DEVELOPMENT AND QUALITY EVALUATION OF EXTRUDED CEREAL FLOURS FORTIFIED WITH GRAIN AMARANTH, BAOBAB AND ORANGE-FLESHED SWEET POTATO

By

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2020

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DEDICATION

This work is dedicated to the memory of my late father who always believed in me and tomy son Kylian.

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ABBREVIATIONS AND ACRONYMS

AOAC	Association of Official Analytical Chemists
AAS	Atomic Absorption Spectrophotometry
ANOVA	Analysis of Variance
ASLT	Accelerated Shelf Life Testing
CDC	Center for Disease Control
CFU	Colony Forming Units
FAO	Food and Agriculture Organization
FFA	Free Fatty Acids
HPLC	High-Performance Liquid Chromatography
ISO	International Organization for Standardization
LSD	Least Significant Difference
OFSP	Orange Fleshed Sweet Potatoes
RDA	Recommended Daily Allowance
SSA	Sub-Saharan Africa
WFP	World Food Program
WHO	World Health Organization

OPERATIONAL DEFINITIONS

Composite flour: a mixture of flours from cereals, tubers and vegetables with or without wheat.

Extrusion: a high-temperature short-time food processing method in which a moistened food material is plasticized and cooked in a barrel under high pressure and temperature and discharged at atmospheric pressure through a die.

Shelf life: length of time a product is stored without becoming unfit for use.

Sensory evaluation: use of human senses to provide information on how food products are perceived.

GENERAL ABSTRACT

Micronutrient malnutrition continues to be a problem in developing countries. Food to food fortification which includes composite flour formulation is one of the methods used to reduce the problem. However, the effectiveness of the composite flours is questionable due to the presence of antinutrients in the raw materials used. Therefore, the current study aimed at developing a nutritious, shelf-stable porridge composite flour from maize, sorghum, grain amaranth, baobab and orange-fleshed sweet potatoes.

Nutrient composition data of each ingredient was generated using standard analytical methods and used in the formulation of the composite flours using Nutrisurvey software. To produce various formulations of composite flours with maize and sorghum as the cereal base and grain amaranth, baobab and OFSP as the fortifiers, a completely randomized study design in factorial arrangement with ingredient ratio and extrusion as variables and seven levels was used. Half of each of the formulations were extruded at 160 °C. Nutritional and anti-nutritional profiling followed by sensory evaluation of the composite flours was done. A comparative analysis of acceptability was performed between the best formula and the commercial composites. Stored in kraft paper and plastic containers, the two best formulations, extruded and non-extruded were subjected to real-time shelf-life studies at 20 °C, 30 °C and 40 °C. The data were analyzed using the *R* Project for *Statistical*Computing, R-3.6.3 and inferential statistics done by ANOVA and the means separated using Turkey’s HSD test.

The protein content (8.99 ± 1.03 g/100 g), beta-carotene content (895.90 ± 346.85 µg/100 g), iron content (11.81 ± 9.73 mg/100 g) and zinc content (1.74 ± 0.18 mg/100 g) were improved by fortification of maize-sorghum blends on average. An increase in grain amaranth increased the phytate content. The protein content, beta-carotene content and antinutrients were decreased by

4.7%,40.9% and 35% respectively by extrusion. Consumer acceptability studies showed that the colour, flavour and overall acceptability of the composites (mean 5.7-7.4 on a 9-point hedonic scale) were affected by formulation, with the most acceptable being those containing more sorghum. The comparative study showed that the new formulations had the potential of being accepted.Compared to plastic, the formulated composites stored in Kraft paper were found to degrade faster. The formulations can retain their quality and safety for six months in various ecological zones in both kraft and plastic.

Therefore, locally available ingredients such as grain amaranth, Baobab and OFSP improves the nutrient composition of maize-sorghum composites while processes such as extrusion reduce antinutrients and have a detrimental effect on beta carotene.

Extrusion should be used in baby food. Bioavailability studies on zinc and iron should be conducted to ascertain the effectiveness of extrusion on the reduction of antinutrients. Refortification of extruded flours with beta carotene is recommended.The Ministry of Agriculture, Livestock and Fisheries in collaboration with the Ministry Health can promote these local crops for consumption by the Kenyan population to reduce micronutrient malnutrition.

CHAPTER ONE: INTRODUCTION

1.1 Background

Micro-nutrient malnutrition is one of the major contributors to the global disease burden and affects more than 2 billion people (CDC, 2018). Vitamin A deficiency, iron deficiency and other minerals, such as zinc, are the most common types of micronutrient malnutrition in Africa. Some of the approaches used to help mitigate the issue are fortification and composite flours, but fortified foods are expensive, whereas composite flours are inexpensive alternatives.

Composite flour refers to a mix of cereals such as maize, millet or sorghum, legumes such as soybeans, peas or beans, root tubers such as yams and sweet potatoes and vegetables with varying concentrations of wheat or non-wheat flours (Ekunseitan et al., 2017). FAO introduced the composite flour technology in 1964 intending to replace wheat with other crops such as maize, yam, and cassava (Noorfarahzilah et al., 2014), among others. Owing to their good nutritional composition and ability to boost the functional properties of other products, they are being replaced with other cereal flours (Ndagire et al., 2015). While composite flours are nutritionally rich, the nutrients in some of the raw material used may not be accessible due to anti-nutrients. Extrusion not only decreases antinutrients but also increases iron bioavailability and kills microorganisms. The shelf life of the end product can be impacted by materials that have a high-fat content. Products with higher nutrients than the recommended dietary allowance (RDA) may also be developed if the formulations are not carefully done.

Cereals are the Poaceae family's edible grains and are a staple food and the main source of carbohydrates for most countries. In Kenya, maize, for example, is the staple food (Singh & Kumar, 2016). Flours of sorghum and maize are used in Kenya to make porridge and ugali. Sorghum is a rich source of protein, fibre and the vitamin B complex but also contains antinutrients such as tannins. Compared to sorghum and wheat, maize is high in fatty acids and higher quantities of lysine, which makes it a preferable cereal.

Amaranth (*Amaranthusspp*) is a leafy vegetable that originated in the United States and is one of the earliest domesticated food crops. The grains are rich in the amino acid lysine that is lacking in other grains. Grains are also a good source of protein, fibre, calcium, iron and vitamin C, as well as a good source of potassium, vitamin A, riboflavin and niacin. (Department of Agriculture, 2010).

The Baobab or monkey tree (*Adansoniadigitata*) is a deciduous tree widely distributed in sub-Saharan Africa (Abdulkarim&Bamalli 2014). Its fruit is rich in vitamin C,pro-vitamin A and minerals such as iron, magnesium, manganese, calcium, zinc, sodium, and phosphorus (Adubiaro et al., 2011). This makes it a good fortifying agent to fix micronutrient malnutrition.

Sweet potatoes are nutritious root tubers. One of the varieties, the orange-fleshed sweet potato (OFSP) is a great source of beta-carotene which is a vitamin-A precursor and polyphenols (Rodrigues et al., 2016) and can therefore be used as a sustainable method of preventing blindness (Honi et al., 2018).

To improve the quantity and quality of cereal nutrients, a great deal of effort has been made. Some of the strategies used to enhance the nutritional quality and organoleptic properties of foods based on cereals are fortification and supplementation. While they are rich in minerals, cereals and legumes have anti-nutritional factors that make them inaccessible. Extrusion is one of the methods of food production that can help minimize the anti-nutrients but also damage the nutrients. It induces protein denaturation, for example, and research is therefore required to determine the conditions of extrusion that can create a healthier product with fewer anti-nutrients.

1.2 Statement of the problem

Micronutrient deficiency is one of the major contributors to the global health burden, with vitamin A, iron and zinc being the most important and 2 billion individuals being affected (CDC, 2018). Composite flours have been used to help alleviate the problem, but because of antinutrients such as tannins and phytates contained in the raw materials used, their effectiveness is questionable. The antinutrients form metal-ion complexes that make them biologically unavailable, especially Zn, Ca and Fe. Some of the techniques traditionally used to minimize the amount of antinutrients in cereals are milling, roasting, germination, fermentation, boiling and soaking, but they affect the nutrient composition of the composite flours. Extrusion is a better choice because, if performed under the correct processing conditions, it not only decreases antinutrients but also increases protein digestibility and iron bioavailability. Therefore, this project seeks to develop a nutritious extruded porridge composite flour that will help reduce micronutrient malnutrition in children below the age of five.

1.3 Justification

If adopted, this project will provide processors and producers with details on the specifications for the extrusion process that produces nutritious composite flours. When adopted by farmers, diversification in the use of maize, sorghum, baobab fruit, grain amaranth and OFSP will be enhanced. Government agencies would also benefit from using this data to make policies to minimize anti-nutrients, such as declaring the extrusion of all baby flours. The project would enhance the awareness of researchers interested in the formulations of composite flour.

1.4. Objectives

1.4.1 Overall Objective

To develop and evaluate the quality of extruded cereal flours fortified with, grain amaranth, baobab andOFSP.

1.4.2 Specific Objectives

i. To determine the nutrient and anti-nutrient composition of cereal flours fortified with Grain Amaranth, Baobab andOFSP
ii. To determine the acceptability of the formulated composite flours as compared to the market composites
iii. To determine the shelf stability of the most acceptable composite flours

1.5 Hypothesis

i. There is no difference in the Nutrient composition and antinutrient composition of extruded and non-extruded cereal flours fortified with Baobab, OFSP and Grain Amaranth.
ii. There is no difference in the acceptability of the formulated composites compared to the market composites.
iii. There is no difference in shelf stability of extruded and non-extruded cereal flours fortified with Baobab, OFSP and Grain Amaranth.

CHAPTER TWO: LITERATURE REVIEW

2.1 Maize production and chemical composition

2.1.1 Origin, classification and maize production

Maize, or as it is often referred to as corn, is one of the most commonly produced and used cereal grain globally. The Native American Indians and the people of southern Mexico are thought to have first grown it before spreading to Columbus just around the time of the Maya civilization, (Tenaillon&Charcosset, 2011).After this, its cultivation was widely spread to Spain and other countries in Europe and later introduced by the Portuguese in most of sub-Saharan Africa (Kumar et al., 2016). It is suspected that it has its ancestral origins from the *teosinte* plant and that gene modification is likely to cause the differences in plant structures. Nikolai Vavilov claimed that maize was a more domesticated version whose closest wild relative was the plant teosinte from the genus Euchlaena (Kumar &Jhariya, 2013).

Maize is scientifically classified as Kingdom Plantae, a grouping of monocotyledons (Liliopsida) of the genus *Zea*L. of the order Cyperales, a family of grasses (*Poaceae*). Hence the genus *Zea mays* L. In addition to the above, based on the characteristics of the kernels, farmers have also further divided maize into around 8 classes (Singh & Kumar, 2016). As below, the classes are;

- Dent corn-The white or yellow kernels contain both soft and hard starches within the endosperm.
- Flint corn, in the middle, has a soft and starchy endosperm.
- Popcorn- With high popping characteristics and a hard endosperm, the kernels are thin.

- Flour corn, with soft endosperm and kernels, resembles flint corn. Although white and blue are the most common, they can be in all colours.
- Sweet maize-starch and sugar are the main components of the endosperm and are responsible for its sweet taste.
- Waxy corn is thought to originate in China. The endosperm looks waxy,
- Pod corn- a primitive form where the kernels are each found in pods or husks.
- Baby corn- a form whose endosperm is rich in minerals and vitamins and is grown for use in vegetables and salads.

As at present, maize has a large global production of over 700 million tons and tends to grow best in moderately warmer temperature regions. With over 30% of maize coming from the country, the United States is ranked as the highest producer (Kumar &Jhariya, 2013). In 2019, for example, figures showed the world's total maize output at 1.05 billion tons, with the United States generating 33.04 % of the total maize production that year.

2.1.2 Nutritional value and uses of maize

Maize is the largest grown cereal crop globally, and this could be attributed to its nutritional value that is appreciated overall. The grains are rich in carbohydrates, otherwise referred to as starch and calories which are important sources of energy. In addition to these, the grains also contain about 3%- 9% proteins and water which is useful in the bodybuilding and tissue formation processes. (Saldivar & Perez-Carrillo, 2015). Vitamins A, C, and E, dietary fibre as well as other essential minerals such as magnesium and phosphorus are also contained within the maize grains and highly improve the nutritive value.

The most common and vast use of maize or corn is for human consumption. The grains can be used as they are and form a huge part of many meals all over the world. However, most of the time, the grains are ground to produce maize flour which is then used in a variety of ways. The

Mexicans for example, have cornmeal as a vital ingredient in their meals and use the flour mixed with lime water, Masa, to make tortillas, atole, tamales among others,(Orhun, 2013). The maize meal is used in many parts of Africa to produce a dense porridge, the South African mealie*pap*, and East African *ugali*. The cobs can also just be boiled as they are or roasted over a fire or a grill and enjoyed or the popcorn can be made by heating in little fat. Another enjoyed snack is the cornflakes that are very commonly used as a breakfast cereal. Other uses of maize revolving around human consumption include the production of corn oil, high-fructose corn syrup used as a sweetener and also for grain alcohol when it is fermented and distilled (Torres et al., 2016).

Except in most African countries and perhaps in Mexico, most countries especially the United States use only about a quarter of their maize production for human consumption. Most of it is grown to be used as livestock forage, or the cornstalks are fermented to produce silage which is usually very good especially for ruminants (Rouf Shah et al., 2016). The kernels can also be dried and fed to the livestock directly as grain feed. Commercial chicken feeds also have maize as a large component, as do feeds for farmed catfish.

Maize cobs are also used as a good and unique source of biofuel used for specialized corn stoves in the West and for supplements of wood pellets for creating open fires in many lower-income countries. Its use could be increased by the fact that it is cheap and rather easily available. In other countries such as Germany, efforts are being made to utilize the whole plant by shredding the maize, having it in silage clamps which are then fed into the biogas plants. Other uses of maize can be considered as an export commodity to earn revenue for countries that produce it in overly large amounts,(Torres et al., 2016). The stigmas from the female plant can be used as herbal medicine; the kernels are also used as fish bait in the form of dough balls especially in Europe.

2.1.3 Maize anti-nutrients

Anti-nutrients are natural or synthetic elements that are found in food compounds and interfere with nutrient absorption. For cereals and grains such as maize, the main anti-nutrients are phytic acid and polyphenols. (Amir et al., 2015). Phytate is an organic phosphate that has a combination of calcium and magnesium salts found in abundance in many plants especially legumes and cereals. It is very important especially in terms of nutrition because it is a chelator of metal ions. It accumulates in the seeds of the growing plant until it matures and is responsible for up to 60% of phosphorous content in the said plants(Kitta, 2013). The human digestive system, as well as that of monogastric animals, is unable to breakdown phytate, as such, it accumulates in the system. Its presence greatly interferes with the absorption of certain essential minerals including, magnesium, zinc, copper, and iron among others and thus reduce their bioavailability. (Vikas& Amit 2018). If taken as a staple food over a long period, this could lead to malnutrition with deficiencies of essential nutrients.

2.2. Sorghum production and chemical characteristics

2.2.1 Origin, distribution, and production of sorghum

Sorghum is ranked fifth in the list of important cereal crops grown globally and is the 3rd most grown in the United States. Its main advantage that increases its cultivation is its ability to adapt to very harsh and dry climatic conditions and become somewhat drought resistant. (Hariprasanna & Patil, 2015). It becomes of great significance to the semi-arid areas particularly in Africa and Asia where it becomes a reliable source of food. Its origin is alluded to the North-Eastern part of Africa, in a place called the Nabta Playa found along the Egyptian- Sudanese border. This is where the initial crop is believed to have been grown before spreading to Asian countries such as India and China. (Patil& Mishra 2014). It, later on, found its way to Australia before the first use of the crop in the United States for making brooms was reported around 1757.

Among the top producers of sorghum worldwide include the United States, India, and Nigeria. Africa however, as a continent might solely account for a large amount of sorghum produced although there it is mainly grown for purposes of human consumption. Large exporters of the crop would include the United States, Australia, and Argentina among others. According to reports of an article, there was a total biomass of sorghum distribution worldwide that amounted to over 42 million metric tons. Out of these, the distribution was greatly varied. However, the top producers were ranked as India with 16%, Nigeria producing 16% of the total, the United States at 13% production, Argentina at 10% and Ethiopia with 9% of the total biomass (Hariprasanna & Patil, 2015) Other countries that contributed greatly to the global biomass included China, Australia, Brazil, and other African countries, in no particular order.

Sorghum, in the true sense of the word, is a genus of the grass family Poaceae that contains over 30 species, the most commonly cultivated being *S. bicolor* or *S.vulgare*. It belongs to the same family as maize, wheat, and rice. They have certain characteristics in common including having a head, grains which are the fruit or the edible seeds that can be in the form of spikelets or panicles (Danovich, 2015). The most commonly recognized forms of sorghum are the grain sorghum and the sweet sorghum. The two have a similar growth process except with subtle differences in the height of the plants where the sweet form grows taller to about 10 feet tall while the grain sorghum only at 3-5 feet tall.

The flag leaf is usually the last to form, and the head is sure to emerge in about seven days once the flag leaf is visible. Flowering begins at the top of the head in a downward movement between 3 to 7 days after the head emerges and beyond that the grains start being produced.

(Prakasham et al., 2014). The grains mature by transforming from the soft, light green forms to harder and usually red, tan, bronze or white grains. After this maturation, the crop is ready for harvesting usually done by a combine harvester for the grain sorghum or by cane harvester or hand for the sweet form of sorghum. Sorghum is considered a cross-pollinating plant that adapts rather impressively to dry and semi-arid conditions (Hariprasanna & Patil, 2015). Some of its features that aid in this ability to survive harsh weather includes, having a deep and extensive root system that gathers water from deep water beds in the soil, a waxy coating on the leaves to prevent loss of moisture and a prolonged time for seed and grain production.

2.2.2 Uses of sorghum

Sorghum is an important cereal crop with a wide array of uses ranging from food, fuel, for alcoholic beverages, as broomcorn, pasture, and hay. Again because of its ability to withstand harsh weather and climatic conditions, its importance is heavily felt in the semi-arid tropics of Latin America, Asia and Africa where it is among the main food grains (Hariprasanna & Patil, 2015). The grain sorghum is what is mainly used as food for people, livestock and even for poultry. Countries such as the United States appreciates sorghum due to its large use as livestock feed. Due to the closely related and very similar nutritional composition and value, it is used easily as a substitute for maize feed, (Dahlberg et al., 2011). Grass sorghum, another form of the crop is also grown and used to make pasture and hay that also feeds livestock.

Common foods that are produced from sorghum include porridges, ground flour that is used to make cakes, cookies, and bread. The sweet sorghum is used to form a syrup that serves as a sweetener for bread, cookies, pancakes, baked beans, and hot cereals. Sorghum is also used in China and many other African countries such as South Africa, Kenya, Nigeria to form distilled beverages enjoyed as alcohol or beer, (Danovich, 2015). For brewing of the African sorghum beer, the sorghum grain is malted and ground, then subjected to souring through lactic acid fermentation before being allowed to continue fermenting as alcohol.

Other uses of sorghum include the use of the straw to make brooms, brushes, baskets and other packaging materials even for sensitive electronic equipment. The sorghum plant has also been used a great construction material especially for grass- thatching of roofs of houses. The stalks, which are thought to contain ethanol in the sap are reportedly good sources of bio-fuel.

2.2.3 Nutritional composition and anti-nutrients in sorghum

As mentioned earlier, sorghum is the fifth most important crop grown worldwide behind wheat, maize, rice, and potatoes. These five crops collectively form at least 85% of the world's dietary intake for energy (Food & Agricultural Organization 2017). It is in some places thought to be a powerhouse of nutrition due to its high nutritional products and value. It is a rich source of proteins, with about 10% of proteins which are useful in forming the building blocks of components of the body including enzymes, skin, muscle, and bone. With its high content of over 75% carbohydrates, it becomes a grain that is a rich source of energy for the body like its counterpart, maize. It is as good as wheat regarding energy supply and is preferred particularly for those with celiac disease as it is gluten-free, (Queroz et al.,2015).

It has decent amounts of iron and niacin which are boosters of the immune system and improve the circulation of blood by increasing the overall oxygen-carrying capacity of the blood. Vitamin B complex and especially, Vitamin B1, B2 and B6 are also contained in sorghum and these help in the synthesis of antibodies and very important in improved nerve function. Sorghum also contains varying amounts of essential minerals and metals such as phosphorus, iron, magnesium, and calcium that are necessary for good bone formation and strength as well as temperature regulation, (Danovich, 2015). The high content of dietary fibre is very good at improving the digestion process. Sorghum also is loved due to its anti-oxidant content which greatly reduces the risk for common lifestyle diseases such as cardiac diseases, diabetes and even certain cancers, (Kayodé et al., 2011).

