EFFECT OF PACKAGING AND STORAGE CONDITIONS ON QUALITY OF AMARANTH SORGHUM GRAINS COMPLEMENTARY FLOUR

GICHAU ANNE WANJIRU

MASTER OF SCIENCE

(Food Science and Technology)

JOMO KENYATTA UNIVERSITY OF

AGRICULTURE AND TECHNOLOGY

2020

Effect of Packaging and Storage Conditions on Quality Attributes of Amaranth Sorghum Grains Complementary Flour

Gichau Anne Wanjiru

A thesis submitted in partial fulfillment for the Degree of Masters of Science in Food Science and Technology in the Jomo Kenyatta University of Science and Technology

2020

DECLARATION

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

Signature……Date…….

Gichau Anne Wanjiru

This thesis has been submitted for examination with our approval as university supervisors.

Signature……...……Date…….....…

Dr. Judith Okoth, PhD

JKUAT, Kenya

Signature……...……Date…….....……

Prof. Anselimo Makokha , PhD

JKUAT, Kenya

DEDICATION

This thesis is dedicated to Almighty God for being my sufficiency, to my friend and husband Francis Mungai, our daughters Tahlia and Tracy, my friend Mirriam, for their constant love and support.

ACKNOWLEDGEMENT

I thank Almighty God for his provision, unfailing love, favour and renewing my strength throughout the period of this study. My sincere appreciation goes to Jomo Kenyatta University of Agriculture and Technology for financial support, providing research facilities and environment to carry out the research.To my supervisors; Dr. J.Okoth and Prof. A.Makokha, I am grateful. Your advice and continuous guidance and unwavering support were fundamental to the success of this work. I also appreciate my colleagues at the university and laboratory technicians, your love, cooperation and support are highly appreciated.

I thank the management and staff of Kenya Institute of Research and Development (KIRDI) and Jomo Kenyatta University of Agriculture and Technology Enterprise Services for their assistance, most especially in the processing of Amaranth Sorghum complementary food for this research. My hearty gratitude goes to my husband Francis Mungai Mwangi; your love, constant encouragement and support gave me the drive to successfully conclude this study.

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

ABBREVIATIONS AND ACRONYMS

A	Aluminium Pouch
AOAC	Association of Official Analytical Chemist
ASCF	Amaranth Sorghum Grain Complementary Flour
^{a}w	Water activity
BET	Brunauer–Emmett–Teller
EAS	East African Standard
FAO	Food and Agriculture Organization
GAB	Guggenheim-Anderson-de Boer Model
HCL	Hydrochloric Acid
ISEO	Institute of Shortening and Edible oils
IYC	Infant and Young Children
JKUAT	Jomo Kenyatta University of Agriculture and Technology
JKUATES Limited	Jomo Kenyatta University of Agriculture and Technology Enterprise
K	Kraft Paper
KDHS	Kenya Demographic Health Survey
KEBS	Kenya Bureau of Standard
KIRDI	Kenya Industrial Research Development Institute
KL	Kraft paper with lining
Meq	Equilibrium Moisture Content
P-AV	P-anisidine Value

PCA	Plate Count Agar
PDA	Potato Dextrose Agar
PS	Physiological Solution
PV	Peroxide Value
RH	Relative Humidity
T_g	Transition Temperature
UNICEF	United Nations Children's Fund
WFP	World Food Program
WPO	World Packaging Organization
WVTR	Water Vapour Transmission Rate

ABSTRACT

Complementary food is food meant for infant and young children with the goal of bridging the gap between their daily energy and nutrient requirement and the amount obtained from breast milk. Complementary foods are characterised by high energy and nutrient density, shelf life stability and safety.1.3 million tons of food is lost yearly in the globe, 25-50% being attributed to poor packaging and storage conditions. Amaranth sorghum grains complementary flour is a blend of 90:10 steeped and germinated amaranth to sorghum grains respectively, dried, extruded and milled. It is a dry product with 8.8% fat content, 70.4% being unsaturated. This makes the product susceptible to lipid oxidation and moisture absorption from the storage environment. Information on the quality stability of ASCF under different storage condition in different type of packaging material is limited.In this study the effect of storage condition and type of packaging on the quality of the flour were examined. The flour was packaged in kraft paper, kraft paper with laminate and aluminium pouch and were then stored at either ambient conditions, 25°C, 60% relative humidity or 35°C,75% relative humidity . Quality assessments were done every 45 days from day zero as the baseline until the 180^{th} day. Lipid oxidation was monitored through determination of peroxide value and p-Anisidine value. Microbial quality was determined through total viable count, and yeasts and moulds counts. Moisture adsorption isotherm was determined gravimetrically and fitted in different models. Water vapour permeability of the packaging materials was determined gravimetrically at 25°C, 75% relative humidity and 35°C, 75% relative humidity. Samples stored at 35°C, 75% relative recorded the highest peroxide value of 2.3meq O_2 / kg at 180^{th} day storage period. The interaction effect between storage conditions, storage time and type of packaging significantly ($p = 0.000$) affected fat oxidation which was seen to increase with storage time, high-temperature storage and kraft paper package. Total microbial count detected at the baseline level and over the storage period showed an insignificant increase ($p = 1.000$) except for samples packaged in kraft paper and stored at 35°C and 75% relative humidity where increase was by

6.6% log cfu/g at 180th day storage period. The product exhibited type two sorption isotherm according to Brunner classification, an indication of monolayer-multilayer sorption behavior. By use of Heiss-Eichner model, the flour is predicted to keep for 1991 days at 25°C and 60% relative humidity having it packaged in aluminium pouch. Based on the results, it can be concluded that aluminium pouch and low storage temperature and relative humidity favours quality stability of the flour during storage.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Complementary food is that which is fed to infants above six months of age and young children with the aim of bridging the gap between their daily energy and nutrients requirement and that which is provided by breast milk. Good complimentary food is characterised by high nutrients and energy density. In Kenya, the proportion of stunted, underweight and wasted children between the ages of 6-59 months according to the Kenya Demographic Health Survey of 2014 was 26.2%, 11.8% and 4.2%, respectively (KDHS, 2014). The prevalence of under-nutrition among children under 5 years continues to be high especially in developing countries with the level of wasting peaking at the period of introduction of complementary foods (KDHS, 2014). Among the causes is the inadequacy of the complementary diet both in quality and quantity causing them to be more vulnerable to diseases (KDHS, 2014; Konyole, 2014). Availing complementary foods that are able to meet the nutrition need of infants and young children is, therefore, key in reducing the occurrence of child under-nutrition among infants and young children (IYC).

Amaranth sorghum grains complementary flour (ASCF) was developed from 90% amaranth grains and 10% sorghum grains (Okoth *et al.,* 2017). This formulation had 5.0±0.2 kcal of energy per gram on dry weight basis which is significantly higher than those of foods used by World Food Program to rehabilitate moderately malnourished children (Okoth *et al.,* 2017). Its protein content was 14.4g/100g which is higher than the recommended 9.1g/d protein required to be provided by complementary foods for children between age12–23 months (Okoth *et al.,* 2017). Protein in ASCF gave 11.5% kcal which was higher than the minimum requirement of 6.9% protein energy contribution recommended by World Health Organization/ Food and Agriculture

Organization (WHO/FAO) (Okoth *et al.,* 2017, WHO, 2007). In addition the product contained, 14.8 g/100g, 9.3 g/100g and 12.3 g/100g of glucose, fructose and sucrose, respectively, which are the key mono-and disaccharides beside lactose (Okoth, *et al.,* 2017). These sugars resulted from the breakdown of starch by amylase enzymes during germination (Kanensi *et al.,* 2011). This is reported to lower viscosity due to increased soluble components hence increased nutrient density of resulting gruels which is advantageous in infant's formulation (Ojha *et al.,* 2018). The level of anti-nutrients in the product was significantly low which was evidenced by inability to detect the anti-nutrients, improved in vitro iron and zinc bioavailability and inability to detect an indigestible protein (Okoth *et al.,* 2017). The product's fat content was 6.8% supplying energy content of 12.2% of total energy of which 7.4% was from essential fatty acids (Okoth *et al.,* 2017). Amaranth is also rich in iron and zinc among other micronutrients (Mburu *et al.,* 2011).

Food products normally undergo deterioration and spoilage during storage resulting from growth and activities of microorganisms, food enzymes activities and chemical reactions within the food (Gernah *et al.* , 2011). These changes are majorly influenced by intrinsic factors like water activity and nutrient content or extrinsic factors such as temperature and relative humidity (Singh and Cadwallader, 2004). Consumption of contaminated foods is the major cause of diarrheal disease which account for 1 in 9 deaths in children below 5years of age globally (WHO, 2009). In Kenya it accounts for 6.8% deaths for children under the age of 5 years (You et al. 2015). Children under the age of 5 years are more susceptible to foodborne illness due to undeveloped immune system, less stomach acid production which kills harmful bacteria and children's exploratory behavior that make them ingest nonnutritive materials (WHO 2009). More so children consume more food per their body weight in comparison with adults. This is due to their rapid growth beside homeostasis maintenance. Therefore, if the food they consume is harmful, they will receive more contaminants relative to their size than adults. The incidence of diarrheal disease in children is reported to be higher after

complementary feeding is initiated (Ogbo 2018). According to Kenya Demographic Health Survey (KDHS 2014) 12.9% of children below six months had diarrhea. This more than doubled in children between the age of 6 and 11 months (26.6%) when complementary food is introduced (KDHS 2014, 150). Diarrheal diseases also increase the risk of malnutrition (Unicef, 2013). Complementary food should therefore be safe and shelf life stable to avoid causing food borne illnesses to the end consumers.

Food packaging is considered the cheapest and easiest way of preventing spoilage of food by acting as a barrier from environmental contamination and maintaining the good attributes of the packaged product (Opara, 2013). According to the World Packaging Organization (WPO), an approximation of over 25% of food is wasted due to poor packaging (Olsmats and Wallteg, 2009). Studies have shown that proximate composition of stored products can be altered by packaging materials used based on their relative permeability of either water vapour or other gases from the storage environment (Butt *et al.,* 2004). Most packaged dry products are susceptible to moisture absorption from the environment through packaging when stored in humid environments which can cause undesirable effects on shelf life (Dalpe and Lloyd-George, 2012). For example, products that are in form of powder or are crystalline can be clumped and biologically active chemicals hydrolyze after absorbing moisture. The key characteristics of packaging material for dried foods are barrier properties to water vapour, oxygen, and light in addition to physical strength required to maintain the integrity of the package (Dalpe and Lloyd-George 2012). Packaging which permeates oxygen or light could initiate oxidation resulting in rancidity and related objectionable flavors in dry products with a high-fat content (Robertson, 2009). Kraft paper (K), kraft paper with laminate (KL) and alluminium pouch (A) are the commonly used packaging materials commercially for complementary dry flours in Kenya.

Quality stability tests such as peroxide value (PV) and p – Anisidine (p-AV) value is key for foods which are high in unsaturated fat as it gives the total lipid oxidation status of the product. Moisture adsorption isotherm determination is necessary to assess the

product's stability at different a_W since products are exposed to different temperatures and humidity during storage and distribution. Analysis of water vapor transmission rate (WVTR) of packaging material is essential in predicting the shelf life of the packaged product based on the package's barrier properties. Analysis of microbial stability is paramount as microbial deterioration is considered the highest food safety risk which is largely influenced by factors like moisture, nutrients and temperature among others (Singh and Cadwallader, 2004).

1.2 Problem Statement

About 1.3 billion tonnes of food is reported to be wasted yearly during production, distribution, and at house hold level (Opara, 2013). This makes it a major contributor of nutrition and food insecurity (Opara, 2013).Among other causes of food losses in terms of quality and quantity are inappropriate processing and packaging which are attributed to 25 to 50% food loss (Opara, 2013).Children under the age of 5 years are vulnerable to food borne illnesses (WHO,2009). Consumption of contaminated food is the major cause of diarrhea diseases which is reported to cause 1 in 9 deaths of children globally.

Food products normally undergo deterioration and spoilage during storage resulting from the growth and activities of microorganisms, food enzymes activities and chemical reactions within the food (Gernah *et al.*, 2011). Spoilage susceptibility is high in foods with unsaturated fatty acids due to the presence of double bonds which are the most active sites on the fatty acid chain (Sewald and DeVries*et al.*, 2003). ASCF has 70.4% unsaturated fat hence may be susceptible to oxidative rancidity which leads to development of off flavor in low moisture foods. Dry products 'storage stability is largely dependent on their water activity (a_w). They are likely to absorbing moisture from the environment hence increasing their a_w .This may cause quality instability by making them susceptible to microbial growth like moulds which releases mycotoxins which are lethal to the health of the consumer. Further, such product clumps, after

absorbing moisture from the storage environment decreasing their solubility which is perceived negatively by the consumer (Dalpe and Lloyd-George 2012).

Quality stability and safety of complementary foods are paramount and should be ensured. The quality stability of food vary with storage environment and packaging materials used. External factors like temperature and relative humidity and packaging barrier properties are key in maintain the quality attributes of food.There is limited information and research regarding the food safety aspect of the ASCF product during storage hence the study.

1.3 Justification

ASCF is nutritionally superior to the commonly used complementary foods in Kenya and can be used to rehabilitate moderate malnourished children. In a study on the efficacy ASCF porridge in the rehabilitation of moderately acute malnourished children in a low-resource setting in Kenya, ASCF was found to reduce the percentage of moderately malnourished children (Okoth *et al.*, 2017).

Poor packaging and storage conditions is attributed to almost half of food lost globally (Opara, 2013).Different packaging materials and storage conditions affects the quality stability of stored products. Packaging materials differ in their barrier properties to environmental factor such as water vapour transmission, light, oxygen and other gases. Packaging is vital in preventing food spoilage by maintains product wholesomeness and quality hence prolonging its shelf-life (Inyang *et al.*, 2006; Opara, 2013; Uchechukwu-Agua, 2015).

Storage environment with high humidity and temperature can lead to caking of the flour product characterized by the product sticking/adhering on the package surface due to increase in friction (Iqbal and Fitzpatrick, 2006). High storage humidity can also increase the a_W of the stored product hence microbial instability. The rate of lipid

oxidation is reported to be enhanced to some degree by a rise in temperature (Kilcast, 2001).Lipid oxidation release products which give undesirable biological and sensory effects affecting quality parameters and nutritive value of foods. It is considered one of the major causes of spoilage in natural and processed foods hence, a huge economic concern in food manufacturing (Frankel, 1980). Appropriate packaging material and storage condition maintains the quality attributes of the product until it gets to the end user

Moisture adsorption isotherm gives knowledge on hydration process of the product. This knowledge is crucial for the design and optimization of drying equipment's, design of packages, predictions of quality stability, shelf-life and for calculating moisture changes that may occur during storage (Jha *et al.,* 2014). Understanding the moisture adsorption behaviour of ASCF will assist in selecting appropriate packaging and storage conditions based on empirical knowledge of its hydration behaviour to optimize its shelf life.Maintaining ASCF nutrition quality will result to healthy IYC void of diarrhea caused by contaminated foods.

1.4 Objectives

1.4.1 Main objective

To establish the effect of packaging and storage condition on quality of an amaranth and sorghum grains complementary food.

1.4.2 Specific objectives

(a) To determine the effect of storage condition and duration on lipid oxidation and microbial quality of an amaranth sorghum grains complementary flour.

(b) To evaluate the effect of selected packaging material on lipid oxidation and microbial the quality of an amaranth sorghum grains complementary flour.
(c) To assess the moisture adsorption behavior of an amaranth sorghum grains complementary flour.
(d) To estimate the shelf life of the amaranth sorghum grains complementary flour.

1.5 Hypotheses (Ho)

(a) The quality attributes of the amaranth sorghum grains complementary flour are not significantly affected by storage conditions and storage duration.
(b) The packaging material do not significantly affect the quality attributes of amaranth sorghum grains complementary flour during storage.
(c) The moisture adsorption behavior of amaranth sorghum grains complementary flour cannot be fit sorption models.
(d) Amaranth sorghum grains complementary flour shelf life cannot be estimated.

1.6 Scope and the Limitation of the study

The study considered fat oxidation as the chemical deterioration that would take place in the flour. Assessment of microbial stability only considered total microbial count and yeast and mold without isolating individual microbes for identification. Selection of appropriate packaging material only considered its permeability to water vapor but not light and other gases. Cost of packaging material was not also considered. This was due to limitation of funds and equipment for determining permeability of other gases to the packaging.

CHAPTER TWO

LITERATURE REVIEW

2.1 Malnutrition in Children

Malnutrition is a worldwide concern affecting over 2 billion people (KDHS, 2014). In general, it refers to both over-nutrition and under-nutrition (Blössner *et al.,* 2005). The most common type of malnutrition in developing countries is under- nutrition. Child under-nutrition is evaluated by determining weight and height and screening for clinical manifestations and biochemical markers (Unicef, 2013; WHO,2009). Indicators based on weight, height and age are equated to international standards to give the nutritional status of a population. Stunting shows early chronic exposure to under-nutrition indicated by inadequate length/height for age; wasting shows acute malnutrition indicated by low weight for height while underweight is a combined indicator of stunting and wasting given by inadequate weight for age (Unicef, 2013; WHO, 2009).

Under-nutrition increases infection susceptibility and aggravates their severity hence categorized as the most key risk factor for illness and death in children in developing countries (Blössner *et al.,* 2005). Although under-nutrition is hardly the direct cause of death except in situations like famine, child malnutrition was linked to nearly half of all deaths in children under the age of 5 years globally (Unicef, 2018). Incidences of under-nutrition among children under 5 years in Sub-Saharan Africa are persistens evidenced by 32.1% stunting and 14.7% wasting according to data by Unicef (2018).

2.2 Complementary Feeding and Under-nutrition in Children

Based on life cycle, 1000 days which include the period of pregnancy and child's second year is the most crucial time to meet the nutrition requirement of a child (WHO, 2009). At this period, vital organs like brain and nervous system are developing hence, timing, severity and length of nutritional deficiencies may result in irreversible effects (Unicef,

2013).Enhancement in nutrition after 2 years of age may not ordinarily result in regaining lost potential (Unicef, 2013).

Complementary feeding falls between the age of 6 months and 23 months when most infants reach common and neurological development stages where they are able to feed on other foods other than breast milk (Dewey, 2013; WHO, 2009). Complementary foods should be particularly designed to meet the nutritional or physiological needs of infants with the goal of bridging the gap between the daily energy and nutrient requirement of infants and young children and the amount obtained from breastfeeding (WHO, 2009). Complementary feeding, however, is a challenge to good nutrition in children aged 6-23 months in several parts of the developing countries (Abeshu *et al.,* 2016). This is majorly caused by feeding them plant based foods low in energy and nutrient density and high in anti-nutrients. The bulkiness of plant-based complementary food limits the quantity weanling consumes due to their tiny stomach hence inadequate nutrients and energy to meet their requirements (Okoth *et al.,* 2017; Lalude and Fashakin, 2006). High anti-nutrients in these foods limit level of absorption of essential micronutrients like zinc and iron and may lead to micronutrient deficiencies in children (Okoth *et al.,* 2017; Asma *et al.,* 2006; Gibson *et al.,* 1998).

2.3 Complementary Feeding as an Intervention to Address Under-Nutrition in Infants and Young Children

Appropriate feeding with adequate and safe complementary foods have been shown to improve health and growth in children from the age of 6 months onward (Unicef, 2013; Bhutta *et al.,* 2008). Good complementary feeding practices have been reported to significantly reduce stunting in children especially in vulnerable populations (Unicef, 2013). Use of supplements fortification of foods with limiting micronutrients, addition of sugar and oil are some of the approaches that have been used to improve the nutrition status IYC.

Use of nutrient-rich but acceptable local foods together with simple processing methods has been found to be more sustainable and should be considered in fighting micronutrient deficiencies and improving infants' growth (Konyol, 2014) Amaranth grain is nutritionally promising and has been recommended in complementing other cereals like sorghum. This is because of its high-quality protein, rich in lysine which is the limiting amino acids in cereals. Amaranth grain also has elevated levels of sulfur amino acid content than most important legumes (Okoth *et al.*, 2017; Konyole, 2014). The total mineral content in amaranth grains is higher than that of conventional grains with iron being at least twice that of wheat grains (Konyole, 2014; Pedersen *et al.*, 1987).

2.4 Amaranth Grain and its Nutritional Quality

Amaranth belongs to the order Caryophyllales, family Amaranthaceae, and genus Amaranthus (Mlakar *et al.*, 2009). Amaranthus is coined from the Greek word "Anthos" which means everlasting or unfading. It is currently referred to as the third-millennium crop plant (Rastogi and Shukla, 2013). Amaranth is among the few superior crops whose both grains and vegetables are of high nutritional quality and can be used as food and feed (Algbejo, 2013). Grain amaranth is a pseudo cereals gluten-free grain originally cultivated in ancient Mexico (Rastogi and Shukla, 2013). Amaranth species are about 400 in the genus Amaranthus though most of the species are characterized as weeds and are linked with difficulties in farming practices (Rastogi and Shukla, 2013). The species are categorized based on their use whether vegetable amaranth, food grains, ornamentals and forage (Algbejo, 2013; Mlakar *et al.*, 2009; Sauer, 1967). The species associated with Grain amaranth are four namely; A. cruentus, A. hypocondricus, A.caudatus, and A. edulis (Mburu *et al.*, 2011; Pal and Khoshoo, 1974). A. lividis is for vegetable amaranth while A. tricolor is both for vegetable amaranth and ornamental (Rastogi and Shukla, 2013).

Like maize and beans, grain Amaranth was one of the basic foods of the New World in Pre-Columbian time (Mlakar *et al.,* 2009). It was used in beverages, porridges, and sauces by Indians. The grains were popped like maize or milled into flour. The grains were also used for medicinal purposes (Rastogi and Shukla, 2013). Indians also used amaranth for religion purposes where they made idols out of amaranth dough which was paraded and eaten ritually as a sign of communion with god (Mlakar *et al.,* 2009). However because of similarity to Holy Communion of Catholics, there was a ban from cultivating amaranth by Cortex in 1519 (Mlakar *et al.,* 2009). The introduction of new crops from Europe also lead to high reduction in Amaranth production as there was drawback of amaranth seed being too small making its commercialization a challenge (Mlakar *et al.,* 2009). In 16th century, crop amaranth was introduced in Spain and spread the whole of Europe. By 19th century, it had spread to African and Asia where it is majorly grown as a vegetable plant and is considered a minor cereal food in the Himalayan region of Asia (Mlakar *et al.,* 2009).

