

**NUTRITIONAL, SENSORY AND STORAGE PROPERTIES OF SNACK
PRODUCED FROM MAIZE (*Zea mays* Linn) AND AFRICAN YAM BEAN SEED
(*Sphenostylis stenocarpa* Hochst Ex A. Rich) FLOUR BLENDS**

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A thesis in the Department of Food Technology

Submitted in Partial Fulfilment of the Requirement for the Degree of

Doctor of Philosophy (Ph. D)

of the

University of Ibadan

2014

CERTIFICATION

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DEDICATION

This report is dedicated to the Trinity, God the Father , God the Son and God the Holy Spirit for being my all in all.

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ACKNOWLEDGEMENTS

I am most grateful to the Almighty God, the Father of light and glory for loving me so dearly and giving me the knowledge, wisdom and strength to carry out this study.

My supervisor, Prof. O.C. Aworh for his expert direction and mentorship, I thank him.

My profound gratitude is extended to all members of staff of the Department of Food Technology, University of Ibadan, Dr. R. Akinoso, Prof. G.O. Adegoke, Prof. K.O. Falade, Mrs. P. Uzo-Peters, Dr. O. Ezekiel, Dr. A.O. Olapade, Dr. Aruyela, Mrs. Ojo, Mr. Oyedokun, Mr. Babarinde, Mr. Ogugua, Mr. Adufe, Mr. Peter and all others.

I express my appreciation to the management and members of staff of the International Institute of Tropical Agriculture for immense contribution to the success of this research work.

Supports of the staff and management of Redeemer's University are highly commendable. My appreciation also goes to the members of the Redeemed Christian Church of God for your prayers and moral support. I also like to acknowledge Prof. T.N. Fagbemi for his support since he was my lecturer at undergraduate level.

I am grateful to my husband, Engr. 'Gbenga Idowu for his relentless effort towards the success of this work, to all my children for their love and understanding. My gratitude also goes to my parents, Elder and Mrs. J.B. Ojurongbe, my siblings, their spouses and all our friends and relatives, God bless them all.

ABSTRACT

Low nutritional value and inconsistent sensory qualities arising from crude and non-standardised processing operations characterise most Nigerian maize-based snacks including *kokoro*. African Yam Bean Seed (AYB) is an underutilised crop with high nutritional value, but literature is sparse on its utilisation to enrich maize snacks. This study was designed to improve nutritional value of *kokoro* by incorporating AYB Flour (AYBF). Varieties of maize (BR-9928-DMR-SY and TZL-Comp-4C2) and AYB (Tss-9 and Tss-30) were tested for processing suitability using physical and chemical properties. The better quality maize and easier to dehull AYB were processed into flours at ratios 100:0, 80:20, 70:30, 60:40 and 0:100. Proximate composition, Trypsin Inhibition Activity (TIA), beta-carotene and amylose contents, functional and pasting properties of the flour blends were determined by standard methods. Using Response Surface Methodology (RSM) experimental design, batters produced from blends of maize and AYBF at ratios 80:20, 70:30 and 60:40 were deep fried at varied temperatures (150, 160 and 170°C) and time (8, 10 and 12 min.) according to 17 combinations associated with three independent variables. Processing conditions including frying temperature, frying time and quantity of AYBF in the flour blends were independent variables while products' qualities were dependent variables. Proximate composition, TIA and texture of snacks were determined using AOAC methods. *Kokoro* with highest products' qualities were obtained from RSM as the optimum processing conditions. *Kokoro* was prepared at these optimum conditions and subjected to rancidity test weekly for fourteen weeks to determine its storage life using free fatty acid test. The *kokoro* and casein diet were separately fed to male wistar rats (90-110g) for 28 days using casein diet as standard to determine its protein availability. Sensory attributes of

the products were determined using semi-trained panelists. Data were analysed using ANOVA at $p=0.05$.

The BR-9928-DMR-SY maize was selected for its higher nutrient density (beta-carotene, $1.8\mu\text{g/g}$). The AYB (Tss-30) was chosen based on its better ease of dehulling. Crude protein (10.5-15.7%), total ash (1.5-2.2%), crude fibre (1.3-4.1%), sugar (4.1-5.3%), TIA (2.9-6.7%) increased, while crude fat (4.9-3.9%), starch (66.6-51.2%), amylose (26.5-24.8%) and beta-carotene ($1.8\text{-}0.9\mu\text{g/g}$) decreased with increase in AYBF in the flour blends. Functional parameters showed no significant change among the flour blends except oil absorption capacity (80.1-57.1%). Peak viscosity (479-580cp) increased but pasting temperature ($89.8\text{-}82.1^{\circ}\text{C}$) decreased with increase in AYBF. While crude protein content was not adversely affected with higher frying temperature and time, TIA (6.7-2.9%) decreased significantly, but crude fibre, crude fat, sugar and starch contents increased. Texture increased with increase in frying time. The best product was obtained from blend of Maize-AYB at ratio 70:30, fried at temperature, 155°C and time, 11.5min. Level of rancidity of the snack was tolerable up to 12 weeks. Protein availability of the *kokoro* was not significantly different ($p<0.05$) from that of casein. The *kokoro* produced was acceptable to panelists up to 10 weeks of storage.

Addition of African yam bean seed flour to *kokoro* improved its nutritional content, creating a novel use for African yam bean seed. Standard processing conditions for producing *kokoro* of consistent sensory qualities was established.

Keywords: African yam bean seed flour, Maize flour, *Kokoro*, Nutritional quality.

Word count: 498

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CHAPTER ONE

1. INTRODUCTION

1.1 Background of the study

Some traditional food products in Nigeria are characterised by low nutritional value, variable sensory quality and short shelf life. The poor packaging and storage techniques make them prone to pest and rodent attack and susceptible to microbial spoilage (Aiyeleye and Eleyinmi, 1997). According to Sobukola *et al.* (2009), the technologies employed in the processing, distribution and storage of indigenous snack foods are based on the traditional knowledge. Therefore, there is need for upgrading these technologies as a way of improving nutritional, sensory and storage properties of these products. Most often snacks do not provide nutrients in adequate quantities needed by the body (Omueti and Morton, 1996); this may be due to their composition or the production process. Thus, it is necessary to ensure that every food consumed by an individual contains required nutrients in adequate amounts. This is especially important due to the fact that many people now work outside their homes and are becoming more dependent on snacks for the supply of part of their daily food requirements.