As was earlier noted in maize, sorghum also contains phytate as an anti-nutrient. Phytate is a metal chelator that has a high affinity for binding to certain metals once it is consumed. The bond formed is insoluble and cannot be broken down in normal physiological pH of the body, and as such, the minerals bound become biologically unavailable and thus unusable to the human or animals. Polyphenols are other anti-nutrients that are largely present in many crops (Danovich, 2015). Grain crops such as sorghum have relatively high amounts of such compounds because of the advantages they pose to the crop itself. Among these include prevention of premature germination of the destruction of the grain by inhibiting the growth of micro-organisms and preventing damage as a result of mould growth, (Ahmed et al., 2014).

Tannin is an example of the polyphenols largely found in the sorghum grain. It is an anti-nutrient in the sense that it binds to various structural and functional proteins including enzymes of the digestive tract and impairs their function. It also imparts a strange taste to the grain if in very high concentrations which reduces its intake. These properties inhibit growth, food efficiency and overall metabolic utilization of the foodstuff that is metabolized in the body, (Kayodé et al., 2011). Other anti-nutrient factors present in sorghum include a variety of inhibitors of enzymes such as amylase, trypsin, and proteases which are essential in the process of digestion, (Mohapatra et al., 2019). Goitrogens and dhurrin which is a cyanogenicglucoside and mycotoxins are also other minor anti-nutritive compounds present in the sorghum grain.

Certain processes can be applied to reduce the content of anti-nutrients in the cereal grains to improve or maintain the nutrition value. These include treatment or fermentation with lactic acid bacteria for about 3-5 days to allow for breakdown and reduction of the anti-nutrients, (Adeyemo et al., 2018). Soaking in water is another simplified way of minimizing the inhibitory effects of the above anti-nutrients.

2.3 Grain amaranth production, uses and chemical composition

2.3.1 Origin, distribution, production, and uses of grain amaranth

The origin of the Amaranth plant date back to more than 8,000 years ago where they are cultivated for their grain. The crop was first recognized in areas of Mexico and other parts of North, Central and South America, (Rastogi & Shukla, 2013). *Amaranthuscaudatus*L.,*Amaranthuscruentus* L. and *Amaranthushypochondriacus* L. are the three main species widely used for the amaranth grain. Grain crop production started in the 1970s in the United States, although it is grown on a much smaller scale compared to other grain crops.

However, over the years, the production of the crop has been fluctuating much influenced by the demand and market costs. Many other countries are emerging as growers of the crop, and these include Nepal. India, China, Kenya, Peru, and Russia. The Amaranth crop also grows like a weed especially in hot, humid areas although this is mostly used as a green, leafy vegetable. (Chemeda, 2018). Many species of the grain are noted to be very resistant to changes in temperature, soil pH, drought and salt content. *Amaranthuscaudatus* is the most common species that is very widely adaptive and is commonly distributed in areas of South America and Asia.

The amaranth plant, in general, has a lot of uses and most importantly for human consumption. The grains are a staple food used in most countries and even hold some cultural importance in Mexico. It is ground to produce flour that is used in making of tamales and tortillas, a very common delicacy in Mexico as well, (Rastogi & Shukla, 2013). The plant in itself is also consumed as a vegetable, and this is common in the semi-arid tropics of Africa and Asia. Other uses of amaranth plant and seeds include the formation of oil that has both cosmetic value and useful in dietary supplementation. The flowers of the plant can be ground to produce a characteristic dye. It also has ornamental value in some areas of the world.

2.3.2 Nutritional composition and anti-nutrient factors of amaranth grain

The amaranth, as are all other cereal crops, is a good source of energy in the form of carbohydrates. It has about 12-17% protein that are essential building blocks of tissues within the body and high dietary fibre that aids in the digestion process. Because it is gluten-free, like sorghum, it is preferred and highly bought by people suffering from celiac disease. It is a food highly valued by the new healthier markets and community due to the reduced amount of saturated fats that it contains,(Chemeda, 2018). Unlike other cereal crops, it contains a higher amount of the amino acid lysine but is low in leucine and threonine. Essential minerals are also present in the grain, and these include manganese, magnesium, phosphorous, potassium, zinc, iron and calcium. There are also reports that it may contain anti-oxidants and perhaps plays an anti-inflammatory role in the body when consumed.

Of the anti-nutrients, it is said to contain include, oxalates, saponins and phenol compounds as in other cereal crops. These impair absorption of nutrients and is common with such grains mostly with inadequate preparation. Saponins, in particular, can destroy the inner cellular lining of the intestines and this limits absorption capacity as well as increasing the risk of bacterial contamination of the gut, (Chemeda, 2018). Proper and adequate methods of cooking after soaking of the seeds has been shown to reduce the anti-nutrient content in the seeds and make them useful regarding their nutritive value.

2.4 Origin, distribution, uses and nutrition value of baobab tree fruit

The baobab tree is of the Adansonia genus that is home to nine other species of trees including the hibiscus and the mallow. Its scientific name is *Adansoniadigitata*, and the name baobab is derived from the Arabic language, and it means ‘father of many seeds.' The tree has its origins in Africa with the first specimen reportedly identified in Senegal by scientist Michel Adanson, from where it derived its name, (Abdulkarim & Bamalli, 2014). The trees are deciduous in that they shed their leaves during the dry season and remain without leaves for up to 9 months in a year. The trees, which grow solitary are commonly found in the scrubland or savannah vegetation.

The trees tend to be large with a wider girth and reaching heights of up to 12-18 meters depending on the species. The wideness of the trunk is considered an adaptive mechanism to the harsh and dry climate in the areas that they grow as it acts as a water reserve. It usually appears as though it is upside down due to the nature of branching off of the leaves and branches from the trunk, (Lisao et al., 2017). They are usually pollinated by fruit bats to produce fruit with hard, black seeds that are kidney-shaped. The fruit is shaped like a gourd and contains a tasty pulp that can be used to make refreshing drinks. The leaves are also edible when they are still young and soft and in some places are used for their herbal effects, (Gebauer et al,2016).

The barks produce strong fibre that has been used in the making of rope and cloth as well as act as raw materials in tools used for hunting and fishing. In many countries especially in Africa, the tree also holds religious and cultural importance where the wide, hollow trunks are used as prisons or burial and ritual sites and temporary shelters, (Abdulkarim & Bamalli, 2014). The baobab fruit and its powder are considered of great nutritive value having the highest anti-oxidant content in comparison with other fruits. It also has a high content of vitamin C and dietary fibrewhich are useful in the promotion of digestion. It is considered a super fruit with high contents of essential minerals such as potassium, calcium, iron, and magnesium, (Adubiaro et al., 2011).

2.5. Orange-fleshed sweet potatoes production, uses and chemical characteristics

2.5.1 Origin, distribution, and production of orange-fleshed sweet potatoes (OFSP).

OFSP stands for the orange-fleshed sweet potato in its entirety. It is a dicotyledonous plant largely known as a root vegetable because of the large tuberous roots that are used for their starch content. Scientifically, they belong to the family Convolvulaceae and are named *Ipomoeabatatas*. The origin of the sweet potato is believed to be from parts of Central and South America about 5,000 years ago. In the early 1600s, the crop was introduced and cultivated in various Asian countries including China, Japan, and Korea mostly as a substitute for when the harvests were not as plentiful,(Mekonnen et al., 2015). With the Columbian exchange, the crop was later introduced to parts of Europe before spreading to countries in other continents of the world.

Globally, sweet potatoes are ranked number seven among the important food crops in production. Developing countries alone account for up to 95% of the world's total, with Asia generating 85%, Africa 15%, leaving only 5% for other countries in the world. In most African countries, the crop is considered a poor man's crop and the countries largely producing it depend on it heavily for consumption. In 2016, for example, total global production was about 105 million tons with an average yield of 13 tons per hectare with China leading with 67% followed by Nigeria and Tanzania, (Wanjuu et al., 2018).

The plant’s growth is favoured by warmer temperatures, adequate sunshine and moderate amounts of rainfall. They can grow on varied types of soil as long as they are well aerated and usually require little or no fertilizer. Instead of seeds, they are grown by sowing vine cuttings that grow rapidly and form some form of shade over weeds and as such not much weeding is required in the time of growth, (Mekonnen et al., 2015).

2.5.2 Uses, nutritional composition and anti-nutrients of OFSP

By far, the tuberous roots are the most important part of the crop and are the most used worldwide for their starch. They are usually prepared by boiling and enjoyed mostly with beverages for breakfast and are considered a healthier source of sugar. In the Asian countries, the roasted form of the sweet potato is a common delicacy prepared by the streets, (Okello et al., 2015). They can also be ground to produce flour that either substitutes or is mixed with wheat flour and used to bake various bread, cakes, cookies, and pastries. It is sometimes also prepared as a vegetable dish and served on the side of various meat dishes. Other non-culinary uses of OFSP include in ceramics, to form cloth dye by a combination of its juice with lime and as natural food colouring, (Mekonnen et al., 2015). The plant itself is used as animal feeds as well.

Sweet potatoes are rich in complex carbohydrates in addition to the simple starches and dietary fibres that are good for digestion. A unique feature of the orange-fleshed sweet potatoes is the higher content of carotene or vitamin A, which is very useful in maintaining eyesight and preventing night blindness, (Wariboko&Ogidi, 2014). Vitamins B and C, as well as other micronutrients such as manganese, magnesium, potassium, and zinc, are also found in moderate amounts. However, when compared to other crops such as maize and sorghum above, they contain a less amount of carbohydrates and proteins.

Sweet potatoes are reported to have moderately high levels of oxalates as anti-nutrients. This is because they prevent the absorption of certain minerals and especially calcium which is useful in the formation of strong bones and teeth, (Owade et al., 2018). The oxalates, especially in high concentrations have been associated with the formation of kidney and gallstones. These oxalates can be reduced by boiling and draining the water or by supplementing magnesium and zinc to bind them and lower their absorption in the gut.

2.6 Composite flours

2.6.1 History, shelf-life and nutritional composition of composite flour

Composite flours can be considered in two forms; first, that they are blends of other flours say from potato or rice, mixed with wheat flour to make unleavened bread, porridge or other snacks. Secondly, it can also refer to the use of other blends of flours that are not wheat. The mixing of other flours with wheat or total replacement is done for several reasons among them being, economic reasons and for a nutritional boost, (Abdelghafor et al., 2011). In the 1960s the Food and Agricultural Organization introduced the idea of composite flours mainly for the above two reasons. There was a need to boost the nutritional value of the flours used especially for the developed countries as well as creating variety. In the developing countries, however, the main reason was to lower the wheat imports that were rampant at that time to reduce the economic burden.

The shelf life, as well as the functional properties of the composite flours, is highly determined by the individual components added or used in the place of wheat flour. The shelf life is perhaps a bit reduced because most of the added flour is from cereals or tubers which are vegetables that tend to be more perishable, (Chandra et al., 2015). The nutritional value of composite flour is increased especially by the starch from the tuber crops and the protein added from foods such as soybeans. The addition of crops such as the OFSP adds lysine content to the flours. The essential minerals are also increased in the composite flours due to the individual additions in comparison to that in wheat flour on its own, (Abdelghafor et al., 2011).Composite flours not only improve the rheological factors, but also the taste and palatability as well as consumer satisfaction,(Chandra et al., 2015).

2.7 Extrusion cooking

Extrusion is a cooking method that was started in the mid-1980s. It is a short-time cooking technique at a high temperature. It combines the operations of mixing, cooking and shaping of raw materials to produce semi-edible products,(Fellows,2017). A moistened food material is plasticized and cooked in a barrel under pressure and high temperature. The food undergoes mechanical shear resulting in both chemical and molecular transformations. It is a preferred method of processing because it is a non-continuous process and there is significant retention of nutrients. Some of the extruded products include breakfast cereals, infant formula foods, textured protein vegetables and precooked starches. The extrusion process can be both detrimental and beneficial. For instance, it eliminates microorganisms, antinutrients, increases the soluble fibre and improves the bioavailability of iron, (Kumar et al., 2019).

2.7.1 Effects of extrusion on nutrient composition

Eribaset al, 2015 found that the extrusion treatment did not change the total protein content, but the soluble protein was reduced by 61-86%. Invitro protein digestibility was also increased by 88-95%. (Singh et al., 2007) attributed this to protein transformation including denaturation as well as reduction of antinutritional factors which impair digestion and also the activation of enzyme inhibitors which expose new sites for enzyme action. The influence of extrusion cooking on the nutritional, antinutritional, antioxidant activity and structural properties of snacks was investigated by Wani and Kumar, 2015. They also concluded that extrusion increased the protein content. Variables such as feed ratio, temperature and the materials involve d also affect the protein content.

The feed ratio increases protein digestibility. Temperatures of 100-140 increased the protein digestibility due to inactivation of protease inhibitors, (Singh et al., 2007) this is also due to the ability of the process to inactivate phytates, trypsin inhibitors, hemaglutinins and tannins which inhibit protein digestibility.

Lysine is one of the essential amino acids in cereals and can be lost during extrusion. The extrusion process should therefore be aimed at retaining lysine. Some of the equipment settings during the extrusion may affect the retention of lysine. For instance, an increase in screw speed increased the retention of lysine. Higher amounts of sweet potatoes also increased retention. Temperatures of below 180°C and low moisture content of 15% are also known to retain lysine. Due to the Maillard reaction, lysine is also lost. This is a chemical reaction that forms brown compounds between sugars and amino acids but improves flavour as well. The reaction occurs at high temperatures and affects lysine and other essential amino acids. Therefore, the extrusion temperatures should be below 180°C and the moisture should be below 15%.

Carbohydrates range from simple sugars to starch and fibre. Research shows that extrusion at higher temperatures of between 170°C -210°C reduces sugars. Raffinose and stachyose which induce flatulence are reduced during extrusion improving the quality of legume extrudates. Starch is the major storage of energy for most plants. Monogastric species such as humans can't digest gelatinized starch. During extrusion, gelatinization occurs at lower moisture contents 12-22%. Molecular weights of amylose and amylopectin are reduced in corn flour. Starch-lipid complexes also form during extrusion, (Camire, 2010).

Dietary fibre which is the edible part of plants resistant to digestion is also affected by extrusion. Eribaset al, 2017 found out that dietary fibre reduced by 30%. A rise in the dietary fibre of extruded barley was also observed. This was due to a change from insoluble dietary fibre to soluble dietary fibre, and indigestible enzyme and starch resistant glucans were created. They concluded that moderate extrusion temperatures don’t affect dietary fibre.

Lipids affect the extrusion process. High lipid foods are difficult to extrude and they tend to lose some fat due to the high temperatures. In low-fat foods, extrusion results in a product with good texture. The extrusion process also denatures hydrolytic enzymes preventing the release of FFA thereby extending the product's shelf life. Iron and peroxide value also increased in extruded rice products. Shradvan concluded that extrusion improved sensory and nutritional quality of food by preventing lipid oxidation (Wang et al., 2016).

Vitamin A and E are not stable under oxygen and heat. Beta carotene is the most affected due to thermal degradation. It was concluded that the retention of vitamins during extrusion depended on temperature, screw speed and energy input. There is an inverse relationship between these factors and vitamin contents after extrusion. ShushilVarma observed a reduction in vitamin A in extruded flour products after storage for six months.

Most minerals are heat stable and they are not affected by extrusion. By reducing antinutrients such as phytates and tannins by hydrolysis and destroying polyphenols during processing, the process enhances the bioavailability of minerals such as iron. Singh et al,2007 suggested that further studies be done on the effect of extrusion on other essential minerals.

Cereals are considered to be nutritious especially since they are high in starch, have a high amount of carbohydrates and others are high in proteins. However, the nutrition value could be altered as described above. By creating composite flours, the nutrition value is increased whereas by undergoing processes such as extrusion, the nutrition value can be diminished, (Brennan et al., 2012). Malnutrition can occur as well if these are the only foods taken without others to supplement other nutrients lacking such as other types of proteins, vitamins, and proteins,(Bakare, Osundahunsi, & Olusanya, 2016). This is especially a big hurdle in developing countries including Africa and Asia. Anti-nutrients, another inherent factor in cereals, especially in high concentrations,can lead to malnutrition. There are some processes which when avoided, e.g., cooking by boiling, or poor storage are known to increase the concentration of these anti-nutrients. This could explain why malnutrition can still occur even when the highly nutritive cereals are included in the diet.Therefore, the current study aimed at formulating a nutritious, acceptable and shelf-stable extruded porridge composite flour from maize, sorghum, grain amaranth, baobab and orange-fleshed sweet potatoes.

CHAPTER THREE: NUTRIENT AND ANTI-NUTRIENT COMPOSITION OF EXTRUDED CEREAL FLOURS FORTIFIED WITH GRAIN AMARANTH, BAOBAB AND ORANGE-FLESHED SWEET POTATO POWDER

Abstract

Cereal-based flours are used by most households in Sub-Saharan Africa in the preparation of most of their staples. However, with higher antinutrient levels, the micronutrient content of these cereal-based flours is low. As an alternative to raising the micronutrient content of cereal-based flour, food to food fortification is used. Using sorghum and maize as the cereal base and baobab, grain amaranth and orange-fleshed sweet potatoes as the fortifiers, this study sought to produce a nutritious extruded composite flour. To create various formulations of the composites, a completely randomized design in factorial arrangement was used with ingredient ratio and extrusion as factors and seven levels. The nutritional and antinutritional profiling of the raw materials and the composite flours was done using official analytical methods. The data wereanalysed usingthe *R* Project for *Statistical*Computing, R-3.6.3. The protein content, beta-carotene, iron and zinc content, on average, 8.99 ± 1.03 g/100 g, 895.90 ± 346.85 µg/100 g, 11.81 ± 9.73 mg/100 g and 1.74 ± 0.18 mg/100 g, significantly ($p<0.05$) increased after the sorghum-maize blend was fortified with amaranth, baobab and orange-fleshed sweet potato powder.

In comparison, composite flour extrusion significantly ($p<0.05$) decreased the content of protein and beta-carotene by 4.7%and 40.9%, respectively. The nutritional profile and antinutritional profile of the composite flours were significantly ($p<0.05$) influenced by extrusion and its association with the ingredient ratio. Both fortifying agents and extrusion play a role in the

reduction of antinutrients. Baby foods should be extruded to reduce antinutrients. Extruded flours should be refortified with beta carotene.

3.1 Introduction

Food to food fortification is one of the nutrition interventions and strategies employed to alleviate micronutrient deficiencies in resource-poor countries (Chadare et al., 2019; De Groote et al., 2020). Micronutrient deficiency in Sub-Saharan Africa (SSA) is as high as 49% among households (Fraval et al., 2019). Harika et al., (2017) estimated that children below the age of five years had a 35-63%, 32-63% and 15-35% zinc, iron and vitamin A deficiencies respectively. 24% of Kenyan children below the age of five are stunted due to poverty and poor nutrition (Ndemwa et al., 2017). Milk, eggs, fish and meat are good sources of protein but their high costs affect their availability in most developing countries households hence the need to improve the nutrient composition of readily available cereals (Manary & Callaghan-Gillespie, 2020).

Considering that the diets of most of the communities in sub-Saharan Africa (SSA) are cereal-based (Ekpa et al., 2019; Van Ittersum et al., 2016), nutrients such as protein, iron, zinc, beta carotene and folate have been incorporated in them for delivery to the population. Fortification of wheat and maize flour has been necessitated by insufficient levels and limited bioavailability of these micronutrients (Aslam et al., 2018). The ever-evolving nature and diversified processing seeking to address various gaps in the nutritional and physicochemical quality preferences by various populations also necessitates fortification of these cereal flours (Asaam et al., 2018; Mitchell et al., 2019).

According to Brown et al., (2010), the recommended zinc fortification levels for cereal flours across the globe were 1.4-3.3 mg $100g^{-1}$. The blending of flours rather than the use of mineral supplements has been proposed as one of the cost-effective and sustainable techniques of food fortification in SSA. Other than improving micronutrient levels in the flours, blending also induces other nutritional and health benefits such as improved protein and fibre contents. Adeyeye, 2016 reported that whereas wheat had a fibre content of 1.42 ± 0.05%, sorghum recorded a higher content of 2.32 ± 0.14% which makes it a good fortificant of fibre. Since sorghum starch has been shown to have poor digestibility (Kulamarva, Sosle, & Raghavan, 2009), compositing it with other flours promotes its acceptability and sensory attributes. According to Stefoska-Needham et al., (2015) and Vila-Real et al., (2017), incorporation of other foods into cereal flours is necessary due to the low level of mineral and protein contents.