Most crop species that are produced in the world today are poor in nutrition value and have led to genetic erosion hence the rising interest in alternative crops that are rich in nutrition value (Macharia-Mutie *et al.,* 2012; Mlakar *et al.,* 2009). Grain Amaranth has drawn great interest due to its high nutrition composition and adaptability. Its agronomic performance is exceptional considering its resistance to heat, drought, and diseases (Mburu *et al.,* 2011; Paredes-López *et al.,* 1990). It is a low-cost production and a fast-growing crop that grows under wide-ranging agro-climatic conditions and soils (Rostagi and Shukla, 2012; Katiyar *et al.,* 2000). Use of amaranth as a grain and vegetable has been emphasized as a rich and affordable source of protein and other important nutrients for the low resource setting communities (Rostagi and Shukla, 2012; Prakash *et al.,* 1995).

Despite maize being the staple food in Kenya, its supply has been very inconsistent with the demand which has intensified food insecurity in the country. Grain amaranth can be a substitute to maize farming, especially in drylands in Kenya or during dry seasons as it

requires little rain. After an extended intense drought which was experienced in East Africa, research on amaranth was done in Kenya during the 1984-1985 growing season (Gupta and Thimba, 1992). Amaranth and sorghum yielded in areas where maize failed due to shortened rain (Gupta and Thimba, 1992). In addition to amaranth showing tolerance during drought, the grain amaranth is of more nutrition value compared to sorghum hence is recommended to substitute maize especially in areas with low rainfall (Gikonyo *et al.,* 2011; Gupta and Thimba, 1992).

Grain amaranth was officially registered as a crop in Kenya by the Ministry of Agriculture in 1991(Gupta and Thimba, 1992). Figures 2.1 and 2.2 shows a picture of amaranth plant and amaranth grains respectively. Its spread, however, has been slow though it is currently emerging to be the solution in areas with challenge of finding a suitable crop. It is being promoted as a cash crop by Poverty Eradication Commission to assist in fighting poverty (Mburu *et al.,* 2011; Sustainet, 2006). Grain amaranth is sold in small quantities in major towns in Kenya. Important institutions like HIV/AIDS orphaned children's homes and the private wing of Kenyatta National Hospital have also mentioned amaranth grain consumption where patients on special diets are recommended to consume. In Uganda, amaranth grain has been promoted for feeding children suffering from malnutrition by the Sustainable Rural Livelihoods program. It is normally blended with other staple grains like maize, millet, and sorghum. Its consumption has shown a direct relation to childhood malnutrition recovery (Mburu *et al.,* 2011).

Figure 2.1: Amaranth plant

Figure 2.2: Amaranth grains

Grain amaranth has been reported to contain an average of 13.1 to 21.0 % crude protein; 48 to 69% starch, 5.6 to 10.9 % crude fat, 3.1 to 5.0 % dietary fibre and 2.5 to 4.4% of ash. Its proximate composition varies among species (Mlakar *et al.*, 2009).

Starch is the major component of amaranth grain and it ranges between 48 to 69% depending on the species. It is waxy with high level of amylopectin (88.9-99.9%), high viscosity and gelatinization at higher temperatures as compared with normal starch

(Mlakar *et al.,* 2009). The starch granule of amaranth grains is very small compared to other cereal grains like rice, maize, and wheat. Due to the small size of the starch granule, its water-binding capacity is higher hence greater swelling power/absorbance capacity, lower solubility and increased resistance to amylase which are suitable characteristics for gelatinisation and freeze/thaw stability valued in food industry (Mlakar *et al.,* 2009; Resio *et al.,* 1999; Qian and Kuhn, 1999).

The protein content of amaranth grains is higher than other common cereal grains (Rastogi and Shukla, 2013; Bejosano and Corke, 1998). Its protein digestibility is also high and is approximated to be 90%. Grain amaranth is known to be rich in lysine which is the limiting amino acids in other grains and it ranges between 4.9 to 6.1 g per100g proteins. It has been shown to be comparable with egg protein hence can be used as a protein source (Rastogi and Shukla, 2013 Pisarikova *et al.,* 2005). It also has elevated levels of the sulfur-containing amino acid content of 2-5%, which is commonly the limiting amino acid in legumes. The amino acid composition of amaranth grains is very close to the FAO/WHO recommendation for humans' balanced diet (Rastogi and Shukla, 2013). 65% of the protein in grain amaranth is found in the embryo while 35% is in the perisperm (Mlakar *et al.*, 2009).

Amaranth grain is rich in fat. Its fat is characterized by a high content of unsaturated fatty acids which is approximated at 77% (Algbejo, 2013). The fatty acid composition of amaranth grains variety grown in Kenya was reported to be as follows: 0.5 to 0.6% lauric acid, 0.1 to 0.2 % myristic acid, 19.6 to 20.7% palmitic acid, 1.5 to 2% stearic acid, 38.2 to 39.3% oleic acid, 1.6 to 1.9% linolenic acid and 36.4 to 37.8 % linoleic acid (Kariuki *et al.,* 2013). It is mainly composed of triglycerides (non-polar lipid compounds) which ranges between 80.3 to 82.3% and low amount of phospholipids in the range of 9.1–10.2%. (Rastogi and Shukla, 2013; Gamel *et al.*, 2007). The oil is a good source of omega series of fatty acids and its digestibility corresponds to that of cotton oil (Rastogi and Shukla, 2013). It also has tocols (1.5 g/kg) and squalene an

antioxidant up to 6.8%, unlike wheat germ oil whose content is 0.1to 1.7% (Rastogi and Shukla, 2013).

Amaranth grain is reported to be rich in micronutrients which are considered essential to the human body. Its minerals composition is; iron 72 to 174 mg/ kg, calcium 1,300 to 2,850 mg/ kg, sodium 160 to 480 mg/ kg, and magnesium 2,300 to 3,360 mg/ kg and zinc 36.2 to 40 mg/ kg. The vitamin composition includes riboflavin, ascorbic acid niacin, and thiamine in the ration of 0.19 to 0.23mg/100g, 4.5 mg/100g, 1.17to 1.45 mg /100g and 0.07 to 0.1 mg/100g respectively (Mlakar *et al.,* 2009). Grain amaranth contains 8-16 % of dietary fibre with 18-40% being soluble fibre (Schnetzler and Breene1994). The dietary fibre content in grain amaranth varies with species.

In addition, amaranth grains contain anti-nutritional factors like phytate which can limit the absorption of other essential nutrients in the body. Amaranth grain grown in Kenya was found to have 7.9 mg per 100g of phytate which was significantly reduced to an undetectable level after germination (Okoth *et al.,* 2017). The level of phytic acid of grain amaranth is however lower than that of other cereals like maize and wheat but higher than that in rice and millet. The grain amaranth also contains important antioxidants in form of polyphenols like tannin content of 0.8% catechin equivalent whole levels are lower than in sorghum, millet and other pseudocereals like quinoa (Mlakar *et al.,* 2009).

2.5 Sorghum Grain

Sorghum is grown globally and is majorly used for animal feeds in developed countries while in Asia and Africa the grain is used both for human nutrition and animal feeds. Sorghum is rated fifth after wheat, maize, rice, and barley, which are among the most important cereal in the world agricultural economy (Dicko *et al.,* 2006). It is, however, the second in sub-Saharan Africa after maize. In 2013 worldwide sorghum production was 61.5 million metric tonnes where 42.3 million hectares were planted with sorghum

globally USA, Nigeria, Mexico, India, and Ethiopia being the main producers (Proietti *et al.,* 2015). In Kenya, sorghum is grown principally in the often drought-prone marginal agricultural areas of Eastern, Nyanza and Coast Provinces. Consumption of sorghum is similarly localized to these growing areas. The crop performs well in areas between 500 meters and 1700 meters above sea level, with seasonal rainfall of 300mm and above.

Sorghum grains are a key source of energy and protein for about one billion people in the semi-arid region of tropics. In developing countries, sorghum is part of the people's staple food hence a major source of energy and nutrients for the majority of rural communities in areas susceptible to drought (Proietti *et al.,* 2015; Belton and Taylor. 2004). More than 300 people in developing countries are estimated to depend on sorghum as their source of energy (Dicko *et al.,* 2006; Godwin and Gray, 2000). An extensive range of traditional food recipes and products are sorghum-based which include boiled sorghum like rice, beer from brewed sorghum, flatbreads, and porridge. Apart from providing calories, sorghum contains protein, vitamins B group E, D and K in addition to minerals, such as zinc, phosphorus, and iron. The protein content of sorghum grain is equivalent to most of the other cereal grains based on composition. Amino acid like lysine, arginine, and threonine are limiting in sorghum just like in wheat and maize (Proietti *et al.,* 2015). The main storage protein in sorghum is kafirins which lack essential amino acid lysine; hence its abundance in a given sorghum variety impacts negatively on its nutritional value. Due to its unique nature of tolerance to drought, water lodging, saline-alkali infertile soil and high temperatures and is a commonly used traditional staple cereal in Kenya, it was used to complement amaranth in developing nutrient-dense food targeting communities in low resource settings (Okoth *et al.,* 2017).

2.6 Processing Treatment of ASCF

Different treatments are employed to enhance the quality of complementary foods based on locally available food materials. These include, soaking, germination, fermentation,

mechanical processing, thermal processing and addition of animal source foods to plant based foods. The following treatments were used in preparation of ASCF

2.6.1 Soaking

Soaking grains before cooking is a practice that is common in many communities especially in Kenya. Soaked grains are said to cook faster hence saving fuel and time. Soaking has also been reported to reduce the level of water soluble phytates in legumes by decanting after they passively diffuse in water (Hotz and Gibson, 2007). Some polyphenols and oxalates that inhibit iron and calcium absorption in the body are also reduced by soaking. The level of reduction, however, is dependent on the species, pH, duration, and conditions of soaking (Hotz and Gibson, 2007, Erdman and Pneros-Schneier, 1994). A soaking procedure that is simple to use in rural subsistence households was developed and shown to reduce phytates in unrefined maize flour by approximately 50%. This is key as research has shown improvement in the absorption of zinc, iron, and calcium in both adults and infants after consuming food based on cereals with reduced phytate contents. Soaking may also result in the reduction of oxalates and polyphenols that inhibit the absorption of calcium and iron. In preparation of ASCF, amaranth grains were steeped for 5 h while sorghum grains were steeped (Okoth et al. 2017). This was found to be the optimum time for steeping amaranth grains based on dry matter loss and reduction in anti-nutrient levels (Kanensi et al., 2011).

2.6.2 Germination

Germination has been reported to increase the activities of endogenous phytase in cereals, oilseeds and legumes by activation of intrinsic phytase or and de novo synthesis hence hydrolyzing the phytates. The level of hydrolysis depends on the species and variety of the grains and other factors like the stage of germination, pH, moisture content, temperature, solubility of phytate, and the presence of certain inhibitors (Egli *et al.,* 2002). Degradation of phytates increases bioavailability of both iron and zinc

(Konyole. 2014). Other than phytates, the process of germination also reduces tannin and other polyphenols in legumes and red sorghum through the formation of polyphenol complexes with proteins in addition to slow degradation of oligosaccharides (Konyole, 2014). Starch in cereals is also hydrolyzed to dextrins and maltose during germination by α-amylase activity resulting in amylase rich flour characterized by reduced bulkiness hence more flour is used resulting in improved energy and nutrient densities of the porridge and thus enhanced child growth (Haug *et al.*, 2010). Amaranth grains that were germinated for 24 hours while sorghum grains were germinated for 72 h, were ground into flour (Okoth et al., 2017). Based on dry matter loss and reduction in anti-nutrient levels, germinating amaranth grains for 24 hours was the optimum processing time (Kanensi et al., 2011)

2.6.3 Thermal Processing

This involves heat treatment which may result in improved micronutrients bioavailability like iodine and thiamin through destruction of some anti-nutritional factors such as thiaminases and goitrogens (Yadav and Sehgal, 2002). Degradation of phytates which inhibits absorption of zinc, iron and calcium is dependent on other factors like pH, plant species and temperature. Blanching of green leaves and boiling of tubers have been reported to reduce phytic acid by 5-15% (Hotz and Gibson 2007; Yeum and Russel, 2002; Erdman and Pneros-Schneier, 1994). It has also been reported to cause release of vitamin B-6, thiamin, folate, niacin and carotenoids from entrapment in the plant matrix hence increasing their bioavailability. Beside improvements in bioavailability however, heat treatment is reported to cause losses in activity of water soluble and heat labile vitamins like vitamin C, thiamin, folate, riboflavin hence there should be a balance of extent of heat treatment to improve bioavailability of nutrients and preserve the heat labile nutrients (Hotz and Gibson, 2007).

Extrusion cooking is also a processing method practiced in developing countries since the 1950s to process cereal-soy blends like Pronutro (South Africa), Faffa (Ethiopia),

and Multipurpose Food (India) among others (WHO, 2003). Uncooked food materials are fed in the extruder and subjected to intense mechanical shear into viscous, plastic-like dough at150 - 180 º C, 25 MPa for 60–120 seconds before being forced through a die (WHO, 2003). This improves protein and starch digestibility, bioavailability of sulfur-amino acids, enhances palatability and texture as well as reducing microbial count (Owino *et al.,* 2007). Extrusion leads to thermal hydrolysis of starch and reduction in moisture content, hence it may reduce dietary bulk and anti-nutrients Alonso *et al.,* (2000), which is a good attribute for complementary foods. In addition, it reduces cooking time during food preparation as the material is already precooked (Mlakar *et al.,* 2009). In processing ASCF, germinated amaranth and sorghum grains were dried in an oven at 60°C for 48 h and the rootlets were removed, then taken through dry extrusion.

2.6.4 Mechanical Processing

This involves processes such as household pounding reducing the grains into small sizes and removing the bran and or the germ from cereals hence reducing phytate contents found in the outer valeurone layer or germ. Although the mineral content and some vitamins of the pounded cereals are simultaneously reduced, their bioavailability may be enhanced. Milled cereal flours in some industrialized countries are enriched to recompense for the lost micronutrients during processing (Hotz and Gibson, 2007). Mechanical processing of vegetable results in disruption of the subcellular membranes where carotenoids are bound hence may assist in improving their bioavailability (Hotz and Gibson, 2007). In processing ASCF, extruded amaranth and sorghum grains were milled to make flour.

2.7 Nutrient Composition of ASCF

Amaranth grain has higher protein level than other staples like maize, sorghum, rice and wheat (Mlakar *et al.,* 2009). The grain’s protein is also of high quality and similar to milk protein being close to the ideal amino acid balance recommended by Food and

Agriculture Organization (FAO) for human consumption (Okoth *et al.,* 2017; Kauffman and Weber, 1990). Amaranth is recommended for infants due to its high protein digestibility, absorption, and retention by the baby's body system (Mburu *et al.,* 2011; Kauffman and Weber, 1990). Its lysine and tryptophan contents are also satisfactory according to FAO/WHO standards. This makes amaranth a suitable complement to cereal grains like maize or sorghum which are inadequate in amino acids lysine and tryptophan, respectively (Okoth *et al.*, 2017; Alegbejo, 2013). It has been shown to yield highly in both arid and semiarid areas (Alegbejo, 2013). Sorghum is a drought-tolerant traditional staple cereal that is widely used in Kenya hence was used with amaranth grain to develop a complementary flour. Due to the high level of anti-nutrients in plant-based food, sorghum and amaranth grains were steeped and germinated to reduce anti-nutrients levels and increase the bioavailability of minerals in the grains (Ertop and Bektas, 2018*;* Okoth *et al.,* 2017).

Okoth *et al*. (2017) used different formulations of amaranth to sorghum grains (90:10, 80:20, 70:30, 60:40) with different treatments aiming at developing a product that provided adequate energy, protein content and minimized anti-nutrients content. On the basis of energy, 90:10 steeped and germinated amaranth to sorghum grains formulation was chosen for the complementary food. Figures 2.3, 2.4, 2.5, 2.6, 2.7 and 2.8 shows steeped amaranth grains, steeped sorghum grains, germinated sorghum grains, germinated amaranth grains, extruded amaranth and sorghum grains and ASCF respectively. The protein content had no significance difference among different formulations while the anti-nutrients in the formulations could not be detected. (Okoth *et al.,* 2017). This formulation contained 5.0 ± 2 kcal / g dwb which was significantly higher than those of foods used by World Food Program to rehabilitate children that are moderately malnourished (Okoth *et al.,* 2017).

ASCF protein content was 14.4g/100g intake which was higher than the recommended 9.1g/d protein required to be provided by complementary foods for children between

age12–23 months (Okoth *et al.,* 2017). Its protein provided 11.5% kcal of energy which was way higher than the minimum requirement of 6.9% protein energy contribution recommended by World Health Organization/ Food and Agriculture Organization (WHO/FAO) (Okoth *et al.,* 2017; WHO, 2007). In addition, the product contains, 14.8 g/100g, 9.3 g/100g and 12.3 g/100g of glucose, fructose and sucrose respectively, which are the key mono-and disaccharides in addition to lactose (Okoth *et al.,* 2013). These sugars resulted from the breakdown of starch during germination and are characterized by high nutrient density and low dietary bulk which is advantageous in infant's food formulation (Ojha *et al.,* 2018; Okoth *et al.,* 2017; Okoth *et al.,* 2013; Nelson-Quartey *et al.,* 2007).

Figure 2.3: Steeped amaranth grains

Figure 2.4: Steeped sorghum grains

Figure 2.5 : Germinated sorghum grains

Figure 2.6: Germinated amaranth grains

Figure 2.7: Extruded amaranth and sorghum grains

Figure 2.8: ASCF

The level of anti-nutrients in the product was significantly low which was evidenced by the inability to detect protein indigestibility (Ojha *et al*., 2018, Singh and Cadwallader, 2004). The product's fat content was 6.8% supplying energy content of 12.2% of total energy of which 7.4% was from essential fatty acids (Okoth *et al* ,. 2017). Its 1ron content was 6 mg/100 g with bioavailable of 5.5 mg per 100 g translating to11.2 mg per 1000 kcal, while zinc content was 3.2 mg per 100 g with the bioavailability of 2.8 mg per 100 g giving 5.6 mg per 1000 kcal based on HCL extractability.

2.8 Storage Stability of Food Products

Food products normally undergo deterioration and spoilage during storage resulting from growth and activities of microorganisms, food enzymes activities and chemical reactions within the food, and pest infestation (Gernah *et al*., 2011). These changes are majorly influenced by intrinsic factors like water activity and nutrient content or extrinsic factors such as temperature, moisture, oxygen, light and physical stress (Singh and Cadwallader, 2004). ASCF like other foods can undergo spoilage during. It being a dry product inform of flour is prone to moisture absorption from the environment which can lead to microbial spoilage. The product is also high in unsaturated fatty acid hence susceptible to fat oxidation which may result in rancidity causing off-flavor and taste.

2.8.1 Microbial Stability and Shelf life of Food Products

Microbiological deterioration is considered the highest food safety risk which is largely influenced by factors like moisture, nutrients, temperature, pH, and time (Singh and Cadwallader, 2004). A high percentage of spoilage microorganisms are air-borne contaminating the product in the farm. In cases where the raw grains have mycotoxins from fungi, the resulting product will also contain mycotoxins which are difficult to eliminate through processing treatments. Spoilage microorganisms can also be

introduced in the product during processing, handling, and storage (Braide *et al.,* 2011). Certain strains like *Escherichia coli* and Aspergillus are toxin and mycotoxin producers that present health risks when consumed. *Mucor* species are responsible for food deterioration and eventual spoilage (Braide *et al.,* 2011).

Dried cereal products like flour, breakfast cereals, infant cereals, snack food, and oatmeals normally have water activity < 0.5 (Serna-Saldivar, 2012) which can inhibit proliferation of most microbes excepts molds thus decreasing the possibility of microbial hazard occurrence (Eskin and Robinson, 2000). In regard to that, cereals related foodborne illnesses are less common with proper storage practices that exclude water adsorption, insect's infestation and rodent's contamination (Legan, 2000). However, if the water activity is favorable, microbial growth will be evident especially *Bacillus* species. (Jay *et al.,* 2005). ASCF is rich in nutrients such as protein, fat, vitamins and minerals (Okoth *et al.,* 2017). It can therefore be a suitable grounds for microbes to flourish and proliferate when the environment is conducive.

ASCF is extruded before milling and packaging. The heating step reduces microbial flora, water activity and enzymatic spoilage (Klunder *et al.,* 2012). Storage temperature and moisture have to be carefully monitored to ensure safety of the product. Most microorganisms cannot grow in an environment with water activity below 0.6 hence products should be dried preferably to a water activity of less than 0.6. Apart from molds and bacteria, insects (weevils) are also responsible for the quality deterioration of cereal products (Indiramma, 2008). Storage should be done in suitable packages that can protect the product from environmental contamination.

2.8.2 Chemical stability and Shelf life of Food Products

Nutrient losses in foods might occur during various stages of processing from the point of harvesting, transportation, processing, and handling or during storage. This is influenced by their sensitivity to factors like oxygen, light, heat or a combination of

these (Severi *et al.*,1997). Chemical changes are majorly attributed to the reaction between food components or reaction between food and other external agents such as oxygen (Kilcast, 2001). Spoilage based on chemical reactions results from enzymatic and non-enzymatic degradation and lipid oxidation. These reactions can concurrently happen in food systems and the rate of reaction is enhanced to some degree by a rise in temperature (Kilcast, 2001).

Oxidation of lipids is one of the major causes of spoilage in natural and processed foods. It constitutes a complex series of reactions first yielding peroxides which are the primary oxidation products that upon exposure to prolonged oxidation conditions releases secondary oxidation products like ketones, hydroxyl compounds, aldehydes, epoxides polymers and oligomers (Barriuso *et al.,* 2013). Most of the released products give undesirable biological and sensory effects affecting quality parameters like color, flavor and nutritive value of foods. Lipid oxidation is, therefore, a huge economic concern in food manufacturing hence its control is very key (Frankel, 1980). Determination of the extent of lipid degradation in processed foods which are high in fat content is one of the main method used to predict their shelf life (Singh and Cadwallader, 2004). Fats consist of fatty acids that are chemically combined to glycerol. These fatty acids can either be saturated or unsaturated and their distinctive combination impacts the chemical and physical characteristics of fat. Saturated fatty acids are known to be stable due to their lack of double bonds between their carbon atoms while unsaturated fat is subjected to variety of reactions due to the presence of double bonds which are the most active sites on the fatty acid chain (Sewald and DeVries, 2003).

Lipid oxidation follows different pathways which include: autoxidation also known as radical mechanism, photo-oxidation is also known as singlet oxygen mediated mechanism and enzymatic oxidation by lipoxygenases (Barriuso *et al.,* 2013). Fat degradation through enzymatic reaction is where triglycerides are hydrolyzed by lipolytic enzyme lipase releasing free fatty acids and glycerol. The liberated compounds may have detectable flavors which can render the product unacceptable. Lauric acid as

low as 0.3% is highly noticeable due to its strong soapy flavour. Besides the change of flavour, the hydrolysis of fat also imparts bitter taste on the product (Sewald and DeVries, 2003). High temperatures favour the rate of hydrolysis reaction. The process hardly occurs during normal storage conditions unless when lipolytic enzymes are present like in palm fruit and coconut oil. The enzymes are normally inactivated through heat treatment during processing.