According to Tettweiler (1991), a snack is a small meal in the broadest sense “and snacking is the consumption of easy- to- handle food products in either solid or liquid form, with little or no preparation. It is eaten in small amount usually between main meals or instead of a meal. A snack is commonly used as convenience food. The need for convenience food is borne out of the need to spend less valuable time and energy in the kitchen preparing meals (Idowu *et al.*, 2010). Snacking is on the increase worldwide and this results primarily from factors such as increase in one person households, a higher proportion of working mothers and more school aged children obtaining their own meals and refreshments.

It would therefore be worthwhile if an acceptable snack with high nutritional quality that could be useful in nutritional programmes to combat malnutrition and nutrient deficiencies could be developed (Rosa *et al.*, 2003). In the tropics, maize is a common cereal crop, of good source of carbohydrate, vitamin and minerals that can be processed into a wide range

of food items and snacks. Maize grains are abundant, stable crop in Nigeria with different varieties. Nutritional contents of the maize varies from one variety to another, while some maize varieties contain β -carotene, other do not. Punita (2006) investigated the nutritional composition of three maize varieties and attributed the differences in chemical composition to their genetic composition, environmental factors and agronomic practices. Therefore, appropriate variety of maize can be chosen in production of snacks to attain increased nutritional contents. Some of the maize-based local snacks include; *aadun* (maize snack), *kokoro* (corn cake) and *donkwa* (maize-peanut ball). Although, these snacks and appetizers are popular food items, with a long history of consumption especially among the low income populace, there exists a paucity of information on their nutritive and functional attributes (Aletor and Ojelabi, 2007). Like most cereal-based foods, *kokoro* is rich in carbohydrate, but low in protein and deficient in some essential amino acids, particularly lysine (Uzo-Peters *et al.*, 2008, Ihekoronye and Ngoddy, 1985). This makes the product nutritionally deficient thus necessitating its enrichment. Improving these snacks therefore will involve the understanding of the production processes and optimisation of these production processes. It will also involve inclusion of nutrient-dense materials such as African yam bean and storage studies to know the appropriate storage conditions for the product.

Since these maize-based snacks particularly *kokoro* are commonly consumed among adults and children especially the school-aged children as refreshment in South-Western Nigeria, it becomes necessary to improve its protein and micronutrients contents. Vitamin A deficiency (VAD) has been reported as a severe public health problem as 29.5% of children (<5years) are vitamin A deficient with serum retinol <0.70 μ m\L (Maziya-Dixon *et al.*, 2006). Recommended Dietary Allowance (RDA) of vitamin A for children is 300-600 μ g (1200IU-2400IU) and 800-1000 μ g in adults (Smolin and Grosvenor, 2003). Vitamin A activity in food is mainly due to all trans-isomer of retinol, which is the most abundant and biologically active member of the vitamin A group. In diet, β -carotene and other carotenoids provide most of the vitamin A. The labeling of food regulations require that vitamin A is calculated as micrograms of retinol or retinol equivalent on the basis that 6 μ g of β -carotene equals 1 μ g of retinol equivalent. Therefore 6 μ g of beta-carotene is equivalent to 1 μ g of vitamin A (Smolin and Grosvenor, 2003, Pearson, 1976). Food materials or commodities

high in β -carotene such as yellow maize, sorghum could be used in *kokoro* production to enhance its vitamin A content.

Legume crops such as soybean, cowpea, groundnut, African yam bean, have very high protein content. It is well documented that most leguminous plant seeds are rich in nutrients such as digestible protein with a good array of amino acids and minerals (Fagbemi *et al.*, 2004; Agbede and Aletor, 2003).

African Yam Bean (AYB) (*Sphenostylis stenocarpa*) is one of the less utilised legumes that are gradually going into extinction (Klu *et al.*, 2001); this may be due to the long cooking time it requires and its taste. However, it can be used in preparation of other food products. According to Adewale *et al.*, (2010), the vast genetic and economic potentials of AYB have been recognized; especially in reducing malnutrition among Africans and the crop has not received adequate research attention. Up till now, it is classified as a neglected underutilised species (NUS) (Bioversity, 2009). Its intended use in improving nutritional quality of maize-based snacks will add value to these maize-based snacks as well as provide more utilisation for the legume.

Although plant proteins are considered inferior to animal proteins, the former are becoming the choice of the populace due to absence of cholesterol and other components of animal protein that pose health risks to consumers. Apart from the use of soybean as an alternative to animal protein, protein from other plant sources should be exploited. Nutritionally, AYB competes with cowpea and soybeans in terms of protein and amino acids contents (NAS, 1979). The protein content in AYBS grains ranged between 21 and 29% and in the tubers, it is about 2 to 3 times the amount in potatoes (Uguru and Madukaife, 2001, Okigbo, 1973). During nutritional evaluation of 44 genotypes of AYB, the crop was reported to be well balanced in essential amino acids and has higher amino acid content than pigeon pea, cowpea, and bambara groundnut (Uguru and Madukaife, 2001). Like most legumes, AYB will improve both protein quantity and quality of maize, since the methionine-containing maize will be complemented by lysine-containing AYB, providing a better balance of amino acids, especially the essential amino acids. Amino acid analyses indicated that the lysine and methionine levels in the protein of AYB are equal to or better than, those of soybeans (Evans and Boutler, 1974). FAO/WHO/UNU (1992) recommended dietary intake states

that an average man requires 65g of protein and 2500kcal of energy per day while a child between age 4 and 7 requires 20g of protein per day and 1830kcal of energy per day. This nutritional requirement has to be borne in mind while formulating products of improved nutritional qualities.

Frying (for example, *kokoro*, *kulikuli*) and roasting (for example, pop corn, groundnut) are commonly used in processing of traditional snacks. Despite acrylamide scare and other limitations associated with fried products, the market of fried products is still growing. Frying is commonly used to cook food. A fried product tastes good, has a good flavour and is prepared within few minutes. Even though frying is an old process of manufacturing food products worldwide, optimisation of its processing parameters may improve fried products' quality. It will have effect on the oil content, texture (crispness), color, and nutritional value of the final product (Lui-ping *et al.*, 2005).