Foods rich in micronutrients such as orange-fleshed sweet potato (OFSP), baobab pulp and grain amaranth have been composited with cereal flours in efforts to improve the micronutrient contents. The beta-carotene rich property of OFSP has enabled its utilization for mitigation of vitamin A deficiencies (VAD) through its incorporation into other foods (Owade et al., 2018). Although Abong' et al., (2020) indicated that the leaves of OFSP have higher beta carotene content than the roots, the roots are still the most consumed edible part of the OFSP. Incorporation of OFSP flour in maize increased the crude fibre and carotenoids by 2.19-2.69% and 160.26-205.22 µg/g respectively (Ukom et al., 2019).

Baobab, a deciduous tree that originated in Africa is mainly found in scrubland and savannah vegetation (Abdulkarim et al., 2014). The baobab fruit is rich in vitamin C, fibre, potassium, calcium, iron and magnesium (Muthai et al., 2017). Studies on flour blends using baobab pulp powder have shown improved rheological and mineral content of cereal flours (Mounjouenpou et al., 2018). According to Tanimola et al., (2016), grain amaranth is rich in micronutrients such as zinc (6.27 mg/100g), iron (11.00 mg/100g) and calcium (33.29mg/100g), of which their deficiency is of great public health importance. Easy availability of OFSP, baobab and grain amaranth in SSA makes their utilization in composite flour formulation viable. However, evaluation of the three ingredients as possible fortificants of cereal flours has not been extensively done. The current study evaluates a novel product developed through fortification of cereal flours for improved nutritional composition

3.2 Materials and methods

3.2.1 Raw material acquisition

All the raw materials were purchased from different parts of Kenya. White maize was purchased from Eldoret market. Pale-red sorghum (E91) from Busia was purchased through McKnight Sorghum research team and pale cream grain amaranth was purchased from Bungoma. Baobab powder was purchased from Mombasa and stored at the University of Eldoret Food Processing Centre at 25 ℃. Sweet potato puree, was purchased formOrgani Ltd in Homabay and stored in a deep freezer at -20 ℃. Each of the raw materials was purchased in packs of 10 kg

3.2.2 Sample preparation

The grains were washed and dried in a forced draft oven at 60 ℃ for 24 h to a moisture content of 12%. The frozen orange-fleshed sweet potato puree was thawed in warm water before drying it in the oven at 60 ℃ for 6 h. They were then milled in a hammer mill fitted with 800 μm sieve to obtain whole-milled flours and stored in clean buckets.

3.2.3 Formulation of the composite flours

A completely randomized design in factorial arrangement with extrusion and formulation as main factors was used in the production of the flours. The formulations were based on findings of the nutritional profile of each of the ingredients to meet the Recommended Dietary Allowance of children below the age of five as per World Health Organization recommendations. Nutrisurvey linear programming software embedded with WHO RDA for children was used in the formulation of the flours. The formulations targeted 25 % of beta-carotene, iron and zinc contents and 15% of protein. Seven different formulations with varying ratios of maize, sorghum, grain amaranth, baobab and orange-fleshed potatoes were arrived at using Nutrisurvey as presented in (Table 3.1). The raw materials were mixed and half of each mixture stored separately for use in comparison to the extruded mixture. The moisture content of the other half was raised to 35% by adding water and mixing thoroughly before being extruded at 160 ℃ in a single screw extruder (TechnoChem, USA) with a screw speed of 800rpm, dried at 100 ℃ for 4 h, milled and packaged.

Table 3.1: Composite flour formulations

Formulation	**Ingredient proportion (%)**					**Description**
	Maize	**Sorghum**	**Amaranth**	**OFSP**	**Baobab**	

A	30	35	20	10	5	Varying cereals and fortificants
B	42.5	22.5	5	15	15	More maize than sorghum with constant fortificants
C	22.5	42.5	5	15	15	More sorghum than maize with constant fortificants
D	32.5	32.5	5	15	15	Equal maize and sorghum, constant fortificants
E	65	0	5	15	15	Maize plus constant fortificants
F	0	65	5	15	15	Sorghum plus constant fortificants
G	20	45	5	15	15	Variant of formulation C

3.2.4 Analytical methods

3.2.4.1 Proximate composition determination

Proximate composition was determined according to AOAC 2012 (AOAC, 2012) methods. Ash content was analyzed as per method number AOAC 942.05:2012, moisture content was according to method number AOAC 976.08:2012, the nitrogen content was done by Kjeldahl method number AOAC 988.05:2012 and converted to protein by multiplying with a factor of 6.25. The crude fibre was determined by gravimetric method according to method number AOAC 958.06:2012 while fat content was determined by Soxhlet method number AOAC 942.05:2012.

Carbohydrates were determined by the difference:

Carbohydrates= (100%-[%protein+%fat+%moisture+%ash+%Fiber])

Energy content was determined by the WHO/FAO(WHO/FAO, 2003) factor; Energy= 4 kcal/g (protein)+ 9 kcal/g (fat)+ 4 kcal/g (carbohydrates).

3.2.4.2 Determination of beta-carotene content

Beta-carotene was done according to a method described by Biswas et al(Biswas et al., 2011). A 2 g sample was extracted using a pestle and motor with small portions of acetone until the residual turned colourless. All the extract was then combined in a 100 mL volumetric flask. Approximately 25 mL of the extract was transferred to a 50 mL round-bottomed flask and evaporated to dryness in a rotary evaporator at 60 ˚C. Petroleum spirit, approximately 1 mL, was added to dissolve the β-carotene. The β-carotene was then eluted through a packed column and the absorbance of the eluent read at 450 nm. The β-carotene content was then calculated from the β-carotene standard curve.

3.2.4.3 Determination of iron and zinc contents

Iron and zinc were determined by a modification of the method described by Puwastien et al(Puwastien et al., 2011) using Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES). Approximately 0.5 g of the samples, blanks, positive control and negative controls were weighed into Teflon tubes and 6 mL of concentrated HNO_3 and 3 mL of H_2O_2 added. The vessels were left to vent in a fume hood for 30 min and then capped. They were then placed on the rotor and inserted into the microwave oven (Anton Paar MULTI WAVE PRO 50 HZ 10HF100, St Albans, UK) with the microwave power set at a maximum of 1500W and digested according to manufacturer's instructions until a clear digest was obtained. They were then transferred to centrifuge tubes and the volume adjusted to 50 mL.The samples and quality control standards were then placed on the autosampler (Agilent Technologies 5110) for analysis and the results read. The

Zn and Fe content of the sample expressed in mg/Kg of the product was calculated using the formula:

Zn/Fe content= $(\boldsymbol{ci} - \boldsymbol{cb}) * \boldsymbol{v} * \boldsymbol{d.f/w}$

Where;

ci = Zn or Fe content of test solution expressed in mg/l read from the calibration curve.

cb = Content of blank solution in mg/l read from calibration curve

v = The final volume

d. f = Dilution factor

w= Sample weight

3.2.4.3 Determination of phytate content

Phytic acid content was determined using the method by Latta et al (Latta & Eskin, 1980). Approximately 1g of the sample was defatted by addition of 10 mL of petroleum ether and left to stand for two hours. The supernatant was discarded and the samples allowed to dry; 10 mL of 10% hydrochloric acid was added and the suspension centrifuged (DrNgerber, K. Schneider & Co, Zurich, Switzerland) at 482.97g for 10 min and the supernatant transferred into 100 mL volumetric flask. This was repeated 4 times. Approximately 2 mL of the sample was transferred to a 50 mL volumetric flask and 2 mL of Wade reagent (0.03% iron chloride+ 0.3% Sulfosalicylic acid) added and topped up to 10 mL with water. Absorbance was read using a single-beam spectrophotometer (Spectronic 1001, Milton Roy Company, USA) at 500 nm wavelength. The phytate content was calculated using the phytic acid standard curve and the results expressed in g/100g dry weight.

3.2.4.4 Determination of tannin content

Tannin content was analyzed according to AOAC (2012) method number 952.0:2012. Briefly,0.5g of the sample was weighed and 50 mL of water added and vortexed for 5 min and allowed to settle. The supernatant was then decanted into a clean conical flask. Approximately 2 mL of Folin Denis reagent, prepared according to Ferreira et al., (2004) was added to 75 mL of distilled water followed by the addition of 2 mL of the sample and 5 mL of concentrated sodium carbonate. The volume was then adjusted to 100 mL by addition of distilled water and allowed to stand for 40 min. Absorbance was then read at 725nm using a single beam spectrophotometer (Spectronic 1001, Milton Roy Company, USA) at 500 nm wavelength and the results expressed in g/100g dry weight.

3.2.5 Statistical analysis

Statistical analysis of the data was done in the *R* Project for *Statistical*Computing, R-3.6.3 (R Core Team, 2019). The nutrient and antinutrient contents were converted to dry weight basis (dwb) and descriptive statistics including the mean and the standard deviation obtained. Normality of the data was tested using the Wilk's Shapiro test. Exploratory analysis of the data was done using the Pearson correlation. Inferential statistics were done by ANOVA, whereby means that were statistically different were separated using the Tukey'sHSD test. Significant differences were tested at $p<0.05$.

3.3 Results

3.3.1 Proximate composition of the raw materials

Proximate composition of the raw materials was statistically ($p<0.05$) different as shown in Table 3.2. Grain amaranth had a fat, protein and fibre content of 9.03 ± 0.23, 15.26 ± 0.34 and 9.28 ± 0.44 g/100g dwb, respectively, which was significantly ($p<0.05$) higher than the combination of these nutrients reported in other raw flour samples whereas the baobab powder had the highest ash content (9.25 ± 0.09 g/100g dwb).

The micronutrient content of the raw flour significantly ($p<0.05$) differed from each other (Table 3.3). Iron, zinc and beta-carotene contents were the micronutrients of interest in this study and they were highest in sorghum, grain amaranth and orange-fleshed sweet potatoes respectively.

3.3.2 Antinutrient content of raw flours

Tannins were highest in sorghum flour (335.08 ± 16.53 mg/100g) whereas the phytates were highest in baobab powder (191.95 ± 0.41 mg/100g), $p<0.05$ (Figure 3.1).

Table 3.2: Proximate composition of raw flours used in formulating composite flours

Raw flour	Moisture (g)	Protein (g)	Fat (g)	Fibre* (g)	Ash* (g)	Carbohydrates* (g)	Energy value (Kcal)
Maize	13.76±0.60^{b}	4.89±0.07^{c}	4.56±0.12^{c}	2.33±0.03^{b}	1.35±0.02^{e}	86.87±0.00^{a}	408.06±0.78^{b}
Sorghum	11.06±0.15^{c}	7.19±0.05^{b}	6.32±0.91^{b}	3.03±0.07^{b}	1.68±0.00^{d}	81.79±0.92^{b}	412.77±4.30^{a}
Amaranth	12.30±0.69^{bc}	15.26±0.34^{a}	9.03±0.23^{a}	9.28±0.44^{a}	2.95±0.05^{c}	63.48±0.17^{d}	396.25±2.74^{c}
Baobab	17.42±0.62^{a}	0.42±0.01^{d}	0.70±0.06^{d}	10.48±0.41^{a}	9.25±0.09^{a}	79.15±0.43^{c}	324.58±2.31^{e}
OFSP	14.06±0.02^{b}	6.09±0.02^{c}	0.54±0.03^{d}	2.89±0.03^{b}	5.03±0.01^{b}	85.46±0.09^{a}	371.00±0.01^{d}
%CV	16.7	75.7	82.6	65.0	72.7	11.1	11.2
p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

The values are mean of duplicate samples ± SD. Values with different letters in the superscript along a column are statistically different at $p<0.001$. All the values are in g/100g dwb except moisture values which are in g/100g wet weight basis (wwb).

Table 3.3: Micronutrient composition of raw flours used in formulating blended flours

Raw flour	**Iron (mg)**	**Zinc (mg)**	**Beta-carotene (mg)**
Maize	2.09 ± 0.02^{e}	0.20 ± 0.02^{b}	Nd
Sorghum	18.57 ± 0.03^{a}	0.14 ± 0.00^{c}	Nd
Amaranth	12.30 ± 0.14^{c}	0.62 ± 0.01^{a}	Nd
Baobab	16.07 ± 0.27^{b}	0.12 ± 0.00^{c}	Nd
OFSP	6.50 ± 0.02^{d}	0.16 ± 0.00^{c}	3268.45±6.64
%CV	58.1	81.8	N/A
p-value	<0.001	<0.001	

The values are mean of duplicate samples ± SD. Values with different letters in the superscript are statistically different at $p<0.05$. Nd- not detected. All the values are in g/100g dwb.

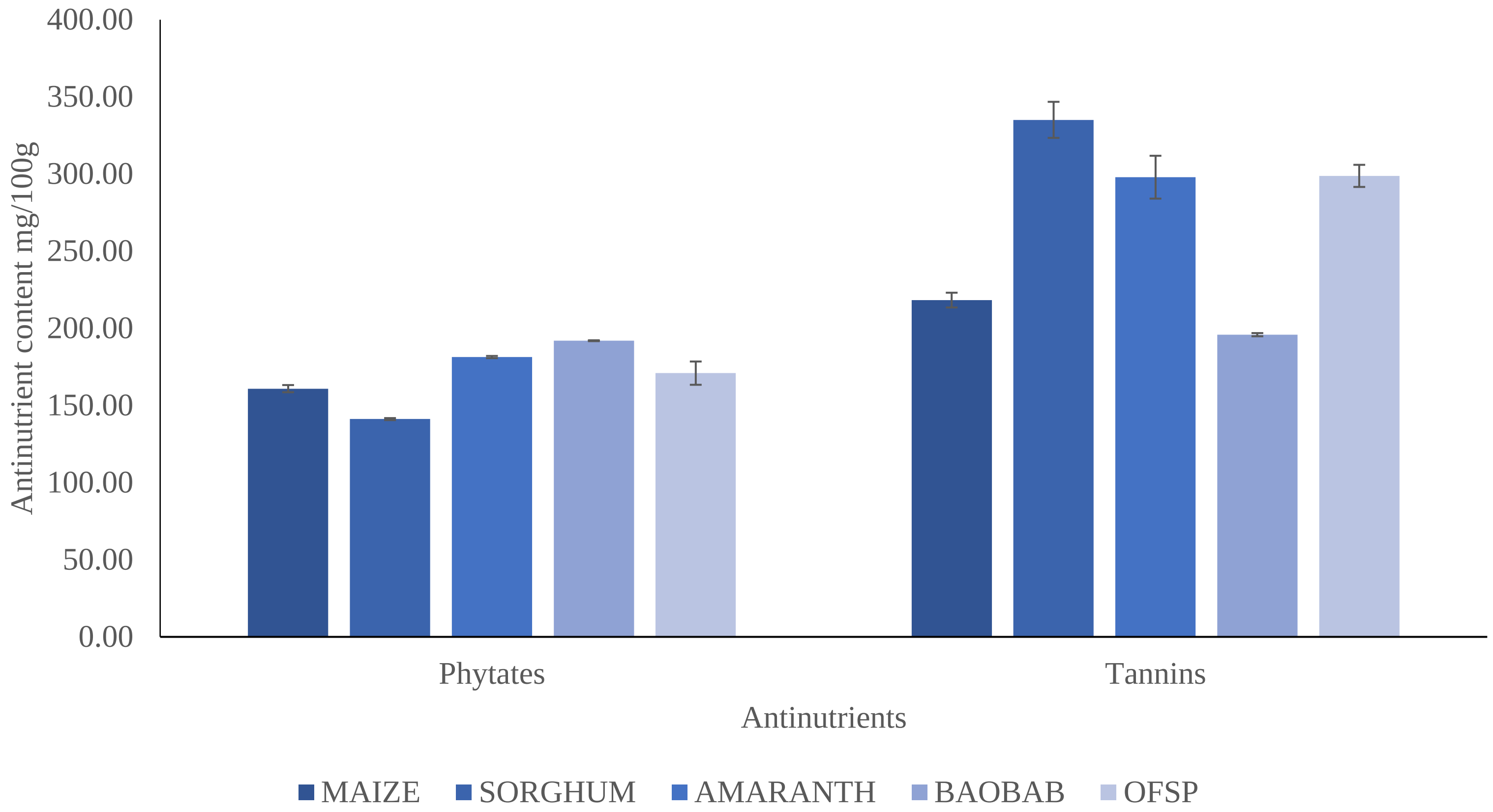


Figure 3.1: Antinutrient content of raw flours used in formulating blended flours. The values are mg/100g dry weight.The error bars indicate the standard error of the mean.

3.3.3 Proximate composition of composite flours

Proximate composition of flours was significantly affected by the ratio of the ingredients, extrusion and the interaction of the two factors. Addition of the fortificants significantly ($p<0.05$) affected the protein, ash and carbohydrate contents (Table 3.4)**.** Sample A in which the highest content of amaranth grain (20%) was added had significantly ($p<0.001$) high contents of protein and ash. Increasing maize levels resulted in significantly ($p<0.05$) higher carbohydrate contents compared to sorghum.

Extrusion of the composite flours significantly improved the fat and fibre contents and the energy values while reducing the ash, moisture, protein, fibre and carbohydrate contents as shown in Figure 3.2.

Table 3.4: Effect of fortification of cereal flours with baobab, orange-fleshed sweet potato and amaranth grain powders on their proximate composition

Blended flours	**Moisture (g)**	**Protein (g)**	**Fat (g)**	**Fibre (g)**	**Ash (g)**	**Carbohydrates (g)**	**Energy values (Kcal)**
A	10.59±3.39^{a}	10.36±0.89^{a}	4.33±0.99^{a}	0.87±0.04^{a}	0.72±0.02^{a}	84.08±0.30^{ab}	416.70±5.13^{a}
B	8.82±4.96^{a}	8.85±0.33^{b}	4.16±1.56^{a}	0.98±0.16^{a}	0.61±0.05^{b}	85.41±1.77^{ab}	414.42±8.29^{a}
C	9.23±4.38^{a}	9.24±0.04^{ab}	3.87±1.24^{a}	0.95±0.08^{a}	0.63±0.04^{b}	85.32±1.14^{ab}	413.04±6.63^{a}
D	11.68±1.85^{a}	8.35±0.78^{b}	3.91±1.29^{a}	0.91±0.19^{a}	0.63±0.03^{b}	86.36±0.24^{ab}	414.05±8.02^{a}
E	11.22±3.11^{a}	7.24±0.33^{c}	3.74±1.15^{a}	0.97±0.24^{a}	0.56±0.01^{c}	87.62±0.52^{a}	413.10±7.36^{a}
F	10.55±2.34^{a}	9.41±0.41^{ab}	3.77±1.45^{a}	0.91±0.10^{a}	0.62±0.02^{b}	85.29±0.96^{ab}	412.71±7.58^{a}
G	7.84±5.57^{a}	9.45±0.27^{ab}	3.88±1.69^{a}	1.07±0.01^{a}	0.54±0.01^{c}	85.05±1.95^{ab}	412.95±8.49^{a}
%CV	36.6	11.5	30.7	14.4	9.6	1.7	1.7
p-value	0.790	<0.001	0.996	0.576	<0.001	0.012	0.989

Values are means of triplicates for a sample. All values are in g per 100g. All the values are in dry weight basis (dwb) except for moisture (wwb). The error bars represent the standard deviation.