Autoxidation and photo-oxidation result in similar peroxides that only differ in stereoisomerism and in position. Autoxidation is initiated by removal of hydrogen atom by activation energy enhanced by presence of double bonds and high temperature (Barriuso *et al.,* 2013). Photooxidation, on the other hand, is triggered by highly reactive singlet oxygen species formed by excitation of triplet molecular oxygen under presence of photosensitizers and exposure to light (Choe and Min 2005; Min and Boff, 2002). Peroxides especially hydroperoxides are the first compounds that are formed during the process of oxidation. Besides them being intermediate compounds of the process of lipid oxidation, they are fairly stable and their stability is dependent on the structure of lipid hence can be used to determine oxidation status of food (Barriuso, Astiasarán, and Ansorena, 2013). Peroxides may continue with the oxidation reaction forming secondary oxidation products like ketones, hydrocarbons, alcohols, and aldehydes. Metals like iron and copper promote the process of autoxidation which is why manufacturers treat oils and fats with chelating agents to complex the metals (Institute of Shortening and Edible oils (I.S.E.O) 2006). Lipid oxidation results in the development of off-flavors and odors a condition identified as oxidative rancidity (Kilcast, 2001). Some fats are more susceptible to this change while others resist the change to a significant degree depending on the level of unsaturation, presence of antioxidants among others.

The oxidation reaction process can be progressively measured by determining peroxide value or by n-hexanal and aldehydes quantification in food products during storage. Most peroxides have no noticeable taste or odour but further reacts forming

objectionable compounds (Padmashree, Sharma and Govindaraj, 2013). Different peroxides formed during oxidation process differ in their stability. Some of the formed products like those that contain conjugated double bonds are highly unstable and cannot be measured through iodometric method for peroxide value (Barriuso Astiasarán, and Ansorena, 2013).However others like those that comprise of methylene interrupted double bonds are stable for weeks or even months at room temperature. When Active Oxygen Method or Oil Stability Index is used in determining oxidation of refined oil, it confirms that peroxide value increases speedily after the induction period. With high stable oil, there is insignificant change in peroxide value during initiation period and this can last for some time. With further oxidation, rate of peroxide formation with respect to decomposition decreases and eventually the formation becomes lower than decomposition. PV value as a measure of rancidity is not applicable for food that contains substances that enhance decomposition of peroxides such as metal ions or enzymes. The method is suitable for measuring the quality of fresh oil whose PV is ≤1 but not for those with higher PV value above 5 like shortenings.

Once hydroperoxides are broken down, varied compounds are formed like aldehydes and can be used to track fat/oil degradation with time. Various methods can be used to determine the aldehydes. One of the chemical analysis methods used is p-Anisidine Value which determines the number of aldehydes (principally 2-alkenals and 2, 4-dienals) in animal and vegetable oils and fats by reaction of these compounds with the p-Anisidine (Barriuso, Astiasarán, and Ansorena, 2013). This reaction highlights the concentration of the number of aldehydes and ketones, giving the dimension of the secondary oxidation of the fat matrices (Labrinea, Thomaidis, and Georgiou, 2001). ASCF had 6.8% fat content with 70.4 % being unsaturated (Okoth *et al.,* 2017). Due to its high level of unsaturation it may be susceptible to oxidative rancidity. The degree of unsaturation in fat is based on presence of double bonds on fatty acid. The more the double bonds the higher the degree of unsaturation, therefore, the instability of fat due to more reaction sites. Rancidity leads to development of off flavour in low moisture foods

resulting in rejection of processed foods by the consumer (Hu, 2016). Objectionable flavors result from the decomposition of peroxides forming volatile like aldehydes, ketones, and esters among others having low flavour threshold values. The rate of lipid peroxidation was monitored by determining peroxide value changes as well as p-anisidine value in ASCF packaged in different packaging materials and stored in three different storage conditions.

2.9 Moisture Sorption Isotherms

Water impacts strongly on product attributes such as safety and quality. Understanding the relationship of water in products requires the knowledge of moisture content that can be held at a given water activity. Water activity (a_W) is a physicochemical parameter that shows the state of moisture within a solid material and is defined as the ratio of the vapour pressure of water in the material to vapour pressure of pure water at the same pressure and temperature (Yaptenco, 2017). It indicates the availability of water in a food matrix for microbial growth, chemical and biochemical reactions hence a major determinant of stability and safety of dried foods. Maintaining a_W below 0.7 slows down microbial activity and is expected to completely stop at a_W below 0.6 though chemical reactions like lipid oxidation, non- enzymatic and enzymatic browning may still happen (Yaptenco, 2017). For dried foods to be successfully preserved the a_W is maintained at 0.3 or below (Yaptenco, 2017).

Sorption isotherms were classified according to their shape and processes by Brunauer *et al.,* (1940) establishing five different types; as shown in Figure 2.9. Type 1, present a characteristic increase in a_W related to increasing moisture content; the curve is convex upwards and the first derivative of the plot increases with moisture content. Type 1 is normally applicable in the process of filling the water monomolecular layer at the internal surface of a material. Type 2, is sigmoidal sorption isotherms, in which the

curves are concave upwards; it takes into account the existence of multilayers at the internal surface of a material. Type 3, is J shaped and accounts for a solvent or plasticizer such as glycerol above the glass transition temperature. Type 4, describes the adsorption of a swell-able hydrophilic solid until a maximum of site hydration is reached. Type 5: the Brunauer-Emmett-Teller (BET) multilayer adsorption isotherm, it is the one observed in the adsorption of water vapour on charcoal and it is related to the isotherms type 2 and 3. Type 2 and 4 are most frequently found in food products (Andrade *et al.*, 2011)

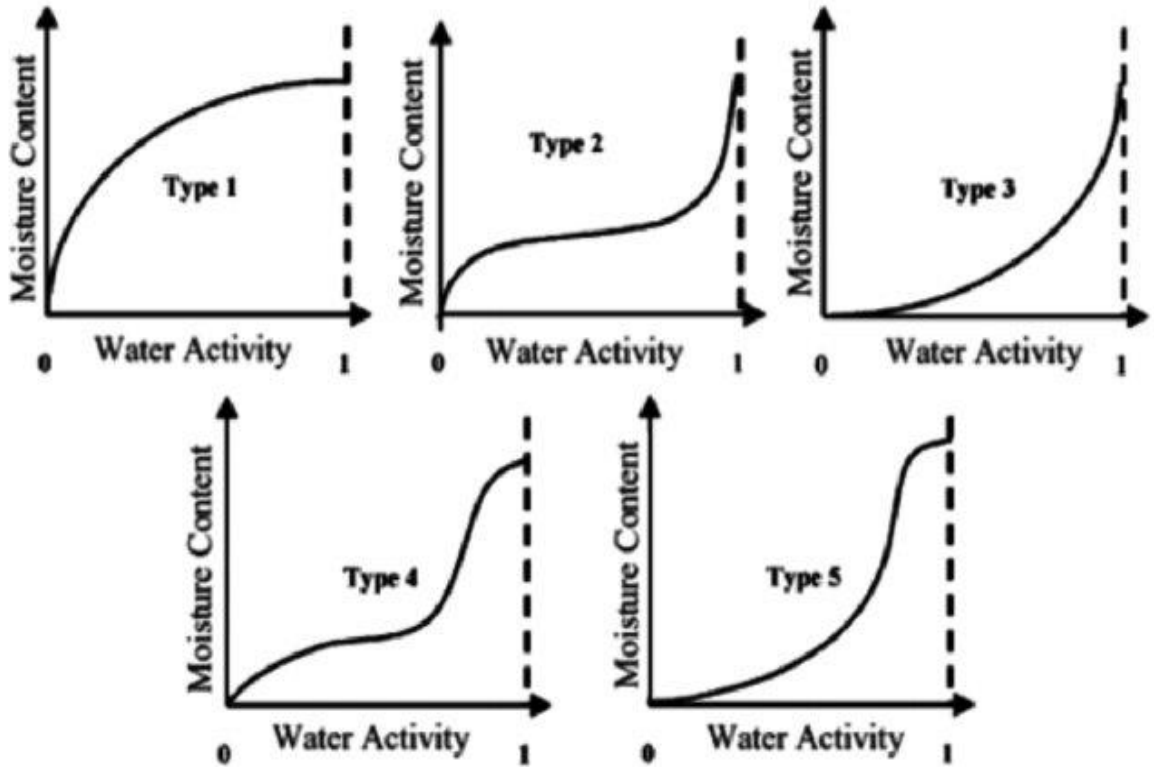

Figure 2.9: Types of isotherms described by Brunauer (Andrade *et al.*, 2011)

Different researchers have proposed over 200 models for simulation of sorption behaviour aiming at expressing the relationship between a_W and equilibrium moisture content of a material at a constant temperature and pressure (Lemus . 2011). Some are

theoretically based, others are empirical while others are a simplification of more sophisticated models. The amount of water absorbed by a product is dependent on its physical-chemical state, chemical composition and physical structure making the isotherm shape unique for each product type (Al-Muhtaseb *et al.,2002)*. Therefore, the model that is suitable for a particular food product may not be suitable for another (Al-Muhtaseb *et al*., 2002).

Fitting the most suitable models helps describe the sorption behaviour of food hence the state of water and its interaction with food components. The knowledge and understanding of sorption isotherms in food are key for the design and optimization of drying equipment, design of packages, predictions of quality stability, shelf-life and for calculating moisture changes that may occur during storage (Jha *et al.,* 2014).

Moisture sorption properties of ASCF has not been studied yet. It being a dry product, its storage stability is largely dependent on its moisture hence a_W . Dry products become unstable at high a_W in the range of 0.6-0.85 making the product susceptible to microbial growth hence reduced shelf life. It is, therefore, necessary to control the a_W in dried products for their safety. Determining ASCFs adsorption isotherm is necessary to assess its stability at different a_W since products are exposed to different temperatures and humidity during storage and distribution. Understanding its moisture sorption behaviour will assist in selecting appropriate packaging and storage conditions based on empirical knowledge of its hydration behaviour to optimize its shelf life.

2.10 Product Packaging and Shelf-Life Stability

Food packaging is a harmonized system of preparing food for transport, distribution, storage, retailing and end-use with optimal cost to satisfy the ultimate consumer (Coles *et al.,* 2003). It is considered the cheapest and easiest way of preventing spoilage of food through undesirable organisms and extraneous matter that may have an effect on product

quality. This is by acting as a barrier (Opara, 2013). According to the World Packaging Organization (WPO), an approximation of over 25% of food is wasted due to poor packaging (Olsmats and Wallteg, 2009). Studies have shown that proximate composition of stored products can be altered by packaging materials used based on their relative permeability of either water vapour or other gases from the storage environment (Butt *et al.*, 2004). Poor and inadequate packaging results in shelf life instability hence food losses (Ogiehor and Ikenebomeh, 2006).

Optimal packaging keeps the product readily available to the consumer in good quality and prevents deterioration caused by microorganisms, chemical reactions, and environmental contamination hence lessen the significant amount of food wasted (Olsmats and Wallteg, 2009). In addition, there is increased impact of food packaging due to the current consumer demand for convenient and high-quality food products (Opara, 2013; Chuzel and Zakhia,1991) Most packaged dry products are susceptible to moisture in the environment. They absorb moisture through packaging when stored in humid environments which can cause undesirable effects on shelf life (Dalpe and Lloyd-George, 2012). For example, products that are in form of powder or are crystalline can be clumped and biologically active chemicals hydrolyze after absorbing moisture. Such products that tend to absorb moisture from the environment are called “hygroscopic” (Dalpe and Lloyd-George, 2012).

The key characteristics of packaging material for dried foods are barrier properties to water vapour, oxygen, and light in addition to physical strength required to maintain the integrity of the package (Dalpe and Lloyd-George 2012). This is because of their sensitivity to water absorption which causes significant changes in quality and texture. Packaging which permeates oxygen or light could initiate oxidation resulting in rancidity and related objectionable flavors in dry products with a high-fat content (Robertson, 2009). To combat this challenge, many improvements have been done on packaging materials to lower their water vapour transmission rates. Dependent on the product that is packaged and the degree to which it is vital to minimize water vapour transmission

rate, the cost of investment in package production varies. For example, dry foods like sugar and flour are hygroscopic but inexpensive and have high turnover rate, hence do not necessitate a very long storage period. Partial migration of moisture to the package does not usually result in significant problems of the packaged product. These inexpensive products, therefore, are packaged in multilayers of coated kraft paper which are affordable and considered to provide adequate protection (Dalpe and Lloyd-George, 2012).

A water vapour transmission rate of 6.0 grams of water per 100 square inches of packaging material per day at the standard testing conditions (90% RH, 38°C) is considered acceptable commercially for dry products (Dalpe and Lloyd-George, 2012). However, some products which are rich protein and fat are more sensitive to moisture migration as it tends to increase their spoilage. Further, such product clumps, decreasing the ability to flow which is perceived negatively by the consumer.

Manufacturers tend to use packaging with lower rates of water vapor transmission in the range of 0.4-0.8 gram per 100 square inches per day in standard testing conditions (Dalpe and Lloyd-George, 2012). Such packaging materials normally comprise polyethylene laminated to kraft paper or whole plastic laminates as such materials are fairly affordable and their water vapour transmission rate is sufficiently low. This achieves the overall goal of the package to provide sufficient protection to the hygroscopic product and in addition is economically feasible to produce. Another effective barrier material used due to its low vapour transmission rate is aluminum foil (Dalpe and Lloyd-George, 2012). Foil is normally used as a protective layer when packaging hygroscopic products. However foil can easily tear due to its being brittle thus, it is lined with plastic layer to make it resistant to puncture (Dalpe and Lloyd-George, 2012).

Another necessity for packaging is sealability. The selection of appropriate packaging material is key to the food market. The package should be adequately versatile to bear

handling process forces without compromising its chemical, physical integrity and proper barrier properties from environment influence. The uniqueness of each product composition makes them interact differently with packaging material making each to have a particular packaging material that is appropriate for it (Robertson, 2009).Commonly used packaging materials for the storage of flour products include plastic containers, polymeric and paper bags (Uchechukwu-Agua, 2015).

The type of package and storage conditions used has an impact on the quality, shelf-life, and safety of food products by their influences on moisture content, water activity and nutrient compositions of the food product (Opara, 2013). Storage environment with high humidity and moisture content can lead to caking of the flour product characterized by the product sticking/adhering on the package surface due to increase in friction (Iqbal and Fitzpatrick, 2006). Thus, packaging is vital in food systems because it adds value, reduces losses, and maintains product wholesomeness and quality hence prolonging its shelf-life (Inyang *et al.,* 2006; Opara, 2013; Uchechukwu-Agua, 2015).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Experimental Material

Amaranth (*Amaranthus cruentus*) and sorghum (*Sorghum bicolor* (L.) Moench) grain samples were purchased from the Bondo Farmers Marketing group in Siaya county, western Kenya. The grains were first sorted separately by hand picking as well as sieving to remove foreign matter and other materials other than the amaranth grains or sorghum grains. The sorted grains were then cleaned using running water until the washing water was void of dirt. The clean grains were then steeped, germinated and dried as described by Okoth *et al.*, (2017). This was carried out at Kenya Industrial Research Development Institute (KIRDI) located in South C Nairobi Kenya. The dried grains were packaged white polypropylene sacks and transported to Jomo Kenyatta University of Agriculture Technology Enterprise Limited (JKUATES) where extrusion and milling was carried out to get ASCF. Blending of the grains in the ratio of 90:10 amaranth to sorghum grains was done before extrusion. This formulation was chosen as it gave the highest energy in comparison to other blends and its protein content was satisfactory (Okoth et al., 2017). Storage and analysis was done in Jomo Kenyatta University of Agriculture Technology (JKUAT)

3.2 Experimental Design

A randomized block design was applied in the study. The samples were divided into four homogeneous sub groups or blocks. Three sub groups were subdivided into twelve (12) 200g samples and packaged either in K, KL or A (Figure 3.1). The fourth subgroup sample was stored in sealed cellophane bags in a cold room at 4°C awaiting analysis as a fresh sample. Four samples in each package were then randomly selected and conditioned at either ambient condition (control), 25°C/60%RH and 35°C/RH 75%.

3.3 Packaging And Sampling of ASCF

About 200g of ASCF was randomly filled into K, KL and A. KL and A were sealed using a heat sealing machine while the K was sealed using a sealing tape as shown in Figures, 3.1. 12 packages including 4 K, 4 KL and 4 A packages were randomly selected and stored in each of the storage environments, ambient condition (between 23-25°C/60%RH) (control), 25°C/60%RH and 35°C/RH 75%. The conditions for 25°C/RH 60% were achieved using saturated sodium bromide solution and 35°C/RH 75% using saturated sodium chloride solution. The conditions were selected to represent conditions usually encountered at retail shops and warehouses that is 25 °C at a humidity of 60 % in Nairobi and 35 °C at a humidity of 75 % in the Coast (Kuong et al., 2016).

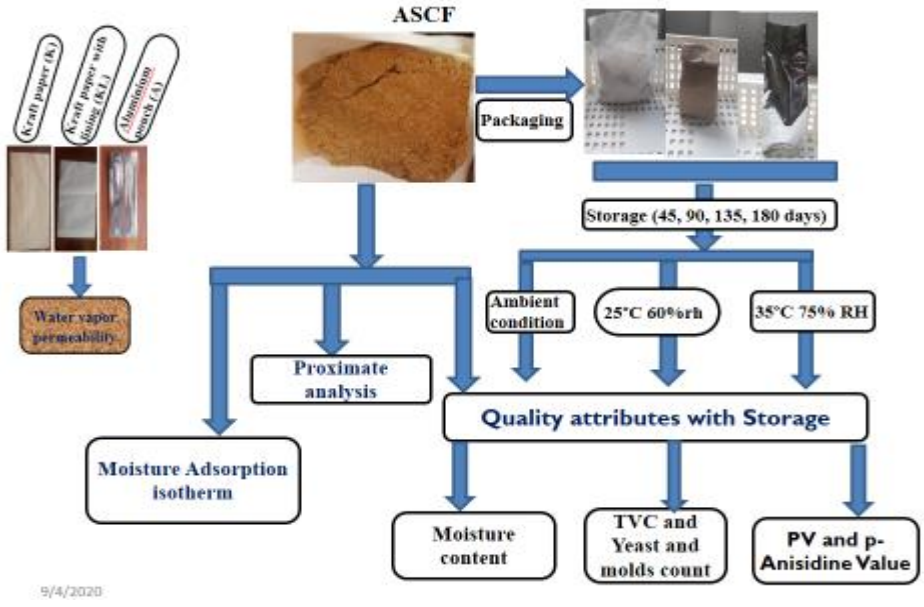

Figure 3.1: Flow diagram of the methods used in determining the effect of packaging and storage conditions on ASCF

The sample in the sealed cellophane stored in a cold room at 4°C was used for trials to establish the baseline and also confirm the suitability of the selected analytical methods.

To obtain samples for the assessment of different parameters at every sampling stage (after 45 days storage), each of the different types of packages from the two different storage environments were taken, thoroughly mixed, and divided into two portions for the different quality stability analysis; chemical quality stability (PV and p-AV), and microbial stability (TVC and yeast and moulds). Every experiment was carried out on thoroughly mixed sample to ensure homogeneity and were done in triplicate. Moisture adsorption isotherms were also determined gravimetrically at the beginning of the experiment.

3.4 Proximate Analyses

3.4.1 Determination of Moisture Content

Moisture content was determined by oven drying according to the Association of Official Analytical Chemist (AOAC, 2012).The moisture dishes were washed and placed in an oven drier at 105°C for one hour. They were then placed in a desiccator to cool and the initial weight of the dishes recorded (W_1). Three grams of the sample was weighed into the moisture dish and weight recorded (W_2). The dishes were then placed in an oven drier for 3 hours when the weight was monitored through weighing until it was constant. After drying the moisture dishes with dried samples were removed from the oven drier, cooled in a desiccator and the final weight recorded (W_3). The moisture contents of the samples were calculated as shown below;

$$Moisture\ content\ (\%) = \frac{(W_3 - W_1)}{(W_2 - W_1)} X100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad 3.1$$

Where;

W_3= Weight of dried sample and moisture dish

W_2=Weight of the fresh sample and moisture dish

W_1=Weight of empty dish

3.4.2 Determination of Protein Content

Protein content was determined using Kjeldahl method; 1g of the sample was weighed into a digestion flask together with a catalyst composed of 5 g of K_2SO_4, 0.5 g of $CuSO_4$ and 15ml of concentrated H_2SO_4. The mixture was heated in a fume hood till the digest color turned blue signifying the end of the digestion process. The digest was cooled, transferred into a 100 ml volumetric flask and topped up to the mark with distilled water. A blank digestion with the catalysts and acid was also made. The digest containing ammonium sulphate and carbon dioxide was diluted with 40 mL distilled water before neutralizing with 35% sodium hydroxide through a distillation unit for 4 minutes. The digest was distilled into boric acid solution containing methyl red and bromocrescyl green indicator (Merck, South Africa). Lastly, the greenish boric acid solution was titrated against 0.02 N HCl until a stable light pink colour was observed signifying the endpoint. Protein content was calculated from % nitrogen (N) using a conversion factor 6.25 (N × 6.25) methods 920.152 AOAC (2006) as follows:

$$Nitrogen\ \% = (V_1 - V_2)X\ N\ X\ FX\ 0.014\ X\left(\frac{100}{V}X\frac{100}{S}\right) \ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad 3.2$$

Where; V_1= Titre for the sample (mL); V_2 =Titre for the blank (mL)

N=Normality of standard HCL solution

F= Factor of standard HCL solution

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

S= Weight of the sample taken (g)

Protein content was calculated from % nitrogen (N) using a conversion factor 6.25 (N × 6.25) methods 920.152 AOAC (2006) as follows:

$$Protein\ \% = Nitrogen\ x\ Protein\ factor \quad \ldots\ldots\ldots\ldots\ldots \quad 3.3$$

3.4.3 Determination of Crude Fat

Fat content was determined by the soxhlet extraction method (AOAC, 2006) using petroleum ether as the extraction solvent. Five grams of samples were weighed into extraction thimbles and the initial weights of the extraction flask taken. Fat extraction was done for 16 hours and pre-weighed extraction flasks were weighed again after extraction and evaporation of petroleum ether.

Calculations were done using the formula below;

$$\text{Crude fat } (\%) = \frac{(W_2 - W_1)}{W_0} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad 3.4$$

Where;

W_0= Weight of sample before extraction

W_1=Weight of the flask with the extract

W_2=Weight of the empty flask before extraction

3.4.4 Determination of Ash

Ash contents were determined according to the Association of Official Analytical Chemist (AOAC,2012) methods, 923.03. Crucibles were dried for 2 hours at 100°C in an oven then put in a desiccator to cool. Their weight was taken and recorded after cooling (W_a). The muffle furnace was programmed to 550°C. About 2 grams of the sample was weighed (W_b) in the cooled crucibles and first charred by flame to eliminate

organic material before being incinerated. After the furnace reached the set temperature of 550°C the charred samples were put in the furnace for incineration to the point of white ash. The furnace was allowed to cool below 200°C when the samples were transferred to a desiccator with stoppered lid. This was allowed to cool to room temperature then weight was taken (W_c).

