Figure 2.2: Main stages in Maillard reaction proposed by Hodge (adapted from Nursten, (2005)

2.8.4 Maillard reaction and desirable characteristics in food

Color formation is the primary characteristic of the Maillard reaction. Brown color development during processing and storage is desirable for many products such as baked foods, coffee, cookies while undesirable in some kinds of food products orange juice, white chocolate, milk and powder egg (Bastos *et al.*, 2012). Predicting and controlling food color development are particularly important for companies to satisfy consumer preference, since a complex array of melanoidins produced by the Maillard reaction is strongly dependent on the food matrix composition as well as the technological conditions of the reaction (Wang *et al.*, 2011).

Flavor and aroma development due to the Maillard reaction depends on the reaction temperature, time, pH, water content and on the type of sugars and amino acids involved (Van Boekel, 2006; Yu and Zhang, 2010). In most cases, the first factor mentioned influences the kinetics parameters, while the second factor determines the type of flavor compounds formed. The intermediate and final stages of the Maillard reaction are the most important to flavor development, especially the so-called Strecker degradation step, in which amino acids are degraded by dicarbonyls formed previously in the reaction, leading to the amino acids deamination and decarboxylation (Rizzi, 2008).

Working on biscuits, Serrem, De Kock, and Taylor (2011) found that Maillard reaction enhanced the colour, flavour and aroma characteristics of sorghum and bread wheat biscuits. This is suggested by the positive correlation between protein content and colour intensity and roasted flavour. A study by Ng'ong'ola-Manani, Mwangwela, Schüller, Østlie, and Wicklund (2014) showed that frying of fermented Soy bean-maize paste produced a snack that had a desirable colour and flavour. However, on the other hand, Maillard reaction has some demerits.

For example, Van Barneveld, Batterham, and Norton (1994) reported that Maillard reaction affects the nutrient quality, particularly with reference to lysine, an essential amino acid, making it unavailable for the body.

2.9 Sensory evaluation methods

According to Rousseau (2004) , in descriptive sensory evaluation, an instrument is developed to measure a set of attributes in the food under examination to complement results from chemical and traditional instrumental analyses by use of a highly trained panel. This method has been used to evaluate a Soy bean fortified fermented maize snack. For instance, Ng'ong'ola-Manani *et al.*, (2014) evaluated the sensory characteristics of natural and lactic acid bacteria-fermented pastes of Soy beans and Soy bean–maize blends using ten panelists who were trained to rate attribute intensities of the six products using a 15-point unstructured line scale. Studies by Kamau *et al.*, (2015) and Agengo *et al.*, (2015) used a 9 point hedonic scale (1= dislike extremely, 5 = neither like nor dislike and 9 = like extremely) to evaluate consumer acceptability of Soy bean fortified porridges. Results showed that the Soy bean fortified porridges were liked as much as the conventional porridge types.

2.9.1 Methods for evaluation with children

According to Guinard (2000), food products developed specifically for children must be tested with children. Studies documenting sensory perception of children are scanty and this limitation can be attributed to lack of methodologies to measure their food preferences (Levin and Hart, 2003). However Leon, Couronne, Marcuz, and Köster (1999) asserts that methods used to conduct sensory evaluation in children should be simple enough to be understood and reliable enough to measure preference. Furthermore, sensory testing with children should be performed with care taking into

account the range of sensory and cognitive abilities of children (Guinard, 2000). A study by Leon, Couronne, Marcuz, and Köster (1999) used three nonverbal methods, paired comparison, ranking by elimination and hedonic categorization, to assess food preference in children aged 4 to 10 years. Five biscuits dressed with different types of jams were used. They found that the products were more discriminated with hedonic categorization than the comparative methods. They concluded that the reliability of a method is linked to the age of the child and the more distinguishable the product, the more reliable the method. Facial scales are often used when conducting acceptability tests with preliterate children because at this level, children cannot read well and may not fully understand complex words but may understand more about facial expression (Popper and Kroll, 2005). A study by Serrem, De Kock, and Taylor (2011) used a five point hedonic facial scale to assess preference of four Soy bean fortified biscuits in children aged 8-9 years. Results showed that all biscuits had a high hedonic rating of above 80%, and the soy biscuits were liked as much as whole wheat biscuits.

2.9.2 Evaluating long term acceptability of foods

Vickers and Holton (1998) assert that a food with long term acceptability can be eaten repeatedly even when other foods are available to the consumer. The acceptance of a new food may increase with repeated exposure, while acceptance of familiar foods may decrease with time. A study by Vickers and Holton (1998) found that the intensity level of stimuli influenced acceptance over repeated exposure. Tea with low flavour intensity was gradually preferred to tea with higher intensity over 20 consumptions. Chung and Vickers (2007) using repeated exposure found that the liking of tea with low sweetness was preferred over time to low sweet tea.

Different methods have been used to assess the long term acceptability of a food by children. A study by Serrem, De Kock, and Taylor (2011) used repeated exposure to

assess the long term acceptability of Soy bean fortified biscuits among young children in Africa. Results showed that repeated exposure did not change the children's liking with the hedonic scores of 80% being sustained throughout the study period. This showed that the children did not become bored with the biscuits and hence would eat them daily over a long period of time.

2.10 Summary of literature review and gaps in knowledge

This review has shown that diets fed to school going children in developing countries are inadequate both in their protein and energy requirement for growth and development. Furthermore, it is recommended that snacks fed to children should be nutritious, easy to prepare and with a long stable shelf life. Therefore, this study sought to fill the gap through development of a snack that meets the previously mentioned requirements. Furthermore, there are limited studies that document long term consumer acceptability of foods meant for children. This study sought to fill this gap too.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

All the ingredients, used in this study were commercially available in Eldoret, Kenya from where they were purchased. They included Soy bean (*Glycine max*) and maize (*Z. mays*) from the Eldoret Municipal market. Sugar (Nzoia Sugar Company Ltd, Nzoia, Kenya) and vanilla essence (Pradip East Africa Ltd, Nairobi) were both purchased from a Supermarket in Eldoret town.

3.2 Experimental design

The physicochemical experiments were conducted in triplicate and the average value used for the study data. The experiment had five physical and six chemical treatments with the following factors: Titratable acidity, pH, water holding capacity, bulk density and oil holding capacity. Moisture content, crude protein, crude fat, ash, carbohydrate and energy contents for proximate analyses were the chemical treatments.

The Descriptive sensory evaluation was based on a Randomized Complete Block Design (RCBD) that involved the assessment of the 9 variations of soy fortified fermented snack samples as the treatments which were randomized and replicated three times with evaluators as the blocks.

The Completely Randomized Design (CRD) was used for the consumer acceptability studies among adults and children. Randomized three digit codes were used to blind each snack and the sample arrangements on the trays randomized for each panelist. The evaluation process was also randomized with the evaluators entering the room at random.

3.3 Processing of maize and Soy bean to flour

The Soy beans and maize were sorted to remove those with damaged seed coats or infested by pests. Both grains were winnowed to manually separate the chaff and the grains. The soy beans were roasted for 20 minutes in an oven at 180⁰C, while stirring occasionally. The purpose of the heating process was to reduce the levels of antinutritional factors, inactivate lipoxygenase enzymes and improve flavour. Both the grains were then cooled at room temperature. Maize and Soy bean grains were later milled separately using a commercial hammer mill (Powerline®, BM-35, Kirloskar, India) in Eldoret, fitted with a 2.0 mm opening screen.

3.4 Formulation of the snack

The concept for formulating the snack targeting school aged children was adopted from Serrem *et al.*, (2011), to provide at least half the protein requirement of 3 to 10 year old school children. To prevent chronic diseases such as PEM, the Acceptable Macronutrient Distribution Range (AMDR) for protein-energy for this age group is 10 to 30 g protein per day (Institute of Medicine, 2005). The study therefore aimed at providing at least half, 14 g protein in one serving of 100 g of the snack

Three variations of soy fortified snacks were formulated and constituted 100% maize (control), and the next two with soy: maize at ratios 30:70 and 50:50, respectively. For all the samples, Sugar and vanilla was added at 10% (100 g) and 2.5% (25 g), respectively. Water added to each treatment varied based on results of preliminary experiments which established that substitution with soy meal made the slurries dry, crumbly and difficult to manage requiring more water. Each variation was then subjected to different fermentation days, 0, 3 or 5 increasing the samples to 9. Table 3.1 shows the 3 composites with their basic ingredients.

Table 3. 1 : Formulation of the Soy bean fortified fermented maize meal snack pastes

Ingredients	Soy bean: Maize		
	0:100	30:70	50:50
Maize flour (g)	1000(78.4)	700 (52.8)	500 (35.1)
Soy bean flour (g)	0	300 (22.6)	500 (35.1)
Water (g)	150 (11.8)	200 (15.1)	300 (21.1)
Sugar (g)	100 (7.8)	100 (7.6)	100 (7.0)
Vanilla essence (g)	25 (2.0)	25 (1.9)	25 (1.7)
Total paste weight (g)	1275 (100)	1325 (100)	1425 (100)

Figures in parentheses are percentages

3.5 Preparation of the snacks

The dry ingredients, flour and sugar were sieved into plastic containers and mixed with a wooden spoon for about 4 minutes. Water was added and mixing continued for another 3 minutes. Three sets of each of the three variations, soy: maize 0:100, 30:70 and 50:50 were prepared. One of each of the three variations was dry fried on a wide flat tray at medium heat 100⁰ C on an electric hotplate for about 15 minutes with continuous stirring until light brown in colour. The semi-dried sample was then transferred to a tray into a pre-heated oven at 50⁰C for 20 minutes before sun drying until they were gritty when felt between fingers. They were then packaged in airtight zip lock plastic bags. The second and third sets of the three variations in plastic containers were covered (airtight) and fermented for 3 and 5 days, respectively before being dry fried, oven and sun dried. The samples for chemical analyses were ground using a mortar and pestle to a particle size of \leq 1 mm and stored at 40C until required. The procedure for preparation of the snack is illustrated in Figure. 3.1.

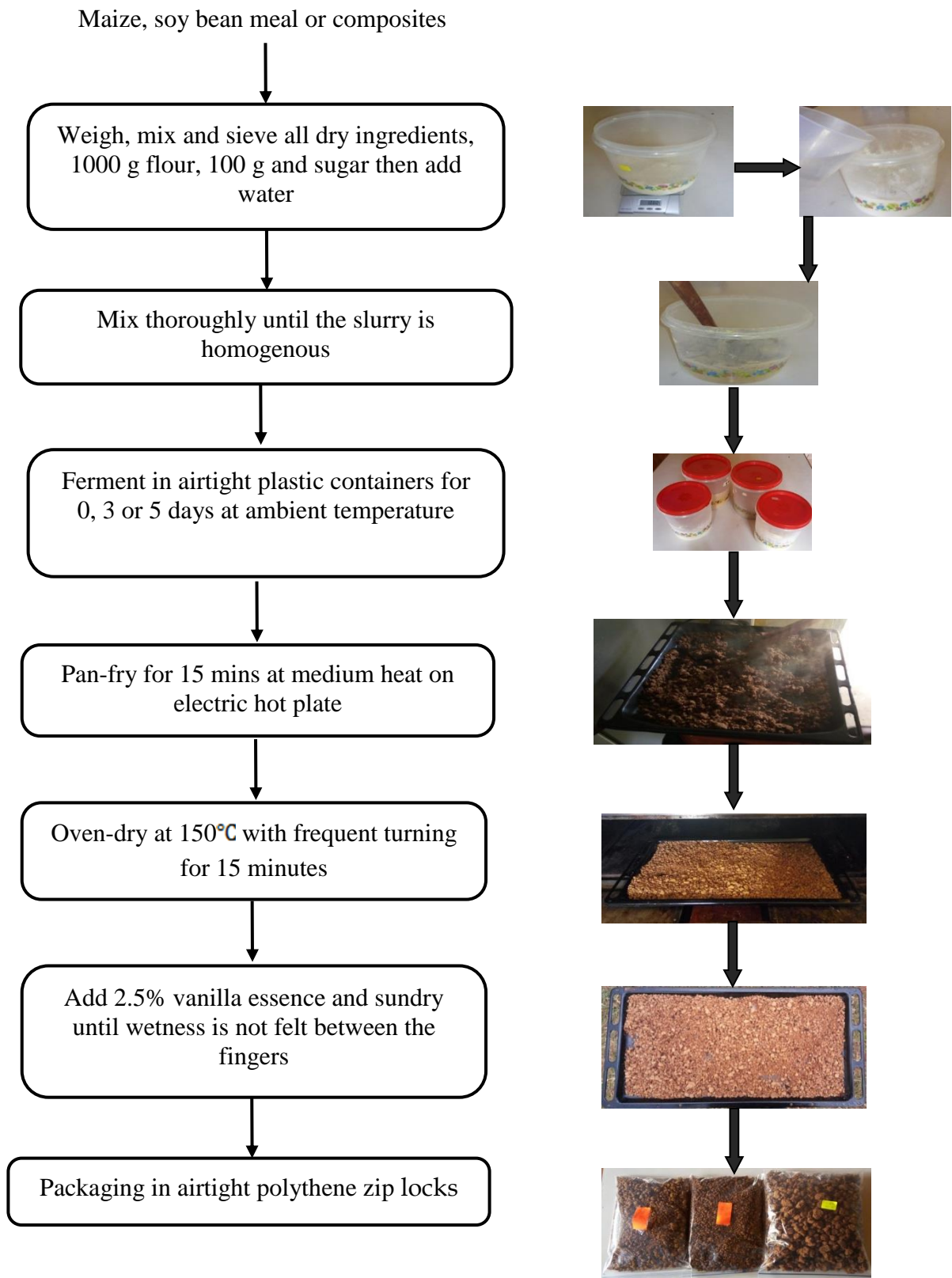


Figure 3. 1: Flow diagram for preparation of Soy bean fortified fermented maize meal snack

3.6 Proximate analyses

Moisture Content

Moisture content of the fermented soy fortified snacks and flours was determined using an oven (Model UNB 300 Schutzart, Memmert GmbH and Co. KG, Germany) drying procedure (AOAC International, 1995) Method 934.1. About 2 g of the sample was oven dried at 105⁰ C for 3.5 hours then cooled in a dessicator and weighed. The moisture content of the sample was expressed as a percentage of the initial weight of the sample using the following formula:

$$\% \text{ Moisture content} = \frac{\text{Weight of wet sample} - \text{Weight of Dry Sample}}{\text{Weight of wet sample}} \times 100$$

Crude Protein

Crude protein was determined by the micro Kjeldahl method (AOAC International, 1995) Method 992.23. A sample of 0.3 g of each of the flours was digested in a heating block (Model DK series 20 digester unit, 115 V / 50 - 60 Hz, manufactured by VELP Scientifica Srl, Milano Italy) at 370 ± 5⁰ C for about 60-90 minutes or until the contents became clear. In 0.2 ml of the digested sample, 5ml of a previously prepared N1 mixture was added and allowed to stand for about 15 minutes before 5 ml of N2 was added. The mixture was allowed to stand for one hour during which it developed a blue color whose absorbance was read off a spectrophotometer (Spectronic 21D AKIU®, Milton Roy, Germany) at 650 nm. The %N in the sample was calculated using the formula:

$$\% \text{ Nitrogen} = \frac{(a - b) \times v \times 100}{1000 \times w \times a l \times 1000}$$

Where

Where

a = Concentration of N in the solution

b = Concentration of N in the blank

v = Total volume at the end of analysis procedure

w = Weight of the dried sample and

al = Aliquot of the solution taken.

The crude protein was then attained by multiplying the % nitrogen by a factor (6.25).

Crude Fat

Crude fat content was determined using the Soxhlet extraction method (AOAC International, 1995) Method 920.29. Samples of 2 g were weighed into a thimble and oil was extracted using petroleum ether ($55 \pm 5^{\circ}$ C) for 8 hrs. The extract was oven-dried at 105° C for about 30 minutes, cooled in desiccators, and weighed. The oil content was determined using the following formula:

$$\% \text{ Crude Fat} = \frac{\textit{Weight of fat}}{\textit{Weight of Sample}} \times 100$$

Ash content

Ash was determined using (AOAC International, 1995) Method 923.03. Samples of 2 g were burned at 550° C for 6 hours in a muffle furnace (Carbolite 530 2 AU, Bamford, Sheffield, England) to constant weight. The samples were cooled in desiccators and weighed. Ash content was determined using the following formula:

$$\% \text{ Ash} = \frac{\textit{Weight of Ash}}{\textit{Weight of Sample}} \times 100$$

Carbohydrate content

Carbohydrate content was determined by subtracting the sum of weights of protein, lipid, ash, and moisture from the total wet matter basis (FAO, 2003)

$$\% \text{ Carbohydrates} = 100 - (\% \text{ Fat} + \% \text{ moisture} + \% \text{ ash} + \% \text{ proteins})$$

Energy content

Energy content was determined by multiplying the mean values of crude protein, crude fat and total carbohydrate by Atwater factors of 16.736 kJ, 37.656 kJ and 16.736 kJ respectively. Results were expressed as kilojoules per 100 g sample (FAO, 2003).

3.7 Functional properties

Bulk density

Bulk density was determined using the method described by Narayana and Rao (1984). An empty calibrated centrifuge was weighed. The tube was filled with a sample to 5 ml by constant tapping until there was no further change in volume. The weight of the tube and its contents was taken and recorded. The weight of the sample alone was determined by difference. Bulk density was calculated from the values obtained as follows:

$$\text{Bulk density} \left(\frac{g}{ml} \right) = \frac{\text{Weight of Sample}}{\text{Volume Occupied}}$$

Water absorption capacity

Water absorption capacity which gives an indication of the amount of water available for gelatinization was determined according to the method of Sosulski, (1962).