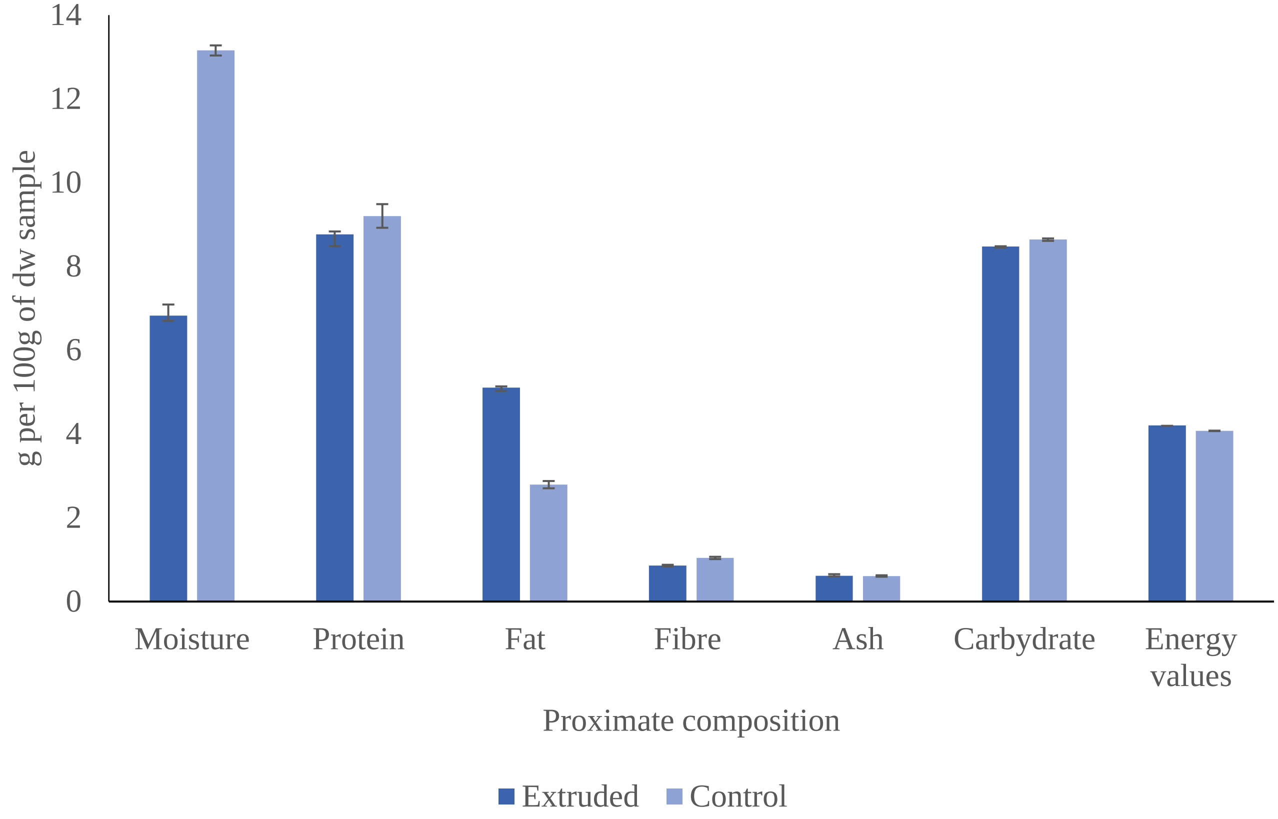


Figure 3.2: Effect of extrusion on the proximate composition of fortified cereal flours.

All values are in dwbexcept moisture which is on a wwb. Carbohydrate and energy values are to be multiplied by a factor of 10 and 100, respectively. The error bars represent the standard error of the mean.

Extrusion and ingredient ratio interaction significantly ($p<0.05$) affected the proximate composition of the composite flours as shown in Table 3.5.Extrusion significantly ($p<0.001$) increased the energy contents of high sorghum formulations. Increasing grain amaranth in the formulations resulted in significantly ($p<0.001$) higher protein and ash contents in both the extruded and non-extruded treatments. Extrusion induced higher fat contents while decreasing the moisture contents in all the formulations as compared to the non-extruded samples ($p<0.001$). The formulations that underwent extrusion all had significantly ($p<0.05$) lower carbohydrate contents notwithstanding the ratio of ingredients incorporated. An increasing proportion of maize in the formulation resulted in lower levels of fibre both in the extruded and non-extruded formulations ($p<0.05$).

Table 3.5: Effect of interaction between formulation and extrusion on the proximate composition of the composite flours

Formulation		**Moisture content(g)**	**Protein(g)**	**Fat (g)**	**Fibre (g)**	**Ash(g)**	**Carbohydrate(g)**	**Energy values (Kcal)**
A	Extruded	7.65±0.01^{d}	9.63±0.01^{e}	5.18±0.02^{e}	0.90±0.03^{d}	0.70±0.02^{fg}	83.94±0.43^{b}	420.90±1.90^{d}
	Control	13.53±0.01^{l}	11.09±0.51^{f}	3.47±0.00^{c}	0.84±0.02^{bc}	0.73±0.02^{g}	84.22±0.04^{c}	412.50±1.09^{b}
B	Extruded	4.53±0.03^{b}	9.12±0.11^{d}	5.51±0.03^{f}	0.84±0.00^{bc}	0.65±0.03^{e}	83.88±0.11^{b}	421.60±0.30^{d}
	Control	13.12±0.04^{j}	8.57±0.06^{c}	2.80±0.01^{b}	1.12±0.01^{f}	0.57±0.01^{abc}	86.94±0.09^{g}	407.20±0.00^{a}
C	Extruded	5.44±0.03^{c}	9.24±0.07^{d}	4.94±0.01^{d}	0.89±0.07^{c}	0.59±0.01^{bcd}	84.33±0.12^{cd}	418.80±0.20^{c}
	Control	13.03±0.02^{i}	9.24±0.01^{d}	2.79±0.03^{b}	1.01±0.01^{e}	0.66±0.01^{ef}	86.30±0.06^{ef}	407.30±0.10^{a}
D	Extruded	10.08±0.01^{g}	7.68±0.13^{b}	5.02±0.20^{d}	0.75±0.05^{a}	0.63±0.02^{de}	86.24±0.33^{e}	420.90±2.60^{c}
	Control	13.28±0.03^{k}	9.03±0.04^{d}	2.80±0.00^{b}	1.08±0.01^{f}	0.62±0.04^{d}	86.47±0.09^{f}	407.20±0.20^{a}
E	Extruded	8.52±0.03^{e}	6.96±0.06^{a}	4.73±0.02^{c}	0.77±0.01^{a}	0.57±0.02^{abc}	87.25±0.49^{h}	419.40±1.60^{c}
	Control	13.91±0.01^{m}	7.53±0.00^{b}	2.74±0.02^{b}	1.18±0.01^{g}	0.55±0.01^{ab}	88.00±0.03^{h}	406.80±0.00^{a}
F	Extruded	8.53±0.00^{e}	9.06±0.10^{d}	5.02±0.01^{d}	0.83±0.01^{b}	0.63±0.00^{de}	84.46±0.12^{d}	419.30±0.00^{cd}
	Control	12.58±0.01^{g}	9.76±0.01^{e}	2.51±0.01^{a}	1.00±0.02^{e}	0.60±0.01^{cd}	86.13±0.05^{e}	406.1±0.10^{a}
G	Extruded	3.02±0.03^{a}	9.68±0.02^{e}	5.35±0.01^{f}	1.07±0.00^{f}	0.54±0.00^{ab}	83.36±0.03^{a}	420.30±0.00^{d}
	Control	12.67±0.02^{h}	9.22±0.00^{d}	2.42±0.02^{a}	1.06±0.01^{ef}	0.54±0.01^{a}	86.75±0.05^{g}	405.60±0.10^{a}

Values are means of triplicates for a sample. Values with different letters in the superscript along a column are statistically different at $p<0.05$. All the values are in dry weight basis (dwb) except moisture which is on wwb. Formulation A had varying fortificants, formulation B had more maize and equal fortificants, formulation C had more sorghum and equal fortificants, formulation D had equal maize and sorghum with constant fortificants, formulation E had maize and fortificants, formulation F had sorghum and the fortificants and formulation G was a variant of formulation C

3.3.4 Micronutrient and antinutrient content of blended flours

Beta carotene, phytates and tannin contents in the formulations were significantly ($p<0.01$) affected by the ratio of the ingredients, extrusion and the interaction of the two factors. However, the zinc content of the flours was only significantly ($p<0.001$) affected by the ratio of the ingredients and the interaction between the ratio of the ingredients and extrusion. Neither the incorporation of the fortificants nor the process of extrusion significantly ($p>0.05$) affected the iron content of the formulations.

The beta-carotene content of the fortified cereal flours was significantly predicted by the moisture content at $p<0.05$ with a variance of 53.1%. The regression equation of the predictor model was as shown in equation 1.

$$y = 3 + 0.01x \tag{1}$$

whereby y is the beta carotene content and *x* is the moisture content. $R^2=0.53$

Whereas extrusion significantly ($p<0.001$) reduced the beta-carotene content of the cereal flours, it had no significant ($p>0.05$) effect on the iron and zinc contents. Treatment with the highest proportion of amaranth (20%) had the highest level (1.97 ± 0.12 mg/100g dwb) of zinc content at $p<0.001$. The trends did not change with the inclusion of the second factor (extrusion) as the incorporation of the amaranth grain significantly increased the zinc content (1.88-2.06 mg/100g dwb). Incorporating OFSP and amaranth grain powder significantly increased the beta carotene content of the cereal flour (Table 3.6). Extrusion resulted in higher degradation of beta-carotene in cereal flour with higherOFSP as compared to that with high amaranth grain, $p<0.05$.

There was significantly ($p<0.001$) higher phytate and tannin contents in non-extruded cereal flours than the extruded(Table 3.7). A higher proportion of sorghum significantly ($p<0.05$) increased the tannin and phytate contents with the samples with 65% sorghum having the phytate and tannin contents of 11.47 ± 2.17 and 1329.9 ± 265.2 mg/100g dwb, respectively. The interaction of extrusion and formulation significantly ($p<0.05$) affected the phytate and tannin contents of the blended flours (Figures 3 and 4). The phytate contents of cereal flour with the higher proportions of sorghum than maize was found to reduce whereas those with more maize than sorghum recorded lower tannin contents on extrusion at $p<0.001$. In all the formulations, the tannin content reduced when extrusion was done. Zinc was positively correlated with the phytate content in the cereal flours ($p<0.05$) as shown in Table 3.8.

Table 3.6: Effect of extrusion and formulation of cereal flours with baobab, orange-fleshed sweet potato and grain amaranth powder on their micronutrient content (mg/ 100 g dwb)

Formulation		**Micronutrient content (mg/100g)**		
		Beta carotene content	**Iron content**	**Zinc content**
A	Extruded	1074.4 ± 1.0^{Ah}	7.35 ± 0.10^{Da}	1.88 ± 0.12^{Ge}
	Control	1301.5 ± 13.6^{Ahi}	5.26 ± 0.01^{Da}	2.06 ± 0.00^{Gf}
B	Extruded	385.8 ± 12.0^{Ab}	14.40 ± 0.09^{Da}	1.46 ± 0.09^{Ga}
	Control	1277.8 ± 20.8^{Ah}	4.25 ± 0.10^{Da}	1.68 ± 0.16^{Gbcd}
C	Extruded	280.3 ± 1.3^{Aa}	14.35 ± 0.02^{Da}	1.89 ± 0.05^{Ge}
	Control	1324.7 ± 20.5^{Ai}	5.15 ± 0.13^{Da}	1.62 ± 0.07^{Gbcd}
D	Extruded	693.1 ± 1.2^{Ad}	22.12 ± 0.07^{Da}	1.59 ± 0.03^{Gabc}
	Control	1320.8 ± 13.1^{Ai}	6.70 ± 0.02^{Da}	1.68 ± 0.04^{Gbcd}
E	Extruded	772.3 ± 1.4^{Ae}	15.64 ± 0.07^{Da}	1.68 ± 0.03^{Gcd}
	Control	1043.9 ± 12.5^{Ag}	4.29 ± 0.02^{Da}	1.86 ± 0.08^{Ge}
F	Extruded	601.9 ± 19.2^{Ac}	19.88 ± 0.09^{Da}	1.76 ± 0.02^{Gde}
	Control	885.0 ± 0.7^{Af}	6.35 ± 0.06^{Da}	1.89 ± 0.05^{Ge}
G	Extruded	577.9 ± 1.3^{Ac}	11.49 ± 0.02^{Da}	1.88 ± 0.04^{Ge}
	Control	1003.1 ± 7.0^{Af}	28.19 ± 2.67^{Da}	1.49 ± 0.02^{Gab}
%CV		38.7%	82.3	10.2
p-value		<0.001	0.219	<0.001

Values with a similar uppercase letter followed by different lowercase letters in the superscript are statistically different at $p<0.05$ whereas values with a similar uppercase letter followed by similar lowercase letters are statistically similar at $p<0.05$. Values are means of triplicates for a sample.

Table 3.7: Main effect of extrusion and formulation on tannins and phytate contents of composite flour

Formulation	**Phytates mg/100g**	**Tannin mg/100g**
A	12.85±1.70^{Aab}	757.7±149.4^{Aa}
B	6.25±2.26^{Aabc}	873.3±385.6^{Aa}
C	8.61±1.04^{Abcd}	1109.0±381.7^{Abc}
D	10.07±1.61^{Abcd}	1078.8±213.5^{Ac}
E	7.57±1.91^{Aa}	570.7±63.0^{Aab}
F	11.47±2.17^{Ad}	1329.9±265.2^{Ad}
G	8.01±1.08^{Acd}	1163.0±225.0^{Ab}
%CV	18.8	26.8
LSD	2.56	387.9
Extrusion		
Extruded	8.74±3.21^{Ba}	775.0±215.3^{Ba}
Control	9.78±1.98^{Bb}	1191.5±314.6^{Bb}
%CV	28.8	27.4

Values with a similar uppercase letter followed by different lowercase letters in the superscript in a column are statistically different at $p<0.05$ whereas values with a similar uppercase letter followed by similar lowercase letters are statistically similar at $p<0.05$. Values are means of triplicates for a sample and all the results are on a dry weight basis. The error bars represent the standard error.

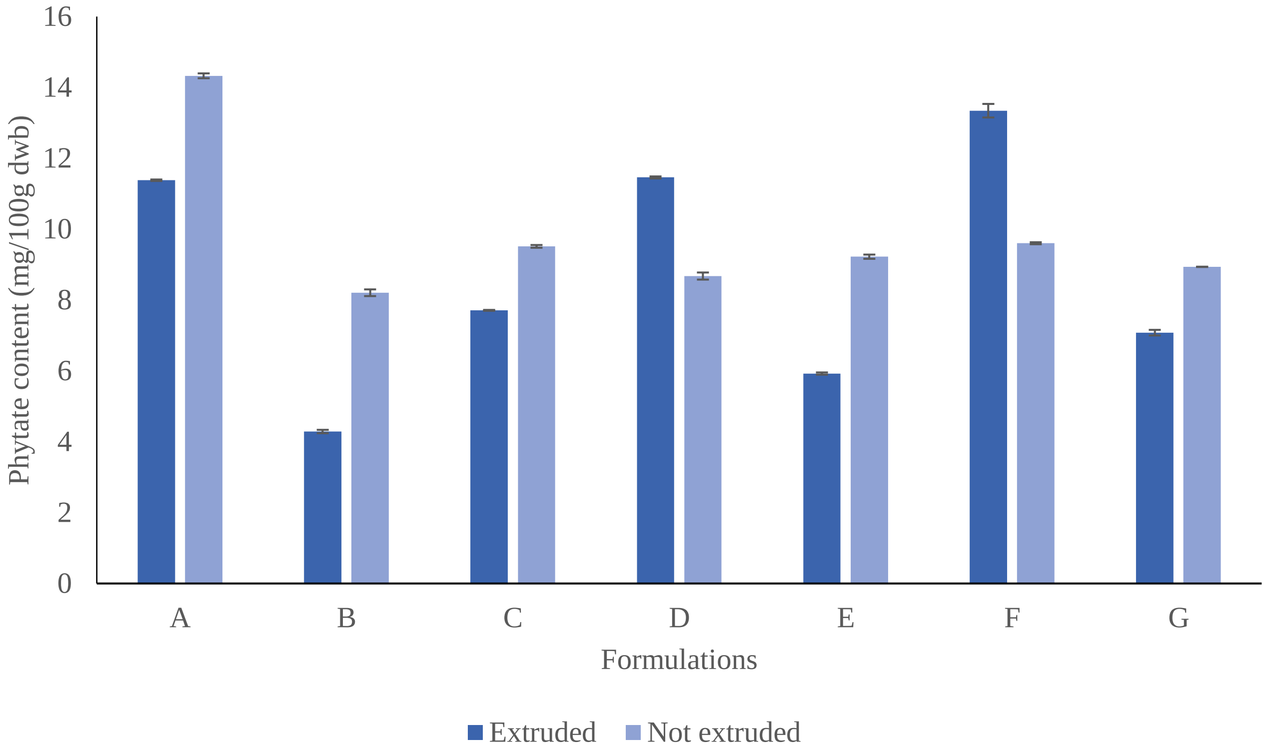


Figure 3.3: Effect of the interaction between extrusion and formulation on the phytate content of blended flours. Values are means of triplicates for a sample. The error bars represent the standard error of the mean.

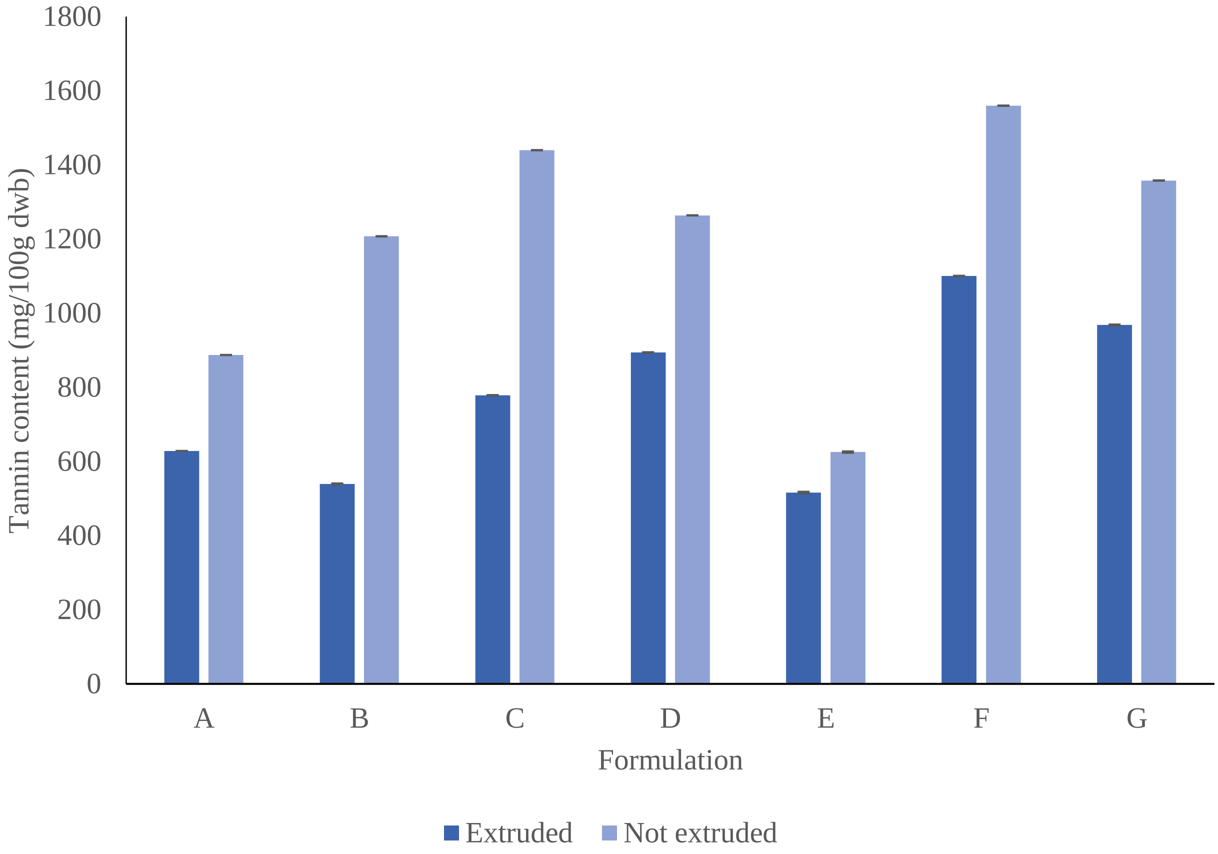


Figure 3.4: Effect of the interaction between extrusion and formulation on the tannin content of blended flours. Values are means of triplicates for a sample. The error bars represent the standard error of the mean.