Ash determination was done as shown below;

$$\text{Crude ash } (\%) = \left(\frac{W_{c\ (g)} - W_{a(g)}}{W_{b\ (g)}}\right) \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.5$$

3.4.5 Determination of Crude Fiber

The crude fiber was determined according to AOAC, (2006) method. Two grams of sample was weighed into a 500 ml conical flask. Then 200 ml of boiling 1.25% H_2SO_4 was added and boiling done for 30 minutes under a reflux condenser. Filtration was done under a slight vacuum with Pyrex glass filter and the residue washed with boiling water to completely remove the acid. Then 200 ml of boiling 1.25% NaOH was added to the washed residue and boiling done under reflux for another 30 minutes. Filtration was done using the same glass filter previously used with the acid. The residue was rinsed with boiling water followed by 1% HCL and again washed with boiling water to rinse acid from the residue. The residue was washed twice with alcohol and thrice with ether. It was then dried in an oven at 105°C in a porcelain dish with a constant weight (W_i). Incineration was done in a muffle furnace at 550°C for 3 hours. The dish was then cooled in a desiccator and the final weight (W_{ii}) taken.

Determination of crude fibre was done as shown below:

$$\text{Crude fibre } (\%) = \left(\frac{W_i - W_{ii}}{W}\right) \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.6$$

Where;

W_1= Weight of acid and alkali digested sample

W_2= Weight of incinerated sample after acid and alkali digestion

W= Weight Of Sample

3.4.6 Percentage Carbohydrates

Percentage carbohydrate content was calculated by difference as shown below:

$$\%Carbohydrate = 100 - \%(protein + fat + Moisture + Ash \ \ldots\ldots\ldots\ldots\ldots\ldots\ 3.7$$

3.4.7 Energy Determination

This was done using a bomb calorimeter model (Jessup,1960). Two ml distilled water was measured and poured into a bomb cylinder. 0.5 g of the sample was weighed and placed in a crucible suspended into the bomb heater. 5.5 cm nichrome wire and 10cm thread were measured and cut then fitted on either side of bomb head into the crucible ensuring that the wire did not touch the crucible. The bomb heater was placed into bomb head cylinder and closed tightly. The bomb was connected to oxygen supply purge and set at 30-atmosphere pressure. Using adjustable spanner, the nut was opened to release pressure. The bomb was put inside the colorimeter basket by attaching the lifting handle to the screw cap and lowering the bomb with its feet spanning the cylinder bass at the bottom of the basket. Then the thermometer and agitator were inserted and the stirrer started. The temperature change was recorded and calorific value determined. One calorie equals 4.1868 absolute Joules and is roughly equivalent to the heat energy required to raise the temperature of 1g of water one degree Celsius at 15°C.

3.5 Determination of quality stability of ASCF Packaged in Different Packaging Material and Stored in differnt Storage Conditions

Oxidation of fat in ASCF was analyzed by determining PV and p-AV while microbial stability was done by analyzing for TVC and Yeast and Molds from day zero as the baseline then analysis of the stored samples was done at 45 days storage interval until the 180th day.

3.5.1 Peroxide Value Analysis

This was determined through iodometric titration according to standard methods for the oils analysis AOCS (1998), and the results were expressed in meq O_2/kg oil. 2 grams of ASCF oil samples were weighed into 250 ml stoppered conical flask. 30 ml of acetic acid chloroform solvent mixture (3:2) was added in each and swirled to dissolve. Then 0.5 ml saturated potassium iodide solution was added with a mohr pipette and left to stand for 1minute in the dark with occasional shaking then added about 30ml of distilled water. This was titrated with 0.01 N sodium thiosulphate solution, with vigorous shaking until yellow color was almost gone. 0.5 ml starch solution was added as an indicator and titration was continued until the blue color disappeared indicating the endpoint. Calculation of the peroxide value was done as below:

$$PV = \frac{TitrexNx100}{W} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad 3.8$$

Where W is the weight of the sample, Titre = ml of Sodium Thiosulphate used N = Normality of sodium thiosulphate solution.

3.5.2 P-Anisidine Value Analysis

The p-anisidine value (p-AV) a measure of carbonyl content in the oils or fats was determined by the standard method according to America Oil Chemists' Society (AOCS) (1998). It is based on the reactiveness of the aldehyde carbonyl bond on the p-

anisidine amine group, leading to the formation of a Schiff base that absorbs at 350 nm wavelength. Approximately 2 g of ASCF oil samples were dissolved in 25 mL isooctane and absorbance of this oil solution measured at 350 nm against a blank of isooctane. Then 5 ml of these solutions and 5 ml of isooctane each were transferred to tubes. Each was added a 1ml anisidine solution (0.25% g/v glacial acetic acid). This was kept in the dark for 10 minutes, then absorbance (A_2) was measured at 350 nm against isooctane containing p-anisidine. p-AV was calculated according to the formula below:

$$p - AV = 25\frac{1.2xA_2 - A_1}{W} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.9$$

Where p-AV is the anisidine value, W is weight of the oil sample (g), A_2 is absorbance of the sample containing anisidine solution and $A_1$is absorbance of sample solution without the anisidine solution.

3.5.3 Enumeration of Total Viable Counts

TVC growth was done by spread plate technique using Plate Count Agar (PCA), as per American Public Health Association (1992). Physiological solution containing 9 g of NaCl in 1000 mL distilled water was used for serial dilutions. Then 5g of ASCF flour was aseptically taken and homogenized in 45 ml of previously sterilized physiological solution. From homogenized flour solution, 1 ml was taken and transferred into 9 ml physiological solution and dilution was done to fivefold. 0.1ml of the dilutions were then plated out in triplicate on already solidified agar. It was then spread over the agar plate using a sterile glass spreader. Plates were placed in an inverted position to avoid water condensing into the medium. The PCA plates were incubated at 37 °C for 48 hours. After the incubation period the observed colonies were counted.

3.5.4 Enumeration of Yeasts and Molds

The determination of yeasts and molds was done by spread plate technique using Potato Dextrose Agar (PDA). Physiological solution (PS) containing 9 g of NaCl in 1000 mL distilled water was used for the serial dilutions. Then 5 g of ASCF flour was aseptically taken and homogenized in 45 mL of previously sterilized physiological solution. From homogenized flour solution, 1 mL was taken and transferred into 9 mL PS and dilution done to fivefold. 0.1ml of the dilutions were then plated out in triplicate on already solidified PDA agar. It was then spread over the agar plate using a sterile glass spreader. Plates were placed in an inverted position to avoid water condensing into the medium. The PDA plates were incubated at 25 °C for 5 days. After the incubation period the observed colonies were counted (AMPH, 1992).

3.7 Moisture Adsorption Behaviour.

The moisture adsorption behaviour of ASCF was determined using standard gravimetric method as described by Bell (2002). Nine reagent grades saturated salt solutions in a_W range of 0.11-0.97 were used to create an atmosphere known a_W at temperature studied as recommended by Greenspan, 1977 (Table 3.1). Each salt was placed in a separate airtight desiccator with a lid and the respective a_W labelled. About 0.5 grams of ASCF was weighed in pre-weighed aluminium dishes with open top. These were first dried in an oven at 105^0C for 24 hours and weight of the dried sample taken (w_1). Three of the weighed samples were put inside separate vacuum desiccators in which saturated salt solutions were placed to maintain respective a_W. Saturation of each salt solution was ensured by maintaining undissolved crystals in the solution. The desiccators were then placed in an incubator maintained at either 25°C, 30°C or 35°C. Equilibrium was monitored by weighing the samples at intervals of 24 hours until the weight of three consecutive readings showed a change of 0.001g or less (w_2).

Table 3.1: The water activity of saturated salts at specified temperature

Materials/Salts	a_W at Set Temperature		
	25^0C	30^0C	35^0C
Lithium Chloride (LiCl)	0.11	0.11	0.11
Potassium Acetate (CH_3CO_2K)	0.23	0.22	0.22
Magnesium Chloride ($MgCl_2$)	0.33	0.32	0.32
Potassium Carbonate (K_2CO_3)	0.43	0.43	0.43
Magnesium Nitrate ($Mg(NO_3)_2$)	0.53	0.51	0.50
Potassium Iodide (KI)	0.69	0.68	0.67
Sodium Chloride (NaCl)	0.75	0.75	0.75
Potassium Chloride (KCl)	0.84	0.84	0.83
Potassium Sulphate (K_2SO_4)	0.97	0.97	0.97

Source; (Greenspan 1977, 91-94)

Mathematical Modelling and Analysis of Sorption Isotherm Data

The obtained experimental data corresponding to the a_W at the temperature studied was fitted to six mathematical models namely; Brunauer-Emmet-Teller (BET), Guggenheim-Anderson-de Boer (GAB), Oswin, Smith, Kuhn, and Caurie models. Mathematical expressions, equations 3.10-3.15 of these models are presented in Table 3.2 Model equation coefficients were obtained by linear and nonlinear regression statistical analysis using Solver add-in of Microsoft Excel 2013 spreadsheet software.

Table 3.2: Mathematical models that were used to fit experimental data

Model	Mathematical expression	a_W applicability	Equation no.

		range	
Brunauer-Emmet-Teller (BET) (Brunauer *et al.*,.1938).	$M_{eq} = \frac{M_o \times C_b \times a_W}{(1-a_W)(1+(C_b-1)a_W)}$	<0.43	3.10
Guggenheim-Anderson-de Boer (GAB) (Anderson, 1946).	$M_{eq} = \frac{M_o \times C_g \times K_g \times a_W}{(1-K_g a_W)(1+(C_g-1)K_g a_W)}$	<0.95	3.11
Oswin (Oswin, 1946).	$M_{eq} = A\left[\frac{a_w}{1-a_w}\right]^B$	<0.98	3.12
Smith (Smith, 1947).	$M_{eq} = A + B\ln(1-a_W)$	>0.6	3.13
Kuhn (Kuhn, 1967).	$M_{eq} = \frac{A}{\ln a_W} + B$	<0.75	3.14
Caurie (Caurie, 1978).	$\frac{1}{Meq} = \frac{1}{C_c M_o}\left(\frac{1-a_W}{a_W}\right)^{(2C_c/M_o)}$	<0.98	3.15

Meq: Equilibrium moisture content (g/100g), $M_{o:}$ monolayer moisture content of BET, GAB and Caurie model, C_b, C_g, K_g, A, B, C_c are model constants.

The quality of the fitting for each proposed model was evaluated through the correlation coefficient (R^2) and the root mean square error (RMSE).

$$RMSE\ (\%) = 100X\sqrt{\left[\frac{\sum_{i=1}^{n}\left(\left(M_{obs(i)} - M_{est}\right)/M_{obs(i)}\right)^2}{N}\right]} \ldots\ldots\ldots\ldots\ldots\ldots 3.16$$

Where M_{obs} and M_{est} are the equilibrium moisture contents determined experimentally and predicted, respectively, from the models at $a_{W\ (i)}$ and N is the number of experimental data.

The Clausius-Clapeyron equation was used to estimate the net isosteric heat of sorption by plotting In (a_W) versus the inverse of absolute temperature (1/T) at constant moisture as shown below:

$$In\left(a_W\right) = -\left(\frac{q_{st}/R}{1/T}\right) + K \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.17$$

Where q_{st} is the net isosteric heat of sorption in J mol^{-1}, T is the absolute temperature in K, R is the universal gas constant (8.314J/mol /K) and a_W is water activity which is dimensionless.

3.8 Water Vapor Permeability of the Packaging Materials

Water vapor permeability of the packaging material was determined using gravimetric method (Gaikwad, 2015). Pouches of the three types of packaging material namely kraft (K), kraft with polythene lining (KL) and aluminium pouch (A) were used in the experiment. The area, thickness, and weight of empty pouches were measured. Then some of the pouches were filled with desiccants and others left empty, they were all sealed and weight taken. Both pouches with and without desiccants were put in a conditioned chamber of either 25°C and 75%RH or 35°C and 75% RH in triplicate and

weight changes were taken at an interval of two days until the weight stabilized. The collected data was used to determine the water vapour transmission rate (WVTR) by applying the equation below (ASTM International, 2013).

$$WVTR = \frac{G}{tA} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.18$$

Where G is the weight change (g), t is time (days) and A is the area of the packaging material for mass transfer (m^2).

Permeability of each packaging material was determined from WVTR and film thickness (T) using the equation below.

$$P_P = \frac{(WVTR)T}{P(RH)} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.19$$

Where P_p is the packaging permeability (g H_2O µm /m^2 /day /mmHg), *WVTR* is the water vapor transmission rate of the package (g $m^{-2}day^{-}$), T is the thickness of the material (µm), P is the water vapour pressure at storage temperature (mmHg), and *RH* is the relative humidity.

Shelf Life Estimation

Heiss Eichner model Heiss and Eichner, (1971), was used to predict the shelf life of ASCF in relation to possible packaging and storage conditions. Kraft paper (K), Kraft paper with polythene lining (KL) and aluminium pouch (A) are common packaging materials for complementary flour in Kenya hence were used in this study. Temperature range between 25-35°C was considered as representing ambient conditions in Kenya while 75 % relative humidity was used as the highest relative humidity in high temperature areas like coast of Kenya. Based on the model, shelf life is estimated by applying the equation below:

$$s_f = \frac{ln\left[\frac{(x_e - x_i)}{(x_e - x_c)}\right]}{p_c\left(A/W_s\right)\left(\frac{P_O}{S}\right)} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad 3.20$$

Where s_f is the estimated shelf life, X_e is the equilibrium moisture content (g/g d.b) of the unpacked product left in contact with the atmosphere (influenced by temperature, relative humidity, and product adsorption isotherm); X_c moisture content of the product corresponding to the safe storage a_W (g/g d.b)(a_W of 0.7 is generally used for most food products); P_c is the permeability constant of the package to moisture vapour (kg H_2O µm/m^2/day/mmHg); P_o is vapour pressure at storage temperature (mmHg); A is the surface area of the package (m^2); W is the weight of the product (dry matter) in the package (kg); and S is slope of the product isotherm (between X_e and X_c which is assumed to be linear).

3.9 Statistical analyses

All the data was subjected to analysis of variance using Stata SE version 12 (StataCorp LP, Texas, USA). Furthermore due to the inherent limitations of ANOVA in describing differences in progression of variables over time, the analysis of covariance (ANCOVA) which combines features of both ANOVA and regression was also applied to test effects of storage duration, storage environment type of package and the interaction effects. When the coefficient of the interaction term was significant ($P<0.05$), it was concluded that there was a significant difference between treatments over the storage period. Means were separated using Boniferroni adjustment at 95% confidence level

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Proximate Composition of ASCF

The proximate composition of ASCF is shown in Table 4.1.The energy content was 5.1kcal per gram on dry weight basis. This is comparable to 5.2 kcal/g that was reported by Okoth *et al.,* (2017) of a similar product at 90:10 amaranth to sorghum grain formulation. The product has higher energy in comparison with those complementary foods used by World Food Program to rehabilitate children with moderate malnutrition whose energy ranges between 3.7 and 3.8 kcal/g (Okoth *et al.,* 2017). Its protein content was 17.2 g/100g giving 15% protein energy contribution. This is within the recommended limit of 14% by East African Community standard on processed cereal-based foods for infants and children (EAS 72:2013). It was also higher than the minimum requirement of 6.9% protein energy contribution recommended by World Health Organization/ Food and Agriculture Organization (Okoth *et al.*, 2017).

Table 4.1: Proximate composition of ASCF (g/100g dwb)

Parameter (%)	Quantity
Protein	17.2±0.1
Fat	8.8±0.1
Crude Fibre	2.5±0.1
Ash	2.5±0.0
Carbohydrate	69.1±0.1
Energy (kcal per g)	5.1±0.2

Means ± standard deviation of the triplicate sample.

The product's fat content was 8.8% supplying energy content of 18.8% of total energy out of which 7.4% was from essential fatty acids. This meets the requirements for processed cereal-based foods for infants and young children of 8.5% minimum fat by mass according to East African Standards (EAS 72:2013). This, therefore, confirms that the product is a good source of protein and energy as reported by Okoth *et al*. (2017).

The crude fiber was 2.5 g/100g on dry weight basis thus within the maximum limit of 5 g/100g according to Kenyan standards on complementary foods (KS 2515:2013). Insoluble fibers increase bulk and decrease gastrointestinal transit hence their amount is recommended to be low in the diet (Okoth *et al.,* 2017; Michaelsen *et al.*,2009). The product's total iron was 14.9 mg/g which was above the minimum recommended composition requirement of 9.3 mg/100g on complementary food (Koletzko *et al.,* 2005).

According to Okoth *et a*l. (2017) a total of 60.1% of ASCF fat content was unsaturated (linoleic acid 231 mg/100g dwb, oleic 176.8 mg dwb/100g and linoleic 10.2 mg/100g dwb) making it susceptible to fat oxidation during storage. The moisture content of the flour was 3.6 % making the product prone to moisture absorption from the storage environment. Low moisture food are prone to absorbing water from the environment. This can be attributed to varying partial vapor pressure difference resulting in concentration gradient between the storage environments and the packaged product causing migration of water until an equilibrium is achieved (Siracusa, 2012).

4.2 Effect of Packaging Material and Storage Condition on the Moisture Content of ASCF

The moisture content of ASCF increased with increase in storage period irrespective of the packaging materials (Figure 4.1). The likely reason for the increase in moisture content of ASCF during storage is due to the ingress of water vapor through the packaging material and initial low moisture content in ASCF rendering the samples to

absorb moisture. Muzaffar and Kumar (2016) reported increase in moisture content of packaged tamarind pulp powder with storage. The increase in moisture content with storage varied with storage conditions whereby high storage humidity and temperature resulted in high moisture content.

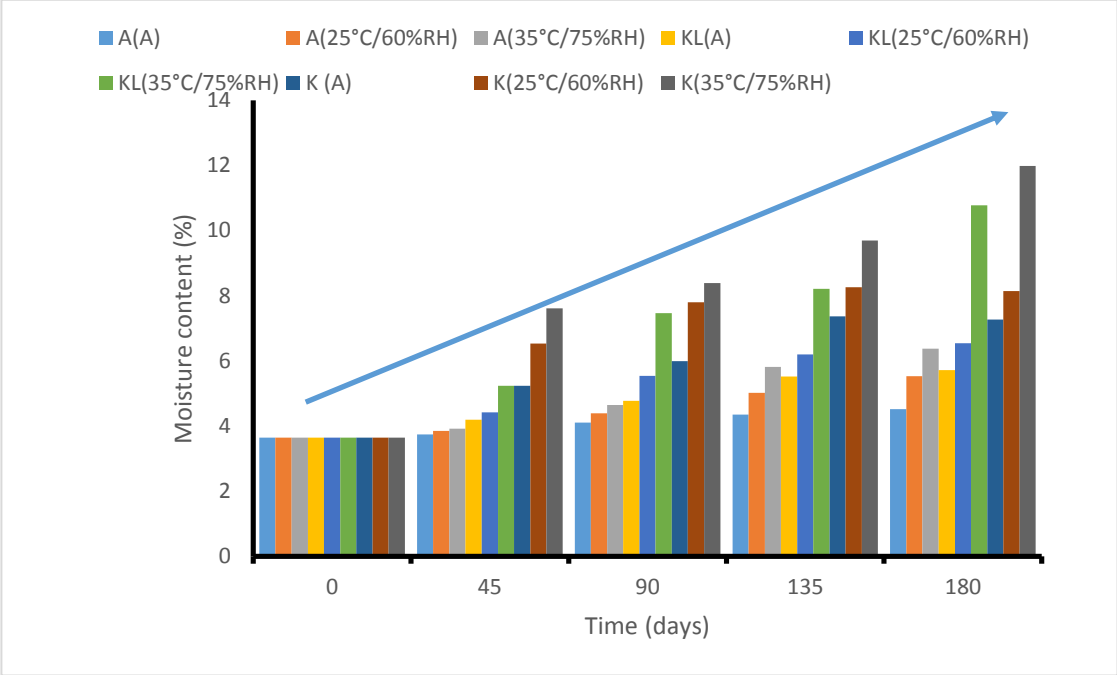

Figure 4.1: Change in moisture content of ASCF at different storage conditions and packaging material

Key: A (A), A (25°C/ 60%RH), A (35°C/75%RH) stands for Aluminium pouch stored at ambient, 25°C/60% RH, and 35°C/75% RH respectively, KL(A), KL(25°C/60%RH), KL(35°C/75% RH) stands for Kraft with polythene lining at ambient, 25°C/60%RH, and 35°C/35%RH respectively, while K(A), K(25°C/60%RH), K(35°C/75%RH) stands for Kraft paper at ambient, 25°C/60%RH, and 35°C/75%RH respectively.

Moisture increased by 8.4%, 4.5% and 3.8% in samples stored at 35°C and 75% RH, 4.5%, 2.9% and 1.5% in samples stored at 25°C and 60% RH and 3.6%, 1.8% and 0.6% in samples stored at ambient condition and packaged in K, KL and A respectively after

a storage period of 180 days. Uchechukwu-Agua (2015), reported moisture increase in cassava flour with storage time under high relative humidity storage condition. The findings are comparable with a study that was carried out on 3 powders which included wheat flour, tea powder and whey permeate where moisture increased significantly as the storage relative humidity increased (Iqbal and Fitzpatrick, 2006).This can be attributed to varying partial vapor pressure difference resulting in concentration gradient between the storage environments and the packaged product hence causing migration of water until an equilibrium is achieved (Siracusa, 2012). There was also variation in moisture content among the packaging materials. Samples of ASCF packaged in K increased in moisture content by 99.7% at ambient condition, 123.6 % at 25°C; 60% RH and 226.9 % at 35°C; 75% RH after 180 days storage. Samples packaged in A had the least moisture content increase with its highest increasing by 3.8%. The variation in moisture content of these products could be directly related to the water vapour permeability of the packaging materials. Packaging materials differ in degree of permeation to small molecules like water vapor, and gases (Muzaffar and Kumar, 2016).The transfer of the small molecules through the package, therefore, ranges from high to low depending on the barrier properties of the material (Siracusa, 2012). Similar variation with packaging has been reported in previous studies done on tamarind pulp powder, cassava flour and wheat flour (Muzaffar and Kumar, 2016; Uchechukwu-Agua, 2015; Butt *et al.*,, 2004)

High storage humidity results in high moisture content, which favors enzymatic hydrolysis of the fat and microbial growth in food products (Mridula *et al.,* 2010). Butt *et al.,* (2004) also reported that the moisture content was affected significantly by storage, treatments, packaging and their interaction. Moisture migration can be stopped by either reducing the environmental storage humidity or using moisture-barrier packaging material for storage of products in the environment of fluctuating atmospheric conditions (Hotchkiss, 1997). This was after a storage period of 180^{th} day during the fourth sampling stage.