One of the most popular methods used in food product and process optimisation in the last two decades is Response Surface Methodology (RSM). RSM is a collection of statistical and mathematical techniques useful for developing, improving and optimising process in which a response of interest is influenced by several variables and the objective is to optimise the process (Myers and Montgomery, 1995). RSM has important application in the design, development and formulation of new products, as well as in the improvement of existing product design (Bas and Boyaci, 2007).

RSM has been very popular for optimisation studies in recent years in the area of Food Science and Technology. These include the optimisation of roasting temperature and time during oil extraction from orange (Akinoso *et al.*, 2011); development of complementary foods from extruded cowpea (*Vigna unguiculata* (L) and Acha (*Digitaria stapf*) blends (Olapade, 2010); optimisation of frying conditions during deep fat frying of yam slices (Sobukola *et al.*, 2008); optimisation of process variables for the preparation of expanded finger millet (Ushakumari *et al.*, 2007) and optimisation of vacuum drying conditions of carrot chips (Lui-ping *et al.*, 2005);.

Use of better quality maize, enrichment with African yam bean as well as use of response surface methodology for standardisation of the processing methods and storage studies of

the maize-based snacks will suggest the appropriate processing and storage conditions. This will result in improved sensory, nutritional and storage properties of this Nigerian maize-based snacks (*Kokoro*).

1.2 Objectives of the Study

The main objective of this study was to develop and optimise *kokoro* from blends of maize and AYB to attain improved nutritional value, better storage and consistent sensory qualities. While the specific objectives of this study were to:

- i. determine the properties of selected maize and African yam bean seeds cultivars for processing suitability,
- ii. evaluate the effect of some processing parameters (frying temperature, frying time and quantity of AYB flour) on the physico-chemical, functional, nutritional and sensory properties of the flour blends and the maize-based snacks.
- iii. determine storage properties and adsorption isotherm characteristics of the products and
- iv. assess the consumers' acceptability of the products developed.

1.3 Justification of study

There is little information on the utilization of African yam bean seed, a nutrient dense but neglected underutilized species (NUS) of legume) to enrich maize snacks. The development of these maize-based snacks through value-added processes will establish appropriate and optimum conditions for improving the product quality. It will offer wider utilisation to AYB, leading to job creation both at farm and industrial level. Also, the crude, non-standardised processing operations associated with this Nigerian snack will be upgraded as a way of improving its nutritional, sensory and storage properties. This research work is in line with national policies of food security and upgrading traditional food processing techniques, thus adding value to Nigerian snacks.

CHAPTER TWO

2. LITERATURE REVIEW

2.1 Cereals and Legumes

Cereal grains and legumes have been the most friendly food resources for man from antiquity. This is because of their liberal agronomic and conservation requirements coupled with their nutritional value as major sources of calorie, protein, minerals, vitamins and roughages (Okaka, 1997). Cereal grains remain the most important source of calories, for most of the world's population. Legumes, in many developing countries, supply most of the plant proteins which are not only main, but also relatively the cheapest source of dietary proteins in areas where animal protein is scarce and too expensive (Adewale, 2010). The quantities of cereal grains and legumes produced and consumed vary considerably within the tropical world. However, as a result of their ability to mature rapidly, and their nitrogen fixing ability, grain legumes feature both in the intensive multi-cropping systems, and in crop rotation patterned on the modern cropping systems typical of temperate agriculture (Adewale, 2010) which increase their gross availability for consumption.

Legumes play a very important role in the local diets and weaning foods. Some legumes are over-utilised while some are under-utilised in our diets due to negligence and ignorance (Aletor and Aladetimi, 1989). African yam bean, *Sphenostylis stenocarpa*, Hochst ex A. Rich, (family Leguminosae, sub-family *Papilionaceae*) is one of the under-utilised legumes in Nigeria (Klu *et al.*, 2001). It is one of the neglected pulses of tropical origin that has attracted research interest in recent times due to its nutrient content (Eneche, 2005).

2.2 Maize: Production and Classification

Maize (*Zea mays* L.) is the third most important cereal in the world after rice and wheat and ranks fourth after millet, sorghum and rice in Nigeria (FAO, 2009). Maize or corn is the most important cereal crop in sub Saharan Africa (Akingbala *et al.*, 1987). It is mostly used and traded as a leading feed crop but is also an important food staple. In addition to food and feed, maize has a wide range of industrial applications ranging from food processing to manufacturing of ethanol (FAO, 2006).

Global statistics for cereal consumption indicate that the average total consumption in the African diet is 291.7g/person/day, including an average maize consumption of 106.2g/person/day (FAO, 2009).

Maize is known and called by different vernacular names in Nigeria depending on locality like *agbado*, *igbado* or *yangan* (Yoruba); *masara* or *dawar masara* (Hausa); *ogbado* or *oka* (Ibo); *apaapa* (Ibira); *oka* (Bini and Isha); *ibokpot* or *ibokpot union* (Efik) and *igumapa* (Yala) (FAO,1992).

2.2.1 Origin of maize

Maize is one of the oldest human-domesticated plants. Its origin is believed to date back to at least 7000 years ago when it was grown in the form of a wild grass called *teosinte* in Central Mexico. Recognizing its early potential as a major food crop, over time the *Mesoamerican* natives managed to improve the crop, by systematically selecting certain varieties for their desired traits. This process led to the gradual transformation of *teosinte* to its present day form known as maize, a name which is a likely derivative of "*mahis*", meaning "source of life" for *Tanio* people, the natives known to have mastered its cultivation. Maize is also known as corn, which is the name that has come into common usage primarily because it is used in the United States, the world's largest producer, consumer and exporter of maize. Maize is an annual plant with high productivity which also enjoys exceptional geographic adaptability, an important property which has helped its cultivation to spread throughout the world. Its gradual expansion in the Americas by the Natives was rapidly propagated in the 16th century following the return of Columbus to Europe. Colonial conquests and trade played a central role in the spread of maize cultivation well beyond the European continent, to Africa and Far East Asia (FAO, 2006). There exist several hybrids of maize, each with their own specific properties and kernel characteristics; the most common ones include: dent (or field maize, used for livestock feeding and can be yellow or white), flint (or Indian maize, grown in Central and South America), and sweet (or green maize). Depending on their colour and taste, maize grown around the world is generally categorized into two broad groups: yellow and white. Yellow maize constitutes the bulk of total world maize production and international trade (FAO, 2006). It is grown in most northern hemisphere countries where it is traditionally used for animal feed. White maize, which requires more favourable climatic conditions for growing, is produced in only

a handful of countries, the United States, Mexico and in southern Africa. White maize is generally considered a food crop. Market prices are usually higher for white maize compared to the yellow type but the premium can vary depending on local supply and demand conditions.