Table 3.8: Correlation between micronutrients and antinutrients in cereal flours

Micronutrient	**Antinutrient**	
	Phytates	**Tannins**
Iron	-0.04	-0.03
Zinc	0.45*	-0.10

*Value significant at $p<0.05$

3.4 Discussion

3.4.1 Nutrition composition of raw material

The findings in the present study showed amaranth and OFSP are the most nutritious fortificants of protein, beta-carotene and zinc in the formulations. The protein content for the amaranth grain found in this study was comparatively within the range of 13.37 to 23.28% reported by Kachiguma et al(Kachiguma et al., 2015) for various accessions grown in Malawi. However, Ayo, (2001) reported slightly a higher figure of 13.65% for the protein content. The range for the protein level reported by that particular study was 13.37% to 21.50%. In proximate composition, baobab had the highest ash content pointing to higher mineral composition. OFSP was the only fortificant that had beta-carotene. In as much as drying of the OFSP roots deteriorates the level of beta-carotene through oxidation (Owade et al., 2018), the level of beta-carotene found in the current study was still higher than the levels reported by Aywa et al(Away et al., 2013) in some of the raw roots of some ascensions grown in Kenya. An earlier study by (Tadesse et al., 2015)found that maize flour had undetectable levels of beta-carotene, thus the 1987±0.05 µg RAE/100 g of vitamin A in OFSP flour makes it ideal as a fortificant. The two kinds of cereals in this study had the highest contents of carbohydrates and energy values. Additionally, the drying of the fresh OFSP roots and grinding into flour has been shown to increase the carbohydrate contents of these ingredients seven-fold (Hacineza et al., 2007).

Considering that cereal flours are high in carbohydrate, this finding justifies the need to fortify the blended flours to increase their protein content.

3.4.2 Antinutrient content of raw materials

Fortification of cereal flours that targets to increase their mineral content seeks to limit the antinutrient content of the specific flours. This is because of antinutrients such as phytic acid form complexes with micronutrients making them less bioavailable (Coulibaly et al., 2011). Evaluation of the cereal grains have shown that the content of antinutritional factors could be as high as 40-60% of the total caloric intake among the populations in SSA(Gupta et al., 2015); posing the risk of low bioavailability of micronutrients in the food taken. Sorghum and maize flours were found to have a higher content of tannin and phytate, respectively, than the fortificants. Increasing the tannin content of the cereal flour blend resulted in declining zinc content. This implies that improving the micronutrient content should not just be limited to the formulation of cereal flour blends with ingredients rich in zinc, additional treatment to address the antinutritional factors has to be applied.

3.4.3 Proximate composition of blended flours

Incorporation of amaranth into the blended flours significantly improved the protein and ash content of the flours. The high protein content of the amaranth grain has been a selling point for its incorporation in food and feed whereby the augmentation of the feed and the food is the target (Ayo, 2001; Pisarikova et al., 2006). However, increasing the maize content had a reverse effect on the fibre content as it was decreasing. This is explained majorly by the low content of fibre in the maize grain compared to the other ingredients. The whole grain cereals are adjudged to be rich in fibre (Sarwar, 2013). Subsequently, incorporation of the fortificants into cereal flours resulted in the decreased energy density of the flours. The average energy values achieved in the extruded cereal flour blends was higher than the average energy values per gram of carbohydrate of 4 KCal.

Most flour manufacturers usually add fats to increase the energy density of the flours (Okoth et al., 2017); however, in the present study, there was no addition of fat to the flour blends. Addition of fat in flour blends has a deleterious effect on the keeping quality of the flour as it decreases the shelf-life of the flour.

Extruded flour had a higher content of fat whereas reducing the ash, protein, carbohydrates and fibre contents. In their evaluation of flour fortification for the preparation of breakfast cereal, Santos et al(Santos et al., 2019) reported a threefold increase in the fat; increase that is higher than what was reported in the current study. This is attributed to the utilization of legumes which are known to be richer in oils in their study. The greatest decline in the protein content due to extrusion was seen in treatments with the highest amaranth content whereas the carbohydrates and energy values increased in samples with higher proportions of sorghum. The impact of extrusion on the proximate composition of extruded cereal flour has varied depending on the ingredients incorporated into the cereal flour. Whereas Yusuf et al(Yusuf et al., 2018)reported a decline in fibre as reported in this study, he reported contrary findings of an increase in the carbohydrate content of an extruded groundnut-sorghum flour blend. On the other hand, Tadesse et al., (2019)reported a decline in the carbohydrate content of an extruded soy-sorghum flour blend.

3.4.4 Micronutrient and antinutrient content of blended flours

In both extruded and non-extruded blended flours, the minimum beta-carotene level for fortified flours, 500 µg/100g (Owade et al., 2018), was achieved. Incorporation of amaranth into cereal flour was reported to improve the iron and zinc whereas not achieving significant levels of vitamin A and its equivalents (Akande et al., 2017). Beta carotene was significantly reduced as a result of the extrusion process. Similar trends were reported by Akande et al., (2017) in their study that evaluated the effect of extrusion conditions on the nutrient composition of cereal flours. With an increasing temperature of extrusion, it was reported that the vitamin A levels of the extruded flour declined. Cereal flour blends with OFSP rather than amaranth grain powder had higher degradation of the beta-carotene due to extrusion. Exposure of the OFSP to thermal treatment has been shown to result in degradation of the beta-carotene content through oxidation (Owade et al., 2018). Additionally, reduction in the moisture contents of the cereal flour blends resulted in a decline in the beta-carotene contents. This can be explained by the first order-kinetics of reduction of beta-carotene in dehydrated foods (Neto et al., 1981), whereby in low moisture beta-carotene deteriorates due to discolouration (Chou & Breene, 1972; Pénicaud et al., 2011).

One of the greatest limitations of cereal-based flours in SSA is the bioavailability and content of the micronutrients (Tadesse et al., 2015). Utilization of locally available ingredients to develop nutrient dense formulations of the of cereal-based composite flour has been recommended as one of the affordable strategies to fight malnutrition especially among the under five years old children to whom porridge constitutes a great part of the diet (Akande et al., 2017). The levels of iron, zinc and beta-carotene achieved in the formulations were 4.29 ± 0.02 to 28.19 ± 2.67**,** 1.46 ± 0.09 to 2.06 ± 0.00 and 280.3±1.3 to 1320.8±13.1 mg/100g dwb, which were higher than some of the levels reported for most rich sources including the indigenous vegetables such as cowpea leaves vastly consumed in SSA(NutriSurvey, 2007b, 2007a; Owade et al., 2019). The formulations in the present study thus serve as major food vehicles for the respective micronutrients especially to the most vulnerable population in SSA which are the children under the age of five years.

Extrusion lowered the antinutrient contents of the cereal-based composite flours by 10.6-35.0%. The findings in this current study lend support to previous works by Gürbilek (Gürbilek, 2016) who reported a 16.55 –50.85% decline in the antinutritional factors in sorghum flour blended cereal foods. Extrusion results in the destruction of inhibitory antinutritional factors such as phyticacids which lower the bioavailability of micronutrients in the cereals blended foods (Omosebi et al., 2018). On the other hand, increasing the sorghum ration in the cereal flours resulted in higher antinutrient content. The phytate content of the cereal flour blends with high sorghum significantly reduced on extrusion. Phytate contents reported in cereals has been estimated at 0.18 to 6.39% with higher intake among those consuming whole wheat cereal; for phytic acid has a higher concentration in the bran (Gupta et al., 2015). These levels are higher than those in the formulation of the cereal flour blends which ranged between 0.006 and 0.012%. Extrusion of the flour lowered the level of the phytate content by a further percentage. This is because extrusion hydrolyses phytic acid to phosphate molecules, thus destroying it (Wani & Kumar, 2016). In the formulation of the cereal flour blends, precooking is thus recommended as a measure of improving the bioavailability of the zinc and iron (Gupta et al., 2015).

The extrusion also achieved a decline of 35.0% in the tannin content of the composite flours and an average tannin content of 0.78% was achieved. In animal studies, it has been established that serially increasing the tannin content in feeds from 0.00% to 0.02% showed a linear reduction in the haemoglobin and hepatic iron concentration (Delimont et al., 2017). Fortification seeks to minimize the antinutritional factors while increasing the micronutrient contents of the composite flours. With the increasing zinc contents, the phytic acid contents of the cereal flour blends also increased.

3.5 Conclusion

Incorporation of OFSP, baobab and amaranth in the maize-sorghum cereal flour blend increases the zinc, iron, protein and beta-carotene contents while reducing the tannin content of the composite flours. However, the phytic acid content of these flours increases with increasing proportions of the fortificants and reduction of the cereal flours. Fortification aims to improve the overall bioavailability of the nutrients in the formulation. To this end, extrusion of the flour blends reduces the level of antinutritional factors. In as much as the beta-carotene is degraded on extrusion, the minimum content of 500µg/100g for fortified foods is still achieved. This study provides input to the nutritional programmes and the ever-evolving dietary practices on the most cost-effective ways to alleviate micronutrient and protein deficiencies in SSA. Considering the value-chains of the ingredients used as fortificants in this study, the output of this study can be promoted as one of the possible ways for commercialization of these value chains.

3.6 Recommendation

More formulations with lower levels of the fortificants can be done to reduce the level of phytates and tannins. Baby foods should be extruded to reduce antinutrients. Bioavailability studies of iron and zinc should be conducted to ascertain the effectiveness of extrusion on the reduction of antinutrients. The flour development process should be adjusted to include refortification with beta-carotene after extrusion.

CHAPTER FOUR: CONSUMER ACCEPTABILITY OF EXTRUDED MAIZE-SORGHUM COMPOSITE FLOURS FORTIFIED WITH GRAIN AMARANTH, BAOBAB AND ORANGE FLESHED SWEET POTATOES

Abstract

Porridge is a popular cereal flour-based food product for children in Sub-Saharan Africa. Compositing of cereal flours to improve their nutritional composition is being done, however, the enrichment of such flours with naturally nutrient-rich plant products is poorly developed. A study was conducted to evaluate the acceptability and sensory attributes of newly formulated extruded composite flours containing maize, sorghum, grain amaranth, baobab and orange-fleshed sweet potatoes. Seven extruded formulations optimized for nutritional composition were developed. The cross-sectional study design was used and purposive sampling used in collecting the market composites. Twelve trained panellists were used to evaluate the sensory attributes and overall acceptability of the composite flours determined on a 9-point hedonic scale. The data were then subjected to analysis of variance (ANOVA) using the R statistical package version 3.6.3, the means separated using Tukey'sHSD test at $p<0.05$ and data explored using clustering and principal component analysis. Formulation of the composite flours significantly ($p<0.05$) affected scores on colour, flavour and overall acceptability with the mean score ranging between 5.7 and 7.4. There was no significant difference ($p>0.05$) on overall acceptability between extruded and non-extruded composite flours but extruded flours had significantly higher scores on texture ($p<0.05$). The comparison of the newly formulated composite flours with the conventional flours showed no significant difference ($p>0.05$) in the overall acceptability, and therefore, they have the potential of being adopted. Mouthfeel and flavour had a higher relationship to overall acceptability, which concludes that these attributes are desirable characteristics of any new naturally fortified cereal formulation. Baby foods should be extruded to improve their texture.

4.1Introduction

Micronutrient, especially iron, zinc, vitamin A and iodine deficiency is prevalent in developing countries despite the employment of different strategies to help reduce the challenge (Faber at el., 2014; Osendarp et al., 2018). Food fortification methods employed to address the deficiencies include food to food fortification, industrial fortification with inorganic compounds, oral supplements and biofortification through plant breeding and genetic engineering (Mannar and Wesley, 2016). Food insecurity in developing countries and the high cost of commercially fortified food hinders the reduction of micronutrient deficiencies (Akinsola et al., 2018), therefore, there is a need for the development of cost-effective and sustainable strategies of improving the nutritional status of food.

Food to food fortification is one of the cheapest interventions used to alleviate micronutrient malnutrition in developing countries (Chadare et al., 2019; De Groote et al., 2020; Morilla-Herrera et al., 2016). More attention is being focused on composite flours for making porridge because it is the most consumed food in low-income families in most African countries (Ajifolokun et al., 2019; Udomkun et al., 2019). Porridge is commonly prepared from cereals, which are low in most nutrients hence the rise in the formulation of composite flours from different locally available foods (Elina et al., 2016). Formulated products have different sensory characteristics which can be caused by the different ingredients and the changes they undergo during processing (Adedola et al., 2019; Gitau et al., 2019), therefore there is a need to assess the acceptability of the new formulations before releasing them to the market.

Flour blending has been adopted as one of the techniques to improve the micronutrient composition of cereal flours. In addition to augmentation of the nutrient composition, flour blending improves the rheological, physical and sensory properties (Adedola et al., 2019). However, some balance needs to be achieved in compositing: for example, composite flours containing sorghum is acceptable to a point but as the amount of sorghum increases, the liking of the colour decreases because of dark pericarps found in red sorghum varieties that may impart undesirable dark colour in food products (De Groote et al., 2020; Omwamba & Mahungu, 2014). Baobab pulp is rich in ascorbic acid, improves the flavour of food products and their acceptability (Mounjouenpou et al., 2018; Netshishivhe et al., 2019). In as much as grain amaranth flour flavour is not preferred on its own, Joshi et al., (2019) found that composite flours containing up to 25% of grain amaranth were acceptable. Orange fleshed sweet potatoes impart colour and flavour to the food which makes it desirable in composite flour formulations (Pereira et al., 2019).

Extrusion is a food processing method that has been widely used in the production of pasta, breakfast cereals and snacks (Leonard et al., 2020). It is currently being used in the production of nutritious instant composite flours with better sensory attributes (Otondi et al., 2020). Extrusion temperature and feed moisture are known to affect the colour and texture of the extrudates(Gbenyi et al., 2016).

Composite flours containing maize, sorghum, grain amaranth, OFSP and baobab have been formulated but the effect of formulation and extrusion on their sensory attributes has not been studied, therefore, this study aimed to conduct a sensory evaluation of newly formulated extruded composite flours.

4.2 Materials and methods

4.2.1 Sample acquisition

The raw materials were sourced from different parts of Kenya. Simple random sampling method was used in obtaining the raw materials. Maize was obtained from Eldoret market located in the Rift valley. Pale-red sorghum (E97) and pale cream grain amaranth were obtained from Busia and Bungoma respectively, in western Kenya. Baobab powder obtained from Mombasa and the sweet potato puree obtained from Organi® Ltd in Homabay. All the dry foodstuffs were stored at 25 °C and the potato puree was stored in a deep freezer at -20 ℃.

4.2.2 Sample preparation

The grains were cleaned and dried in a forced-air draft oven at 60 °C for 24 h to a moisture content of about 12%. Frozen orange-fleshed sweet potato (OFSP) puree was thawed in hot water and oven-dried at 60 °C for 6 h. The dry grains and OFSP were separately milled in an 800 µm sieve-hammer mill and whole-meal flours obtained.

4.2.3 Formulation of the composite flours

The formulations were based on findings of the nutritional profile of each of the ingredients (unpublished work) to meet the Recommended Dietary Allowance (RDA) of children below the age of five years per World Health Organization (WHO) recommendations. Nutrisurvey linear programming software embedded with WHO RDA for children was used in the formulation of the flours (NutriSurvey, 2007a). The formulations targeted 25 % RDA of beta-carotene, iron and zinc contents and 15 % RDA of protein. Seven formulations were developed as indicated in (Table 4.1).

Table 4.1: Composite flour formulations

Formulation	**Ingredient proportion (%)**					**Description**
	Maize	**Sorghum**	**Amaranth**	**OFSP**	**Baobab**	
F1	30	35	20	10	5	Varying cereals and fortificants
F2	42.5	22.5	5	15	15	More maize than sorghum with constant fortificants

F3	22.5	42.5	5	15	15	More sorghum than maize with constant fortificants
F4	32.5	32.5	5	15	15	Equal maize and sorghum, constant fortificants
F5	65	0	5	15	15	Maize plus constant fortificants
F6	0	65	5	15	15	Sorghum plus constant fortificants
F7	20	45	5	15	15	A variant of formulation C

The flours were thoroughly mixed and half of each mixture stored separately as the control whereas the other half was processed through extrusion. The moisture content of the other half was raised to 35% by adding water and mixing thoroughly and extruded at 160 °C in a single screw extruder (Techno Chem, Indiana, USA) with a screw rotation of 800 rpm. The extruded products were dried at 60 °C for 4 h, milled and vacuum-packed in polythene bags and transported in boxes to Nairobi for sensory evaluation. The best flour was selected for comparison with commercial flours.

4.2.4 Acquisition of commercial composite flours for comparison

Commercial composite flours containing maize and sorghum for comparison were obtained from different supermarkets in Nairobi Kenya as described in (Table 4.2).

Table 4.2: Commercial composites for comparison

MARKET COMPOSITES	**COMPOSITION**
S1	Formulated flour containing sorghum, maize, baobab, grain amaranth and orange-fleshed sweet potato (the best formulation)

S2	Millet, sorghum, lemon and souring agent blended flour
S3	Finger millet, maize, wheat, amaranth, soya and sorghum cereal flour blend
S4	Finger millet, maize, sorghum and souring agent cereal flour blend
S5	Sorghum, maize, vitamins and minerals flour blend
S6	Soya beans, groundnuts, beans, finger millet, cassava, maize and silverfish flour blend
S7	Maize, millet, sorghum, soya and groundnuts flour blend
S8	Maize, sorghum and soy flour blend

4.2.5 Porridge preparation

Thin porridge was prepared according to a modification of the method described by Onyango et al., (2020). Briefly, 40 g of the composite flour was added to 200 ml of cold water and thoroughly mixed with a wooden ladle and transferred to a stainless-steel pot containing 640 ml of boiling water and stirred continuously for 5 min, boiled for 10 min and transferred to vacuum flasks.

4.2.6 Sensory evaluation

Sensory evaluation was carried out at Kenya Bureau of standards laboratories in Nairobi by a modification of the method described by Onyango et al., (2020). Twelve sensory evaluation panellists were recruited, trained on sensory attributes for 6 h using sample porridges with different attributes. The first three hours involved attribute generation while the rest of the time was used to identify references that match with the attributes (Table 4.3) and how to rate them on a 100 mm unstructured scale.

During the actual evaluation, coded clear plastic cups with 50 g of porridge were served to the panellists. A cup of drinking water was also provided for the panellists to rinse their mouths between samples. All the attributes of each sample were fully evaluated using the provided questionnaire before the next sample was served and the results recorded in duplicate.

Table 4.3: Descriptive sensory lexicon developed by the sensory evaluation panel to evaluate the quality of porridge.

ATTRIBUTE	**DESCRIPTION**	**REFERENCE AND RATING SCALE**
Appearance Colour	Discernment of colour ranging from white to dark brown	Corn starch (10% w/v) stirred in hot water = 0 (white) [a]Dairy land dark compound chocolate = 10 (dark brown)
Brown and dark specks	Quantity of brown and dark specks in porridge when smeared on a white surface	Corn starch (10% w/v) stirred in hot water = 0 (no dark specks)

		Whole milled sorghum flour (30% w/v) stirred in hot water = 10 (many dark specks)
Flavour		
Maize flavour	Flavourcharacteristic of maize flour in hot water	Whole-milled maize flour (30% w/v) stirred in hot water = 10 (very intense)
Sorghum flavour	Flavour characteristic of sorghum flour in hot water	Whole-milled sorghum flour (30% w/v) stirred in hot water = 10 (very intense)
Grain amaranth flavour	Flavour characteristic of grain amaranth flour in hot water	Whole-milled grain amaranth flour (30% w/v) stirred in hot water = 10 (very intense)
Baobab powder flavour	Flavour characteristic of baobab fruit pulp flour in hot water	Whole-milled baobab pulp flour (30% w/v) stirred in hot water = 10 (very intense)
OFSPflavour	Flavour characteristic of OFSP flour in hot water	Whole-milled OFSP flour (30% w/v) stirred in hot water = 10 (very intense)

[a]Topserve Kenya Limited

Table 4.3: Cont………………..

Descriptive sensory lexicon developed by the sensory evaluation panel to evaluate the quality of porridge.