Figure 4.9: Caked sample of ASCF packaged in kraft paper, stored at 35°C and 75%RH

Storage of powder products in environments with high relative humidity increases absorption of water vapour from the environment resulting in increase in moisture content. It is reported that increase in moisture content of powder products during storage, increases the cohesiveness of the powder leading to decrease in flowability (Muzaffar and Kumar, 2016; Janjatović *et al.*,2012). An increase in cohesiveness of powder particles results in formation of lumps in the product. Iqbal and Fitzpatrick (2006), reported formation of a hard cake on tea powder stored under high relative humidity conditions.

A study that was done on shelf life of a savoury powder used as a topping on food known as " koya" reported that the powder had an initial moisture content of 4.31% but its critical moisture content based on caking score of 3 was 7.6% (Anandito *et al.*,2017). This is an indication that increase in moisture of powder products may result in caking. Caking in flour products causes them to adhere to the surface of the package (Iqbal and Fitzpatrick, 2006). It is a major problem for dry powdered product manufacturers. It is said to affect solubility of the product and accelerates chemical reactions like enzymatic activities and lipid oxidation (Chung *et al.*, 2000). It is also said to cause loss of

crispness and flavor, enhance organoleptic quality deterioration hence decreasing product shelf life and quality.

Caking of powdered products to majority of consumers is an indication of poor quality (Hartmann and Palzer, 2011). It is normally as a result of water absorption or redistribution in the powdered product during processing and storage(Chung *et al.*, 2000). Water mobilizes the powder surface making them sticky resulting in inter-particle binding and formation of clusters hence caking. Temperature also influences caking and the magnitude of its effect is strongly dependent on moisture (Saltmarch and Labuza 1980).

Caking is aggravated by moisture which acts as a plasticizer and lowers the glass transition temperature (Hartmann and Palzer, 2011; Aguilera *et al.*, 1995). The formation of inter-particle bridges process is a function of viscosity, which dramatically changes around glass transition temperature (Tg). Below Tg, the product is considered to be in glassy state, characterized by very high viscosity and very low mobility. Therefore, the plasticizers take long to mobilize the particles lengthening the contact time between particles. This protects the product from caking in the course of its shelf lifetime (Chung *et al.*, 2000). If the storage temperature of the product is above its Tg, polymer segmental viscosity decreases drastically while the mobility increases, therefore, reducing the contact time which supports sticking and caking (Zafar *et al.*, 2017). High humidity also leads to decrease in polymer segmental viscosity and reduces the Tg therefore favoring caking. This explains why caking was observed in the sample that was stored at high temperature and relative humidity. The high temperature decreased the viscosity of the particles as it increased its mobility which was favored by high storage humidity. K also favored the process due to its poor barrier properties to water vapor resulting into high permeability and therefore caking.

4.3 Effect of Storage Condition and Type of Package on the PV and p-AV of ASCF

In the present study, PV) and p-AV were used to monitor the ASCF oil quality at different storage condition and different packaging material. Changes in PV of stored ASCF are shown in Figure 4.3. Results show that oil of ASCF before storage had a PV of 0.487 meq O_2/kg. It is an indication that some primary oxidation had taken place before storage of ASCF. This may have been due to heat processing during drying and extrusion and/or exposure of the grains to oxygen when drying (Kaleem *et al.,* 2015). Variation in initial PV may be due to difference in grain processing and also the oil extraction process. Fresh coconut oil was found to have PV between 0.24–0.49 O_2meq/kg (Moigradean *et al.,* 2012). Similarly, ASCF contained approximately 7.8% fat. According to Okoth *et al.,* (2017), 70.4% of the fat in ASCF is unsaturated, therefore susceptible to oxidative deterioration during storage depending on the surrounding conditions.

As shown in Figure 4.3, PV of ASCF samples continually increased during the entire storage period regardless of the packaging and storage condition. PV is a measure of primary oxidation in oils and increases with an increase in peroxides (Kaleem *et al.,* 2015).

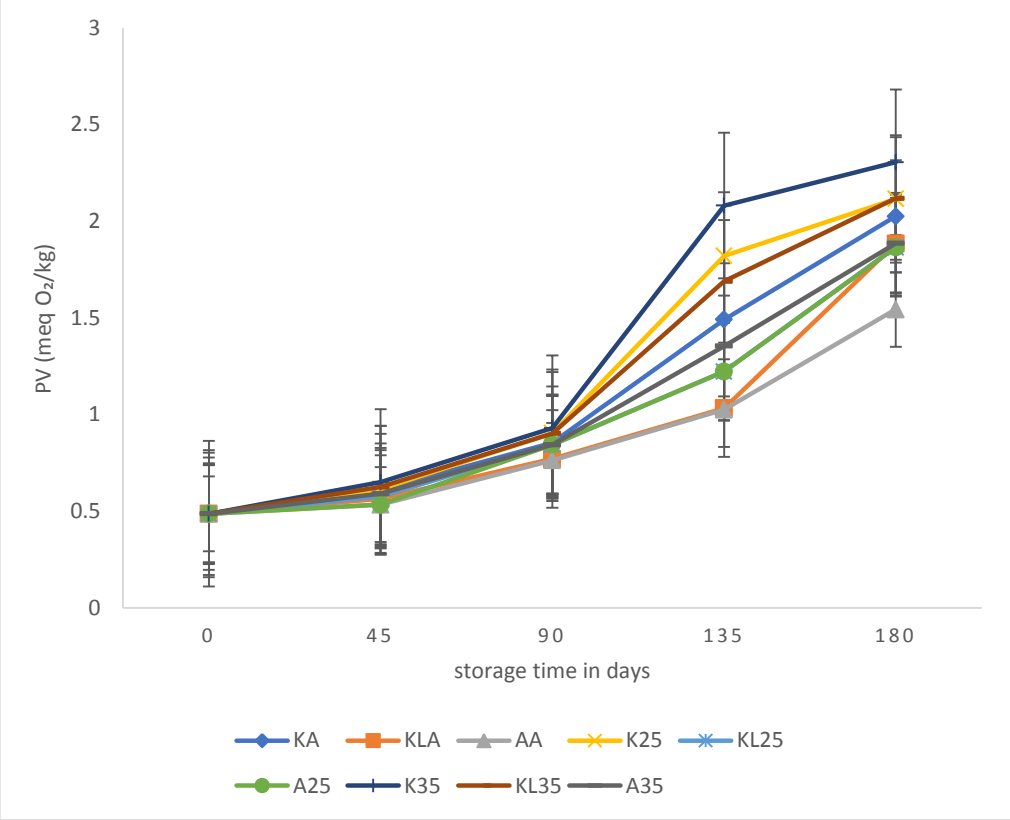

Figure 4.10: Change in peroxide value (PV) of ASCF with storage time under different storage conditions and packaging material

Key: KA,KLA, AA,K25,KL25,A25,K35,KL35,A35 is Kraft paper, kraft paper with polythene lining and aluminium pouch at ambient condition, 25°C and 35°C respectively.

Lipid oxidation rates are directly related to temperature (Flick *et al.*, 1992). In this study, PV increase in samples stored at 35°C at different storage periods was in the range of; 22.9-35.4% at 45 days, 75-93.6% at 90 days, 181.3-333.3% at 135 days and 293.6-379.2% at 180 days. Increase in PV in samples stored at 25°C was in the range of; 10.4-27.1% at 45 days, 70.8-87.5% at 90 days, 116.7-300% at 135 days and 260.4-341.7% at 180 days. Increase in PV in samples stored at ambient condition was in the range of; 10.4-33.3% at 45 days, 58.3-77% at 90 days, 112.5-293.8% at 135 days and 220.8-

320.8% at 180 days. At all the different sampling stages in the three storage environments, the level of PV based on packaging material was in the order K> KL>A. Packaging materials differ in permeability to environmental factors that may affect the rate of fat oxidation.. a_W influences the rate of oxidation whereby from 0.3-0.8a_W the rate of oxidation increases due to dissociation of catalysts as well as the mobility of oxygen and metal ions hence increasing the rate of oxidation of lipids (Bell, 2002). K had higher permeability rate followed by KL while A had the least. Analysis of variance showed that the interaction between the effect of package and storage condition on the PV was not significant (P=0.9902). However the storage period had a significant effect on PV (P=0.000) whereby PV was seen to increase significantly with increase in storage period regardless of the storage condition and package used.

Autoxidation of fat takes place at the double bonds of unsaturated fatty acids. Heat, light or high energy radiation or metal ions act as catalysts in initiating autoxidation process through free radical route (Flick *et al.,* 1992). In addition the packaging materials barrier properties may be affected by environmental factors like temperature and relative humidity. As a result, the transfer of gases like oxygen ranges from high to low hence affecting the product at varying levels (Siracusa, 2012). This, therefore, explains the difference in peroxide value at different storage conditions and packaging material.

Although there was continuous increase in PV with storage time of ASCF, results revealed that the product had good oxidation stability. This is because 2.3meq O_2/kg was the highest PV obtained after 180 days storage. A product with PV below 5 meq O_2/kg is classified at low oxidation state; that between 5 and 10 meq O_2/kg at moderate oxidation and above 10 meq O_2/kg is classified at high oxidation state. The PV limit of refined oil according to East African Standard is 10 meq O_2/kg (EAS 795: 2013). Codex gives a general PV limit of 15 meq O_2/kg for virgin oils (Codex, 2001). Therefore at 180 days storage even at 35°C, ASCF can be classified at low oxidation state as its PV was not above the limit. Amaranth seed oil was considered to be very stable even without

addition of antioxidants according to a study that was carried out on the oxidation stability of amaranth oil (Santiago *et al.,* 2012). Gamel *et al.,* (2007), found that amaranth oil had good oxidation stability better than sunflower oil.

p-AV represents secondary oxidation product contents that are able to react with p-anisidine reagents such as α and β alkenals. The p-AV was not detected in fresh flour and subsequent samples after storage. This is an indication that secondary oxidation did not take place or the levels were below detection limit during processing and after the 180 days storage period. According to Guilenn (2002), the onset of secondary oxidation product formation from the primary oxidation products differs with the type of oil (Choe and Min, 2005, Guillen and Cabo, 2002). In some oils, secondary oxidation products start almost simultaneously with hydroperoxide generation while in others, degradation of hydroperoxides begins when its concentration is appreciable (Guillen and Cabo, 2002). In reference to a study that was done on oxidation stability of edible oils, safflower and sunflower oils secondary oxidation products formed after the hydroperoxide concentration was substantial (>50 meq O_2/kg) while in olive and rapeseed oils it was immediate after hydroperoxides formation (Guillen and Cabo, 2002). This, therefore, explains why there were no secondary oxidation products detected in ASCF after six months storage. The concentration of the primary oxidation products may not have been substantial enough to cause secondary oxidation products formation. In addition, amaranth seed contains significant amount of antioxidant compounds, Barton *et al.,*.(2011), with squalene ranging between 5%-7% in amaranth oil (Santiago *et al.,* 2012).

Squalene was reported to act as a peroxyl radical scavenger in temperature-dependent autoxidation by Amarowicz, (2009), with antioxidant activity of 0.74 mmol Trolox equivalents/g (Tikekar *et al.,* 2008). The rate constant of quenching singlet oxygen by squalene was reported to equal that of butylated hydroxytoluene (Amarowicz, 2009). At a molar ratio of 1:7 polyunsaturated fatty acid, squalene inhibited oxidation of docosahexaenoic arachidonic and linolenic acid by 50% while that of linoleic was 22%.

It was also found to significantly reduce the lipid peroxidation levels in heart tissue of rats after being administered with polyunsaturated fatty acids concentrate. Squalene was also reported to be stable with storage and technological processes (Tikekar *et al.*,2008). Besides squalene, amaranth extracts were found to have substantial antioxidant activity. The presence of these natural antioxidants in amaranth flour may have contributed to its shelf life stability in terms of fat oxidation.

According to Kenya Bureau Standards (KEBS) on formulated complementary foods for older infants and young children should be free from rancid or musty odor or flavor (KEBS, 2013). The rancid flavor may be due to secondary oxidation products that were not detected in ASCF product over the storage period of 180 days. Egan (1981) reported that acidity and a rancid taste often started to be noticeable in foods when peroxide values were between 20 to 40 meqO_2/kg. In this study, the highest was 2.3meq O_2/kg. To predict the shelf life of the flour, 10 meqO_2/kg was taken as a quality reference estimation of a maximum acceptable PV. This limit was established using the East African Community Standard 795: 2013 (2013), applicable for refined oils.

Results showed that ASCF packaged in kraft paper and stored at 35 °C could last for 860 days; at 25 °C, 980 days, and at ambient condition, 1102 days. Samples packaged in kraft paper with polyethylene lining could store for 1010 days at 35°C, 1237 days at 25°C and 1329 days at ambient condition. Those packaged in aluminium pouch could last for 1225 days at 35°C, 1259 days at 25°C and 1663 days at ambient condition. In terms of fat oxidation, it can be concluded that ASCF is shelf life stable considering that it can store for 860 days having been stored at 35°C and packaged in kraft paper.

4.4 Effect of Storage Condition and Type of Package on the Microbial Stability of ASCF

The effect of storage temperature and relative humidity, packaging, and duration on the microbial stability of ASCF were monitored for 180 days. The evaluation was conducted

after every 45 days storage starting at day 0 as the baseline. Samples were stored at ambient conditions, 25°C, 60%RH and 35°C, 75%RH and packaged in K, KL, and A. The total viable count for ASCF at the beginning of the experiment was found to be 3.81 log colony-forming unit (cfu)/gram (g). This was within the acceptable limit of cereal-based infant foods requiring further cooking where acceptable limit is 5.0 log cfu/g (Mossel and Van, 1991).

Yeast and molds were not detected in ASCF before storage and during the entire storage period of the samples. Bacteria presence even after processing can be attributed to contamination during processing, handling, packaging, and storage (Braide *et al.*, 2011; Mujuru *et al.*, 2014; Opara, 2013). The process of extrusion employs high heat, high pressure, and mechanical shear simultaneously and is reported to reduce microbial levels substantially. The process of extrusion was reported to reduce the aerobic mesophilic bacteria in a range of 2.2 to 4.1 log cfu/g when semolina was being processed to spaghetti while yeast and mold reduction ranged from 0.1-1.7 log cfu/g (Sabillón,2014, Manthy, 2004). Generally, post-process contamination is responsible for heat-processed product spoilage.

Although heat applied during drying and extrusion destroys microbes, heat processed products may be subjected to recontamination from the air, equipment surfaces, and handling during packaging operations (Sabillon, 2014). The presence of 3.8 log cfu/g aerobic mesophilic microorganisms in ASCF after extrusion and milling may have been due to contamination by milling equipment and handlers as the process was not done aseptically. Variation in the total viable count of ASCF with storage is as shown in Figure 4.5;

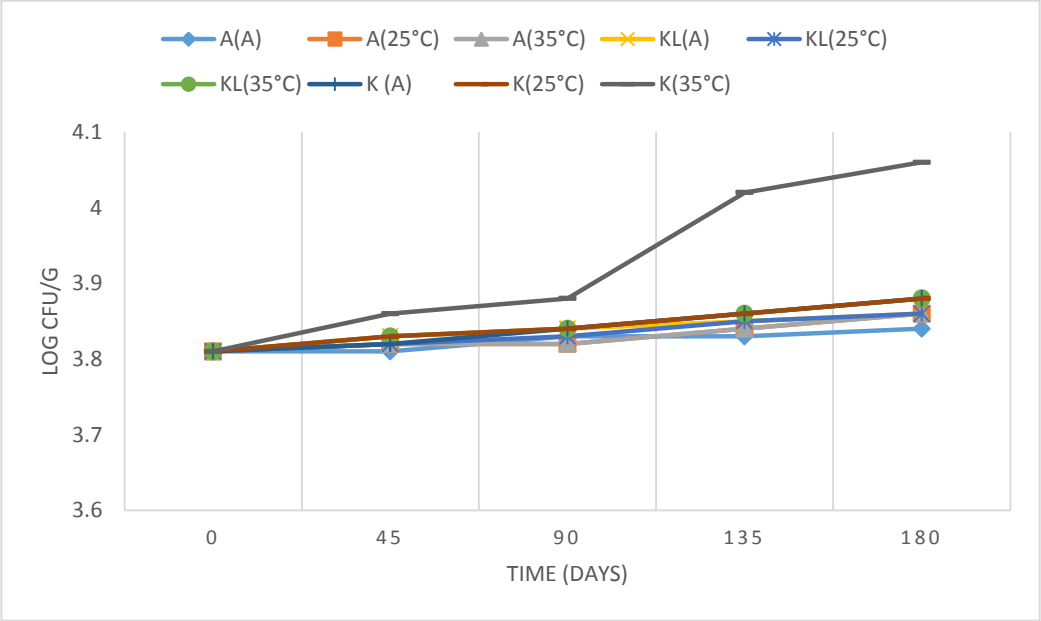

Figure 4.4: Change in total viable count in ASCF with storage time

Key: A (A), A (25°C), A (35°C) stands for Aluminium pouch stored at ambient, 25°C, and 35°C respectively, KL(A), KL(25°C), KL(35°C) stands for Kraft with polythene lining at ambient, 25°C, and 35°C respectively, while K(A), K(25°C), K(35°C) stands for Kraft paper at ambient, 25°C, and 35°C respectively.

The total aerobic mesophilic microorganisms increased slowly in all the packages throughout the storage period. This was with exception of samples packaged in K and stored at high temperature and relative humidity (35°C; 75%) where the increase of total aerobic mesophilic microorganisms was more rapid. ASCF is nutrient-rich and can provide an environment that is suitable for the growth and multiplication of diverse microorganisms. Microbial growth increased from 3.8 log cfu/g in ASCF before storge to 4.06 log cfu/g after storing for 180 days which was still within the acceptable limit of 5 log cfu/g.

Growth rates of most microorganisms are directly proportional to temperature and moisture and increase as temperature and moisture increase (Mossel and Van, 1991). In

addition, packaging materials have been reported to influence shelf-life stability of products due to their difference in permeability (Hotchkiss, 1997). Uchechukwu-Agua (2015), reported increase in total microbial growth with an increase in moisture content in cassava flour. Samples packaged in K recorded higher moisture content in comparison to the other packaging materials which translates to higher water activity hence higher microbial growth.

Though thermal treatment simultaneously reduces microbial population it is reported to have minimal impact on mycotoxin level if it was initially present in the raw material. Therefore, for safety to be ensured, microbial load and mycotoxins should be reduced to the minimum in cereal-based products. This can be achieved by preventing microbial establishment and growth within the mill and whole production chain through proper sanitation of production equipment, proper temperature control during heat treatment, raw material testing and resulting products, and hygienic packaging and shipment procedures (Sabillón, 2014).

Microbial spoilage in dried cereal products is influenced by water activity. Low water activity in these products (<0.6) prevents the growth of bacteria, molds, and yeast hence the reason why shelf life can be long in comparison with other food products. Insignificant microbial growth in ASCF during storage may be attributed to low water activity in the product. It can, therefore, be considered shelf-life stable if stored in airtight packages. Proper storage conditions and packaging are key for maintaining low levels of water activity in dry products to prevent microbial spoilage. Aluminium pouch, in this case, offered better shelf-life stability than the other materials based on water vapour permeability through the package into the product which directly relates to microbial stability.

4.5 Water Adsorption Properties and Shelf Life Estimation of ASCF

4.5.1 Sorption Isotherm

The time required for the samples to equilibrate in terms of weight varied with temperature and water activity. It took samples 7 to 28 days for them to equilibrate which was through adsorption of moisture since the initial samples were fully dried. Experimental sorption isotherm of ASCF at 25°C, 30°C and 35°C is shown in **Figure** 4.6.

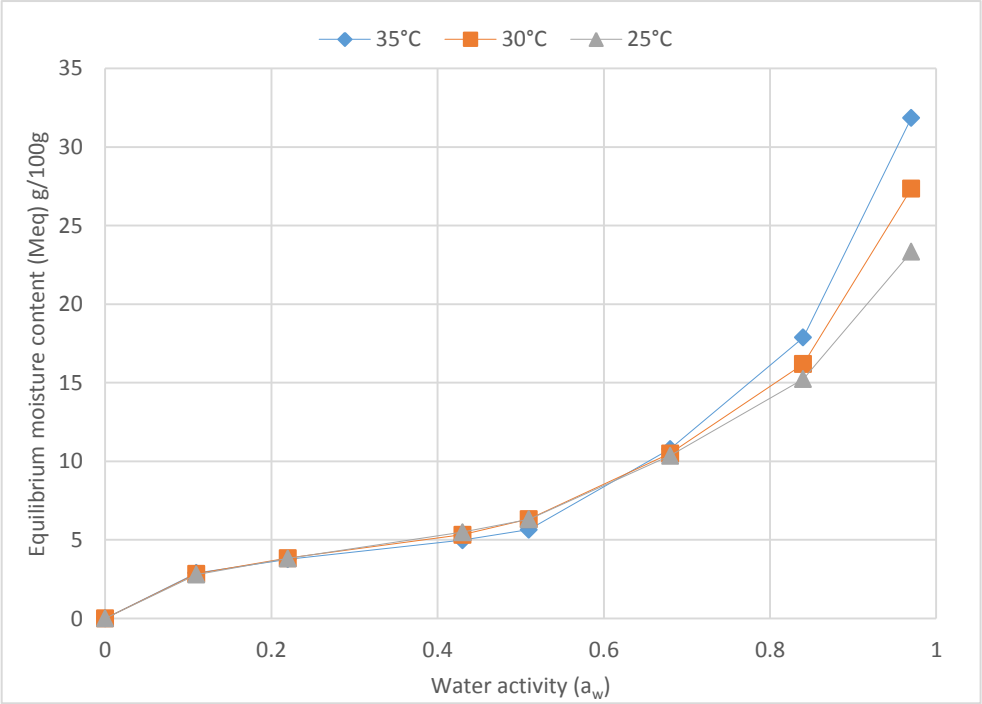

Figure 4.11: Moisture adsorption isotherm of ASCF at 25°C, 30°C and 35°C

The moisture sorption isotherm obtained exhibited sigmoidal shape for all the temperatures corresponding to the type II sorption isotherm based on Brunauer's classification (Lemus 2011). This is due to differential water attachment in the product known as monolayer-multilayer sorption mechanism (Brunauer *et al.*, 1940). It was

observed that the Meq of ASCF increased with a_W at constant temperature. This is due to hygroscopic nature of ASCF (Balderrama and Cadima, 2014; Malliaris, 2013; Timmermann *et al.,* 2001). The sorption isotherm also revealed that equilibrium moisture content increased linearly at low and intermediate a_W, while there was a sharp increase at high a_W a region known as capillary condensation. This trend has been observed in many food stuffs as reported in literature (Al-Muhtaseb *et al.,* 2002; Sandoval *et al.*,2009).