Two and a half grams of each sample were added to 30 ml distilled water in a weighed 50 ml centrifuge tube.

The tube was agitated for about 5 min before being centrifuged (D72, Andreas, Hettich, Germany) at 4000 rpm for 20 min. The mixture was decanted, the liquid decant collected and measured, including the new weight of flour and water absorbed. Water absorption was calculated using the difference between the new and previous weight then expressed as the weight of water bound by 100 g dry flour.

Oil absorption capacity

Oil absorption capacity was determined according to the method of (Beuchat, 1977). One (1) gram of the sample flour was mixed with 10 ml oil (pure Soy bean oil) in a 25 ml centrifuge tube and stirred for 2 min. The samples were allowed to stand at room temperature for 30 min, centrifuged at 5000 rpm using a centrifuge (D72, Andreas Hettich, Germany) for 30 min, and the volume of the supernatant was noted in a 10 ml graduated cylinder. The difference in volume was taken as the oil absorbed by the sample. Density of oil was taken as 0.895 g/ml.

Titrateable acidity

Titrateable acidity was conducted according to AOAC (2000) Method 942.15. Ten ml of sample was titrated with a standard alkali solution of 0.1N NaOH to 3 drops phenolphthalein endpoint until a constant light pink color was achieved. The titrateable acidity was calculated as:

$$\% \text{ Acid } \left\langle \frac{\text{Weight}}{\text{Volume}} \right\rangle = \frac{N \times v_1 \times \text{Eq. wt}}{v_2 \times 10}$$

Where:

Where:

N = Normality of titrant (NaOH) (mEq./ml)

V1 = Volume of titrant used (ml)

Eq.Wt. = Equivalent weight of predominant acid (g); The predominant acid in this case is lactic acid

V2 = Volume the sample (ml)

1/10 is the factor relating milligrams to grams (100/1000)

pH

Potentiometric pH measurements were obtained with the pin electrode of a pH meter (Tester Accumet, model 10) inserted directly into the fermenting slurry samples and readings of values from digital pH meter.

3.8 Descriptive Sensory Analysis

3.8.1 Recruitment and screening of panelists

Students of the University of Eldoret who normally consume fermented maize snack and did not suffer from food allergies were invited to apply to train for a descriptive sensory panel through an advert on notice boards, phone calls and email. Thirty five (35) individuals who responded attended an orientation session and were subjected to three different screening tests to determine their sensory acuity. Three types of screening tests used in this study included the basic taste test as described by Lawless and Heymann (2013), an aroma identification test and an exercise to describe differences in attributes related to appearance, texture, odour and flavour among the fermented snacks.

The five basic tastes, bitter, sweet, sour, salt and umami were presented to panelists as taste solution impregnated filter papers of different shapes. The aroma compounds used in the identification test were pineapple, orange, vanilla, lemon and strawberry. Thirty five applicants underwent the test, and twelve (12) passed the screening test. The final panel selected constituted five males and seven females, aged between 20 and 26 years. Before the tasting exercise, the panelists filled in a consent form that informed them about the nature of the samples they would evaluate.

3.8.2 Training of the Panel

The panelists were trained in six sessions of 2 hours each over a period of 2 weeks. The generic descriptive method described by Einstein (1991) was used to perform the descriptive sensory profiling of the nine snack samples (Figure 3.2). During the training, the panelists described the differences that existed among samples and food items were used as references to clarify sensory attributes. Panelist agreement was evaluated through several tests during the training. The panelists generated and reached consensus for 34 descriptive terminologies that were grouped under appearance, aroma/smell, flavour, texture and after taste, with their definitions and references standards to anchor the scale ends (Table 3.2).

3.8.3 Evaluation of fermented soy fortified snack samples

After the training, evaluation of the snacks was carried out over a period of 3 days in 3 sessions of 1 hour a day, using a randomized complete block design. During evaluation of the fermented snack samples, each panelist was given approximately 20 g of each sample in a disposable white cup. Twenty (20) ml of water was added to each, 5 minutes before the evaluation session commenced.

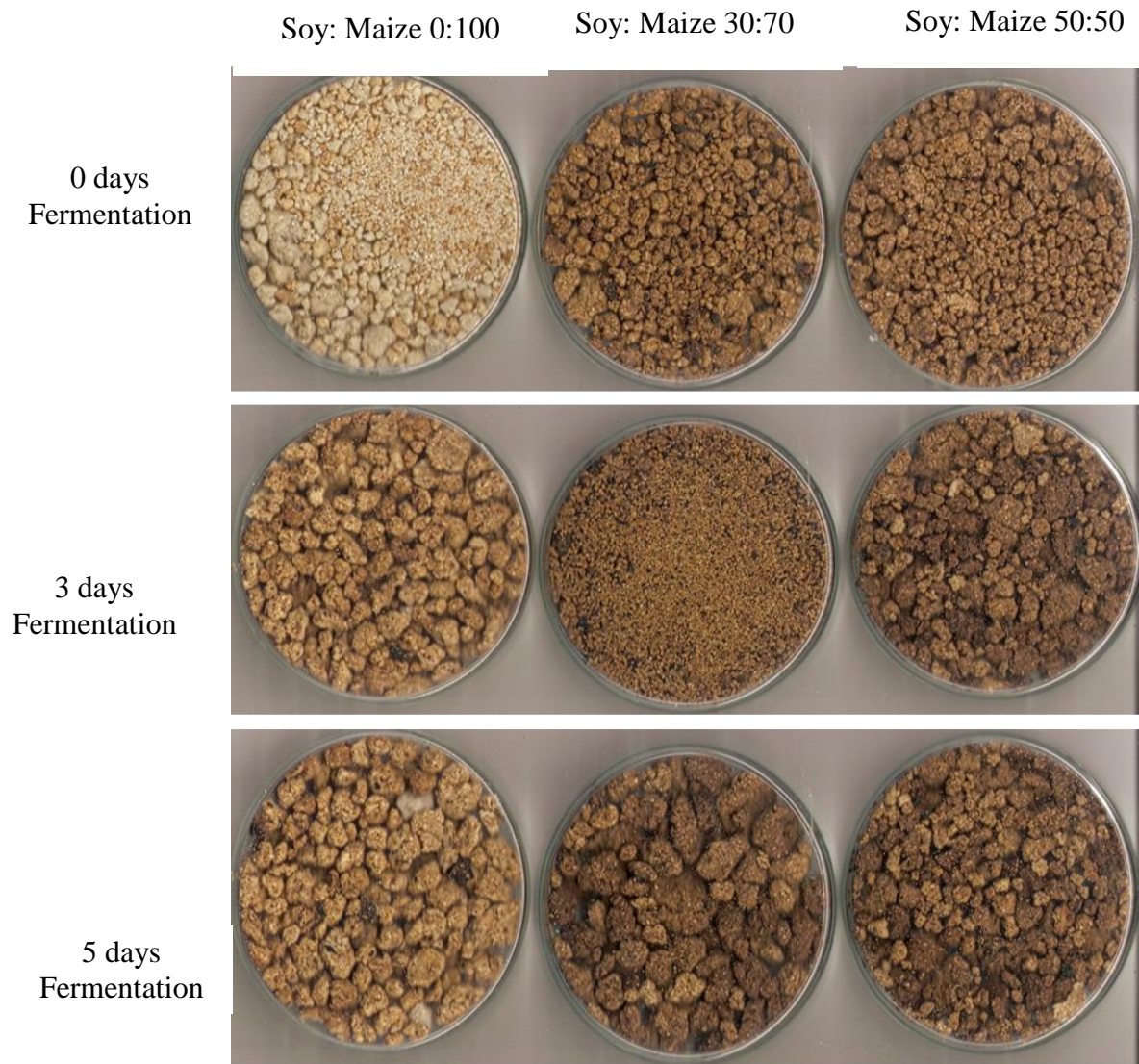


Figure 3.1: Soy fortified fermented maize meal snack



Figure 3.3: Tray set up for sensory evaluation

The white tray on which the samples were presented also had carrot wedges and a plastic tumbler filled with distilled water for cleansing the palate between tasting of the samples as well as a tooth pick and a serviette (Figure 3.3).

Table 3.2: Descriptive sensory attributes used by the trained panel to evaluate Soy bean fortified fermented maize meal snacks

Sensory Attribute	Definitions	References to clarify and anchor sensory attributes	Rating Scale
Appearance			
Dry	Degree of freedom from moisture	Maize flour=0 Maize flour paste=10	Not moist=0 Very moist=10
Brown	Colour intensity of <i>Mkarango</i> ranging from light brown to dark brown	White bread crumb (light) = 0 Dark chocolate cake (dark) = 10	Not dark = 0 Very dark brown=10
Cream	Intensity of appearance associated with skimmed milk powder	Dark chocolate cake=0 Milk powder=10	Not creamy=0 Very creamy= 10
Granular	Degree of similarity to granules	Maize flour=0 Millet grains=10	Not granular=0 Very granular=10
Varying sizes	Degree of lack of uniformity in size	Choco rice =0 Sample 100F1	Not varied=0 Very varied=10
Coarse	Degree of a food product being composed of large particles	Maize flour=0 sample =10	Not coarse=0 Very Coarse=10
Rough	Degree of abrasiveness of products surface perceived by sight	White bread (super loaf) = 0 Rock cake = 10	Not rough = 0 Very rough = 10
Irregular shapes	Degree of lack of uniformity in shapes	Choco rice =0 sample =10	Very regular=0 Very irregular=10

Table 3.2: Descriptive sensory attributes used by the trained panel to evaluate Soy bean fortified fermented maize meal snacks (Cont')

Sensory Attribute	Definitions	References to clarify and anchor sensory attributes	Rating Scale
Aroma			
Roasted maize flour	Intensity of aroma associated with roasted dried maize flour	Maize meal flour=0 Roasted maize flour=10	No intense roasted aroma =0 Intense roasted aroma=10
Fermented	Intensity of aroma associated with a fermented cereal	Unfermented maize flour= 0 Fermented maize flour slurry=10	No intense fermented maize aroma= 0 Intense fermented maize aroma= 10
Baked	Intensity of aroma associated with a baked product with yeast	Pancake =0 White wheat bread=10	Not intense = 0: Intense baked aroma =10
Soy bean	Degree of aroma associated with parboiled Soy bean grains	Stiff porridge=0 Parboiled Soy bean grains=10	No intense Soy bean aroma=0: Intense Soy bean aroma= 10
Vanilla	Degree of aroma associated with vanilla	Stiff porridge=0 Vanilla Essence =10	No intense vanilla flavour= 0: Intense vanilla flavour=10
Fermented maize	Intensity of aroma associated with fermented maize flour	Unfermented maize flour=0 Fermented maize flour slurry=10	No intense fermented maize aroma=0 Intense fermented maize aroma=10
Sorghum flour	Intensity of aroma associated with sorghum flour	Stiff porridge=0 Ground sorghum flour=10	No intense sorghum flavour=0 Intense sorghum flavour=10
Stiff porridge	Aroma associated with stiff porridge	Ground maize flour=0 Stiff porridge=10	No intense stiff porridge aroma=0 Intense stiff porridge aroma=10

Table 3.2: Descriptive sensory attributes used by the trained panel to evaluate Soy bean fortified fermented maize meal snacks (Cont')

Sensory Attribute	Definitions	References to clarify and anchor sensory attributes	Rating Scale
Flavour Fermented	Intensity of flavour associated with fermented maize meal.	Stiff maize meal porridge = 0 Fermented maize meal flour = 10	No fermented maize meal flavour = 0 Intense fermented maize meal flavour = 10
Vanilla	Intensity of flavour associated with vanilla	No intense vanilla flavour=0 Vanilla Essence =10	No vanilla flavour=0 Intense vanilla flavour=10
Sweet	Intensity of flavour associated with taste sensation typical of sucrose solution	Spring water without sucrose = 0 5% sucrose solution in spring water =10	No sweet flavour = 0 Intense sweet flavour = 10
Sour	Intensity of taste sensation associated with organic acids	Spring water without citric acid =0 5% citric acid solution in spring water= 10	No sour flavour = 0 Intense sour flavour = 10
Burnt	Intensity of flavour associated with burnt maize flour	Maize flour=0 Burnt maize flour=10	No burnt taste=0 Intense burnt taste=10
Roasted maize	Intensity of flavour associated with roasted maize flour	Maize meal flour=0 Dry stiff porridge morsel=10	No intense roasted maize flavour=0 Intense roasted maize flavour=10
Soy bean	Degree of aroma associated with parboiled Soy bean	Stiff porridge=0 Parboiled Soy bean grains=10	No intense Soy bean flavour=0 Intense Soy bean flavour=10

Table 3.2: Descriptive sensory attributes used by the trained panel to evaluate Soy bean fortified fermented maize meal (Cont')

Sensory Attribute	Definitions	References to clarify and anchor sensory attributes	Rating Scale
Flavour continued			
Stiff porridge	Flavour associated with stiff porridge	Ground maize flour=0 Stiff porridge=10	No intense stiff porridge flavour=0 Intense stiff porridge flavour=10
Baked product	Intensity of flavour associated with baked product with yeast	Pancake =0 White wheat bread=10	No intense baked product flavour= 0 Intense baked product flavour =10
Texture			
Rough	Degree of abrasiveness of products surface perceived by lips and tongue during mastication	White bread crumb = 0 Rock cake = 10	Not rough = 0 Very rough = 10
Grainy	Degree to which mouth contains small particles after sample has been swallowed	Pancake = 0 Millet grains=10	Not grainy=0 Very grainy=10
Soily	Intensity of texture associated with soil	Pancake=0 Millet grains=10	Not soily =0 Very soily=10
Crunchy	Intensity of noise made in the first bite of the sample between the molars	Pancake=0 Paul's cookies =10	Very crunchy=0 Very crunchy=10

Table 3.2: Descriptive sensory attributes used by the trained panel to evaluate Soy bean fortified fermented maize meal snacks (Cont')

Sensory Attribute	Definitions	References to clarify and anchor sensory attributes	Rating Scale
Aftertaste			
Grainy	Degree to which mouth contains small particles after sample is swallowed	Pancake = 0 Millet grains = 10	Not grainy =0 Very grainy=10
Sour	Intensity of taste sensation associated with organic acids	Pure spring water=0 5% citric acid in spring water= 10	No sour taste=0 Intense sour taste=10
Sweet	Intensity of sensation typical of sucrose solution	Spring water without sucrose = 0 5% sucrose solution in spring water =10	No sweet taste = 0 Intense sweet taste = 10
Fermented	Intensity of flavour associated with fermented cereal	Pancake = 0 Fermented maize flour slurry = 10	No fermented taste=0 Intense fermented taste=10

White bread, Supa Loaf (Mini Bakeries, Nairobi, Kenya), Vanilla essence (Pradip East Africa Limited, Nairobi, Kenya), Choco rice (Proctor and Allan, Nairobi, Kenya), Paul's Cookies (Paul's Bakery, Eldoret, Kenya)

During each session, the 9 samples were randomly presented to each panelist. However, to avoid fatigue five variations of the snacks were evaluated first followed by a 15 minute break before evaluating the next four variations. Panelists were also provided with Table 3.1 showing the attributes and their definitions and had access to the reference foods, located at a central table, throughout the evaluation. The evaluation session was conducted at the research room in the foods laboratory of the University of Eldoret. Panelists were seated at individual stations where they could not see each other and evaluated the samples at ambient temperature. The 34 descriptors were used to rate the nine samples on a 10-point graphic rating scale to measure the intensity of the individual attributes. Responses were entered manually in the ballot.

3.9 Consumer evaluation by adults

Two panels, first using adults then children were used to evaluate the consumer acceptability of the fermented snack.

3.9.1 Recruitment and screening of adults

Consumers who normally consume maize and soy bean were recruited through an advert on the notice boards at the University of Eldoret, Kenya to invite 55 panelists among the staff and student population. Those who responded to the advert were asked to fill in a consent form informing them about the ingredients in the samples and to ascertain their personal commitment in participating on the consumer panel to evaluate the nine variations of snacks (Appendix I and II). Only those who were not allergic to any foods and did not participate in the descriptive panel were allowed to participate. A random number of twenty four males and thirty one females, aged between 18 and 34 years were selected (24.9 ± 6.8 years).

3.9.2 Evaluation session

Each consumer was provided with all the nine variations of snacks, each served in white disposable cups on a white tray accompanied by a carrot and a glass of distilled water to cleanse their palates before and in between the tasting. The consumers were asked to rate their degree of liking for appearance, aroma, flavour colour and texture on a nine-point hedonic scale where 1 = dislike extremely, 5 = neither like nor dislike and 9 = like extremely. The evaluation was carried out in one session, each session lasted 25 minutes. Evaluation was conducted using a Completely Randomized Design (CRD). The samples were blinded using a three digit code. The evaluation was done at the Department of Family and Consumer Sciences Food Laboratory at University of Eldoret where the panelists assessed the samples seated in individual stations in the laboratory. The method of sample preparation was the same as that for descriptive sensory evaluation. (Appendix V)

3.10 Consumer acceptability by children

3.10.1 Sample preparation

In this study, four variations of snacks were evaluated. The three experimental samples soy meal: maize, 30:70 (5 days fermentation), 30:70 (3 days fermentation) and 50:50 (3 days fermentation) considered best by the adult consumer acceptability panel were selected for children's acceptability test. A fourth sample, 0:100 soy meal: maize (3 fermentation days) was used as the control as it is the conventional snack commonly consumed by rural children in Kenya.