Taste Sour taste	The taste associated with lemon juice	[b]Quencher mineral water = 0 (not sour) Whole-milled finger millet porridge (10% w/v) containing 1% w/v citric acid solution = 10
Texture Viscosity	Resistance to flow when the porridge is poured in another cup	[c]KCC gold crown milk (fat content 3.5%) = 0 (thin) [d]Daima thick yoghurt= 10 (thick)

Coarseness	The extent to which particles are perceived in the mouth in the process of chewing	Honey = 0 (not perceived) Fresh blended, unsieved watermelon juice = 10 (intensely perceived)
Mouthfeel	Physical sensation in the mouth	[e] Krackles crisps= 0 (crispy) [d]Daima thick yoghurt= 10 (smooth)
After swallow		
Sour aftertaste	Perception of lingering sourness in the mouth after chewing and swallowing	10= Strong after taste
Residual particles	Perception of particles in the mouth after swallowing porridge	Fresh blended, unsieved watermelon juice = 10 (many residual particles)

[b]Excel chemicals limited, Nairobi, [c]New Kenya Co-operative Creameries, Nairobi Kenya
[d]Sameer Agriculture, Kenya and [e]Propack Kenya Limited, Nairobi

4.2.7 Data analysis

The data were analysed using the *R* Project for *Statistical* Computing, R-3.6.3(R Core Team, 2019). The normality of the data was first tested using Wilk's Shapiro test with the data that was not normal transformed to z-distribution before inferential analysis. Descriptive statistics, including mean and standard deviation, were determined for the formulations and treatments. Analysis of variance (ANOVA) was used to establish significant differences in the mean sensory scores of attributes with different means separated using Tukey's HSD test at $p<0.05$. The product with the highest sensory scores was evaluated against commercial conventional blended flours retailed in the market and their means compared using ANOVA. Data exploration was done using clustering and principal component analysis.

4.3Results

4.3.1 Sensory attributes of blended cereal flours

Formulation, extrusion and their interaction affected sensory attributes of the formulated composite flours significantly ($p<0.05$). The scores of the new formulations in terms of the colour, texture, mouthfeel, flavour and overall acceptability were all acceptable (Table 4.4). Based on colour, F7 was the most acceptable (7.4±1.38) while F2 was the least acceptable (6.13±1.70). F7 was also the most acceptable for texture (6.88±1.54) as compared to F1 which scored the least (6.05±1.83). Based on the mouthfeel, F3 was the most acceptable (7.40±1.34) while F2 had the lowest score (6.25±2.26). F7 was the most acceptable based on flavour and overall acceptability (7.55±1.06 and 7.43±1.24) respectively. With a lower proportion of sorghum, the liking of the colour of the blended flour significantly ($p<0.05$) increased. Formulations with the highest proportion of amaranth grain powder had the least scores for flavour and overall acceptability ($p<0.01$).

Evaluation of extrusion, too, as the main effect found that extruded flour had significantly ($p<0.05$) higher scores for texture than the non-extruded flours (control) as shown in Figure 4.1. The extruded composite flours had slightly higher scores on colour (6.8), texture (6.9) and mouthfeel (6.8) compared to the non-extruded flours (control) which were better in flavour and overall acceptability with the scores ranging between 6.5 and 7.4.

Interaction of extrusion and the formulation as treatments resulted in significant ($p<0.05$) differences in the sensory scores of texture and flavour (Table 4.5). Control (Non extruded) formulations with higher amaranth content and those that were extruded with higher maize content had significantly ($p<0.05$) least scores for texture (5.35±1.42 and 5.65±1.69) respectively. For flavour, the extruded formulation with a higher content of amaranth had significantly ($p<0.05$) the least sensory scores. The principal component analysis showed that all the four attributes contributed to the overall acceptability of the formulations with mouthfeel and flavour having a higher relationship to the overall acceptability as shown in Figure 4.2.

Table 4.4: Main effect of formulation on the sensory attributes of non-extruded composite flours

Formulation	Colour	Texture	Mouthfeel	Flavour	Overall acceptability
F1	7.08 ± 1.61^{ab}	6.05 ± 1.83^{a}	6.30 ± 2.05^{a}	5.90 ± 2.01^{a}	5.65 ± 1.63^{b}
F2	6.13 ± 2.05^{a}	6.83 ± 1.58^{a}	6.25 ± 2.26^{a}	6.78 ± 2.14^{ab}	6.98 ± 1.73^{a}
F3	6.85 ± 1.70^{ab}	7.08 ± 1.31^{a}	7.40 ± 1.34^{a}	6.95 ± 1.48^{ab}	7.23 ± 1.19^{a}
F4	6.68 ± 1.51^{ab}	6.83 ± 1.55^{a}	6.90 ± 1.53^{a}	6.95 ± 1.13^{ab}	6.75 ± 1.43^{a}
F5	6.18 ± 2.32^{a}	6.05 ± 1.85^{a}	6.58 ± 1.69^{a}	6.65 ± 1.90^{ab}	7.03 ± 1.46^{a}
F6	6.73 ± 1.87^{ab}	6.78 ± 1.85^{a}	6.78 ± 1.87^{a}	6.70 ± 1.76^{ab}	6.68 ± 1.56^{a}
F7	7.43 ± 1.38^{b}	6.88 ± 1.54^{a}	6.88 ± 1.80^{a}	7.55 ± 1.06^{b}	7.43 ± 1.24^{a}
%CV	27.3	25.3	27.3	25.1	22.8
p-value	0.013	0.116	0.083	0.002	<0.001

The values are mean ± sd. Values with the same superscript in a column are not statistically different at $p>0.05$. The samples (F1-F7) are as described in Table 4.1.

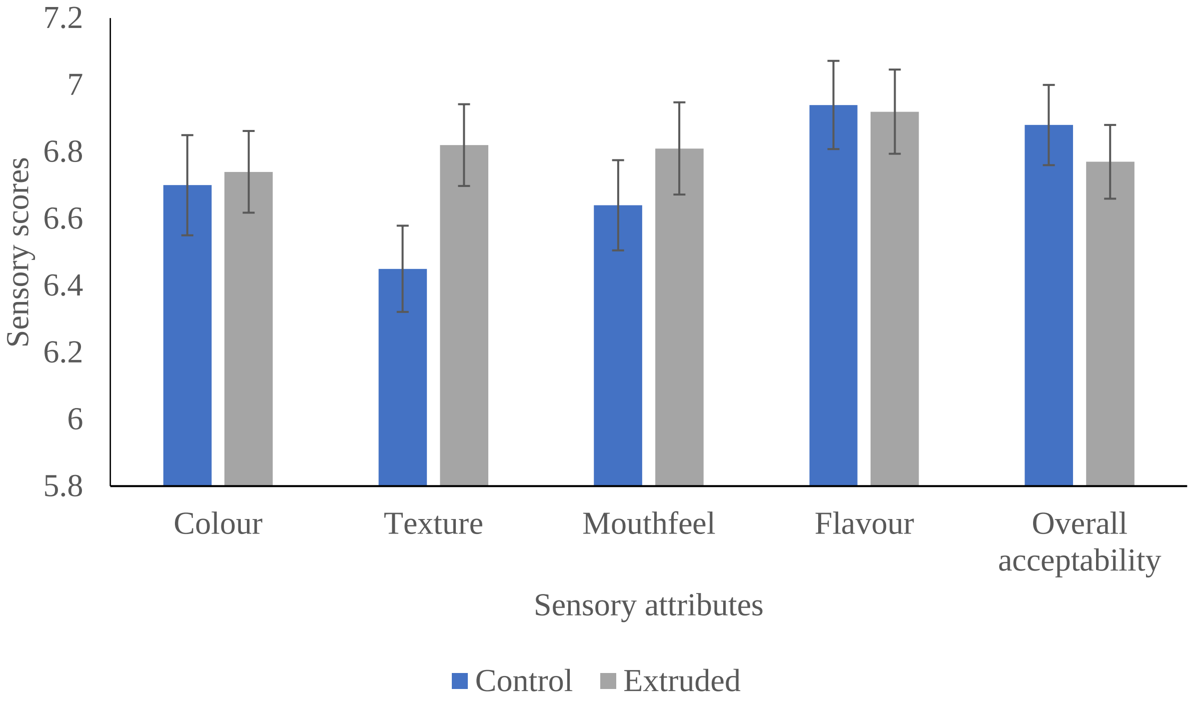


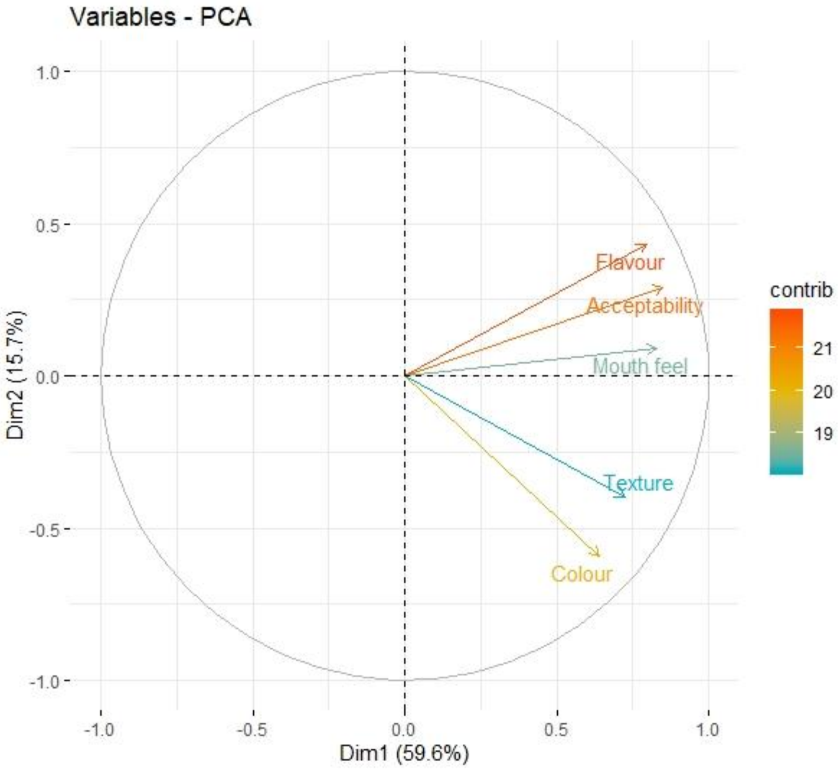
Figure 4.1: Main effects of extrusion on the sensory scores of blended cereal flours. The error bars represent the standard error of the mean.

Table 4.5: Effect of the interaction between extrusion and formulation on sensory attributes of blended cereal flours

The values are mean ± sd. Values with the same superscript in a column are not statistically different at p>0.05. The samples (F1-F7) are as described in Table 4.1

Sample	Treatment	Colour	Texture	Mouthfeel	Flavour	Overall Acceptability
F1	Extruded	6.55±1.85^{a}	6.75±1.94^{a}	6.40±2.62^{a}	5.05±1.79^{b}	5.25±1.83^{a}
	Control	7.60±1.14^{a}	5.35±1.42^{b}	6.20±1.32^{a}	6.75±1.89^{ab}	6.05±1.32^{a}
F2	Extruded	6.26±1.48^{a}	6.50±1.82^{a}	6.20±2.04^{a}	6.70±2.00^{ab}	6.75±1.59^{a}
	Control	6.00±2.53^{a}	7.15±1.27^{a}	6.30±2.52^{a}	6.85±2.32^{ab}	7.20±1.88^{a}
F3	Extruded	6.80±1.67^{a}	7.30±1.22^{a}	7.60±1.05^{a}	7.35±1.04^{ab}	7.55±0.94^{a}
	Control	6.90±1.77^{a}	6.85±1.39^{a}	7.20±1.58^{a}	6.55±1.76^{ab}	6.90±1.33^{a}
F4	Extruded	7.05±1.36^{a}	7.40±1.14^{a}	7.10±1.45^{a}	6.95±1.10^{ab}	6.85±0.88^{a}
	Control	6.30±1.59^{a}	6.25±1.71^{a}	6.70±1.63^{a}	6.95±1.19^{ab}	6.65±1.84^{a}
F5	Extruded	6.15±1.69^{a}	5.65±1.69^{b}	6.25±1.68^{a}	6.30±1.49^{ab}	6.90±1.12^{a}
	Control	6.20±2.86^{a}	6.45±1.96^{a}	6.90±1.68^{a}	7.00±2.22^{ab}	7.15±1.76^{a}
F6	Extruded	6.80±1.94^{a}	7.00±1.49^{a}	6.70±1.92^{a}	6.35±1.87^{ab}	6.55±1.61^{a}
	Control	6.65±1.84^{a}	6.55±2.16^{a}	6.85±1.87^{a}	7.05±1.61^{ab}	6.80±1.54^{a}
F7	Extruded	7.60±1.14^{a}	7.15±1.31^{a}	7.40±1.50^{a}	7.65±0.99^{a}	7.45±1.10^{a}
	Control	7.25±1.59^{a}	6.60±1.73^{a}	6.35±1.95^{a}	7.45±1.15^{ab}	7.40±1.39^{a}
%CV		27.3	25.3	27.3	25.1	22.8
p-value		0.428	0.016	0.519	0.030	0.411

Figure 4.2: Principal components explaining data variability of the sensory scores for the formulated flour blends



4.3.2 Comparative sensory quality of blended flours to the conventional flours

The formulated flour (S1) had significantly ($p<0.001$) lower scores for colour compared to the commercial conventional flours as shown in (Table 4.6). The overall acceptability of the formulated cereal flours had no significant difference ($p>0.05$) with the most acceptable cereal flour blends on the market.

Table 4.6: Comparison of the formulated flour against conventional flour retailed in the market

Sample	**Colour**	**Mouthfeel**	**Flavour**	**Acceptability**
S1	5.58 ± 1.82^{c}	5.63 ± 1.74^{ab}	6.21 ± 1.89^{a}	6.33 ± 1.66^{ab}
S2	8.04 ± 0.81^{a}	6.42 ± 2.02^{a}	6.21 ± 1.86^{a}	7.25 ± 1.26^{a}
S3	7.46 ± 1.25^{ab}	6.75 ± 1.59^{a}	6.63 ± 1.53^{a}	7.29 ± 1.55^{a}
S4	5.71 ± 1.12^{c}	6.46 ± 1.67^{a}	6.75 ± 1.65^{a}	6.79 ± 1.50^{a}
S5	3.25 ± 1.75^{d}	4.42 ± 1.86^{b}	4.33 ± 2.06^{b}	4.46 ± 2.13^{c}
S6	7.96 ± 1.04^{b}	6.67 ± 1.24^{a}	6.63 ± 1.41^{a}	7.13 ± 1.26^{a}
S7	6.04 ± 2.37^{bc}	5.63 ± 2.04^{ab}	6.04 ± 1.90^{a}	6.46 ± 1.98^{a}
S8	3.17 ± 2.10^{d}	4.79 ± 2.26^{b}	5.29 ± 2.65^{ab}	4.88 ± 2.27^{bc}
%CV	40.7	33.8	33.6	31.4
p-value	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$

The values are mean ± sd. Values with the same superscript in a column are not statistically different at $p>0.05$. The samples (S1-S8) are as described in Table 4.2.

4.3.3 Clustering of sensory qualities of the blended flours

The sensory attributes of the flour blends are maximally explained by two clusters explaining 87.3% in the data variability. The cluster 1 had higher scores in all the four sensory attributes evaluated (Figure 4.3); colour, mouthfeel, flavour and overall acceptability. Except for the flour blends that had limited ingredients that are majorly constituted cereals as the ingredients, all other flour blends were in cluster 1, Figure 4.4. All the three attributes contributed towards determining the overall acceptability of the cereal flour blends (Figure 4.5). However, mouthfeel and flavour had a closer relationship with the overall acceptability.

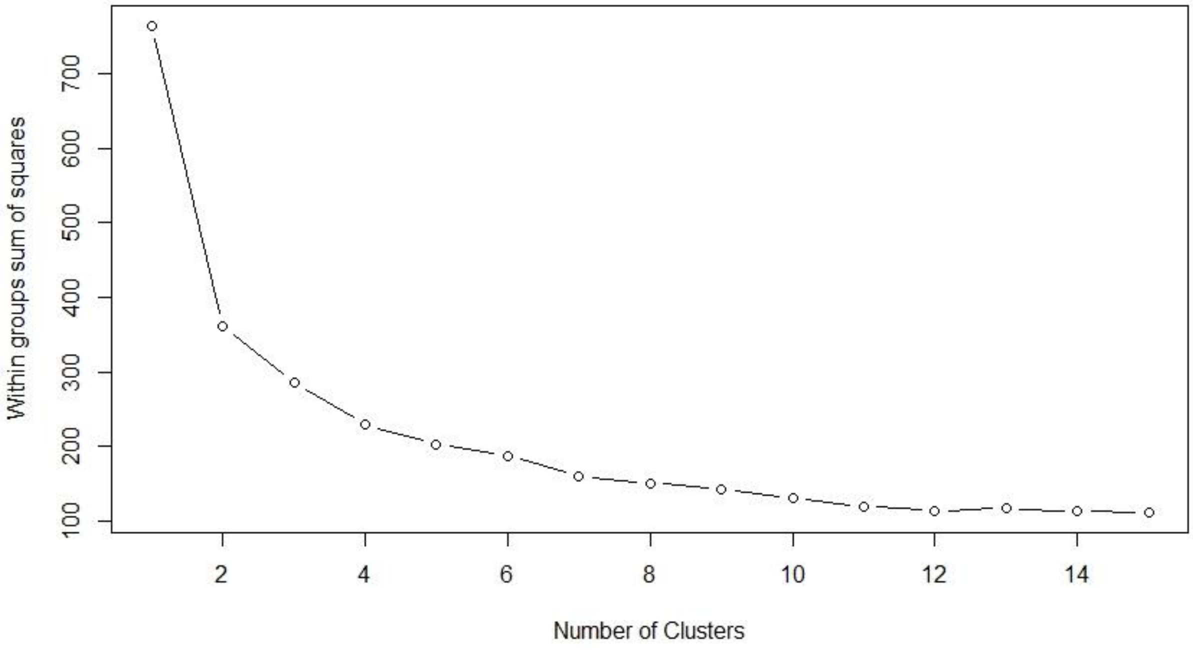


Figure 4.3: WSSplot for clustering of the sensory attributes of cereal flour blends

Figure 4.4: Clustering of the sensory attributes of blended flours (both formulated and conventional ones). The values have been transformed into z-distribution where the mean is 0 and the standard deviation is 1.

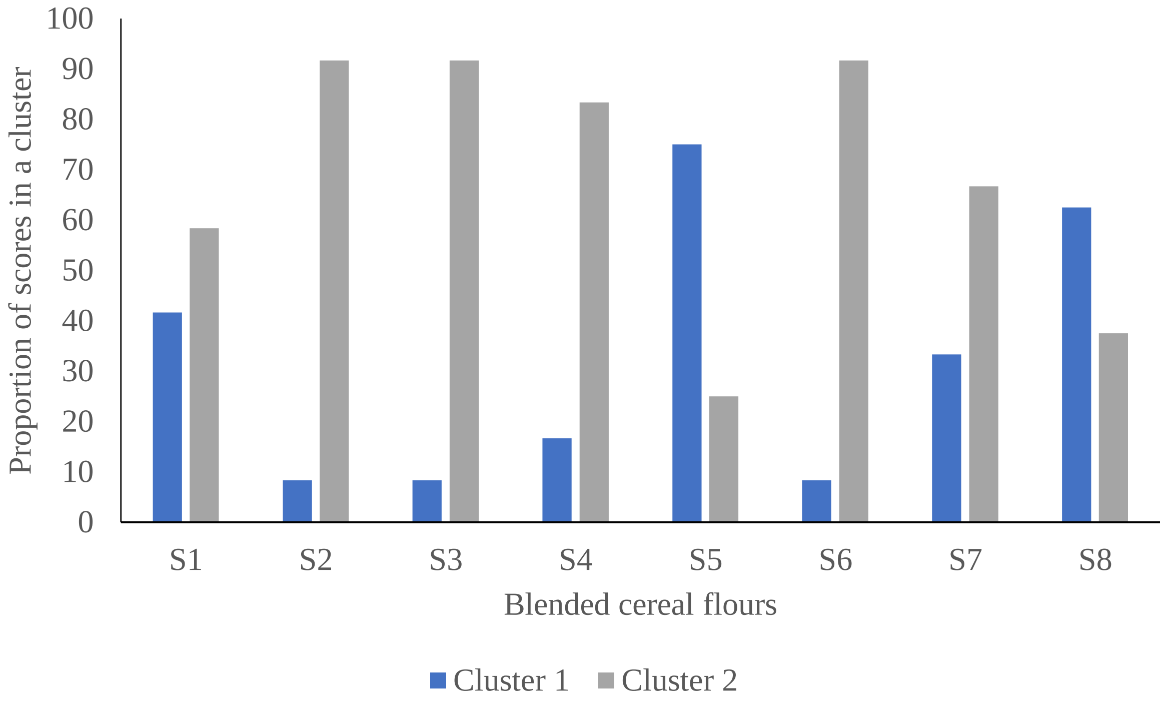


Figure 4.5: Proportion of the sensory scores of blended flours loading into the clusters.