2.2.2 Advances in global maize production

The term *Green Revolution* refers to the transformation of agriculture which resulted in significant gains in cereal production between the 1940s and 1960s in the developing countries. The novel technological development of the Green Revolution was the production of high yielding varieties of maize, wheat, and rice. At around 700 million tonnes, world maize production represents over one-third of world cereal output (FAO, 2006). Over the past two decades, global maize production has increased by nearly 50 percent, or 1.8 percent annual compound growth rate (FAO, 2006). Most of the increase in world maize production during the past decade can be attributed to a rapid expansion in Asia (FAO, 2006). Asian maize production grew by nearly 35 percent during the past decade, accounting for almost 30 percent of the global increase (FAO, 2006). Both area and yield increases contributed to this high level of growth, with China making the most significant advance by contributing to as much as 60 percent of the total gains in Asian maize production over the past decade (FAO, 2006). In spite of the advances attributed to the *Green Revolution* and the introduction of high yield maize varieties, the possibilities for maize yield improvements in many countries has remained large as the degree of production efficiency, especially in the developing countries, still falls below major commercial producers. Average maize yields among the developing countries, as an aggregate, are about one-third of the amount of the major maize producers which include: United States, China and Republic of South Africa (FAO, 2006).

2.2.3. Chemical composition of maize

Generally, whole maize contains 362 Kcal/100g; 8.1% crude protein; 72% starch, 5% fat, 1.3% ash, 1.2% fibre; 60 ppm calcium, 35 ppm iron; 1.8 ppm Zinc; 3.9 ppm Thiamine; 2.0 ppm Riboflavin; 36ppm Niacin; 3.0ppm pyridoxine; 0.25 ppm folates; 241 mg/100g phosphorous; 0.16 ppm selenium (Bressani, 1972).

Starch: is a major chemical component of the maize kernel, it provides up to 72 to 73% of the kernel weight. Other carbohydrates are simple sugars present as glucose, sucrose and

fructose in amounts that vary from 1 to 3% of the kernel. The starch in maize is made up of two glucose polymers: amylose (an essentially linear molecule), and amylopectin (a branched form). The composition of maize starch is genetically controlled. In common maize, with either the dent or flint type of endosperm, amylose makes up 25 to 30% of the starch and amylopectin makes up 70 to 75%. Waxy maize contains a starch that is 100 % amylopectin. An endosperm mutant called amylose-extender (ae) induces an increase in the amylose proportion of the starch to 50% and higher. Other genes, alone or in combination, may also modify the amylose-to-amylopectin ratio in maize starch (Zarkadas *et al.*, 2000; Boyer and Shannon, 1987).

Protein: After starch, protein is the next largest chemical component of the kernel. Protein content varies in common varieties from about 8 to 11% of the kernel weight, with most of it found in the endosperm. The protein in maize kernels is made up of at least five different fractions: albumin (7%), globulins (5%) and non-protein nitrogen (6%) amounting to about 18% of total nitrogen as well as the prolamine fraction (52%) and glutelin fraction (25%) of the total protein in the kernel. Usually a small amount, about 5%, is residual nitrogen (Afoakwa *et al.*, 2002; Landry and Moureaux, 1982). The nutritional quality of maize as a food is determined by the amino acid make-up of its protein. In common maize, deficiencies in lysine and tryptophan are evident as confirmed with Quality Protein Maize (QPM) (Punita, 2006). An additional important feature of maize composition is the high leucine content in common maize and the lower value of this amino acid in QPM (Zarkadas *et al.*, 2000; Mertz *et al.*, 1975).

Oil and fatty acids: The oil content of the maize kernel comes mainly from the germ. Oil content is also genetically controlled, with values ranging from 3 to 18% (Afoakwa, 2007). Maize oil has a low level of saturated fatty acids, i.e. on average 11% palmitic and 2% stearic acid. It also contains relatively high levels of polyunsaturated fatty acids, mainly linoleic acid with an average value of about 24%. Maize oil is relatively stable since it contains only small amounts of linolenic acid (0.7%) and high levels of natural antioxidants. Maize oil is highly regarded because of its fatty acid distribution, mainly oleic and linoleic acids (Mosha and Vincent, 2004; Bressani *et al.*, 1990).

Dietary fibre: Fibres are complex carbohydrate found in large amounts. Dietary fiber consists of the remnants of the plant cells, polysaccharides, lignin and associated substances resistant to digestion by the alimentary enzymes of humans (Prosky *et al.*, 1992). There are basically two types of dietary fibre in the food system: Insoluble (IDF) and soluble dietary fibre (SDF) ((Gupta, 2003; Burkitt *et al.*, 1972). The most important forms of soluble dietary fibre include; pectin, gums, guar and some hemicelluloses. Food sources rich in these types of fibre components include legumes, vegetables, fruits, oat bran and seeds (Oluwatayo *et al.*, 2008). Research shows that decreased risk of coronary heart disease is correlated with increase consumption of DF, typically SDF. Risk of coronary heart disease is also correlated with a significant number of other risk factors which are reduced by SDF, such as diabetes, high serum cholesterol, high levels of low density lipoprotein (LDL) associated cholesterol, and low levels of high density lipoprotein (HDL) associated cholesterol, obesity, and possibly hyperinsulinemia (Anon, 1996). The complex carbohydrate content of the maize kernel comes from the pericarp and the tip cap, although it is also provided by the endosperm cell walls and to a smaller extent the germ cell walls. Maize bran composed of 75% hemicellulose, 25% cellulose and 0.1% lignin on a dry-weight basis (Gupta, 2003; Sandstead *et al.*, 1978).. Bauer and Turler-Inderbitzin (2008), reported that the IDF content of highland maize is 10.94% and the SDF is 1.25% while the IDF of lowland maize is 11.15% and the SDF is 1.64%.