The trend in Figure 4.6, shows that increase in temperature led to a decrease in Meq at a_W approximately <0.6. This is due to decrease in hygroscopic nature of food with increase in temperature (Muzaffar and Kumar, 2016; Al-Muhtaseb *et al.,* 2002). It is attributed to higher energy levels and lower stability of water molecules with increase in temperature causing separation of water molecules from their binding sites within the food matrix (Timmermann *et al.,* 2001). Some water molecules are activated to higher energy levels that cause them to break from their sorption sites at high temperatures, leading to a decrease in the Meq (Balderrama and Cadima, 2014, Timmermann *et al.,* 2001). Temperature effect on sorption isotherm is important since it influences mobility of water molecules and the dynamic equilibrium between the vapour and adsorbed phase (Balderrama and Cadima, 2014; Sandova *et al.,* 2011).

There was a crossover of the ASCF sorption isotherm with increase in temperature at approximately $0.6 a_W$.The cross over behaviour can be attributed to endothermic dissolution of sugars at elevated a_W where they dissolve in water. ASCF has simple sugars as follows; 14.8g/100g glucose, 9.39 g/100g fructose and 12.3g/100g sucrose (Okoth *et al.,* 2017). These soluble components absorb more water at high a_W and this is enhanced by temperature (Sandoval *et al.,* 2009). Another factor that causes cross over

is greater exposure of active sites or hydrophilic groups at high a_W as temperature increases (Sandoval *et al.,* 2009) Presence of low molecular weight food constituents like sugars and salts have been reported to cause deviation of the sorption isotherm behaviour (Al-Muhtaseb *et al.,* 2002). Their solubility in water makes them more hygroscopic with increase in temperature hence the deviation of the trend with the temperature at high a_W (Al-Muhtaseb *et al.,*2002). Similar effects of temperature on the isotherm characteristics have been observed in sugars, sugar alcohol, dried fruits, at a a_W between 0.55–0.7 (Malliaris, 2013; Djendoubi *et al.,* 2012).

This phenomenon of decreasing Meq with increase in temperature at low a_W is because at low a_W values the sorption of water is mainly due to the biopolymers, with an increase of temperature having the normal effect of lowering the isotherm (Malliaris, 2013). However, as a_W increases beyond the intermediate region, the sugars and other low molecular weight constituents start to sorb water offsetting the effect of temperature resulting in deviation of the isotherm. The point of crossover is dependent on the composition of the food and the solubility of the sugar.

4.5.2 Fitting of Sorption Models

Sorption experimental data obtained for the three temperatures studied were fitted to six models namely BET, GAB, Oswin, Caurie, Smith and Kuhn models over the theoretically appropriate ranges at 25°C,30°C and 35°C refer to Figure 4.7 (A),(B) and (C).

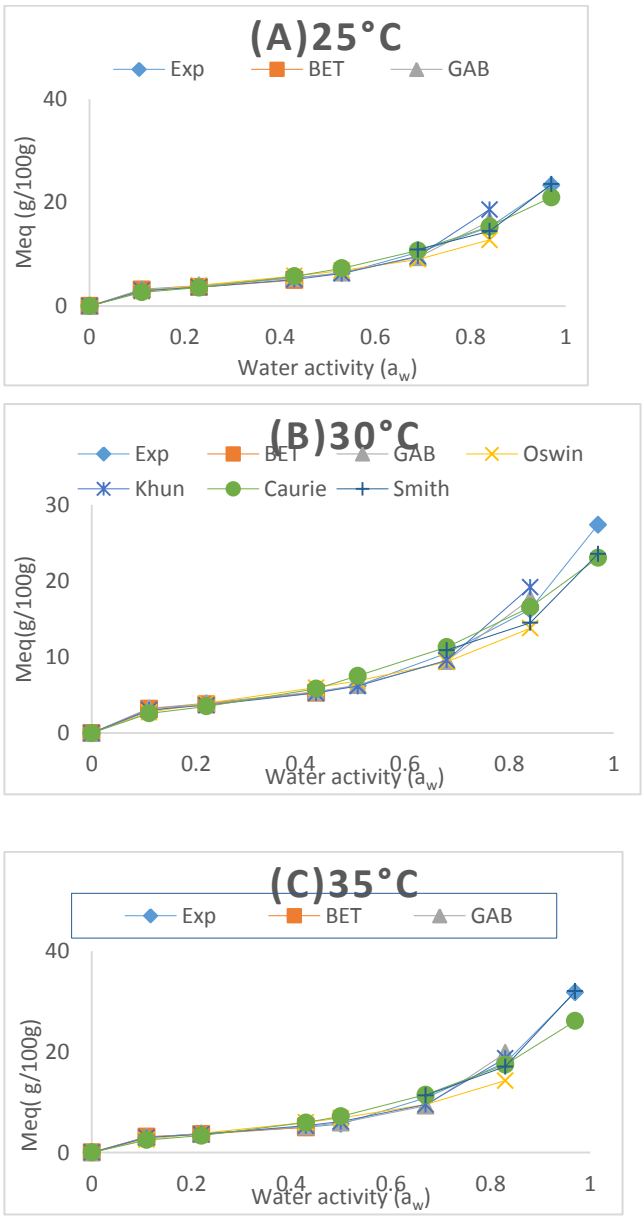

Figure 4.6: BET, GAB, Oswin, Kuhn, Caurie and Smith model fits to experimental data at 25°C (A), 30°C (B) and 35°C (C)

The fitting was done by applying the appropriate equations for each model. The predicted data fitted well in most of the models as shown in Tables 4.2. Only, Oswin and Caurie models failed to fit the the experimental behaviour of the product as their %

RMSE was ≥10%. Smith model gave the best-fit judging from percentage root mean square error (% RMSE ≤4.2%) while BET gave the best fit based on correlation coefficient (R^2 >0.99). Though Smith and BET model gave the best fit for experimental data, they are limited in applicability. Smith's model can only be used in a_W range between >0.6 to < 0.95 and was developed to describe the final curved portion of water sorption isotherm(Lemus, 2011). BET model is applicable in a_W range between >0.05 to < 0.45 and overestimates the sorption of a_W above 0.45 hence its application is limited to monolayer sorption only (Lemus, 2011). Kuhn model fitted data well with % RMSE < 7.6% and R^2 >0.9 but overestimated sorption at a_W <0.2 and >0.75. Therefore, the model that is suitable for a particular food product may not be suitable for another (Al-Muhtaseb *et al*., 2002).

Fitting the most suitable models helps describe the sorption behaviour of food hence the state of water and its interaction with food components. This knowledge of sorption isotherms in ASCF is key in predicting its shelf-life.

Table 3.2: Model parameters and measure of fit at various temperatures

Model	Parameters/measure of fit	Temperature °C		
		25°C	30°C	35°C
GAB	M_o (g/100g)	3.236	3.210	2.903
(<0.9)	C_g	73.486	84.189	186.094
	K_g	0.956	0.974	1.029
	R^2	0.977	0.978	0.9698
	RMSE (%)	8.147	7.548	8.378
BET	M_o (g/100g)	3.146	3.0779	2.8752
(<0.43)	C_b	73.930	112.034	434.75
	R^2	0.995	0.997	0.998
	RMSE (%)	8.072	6.591	5.208
Oswin	A	6.537	6.776	6.884
(<0.98)	B	0.404	0.428	0.463
	R^2	0.977	0.981	0.965
	RMSE (%)	10.349	10.143	15.193
Caurie	C_c	0.721	0.682	0.615
(<0.98)	M_o	2.394	2.526	2.731
	R^2	0.987	0.9741	0.962
	RMSE (%)	8.2515	12.097	16.313
Smith	A	4.552	2.603	1.888
(>0.6)	B	-5.419	-7.114	-8.608
	R^2	0.989	0.996	0.996
	RMSE (%)	4.192	3.222	3.753
Kuhnn	A	-2.968	-3.059	-3.211
(<0.75)	B	1.616	1.638	1.509
	R^2	0.9063	0.9309	0.9188
	RMSE (%)	5.0805	5.1221	7.5789

Meq: Equilibrium moisture content (g.100g), M_{∞} monolayer moisture content of BET, GAB and Caurie model, C_b, C_g, K_g, A, B, C_c are model constants. R^2 : Correlation Coefficient; RMSE: root mean square error.

GAB model fitted the data adequately with %RMSE <8.4% and R^2 >0.96. Its simulation fitted a wider range of a_W up to 0. 9 compared to the other four models that could adequately describe the experimental behaviour of the product. Based on literature, GAB model is considered the most useful in characterizing isotherm in almost the entire range of water activity

4.5.3 Properties of Sorbed Water

The BET and GAB models were rated superior as they have a viable theoretical background and the magnitudes of their parameters have physical meaning (Timmerman *et al.,* 2001; Rao *et al.,* 2006). They are also the most widely used in food systems (Lemus, 2011). The BET and GAB model gave an estimation of monolayer moisture content. In the temperature range of 25-35℃, the water content associated with the monolayer of ASCF varied from 3.23 to 2.90g water /100g dry solids when the GAB model was considered and from 3.15 to 2.67 g water/100g dry solids by BET model. The monolayer water content was seen to be inversely related to temperature irrespective of the equation used in their determination as expected (Table 4.2). According to literature, this has been attributed to the reduced number of active sites and to the increased vapour pressure inside the food at elevated temperatures, which makes water available for reactions (Baptestini *et al.,* 2017). From the study, monolayer moisture contents calculated using the GAB equation were higher than those calculated using the BET equation, which agrees with results observed in various studies by different authors (Brett *et al.,* 2009). The difference in monolayer moisture content on the two models may be due to differences in a_W range coverage as BET model is only applicable in a_W range between >0.05 to < 0.45 while the GAB model fits a wider range of a_W up to 0. 9 (Kamau *et al.,* 2018). GAB model is an improvement of the BET model

and covers a wider range of a_w and is reported to always give higher monolayer value in comparison with BET model (Timmermann *et al.*, 2001).

Monolayer water content is water that is strongly bound whereby water molecules have occupied all the ionic and polar groups of the adsorbent (Muzaffar and Kumar, 2016, Valdez-Niebla *et al.*,1993). It is considered the moisture content for maximum stability of food material as it does not participate in the reactions (Igbabul *et al.*, 2013,). Since the monolayer moisture is strongly bound, product with moisture content lower than the monolayer moisture content are expected to have higher stability based on chemical reactions that depend on solvation (Rao *et al.*, 2006). However lipid oxidation which leads to rancidity may be enhanced by the low moisture content.

The C_b and C_g constants from BET and GAB equations are associated with the net enthalpies of the sorption of the monolayer. They relate to the differences of chemical potentials between monolayer and the upper layers and determines the binding energy of water molecules to the primary binding sites on the product's surface (Muzaffar and Kumar, 2016). The higher the C values the stronger the bond between water molecules in the monolayer and binding site on the sorbent's surface (Baptestini,*et al.*, 2017,Valdez-Niebla *et al.*, 1993) In both models the binding energy C_b and C_g were seen to increase with temperature hence the values were highest at 35°C. This may be due to superficial hardening that occurs when the speed of surface evaporation exceeds the speed of moisture diffusion inside the product (Baptestini *et al.*, 2017, Potter and Hotchkiss, 1995), which results in alteration of the physical structure of the product, blocking their pores. Constant k_g from GAB model relates to multilayer sorption enthalpy (Mutungi *et al.*, 2011). As its value approaches 1, the characteristics of the water molecules become closer to that of pure water (Quirijins *et al.*, 2005, Malliaris, 2013). k_g was seen to have a direct relationship with the temperature. The estimated

coefficients and enthalpies associated with the GAB and BET model for ASCF are presented in Table 4.2.

Adsorption process releases heat, and the measure of the amount of released energy is isosteric heat of sorption. The variation of isosteric heat of sorption with equilibrium moisture content is shown in Figure 4.8. Net isosteric heat of sorption with moisture content was determined for data below the crossover point (>8.6g water /100g dry solid) where trend with temperature was as expected. As shown in Figure 4.8, the net isosteric heat of sorption varied from 16.1 to 2.6 kJ mol^{-1} in a moisture content range of 2 to 8 g water/100 g dry solid.

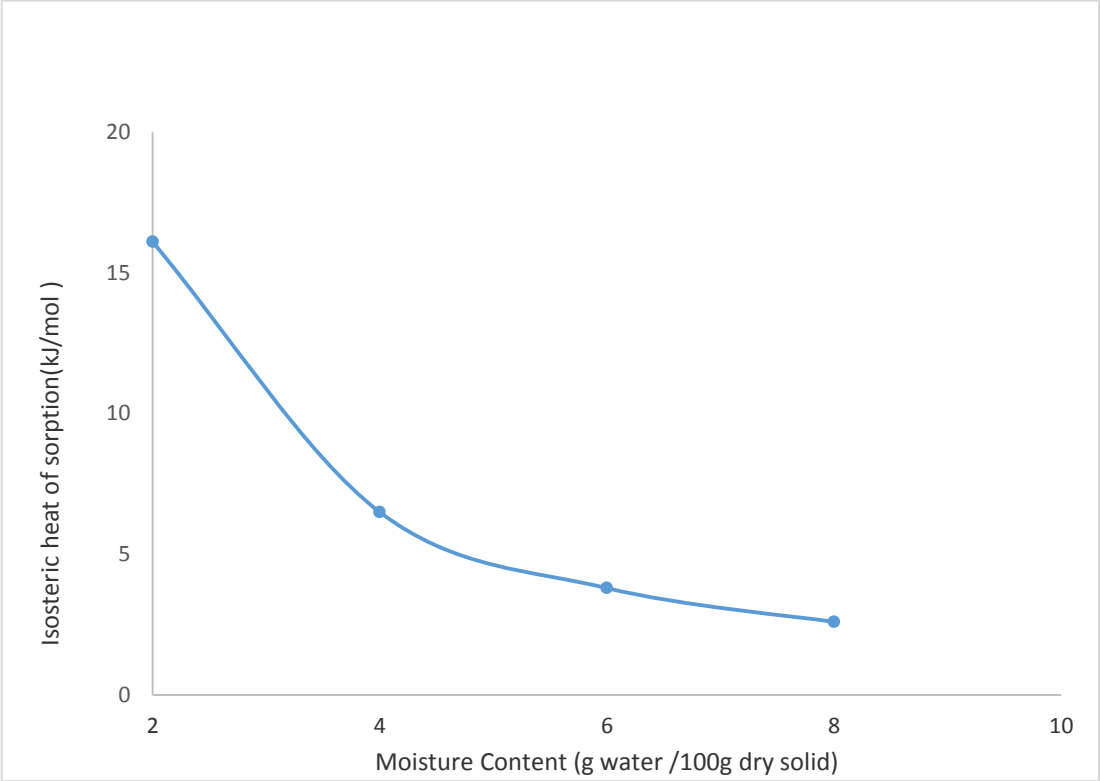

Figure 4.12: Relationship between Isosteric Heat of Sorption and Equilibrium Moisture Content of ASCF

The net isosteric heat of sorption of ASCF decreased rapidly below 4 g water/100 g dry solid (d.s.) as adsorption of water increased. The high energy value at low moisture content indicates high water binding energy of the first layer representative of the monolayer sorption. Above 4 g water/100 g dry solid moisture content, however, there was a change of trend where the net isosteric heat of sorption decreased at a decreasing rate. This is because as the active sites become occupied, sorption occurred on the sites with lower affinity hence the decrease in binding energy. The decline in isosteric heat with increase in moisture content also indicates weakening of interactions between water and solids leading to free water as it approaches zero (Toujani *et al.* 2011).

4.6 Water Vapour Permeability of the Packaging Material

There was an increase in weight in all the packaging materials with desiccants indicating that there was migration of water vapour from the environment into the package. The weight increase with time was linear in all the packaging (Figure 4.9). The rate of increase was highest in Kraft paper followed by kraft paper with polyethylene lining while aluminium pouch had the least. Water vapour transmission rate was obtained by applying equation 10 while package permeability was determined by applying equation 3.19. At 25°C and 35°C, WVTR for K was 0.0023 and 0.0027g/ day / m^2, 0.0016 and 0.0019 g day / m^2 /for KL and 0.0003 and0.00087 g /day/ m^2 for A.

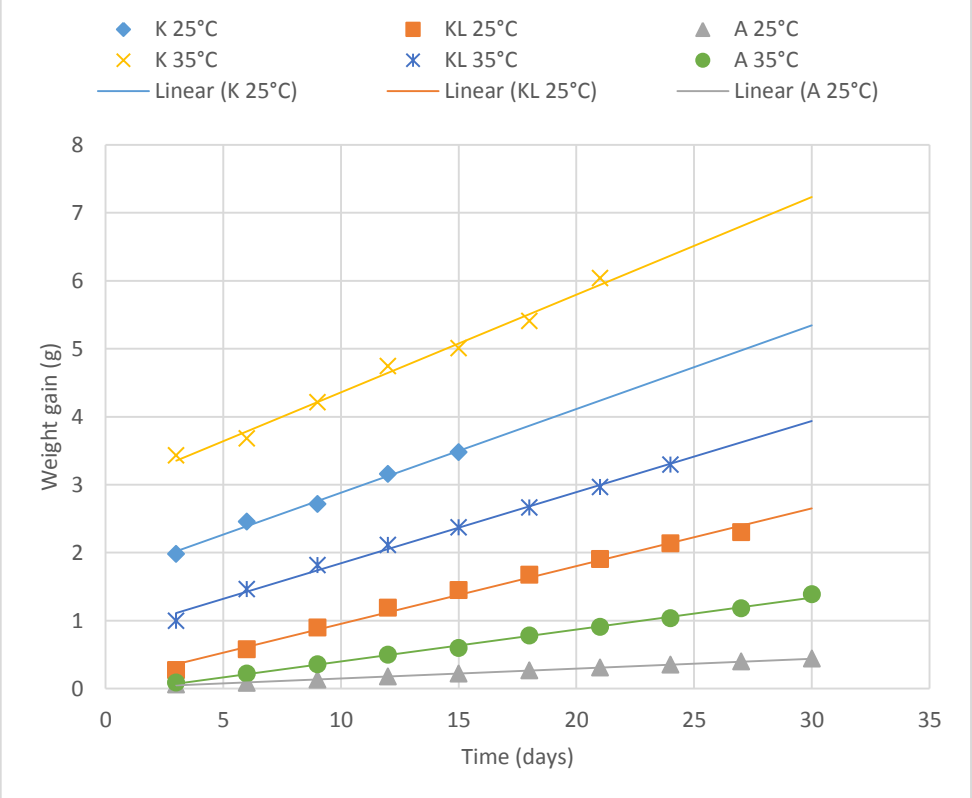

Figure 4.8: Increase in weight of packaged desiccant (g) with time (day)

Key: Kraft paper (K), Kraft paper with polypropylene lining (KL) and aluminium pouch (A) containing desiccants under constant temperature (25°C and 35 °C) and relative humidity (75%). Solid lines represent the best-fit equation for weight gain (g) versus time (d) by linear regression.

Weight was seen to increase with temperature in all the packaging and was in the order of K> KL >A. Gaikwad (2015), reported that temperature had an effect on molecules of the polymeric of packaging material causing more spaces to be generated hence increasing the permeation. Siracusa (2012), indicated that an increase in temperature at 50°C and 60°C increased permeability due to enhanced mobility of the polymer segments and increased energy level of the permeates. Increase in temperature had a greater effect on WVTR of A which increased by 220.5% from 25°C to 35°C, while KL

increased by 23.3% while K increased by 16.7%. The WVTR of packaging varies with packaging material, between film samples from the same and even manufacturers (Yaptenco, 2017). WVTR is key in food packaging as it is the standard measurement for determining the ability of packaging material to resist transmission of moisture (Gaikwad, 2015). Proper packaging should ensure that the attributes of the products are maintained. Improper packaging may result in products rapidly gaining or losing moisture until equilibration with the environmental relative humidity making crispy products to be soggy, and tender products hard and dry (Cooksey, 2004).

4.7 Shelf Life Prediction

Storage conditions influence the shelf-life stability of products especially high ambient temperature and relative humidity is a challenge for storage of dried products. Based on Heiss Eichner model, Heiss Eichner (1971) shelf life of ASCF packaged in K, KL, and A (thickness; 110, 80, 110µm respectively and 0.054 m^2 surface area each) was determined by applying equation 3.20 and the results are as shown in Figure 4.10.

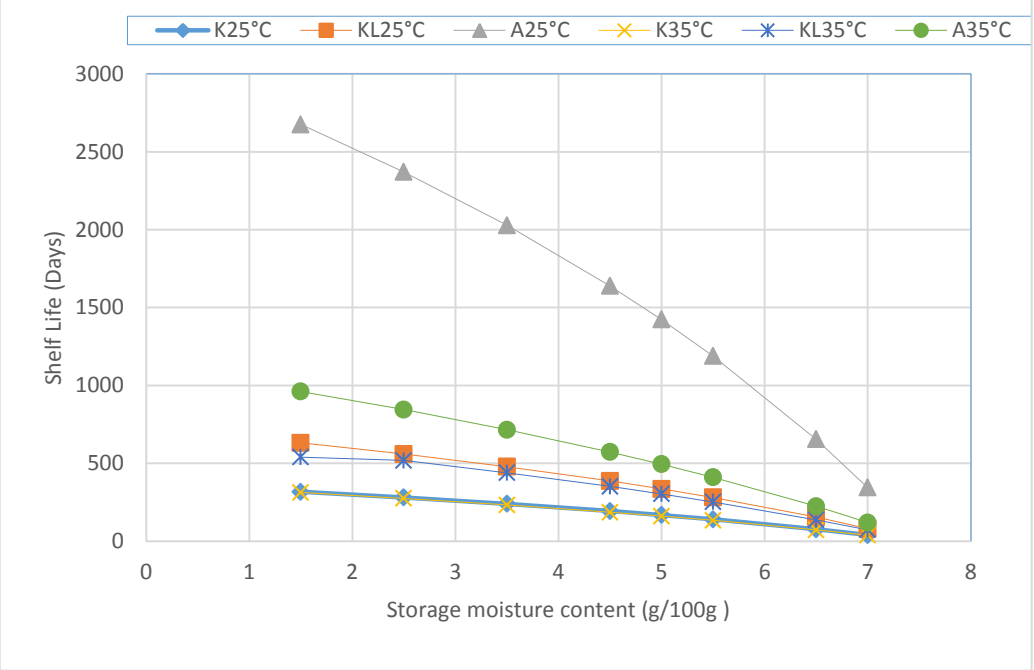

Figure 4.13: Estimated shelf life of ASCF packaged in K, KL and A stored at 25°C and 35°C and 75% RH

A 75% relative humidity (a_W 0.75) was used as the ambient storage relative humidity, representing the highest ambient air humidity that may be experienced at high temperatures in Kenya like coast. GAB model was used to determine the critical storage moisture content (Xc) of ASCF which was 0.075g water/g dry solids corresponding to the safe borderline a_W of 0.6 where microbiological stability is ensured. The model was used to predict the shelf life of ASCF in relation to possible packaging and storage conditions. As expected, the product shelf-life decreased with an increase in initial storage moisture content and storage temperature in all the packaging (Figure 4.10). Storage conditions influence the shelf-life stability of products especially high ambient temperature and relative humidity is a challenge for storage of dried products (Anandito *et al.,* 2017). Other researchers have also used the model in predicting various packaged product shelf life (Cooksey, 2004).