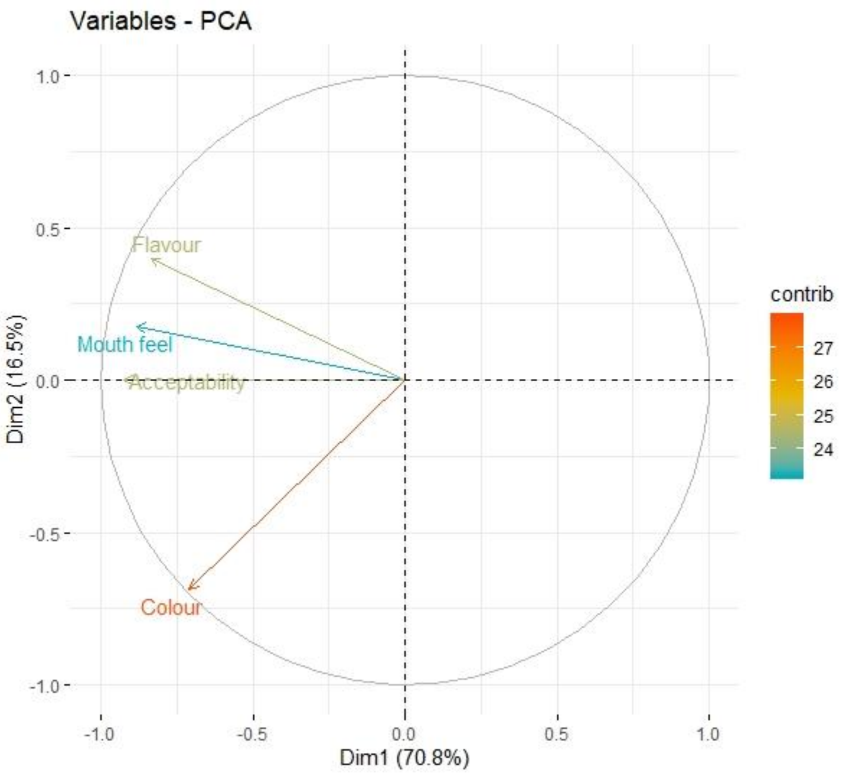


Figure 4.6: Principal Component Analysis plot of the sensory attributes of blended flours

4.4Discussion

The formulated composite flours varied in colour, flavour and overall acceptability. Flours with lower sorghum contents had better scores in terms of colour. This finding is similar to that reported by (Tegeye et al., 2019) who reported that increasing the proportion of fortificants tend to improve the liking of colour among the consumers. An increase in amaranth content in the formulations resulted in a decrease in flavour scores which agrees with other studies. Studies by Joshi et al., (2019), found that composite flours containing 25% amaranth were more acceptable. Akande et al,2017 on the other hand, found that addition of up to 35% amaranth grain in porridge composites left an aftertaste that was not liked by consumers.

Extrusion affected the sensory scores in terms of colour and texture of the composites. This may be due to extrusion temperature and feed moisture which is known to affect the colour of the final product through mallards reaction, non-enzymatic browning and pigment destruction reactions (Adams et al., 2019; Gbenyi et al., 2016). Extrusion imparts a soft texture to extruded products hence the preference in texture (Patil et al., 2016). This may also be attributed to texturization that occurs during extrusion,(Sun et al., 2019). Non-extruded flours had course texture because the flours were wholemeal and only milled once while the extruded ones were milled two times, before and after extrusion (Heiniö et al., 2016). Extrusion reduced the coarse properties of sorghum flour and improved overall acceptability and flavour of sorghum wheat composites (Jafari et al., 2018). This finding implies that consumers will tend to have a higher liking for the extruded flour with a softer texture than the non-extruded ones.

The study established that the attributes of colour, flavour and mouthfeel are essential sensory qualities that contribute towards acceptability of food products. These findings are in agreement with (Eze et al., 2020; Ramírez-Jiménez et al., 2018) who reported that aroma, taste, mouthfeel and colour were the major determinants of acceptability. However, the mouthfeel and flavour were found to be greater contributors in improving the acceptability of the products compared to colour. Therefore in developing product qualities, much more attention should be put in improving mouthfeel and sensory qualities (Elina et al., 2016).

There was no significant difference in the overall acceptability of the new formulation compared to the market blends. This means that all the flours were equally accepted which agrees with the study conducted by Elina et al, (2016). However, it contradicts earlier studies that indicated that extruded products were disliked by consumers due to flavour changes that develop during extrusion (Muoki et al., 2012).

The newly formulated flours scored low on colour which can be attributed to the extrusion process which increases the intensity of the colour. Eze et al., (2020), in their study, found that extruded flours had more intense colour and better mouthfeel and liking compared to conventional flours, therefore, there is need to improve the colour attribute for enhanced acceptability. This also explains the low determinant level in overall acceptability of the cereal flour blends attributable to colour. Mouthfeel was highest for flours containing finger millet, maize, wheat, amaranth, soya and sorghum (6.75). This could be attributed to the use of roasted soya that is known to improve the taste of food (Gitau et al., 2019; Maria and Anuoluwapo, 2018).

4.5Conclusion

Formulation affects the colour, flavour and the overall acceptability of maize, sorghum, grain amaranth, baobab and orange-fleshed sweet potato composite flours with the formulations containing higher sorghum amounts being the most acceptable while extrusion affects the texture scores. The composite flours are all liked thus indicating the potential of the new formulations being adopted. Colour, flavour, texture and mouthfeel contribute to overall acceptability of the composite flours with the major contributors being mouthfeel and flavour. Flavour and mouthfeel are therefore desirable characteristics of any new formulations of cereal flour blends in the food industry.

4.6 Recommendations

Porridge composite flours should contain more sorghum. Baby foods should be extruded to improve their texture. Flavour and mouthfeel should be the attributes of interest when developing new cereal flours.

CHAPTER FIVE: SHELF STABILITY OF EXTRUDED CEREAL FLOURS FORTIFIED WITH LOCALLY AVAILABLE PLANT MATERIALS

Abstract

Hidden hunger and food insecurity are still a problem in developing countries. Compositing of flours using different ingredients is one of the methods used to improve their nutritional composition. However, the shelf life and quality of the new formulations which is important for any new product is challenging to the product developers. The current study aimed at identifying the best storage material, optimal storage temperature and storage time for the newly formulated extruded flour blends fortified with grain amaranth, baobab and orange-fleshed sweet potatoes. The longitudinal study design was used in evaluating the shelf life of the flours. The composite flours were packaged in plastic and kraft per in triplicates and stored at 20°C,30°C and 40°C for real-time shelf-life studies for six months. Moisture content, protein, fat, fibre, ash, Free Fatty Acids (FFA) and yeast and mould were analyzed at a monthly interval. The data were subjected to Analysis of Variance (ANOVA) using the R project for statistical computing and the means separated by the Tukey'sHSD test. The proximate composition of the flours at various storage temperatures was relatively stable. Yeast and moulds decreased as the storage temperature increased. The highest count (3.78 logCFU/g) was recorded at 20°C while the lowest, 3.48 logCFU/g was recorded at 40°C. Storage significantly affected moisture content, protein, fibre, FFA and yeast and mould count ($p<0.05$). Flour stored in plastic containers had better retention of moisture and lower yeast and mould counts compared to kraft paper. Therefore, Plastic is the best storage material for the formulated composite flours regardless of the storage

temperature.Extrusion can be applied to baby food to improve shelf life. A policy of packaging materials for flours should be reviewed.

5.1 Introduction

Perishability of food refers to how fast a food product gets spoilt and foods are classified into three based on this; perishable, semi-perishable and non-perishable. Composite flours are non-perishable but this is dependent on the ingredients used in the formulation of the composite flours, storage temperature, the length of storage, the packaging material and the condition of the food product before storage, (Odeyemi et al., 2020). Therefore, these factors are key in determining the shelf stability of food products (Gitau et al., 2019). During storage, food undergoes chemical, physical and microbiological changes that cause spoilage of food. Food spoilage varies from changes in colour and odour to more serious effects such as severe illnesses or even death (Peleg et al., 2016).

Chemical changes during storage include lipid oxidation, loss of nutrients and absorption of moisture. Lipid oxidation is mainly accelerated by the presence of light, high temperatures and presence of transition elements such as iron and copper which act as catalysts (M. Sahu&Bala, 2017; Wani & Kumar, 2016). The moisture content of most foods increases during storage due to absorption of moisture by the hygroscopic component of food which further lead to loss of nutrients by hydrolytic action. Packaging materials are used as barriers to moisture but they cannot protect food products in their optimal conditions. This could be due to leakages or seal breakage (Steele, 2004)Moulds are the most common microorganisms because they are naturally found in nature and they initiate the spoilage of most foods with low moisture content (Odeyemi et al., 2020). They are a major food safety concern because of their ability to produce mycotoxins (Rico-Munoz et al., 2019).

Shelf life is an important aspect of product development and its introduction to the market with an emphasis on the effect of temperature on the food product (Nicoli, 2012). The shelf life of most foods is based on their sensory and microbiological quality, (Bonat Celli et al., 2016). The two methods of shelf life determination include real-time storage and accelerated shelf-life testing (ASLT), (Manzocco, 2016). Extrusion cooking has shown improvement in the shelf life of food products as it lowers moisture content, (Chanadang & Chambers, 2019).

Maize and grain amaranth contain lipids which might undergo oxidation during storage and therefore, affect the shelf stability of the final product. Sorghum is a good source of iron which acts as a catalyst in lipid oxidation (Danovich, 2015). Baobab fruit pulp contains citric acid which prevents oxidation hence increasing the shelf stability of food (Gurashi et al., 2016).

Therefore, this study aimed at determining the shelf life of cereal flours fortified with grain amaranth, baobab and orange-fleshed sweet potatoes.

5.2 Materials and methods

5.2.1 Composite flour formulation

The composite flours were developed as described in Figure 5.1 below. Seven formulations were developed using Nutrisurvey linear programming software based on data obtained from l profiling the nutrient composition of each of the ingredients with a target of meeting the RDA of children below the age of five as per WHO’s recommendations.

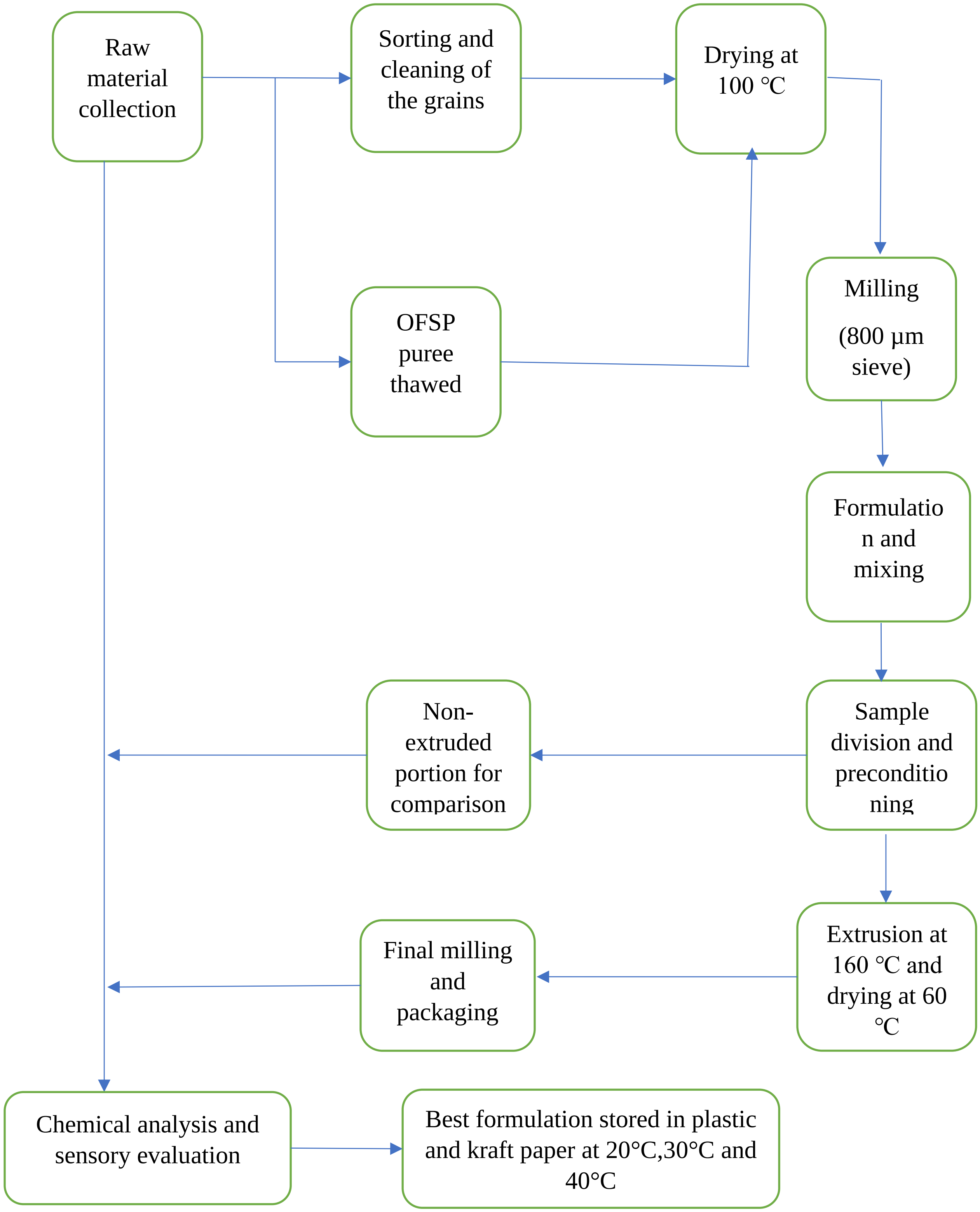


Figure 5.1: Composite flour development flow diagram

5.2.2 Sample collection

Three kg of each formulation was milled, extruded, dried and vacuum packed in polythene papers. The samples were then transported to the Kenya Bureau of Standards in Nairobi for storage and analysis.

5.2.3 Shelf-life determination

The longitudinal study design was used in determining the shelf life of the composite flours. Real-time storage method as described by Adebowale et al., 2017was used. Initial analysis of the samples was done and 500g of each sample packaged in kraft paper and plastic tins. The samples were then stored in incubators set at 20°C,30 °C and 40°C in triplicates for six months. Sampling was done at a monthly interval and the proximate composition, free fatty acids and yeast and moulds determined.

5.2.4 Analytical methods

5.2.4.1 Proximate composition

Proximate composition of the stored flours was determined by the AOAC 2012 (AOAC, 2012) methods. Moisture content, protein content, fibre content, and fat contents were determined by the method numbers AOAC 942.05, AOAC 976.08, AOAC 988.05, AOAC 958.06 and AOAC 942.05 respectively.

5.2.4.2 Free fatty acid determination

FFA was determined by a titrimetric method, AOAC, 965.33 (AOAC,2012). Briefly, 1 ml of phenolphthalein indicator was added (1%) to a mixture of 25ml of diethyl ether and 25ml of ethanol. Followed by neutralization of the mixture with 0.1M sodium hydroxide. 2g of the extracted fat was dissolved in the neutral solution and titrated with 0.1M sodium hydroxide until a stable change in colour was obtained and the results expressed in g oleic acid.

5.2.4.3 Yeasts and moulds determination

Yeast and moulds were determined by the ISO 21527-1:2008 method. Briefly, 30±0.5g of the test sample was aseptically weighed into a sterile stoppered bottle of at least 500ml capacity. 270±5ml of Buffered peptone water was added and then mixed by shaking. 1 ml of the test sample was transferred to sterile Petri dishes in duplicate. Using a fresh sterile pipette tip, 1 ml of the first decimal dilution (10^{-1}) was added to other sterile Petri dishes in duplicate. This was repeated up to the 10^{-3} dilution. 15-20 ml of the acidified Potato Dextrose Agar was poured into each Petri dish. The medium and the inoculum were gently mixed and the mixture allowed to solidify. The dishes were then incubated in an inverted position at 25°C for 5 days. The results were observed and recorded on day 5 and the colonies counted using an illuminated digital colony counter (Stuart® SC6, UK).

5.2.5 Statistical Analysis

The data was analysed using the R project for statistical computing (R Core Team, 2019). Significance in the nutrient composition and yeast and mould counts was tested by the ANOVA test. The choice of the ANOVA model to be used was evaluated using the Akaike information criterion (AIC) model fitting function of the AICcmodavg package. Because of the several factors under consideration, the model explaining the highest variability in the data and with the lowest delta AIC score was chosen for the explanation of variables. Models could be mixed, however; the included model was not to have more than twice the AIC delta score of the previous. Tukey'sHSD function of the Agricolae Package at the significance level of $p<0.05$ was used to separate the significant means.

5.3 Results

5.3.1 Model fitting

The type of packaging, extrusion process and period of storage significantly ($p<0.001$) affected the stability of moisture, protein, fat, fibre ash and free fatty acids contents and yeast and mould counts whereas the temperature of storage only affected the protein, fibre and yeast and mould counts, $p<0.01$ (Appendix 1). The ANOVA model co-opting the interaction factors explained the variance in moisture, protein, fibre, and yeast and mould counts but accounted for low variance in fat, free fatty acid and ash contents (Appendix 2). The individual effect of the interaction factors is expounded in the succeeding sections.

5.3.2 Effect of temperature on the stability of chemical and microbial quality of blended flour

With increasing temperature, the microbial counts significantly ($p<0.05$) decreased (Table 5.3). The proximate composition of the extruded flour was relatively stable at the different storage temperatures; however, moisture content was significantly different at $30^{o}C$ ($p<0.05$) while the protein and fibre contents were significantly different at $40^{o}C$. At higher temperatures, the plastic packaging had significantly ($p<0.01$) higher retention of the fibre than the khaki packaging (Figure 5.1). On the other hand, in Khaki packages protein content had no significant ($p>0.05$) change at different temperatures of storage.

Table 5.1: Effect of temperature on the nutritional composition and microbial quality

Storage Temperature (°C)	**Moisture %**	**Protein %**	**Fat %**	**Fibre %**	**Ash %**	**FFA (oleic acid) %**	**Yeast & moulds count (log CFU/g)**
20	9.71±1.73^{a}	8.44±0.71^{b}	3.18±0.71^{a}	0.88±0.24^{b}	0.52±0.04^{a}	0.06±0.01^{a}	3.78±0.46^{a}
30	9.46±1.66^{b}	8.43±0.64^{b}	3.64±0.98^{a}	0.85±0.23^{b}	0.50±0.04^{a}	0.07±0.05^{a}	3.68±0.48^{a}
40	9.84±1.71^{a}	8.73±0.53^{a}	3.26±0.73^{a}	0.94±0.27^{a}	0.51±0.03^{a}	0.04±0.02^{a}	3.48±0.00^{b}
HSD	0.2	0.1	44.8	0.02	0.02	0.045	0.1
%CV	**4.0**	**3.5**	**13.1**	**6.2**	**5.9**	**70.4**	**7.9**

Mean ± standard deviation with different letters in the superscript are statistically different at $p<0.05$. Moisture, protein, fat, fibre, ash and free fatty acids (FFA) are expressed in dry matter basis.

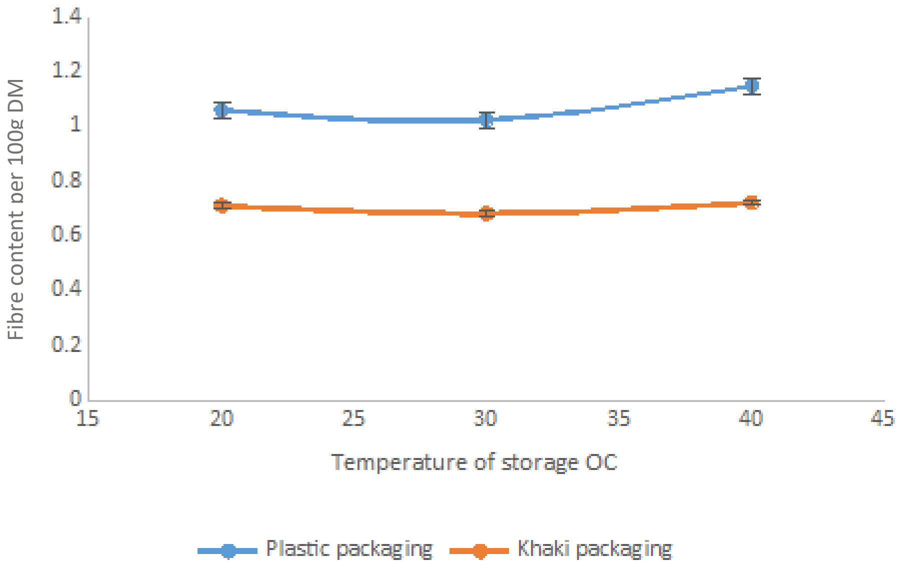


Figure 5.1: Stability of fibre content of blended flour at different temperatures of storage

5.3.3 Stability of the nutritional composition and microbial quality during storage

Storage of blended flour significantly ($p<0.05$) affected moisture, protein, fibre, and free fatty acids contents and the yeast and mould counts. With an increasing period of storage, there was an increase in moisture and free fatty acids contents and yeast and mould counts (Table 5.4). Storing of blended flour in plastic containers significantly ($p<0.05$) resulted in higher retention of moisture and protein contents than in Khaki packages (Table 5.5). On the other hand, the yeast and mould count of blended flour stored in plastic packages were lower across the period of storage than in the Khaki packages.