3.10.2 Recruitment and screening

Children (24 boys and 36 girls) aged 8 to 9 years and who attended University of Eldoret primary school in Eldoret, Kenya evaluated the snacks.

Permission to carry out the study was granted by the Head Teacher of University of Eldoret Primary school. The children's parents were informed about the purpose, procedures, activities, risks and benefits of the study their children would be involved in, in a letter addressed to them (Appendix VII). Only children who voluntarily consented and whose parents signed the consent form were involved in the study.

3.10.3 Orientation

A one hour orientation session was carried out on the first day of the 5 day study in the school's classroom, during tea-break at 10:00 am. The purpose of the orientation was to familiarize and teach the children how to use the score card, a seven point scale with stylized faces (Figure. 3.4). The sitting arrangement was designed to divide the 60 children into 4 groups of 15 each. Each group was allocated a red, yellow, green or orange colour. The children sat at one of the 60 stations with set trays containing evaluation samples and name tags with the child's number and group number. Four research assistants, from the University of Eldoret that can speak both English and Kiswahili were allocated to each group. It was explained to the children that the faces meant that they liked extremely, liked very much, liked a little, not sure, dislike a little, disliked very much and disliked extremely what they were eating. Two fruits, a banana, that children generally liked (sweet) and lemon, they generally did not like (sour) labeled with 3 digit blinding codes were used as test examples.

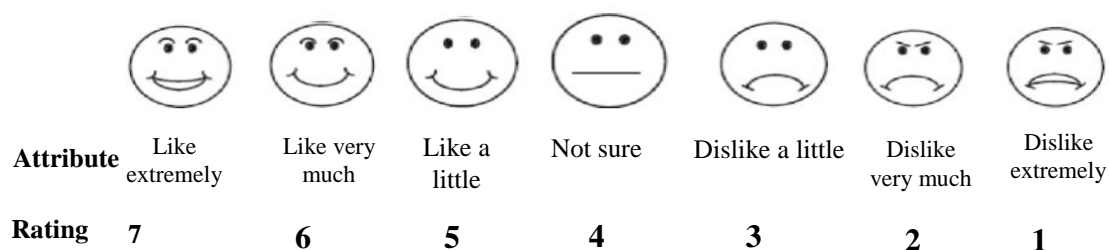


Figure 3.4: Seven point facial scale used by school children for hedonic categorization of Soy bean fortified fermented maize meal snack

The children were instructed to remove the label from the respective fruits and place it above the face corresponding to how they felt about the fruit they had just tasted, with the liked banana label on a happy face and the disliked lemon's label on a sad face. Bottled water to cleanse the pallet before and in between tasting was provided. This session was conducted using both English and Kiswahili languages. The children were also informed that they could withdraw from the study at any point if they wanted to.

3.10.4 Procedure for evaluation

Evaluation was conducted over a period of four days using hedonic categorization and repeated exposure tests to determine children's liking. A seven point hedonic facial scale (dislikes extremely at 1, neither like nor dislike at 5 and like extremely at 7) was used. The children were divided into four groups. Two half-hour evaluation sessions were conducted each day. In the first session, responses for the four snacks presented to each child in a tray labeled with randomized 3 digit codes were entered by the children into a score sheet. They were provided with clean water to cleanse their palate after tasting. The same procedure was repeated for the four days. Therefore, in four days each child evaluated each variation of snack 4 times to establish long term acceptability. The children received a gift of an exercise book at the end of the four days.

3.11 Determination of snack satiating power

3.11.1 Sensory specific satiety

To evaluate sensory specific satiety, the method used by Vickers and Holton (1998) was adopted with modifications. During sensory evaluation, after tasting and rating each of the four snacks in the first session.

The children who had already been divided into four groups were given one of the snacks evaluated earlier in a second session, to consume again and then indicate whether they would like to eat it again tomorrow on a 3-point hedonic scale. Each of the four groups got a different snack on each of the four days, so that at the end of the four days, each group and child had evaluated all the four snacks. The sensory specific satiety was determined by comparing the change in the score between the first consumption and the second consumption of each snack by all the 60 children.

3.11.2 Estimated/predicted Glycemic index and load

To evaluate the potential of the snacks to alleviate short term hunger in school children due to the increase in protein content due to soy fortification, proximate results were used to predict glycemic index and load. Values for glycemic indices for maize and soy were obtained from the International table of glycemic index and glycemic load by Foster-Powell, Holt, and Brand-Miller (2002). The snack foods with composites of maize and soy meal were treated as a meal as the two foods have different glycemic indices. The following formula for calculating a meal GI from the individual food GI by Dodd, Williams, Brown, and Venn (2011) was used:

$$\text{Meal GI} = \frac{\{[\text{GI}_{\text{FoodA}} \times \text{g available carbohydrate (avail VHO)}_{\text{FoodA}}] + (\text{GI}_{\text{FoodB}} \times \text{g avail CHO})_{\text{FoodB}}\}}{\text{Total g available CHO}}$$

After obtaining the glycemic index, it was used to estimate the glycemic load. The glycemic load was predicted using the following formula by Foster-Powell *et al.*, (2002):

$$\text{Glycemic Load} = \frac{\text{Glycemic index}}{100} \times \text{CHO g/serving}$$

The available carbohydrate for each of the 9 variations of soy fortified fermented snacks used in the formula to obtain glycemic load was obtained by subtracting the fiber from the total carbohydrate, of the snack using data from USDA (2018) Tables.

The food products were then classified according to the glycemic load based on the categories shown in Table 3.3. Lowering the glycemic load was considered an indicator that the snack would have a higher satiating effect.

Table 3.1: Glycemic Index categories

Category	Glycemic Load
Low	≤ 10
Medium	11-19
High	≥ 20

Adapted from American Institute for Cancer Research (2013)

3.12 Data analysis

All the experiments were performed in triplicate and the results presented as mean values and standard deviation. The statistical program used was Statistica software Version 8.0 (Statsoft, Tulsa, UK). The descriptive panels' mean scores for sensory attributes were determined by two-way analysis of variance ANOVA with samples as fixed effects and panelists as random effects, of the significant sensory attributes from means across significant panelists was performed using a correlation matrix with the snack samples in rows and descriptors in columns. The data for consumer evaluation were analyzed using repeated measures analysis of variance (ANOVA). Means for all analyses were compared using Fisher's least difference (LSD). Box and whisker plots were used to illustrate consumer hedonic score distributions for the snacks. Significant differences were considered at $P < 0.05$.

3.13 Ethical consideration

Ethical approval was granted by the National Commission for Science, Technology and Innovation (NACOSTI) (Permit Number: NACOSTI/P/17/27159/15643) (Appendix VIII). For the adult consumer study, informed and written consent was sought from the participants before the evaluation commenced (Appendix II and III). Permission to conduct the study was granted by the headmaster of the primary school and only children who voluntarily accepted and whose parents signed a consent form that informed them of the nature of the snack samples and the activities involved in the study were included.

CHAPTER FOUR

RESULTS AND DISCUSSIONS

4.1 Proximate composition on dry matter basis

The proximate composition of the snacks is shown in Table 4.1. The moisture content of the snacks ranged from 4.6-8.8%. The fluctuating moisture content values could be attributed to the fact that the snacks were sun dried and therefore the drying process was dependent on ambient temperature, which varies based on the environmental conditions. However, the moisture content of the snacks is below the recommended 10% for maize-Soy bean blends used for supplementary feeding (World Food Programme, 2018). Low moisture content prevents microbial activity due to low water activity, increasing shelf life (Omemu, Okafor, Obadina, Bankole, and Adeyeye, 2018). Additionally, in their study, Amankwah *et al.*, (2009) found that the removal of moisture generally increased nutrient density and availability.

The lipid content of the snacks was lowest, 6.9 g/100 g in the unfortified, unfermented snack and highest, 13.4 g/100 g in the snack with 50% soy replacement of maize, fermented for 5 days. Fortification of maize meal with soy meal increased the oil content by 46.4 and 78.3% at 30 and 50% soy fortification, respectively compared to the unfermented snack. The increase in lipid content may be explained by the high (10%) fat content of the full fat Soy bean flour used in this study. This could be due to the thermal treatment of legumes, in this study, which disrupted the lipid bodies of the Soy bean expelling more oil from the flour (Kayitesi, Duodu, Minnaar, and De Kock, 2010).

Table 4.1: Proximate composition of the Soy bean fortified fermented maize meal snacks (g/100 g) on dry matter basis

Proximate	Unfermented			3 fermentation days			5 fermentation days		
	Soy bean levels (%)			Soy bean levels (%)			Soy bean levels (%)		
	0	30	50	0	30	50	0	30	50
Moisture	5.6 ^b ±0.1	5.2 ^b ±0.2	4.6 ^a ±0.8	7.1 ^d ±0.3	6.3 ^c ±0.1	5.5 ^b ±0.0	8.7 ^f ±0.0	4.1 ^a ±0.0	7.8 ^e ±0.1
Ash	1.3 ^a ±0.1	3.9 ^c ±0.1	5.0 ^{de} ±0.2	1.5 ^a ±0.1	3.5 ^{bc} ±0.3	5.5 ^e ±0.1	3.1 ^b ±0.3	3.6 ^{bc} ±0.4	4.5 ^d ±0.5
Crude protein	6.1 ^a ±0.0	19.4 ^e ±0.2	21.7 ^h ±0.0	6.3 ^a ±0.0	18.2 ^c ±0.0	21.1 ^f ±0.0	6.8 ^b ±0.0	18.4 ^d ±0.0	21.3 ^g ±0.1
Lipids	6.9 ^a ±0.0	10.1 ^d ±0.0	12.3 ^g ±0.0	8.2 ^b ±0.0	10.3 ^e ±0.0	12.6 ^h ±0.0	9.2 ^c ±0.0	11.0 ^f ±0.0	13.6 ⁱ ±0.3
Carbohydrate ¹	79.9 ^h ±0.2	61.4 ^d ±0.4	56.2 ^c ±0.8	76.7 ^g ±0.3	61.3 ^d ±0.2	55.1 ^b ±0.1	65.2 ^f ±0.4	62.6 ^e ±0.5	52.6 ^a ±0.5
Energy(kJ/g 100g)	1635.1 ^{bc}	1638.4 ^{bc}	1660.7 ^d	1633.7 ^b	1630.1 ^b	1640.7 ^c	1600.4 ^a	1631.9 ^e	1640.8 ^c

Values are means± standard deviation. Values with the same superscript on the same column are not significantly different at (P<0.05) as assessed by Least significant difference

¹Calculated by the difference method (FAO, 2003) where % carbohydrates= 100- (% fat+% moisture+% ash (minerals) + %protein)

²Calculated by multiplying with Atwater's factor (FAO, 2003) where energy (kJ) = (%carbohydrates×16.736) + (% protein×16.736)+ (% oil×37.656)

Soy bean stores its energy as oil (USDA, 2018). Several workers have found increased lipid content in foods fortified with soy beans and other oil seeds. Kayitesi *et al.*, (2010) found that fortification of sorghum with 30% of full fat marama bean increased the lipid content of the sorghum flour significantly from 3.0% to 14.2%. Another study by Glover-Amengor, Quansah, and Peget (2013) showed that fortification of yam flour with Soy bean flour yielded a composite with 143% higher oil content than unfortified yams.

The oil content in the fortified and unfortified snacks increased substantially as the fermentation days increased. Compared to the unfermented snacks fortified with soy at 0, 30 and 50%, fermentation for 3 days increased the oil content by 18.8, 1.9 and 2.4%, respectively, while for 5 days fermentation, the increase was 33.3, 8.9 and 10.5%, respectively. The increase in oil content may be due to increased activity of lipolytic enzymes in the fermentation medium which hydrolyzed fat to glycerol and fatty acid (Achinewhu, 1986). The results in this study are similar to those of (Meseret, 2011) who demonstrated that a maize based snack fortified with 18% full fat Soy bean flour fermented for 48 hours had its oil content increased from 6.93 g/100 g to 10.90 g/100 g. This researcher further postulated that fermentation of cereals decreases the carbohydrate content concentrating the lipids content, hence, the increase. Lipids are an important ingredient used to increase energy density in formulation of fortified blended food for vulnerable populations such as malnourished children (Michaelsen *et al.*, 2009). Fat also provides a medium through which fat soluble Vitamins A which is critical for growth and development and is highly deficient in the diets of children in developing countries (WHO, 2010) is provided. Fat from soy is also rich in essential fatty acids, which promote growth, cognitive development and immune function (Michaelsen *et al.*, 2009).

In addition, fats slow gastric emptying and intestinal motility, improving satiety (Mosha and Vicent, 2005). Incorporation of soy meal into the snacks dramatically increased the protein content in the snacks. Replacement of maize meal flour with 30 and 50% maize meal increased the protein content by 218.1 and 255.7%, respectively compared to the unfortified and unfermented snack. A similar trend was observed in the fermented snacks. These increases may be attributed to the high protein content of Soy bean flour of up to 43% (USDA, 2018). Several workers have reported similar results on substituting cereal with legume flours. Adeyeye, Adebayo-Oyetero, and Omoniyi (2017) showed that increase in the proportion (5-30%) of soy protein isolate in preparation of maize flour cookies significantly increased the protein content by 233%. Similarly, Serrem, De Kock, and Taylor (2011) found that incorporation of defatted soy flour in biscuits at 28.6, 50 and 71.4% substantially increased the protein content by 95, 168, and 234%, respectively. Awadalkareem, Mustafa, and El Tinay (2008) found that sorghum-soy composite food had increased protein contents of 18 to 26% after adding soy concentrate at between 4 to 12% levels.

According to the WHO (2007) Expert Consultation, the protein requirements of children aged 1 to 2 years, 3 to 10 years and 10 to 18 years, are 1.12, 0.73 and 0.7 g/kg/day, respectively. The daily protein requirements for such children, based on FAO (2004) weight for age values, translate to 12 to 13 g/day for 1 to 2 year olds, 11 to 22 g/day for 3 to 10 year olds and 24 to 40 g/day for 10 to 18 year olds. The protein content of snacks fortified with 30% to 50% Soy bean flour in this study was between 6.1 g/100 g and to 21.7 g/100 g and 6.1% to 13.59% for maize based fermented snacks. Fortification of the snacks with 30% and 50% Soy bean increased the protein content of the snacks by 218% and 256% respectively.

Other studies have shown that Soy bean fortified snacks can meet the nutrient requirements of school going children by half. Serrem, De Kock, and Taylor (2011) reported that consumption of 1, 2 and 3 biscuits with a 1:1 ratio of sorghum or bread wheat with defatted soy flour met the target of providing 7 g protein in 28 g biscuit weight, where this would provide half the protein intake for children aged 1 to 2, 3 to 10, and 10 to 18 years, respectively. Mohsen, Fadel, Bekhit, Edris, and Ahmed, (2009) reported that 100 g of wheat biscuits supplemented with 20% isolated soy protein would provide the recommended daily requirement for protein according to WHO (2007). Protein is an essential macronutrient for growth and maintenance of body tissues and is required in great amounts by school going children as they are still growing and that high physical activity in this age group increases their protein requirements.

Substitution of the snack with Soy bean flour significantly reduced the carbohydrate content of the snacks. At 30 and 50% Soy substitution, carbohydrate content of the maize meal snacks reduced by 23.3 and 29.7%, respectively. The decrease can be attributed to the low carbohydrate content of legumes such as Soy bean that have content of up to 29% (USDA, 2018). Therefore compositing Soy bean with the maize might have diluted the carbohydrate content Serrem, De Kock, and Taylor (2011). Several workers have reported similar results. Kamau *et al.*, (2015) found that substituting 30% Soy bean in complementary porridges made from millet, maize, sorghum, cassava and banana significantly decreased the carbohydrate content. Similarly, Kayitesi *et al.*, (2010) while working with sorghum flour fortified with 30% marama beans, which have low carbohydrate content, reported significant decrease in carbohydrates by 22.7%.

There was a significant increase in the energy content of the snack as a result of increase in the Soy bean content at 50%. However, there was no significant increase in the energy content at 30% fortification. In the unfermented sample, there was a 1.56% increase in the energy content. The energy content of the Soy bean fortified snacks ranged from 1723-1775 Kcal/100 g, which is almost four times the recommended minimum quantity of 400 kcal for supplementary feeding for young children (FAO/WHO, 1994). This is explained by the increase in fat content, which provides higher energy density of 37 Kj/g and also the decrease in the carbohydrate content of the snacks.

High dietary energy is important for sparing protein for body building and repairing body tissues avoiding diversion to provide energy (Michaelsen *et al.*, 2009). The FAO/WHO (1994) Codex Alimentarius Commission recommends that protein-energy in foods for pre-school children should not be less than 15%. Therefore one serving of 100 g of the snack would provide 20% of the energy requirements for a 4 to 6 year old child, which is approximately 312 Kcal (FAO, 2004). Similar results have been found by other researchers. Serrem, De Kock, and Taylor (2011) found that inclusion of fat from Soy bean significantly increased the energy density of sorghum based biscuits therefore meeting the ranges for energy requirements for school going children.