Storing at higher temperature was predicted to reduce shelf life due to increase transmission rate of water vapour. Irrespective of the temperature, A was predicted to give a longer shelf life due to its low vapour transmission rate. However, since A showed more sensitivity to temperature changes than K and KL, shelf life reduction was higher for A (65.2%) followed by KL (9.5%) while K was (4.1%) at 35°C storage compared to 25°C storage. A shelf life of 1991days is possible for ASCF with initial moisture content of 3.6g/100g, stored at 25° C and packaged with A. This estimation of the shelf life focused on the permeability of the package to water vapour.

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

a) Storage of ASCF at 35°C/75%RH gave the highest microbial count of 4.06 log cfu/g, highest increase in moisture content of 11.99% and highest PV of 2.3 meq O_2 /kg a sign of shorter shelf life in comparison with the other two storage conditions.

b) Aluminium package samples had the highest quality stability evidenced by low microbial count, low moisture content and low PV. It is the best packaging for longer shelf life of ASCF in comparison with the other two packaging materials tested.

c) The moisture adsorption isotherm of ASCF was sigmoidal an indication of monolayer multilayer mechanism.7.5% moisture content was established as the critical moisture content of ASCF corresponding to the safe borderline a_W of 0.6.

d) The product can keep for 1991 days at 25°C with product initial moisture content of 3.6 g/100 g and packaged in 110 µm thick aluminium pouch.

5.2 Recommendations

5.2.1 Recommendation from This Study;

a) ASCF be packaged in A pouch and stored at normal room temperature conditions.

b) Once opened at house hold level, ASCF should be stored in an airtight clean container at room temperature to avoid moisture absorption and contamination from the environment.

c) Concerned institutions should consider using ASCF to fight child under-nutrition as it is shelf-life stable besides being nutritionally rich.

5.2.2 Recommendation for Future Study

- Future work regarding ASCF should aim at doing the cost analysis of the packaging in relation to its effect on shelf life to harmonise its cost of production.

REFERENCES

Abeshu, M. A., Lelisa, A., and Geleta, B. (2016). Complementary Feeding: Review of Recommendations, Feeding Practices, and Adequacy of Homemade Complementary Food Preparations in Developing Countries–Lessons from Ethiopia. *Frontiers in nutrition*,

Abraham, S. I., Charles, A. C., Oneh, A. J., & Iorwuese, G. D. (2016). Moisture Adsorption and Thermodynamic Properties of Sorghum-Based Complementary Foods. *International Journal of Food Engineering and Technology*, *1*(1), 1.

Adebola, M. O., Dadi-Mamud, N. J., and Nusa Halima, M. K. (2014). The effects of packaging materials of shelf-life stability of garri bought from markets in Lapai Niger State Nigeria. *J. Appl. Environ. Microbiol*, *2*, 12-15.

Aguilera, J., del Valle, J., and Karel, M. (1995). Caking phenomena in amorphous food powders. *Trends in Food Science and Technology*, *6*(5), 149-155.

Akusu, O. M., and Kiin-Kabari, D. B. (2013). Effect of storage period on selected functional, chemical stability and sensory properties of bush mango (Irvingia gabonensis) seed flour. *Africa Journal of Food Science and Technology*, *4*(6), 136-140.

Alegbejo, J. O. (2013). Nutritional value and utilization of Amaranthus (Amaranthus spp.)–a review. *Bayero Journal of Pure and Applied Sciences*, *6*(1), 136-143.

Alimentarius, C. (1991). Guidelines on formulated supplementary foods for older infants and young children CAC/GL08-1991. Rome: Joint FAO/WHO Food Standards Programme Codex Alimentarius Commission.

Alimentarius, C. (2001). Codex standard for named vegetable oils, CODEX STAN 210-1999. *Codex Alimentarius*, *8*, 11-25.

Al-Muhtaseb, A. H., McMinn, W. A. M., & Magee, T. R. A. (2002). Moisture sorption isotherm characteristics of food products: a review. *Food and Bioproducts Processing*, *80*(2), 118-128.

Amarowicz, R. (2009). Sequalene: a natural antioxidant?. *European journal of lipid science and technology*, *111*(5), 411-412.

American Society for Testing and Materials. (2013). Standard test methods for water vapor transmission of materials. ASTM International.

AMPH. (1992). Compendium of methods for the microbiological examination of foods. Washington DC: American Public Health Association.

Anandito, R. B. K., Siswanti, L. P., & Sodiq, H. Shelf-life Determination of Fish Koya using Critical Moisture Content Approach. *B. Life and Environmental Sciences*, 201.206(2017)

Anderson RB (1946) Modifications of the Brunauer, Emmett and Teller equation1. J Am Chem Soc 68(4):686–691

Andrade, R. D., Lemus, R., & Perez, C. E. (2011). Models of sorption isotherms for food: uses and limitations. *Vitae*, *18*(3), 325-334.

AOAC, (2006). Official Methods of Analysis. 18th ed. (edited by W. Horwitz). Gaithersberg, USA: Association of Official Analytical Chemists.

AOAC, (2012). Official Methods of Analysis. 18th ed. (edited by W. Horwitz). Gaithersberg, USA: Association of Official Analytical Chemists.

AOCS, O. (1998). Methods and recommended practices of the American Oil Chemists' Society. *American Oil Chemists' Society, Champaign, IL, USA*.

Asma, M. A., El Fadil, E. B., and El Tinay, A. H. (2006). Development of weaning food from sorghum supplemented with legumes and oil seeds. Food and Nutrition Bulletin, 27(1), 26-34.

Balderrama, J. G. P., & Cadima, S. C. C. (2014). Water Adsorption Isotherms of Amaranth (Amaranthus caudatus) Flour. *Food and Nutrition Sciences*, *5*(02), 153.

Baptestini, F. M., Corrêa, P. C., Vanegas, J. D., Leite, R., Botelho, F. M., & Campos, R. C. (2017). Water sorption kinetics of damaged beans: GAB model. *Revista Brasileira de Engenharia Agrícola e Ambiental*, *21*(8), 550-555.

Barriuso, B., Astiasarán, I., and Ansorena, D. (2013). A review of analytical methods measuring lipid oxidation status in foods: a challenging task. *European food research and technology*, *236*(1), 1-15.

Bejosano, F. P., and Corke, H. (1998). Protein quality evaluation of Amaranthus wholemeal flours and protein concentrates. *Journal of the Science of Food and Agriculture*, *76*(1), 100-106.

Bell, L. (2002). Water Activity and Reaction Rates/Physical Properties/Shelf-Life. In *IFT Fundamentals of Water Activity Short Course presented at the 2002 Annual Conference of the Institute Food Technologists, Anaheim, CA*.

Belton, P. S., and Taylor, J. R. (2004). Sorghum and millets: protein sources for Africa. Trends in Food Science and Technology, 15(2), 94-98.

Bennaceur, S., Draoui, B., Touati, B., Benseddik, A., Saad, A., & Bennamoun, L. (2015). Determination of the moisture-sorption isotherms and isosteric heat of henna leaves. *Journal of Engineering Physics and Thermophysics*, *88*(1), 52-62.

Berghofer, L. K., Hocking, A. D., Miskelly, D., and Jansson, E. (2003). Microbiology of wheat and flour milling in Australia. *International journal of food microbiology*, *85*(1-2), 137-149.

Bhutta, Z. A., Ahmed, T., Black, R. E., Cousens, S., Dewey, K., Giugliani, E., & Shekar, M. (2008). What works? Interventions for maternal and child undernutrition and survival. *The lancet*, *371*(9610), 417-440.

Blossner, M., De Onis, M., & Prüss-Üstün, A. (2005). *Malnutrition: quantifying the health impact at national and local levels*. World Health Organization.

Braide, W., Oranusi, S. U., Udegbunam, L. I., Oguoma, O. I., Akobondu, C., and Nwaoguikpe, R. N. (2011). Mirobiological quality of an edible caterpillar of an emperor moth, Bunaea alcinoe. *Journal of Ecology and the Natural Environment*, *3*(5), 176-180.

Brett, B., Figueroa, M., Sandoval, A. J., Barreiro, J. A., & Müller, A. J. (2009). Moisture sorption characteristics of starchy products: oat flour and rice flour. *Food Biophysics*, *4*(3), 151-157.

Brunauer S, Deming LS, Teller E. On a theory of Van der Waals adsorption of gases. J Am Chem Soc. 1940 Jul; 62 (7): 1723-1732.

Brunauer S, Emmett PH, Teller E (1938) Adsorption of gases in multimolecular layers. J Am Chem Soc 60(2):309–319

Butt, M. S., Nasir, M., Akhtar, S., and Sharif, K. (2004). Effect of moisture and packaging on the shelf life of wheat flour. *Internet Journal of Food Safety*, *4*(1), 1-6.

Caurie M (1981) Derivation of full range moisture sorption isotherms. In: Water activity: influences on food quality: a treatise on the influence of bound and free water on the quality and stability of foods and other natural products, pp 63–87

Choe, E., and Min, D. B. (2005). Chemistry and reactions of reactive oxygen species in foods. *Journal of food science*, *70*(9), R142-R159.

Chuzel, G., and Zakhia, N. (1991). Adsorption isotherms of gari for estimation of packaged shelf- life. *International journal of food science and technology*, *26*(6), 583-593.

Coles, R., McDowell, D., and Kirwan, M. J. (Eds.). (2003). *Food packaging technology* (Vol. 5). CRC Press.

Cooksey, K. (2004). Important factors for selecting food packaging materials based on permeability. In *Flexible Packaging Conference* (pp. 1-12).

Dalpe, J. F., and Lloyd-George, I. (2012). *U.S. Patent Application No. 13/026,893*.

Demographic, K. (2015). Health Survey 2008–2009. 2010. Nairobi: Kenya Service Provision Assessment.

Desobry, S. (2000). Packaging/fatty food interactions. Oléagineux, Corps gras, Lipides, 7(5), 427-430.

Dewey, K. G. (2013). The challenge of meeting nutrient needs of infants and young children during the period of complementary feeding: an evolutionary perspective. *The Journal of nutrition*, *143*(12), 2050-2054.

Dicko, M. H., Gruppen, H., Traoré, A. S., Voragen, A. G., and Van Berkel, W. J. (2006). Sorghum grain as human food in Africa: relevance of content of starch and amylase activities. *African journal of biotechnology*, *5*(5), 384-395.

Djendoubi, N., Bonazzi, C., Boudhrioua, N., Kechaou, N., & Courtois, F. (2012). Influence of sugar composition on water sorption isotherms and on glass transition in apricots. *Journal of Food Engineering*, *111*(2), 403–411. https://doi.org/10.1016/j.jfoodeng.2012.02.001

Draskovic, N. (2010). Packaging Convenience: Consumer Packaging Feature Or Marketing Tool?. *International Journal of Management Cases*, *12*(2), 267-274.

East Africa Community (EAC) (2013). Processed cereal-based foods for infants and children – Specification

Egan, W. F. (1981). Frequency synthesis by phase lock. *wi*.

Eglezos, S. (2010). Microbiological quality of wheat grain and flour from two mills in Queensland, Australia. *Journal of food protection*, *73*(8), 1533-1536.

El-Hady, E. A., and Habiba, R. A. (2003). Effect of soaking and extrusion conditions on antinutrients and protein digestibility of legume seeds. LWT-Food Science and Technology, 36(3), 285-293.

Erdman, J. W., and Pneros-Schneier, A. G. (1994). Factors affecting nutritive value in processed foods. *Modern nutrition in health and disease. Philadelphia, PA, USA: Lea and Febiger*, 1569-78.

Ertop, M. H., & Bektaş, M. (2018). Enhancement of bioavailable micronutrients and reduction of antinutrients in foods with some processes. *Food and Health*, *4*(3), 159-165.

Flick Jr, G. J., Hong, G. P., and Knobl, G. M. (1992). Lipid oxidation of seafood during storage.

Food Fats and Oils. (2006). By the Institute of Shortening and Edible Oils, Inc. 1750 New York Avenue, NW, Washington, DC 20006,http://www.iseo.org/foodfats.htm.

Frankel, E. N. (1980). Lipid oxidation. *Progress in lipid research*, *19*(1-2), 1-22.

Gaikwad KK (2015) Water vapour permeability of packaging materials (LDPE) and fabricated package systems. MS: Packaging, School of Packaging Michigan State University, East Lansing

Gamel, T. H., Mesallam, A. S., Damir, A. A., Shekib, L. A., and Linssen, J. P. (2007). Characterization of amaranth seed oils. *Journal of food lipids*, *14*(3), 323-334.

Gernah, D. I., Ariahu, C. C., Ingbain, E. K., and Sengev, A. I. (2011). Storage and Shelflife evaluation of food formulations from malted and fermented maize (zea mays L.) fortified with defatted sesame (Sesamun indicum L.) Niger. *J. Nutr. Sci*, *32*(1), 45-54.

Gibson, R. S., Ferguson, E. L., and Lehrfeld, J. (1998). Complementary foods for infant feeding in developing countries: their nutrient adequacy and improvement. *European Journal of Clinical Nutrition*, *52*(10), 764.

Gikonyo, N. K., Mburu, M. W., Glaston, M. K., and Mwasaru, A. M. (2011). Properties of a complementary food based on amaranth grain (Amaranthus cruentus) grown in Kenya.

Godwin, I. D., and Gray, S. J. (2000). Overcoming productivity and quality constraints in sorghum: the role for genetic engineering. *Transgenic cereals*, 153-177.

Granum, P. E., and Lund, T. (1997). Bacillus cereus and its food poisoning toxins. *FEMS microbiology letters*, *157*(2), 223-228.

Greenspan, N. (1977). Humidity fixed points of binary saturated aqueous solutions. *Journal of Research of the National Bureau of Standards-A. Physics and Chemistry*, *81*, 89-96.

Guillén, M. D., & Cabo, N. (2002). Fourier transform infrared spectra data versus peroxide and anisidine values to determine oxidative stability of edible oils. *Food Chemistry*, *77*(4), 503-510.

Gupta, V. K., and Thimba, D. (1992). Grain amaranth: a promising crop for marginal areas of Kenya. *Food Reviews International*, *8*(1), 51-69.

Hartmann, M., and Palzer, S. (2011). Caking of amorphous powders—Material aspects, modelling and applications. *Powder Technology*, *206*(1-2), 112-121.

Haug, A., Christophersen, O. A., Kinabo, J., Kaunda, W., & Eik, L. O. (2010). Use of dried Kapenta (Limnothrissa miodon and Stolothrissa tanganicae) and other products based on whole fish for complementing maize-based diets. *African Journal of Food, Agriculture, Nutrition and Development*, *10*(5).

Heiss R, Eichner E (1971) Moisture content and shelf-life. Part 2. Food Manuf 46:37–42

Heiss, R., & Eichner, E. (1971). Moisture content and shelf-life. Part 2. *Food Manufacture*, *46*, 37-42.

Hotchkiss, J. H. (1997). Food- packaging interactions influencing quality and safety. *Food Additives and Contaminants*, *14*(6-7), 601-607.

Hotz, C., and Gibson, R. S. (2007). Traditional food-processing and preparation practices to enhance the bioavailability of micronutrients in plant-based diets. The Journal of nutrition, 137(4), 1097-1100.

Igbabul, B. D., Ariahu, C. C., & Umeh, E. U. (2013). Moisture Adsorption Isotherms of African Arrowroot Lily (Tacca involucrata) Tuber Mash as Influenced by Blanching and Natural Fermentation. *Journal of Food Research*, *2*(3), 79–92. http://doi.org/10.5539/jfr.v2n3p79

Indiramma, A. R. (2008). Packaging Aspects of Wheat, Rice, Milled Products and Sugar. CFTRI, Mysore-570, 20.

Inyang, C. U., Tsav-Wua, J. A., and Akpapunam, M. A. (2006). Impact of traditional processing methods on some physico chemical and sensory qualities of fermented casava flour" Kpor umilin". *African Journal of Biotechnology*, *5*(20).

Iqbal, T., & Fitzpatrick, J. J. (2006). Effect of storage conditions on the wall friction characteristics of three food powders. *Journal of Food Engineering*, *72*(3), 273-280.

Jacques, R., and Ekhard, E. Z. (2006). Protein And Energy Requirements in Infancy And Childhood: Nestle Nutrition Workshop Series: Pediatric Program.

Janjatović, D., Benković, M., Srečec, S., Ježek, D., Špoljarić, I., & Bauman, I. (2012). Assessment of powder flow characteristics in incoherent soup concentrates. *Advanced Powder Technology*, *23*(5), 620-631.

Jessup, R. S. (1960). *Precise Measurement of Heat Of Combustion with a Bomb Calorimeter*. National Bureau of Standards Boulder Co.

Jha, E., Dahiya, V. S., & Singh, A. K. (2014). Moisture sorption characteristics of gluten-free flour. *International Journal of Agriculture and Food Science Technology*, *5*(2), 27-34.

Kaleem, A., Aziz, S., and Iqtedar, M. (2015). Investigating changes and effect of peroxide values in cooking oils subject to light and heat. *FUUAST Journal of Biology*, *5*(2), 191-196.

Kamau, E., Mutungi, C., Kinyuru, J., Imathiu, S., Tanga, C., Affognon, H., ... & Fiaboe, K. K. M. (2018). Moisture adsorption properties and shelf-life estimation of dried and pulverised edible house cricket Acheta domesticus (L.) and black soldier fly larvae Hermetia illucens (L.). *Food Research International*, *106*, 420-427.

Kanensi, O. J., Ochola, S., Gikonyo, N. K., & Makokha, A. (2011). Optimization of the period of steeping and germination for amaranth grain. *Journal of Agricultural and Food Technology*, *1*, 101-105.

Kariuki, S., Sila, D., & Kenji, G. (2013). Nutritional profile of Amaranth grain varieties grown in Kenya. *Journal of Agriculture and Food Technology*, *17*, 19-25.

Katiyar, R. S., Sudhir, S., and Sanjay, R. (2000). Varietal performance of grain amaranths (A. hypochondriacus) on sodic soil. *Proceedings of the National Academy of Sciences India. Section B, Biological Sciences*, *70*(2), 185-187.

Kauffman, C. S., & Weber, L. E. (1990). Grain amaranth. *Advances in new crops*, 127-139.

Kenya Bureau of Standards (KEBS) (2013). Formulated complementary foods for older infants and young children – Guidelines

Kenya Demographic and Health Survey K. (2014). Health Survey (2014) KDHS.

Kenya National Micronutrient Survey 2011. Ministry of Health

Kilcast, D. (2001). Shelf- life Evaluation of Foods Edited by CMD Man and Adrian A. Jones Gaithersberg, USA: Aspen Publishers, 2000. Pp. xv+ 272. ISBN 0- 83421- 782- 1. *International Journal of Food Science and Technology*, *36*(8), 856-856.

Kimura, A. C., Palumbo, M. S., Meyers, H., Abbott, S., Rodriguez, R., and Werner, S. B. (2005). A multi-state outbreak of Salmonella serotype Thompson infection from commercially distributed bread contaminated by an ill food handler. *Epidemiology and Infection*, *133*(5), 823-828.

Klunder, H. C., Wolkers-Rooijackers, J., Korpela, J. M., & Nout, M. J. R. (2012). Microbiological aspects of processing and storage of edible insects. *Food control*, *26*(2), 628-631.

Koletzko, B., Baker, S., Cleghorn, G., Neto, U. F., Gopalan, S., Hernell, O., and Pzyrembel, H. (2005). Global standard for the composition of infant formula: recommendations of an ESPGHAN coordinated international expert group. *Journal of pediatric gastroenterology and nutrition*, *41*(5), 584-599.

Konyole, S. O. (2014). *Effect of improved complementary foods on growth and iron status of Kenyan infants* (Doctoral dissertation, University of Nairobi).

Kuhn I (1967) A generalized potential theory of adsorption: I. The derivation of a general equation for adsorption isotherms. J Colloid Interface Sci 23(4):563–571

Kuong, K., Laillou, A., Chea, C., Chamnan, C., Berger, J., & Wieringa, F. T. (2016). Stability of Vitamin A, iron and zinc in fortified rice during storage and its impact on future national standards and programs—Case study in Cambodia. *Nutrients*, *8*(1), 51.

Labrinea, E. P., Thomaidis, N. S., and Georgiou, C. A. (2001). Direct olive oil anisidine value determination by flow injection. *Analytica Chimica Acta*, *448*(1-2), 201-206.

Labuza, T. P., & Altunakar, B. (2007). Water activity prediction and moisture sorption isotherms. *Water activity in foods: Fundamentals and applications*, *1*, 109-154.

Labuza, T. P., Kaanane, A., & Chen, J. Y. (1985). Effect of temperature on the moisture sorption isotherms and water activity shift of two dehydrated foods. *Journal of Food science*, *50*(2), 385-392.

Lalude, L. O., & Fashakin, J. B. (2006). Development and nutritional assessment of a weaning food from sorghum and oil-seeds. *Pakistan Journal of Nutrition*.

Lemus M, R. (2011). Models of sorption isotherms for food: uses and limitations. *Vitae*, *18*(3), 325-334.

Macharia-Mutie, C. W. (2012). Efficacy of amaranth grain (Amaranthus cruentus) on anaemia and iron deficiency in Kenyan pre-school children.

Malliaris, A. (2013). 001 - Effect of temperature on the water Adsorption isotherms of sultamna raisins - end page missing Effect of Temperature on the Water Adsorption of Sultana Raisins, (May 2014).

Manthey, F. A., Wolf-Hall, C. E., Yalla, S., Vijayakumar, C., and Carlson, D. (2004). Microbial loads, mycotoxins, and quality of durum wheat from the 2001 harvest of the northern plains region of the United States. *Journal of food protection*, *67*(4), 772-780.

Mburu, M. W., Gikonyo, N. K., Kenji, G. M., & Mwasaru, A. M. (2011). Properties of a complementary food based on amaranth grain (Amaranthus cruentus) grown in Kenya. *Journal of Agriculture and Food Technology*, *1*(9), 153-178.]

Michaelsen, K. F., Hoppe, C., Roos, N., Kaestel, P., Stougaard, M., Lauritzen, L., & Friis, H. (2009). Choice of foods and ingredients for moderately malnourished children 6 months to 5 years of age. *Food and nutrition bulletin*, *30*(3_suppl3), S343-S404.