Other carbohydrates: When mature, the maize kernel contains carbohydrates other than starch in small amounts. Total sugars in the kernel range between 1% and 3%, with sucrose, the major component, found mostly in the germ. Higher levels of monosaccharide and disaccharides are present in maturing kernels (Afoakwa, 1996). At 12 days after pollination the sugar content is relatively high, while starch is low. As the kernel matures, the sugars decline and starch increases (FAO, 1992).

Minerals: The concentration of ash in the maize kernel is about 1.3%, only slightly lower than the crude fibre content. Environmental factors probably influence the mineral content. The germ is relatively rich in minerals, with an average value of 11% as compared with less than 1% in the endosperm. The germ provides about 78% of the whole kernel minerals. The most abundant mineral is phosphorus, found as phytate of potassium and magnesium. All of

the phosphorus is found in the embryo, with values in common maize of about 0.90% and about 0.92% in opaque-2 maize (Okaka, 1997). Like most cereal grains, maize is low in calcium content and trace minerals (Bauer and Turler-Inderbitzin, 2008).

Fat-soluble vitamin: The fat-soluble vitamin present in the maize grain are provitamin A, or carotenoids, and vitamin E. Carotenoids are found mainly in yellow maize, in amounts that may be genetically controlled, while white maize has little or no carotenoid content. Most of the carotenoids are found in the hard endosperm of the kernel and only small amounts in the germ. The β -carotene content is an important source of vitamin A, but unfortunately yellow maize is not widely utilised in production of maize-based snack by humans as much as white maize. Studies have shown that the conversion of β -carotene to vitamin A is increased by improving the protein quality of maize (FAO, 1992). Vitamin E, which is subject to some genetic control, is found mainly in the germ. The source of vitamin E is four tocopherols, of which α -tocopherol is the most biologically active. γ -tocopherol is probably more active as an antioxidant than α -tocopherol (FAO, 1992).

Water-soluble vitamin: Water-soluble vitamins are found mainly in the aleurone layer of the maize kernel, followed by the germ and endosperm. Variable amounts of thiamine and riboflavin have been reported and their content is affected by the environment and cultural practices rather than by genetic make-up (Patterson *et al.*, 1980). The water-soluble vitamin, nicotinic acid has attracted much research because of its association with niacin deficiency or pellagra, which is prevalent in populations consuming high amounts of maize (Zarkadas *et al.*, 2000; Chistianson *et al.*, 1968). As with other vitamin, niacin content varies among varieties, with average values of about 20 μg per gram. A feature peculiar to niacin is that it is bound and therefore not available to the animal organism. Some processing techniques hydrolyse niacin, thereby making it available. The association of maize intake and pellagra is a result of the low levels of niacin in the grain, although experimental evidence has shown that amino acid imbalances, such as the ratio of leucine to isoleucine, and the availability of tryptophan are also important (Patterson *et al.*, 1980). Maize has no vitamin B₁₂, and the mature kernel contains only small amounts of ascorbic acid, if any. Other vitamin such as choline, folic acid and pantothenic acid are found in very low concentrations.

2.2.4. Approaches to improving the nutritive value of maize

Because of the great importance of maize as a basic staple food for large population groups, particularly in developing countries, and its low nutritional value, mainly with respect to protein, some approaches have been used to improve its nutritive value: genetic manipulation, processing and fortification.

Genetic approaches has been used to modify the carbohydrates content (Boyer and Shannon, 1987), protein quality and quantity (Bressani *et al.*, 1990), influence fatty acid composition (Leibovits and Ruckenstein, 1983) as well as increase the quality and quantity of other nutrients such as niacin in maize grain.

Processing of foodstuffs stabilizes nutrients in the food, but losses may take place when optimum conditions are exceeded. There are cases, however, in which processing induces beneficial changes in the food; a classic case is the elimination of anti-physiological factors in beans (FAO, 1992). Natural fermentation of cooked maize, results in higher B-vitamin concentration and protein quality (Afoakwa, 2007; Wang and Fields, 1978). Germination of maize grain has also been reported to improve the nutritional value of maize by increasing lysine and to some extent tryptophan (Umerie *et al.*, 2009; Tsai *et al.*, 1975).

Another approach often used to improve the nutritive value of foods, mainly cereal grains, is fortification. Because of the great nutritional limitations in maize, many efforts have been made to improve its quality, and particularly that of its protein, through addition of amino acids or protein sources rich in the limiting amino acids (Awoyale *et al.*, 2011; FAO, 1992). These include:

Supplementation with protein sources: The results from animal and human studies in which limiting amino acids have been added to lime-treated maize have served as the basis for evaluating the ability of different types of protein supplements to improve its protein quality (FAO, 1992). The improvement in quality of protein in tortilla flour is in most cases a synergistic response to lysine and tryptophan enhancement and to a higher level of protein, both provided by the supplement (soybean flour). Since soybean protein in different forms is the supplement to tortilla flour most often tested by different investigators and because it

is almost the only one also tested in children, with results comparable to those in studies with animals (Oloyede and Kolawole, 2004; Bressani and Marengo, 1963).