Fortification with Soy bean flour significantly increased the ash content by 200% and 284.62% for the unfermented samples at 30% and 50 % levels of fortifications respectively. The increase in ash content through complementation with Soy bean is that Soy bean flour has a higher mineral content than maize (USDA, 2018). Soy bean flour contains high amounts of potassium, moderate levels of calcium, phosphorus and magnesium and traces of selenium, iron, zinc and sodium.

Similar results were reported by Serrem, De Kock, and Taylor (2011) where fortification of sorghum biscuits with defatted Soy bean flour ranged from 28.6 to 71.4% relative to cereal flour increased ash (mineral) content of sorghum- and bread wheat-based biscuits by 50 to 136% and 200 to 520%, respectively compared to the 100% cereal biscuits.

4.2 Percent contribution of Energy and Protein content of Soy fortified fermented maize meal snack per 100 g toward RDA of children aged 0.5 to 10 years

Table 3 shows the contribution of the soy fortified fermented maize meal snack to the Recommended Daily Allowance (RDA) of protein and energy for children aged 0.5 to 10 years. Results show that 100 g of the unfortified snack whether fermented for 3 or 5 days (*Mkarango*) or unfermented did not meet half the protein RDA for children of all ages with the highest at 48.5%. Additionally, the contribution reduced to less than quarter (21.8-24.5%) of the RDA as the children's age increased to 10 years. The energy contribution also reduced with age from 45-46.7% at 0.5 to 1 year to 19.1-19.8% at 7 to 10 years. The reduction in energy and protein is due to increased requirements for metabolism, growth and development (Thompson, Manore, & Vaughan, 2011). Compositing maize: soy at 70:30 and 50:50 dramatically increased the snacks contribution ranging from 14 to 55%, respectively above the RDA of protein for children aged 0.5 to 3 years. For children aged 4 to 10 years more than half 65 to 90% of RDA can be met by the fortified snacks. The contribution of protein above the RDA for 0.5 to 3 year olds is within tolerable limits and not toxic as the recommended intake should not be more than twice the RDA (Food and Nutrition Board, 1989). From these findings, the fortified *mkarango* more than adequately meets half of the protein intake for children aged 0.5 to 10 years.

Table 4.2: Percent Contribution of energy and protein content from 100 g of soy fortified fermented maize snack to RDA for children aged 0.5-10 years

Nutrient	Age group (years)	RDA ¹	Percent contribution of soy fortified fermented maize snack (<i>mkarango</i>) to RDA								
			Fermented (0) days			Fermented (3) days			Fermented (5) days		
			% Level of fortification with soy bean flour								
			0	30	50	0	30	50	0	30	50
Energy/kJ/day	0.5-1	3556.4 (850)	46.0	46.1	46.7	45.9	45.8	46.1	45.0	45.8	46.1
	1-3	5439.2 (1300)	30.1	30.1	30.5	30.0	30.0	30.2	29.4	30.0	30.2
	4-6	7531.2 (1800)	21.7	21.8	22.1	21.7	21.6	21.8	21.3	21.7	21.9
	7-10	8368 (2000)	19.5	19.6	19.8	19.5	19.5	19.6	19.1	19.5	19.6
Protein g/day	0.5-1	14	43.5	138.0	155.0	45.0	130.0	150.7	48.5	131.4	152.9
	1-3	16	38.1	121.3	135.0	39.3	113.8	131.9	42.5	115.0	133.1
	4-6	24	25.4	80.8	90.4	26.3	75.8	87.9	28.3	76.7	88.8
	7-10	28	21.8	69.3	77.5	22.5	65.0	75.3	24.3	65.7	76.1

Samples are fortified fermented maize snacks with 0, 30 and 50% soy replacement, fermented for 0, 3 and 5 days.

¹Food and Nutrition Board (1989), figures in parentheses are energy/kcal/day.

4.3 Functional properties

The functional properties of the snack are shown in table 4.2. There was a 3 and 10% reduction in acidity due to fortification at 30 and 50%, respectively of soy replacement of maize. There was also substantial reduction in the pH of snacks as a result of fermentation in both the unfortified and fortified samples. For example, compared to the unfortified sample, pH decreased by 29 and 31% on days 3 and 5, respectively while in the 30% soy replacement sample, the reduction was 33 and 26% for days 3 and 5, respectively. In contrast the titratable acidity increased as a result of fortification with soy and fermentation. Titratable acidity increased by 50 and 75% due to replacement of maize with soymeal at 30 and 50%, respectively. Increased fermentation also increased the titratable acidity. For days 3 and 5 of fermentation, increases in titratable acidity were 50 and 67% (unfortified), 51 and 66% (30% soy replacement) and 49 and 53% (50% soy replacement), respectively.

The reduction in pH and increased titratable acidity may be a result of hydrolysis of carbohydrates followed by increase in the concentration of fatty acids, phosphoric acids, hydrogen ions (H^+) and the carboxyl groups of protein amino acids following the fermentation process (Roger, Ngouné Léopold, and Carl Moses Funtong, 2015). Studies by a number of researchers are in agreement with the findings from this study; Owusu-Kwarteng, Akabanda, and Glover (2010) during Soy bean fortification of *Hausa Koko*, a Ghanaian fermented porridge, found significant reduction in the pH as the quantities of Soy bean in the porridge increased. A similar trend was observed by Omemu et al., (2018) who reported increase in pH from 3.47 to 4.27 and increase in total titratable acidity from 0.47% to 0.54% after 96 hours, in fermentation of maize *ogi* fortified with pigeon pea.

The increase in pH due to fortification could also be attributed to the buffering effect of proteins as a result of the higher content of amino acids contributed by the Soy beans (Plahar, Nti, and Annan, 1997). Lactic acid bacteria whose metabolites result in increase in titratable acidity and reduced pH are inhibitory to many other microorganisms (Adams, 1990). This forms the basis of the increased shelf life and improved microbiological safety of lactic acid fermented foods due to production of organic acids, carbon dioxide, ethanol, hydrogen peroxide, diacetyl, antifungal compounds (free fatty acid), bacteriocins and antibiotics (Powell, 2006). This is of particular benefit to foods used for school feeding and stored at ambient temperatures. The bulk density of the snacks ranged between 0.52-0.8 g/mL. Fermentation decreased the bulk density by 13 and 25% for samples fermented for 3 and 5 days, respectively. It is possible that the reduction is a reflection of the activity of alpha-amylase enzyme activated during the fermentation process and dextrinization of starch to its constituent sub-units, resulting in reduced fiber content (Chelule *et al.*, 2010).

A similar study by Ojokoh and Bello (2014) showed that there was significant reduction in fiber content of millet after fermentation for 72 hours demonstrating that fermentation reduces the fiber content of cereals, which in turn reduces the bulk density of the flour. Similar results have been reported. Alka, Neelam, and Shruti, (2012) also found significant reduction in bulk density of sorghum, pearl millet and maize after 120 hours of fermentation.

Further, fortification of the samples with soy meal at 30 and 50% fermented for 3 days decreased the bulk density by 14 and 29% respectively, whereas snacks fortified with soy meal at 30 and 50% fermented for 5 days had 20 and 35% decreases, respectively.

Table 4.3 Functional properties of the Soy bean fortified fermented maize snack

Maize/soy snack / Fermentation days	pH	Titratable acidity	Bulk density (g/mL)	Water absorption capacity (g/mL)	Oil absorption Capacity (g/mL)
Maize/Soy					
Fermented (0) days					
100:0	5.85±0.01 ^g	0.17±00 ^a	0.85±00 ^h	137.28±00 ^a	2.73±0.00 ^g
70:30	6.09±0.01 ⁱ	0.23±00 ^b	0.75±00 ^f	140.39±00 ^b	1.56±0.01 ^f
50:50	5.92±0.01 ^h	0.45±00 ^d	0.64±00 ^c	142.45±00 ^d	1.25±0.00 ^d
Fermented (3) days					
100:0	4.18±0.02 ^b	0.21±01 ^b	0.77±00 ^g	140.35±01 ^b	4.70±0.01 ^h
70:30	4.63±0.01 ^e	0.42±06 ^d	0.70±00 ^d	142.32±00 ^c	1.46±0.01 ^e
50:50	4.69±0.01 ^f	0.78±00 ^f	0.58±00 ^b	145.43±00 ^f	1.11±0.00 ^b
Fermented (5) days					
100:0	4.07±0.01 ^a	0.30±00 ^c	0.70±00 ^e	142.46±00 ^d	5.32±0.02 ⁱ
70:30	4.43±0.01 ^c	0.60±00 ^e	0.64±00 ^c	144.44±00 ^e	1.18±0.04 ^c
50:50	4.53±0.01 ^d	0.87±00 ^g	0.52±00 ^a	146.43±00 ^g	1.09±0.00 ^a

Values are means± standard deviation. Values with the same superscript on the same column are not significantly different at (P<0.05) as assessed by Least significant difference

The unfortified maize grain had the highest bulk density. This may be explained by the whole meal maize used in this study, hence, the high fiber content (USDA, 2018). The decreased bulk density of the snack is an advantage in the preparation of foods for children as they should have a low bulk density to enhance nutrient and calorie density per feed (Akpata and Akubor, 1999), hence fermentation and fortification are useful traditional methods of preparing low bulk density children's foods (Mbata, Ikenebomeh, and Ezeibe, 2009).

Increase in fermentation days led to a gradual and significant increase in the water absorption capacity of the snack. Snacks fermented for 3 days had 1.4 and 3.6% increase in the water holding capacity at 30 and 50% levels of fortification, while those fermented for 5 days had 1.4 and 2.8% increase at 30 and 50% levels of fortification respectively. Increase in the fermentation days possibly led to increase in the number of micro-organisms, with proteolytic activity resulting in increased availability of protein functional groups in the flour, which may have increased the availability of polar groups in proteins thereby increasing the hydrophilicity of flour proteins (Ohizua *et al.*, 2017).

Fortification also increased the water absorption capacity of the snacks significantly, where at 30% level of soy meal, there was 5.9% increase, while at 50% there was 6.7% increase. Protein enhances water absorption capacity of flours due to its hydrophilic parts, such as polar or charged side chains (Adebowale and Lawal, 2004) accounting for the increased water absorption capacity as the fortification level increased. Water absorption capacity is a functional property that indicates the degree to which water can be added to a food, and also gives an indication of the amount of water available for gelatinization (Ohizua *et al.*, 2017). This is important in this study as the snack requires hydration prior to consumption by the school children.

The oil holding capacity of the snacks ranged from 0.9-1.5 g/mL with the least being the sample fortified with Soy bean, fermented for five days, while the highest was the unfermented maize meal. Fortification significantly increased the oil holding capacity by 25% for 30% fortification and 27% for 50% fortification. The increase in the oil holding capacity may be attributed to the high oil content in soy meal (USDA, 2018). The increase in the oil holding capacity is desirable for foods meant to be used for supplementary feeding because oil is energy dense.

4.4 Descriptive sensory analysis

Analysis of variance of the (*F*- values) for the snack's profile data of the 31 attributes scored by the descriptive sensory panel (12 panelists) showed significant differences ($p \leq 0.05$) among the snack types for 21 attributes (Table 4.3). The data were further analyzed by Principal Component Analysis (PCA) to determine the systematic variation and underlying relationships among the functional and sensory attributes of the snacks made from flours of varying maize-Soy bean ratios and fermented for 0, 3 or 5 days.

Principal component analysis explained 86% of the variation among the snacks resulting from fermentation and fortification. The first two principal components explained 74% of the variation among the 9 Soy bean fortified fermented snacks

Table 4.4 Mean scores for sensory attributes of Soy bean fortified fermented maize meal snack as evaluated by a trained descriptive sensory panel (n=12)

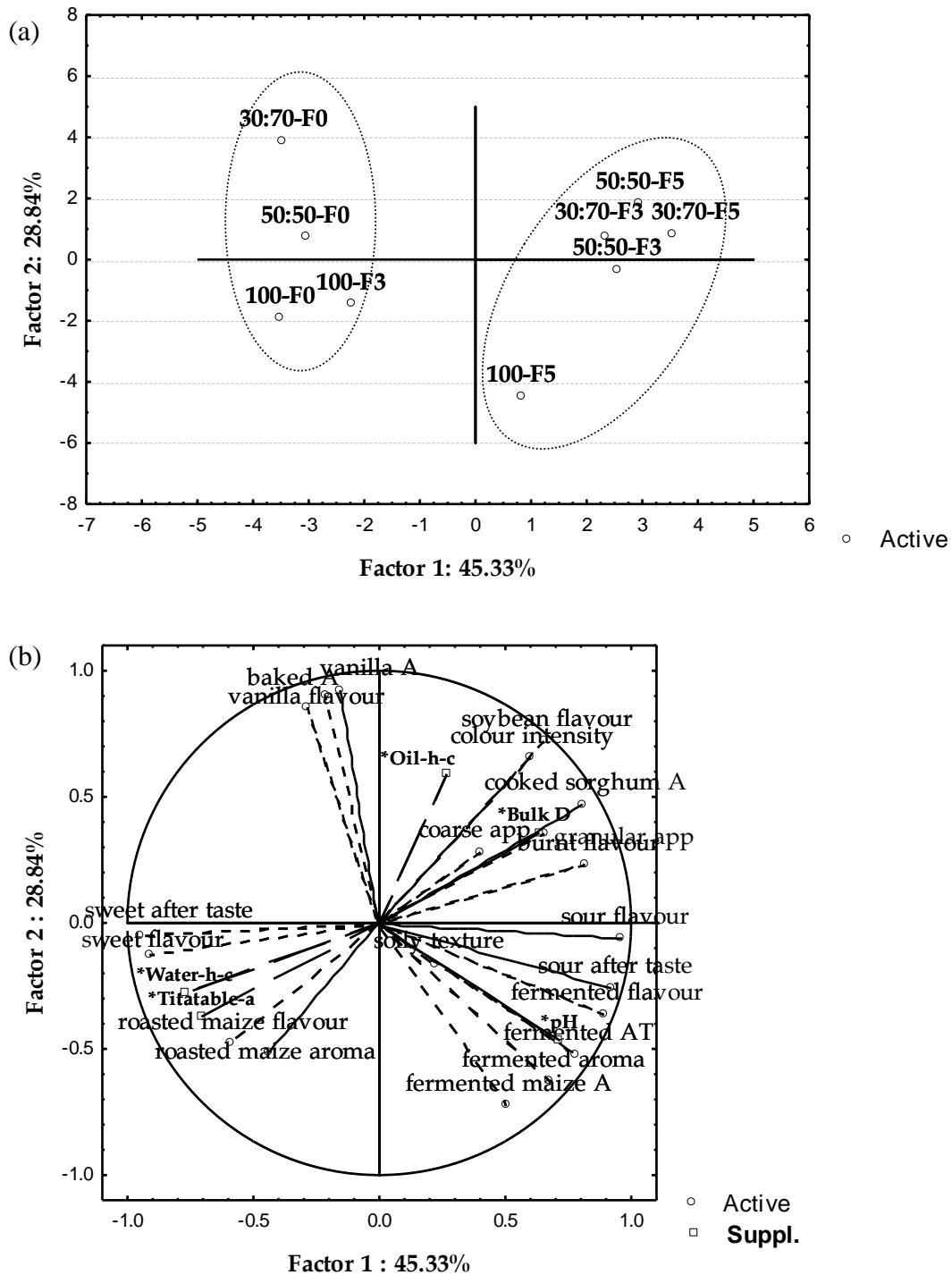
Attributes	Unfermented			3 fermentation days			5 fermentation days			Sample effects
	Soy bean levels (%)			Soy bean levels (%)			Soy bean levels (%)			<i>f values</i>
	0	30	50	0	30	50	0	30	50	
Appearance										
Colour intensity	3.9 ^b ±1.6	6.1 ^c ±1.2	6.7 ^c ±1.2	2.8 ^a ±1.8	8.0 ^d ±1.2	8.0 ^d ±1.1	3.4 ^{ab} ±2.6	7.4 ^d ±1.3	7.8 ^d ±0.9	63.8*
Granular appearance	8.3 ^{abc} ±1.1	8.4 ^{bc} ±1.1	7.7 ^a ±1.7	8.2 ^{abc} ±1.0	8.6 ^c ±1.2	8.3 ^{abc} ±1.4	8.0 ^{ab} ±1.2	8.7 ^c ±1.2	8.3 ^{bc} ±1.2	2.0*
Varying sizes	8.8 ^b ±1.2	8.6 ^b ±1.4	7.6 ^a ±2.0	8.1 ^{ab} ±1.8	8.6 ^b ±1.5	8.2 ^{ab} ±1.4	8.3 ^{ab} ±1.5	8.3 ^{ab} ±1.7	8.2 ^{ab} ±1.5	1.4ns
Coarse appearance	8.8 ^{bc} ±1.0	8.8 ^{bc} ±1.3	8.3 ^{ab} ±1.9	8.1 ^a ±1.3	9.0 ^c ±1.1	8.6 ^{abc} ±1.1	8.4 ^{abc} ±1.3	8.8 ^{bc} ±1.1	8.6 ^{abc} ±1.2	2.3*
Rough appearance	8.9 ^c ±1.1	8.7 ^{bc} ±1.3	8.0 ^{ab} ±1.6	8.5 ^{abc} ±1.4	8.5 ^{abc} ±1.7	8.0 ^{ab} ±1.8	8.3 ^{abc} ±1.5	7.8 ^a ±2.0	8.2 ^{abc} ±1.4	1.3ns
Irregular shapes	8.5 ^a ±1.5	8.7 ^a ±1.4	8.1 ^a ±2.1	8.1 ^a ±1.7	8.7 ^a ±1.4	8.4 ^a ±1.4	8.1 ^a ±1.5	8.4 ^a ±1.7	8.2 ^a ±1.8	0.7ns
Aroma										
Roasted maize aroma	7.4 ^c ±1.6	6.3 ^{ab} ±1.9	6.7 ^{bc} ±2.0	6.6 ^{bc} ±1.8	7.0 ^{bc} ±2.2	6.3 ^{ab} ±2.4	6.7 ^{bc} ±1.8	6.17 ^{ab} ±2.0	5.5 ^a ±2.6	2.3*
Fermented aroma	3.8 ^a ±2.5	3.2 ^a ±2.5	4.0 ^{abc} ±2.3	5.3 ^{de} ±2.7	5.2 ^{cde} ±2.4	4.9 ^{abc} ±2.5	6.3 ^e ±2.4	5.4 ^{de} ±2.0	5.0 ^{cd} ±2.5	5.4*
Baked aroma	3.0 ^{ab} ±1.8	4.4 ^c ±2.7	3.1 ^{ab} ±2.4	3.3 ^{ab} ±1.9	3.4 ^{abc} ±2.3	3.0 ^{ab} ±2.0	2.4 ^a ±2.1	3.3 ^{abc} ±2.4	3.5 ^{bc} ±2.1	2.2*
Soy bean aroma	3.4 ^{ab} ±1.9	4.0 ^{bc} ±1.6	3.2 ^a ±1.5	3.1 ^a ±1.5	3.6 ^{abc} ±1.6	3.8 ^{abc} ±1.7	3.2 ^a ±1.5	3.5 ^{abc} ±1.7	4.3 ^c ±1.6	1.6ns
Vanilla aroma	2.5 ^{ab} ±1.6	4.6 ^d ±2.5	2.9 ^{abc} ±2.0	3.0 ^{bc} ±1.7	3.3 ^{bc} ±2.5	2.8 ^{abc} ±2.0	1.9 ^a ±1.8	2.9 ^{abc} ±2.1	3.5 ^{cd} ±1.7	5.0*
Fermented maize aroma	3.9 ^{ab} ±2.5	3.4 ^a ±2.4	3.9 ^a ±2.1	5.6 ^{cd} ±2.6	5.0 ^{bc} ±2.2	5.0 ^{bc} ±2.4	6.3 ^d ±2.5	4.8 ^{bc} ±2.1	4.5 ^{abc} ±2.3	5.3*
Cooked sorghum aroma	2.8 ^a ±1.5	3.1 ^{ab} ±2.1	3.2 ^{ab} ±2.3	3.2 ^{ab} ±1.8	3.9 ^{bc} ±2.0	4.0 ^{bc} ±1.8	2.7 ^a ±1.8	4.6 ^c ±2.5	4.0 ^{bc} ±2.0	3.1*
Stiff porridge aroma	5.3 ^c ±2.1	4.5 ^{abc} ±2.3	4.3 ^{abc} ±2.1	5.0 ^{bc} ±1.6	4.2 ^{ab} ±2.3	3.9 ^a ±1.9	4.9 ^{abc} ±2.4	4.7 ^{abc} ±2.1	4.2 ^{ab} ±1.8	1.5ns