Mills, C. (2011). The raw truth about consumer eating habits. *Published online at www. conagramills. com/media/Food% 20Habits-% 20of% 20American% 20Consumers% 20Final. pdf. ConAgra Mills, Omaha, NE*.

Min, D. B., and Boff, J. M. (2002). Chemistry and reaction of singlet oxygen in foods. *Comprehensive reviews in food science and food safety*, *1*(2), 58-72.

Mlakar, S. G., Turinek, M., Jakop, M., Bavec, M., & Bavec, F. (2009). Nutrition value and use of grain amaranth: potential future application in bread making. *Agricultura*, *6*(4), 1.

MOH, K. (2012). National Nutrition Action Plan 2012–2017.

Moigradean, D., Poiana, M. A., and Gogoasa, I. (2012). Quality characteristics and oxidative stability of coconut oil during storage. *Journal of Agroalimentary Processes and Technologies*, *18*(4), 272-276.

Mossel, D. A., and Van, P. N. (1991). Microbiological reference values for foods: a European perspective. *Journal-Association of Official Analytical Chemists*, *74*(2), 420-432.

Mridula, D., Jain, R., & Singh, K. K. (2010). Effect of storage on quality of fortified Bengal gram sattu. *Journal of food science and technology*, *47*(1), 119-123.

Mujuru, F. M., Kwiri, R., Nyambi, C., Winini, C., & Moyo, D. N. (2014). Microbiological quality of Gonimbrasia belina processed under different traditional practices in Gwanda, Zimbabwe.

Mutungi, C., Schuldt, S., Onyango, C., Schneider, Y., Jaros, D., & Rohm, H. (2011). Dynamic moisture sorption characteristics of enzyme-resistant recrystallized cassava starch. *Biomacromolecules*, *12*, 660-671.

Muzaffar, K., & Kumar, P. (2016). Moisture sorption isotherms and storage study of spray dried tamarind pulp powder. *Powder Technology*, *291*, 322–327.

Ogbo, Felix Akpojene, Hillary Nguyen, Sabrina Naz, Kingsley E. Agho, and Andrew Page. "The association between infant and young child feeding practices and diarrhoea in Tanzanian children." *Tropical medicine and health* 46, no. 1 (2018): 2.

Ogiehor, I. S., and Ikenebomeh, M. J. (2006). The effects of different packaging materials on the shelf stability of garri. *African Journal of Biotechnology*, *5*(23).

Ojha, P., Adhikari, R., Karki, R., Mishra, A., Subedi, U., & Karki, T. B. (2018). Malting and fermentation effects on antinutritional components and functional characteristics of sorghum flour. *Food science & nutrition*, *6*(1), 47-53.

Okoth, J. K., Ochola, S. A., Gikonyo, N. K., & Makokha, A. (2017). Development of a nutrient- dense complementary food using amaranth- sorghum grains. *Food science & nutrition*, *5*(1), 86-93.

Olsmats, C., and Wallteg, B. (2009). Packaging is the answer to world hunger. (World Packaging Organization (WPO) and International Packaging Press Organization (IPPO)).

Opara, U. L. (2013). A review on the role of packaging in securing food system: Adding value to food products and reducing losses and waste. *African Journal of Agricultural Research*, *8*(22), 2621-2630.

Oswin, C. R. (1946). The kinetics of package life. III. The isotherm. *Journal of the Society of Chemical Industry*, *65*(12), 419-421.

Owino, V. O., Sinkala, M., Amadi, B., Tomkins, A. M., & Filteau, S. M. (2007). Acceptability, storage stability and costing of α- amylase- treated maize–beans–groundnuts–bambaranuts complementary blend. *Journal of the Science of Food and Agriculture*, *87*(6), 1021-1029.

Padmashree, A., Sharma, G. K., and Govindaraj, T. (2013). Development and evaluation of shelf stability of flaxoat nutty bar in different packaging materials. *Food and Nutrition Sciences*, *4*(05), 538.

Pal, M., & Khoshoo, T. N. (1974). Grain amaranths. *Evolutionary studies in world crops: diversity and change in the Indian subcontinent. Cambridge University Press, UK*, 129-137.

Paredes- López, O., Barba De La Rosa, A. P., and Cárabez- Trejo, A. (1990). Enzymatic production of high- protein amaranth flour and carbohydrate rich fraction. *Journal of Food Science*, *55*(4), 1157-1161.

Pedersen, B., Kalinowski, L. S., & Eggum, B. O. (1987). The nutritive value of amaranth grain (Amaranthus caudatus). *Plant foods for human nutrition*, *36*(4), 309-324.

Písaříková, B., Kráčmar, S., & Herzig, I. (2005). Amino acid contents and biological value of protein in various amaranth species. *Czech Journal of Animal Science*, *50*(4), 169-174.

Potter, N. N., & Hotchkiss, J. H. (1995). Irradiation, microwave, and ohmic processing of foods. In *Food Science* (pp. 245-263). Springer, Boston, MA.

Prakash, D., Joshi, B. D., and Pal, M. (1995). Vitamin C in leaves and seed oil composition of the Amaranthus species. *International journal of food sciences and nutrition*, *46*(1), 47-51.

Proietti, I., Frazzoli, C., and Mantovani, A. (2015, March). Exploiting Nutritional Value of Staple Foods in the World's Semi-Arid Areas: Risks, Benefits, Challenges and Opportunities of Sorghum. In Healthcare (Vol. 3, No. 2, pp. 172-193). Multidisciplinary Digital Publishing Institute.

Qian, J., and Kuhn, M. (1999). Characterization of Amaranthus cruentus and Chenopodium quinoa starch. *Starch-Stärke*, *51*(4), 116-120.

Quirijns, E. J., Van Boxtel, A. J., van Loon, W. K., & Van Straten, G. (2005). Sorption isotherms, GAB parameters and isosteric heat of sorption. *Journal of the Science of Food and Agriculture*, *85*(11), 1805-1814.

Rao, G. P. C., Satyaveni, S., Ramesh, A., Seshaiah, K., Murthy, K. S. N., & Choudary, N. V. (2006). Sorption of cadmium and zinc from aqueous solutions by zeolite 4A, zeolite 13X and bentonite. *Journal of environmental management*, *81*(3), 265-272.

Rastogi, A., & Shukla, S. (2013). Amaranth: a new millennium crop of nutraceutical values. *Critical reviews in food science and nutrition*, *53*(2), 109-125.

Resio, A. C., Aguerre, R. J., and Suarez, C. (1999). Analysis of the sorptional characteristics of amaranth starch. *Journal of food engineering*, *42*(1), 51-57.

Robertson, G. L. (2009). *Food packaging and shelf life: a practical guide*. CRC Press.

Robinson, D. S., & Eskin, M. (2000). *Food shelf life stability: chemical, biochemical, and microbiological changes*. CRC Press.

Sabillón Galeas, L. E. (2014). Understanding the factors affecting microbiological quality of wheat milled products: from wheat fields to milling operations.

Sandoval, A. J., Barreiro, J. A., & Müller, A. J. (2011). Determination of moisture adsorption isotherms of rice flour using a dynamic vapor sorption technique. *Interciencia*, *36*(11), 848-852.

Santiago, D. P., Lauro, C.P.A., Torres. T.J., Rodriguez.T.R., Romero. G.D.J., and Martín Del Campo S.T. (2012). Accelerated Shelf Life Tests of Amaranth Oil (Amaranthus hypochondriacus). Conference: IFT12 at: Las Vegas, USA

Sauer, J. D. (1967). The grain amaranths and their relatives: a revised taxonomic and geographic survey. *Annals of the Missouri Botanical Garden*, *54*(2), 103-137.

Schipmann-Schwarze, C., Orr, A., Mulinge, W., and Mafuru, J. (2015). Sorghum and finger millet flour processing in Tanzania, Kenya, and Uganda, Socioeconomics Discussion Paper series 32.

Schmid-Hempel, P., and Frank, S. A. (2007). Pathogenesis, virulence, and infective dose. *PLoS pathogens*, *3*(10), e147.

Schnetzler, K. A., & Breene, W. M. (1994). *Food uses and amaranth product research: a comprehensive review* (pp. 155-184). CRC Press: Boca Raton, FL.

Severi, S., Bedogni, G., Manzieri, A. M., Poli, M., & Battistini, N. (1997). Effects of cooking and storage methods on the micronutrient content of foods. *European Journal of Cancer Prevention*, *6*, S21-S24.

Sewald, M., & DeVries, J. (2003). Food product shelf life. *Medallion Laboratories Analytical Progress*, 1-10.

Shastry, M., and John, E. (1991). Biochemical changes and in- vitro protein digestibility of the endosperm of germinating dolichos lablab. Journal of the Science of Food and Agriculture, 55(4), 529-538.

Silvagni, A., Franco, L., Bagno, A., and Rastrelli, F. (2010). Thermoinduced lipid oxidation of a culinary oil: a kinetic study of the oxidation products by magnetic resonance spectroscopies. *The Journal of Physical Chemistry A*, *114*(37), 10059-10065.

Singh, T. K., & Cadwallader, K. R. (2004). Ways of measuring shelf-life and spoilage. *Understanding and Measuring the Shelf-life of Food*, 165-183.

Siracusa, V. (2012). Food packaging permeability behaviour: A report. *International Journal of Polymer Science*, *2012*.

Smith SE (1947) The sorption of water vapor by high polymers. J Am Chem Soc 69(3):646–651

Standard, A. S. T. M. (2000). E96-00. *Standard test methods for water vapour transmission of materials. Annual book of ASTM standards*, *4*.

Stark, C., McNeil, D. L., and Savage, G. P. (2000). The effect of storage conditions on the stability of peroxide values of New Zealand grown walnuts. In *Proceedings of the Nutrition Society of New Zealand* (Vol. 25, pp. 43-54).

Sustainet, G. T. Z. (2006). Sustainable agriculture: A pathway out of poverty for East Africa's rural poor: Examples from Kenya and Tanzania. *Deutsche Gesellschaft für Technische Zusammenarbeit, Eschborn*.

Teutonico, R. A., and Knorr, D. (1985). Amaranth: Composition, properties, and applications of a rediscovered food crop. *Food technology (USA)*.

Tikekar, R. V., Ludescher, R. D., and Karwe, M. V. (2008). Processing stability of sequalene in amaranth and antioxidant potential of amaranth extract. *Journal of agricultural and food chemistry*, *56*(22), 10675-10678.

Timmermann, E. O., Chirife, J., & Iglesias, H. A. (2001). Water sorption isotherms of foods and foodstuffs : BET or GAB parameters ?, *48*, 19–31.

Tontisirin, K., Nantel, G., and Bhattacharjee, L. (2002). Food-based strategies to meet the challenges of micronutrient malnutrition in the developing world. Proceedings of the Nutrition Society, 61(2), 243-250.

Toujani, M., Hassini, L., Azzouz, S., & Belghith, A. (2011). Drying characteristics and sorption isotherms of silverside fish (Atherina). *International journal of food science & technology*, *46*(3), 594-600.

Uchechukwu-Agua, A. D. (2015). Effects of packaging and storage condition on functional properties and quality attributes of cassava flour (CVS.'TME 419'AND 'UMUCASS 36') (Doctoral dissertation, Stellenbosch: Stellenbosch University, 2015.).

Unicef. (2013). Improving child nutrition: the achievable imperative for global progress. *New York: UNICEF*, 1-114.

Unicef. (2018). *Levels and trends in child malnutrition*. eSocialSciences.

Valdez-Niebla, J. A., Paredes-Lopez, O., Vargas-Lopez, J. M., & Hernandez-Lopez, D. (1993). Moisture sorption isotherms and other physicochemical properties of nixtamalized amaranth flour. *Food Chemistry*, *46*(1), 19-23.

Venskutonis, P. R., and Kraujalis, P. (2013). Nutritional components of amaranth seeds and vegetables: a review on composition, properties, and uses. *Comprehensive Reviews in Food Science and Food Safety*, *12*(4), 381-412.

WHO. Complementary feeding of young children in developing countries: a review of current scientific knowledge. WHO/ NUT/98.1, Geneva, Switzerland. 2003.

World Health Organization, and Unicef. (2009). WHO child growth standards and the identification of severe acute malnutrition in infants and children: joint statement by the World Health Organization and the United Nations Children's Fund.

World Health Organization, and United Nations University. (2007). *Protein and amino acid requirements in human nutrition* (Vol. 935). World Health Organization.

World Health Organization. "Diarrhoea: why children are still dying and what can be done." (2009).

Yadav, S. K., and Sehgal, S. (2002). Effect of domestic processing and cooking methods on total, HCl extractable iron and in vitro availability of iron in spinach and amaranth leaves. *Nutrition and health*, *16*(2), 113-120.

Yaptenco, K. F. (2017). Moisture sorption isotherms and mathematical model selection for dried sandfish (Holothuria scabra). *Agricultural Engineering International: CIGR Journal*, *19*(2), 176-186.

Yeum, K. J., and Russell, R. M. (2002). Carotenoid bioavailability and bioconversion. *Annual review of nutrition*, *22*(1), 483-504.

You, D., L. Hug, S. Ejdemyr, P. Idele, D. Hogan, C. Mathers, P. Gerland, J. R. New, and L. Alkema. "United Nations Inter-agency Group for Child Mortality Estimation (UN IGME). Global, regional, and national levels and trends in under-5 mortality

between 1990 and 2015, with scenario-based projections to 2030: a systematic analysis by the UN Inter-agency Group for Child Mortality Estimation." *Lancet* 386, no. 10010 (2015): 2275-2286.

Zafar, U., Vivacqua, V., Calvert, G., Ghadiri, M., and Cleaver, J. S. (2017). A review of bulk powder caking. *Powder Technology*, *313*, 389-401.

Zebdewos, A., Singh, P., Birhanu, G., Whiting, S. J., Henry, C. J., and Kebebu, A. (2015). Formulation of complementary food using amaranth, chickpea and maize improves iron, calcium and zinc content. *African Journal of Food, Agriculture, Nutrition and Development*, *15*(4), 10290-10304.

APPENDICES

Appendix I: Calculation of Isosteric Heat Of Sorption for ASCF

	35c			e Quadratic for determining isosteric heat o					Plus	subtract			
c		a	b						±	±		Aw	Aw
MC%	**186.094aw**	**_191.46Aw2**	**_555.89Aw**	_b	**b Squared**	**4ac**	**b2-4ac**	**SQRT**	**plus**	**subtract**	**2a**	**plus**	**subtract**
2	372.188	-382.92	-183.702	183.702	33746.42	-3063.36	36809.785	191.859	375.561	-191.86	-765.84	-0.4904	0.25052
3	558.282	-574.38	2.392	-2.392	5.721664	-6892.56	6898.2817	83.0559	80.6639	-83.056	-1148.8	-0.0702	0.0723
4	744.376	-765.84	188.486	-188.49	35526.97	-12253.4	47780.412	218.587	30.1013	-218.59	-1531.7	-0.0197	0.14271
5	930.47	-957.3	374.58	-374.58	140310.2	-19146	159456.18	399.32	24.7396	-399.32	-1914.6	-0.0129	0.20857
6	1116.564	-1148.76	560.674	-560.67	314355.3	-27570.2	341925.57	584.744	24.07	-584.74	-2297.5	-0.0105	0.25451
7	1302.658	-1340.22	746.768	-746.77	557662.4	-37526.2	595188.61	771.485	24.7167	-771.48	-2680.4	-0.0092	0.28782
8	1488.752	-1531.68	932.862	-932.86	870231.5	-49013.8	919245.27	958.773	25.9108	-958.77	-3063.4	-0.0085	0.31298
10	1860.94	-1914.6	1305.05	-1305.1	1703156	-76584	1779739.5	1334.07	29.0188	-1334.1	-3829.2	-0.0076	0.34839
14	2605.316	-2680.44	2049.426	-2049.4	4200147	-150105	4350251.6	2085.73	36.2997	-2085.7	-5360.9	-0.0068	0.38906
18	3349.692	-3446.28	2793.802	-2793.8	7805330	-248132	8053461.8	2837.86	44.0602	-2837.9	-6892.6	-0.0064	0.41173
20	3721.88	-3829.2	3165.99	-3166	10023493	-306336	10329829	3214.01	48.0151	-3214	-7658.4	-0.0063	0.41967
24	4466.256	-4595.04	3910.366	-3910.4	15290962	-441124	15732086	3966.37	56.0034	-3966.4	-9190.1	-0.0061	0.43159
30	5582.82	-5743.8	5026.93	-5026.9	25270025	-689256	25959281	5095.03	68.0951	-5095	-11488	-0.0059	0.44352

Appendix II: Fitting of Experimental Moisture Adsorption Data on GAB Model

GAB Equilibrium moisture content prediction at 35°C										GAB Equilibrium moisture content prediction at 25°C								
Salt Aw	Meq	Aw	Aw/Meq	Mo*Cg*K	(1-KgAw)(1+(Cg-1)		GAB Meq	RMSE		Salt Aw	Meq	Aw	Aw/Meq	Mo*Cg*K	(1-KgAw)(1+(Cg-1)		GAB Meq	
0	0	0	0	0	1		0			0	0	0	0	0	1		0	
0.11	2.89428	0.11	0.03801	57.8947	18.5707		3.11753	0.00595		0	0	0	0	0	1		0	
0.22	3.76967	0.22	0.05836	115.789	31.6249		3.66134	0.00083		0.11	2.79307	0.11	0.03938	24.4445	7.58297		3.2236	0.02376
0.43	4.98043	0.43	0.08634	226.316	44.0049		5.14297	0.00107		0.23	3.8241	0.23	0.06014	51.1112	12.9692		3.94098	0.00093
0.5	5.65667	0.5	0.08839	263.158	44.4736		5.91717	0.00212		0.43	5.48667	0.43	0.07837	95.5557	17.7833		5.37334	0.00043
0.67	10.7904	0.67	0.06209	352.631	37.9973		9.28043	0.01958		0.53	6.31397	0.53	0.08394	117.778	18.239		6.45747	0.00052
0.83	17.8858	0.83	0.04641	436.842	22.0478		19.8134	0.01161		0.69	10.3581	0.69	0.06661	153.334	16.2623		9.42876	0.00805
0.97	31.8638	0.97	0.03044	510.526	0.25352		2013.75	0.00686		0.84	15.2378	0.84	0.05513	186.667	11.3846		16.3964	0.00578
								0.08282		0.97	23.34	0.97	0.04156	215.556	4.78964		45.0046	0.00658
0.11731	-2.04727	-0.0056						8.28244	%RMSE									0.08111
-0.0027	1.98871	-177.11		**Mo= 2.88674 (monolayer moisture content)**														8.11055
0.12	-1.02945			**Kg= 1.02945 (monolayer sorption energy constant)**							0.0909	-1.8877	-0.0139	**Mo=3.22462 (monolayer moisture content)**				
8.33325				**Cg=177.106 (multilayer sorption energy constant)**							-0.00527	1.97222	-72	**Kg=0.95714 (monolayer sorption energy constar**				
2.88674											0.09617	-0.9571		**cg=72.0003 (multilayer sorption energy constant**				
											10.3982							
											3.22462							

Appendix III: Water Vapour permeability of K,KL and A at 25C 75%RH and 25C 75%RH

Weight Increase of Dessicant Packaged in K,KL andStored at 25C 75% RH															
Package	EMPTY	INITIAL	DAY 1	day 2	day 3	day 4	day 7	day 8	9 days	10 days	14th	17th	24th	31DAY	32nd
K															
1	14.9756	66.4647	68.3479	68.7418	68.9466	69.1842	69.7208	69.7128	69.7309	69.7558	69.8431	69.8985	70.0598	70.1348	70.2759
2	14.9834	66.7319	68.3355	68.8209	69.0267	69.1602	69.433	69.5878	69.683	69.765	69.9243	69.9753	70.1013	70.1518	70.1481
3	15.0302	66.1859	67.9425	68.298	68.474	68.6051	68.8252	69.0274	69.12	69.1596	69.2578	69.3019	69.5159	69.5158	69.504
KL															
1	7.7641	57.8614	57.9086	57.971	58.0384	58.1774	58.3842	58.449	58.5264	58.6113	58.8772	59.0216	59.4359	59.7334	59.7824
2	7.8299	57.9186	57.9674	58.0297	58.0848	58.1289	58.4565	58.5256	58.6018	58.6774	58.9383	59.1026	59.5506	59.8864	59.9237
3	7.7427	58.0151	58.0796	58.1437	58.2307	58.3087	58.5914	58.6742	58.7584	58.8362	59.1104	59.2638	59.7383	60.0643	60.1067
A															
1	7.5142	58.6099	58.625	58.6315	58.6448	58.6542	58.6956	58.7085	58.7252	58.7371	58.812	58.8598	58.9617	59.0805	59.0993
2	7.4773	58.4063	58.4164	58.4268	58.4356	58.4498	58.4868	58.4966	58.5079	58.5178	58.5664	58.5933	58.6646	58.7428	58.7527
3	7.5711	58.2415	58.2544	58.2643	58.273	58.287	58.323	58.3331	58.345	58.355	58.402	58.4304	58.5003	58.5803	58.5899
Weight Increase of Dessicant Packaged in K,KL andStored at 25C 75% RH															
	EMPTY	INITIAL	DAY 1	day 2	day 3	day 4	day 7	day 8	9 days	10 days	14th	17th	24th	31DAY	32nd
K															
1	14.9905	66.152	67.7109	68.1731	67.6263	68.2069	69.0508	68.8784	68.8516	68.8934	69.7497	69.7543	69.5076		
2	14.9929	66.444	68.0592	68.4527	67.7983	68.203	69.1744	69.0697	69.2276	69.2624	69.9513	70.1708	69.7166		
3	15.0375	66.285	68.0976	68.4235	67.8061	67.8488	69.0295	69.0559	68.9521	69.1259	69.7306	70.0989	69.3899		
KL															
1	7.8422	58.3986	58.669	58.9146	59.0192	59.2386	60.0074	60.1518	60.3158	60.4592	60.8715	61.2344	61.6633	61.2663	
2	7.799	57.8523	58.03210	58.2725	58.4055	58.6032	59.3347	59.4452	59.6062	59.7502	60.239	60.5503	61.001	60.7319	
3	7.7766	58.3419	58.5163	58.7653	58.8732	59.073	59.782	59.9477	60.1135	60.2775	60.8652	61.2022	61.703	61.3483	
ALUMINIUM															
1	7.5484	57.9545	57.986	58.0273	58.0432	58.0775	58.2155	58.2584	58.3004	58.3422	58.5335	58.6651	58.9542	59.1273	59.1439
2	7.5629	58.0815	58.1087	58.1476	58.164	58.1958	58.3411	58.3821	58.426	58.4689	58.6591	58.8153	59.1072	59.283	59.2981
3	7.4619	58.0229	58.0577	58.101	58.1196	58.1578	58.3134	58.3547	58.4033	58.4519	58.6594	58.8006	59.0981	59.2959	59.3076