2.2.5 Processing and Utilisation of Maize

Maize has a wide range of industrial applications ranging from food processing to manufacturing of ethanol, apart from its use for feed. Maize could be processed into various forms namely: roasted, boiled, fermented, toasted, toasted and milled, toasted, milled and mixed with palm oil and pepper, depending on the region where it is produced. For instance, maize grains are prepared by boiling (*agbado*) or roasting (*elekute*), or fermenting and boiling as paste (*eko*), in Nigeria and *kenke* in Ghana, or as popcorn which is eaten all over West Africa (FAO,1992). Grain is the major part of the maize crop that could be put into various uses as reported by Abdulrahman and Kolawole (2006) which include:

Pap: There are two popular paps in Nigeria; hot-pap and cold-pap. Pap is prepared by soaking maize in water for 2 to 3 days to ferment. Then the grains are washed with clean water and ground to paste. After the grinding of the grains, the ground paste is sieved using clean, white cloth to get very smooth paste. The residue of sieving is used to feed animals. While, the remaining fine paste after sieving is allowed to settle down at the bottom of the container. At this stage, amount of paste desired may be taken, stirred and poured inside boiling water and stirred until a semi-liquid porridge (hot pap) or a semi-solid porridge(cold pap) is obtained. The semi-solid porridge is then put inside wrapping/banana leaves to give a characteristic domed shape. Alternatively, it may be put inside polyethylene bags (nylon), the hot product is allowed to cool down and solidified, and thus become thick porridge (cold pap).

Tuwo: To prepare *tuwo*, testa of the grains is removed by grinding gently inside mortar with pestle. Small water is added to the grains to enhance testa removal. The grains are then ground with local grinding stone or with grinding machine to obtain a smooth, whitish paste. The paste is poured into hot water and stirred with a stirring-stick to make a thick porridge food (*tuwo*). *Tuwo* can be eaten with bean soup or with vegetable soups like sesame (*Sesamum indicum* L.), okra (*Abelmoschus esculentus*), celosia (*Celosia argentea* L., Amaranthaceae) e.t.c.

Donkunnu: This food is an exotic food to Nigeria because it was introduced from Ghana probably by the emigrant Ghanaians or by Nigerians who lived in Ghana. *Donkunnu* is prepared by soaking maize for about two days in cold water. It is then ground into wet paste and left in this state for about two days to ferment (so as to bring out the characteristic sour taste of the finished product). A desired quantity of fermented paste is put inside maize husk and cooked until a thick, solid porridge (*donkunnu*) is obtained. *Donkunnu* is eaten with pepper stew and fried fish.

Maasa: This is a thick fried porridge produced from fermented maize dough. It is prepared by wet milling the fermented maize grains into coarse particle sizes, after which small piles of it are put separately into frying-pan containing vegetable oil. It could be eaten by sprinkling sugar on its surface.

Cous cous: This is prepared by milling the maize grain into flour after the removal of the grain testa. This flour is then mixed with sliced tomato, pepper and onion, before being cooked into a solidified mixture.

Gwate: preparation of *gwate* is similar to *cous cous*. While *cous cous* is solid, *gwate* is semi-solid porridge. Unlike *cous cous*, ingredients like pieces of soft bones, meat, amaranth (*Amaranthus spp.* L., Amaranthaceae) or bitter leaf (*Vernonia amygdalina* Del. Asteraceae) and 'efirin' (*Ocimum spp.* L., Lamiaceae) are mixed with the flour and cooked to make *gwate*.

Popcorn: This is made by putting maize grains inside a hot pan with oil, water and salt or sugar (honey). The heat applied changed the colour of the whitish grains to brownish (*guguru*).

Aadun: Grains are roasted and then ground into coarse flour. This is mixed with palm oil which makes it solidified, or clumps together.

Elekute: Dried maize grains are roasted with hot-charcoal and ground into fine particles. This could be eaten with small amount of granulated sugar.

Kokoro: This is produced by milling the maize grains, pre-gelatinizing, spicing, kneading, and frying (Adegoke and Adebayo, 1994).

Donkwa is a corn and groundnut based snack common among the Hausa of Nigeria. It is referred to as ‘Tarifirin’ among the Yoruba. It has a sweet taste and pleasant aroma and it is often used as light refreshment. It is made by pounding together individually roasted corn and groundnut with other ingredients like dried pepper, local condiments, sugar, and salt before moulding.

Apart from food, maize could also be used as livestock feed, industrial uses for ethanol production, High Fructose Corn Syrup (HFCS) which is a popular substitute for sucrose (found in sugar) and used in soft drinks and other processed foods. It could also be processed into starch for food and industrial use such as paper, textiles, adhesives, plastics, baked goods, condiments, candies, soups and mixes. Other uses include: Distillers’ grains, and maize products used in feed rations; maize gluten meal, maize gluten feed, maize seed cake, maize germ meal, liquid feed syrup (FAO, 2006).

2.3 African Yam Bean

Legumes play a very important role in the local diets and weaning foods. Some legumes are over-utilised while some are under-utilised in our diets due to negligence and ignorance (Aletor and Aladetimi, 1989). African yam bean, *Sphenostylis stenocarpa*, Hochst ex A. Rich, (family Leguminosae, sub-family *Papilionaceae*) is one of the under-utilised legumes in Nigeria (Biodiversity, 2009; Okigbo, 1973). The Yam bean is locally grown in South and Central America, South Asia, East Asia and the Pacific. It is produced in three species which are called the Amazonian, Mexican and Andean (Anon., 2007). The African yam bean (AYB) is one of the neglected legumes of tropical origin that has attracted research interest in recent times (Azeke *et al.*, 2005; Ene-Obong and Obizoba, 1996) due to its nutrient content. It is a tuberous legume, having pulse and tuber as shown in Plates 2.1 and 2.2. The plant is found growing wild throughout tropical Africa, especially in southern Nigeria. It is also reported to be cultivated in Ivory Coast, Ghana, Togo, Gabon, Congo, Ethiopia and parts of East Africa (Wokoma and Aziagba, 2001). African yam bean provides two consumable products -the tuber which grows as the root source and the actual yam bean seeds which develop in pods above ground. Grown in pockets of tropical Central, West and East Africa, the African yam bean has great potential to contribute to overall food security and improve local diets. Like other beans, yam bean seeds are easily-preserved through



Plate 2.1: Tuber yield per stand of AYB accession TSs-96 at Ibadan, 2006. IITA.

Source: Adewale, 2010.



Plate 2.2: Diversity in colour, colour pattern, structure, texture, brilliance of African yam bean seeds

Source: Adewale, 2010.

drying. This means that the crop can provide food security to households and communities in Africa that experience seasonal or unexpected disruptions in agricultural production.