Values are means±standard deviations. Values in a row followed by different letter notations ^(a - e) are significantly different at p ≤ 0.05, *p ≤ 0.05, ns, not significant

Table 4.4 Mean scores for sensory attributes of Soy bean fortified fermented maize meal snack as evaluated by a trained descriptive sensory panel (n=12) (Cont')

Attributes	Unfermented			3 fermentation days			5 fermentation days			Sample effects
	Soy bean levels (%)			Soy bean levels (%)			Soy bean levels (%)			<i>f values</i>
	0	30	50	0	30	50	0	30	50	
Flavour										
Sweet flavour	6.7 ^c ±1.6	6.1 ^{bc} ±1.8	6.2 ^{bc} ±1.6	7.6 ^c ±1.8	4.2 ^{ab} ±2.1	2.7 ^a ±1.7	4.5 ^{ab} ±2.2	3.5 ^a ±1.7	3.3 ^a ±1.6	5.5*
Fermented flavour	3.1 ^a ±2.6	2.8 ^a ±2.5	3.2 ^a ±2.4	4.7 ^b ±2.7	5.6 ^{bc} ±2.3	5.5 ^{bc} ±2.3	6.4 ^c ±2.8	5.7 ^{bc} ±2.3	5.7 ^{bc} ±2.3	10.5*
Vanilla flavour	2.7 ^{ab} ±1.6	4.7 ^d ±2.4	3.2 ^{abc} ±2.0	3.5 ^{ac} ±2.0	3.4 ^{ac} ±2.3	2.6 ^{ab} ±1.8	2.3 ^a ±1.8	3.2 ^{abc} ±2.5	3.9 ^{cd} ±2.1	4.2*
Sour flavour	1.8 ^a ±2.0	5.7 ^c ±2.4	2.8 ^{ab} ±2.3	3.7 ^b ±2.1	5.3 ^c ±2.3	5.8 ^c ±2.0	5.2 ^c ±2.8	5.8 ^c ±1.8	5.4 ^c ±2.4	16.5*
Burnt flavour	2.4 ^a ±2.3	2.4 ^a ±2.2	3.8 ^{bc} ±2.5	2.3 ^a ±1.8	4.5 ^c ±2.1	4.8 ^c ±2.3	2.7 ^{ab} ±1.8	4.5 ^c ±2.7	3.7 ^{bc} ±2.3	7.6*
Roasted maize flavour	6.8 ^c ±2.0	6.0 ^{bc} ±2.2	5.5 ^{ab} ±2.2	5.7 ^{abc} ±2.5	6.11 ^{bc} ±2.3	5.5 ^{ab} ±2.2	6.1 ^{bc} ±2.2	4.7 ^a ±2.7	5.0 ^{ab} ±2.4	2.1*
Soy bean flavour	2.9 ^a ±1.4	3.6 ^{abcd} ±1.6	3.3 ^{abc} ±1.9	3.3 ^{abc} ±1.5	3.9 ^{cd} ±1.7	3.7 ^{abd} ±1.5	3.0 ^{ab} ±1.6	3.8 ^{cd} ±1.6	4.2 ^d ±1.6	2.1*
Stiff porridge flavour	5.4 ^c ±2.2	4.6 ^{abc} ±2.2	4.2 ^{ab} ±1.8	4.5 ^{abc} ±1.9	4.6 ^{abc} ±2.5	3.6 ^a ±2.3	4.8 ^{bc} ±2.6	3.8 ^{ab} ±1.6	4.8 ^{bc} ±1.9	1.7ns
Baked flavour	4.9 ^a ±2.3	4.0 ^a ±2.7	4.0 ^a ±2.2	4.6 ^a ±2.8	3.8 ^a ±2.1	3.9 ^a ±2.4	3.8 ^a ±2.6	3.8 ^a ±2.7	4.2 ^a ±2.1	0.7ns
Texture										
Rough texture	9.0 ^b ±1.2	8.3 ^{ab} ±1.7	8.0 ^a ±2.0	8.5 ^{ab} ±1.3	8.4 ^{ab} ±1.5	8.6 ^{ab} ±1.3	8.6 ^{ab} ±1.4	8.5 ^{ab} ±2.0	8.6 ^{ab} ±1.5	0.9ns
Grainy texture	9.1 ^b ±0.9	8.6 ^{ab} ±1.6	8.5 ^{ab} ±1.7	8.8 ^{ab} ±1.0	8.7 ^{ab} ±1.7	8.6 ^{ab} ±1.1	8.4 ^a ±1.6	8.3 ^a ±1.8	8.7 ^{ab} ±1.3	1.2ns
Soily texture	8.3 ^b ±1.9	7.5 ^{ab} ±2.1	6.9 ^a ±2.8	7.0 ^a ±2.4	7.5 ^{ab} ±1.9	7.7 ^{ab} ±1.9	7.6 ^{ab} ±2.0	7.0 ^a ±2.2	7.8 ^{ab} ±1.9	2.0*
Crunchy texture	9.4 ^c ±0.7	8.8 ^{ab} ±1.3	8.3 ^a ±1.5	8.9 ^{abc} ±1.1	8.7 ^{ab} ±1.4	9.0 ^{bc} ±1.1	8.9 ^{abc} ±1.0	9.1 ^{bc} ±0.9	8.8 ^{ab} ±1.3	1.9ns
After taste										
Grainy after taste	8.6 ^a ±1.9	8.4 ^a ±1.5	8.1 ^a ±2.4	8.3 ^a ±1.9	8.2 ^a ±1.5	8.1 ^a ±1.7	8.6 ^a ±1.7	8.5 ^a ±1.9	8.7 ^a ±1.3	0.4ns
Sour after taste	4.1 ^{ab} ±3.5	3.0 ^a ±2.9	3.5 ^a ±3.0	3.7 ^a ±2.7	5.6 ^c ±2.7	5.1 ^{bc} ±2.6	5.5 ^{bc} ±2.9	6.0 ^c ±2.7	6.0 ^c ±2.5	4.7*
Sweet after taste	5.5 ^e ±2.4	5.4 ^e ±2.8	5.5 ^e ±2.6	5.2 ^{de} ±2.3	3.8 ^{abc} ±2.4	4.4 ^{cde} ±2.7	4.2 ^{bcd} ±2.5	3.2 ^{abc} ±1.8	3.0 ^a ±1.9	3.9*
Fermented after taste	4.5 ^{ab} ±3.1	3.3 ^a ±3.3	3.4 ^a ±2.7	4.6 ^{ab} ±2.9	5.4 ^{bc} ±2.8	5.1 ^{bc} ±2.4	6.3 ^c ±2.8	5.4 ^{bc} ±2.4	5.7 ^{bc} ±2.8	4.3*

Values are means±standard deviations. Values in a row followed by different letter notations ^(a - e) are significantly different at $p \leq 0.05$, * $p \leq 0.05$, ns, not significant



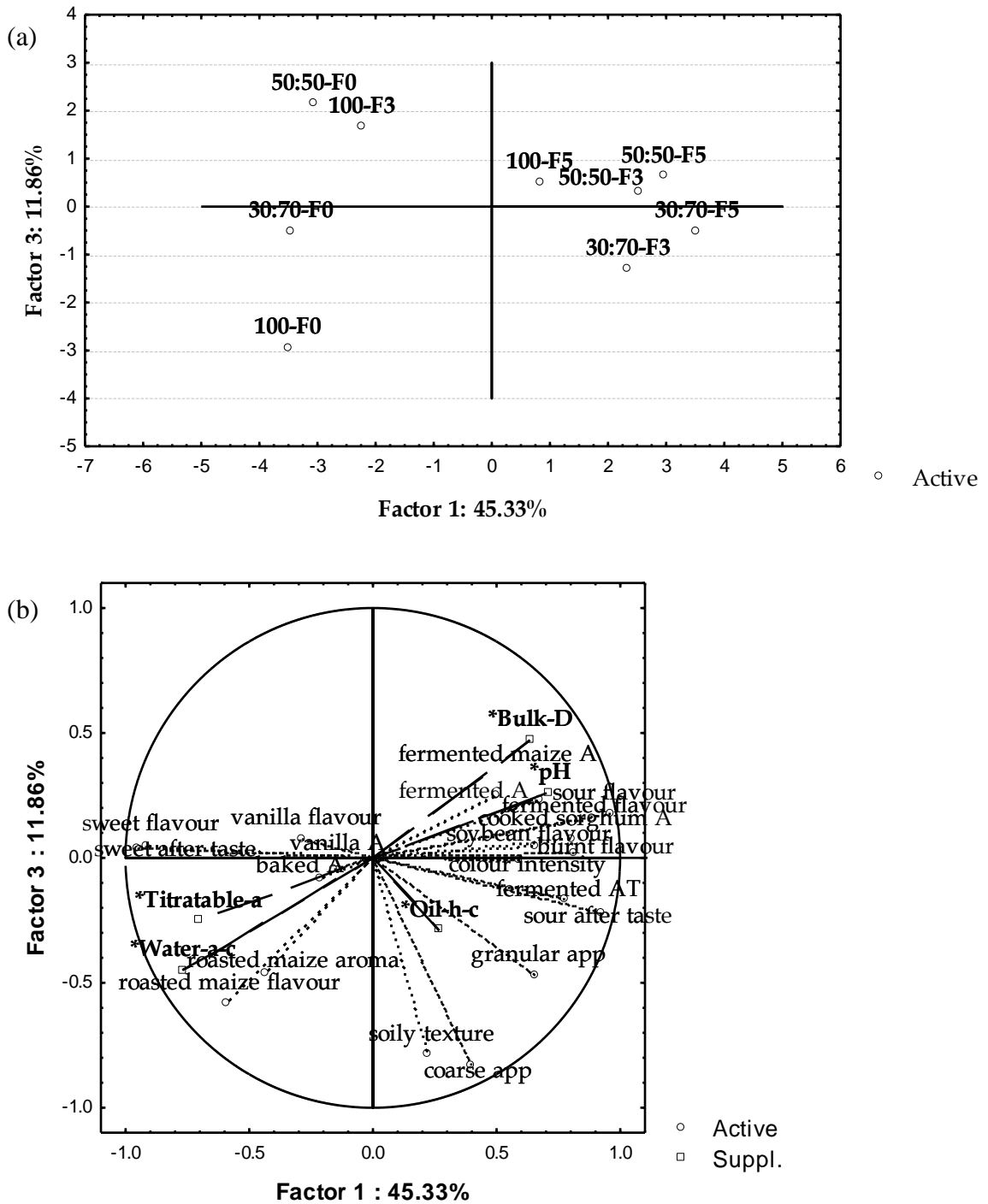


Figure 4.1 Principal component analysis (correlation matrix) of variations of soy fortified fermented maize snack

(a) Plot of the first and third principal component scores of the snacks, (b) Plot of the first and third principle component loading projections of sensory attributes. Acronyms for Soy:maize ratio :(100=maize100%; 30:70=soy30:maize70; 50:50=soy50:maize50). Acronyms for fermentation: F0= 0-days-fermentation; F3= 3-days-fermentation; F5= 5-days-fermentation. A=aroma, T=texture, F=flavour, AP=appearance, AT=aftertaste.

(Figure 4.1). Figure 4.1a shows the first two principal component scores of the fortified fermented snacks. PC1 explained 45% of the variation and separated the snacks based on fermentation with the fermented snacks to the left and the unfermented snacks to the right. PC2 accounted for an additional 29% of the total variation and separated the soy fortified snacks from the unfortified ones with the Soy bean fortified variations at the top and the unfortified pure maize snacks at the bottom. The third principal component was used to further explain the cause of variations since the first two only explained 74%. Figure 4.2 shows the first and third principal components. PC3 explained 12% of the variation, and separated snacks according to the level of fortification with samples fortified with 30% Soy bean at the bottom and 50% Soy bean samples at the top.

The first principal component (PC1) shows that attributes related to the samples fermented for 3 or 5 days, that include, maize aroma, fermented aroma, fermented after taste, sour after taste and sour flavour were positively correlated to each other. These attributes were negatively correlated to those of sweet and vanilla flavours and sweet after taste associated with unfermented samples. These sour and fermented characteristics are due to the accumulation of lactic acid bacteria as a result of fermentation (Narayanan, Roychoudhury, and Srivastava, 2004). The vanilla flavour and aroma was caused by the addition of vanilla essence. Furthermore, these samples were not fermented therefore there was no volatile compounds produced making vanilla flavour dominant. The attributes that were positively correlated in PC2 were Soy bean flavour, colour intensity, burnt flavor as well as coarse and granular appearance and characterized the Soy bean fortified snacks. These were negatively correlated with roasted maize and vanilla flavours and roasted maize aroma. The beany flavour is commonly found in food legumes.

According to Yang, Smyth, Chaliha, and James (2016) enzymatic breakdown of Soy beans by lipoxygenases or autoxidation of polyunsaturated lipids (linoleic and linolenic acids) produces hydroperoxides such as ketones, aldehydes and alcohols that may be responsible for the green, beany, grassy, painty, or cardboardy flavours. These off flavours discourage Soy bean consumption (Agengo *et al.*, 2015). Jeleń, Majcher, Ginja, and Kuligowski (2013) while working with tempeh attributed the beany flavour to a mixture of volatile compounds such as methyl-1-butanol, hexanal, 2, 4-decadienal, dimethyl. Yang *et al.*, (2016) identified hexanal as major among the volatile compounds contributing to the beany/rancid/off-flavour in tofu. In this study the score for the Soy bean flavour increased with increase in the amount of the Soy bean substitution in the snack. This could be attributed to increased availability of polyunsaturated fatty acids from lipids which favoured auto-oxidation considering that full fat Soy bean was used.

Kobayashi, Tsuda, Hirata, Kubota, and Kitamura, (1995) reported that there was a significant increase in the total volatiles in full fat soy meal after 1 week storage at 37⁰C. Yang *et al.*, (2016) showed that use of full fat soy in the production of tofu, increased the amount of hexanal, a compound responsible for the beany aroma. This could have been similar in this study. Studies on sensory description of Soy bean-based products have also found the beanny attribute in products. These include studies by Kustyawati, Nawansih, and Nurdjanah (2017) while working with niwoymodified tempeh prepared by addition of *Saccharomyces cerevisiae* and Serrem, De Kock, and Taylor (2011) who reported that biscuits with 71.4 or 100% soy had roasted soy bean flavour, aroma and aftertaste. Snacks fortified with 30% and 50% Soy bean were associated with burnt flavour.