Table 5.4: *Effect of period of storage on nutritional and microbial quality of blended flour*

Period of storage (Months)	**Moisture %**	**Protein %**	**Fat %**	**Fibre %**	**Ash %**	**FFA (oleic acid) %**	**Yeast and counts log CFU/g**
0	8.74 ± 1.55^{e}	8.91 ± 0.75^{a}	2.96 ± 0.71^{a}	0.83 ± 0.24^{c}	0.53 ± 0.04^{a}	0.03 ± 0.01^{b}	3.28 ± 0.42^{e}
1	8.77 ± 1.55^{e}	8.35 ± 0.71^{c}	2.99 ± 0.66^{a}	0.85 ± 0.25^{bc}	0.50 ± 0.03^{ab}	0.03 ± 0.01^{b}	3.29 ± 0.42^{de}
2	9.12 ± 1.46^{d}	8.63 ± 0.57^{b}	3.20 ± 0.72^{a}	0.86 ± 0.25^{bc}	0.51 ± 0.02^{ab}	0.03 ± 0.02^{ab}	3.73 ± 0.38^{bc}
3	9.61 ± 1.62^{c}	8.66 ± 0.65^{b}	3.22 ± 0.71^{a}	0.87 ± 0.26^{bc}	0.50 ± 0.03^{b}	0.04 ± 0.02^{ab}	4.00 ± 0.30^{ab}
4	9.92 ± 1.55^{c}	8.68 ± 0.55^{b}	3.52 ± 0.79^{a}	0.89 ± 0.25^{b}	0.51 ± 0.03^{ab}	0.08 ± 0.07^{ab}	3.89 ± 0.33^{ab}
5	1056 ± 1.50^{b}	8.58 ± 0.59^{b}	3.31 ± 0.72^{a}	0.94 ± 0.24^{a}	0.51 ± 0.03^{ab}	0.12 ± 0.02^{a}	3.70 ± 0.45^{bc}
6	10.97 ± 1.40^{a}	8.64 ± 0.59^{b}	3.30 ± 0.73^{a}	0.97 ± 0.21^{a}	0.53 ± 0.03^{a}	0.06 ± 0.02^{ab}	3.53 ± 0.00^{cd}
HSD	0.33	0.35	25.4	0.05	0.03	0.08	7.9
%CV	**4.0**	**3.5**	**44.8**	**6.2**	**5.9**	**74**	

Mean ± standard deviation with a different lowercase letter in the superscript are statistically different at $p<0.05$. Moisture and protein are expressed in dry matter basis

Table 5.5: Effect of the interaction between packaging material and period of storage on Moisture, protein and yeast and mould counts

Type of package	**Period of storage (Months)**						
	0	**1**	**2**	**3**	**4**	**5**	**6**
				Moisture %			
Khaki	9.9 ± 1.4^{Abc}	10.0 ± 1.4^{Abc}	10.1 ± 1.5^{Ab}	10.7 ± 1.7^{Aab}	10.8 ± 1.7^{Aab}	11.3 ± 1.7^{Aa}	11.5 ± 1.8^{Aa}
Plastic	7.5 ± 0.1^{Ae}	7.6 ± 0.1^{Ae}	8.2 ± 0.3^{Ade}	8.6 ± 0.5^{Ad}	9.1 ± 0.7^{Acd}	9.8 ± 0.8^{Abc}	10.5 ± 0.7^{Aa}
				Protein %			
Khaki	7.5 ± 0.2^{Bd}	7.7 ± 0.2^{Bd}	8.2 ± 0.5^{B}	8.3 ± 0.5^{Bc}	8.4 ± 0.5^{Bc}	8.5 ± 0.7^{Bbc}	8.6 ± 0.5^{Babc}
Plastic	8.9 ± 0.3^{Bab}	9.0 ± 0.3^{Ba}	9.0 ± 0.4^{Ba}	9.0 ± 0.6^{Ba}	8.9 ± 0.5^{Ba}	8.7 ± 0.5^{Babc}	8.7 ± 0.6^{Babc}
				Yeast and mould counts log CFU/g			
Khaki	3.6 ± 0.4^{Cbc}	3.7 ± 0.4^{Cbc}	4.0 ± 0.3^{Ca}	4.1 ± 0.3^{Ca}	4.0 ± 0.4^{Ca}	3.9 ± 0.5^{Cab}	3.7 ± 0.5^{Cbc}
Plastic	3.0 ± 0.0^{Ce}	3.1 ± 0.2^{Cde}	3.5 ± 0.3^{Ccd}	3.9 ± 0.2^{Cab}	3.7 ± 0.3^{Cbc}	3.6 ± 0.4^{Cb}	3.4 ± 0.4^{Ccd}

Mean ± standard deviation with similar uppercase letters followed with a different lowercase letter in the superscript are statistically different at $p<0.05$. Moisture and protein are expressed in dry matter basis

5.4 Discussion

Shelf stability of food products depends on factors such as the ingredients, the storage temperature, the packaging material and the time of storage (Obadina et al., 2016). Microbial count decreased with an increase in temperature. High temperatures are known to hinder the growth of yeast and moulds. This is in agreement with studies conducted by Gitauet al., (2019). Moisture content was lower in samples stored at 30°C. This could be attributed to the sealing of the samples during storage. Gitau et al., 2019 found that improper sealing of the Kraft paper and plastic container resulted in an increase in moisture content and oxygen that promoted rancidity. This finding contradicts that of Ahmed et al., (2016). They found that an increase in temperature from 27.5°C to 37.5°C increased the water absorption of wheat flour. Protein and fibre content were significantly different at higher temperatures. The increase in protein content can be attributed to bio-conversion of carbohydrates and lignocelluloses into protein which in turn lowers the fibre content (Obadina et al., 2016). This contradicts with studies conducted by Rehman et al., (2017). In their study, protein content, fat and fibre of maize-chickpea-soybean composite flour stored at room temperature decreased in storage. The protein content of the samples stored in kraft paper was not affected by the different storage temperatures. The relative stability of protein can also be as a result of the storage temperatures that were below the protein denaturation temperature of above 41°C.

Moisture content, FFA and the yeast and mould count increased with the increasein storage time. The increase in moisture can be attributed to relative permeability of the packaging materials (Adebowale et al., 2017) and the hydrophilic nature of the different flour ingredients, (Onyango et al., 2020). The findings are similar to those of Akpapunam et al., (2019). Even after six months of storage, the moisture content of the composite flours was still within the acceptable limit of below

14%. The low moisture content and the product expansion, which increases the surface area during the extrusion process makes them susceptible to lipid oxidation, (Honi et al., 2018). The extruded flours are known to have low moisture which increases their shelf life (Adegunwa et al., 2020). The composite flours also contained the iron which is a catalyst for oxidation (Honi et al., 2018). This explains the increase in FFA during storage. Studies conducted by Temba et al., (2017) also found that increase in storage time of maize-groundnut composites at room temperature increased FFA and microbial contamination. Moisture content and the protein content of the flours stored in plastic containers were relatively stable. This can be attributed to the low permeability of plastic. This agrees with studies conducted by Sahu and Patel, (2020). Their study found plastic to be the best protective barrier against moisture. Yeast and mould were relatively higher in Khaki paper as compared to plastic. This might be contributed by the increase in moisture content which promoted their growth.

5.5 Conclusion

Extrusion, storage temperature, packaging material and storage duration affect the shelf stability of extruded cereal flours fortified with grain amaranth, baobab and orange-fleshed sweet potato. Moisture content, FFA and yeast and moulds increase with an increase in the storage time. Though moisture increases in the different packaging material, the increase is higher in the flour stored in Kraft paper. Storage temperature and storage duration do not affect fat and ash contents. Even though there is an increase in the moisture content, itis still below the range of 12-14% recommended by FAO. Therefore, this study provides inputs on best the storage conditions for composite flours containing grain amaranth, baobab and OFSP.

5.6 Recommendations

To improve the shelf life of baby foods, extrusion should be done. Policy changes should be made in regards to packaging materials. A polythene lining can be added in kraft paper to prevent deterioration of the quality of composite flours.

CHAPTER SIX: GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATION

6.1 General Discussion

The different raw materials used in this study had different nutritional and antinutritional composition. The nutrients of interest which included protein, iron, zinc and beta carotene were therefore provided by the different ingredients. For instance, grain amaranth had the highest protein content (15.26 ± 0.34g/100g dwb). The values obtained were comparable with those from other studies, (Kachiguma et al., 2015) who reported a range of 13.37 to 23.38% protein in different varieties of grain amaranth cultivated in Malawi. Baobab powder had the highest ash content which points to a higher content of minerals which agrees with studies conducted by Kinuthia et al, (2017). Zinc content was highest in grain amaranth while the iron content was highest in sorghum. Studies on the nutrient composition and physicochemical properties of grain amaranth have shown that it is a good source of minerals including zinc, (Ayalew and Geremew,2017). Beta carotene was only reported in OFSP, however, Aywa et al, (2013) reported higher values than those reported in this study. The lack of beta carotene in the other raw materials agrees with other studies. Tadesse et al, (2015) in their study did not detect beta carotene in maize. This, therefore, makes OFSP a better fortificant for beta carotene. Antinutrients are a great challenge in most food products with plant materials because they form indigestible metal complexes with minerals in food (Coulibaly et al.,2011). Therefore, studies that target the improvement of iron and zinc should address the issue of antinutrients in the different raw materials to be used.

Incorporation of grain amaranth in the composite flours increased the protein content, zinc and iron contents because it is rich in these nutrients. This is in agreement with studies conducted by Akande et al., (2017). Extrusion reduced protein, carbohydrate, fibre and beta carotene contents of the composite flours and increased the fat content. Yusuf et al., (2018) in their study reported an increase in fibre content which agrees with the findings of this study. The reduction in carbohydrate content is similar studies conducted by Tadesse et al., (2019). This finding, however, contradicts the study conducted by Yusuf et al.,(2018) who found an increase in the carbohydrate content of extruded sorghum-groundnut composites. Extrusion reduced antinutrients in the composite flours by 35%. Studies conducted by Gürbilek, (2016) reported a 16.55 –50.85% reduction in antinutrients. Extrusion is known to destroy phytic acid by hydrolyzing it to phosphate molecule (Wani & Kumar, 2016) and therefore precooking is recommended to improve iron and zinc bioavailability (Gupta et al., 2015).

Formulation of the composite flours affected sensory scores in terms of colour, flavour and overall acceptability. An increase in sorghum negatively affected the colour of the composite flours. This is due to the presence of dark pericarps in some sorghum varieties that impart a dark colour in flours. Grain amaranth affected the flavour of the flours. The flavour of grain amaranth alone is not acceptable but mixing it with other ingredients up to 25% improved the acceptability of the final product, (Joshi et al., 2019). Extrusion affected the colour and texture of the composite flours. Studies have shown that different extrusion conditions are the major contributors to changes in the colour of the final product. Studies by Gbenyi et al., (2016) and Adams et al., (2019) found that the feed moisture and extrusion temperature affected the colour of the final product due to chemical reactions such as the mallard’s reaction and non-enzymatic browning. The improved texture of extruded products can be attributed to the double milling, before and after extrusion as well as texturization during extrusion (Heiniö et al., 2016, Patil et al., 2016 and Sun et al., 2019).

Mouthfeel and flavour were the major contributors to acceptability. Ramírez-Jiménez et al., (2018) found that aroma, taste, mouthfeel and colour contribute greatly to the overall acceptability of flours. Therefore, these attributes should be considered in the development of any new flour formulations. Comparative studies on the acceptability of the new formulation with the market composite showed that it had an equal chance of being accepted. This showed that both extruded and non-extruded flours had equal acceptability. Elina et al., (2016) found that extruded products had equal acceptability with the non-extruded products. This finding, however, contradicts with that of Eze et al., (2020). They found that extruded products had lower acceptability due to flavour changes that occur during extrusion.

Microbial count decreased with an increase in storage temperature. The optimal growth temperature for yeast and mould is 35°C therefore their growth is affected by high temperatures. The findings were similar to those obtained by Gitau et al., (2018). Moisture content, FFA and the yeast and mould count increased with the increase in storage time. This can be attributed to the permeability of the raw materials that increased the oxygen levels of the flours hence promoting lipid oxidation, (Adebowale et al., 2017). Iron is known to be a catalyst of lipid oxidation hence the increase in FFA (Honi et al., 2018).

Plastic was a better storage material due to its lower permeability. Sahu and Patel, (2020) recommended plastic as the best protective barrier of food against moisture which agrees with the findings of this study.

6.2 General conclusion

Grain amaranth, baobab and orange-fleshed sweet potatoes have varying nutritional composition and therefore they can be incorporated into maize-sorghum composites to improve their nutritional composition. The fortificants improve the protein, iron, zinc and beta-carotene composition of the composites. The antinutrient content of the flours depends on the quantity of each ingredient, but the formulation decreases the tannin content while the phytic acid content rises. Therefore, proportioning of the different ingredients is a major factor in the determination of the nutritional quality of new products. The extrusion process decreases the content of protein, fibre carbohydrates, and beta-carotene, but increases the composite flour fat content. Even though the beta-carotene content is reduced, it still meets the minimum allowable limits. Anti-nutrients are also reduced by the extrusion process.

Formulation of composite flours containing maize, sorghum, grain amaranth, baobab and orange-fleshed sweet potatoes affects sensory attributes including colour,flavour and overall acceptability. All the composites in the current study are acceptable, however, those containing high amounts of sorghum are the most acceptable. The key sensory qualities that contribute to the general acceptability of the flours are mouthfeel and flavour. The formulated composites have an equal chance of being accepted in comparison with the market composites containing sorghum.

Extrusion, storage duration, packaging and storage temperature affect the shelf stability of the composite flours. As the storage time increases, the moisture content, FFA and yeasts and moulds also increase. The storage temperature only affects the growth of yeast and moulds. The biochemical and microbial changes are greater in flours stored in kraft paper as compared to those stored in plastic. The moisture content and FFA of the flours are still within the acceptable range.

The formulated composites can be stored safely in both kraft and plastic containers in different ecological zones for over 6 months.

6.3General recommendations

Fortification of the maize-sorghum cereal flours with grain amaranth, baobab and orange-fleshed sweet potatoes improved the nutritional composition, butdigestibility studies of the new formulation should be done to ascertain the absorption of each of the nutrients. Anti-nutrient content of the composite flours can be increased by increasing the quantity of some of the ingredients. More formulations with lower levels of the fortificants can be done to reduce the level of phytates and tannins. Extrusion reduced the beta carotene by a greater percentage. Extrusion cooking reduces antinutrients and therefore its use in baby food is recommended because they are the most vulnerable when it comes to micronutrient malnutrition. Though the antinutrients are reduced by extrusion, Bioavailability studies of iron and zinc should be conducted to ascertain the effectiveness of extrusion on the reduction of antinutrients.

When choosing ingredients to include in new formulations, it is important to know the sensory attributes of each of them because they will affect the flavour and mouthfeel of the final product which will determine its overall acceptability. Porridge composite flours should contain more sorghum. Baby foods should be extruded to improve their texture. Flavour and mouthfeel should be the attributes of interest when developing new cereal flours.

The flours were still safe after six months of storage therefore a longer storage period is recommended. To improve the shelf life of baby foods, extrusion should be done. Policy changes should be made in regards to packaging materials. A polythene lining can be added in kraft paper to prevent deterioration of the quality of composite flours.

The research had informative findings therefore if adopted, it will be beneficial to farmers, grain millers and Food Aid NGO’s.

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8.0 APPENDICES

Appendix 1:F-values for ANOVA test on the nutritional composition of blended flour

Source of variation	Response variables						
	Moisture	**Protein**	**Fat**	**Fibre**	**Ash**	**FFA (oleic acid)**	**Yeast and moulds**
Type of package (A)	50.2***	115.7***	1.0	554.1***	2.0	13.1***	83.4***
Extrusion (B)	34.9***	34.9***	2.1	160.5***	0.7	18.6***	118.1***
Period of storage (C)	22.6***	12.7***	0.3	24.2***	0.2	11.5***	29.9***
Storage temperature (D)	0.5	7.8**	0.0	5.5***	0.7	1.1	145***
A*C	0.03*	68.5***	0.3	4.1	2.3	1.6	9.7**
A*D	0.1	1.9	0.0	19.4***	0.0	0.6	0.5
B*C	0.9	6.7*	0.3	3.7	2.0	3.2	1.9
B*D	0.3	0.0	0.0	0.7	3.2	1.6	2.8
A*B*C*D	0.6	0.6	0.0	0.2	2.1	1.1	1.1

***significant at $p<0.001$, **significant $p<0.01$, *significant at $p<0.05$

Appendix 2: AIC model fitting for ANOVA tests on the nutritional composition of blended flour

Model	**Delta AIC values of variables (variance explained in %)**						
	Moisture	**Protein**	**Fat**	**Fibre**	**Ash**	**FFA (oleic acid)**	**Yeast and counts**
Interactions	0.0 (100)	0.0(100)	22.3(0)	0.0(100)	58.1(0)	5.7(5)	0.0(100)
Factors	32.3(0)	67(0)	0.0(100)	320.3(0)	0.0(100)	0.0(95)	41.6(0)

Appendix 3: Sensory consent form

CONSENT FOR SENSORY EVALUATION PANEL

I volunteer to participate in a research project conducted by **EmmaculateSanya** from the University of Nairobi. I understand that the project is designed to gather information about the academic work of faculty on campus. I will be one of approximately 12 people in the panel of sensory evaluation for this research.

1. My participation in this project is voluntary. I understand that I will be paid for my participation. I may withdraw and discontinue participation at any time without penalty. If I decline to participate or withdraw from the study, no action will be taken against me.

2. Participation involves tasting porridge made from different composite flours. The interview will last approximately 30-45 minutes and the data will be collected in form of questionnaires.

3. I understand that the researcher will not identify me by name in any reports using information obtained from this interview and that my confidentiality as a participant in this study will remain secure. Subsequent uses of records and data will be subject to standard data use policies which protect the anonymity of individuals and institutions.

4. I understand that this research study has been reviewed and approved by the Institutional Review Board (IRB) for Studies Involving Human Subjects: Behavioral Sciences Committee at the University of Nairobi.

5. I have read and understood the explanation provided to me. I have had all my questions answered to my satisfaction, and I voluntarily agree to participate in this study.

8. I have been given a copy of this consent form.

______________________________ __________________________

Signature Date

______________________________ __________________________

Name Signature of the Investigator

Appendix4: Sensory evaluation form

SENSORY EVALUATION FORM FOR COMPOSITE FLOURS FORTIFIED WITH PLANT-BASED PRODUCTS IN NAIROBI ON

Name of the panellist (Optional)……………………………………………Date…………………

Instructions:

1. Please rinse your mouth with water before starting. You can repeat every time you want to test a different sample.
2. Please taste the samples of the porridge in the order presented, from left to right.
3. You may retest the samples once you have tried them all.
4. Rank the samples from most preferred to the least preferred in terms of taste, texture, smell, colour and overall acceptability.

The sensory evaluation is done using a 9-point hedonic score where:

1. Extremely disliked
2. Very much disliked
3. Moderately Disliked
4. Disliked slightly
5. Neither liked nor disliked
6. Slightly liked
7. Moderately liked
8. Very much liked
9. Extremely liked

Samples	Colour	Texture	Mouthfeel	Flavour	General Acceptability
CF1					
CF2					
CF3					
CF4					
CF5					
CF6					
CF7					
CF8					