2.3.1 Potentials of African yam bean

Sphenostylis stenocarpa is an important tuberous legume of tropical Africa. It is a perennial climbing bush grown as annual crop. It belongs to the family, fabaceae and genus, sphenostylis (Potter and Doyde, 1994). It is usually cultivated as a secondary crop with yam in Ghana and Nigeria. The crop flourishes and takes over the stakes from senescing yam. It flowers and begins to set fruits from late September and October. The large bright purple flowers result in long linear pods that could house about 20 seeds. The seed grains and the tubers are the two major organs of immense economic importance as food for Africans. This indigenous crop has huge potential for food security in Africa. However, there are cultural and regional preferences. In West Africa, the seeds are preferred to the tubers but the tubers are relished in East and Central Africa (Potter, 1992). The crop replaces cowpea in some parts of southwestern Nigeria (Okpara and Omaliko, 1995). Uguru and Madukaife (2001) did a nutritional evaluation of 44 genotypes of AYB seeds (AYBS) and reported that the crop is well balanced in essential amino acids and has higher amino acid content than pigeon pea, cowpea, and bambara groundnut. Tables 2.1 and 2.2 show the values of some essential amino acids of tropical food and feeding stuffs, and the nutritional composition of AYBS compared to some tropical legumes, respectively. Areas of cultivation include West Africa and parts of equatorial Africa (Porter, 1992; Tindall, 1986). It is a vigorous vine, which twines and climbs to heights of about 3m and requires staking. The slightly woody pods contain 20 to 30 seeds, and are up to 30cm long and mature within 170 days (Klu *et al.*, 2001). The legume seed can appear white, speckled or marbled with a dark-brown boarder. Highest seed yields are obtained in mixed planting with yams, maize, okra and other vegetables (Philips, 1972). AYBS produces an appreciable yield under diverse environmental conditions (Schippers, 2000; Anochili, 1984). Another positive contribution of the crop to food security is the identification of the presence of lectin in the seeds, which could be a potent biological control for most leguminous pests (Adewale, 2010).

Table 2.1: Essential Amino acid content of some tropical food and feeding stuffs (mg/N)

| Common names | Botanical names | Arginine | Histidine | Iso-leucine | Leucine | Lysine | Phenylalanine | Tyrosine | Cystine | Methionine | Theonine | Tryptophan | Valine |
|---------------------------|--------------------------------|----------|-----------|-------------|---------|--------|---------------|----------|---------|------------|----------|------------|--------|
| Cowpea | <i>Vigna unguiculata</i> | 444 | 194 | 256 | 456 | 394 | 325 | 190 | 106 | 119 | 238 | 60 | 325 |
| Groundnut (whole) | <i>Arachis hypogaea</i> | 775 | 150 | 250 | 438 | 319 | 325 | 220 | 81 | 88 | 244 | 70 | 313 |
| Gnut protein (Arachin) | <i>Arachis hypoagea</i> | 763 | 119 | 413 | 425 | 250 | 344 | 300 | 81 | 38 | 144 | 56 | 244 |
| Gnut protein (Conarachin) | <i>Arachis hypogaea</i> | 744 | 119 | 219 | 363 | 375 | 106 | 156 | 163 | 106 | 113 | 31 | 200 |
| Soybean meal | <i>Glycine max</i> | 519 | 175 | 306 | 488 | 406 | 306 | 200 | 94 | 94 | 244 | 81 | 319 |
| Lima bean | <i>Phaseolus lunatus</i> | 388 | 206 | 350 | 556 | 431 | 400 | 160 | 61 | 119 | 300 | 56 | 363 |
| Bambara nut | <i>Vigna subterranean</i> | 394 | 118 | 275 | 494 | 400 | 350 | 219 | 180 | 113 | 219 | - | 331 |
| Field bean | <i>Dolichus lablab</i> | 456 | 163 | 228 | 525 | 388 | 325 | 220 | 69 | 50 | 225 | - | 244 |
| Common pea | <i>Pisum sativum</i> | - | - | 350 | 520 | 460 | 320 | 250 | 80 | 80 | 240 | 70 | 350 |
| Green gram seed | <i>Phaseolus aureus</i> | - | 188 | 350 | 560 | 430 | 300 | 100 | 40 | 70 | 200 | 50 | 370 |
| Pigeon pea | <i>Cajanus cajan</i> | 419 | 213 | 238 | 475 | 438 | 544 | 210 | 75 | 94 | 213 | 30 | 313 |
| Sunflower seed | <i>Helianthus annuus</i> | 513 | 137 | 356 | 419 | 238 | 313 | 163 | 88 | 213 | 250 | 81 | 331 |
| Geocarpa seed | <i>Kerstingiella geocarpa</i> | 425 | 181 | 275 | 494 | 388 | 369 | 220 | 63 | 94 | 244 | - | 406 |
| Stenocarpa seed | <i>Sphenostylis stenocarpa</i> | 388 | 231 | 275 | 481 | 425 | 331 | 270 | 94 | 119 | 256 | - | 350 |

Source: Ihekoronye and Ngoddy, (1985)

Table 2.2: Proximate composition of the African yam bean seeds compared to some tropical legumes

| Crop | Percent composition | | | | |
|-------------------|---------------------|------|---------------|-------|-----|
| | Protein | Fat | Carbohydrates | Fibre | Ash |
| African yam bean | 19.1 | 0.5 | 61.6 | 5.2 | 2.4 |
| Bambara groundnut | 19.2 | 5.6 | 54.5 | 5.3 | 3.5 |
| Cowpea | 19 | 1.1 | 60.6 | 5 | 3 |
| Mung Bean | 23 | 1.3 | 53.5 | 3.8 | 3.4 |
| Pigeon pea | 19.8 | 1.2 | 55 | 7.8 | 3.2 |
| Winged Bean | 32.2 | 16.5 | 3.2 | 6 | 3.4 |
| Soybean | 32.5 | 19.2 | 29.2 | 4.6 | 4.8 |
| Groundnut | 20.5 | 48.5 | 20 | 2.6 | 2.4 |

Source: Amoatey *et al.*, 2000

The crude protein content in AYBS is lower than that of soybeans, but the amino acid spectrum indicated that the levels of most essential amino acids (lysine, methionine, histidine and isoleucine) in AYB seeds is higher than those of other legumes including soybean (Adewale and Dumet, 2011). The availability of AYBS may be attributed to its ability to adapt to diverse environment which is responsible for its continual existence and survival.