It is possible that the high level of protein contributed by Soy bean readily reacted with sugar in the Maillard reaction (Serrem, De Kock, *and* Taylor, 2011), hence this attribute was negatively correlated with sweet flavour and after taste, roasted maize aroma and flavour associated with samples with no soy. The Maillard reaction involves condensation of reducing sugars with a free amino group such as lysine forming N-substituted glycosylamine. N-substituted glycosylamine is unstable. With increased temperatures because of its instability it undergoes the amadori rearrangement forming ketosamines (Martins, Jongen, *and* Van Boekel, 2000). Further reaction with amino acid forms brown nitrogenous polymers and co-polymers known as melanoidins that may result in undesirable flavours such as bitterness or a burnt aroma and flavour. Furthermore, fermentation increases the susceptibility of the substrate to Maillard reaction probably as a consequence of protein and starch hydrolysis (Yang *et al.*, 2016).

Serrem, De Kock, *and* Taylor (2011) found that a burnt flavour was positively correlated with protein content of Soy bean fortified biscuits. Similarly, Mohsen *et al.*, (2009) reported biscuit-like and burnt odour in biscuits when 2-ethyl-5-methylpyrazine increased after substituting wheat with 20% soy protein isolate.

Snacks with more than 30% Soy bean substitution were described as having high colour intensity. The colour intensity may have been contributed by Maillard reaction as explained earlier. The final products of the Maillard reaction are melanoidins and they are also responsible for the brown colour development (Bastos *et al.*, 2012). Maillard reactions are dependent on time, temperature, reactant concentration and pH (Wong, Abdul Aziz, *and* Mohamed, 2008).

The frying temperature could have had an influence on the brown colour development since it increases the activation energy for the carbonyl-amino acid groups to interact

and react, favouring Maillard reactions (Wong *et al.*, 2008). The colour intensity could have been also attributed to increase in the amount of reducing sugar levels in the LAB fermented maize.

While working with deep fried fermented maize-Soy bean pastes, Ng'ong'ola-Manani *et al.*, (2014) attributed the brown colour in lactic acid fermented maize pastes to caramelization due to the presence of reducing sugars and increased protein content as a result of fermentation. Omoba, Taylor, and De Kock, (2015) while working with biscuits made from sour dough of whole grain sorghum and pearl millet, attributed the brown colour of the biscuits to browning reactions due to the amyolytic action of sough dough fermentation. Furthermore, the browning could have been favoured by the pH of the samples. A pH media of less than 6 offers stability of amino acids during frying in presence of reducing sugars (Ajandouz and Puigserver, 1999).

Furthermore, fortification of cereals with Soy bean protein significantly increases the lysine content (Serrem, De Kock, and Taylor, 2011), an amino acid that plays an important role in brown colour development in baked products. While working with gluten free bread fortified with soy protein isolates (Fujiwara, 2014) found that lysine readily reacts with reducing sugars resulting in brown colour formation. This could have been the case in this study.

The snack was described as having granular and coarse appearance. This could be attributed to the use of whole kernel dry milling method that attempts to grind the kernel to uniform sizes, and does not fractionate the maize kernel into the germ, pericarp and the endosperm components (Rausch and Eckhoff, 2016).

Therefore, since the maize and the Soy beans were processed whole, the granular and the coarse appearance could have been contributed by the presence of pericarps in the

flour. According to Pan, Eckhoff, Paulsen, *and* Litchfield (1996), dry milling of maize increases pericarp yields in the flour due to an incomplete separation of the pericarp and the endosperm.

Ngo'ngo'la-Manani *et al.*, (2014) found that roughness intensity increased in products with maize, which accounted for rough appearance and large particle sizes in a fermented maize-Soy bean based snack. Serrem, De Kock, *and* Taylor (2011) found that the presence of bran fragments, visible as white specs on the surface of sorghum biscuits was positively correlated with rough and coarse characteristics. Similarly, Dlamini (2016) while working with a sorghum-cowpea snack found that compositing 50:50 sorghum-cowpea blended snacks increased their roughness possibly due to high fiber content.

Snacks fortified with 30% and 50% Soy bean and unfermented were described as having baked aroma and roasted cereal aroma and flavour. These aroma and flavours could be attributed to derivatives of Maillard reaction favoured by the high frying temperature. Similar results were reported by Serrem, De Kock, *and* Taylor (2011) where sorghum biscuits with DSF replacement of 50% and below had roasted cereal flavour and aroma. Bredie, Mottram, Hassell, *and* Guy (1998) showed that when pyrazines increased in thermally treated maize and wheat flours, roasted/toasted flavour developed. Attributes positively correlated to 50% soy meal: 50% maize 5 fermentation days shown in PC3 were fermented aroma, fermented maize, soy flavour, cooked sorghum aroma, Soy bean flavour and burnt flavour. These attributes may be explained by the factors related to increased soy and fermentation as explained earlier.

4.5 Consumer evaluation by adults

Consumer perception of the sensory attributes of appearance, smell, flavour and texture for the soy fortified and fermented maize meal snacks are shown in table 4.3. The 100% maize snack fermented for 3 days and the unfermented snacks with 0 and 30% soy bean scored highest for colour while the 50:50 soy: maize snack fermented for 5 days was the least liked.

The low score was probably a result of consumers' unfamiliarity with the intense brown colour due to the increased percentage of Soy bean flour. The traditional *Mkarango* is light brown. The study by Ng'ong'ola-Manani *et al.*, (2014) also established that brown colour in a Soy bean fortified maize based fermented snack was one of the major drivers for dislike. Similarly, Otegbayo, Adebisi, Bolaji, and Olunlade (2018) reported decreased general acceptability of soy enriched bread with increased level of Soy bean substitution.

Table 4.5: Effect of fermentation and soy fortification of maize meal on consumer perception of snack sensory attributes

Attribute	Unfermented			3 fermentation days			5 fermentation days		
	Soy bean levels (%)			Soy bean levels (%)			Soy bean levels (%)		
	0	30	50	0	30	50	0	30	50
Appearance	7.6 ^{cd} ±1.6	7.5 ^{cd} ±1.5	7.1 ^b ±1.5	8.0 ^d ±1.0	6.3 ^b ±1.7	5.9 ^b ±1.7	7.3 ^c ±1.3	6.3 ^b ±1.8	5.1 ^a ±2.3
Smell	7.1 ^{ef} ±1.5	7.3 ^f ±1.6	6.6 ^{cde} ±1.5	7.3 ^f ±1.4	6.3 ^{bcd} ±1.6	6.0 ^{abc} ±2.0	6.7 ^{def} ±1.6	5.8 ^{ab} ±2.1	5.5 ^a ±2.1
Flavour	7.7 ^e ±1.7	7.5 ^e ±1.6	6.5 ^{cd} ±1.7	7.5 ^e ±1.3	5.9 ^{bc} ±2.2	5.6 ^b ±2.0	6.9 ^{de} ±2.0	5.8 ^{bc} ±2.5	4.6 ^a ±2.5
Texture	7.1 ^{bc} ±1.7	7.0 ^{bc} ±1.6	6.7 ^{bc} ±1.9	7.4 ^c ±1.4	7.4 ^c ±1.1	5.6 ^{ab} ±1.9	7.0 ^{bc} ±1.6	5.7 ^{abc} ±2.2	4.9 ^a ±2.3
Rank	6.5 ^c ±2.6	5.9 ^c ±2.3	5.6 ^c ±1.9	6.3 ^c ±2.3	4.1 ^b ±2.0	4.1 ^b ±2.6	5.8 ^c ±2.3	3.9 ^b ±2.2	2.9 ^a ±2.2

Values are mean± SD. Values followed by different letter superscripts in a column are significantly different at $p \leq 0.05$ as assessed by Fisher's least significant test. 9= Like extremely, 8= Like very much, 7= Like moderately, 6= Like slightly, 5= Neither like nor dislike, 4= Dislike slightly, 3= Dislike moderately, 2= Dislike very much, 1= Dislike extremely. Consumers n=60.

Consumer liking of aroma/smell and flavour decreased with increase in soy fortification and fermentation days of the snack. Hence, the highest score (7.3) for smell was for 100% maize and 30: 70 soy: maize, both unfermented, while the lowest score was for 50% soy replacement of maize and fermented for 5 days. This high score may be explained by unfermented snacks which did not produce volatile compounds (Kobayashi et al., 1995) like fermented samples. The highest score (7.7) for flavour was 100% maize unfermented, while the lowest score (4.6) was 50:50 maize: soy fermented for 5 days. The high score could be due to release of flavor compounds from Maillard and caramelization reactions during frying and baking of the snacks (Bastos et al., 2012). The process enhances the acceptability of products made from roasted flour, because of dextrinization, and starch breakdown (Mensah and Tomkins, 2003).

The lowest score for taste was 50% Soy bean: 50% maize meal, 5 fermentation days snack. The low score might have been due to the sourness and bitter after taste associated with fermented foods. Furthermore, the type of Soy bean flour used is likely to determine the amount of lactic acid produced during the fermentation process. Use of full fat Soy bean flour produces high acid content (Griffith, Castell-Perez, and Griffith, 1998) and therefore, the low pH in this snack might have contributed to the low score. Farzana, Mohajan, Saha, Hossain, and Haque (2017) found that there was a decrease in consumer liking of flavour of a vegetable soup as a result of increase in Soy bean content.

Snacks made from maize:soy 50:50, fermented for 3 and 5 days were significantly different from the rest in texture. When ranked, the snack made from 50:50 fermented for 5 days was significantly different from the rest and scored lowest (2.9) while 100% maize snacks and 30:70, unfermented were the highest.

The reason for the high score for the unfermented snack could be because of the sweet taste associated with the unfermented sugars. The reason for the 50% Soy bean: 50% maize meal 5 fermentation days snack being least liked could be attributed to the cumulative unfamiliarity of consumers with the appearance, smell and flavour of the product.

The total quality of the Soy bean fortified fermented maize-meal snacks are shown in figure 4.3.

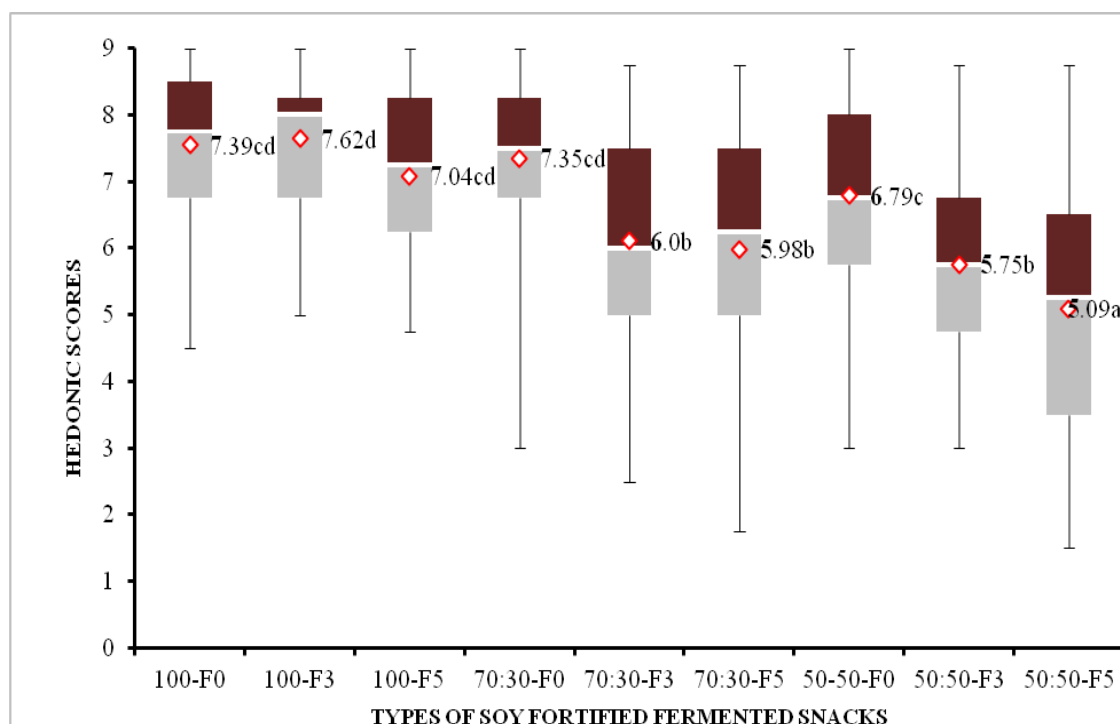


Figure 4.2: Total quality of the Soy bean fortified fermented maize meal snacks

abcd= Mean values with different letter superscripts differ significantly at $p < 0.05$ as assessed by Fisher's least significant test. The dark shaded area is the higher percentile and represents the value above which 75% of the ratings fell. The light shaded area is the lower percentile and represents the area where 25% of the ratings fell. The median is the thin line between the two shaded areas where 50% of the values fell above and 50% below. Overall liking ratings 1=Dislike extremely, 2=Dislike very much, 3=Dislike moderately, 4=Dislike slightly, 5=Neither like nor dislike, 6=Like slightly, 7=Like moderately, 8=Like very much, 9=Like extremely. Consumers $n=60$. The snack made from 100% maize fermented for 3 days was the most acceptable to the consumers with a score of 7.6, while the least accepted was the maize:soy 50:50 snack fermented for 5 days.

4.6 Consumer evaluation by school children

Figure 4.4 shows the liking of the snacks over the four days of evaluation. On the first and the second days, there was no significant difference between likings of the samples. This could be explained by the fact the children may have been excited about the introduction of the snacks. On the third and the fourth days, the liking of the 100% maize 3 days fermentation snack increased compared with the other three samples and this is explained by the leveling of the graph between the second and the fourth day. The 100% maize 3 fermentation days snacks was the most liked over time compared to the rest of the samples. This was the control and is the conventional snack that the children are familiar with compared to the other snacks.

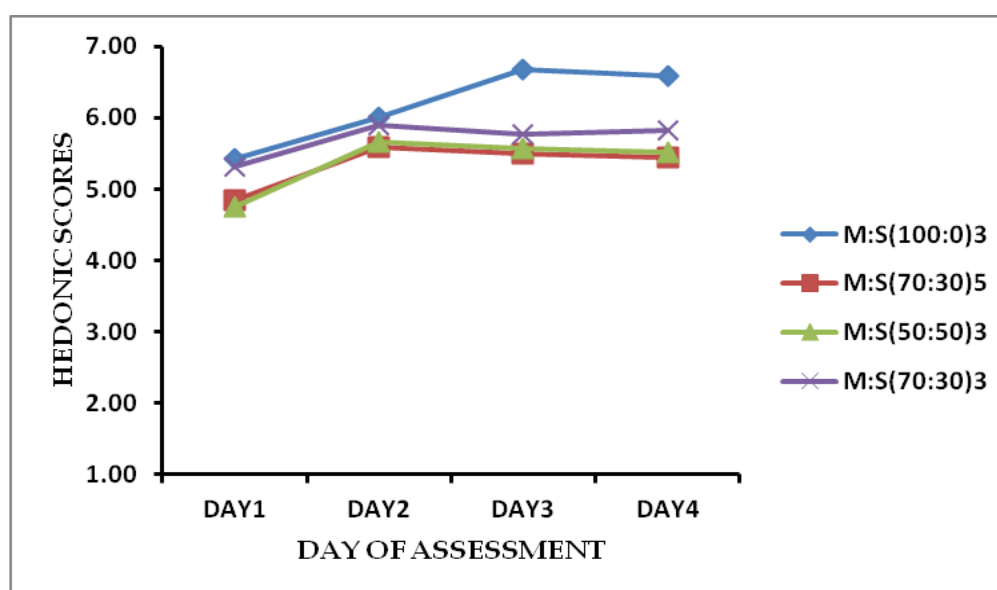


Figure 4.3: Children's' liking of the snacks over time.

M:S (100:0)3-100% maize fermented for 3 days; M:S (70:30) 5-70% maize:30% Soy bean fermented for 5 days; M:S (50:50)3-50% maize:50% Soy bean fermented for 3 days; M:S (70:30) 3-70% maize:30% Soy beans fermented for 3 days

Table 4.5 shows that liking of the snack made from 100% maize fermented for 3 days was significantly higher than all the other snacks when evaluated by 8 to 9-year-old

school children. The reason for the high score may be attributed to the children's familiarity to it as the conventional snack.

The children were probably not familiar with Soy bean as an ingredient in the other three snacks, hence the lower scores. Similarly, Lee (2013) studied the association between the number of unfamiliar vegetables and their choice among elementary school children and found that children had lower preference for the unfamiliar vegetables. Familiarity and familiar associations are pivotal to a child's psychological judgment and physical reaction to foods, novel or otherwise (Aldridge, Dovey, and Halford, 2009). Rejection of a particular food, flavour or dietary choice can be derived from internal or external motivations, hence children like what they know and eat what they like (Cooke, Chambers, Añez, and Wardle, 2011).

Table 4.2: The effect of Soy bean fortification of fermented maize meal on overall liking of snacks by 8 to 9 year old school children

Snacks	Hedonic Score
100% maize 3 days	6.1 ^b ±0.9
30% Soy bean:70% maize 3 days	5.7 ^a ±1.2
30% Soy bean: 70% maize 5 days	5.3 ^a ±1.3
50% Soy bean: 50% maize 3 days	5.3 ^a ±1.2

Values are mean±SD. Values followed by different letter superscripts in a column are significantly different at $P \leq 0.05$ as assessed by Fisher's least significant test. Overall liking ratings 1= dislike extremely, 2= dislike very much, 3= Dislike a little, 4= Not sure, 5= Like a little, 6=Like very much, 7= Like extremely. Consumers n=60.

4.6 Sensory specific satiety

Figure 4.7 shows that the liking for the 50% maize:50% Soy bean snack fermented for 3 days had the highest drop in liking compared to three other samples, following the sensory specific satiety test. According to O'doherty et al., (2000), sensory specific satiety is a decline, in pleasure felt in the course of eating, as reaction to

sensory properties of the currently consumed food. The drop in liking in this study for the 50:50 maize:soy snack fermented for 5 days may be associated with the attributes that made the snack score lowest in both the children and adult hedonic categorization. This may be attributed to the dark colour intensity and intense sour taste characteristics that were found undesirable for *Mkarango* in this study. Conventional *Mkarango* (control) of maize 100% fermented for 3 days is cream in colour and slightly sour. Therefore it is possible that the decrease in pleasure to eat may have developed in the course of eating, when the taste, appearance and other sensory properties became less pleasant to the consumer (Serrem, De Kock, and Taylor, 2011).

Other researchers have also found that consumption of foods with intense sensory characteristics may promote the development of sensory specific satiety. For instance, Vickers and Holton (1998) found a negative association between the flavour intensity of iced tea and intake. Additionally, most children do not like sour taste (Liem and Zandstra, 2009).

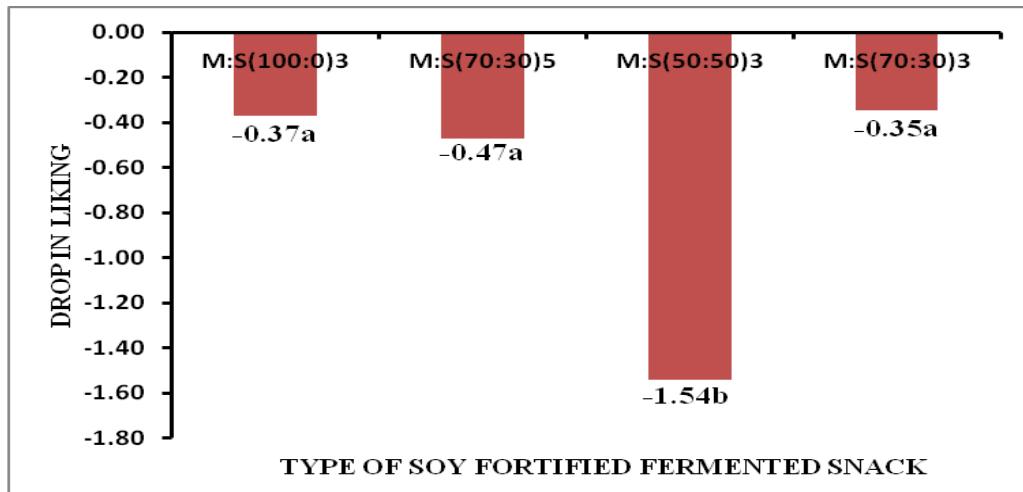


Figure 4.4: Drop in liking of soy fortified fermented maize meal snack during the sensory specific satiety test

M:S (100:0)3-100% maize fermented for 3 days; M:S (70:30) 5-70% maize:30% Soy bean fermented for 5 days; M:S (50:50)3-50% maize:50% Soy bean fermented for 3 days; M:S (70:30) 3-70% maize:30% Soy beans fermented for 3 days

Sensory specific satiety has further been related to the long term acceptability of foods. For instance, Weenen, Stafleu, and De Graaf (2005) found an effect of eating cheese biscuits to satiety in a sensory specific test on liking ratings for cheese biscuits over a period of six day in-a home consumption study. Vickers and Holton (1998) also demonstrated that the amount of tea consumed during an SSS test might be a good indicator of long term acceptability. Therefore in this study, the SSS test results for the 50% maize: 50% Soy bean sample may be an indicator that the children will not sustain its consumption over time. This is important in this study, as it is important that school children continue to like the snack over a long period of time.

4.7 Estimated Glycemic index (eGI) and load of the snacks

Further analysis of the snacks was conducted using results from proximate analyses (carbohydrate content) and values from the International Glycemic Index table by

Foster-Powell, Holt, and Brand-Miller (2002) to establish their potential satiating effect for school children, as one of the aims of school feeding is to alleviate short term hunger. Studies have shown that protein a low glycemic Index and hence glycemic load food is the most satiating (Chambers, McCrickerd, and Yeomans, 2015; Groenen et al., 2017; Leidy et al., 2015). In this study, there was significant decrease in the glycemic load of the snacks as the quantity of Soy bean flour increased. For the unfermented snack, there was a 20% and 58% decrease in the glycemic load with 30% and 50% Soy bean fortification respectively. For the snacks fermented for 3 days, there was a 39% and 65% decrease in the glycemic load with a 30% and 50% Soy bean fortification respectively. Furthermore, in the 5 days fermented snack, there was a 37% and 59% decrease in the glycemic load with 30% and 50% substitution with Soy bean respectively (Table 4.3).

Other workers have shown that increase in the protein content of a starchy food using soy decreases its glycemic index. Quek, Bi, and Henry (2016) studied the effect of five rice based meals; rice alone (control) or rice with fish, egg white, Soy bean curd or chicken. Results showed that rice with Soy bean curd meal had the greatest reduction in glycemic index. Sugiyama, Tang, Wakaki, and Koyama (2003) further demonstrated that adding Soy bean products (miso, natto and ground Soy bean flour) lowered the G.I of white rice by 20-40%. Also, Fujiwara (2014) working with bread fortified with Soy Protein Isolates (SPI) showed that the bread had a lower G.I due to increased protein content.

Table 4.4: Glycemic load of the Soy bean fortified fermented maize snacks

Properties	Unfermented			3 fermentation days			5 fermentation days		
	Soy bean levels (%)			Soy bean levels (%)			Soy bean levels (%)		
	0	30	50	0	30	50	0	30	50
CHO Content	79.9 ^h ±0.2	61.3 ^d ±0.4	56.2 ^c ±0.8	76.7 ^g ±0.3	61.4 ^d ±0.2	55.1 ^b ±0.1	65.2 ^f ±0.4	62.6 ^e ±0.5	52.6 ^a ±0.5
¹ Available CHO Content	72.9 ^h ±0.2	54.5 ^d ±0.4	48.2 ^c ±0.8	69.7 ^g ±0.3	54.7 ^d ±0.2	47.1 ^b ±0.0	58.2 ^f ±0.4	55.9 ^e ±0.5	44.6 ^a ±0.5
Glycemic load	43.0 ^h ±0.1	34.3 ^f ±0.2	17.8 ^c ±0.3	41.1 ^g ±0.1	25.0 ^d ±0.2	17.4 ^b ±0.0	41.1 ^g ±0.1	25.6 ^e ±0.2	16.5 ^a ±0.2
² Glycemic load categories	High	High	Medium	High	High	Medium	High	High	Medium

¹Calculated by subtracting fiber content from carbohydrate content, Fiber content calculated from USDA Reference Tables, Release 26 (USDA, 2018)

²Adapted from American Institute for Cancer Research (2013); Glycemic load categories: Low GL – 10 or less; Medium GL – 11 to 19; High GL – 20 or more

The increase in protein content through soy fortification and decrease in glycemic index and load has the potential to reduce appetite and increase satiety to alleviate short term hunger. A recent systematic review of evidence on the effect of protein intake on perceived fullness by Leidy et al (2016) confirmed that protein intake is related to satiety, and higher protein loads make people feel fuller between meals. Another earlier study by Leidy and Racki (2010) found that increased protein consumption among adolescents who skip breakfast was associated with reduced appetite and increased satiety. Studies among adults have shown reductions in hunger, increase in satiety, and/or reductions in daily food intake after consumption of high-protein snacks compared with those adults not snacking or consuming high fat and/or high-carbohydrate snacks (Ortinou, Culp, Hoertel, Douglas, and Leidy, 2013; Ortinau, Hoertel, Douglas, and Leidy, 2014).

In this study, there was also a significant reduction in the glycemic load as the fermentation days increased. For instance, fermentation for 3 days lowered the glycemic load of the snacks fortified at 30% and 50% by 42% and 60%, respectively. For 5 fermentation days at 30% fortification, the glycemic load decreased by 40%, while 50% fortification the decrease was 62%. In all these instances, the glycemic load moved from high to medium to low. These results are similar to those of Eli-Cophie, Agbenorhevi, and Annan (2017) who established that the glycemic index of five local staples commonly consumed in Ghana, was lowest in fermented snacks and increasing the fermentation days, reduced the glycemic index. Similarly, a study by Liljeberg and Björck (1998) demonstrated that sourdough fermentation of wholegrain bread lowers its glycemic index.

The decrease in the GI of the snack is a pointer that fortification and fermentation of maize, which is a high G.I food, with soy increases the satiation power of the snack by lowering the G.I and load and therefore has the potential to keep children full for a long time, one of the main goals of school feeding programs in developing countries. It is important to make the observation that most studies in which glycemic index and load are determined aim to show their relationship between diet and obesity. In this study, the change in glycemic load of snacks when fortified with soy shows their potential to alleviate short term hunger in school children, a very important aim of school feeding and the achievement of SDG 2. Clearly the snacks at 50% soy replacement of maize changed from high to low showing their potential ability to keep the children satisfied for a longer time compared to the unfortified snacks.

4.8 General Discussion

School children in developing countries are vulnerable to protein deficiency because of their dependence on starchy staples as their sole source of nutrients. *Mkarango* is a traditional 100% fermented maize snack popular among rural school children. This study fortified *Mkarango* with soy to improve the protein quality and density.

There were some limitations in the study. First, the snacks were prepared by dry pan frying with constant stirring as is done conventionally in the households. This may have contributed to inconsistent moisture contents of the snacks as reported in table 4.1. A similar product was developed by Devi, Shobha, Alavi, Kalpana, & Soumya (2014) using millet-soy complement using extrusion technology and found that the snacks were of low moisture content and had a very stable shelf life. Pan frying may also have caused the irregular shapes as reported by the panelists during the sensory evaluation. Uniform cooking and regular shapes could have been obtained through extrusion cooking.

However, this method is expensive given the context of school feeding programs in developing countries. Furthermore, this traditional method of making *Mkarango* can be easily adopted in the villages and cottage industries as it requires very simple equipment and reaching schools in poor, rural underdeveloped communities. This sustains its low cost and affordability for such communities.

Secondly, the total dietary fiber was obtained from published data from the USDA (2018) food composition database. This option was taken to avoid the difficulty involved in sourcing for the apparatus required in performing the total dietary fibre tests. Similar options have been used by other researchers such as Agengo et al., (2015).

Third, the sensory evaluation sessions were conducted in the children's classrooms, with one group of 60 learners each seated in different rows in the class room. A possible limitation of this arrangement instead of individual stations is peer influence. Although efforts were made to separate children who appeared to be friends, it is possible that friendship among some children may have influenced the outcome. Similarly, Birch (1980) demonstrated that children could change their preference for food depending on what they see other children eat and the shift in change could be sustained weeks after, even in the absence of their peers. However, it unlikely that this had an influence on the final results because as explained earlier there was agreement among the children over the scores and results were consistent. Use of school classrooms and arrangement of students has yielded similar results in the study by Serrem, De Kock, & Taylor (2011).

In spite of these limitations, the study did establish that fortification with soy does improve the protein content, nutrient density and sensory properties of the snack, demonstrated from the results of proximate composition and sensory evaluation.

A comparison of the cost of making the Soy bean fortified snacks with reference to *githeri* that is commonly used in school feeding programs shows that the difference in the cost of making the Soy bean fortified snack is between Khs 21-30. This shows that it can be affordable to incorporate Soy bean fortified foods in the school feeding programs in developing countries. Increase in the level of Soy bean in the snack reduced the amount of snack needed to meet half the protein requirements of the school going children by 67% in the 30% and 72% in the 50% Soy bean fortified snacks. Furthermore, with the potential increase in the satiating power of the Soy bean fortified snacks, this means that not only will the snack be able supplement the protein in the diets of the children but also keep the school children full for a long time, a major goal of school feeding programs in developing countries. Therefore this Soy bean fortified biscuits can be fitted well into the HGSFP which was endorsed in some African countries, Kenya included.

Table 4.5: Costing of the snacks compared to (maize:beans) *githeri*

Ingredients for Soy bean snack	Cost (KSh/Kg)					
	Purchasing price ^a	Milling cost				Total
Maize	45	10				55
Soy bean	90	10				100
Vanilla essence	26	-				26
Sugar	13	-				13
Total						194
Cost of making the snacks ^b (Ksh/kg)	Purchasing cost	Milling cost	Vanilla essence	Sugar	Total	Difference ^d
100% maize	45	10	26	13	94	7
30% soymeal:70% maize	59	10	26	13	108	21
50%soymeal:50% maize	68	10	26	13	117	30
<i>Githeri</i>^c	87	-	-		87	0
Snack type	Amount of snacks for 14 g protein (g)					
100% maize	230					
30% soymeal:70% maize	77					
50%soymeal:50% maize	65					

^a Based on the current price of the ingredients and the amount needed to make the snacks

^b Based on the cost ingredients making the snacks in their respective ratios

^c Based on the cost of the ratio of maize: beans in the *githeri* to be 4:1

^d Value obtained from subtracting the cost of making the respective snacks with *githeri*

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

1. Increase in the quantity of Soy bean in the snacks significantly increased the protein, lipid, ash content, with a decrease in the carbohydrate content. Increase in the fermentation time increased the lipid content, while decreasing the carbohydrate content. Therefore fortified cereal based fermented foods have considerable potential for use supplementary foods for increased protein and energy content in the prevention of PEM among school going children in developing countries.
2. Increase in the amount of Soy bean in the snacks and increase in the fermentation time produced desirable qualities in the functional properties of the snack. Both, fortification with Soy bean and increase in fermentation time lead to a decrease in the bulk density, increase in water absorption capacity, decrease in pH.
3. Fortification with Soy bean produced a snack with positive attributes such as baked aroma burnt flavour and beany flavour
4. Fortification of the fermented snacks with Soy bean lead to a significant decrease in the glycemic load of the snacks, with improved sensory specific satiety

5.2 Recommendations

1. **Recommendation for policy:** Institutional Policies on fortification of commonly consumed snacks with Soy bean to combat Protein and Energy Malnutrition among pre-school and school going children
2. **Recommendation for practice:** Raise awareness of the nutritional benefits of Soy bean fortified snacks to increase its adoption and therefore consumption in the households
3. **Recommendation for further research:** It is recommended that further study be carried out to determine the protein nutritional quality of the Soy bean fortified fermented snack using a small animal assay as this is the standard method of determining protein nutritional quality in foods, and also to measure effect on growth and metabolic indicators

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APPENDICES

APPENDIX I: APPLICATION FORM FOR SERVING IN A TRAINED

SENSORY PANEL

1. Full name and surname -----
 2. Your residential address? -----

 3. Telephone or mobile cell No. -----
 4. E-mail address -----
 5. Your age? -----

6. Are you?	Male	Female
7. Your occupation or main activity during 17 th June 2014 to 4 th July, 2014 (e.g. student, technician etc.)?		
8. Are a registered University of Eldoret student?	Yes	No
If yes, course and year of study and hours you are available		
9. Are you a University of Eldoret staff member?	Yes	No
If yes, state the time and day of the week you are available		

10. Please evaluate your ability to read, speak and write English on the following scale:

Poor Fair Average Good Excellent

11. Are you allergic to anything?	Yes	No
If yes, give details.		
12. Please specify any specific food product/s that you prefer not to consume.		
13. Do you smoke?	Yes	No
If Yes, how many cigarettes a day?		
14. Will you be available for the taste panel as explained during the introduction session on 17 th March 2014 to 4 th April 2014	Yes	No
15. Have you ever been on any sensory evaluation panel?	Yes	No
If yes, where/when/to evaluate what?		
19. Will you be able to attend the screening sessions on:		
Wednesday: 15 th June, 2016	Yes	No
20. If you are available for the screening sessions, which of the following time/s would		

be suitable.		
10:00 – 11:00 Hours	Yes	No
13:00 – 14:00 Hours	Yes	No
21 In not more than 20 words, write down why you think we should choose you for our sensory panel		

I declare that the information furnished above is correct and true to the best of my knowledge.

Signature

Date

APPENDIX II: SENSORY PANELIST CONSENT FORM

Sensory evaluation of Makarango

Thank you for your willingness to potentially participate in a sensory evaluation project at the Department of Family and Consumer Sciences, University of Eldoret.

Date of Participation:

Voluntary Nature of Participation: I understand that participation in this project is completely voluntary and I do not have to participate in this sensory project if I do not agree to participate hence I can withdraw my participation at any time.

Risks to the individual: I understand that I will evaluate different varieties of *Mkarango* using descriptive sensory evaluation. I note that people who are allergic to Soy beans should avoid these products.

Medical Liability: I understand that no financial compensation will be paid to me in connection with any physical injury or injury in the unlikely event of physical injury or illness as a direct or indirect result of my participation in this sensory project.

Confidentiality: participants are not required to reveal any confidential information. All responses to questions will be treated in a confidential manner. Responses to sensory questions via the evaluation form are tracked using numbers only. These numbers are not in any way related to the participant's name.

If you have any questions about this sensory project, contact Prisca Linda Rapando. Department of Family and Consumer Sciences, University of Eldoret at 0721 673 871

I HAD THE OPPORTUNITY TO READ THIS CONSENT FORM, ASK QUESTIONS ABOUT THE SENSORY PROJECT AND I AM PREPARED TO PARTICIPATE IN THIS PROJECT.

Participant's Signature

Date

Participant's Name *please print clearly*

Sensory Panel Leader Signature

Date

APPENDIX III: DESCRIPTIVE SENSORY EVALUATION






SCREENING TESTS

TEST 1

Name: _____

Date:

Identify the taste on each of the papers

TEST 2

Name: _____

Date:

Identify the following flavours by smelling. Enter the code of the sample you have identified against the flavour.

Perceived flavour	Code
Lemon flavour	
Caramel flavour	
Almond flavour	
Pineapple flavour	
Chocolate flavour	
Orange flavour	

TEST 3

Name: _____

Date:

You are provided with five samples of fermented cereal products. Please take a sip of water before you start tasting and in between tasting the different samples. Using your own terms, show how the beans are different in taste, flavour, texture and appearance.

	743	692	508	455	122
TASTE					
FLAVOUR					
TEXTURE					
APPEARANCE					

APPENDIX IV: DESCRIPTIVE PANEL EVALUATION SHEET**WELCOME TO THIS TASTING SESSION**

DEPARTMENT OF FAMILY AND CONSUMER SCIENCES

UNIVERSITY OF ELDORET

PANELIST CODE

PANELIST NAME

ENTER TRAY NO.

DATE: 1st August, 2016

Instructions

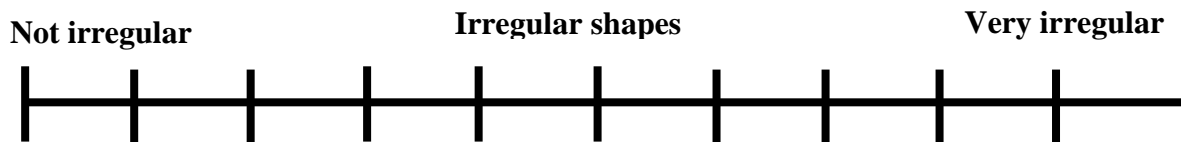
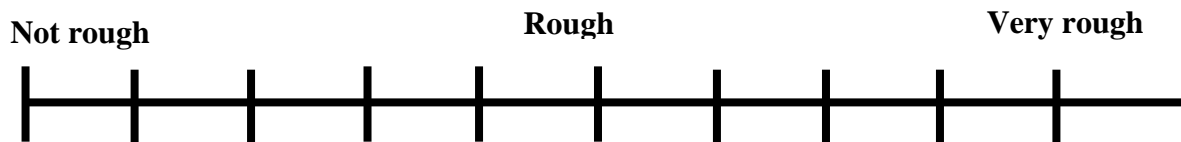
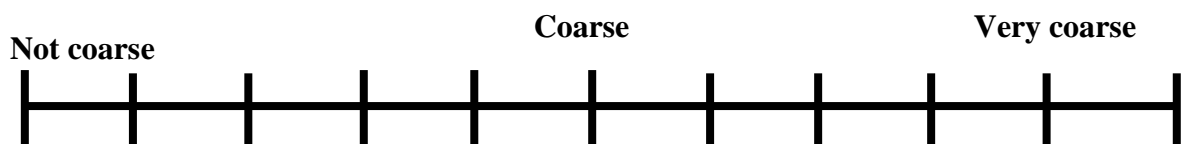
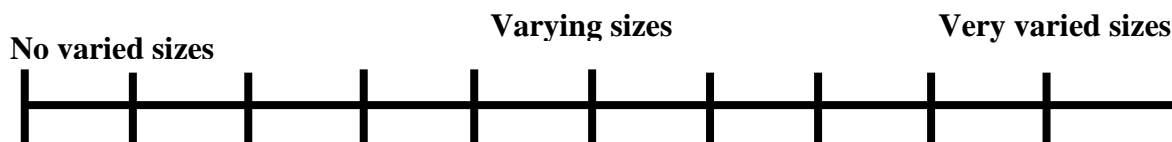
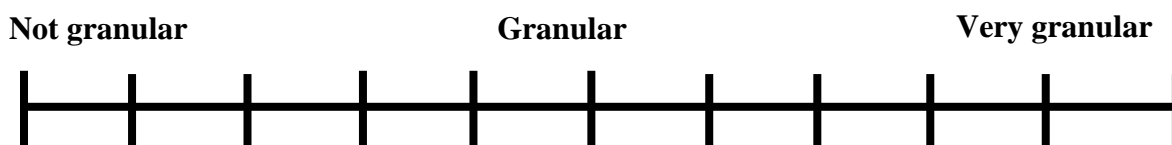
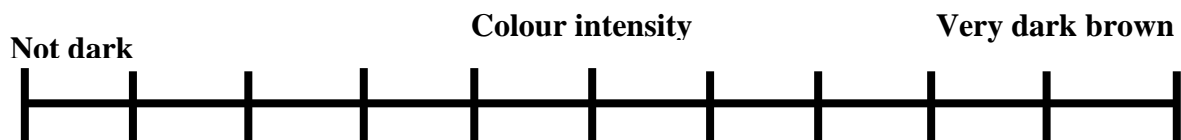
You are provided with (4) samples of *Mkarango*. Please taste the samples in the order presented from left to right. Take a sip of water and eat a piece of carrot before you start tasting and in between tasting the different samples. Circle the relevant bar on the scale provided for each attribute.

SAMPLE

APPEARANCE

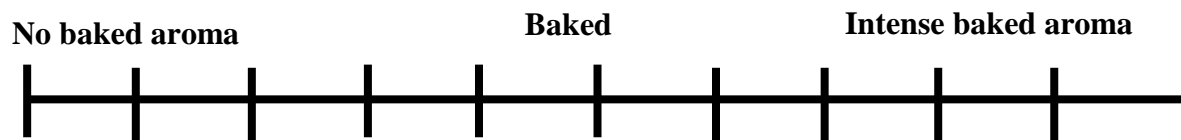
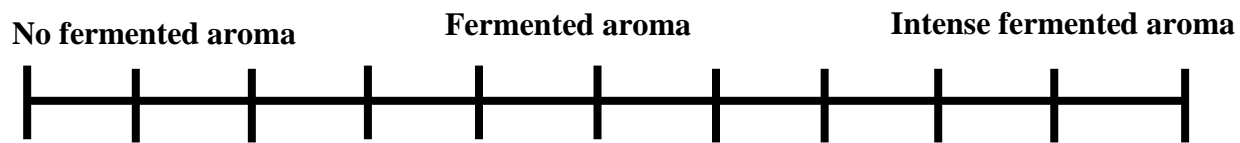
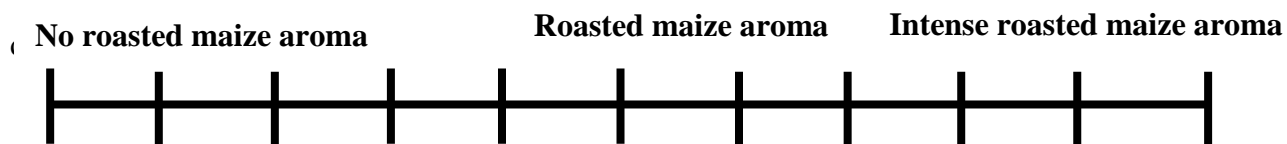
Question 1:

Look at the sample and rate the following appearance descriptors



Question 2:

Smell sampleusing short sniffs and rate the intensity of the following aroma



Question 4:

TEXTURE

Taste sampleand rate the intensity of the following texture descriptors

Not rough	Rough texture	Very rough
----- ----- ----- ----- ----- ----- ----- ----- ----- -----		
Not grainy	Grainy texture	Very grainy
----- ----- ----- ----- ----- ----- ----- ----- ----- -----		
Not soily	Soily texture	Very soily
----- ----- ----- ----- ----- ----- ----- ----- ----- -----		
Not crunchy	Crunchy texture	Very crunchy
----- ----- ----- ----- ----- ----- ----- ----- ----- -----		

Question 5:

AFTER TASTE

Taste sampleand rate the intensity of the following after taste descriptors

Not grainy	Grainy after taste	Very grainy
----- ----- ----- ----- ----- ----- ----- ----- ----- -----		
No sour after taste	Sour after taste	Intense sour after taste
----- ----- ----- ----- ----- ----- ----- ----- ----- -----		
No sweet after taste	Sweet after taste	Intense sweet after taste
----- ----- ----- ----- ----- ----- ----- ----- ----- -----		
No fermented after taste	Fermented after taste	Intense fermented after taste
----- ----- ----- ----- ----- ----- ----- ----- ----- -----		

APPENDIX V: CONSUMER ACCEPTABILITY SHEET

WELCOME TO THIS MAKARANGO TASTING SESSION
 DEPARTMENT OF FAMILY AND CONSUMER SCIENCE
 UNIVERSITY OF ELDORET

Age: Gender:

Tray Number:

PART A - Instructions

You are provided with four (4) samples of soy bean. Please taste the samples in the order presented from left to right. Take a sip of water before you start tasting and in between tasting the different samples. Indicate your liking or disliking by placing a check mark at the relevant bar on the scale provided for each attribute.

Sample No.																
Scale	Ap pea ran ce	S m el l	Fla vo ur	Te xtu re	App eara nce	Sm ell	Flav our	Text ure	Appe aranc e	Sm ell	Flav our	Text ure	Ap pea ran ce	S m ell	Flav our	Tex ture
Like extremely																
Like very much																
Like moderately																
Like slightly																
Neither like nor dislike																
Dislike slightly																
Dislike moderately																
Dislike very much																
Dislike extremely																

APPENDIX VI: CONSENT LETTER TO SCHOOL ADMINISTRATION

School of Agriculture and Biotechnology
Department of Family and Consumer Science
P.O Box 1125-30100,
Eldoret

5th August, 2016

Dear Sir/Madam,

RE: EVALUATION OF NUTRITIONALLY IMPROVED FERMENTED MAIZE MEAL SNACK

The Department of Family and Consumer Sciences at the University of Eldoret would like to carry out evaluation of nutritionally improved fermented maize meal snack suitable for school children, at your school. The snack has the potential to improve the nutritional status, concentration ability and performance of school children. The reason for the evaluation is to determine how acceptable the snack is to young children and whether children will be willing to consume them over the long term. This is part of a project for Prisca Linda Rapando, a MSc student. We would like to kindly request for your permission to conduct the research at your school and also request for your assistance to organize the activity.

We require that a minimum of eighty, eight and nine year old children from your school to participate in this study in a suitable week in the month of September, 2016. We have prepared a consent letter that has be signed by parents/guardians of the designated children. This research will involve the children for approximately 30 minutes daily for 5 days preferably 10:00 to 10:30 am (or during your break time). The children will be asked to taste and eat 4 types of *Mkarango* which contain maize flour, Soy bean flour, sugar and vanilla flavouring. Each child that participates will receive a banana after each tasting session and at the end will be given a small gift as a sign of our appreciation.

The following are the activities children will be involved in during the first five days:

Day 1: The children will be familiarized with and taught how to score the card (attached) to evaluate the snacks.

Children will be asked to remove a label from one food they like and place it on a happy face, and one that they do not like on unhappy face. They will be divided into four groups. Each group will be managed by two research assistants from the University of Eldoret, who are familiar with working with children.

Day 2 to 5: Children will taste and score the acceptability of the five snacks. Each child will be provided with a bowl with each of the samples with a three digit code attached. They will be asked to taste each snack, remove the code and place it on a face that corresponds with how they feel on the score card. Each child will then be provided with each snacks of each of the remaining samples and asked to taste and complete it. Immediately after, the child will again be provided with five samples to evaluate using the score card. The same procedure will be repeated for the four days.

We will share the findings from the research conducted at the school with the school and are willing to share some of the knowledge gained from this project in the form of training/ teaching activity for the school's parents and/or educators.

We look forward to hearing from you. Please contact me should you have any further questions or need further clarification on any aspect of the project.

Yours truly,

Ms Prisca Linda Rapando

MSc Student

Dr. Charlotte Serrem,

Study leader

APPENDIX VII: CONSENT LETTER TO PARENTS

School of Agriculture and Biotechnology
Department of Family and Consumer Science
P.O Box 1125-30100,
Eldoret

Dear parent/guardian

RE: EVALUATION OF NUTRITIONALLY IMPROVED FERMENTED MAIZE MEAL SNACK

The Department of Family and Consumer Sciences at the University of Eldoret would like to carry out evaluation of nutritionally improved fermented maize meal snack suitable for school children. The reason for this work is to determine how acceptable the snack is to young children and whether children will be willing to consume them over the longer term. This is part of a project work for Prisca Linda Rapando, a MSc student. We would like your children in class 4 and 5 at the University of Eldoret Primary School to be part of a five day tasting exercise from September, 2016. This work will take 30 minutes daily for five days. The children will be asked to taste and eat five types of the snack which contain maize flour, Soy bean flour, sugar and vanilla flavouring.

The following are the activities children will be involved in during the five days:

Day 1: The children will be familiarized with and taught how to score card, a seven point scale with five stylized faces. It will be explained to the children that they have in front of them figures that are smiling or putting on airs because they Like extremely, like very much, like, do not like or dislike, dislike, dislike very much and dislike extremely what they are eating. Two types of food, one that they like (banana) and one that they do not like, (lemon) will be used. They will be asked to remove the label from the food they like and place it on the face that corresponds with what they feel about the food they have just tasted. The liked food should be placed on one of the happy faces and the disliked one on one of the unhappy faces.

Day 2: The children will be divided into four groups. Each group will be managed by a research assistant from the University of Eldoret who is familiar with handling children.

First, the children will be provided with five samples of the snack, each with a coding label attached. They will be asked to taste each sample and place the coding label from each snack on the face that corresponds with their feelings for the snack. The evaluated scale and the left over snack sample will be withdrawn.

Second, each child will then be provided with 2 samples of the one of the five types of snack sample and asked to eat and complete it.

Third, immediately after eating, the child will be asked by the research assistant if he/she would like to eat the same snack tomorrow. Then children will again be provided with a score card similar to the one used earlier, and the five samples of the snack to rate.

Day 3 to 5: The same procedure as described for day 2 will be repeated on each of these days.

Each child that participates in the exercise will be given a small gift as a sign of appreciation. If you would like your child to be included in the exercise, please fill the consent form below.

Parent/guardian's consent form

Sensory evaluation of Soy bean fortified fermented maize meal snack

Thank you for your willingness to allow your child to participate in a sensory evaluation project conducted by the Department of Family and Consumer Science, University of Eldoret.

Date of participation: 12th to 16th September, 2016

Volunteer nature of participation: I understand that my child's participation in this project is completely voluntary. I do not have to allow my child to participate in this project. If I do agree to my child's participation, the child can withdraw from participating any time.

Risk to the individual: I understand that my child will evaluate these snacks. The risk involved in eating this snack is no greater than that of eating a similar snack produced at the household level. I understand that the product samples may contain maize, Soy bean, sugar and vanilla and any child that is allergic to soy should not participate.

Confidentiality: The researcher will not reveal any personal information (e.g. name of child) to third parties. All responses to questions will be treated in a confidential manner. Responses to sensory questions via the evaluation form are tracked using numbers only. These numbers are not in any way related to the participant's name.

If you have any questions about this sensory project, contact Prisca Linda Rapando, Department of Family and Consumer Science, University of Eldoret at 0720 871 673

I HAD THE OPPORTUNITY TO READ THIS CONSENT FORM, ASK QUESTIONS ABOUT THE SENSORY PROJECT AND I AM PREPARED TO PARTICIPATE IN THIS PROJECT.

Parent's name.....Telephone/Mobile number.....

Parent's signature.....

Name of the child (*Please print clearly*).....Date.....


Consumer Panel Leader Signature..... Date.....

APPENDIX VIII: RESEARCH PERMIT

THIS IS TO CERTIFY THAT:
MISS. PRISCA LINDA RAPANDO
of **UNIVERSITY OF ELDORET,**
1125-30100 Eldoret, has been permitted
to conduct research in *Uasin-Gishu*
County

Permit No : **NACOSTI/P/17/27159/15643**
Date Of Issue : **17th March, 2017**
Fee Recieved : **Ksh 1000**

on the topic: **DEVELOPMENT OF A
FERMENTED SOYBEAN FORTIFIED MAIZE
MEAL SNACK TO ALLEVIATE PROTEIN
ENERGY MALNUTRITION IN SCHOOL
GOING CHILDREN**



for the period ending:
16th March, 2018

.....
Applicant's
Signature

.....
Sammuti Bwalya
Director General
National Commission for Science,
Technology & Innovation

Generated by Ushahidi

APPENDIX IX: SIMILARITY INDEX/ANTI-PLAGIARISM REPORT

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<p>< 1% match (publications) Kayitesi, Eugénie, Kwaku Gyebi Duodu, Amanda Minnaar, and Henriette L de Kock. "Effect of micronisation of pre-conditioned cowpeas on cooking time and sensory properties of cooked cowpeas : Effect of micronisation of pre-conditioned cowpeas", Journal of the Science of Food and Agriculture, 2013.</p>
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