2.3.2 Chemical composition of African yam bean seeds

Amino acid analysis indicates that lysine and methionine levels of AYBS protein are better than those of soybean (Uguru and Madukaife, 2001). According to NAS (1979), AYBS competes nutritionally with cowpea and soybeans. The seeds have a total carbohydrate of 61.6%, 19.1% crude protein, 0.5% crude fat, 5.2% crude fibre and 2.4% total ash (Amoatey *et al.*, 2000). It is made up of 0.3% phosphorus, 0.5% calcium, 0.16% Magnesium, 2.4µg/g zinc; 1.06µg/g potassium, 14µg/g, copper, 21µg/g manganese and 30 µg/g iron. It is rich in P, Ca, Mg, K, Fe, Zn but low in Cu and Na (Ekop, 2006). Oshodi *et al.*, (1995) reported that the whole AYBS flour contains 0.33% caprylic acid, 0.18% lauric acid, 28.8% linoleic acid, 1.84% linolenic acid, 1.52% escoseneic, 0.60% erucic and 11.39% unknown fatty acid. AYBS contains a very low amount of sodium (between 2 and 8mg/100g), therefore, it is good for hypertensive patients and are digested very slowly resulting in a gentle rise in blood sugar level; therefore it is good for diabetic patients (Arisa and Ogbuele, 2007; Butler and Price, 1980). It also contains anti-nutritional factors such as haemagglutinins, tannins, oligosaccharides (Okeola and Machuka, 2001).

Anti-nutritional factors (ANF) in leguminous products are chemical substances present in products although non-toxic but generate adverse physiological responses in animals that consume them. In most cases, ANF interferes with the utilisation of nutrients in legume products (Nwokolo, 1996). Some anti-nutritional factors such as alkaloids, flavinoids, saponins, lectin, trypsin inhibitors, phytate and oxalate have been identified in the seeds of AYB (Ajibade *et al.*, 2005; Okeola and Machuka, 2001; Azuzu and Undie, 1986). However, the levels of the various ANF in AYBS were found to be lower than those of cowpea (Aletor and Aladetimi, 1989). Processing destroys most of these ANF and thus poses no serious problems. Apata and Ologhobo (1997) reported complete destruction of trypsin inhibitor and haemagglutinins in some tropical legumes by cooking.

2.3.3 Processing and Utilisation of African yam bean seeds

Like cowpea, African yam bean (AYBS) could be eaten as cooked beans, cooked and eaten with rice, cooked and eaten with maize. Also, it could be toasted in a hot frying pan, and testa removed to produce a toasted cotyledon which may be eaten as a snack alone or with fresh coconut or palm kernels (Ishiwu and Onyeji, 2004). It could be fermented and used as sauce (Arisa and Ogbuele, 2007). Other researchers have worked on the processing and functional properties of African yam bean seed (Ajibola *et al.*, 2013). But there is limited literature on the production of African yam bean flour and its utilisation for Nigerian maize-based snack.

As expected from all cereals, maize-based snacks are rich in carbohydrates, but low in proteins and lacking in some essential amino acids, which are found in legumes. Therefore, the use of African yam bean seeds (classified as neglected underutilized species-NUS of legume) to enrich maize-based snack is nutritionally complementary. Like most legumes, African yam bean will improve both protein quantity and quality of maize, since the methionine-containing maize is complemented by lysine-containing African yam bean, providing a better balance of amino acids, especially the essential amino acids. Earlier work has been done on supplementation of maize snacks using soybeans (Henshaw and Craig, 1998; Uzo-Peters *et al.*, 2008) which showed increased protein, fat and ash contents.

2.4 Application of Response Surface Methodology

Response surface methodology (RSM) is a statistical technique for investigating multiple parameters alone or in combination, on response variables. RSM is a collection of statistical and mathematical techniques useful for developing, improving and optimising processes in which a response of interest is influenced by several variables which objective is to optimise the process. It can be used to define the relationships between the response and the independent variables. The independent variables can be defined alone or in combination, on the process and also generate a mathematical model (Myers and Montgomery, 1995). RSM has important application in the design, development and formulation of new products, as well as in the improvement of existing product design.

The relationship between the response and the variable is in equation

$$Y=f(x_1, x_2, \dots, x_n) + \sum \dots \dots \dots 1$$

Where Y is the response, f is the unknown function of response,

x_1, x_2, \dots, x_n denote the independent variables,

n is the number of independent variables and

\sum is the statistical error that represents other sources of variability not accounted for by f, which include measurement error. It is generally assumed that \sum has a normal distribution with mean zero and variance.

RSM have been utilised to optimise various food systems by earlier workers (Akinoso *et al.*, 2011; Ushakumari *et al.*, 2007; Corzo and Gomez, 2004; Rastogi *et al.*, 1998).

2.5 *Kokoro* (Maize rings)

Kokoro is a snack produced from wet-milled maize, which is seasoned and deep fried in oil. *Kokoro* is produced in some cities in South-western part of Nigeria and its main cottage industry is located in the Imasayi-Ibora areas of Ogun State, Nigeria.

It is widely acceptable and consumed by children and adults, especially in the southwestern part of Nigeria (Adelakun *et. al.*, 2004). The traditional process for *Kokoro* production (Figure 2.1) involves; cleaning of dried maize kernel, followed by parboiling of the maize in salted water, for 15-20 min. Parboiled maize is steeped in water overnight and wet-milled the following day. The maize dough resulting from the milling is then seasoned, moulded manually on wooden boards into a characteristic loop shape, deep fried in oil and packaged in polyethylene. It is shelf stable and was reported to have an estimated shelf life of 60 days when packaged in Polyethylene (Henshaw and Ihedioha, 1992).

The technology of *kokoro* production (Figure 2.2) involves mixing maize flour with boiled water to form paste and seasoned with salt and sugar or onion and salt depending on the individual. The dough is kneaded and cut into shapes and then deep-fried in vegetable oil for about 5min to produce a golden-yellow, hard textured, low-moisture product. *Kokoro* made from whole maize grain nutritionally contains 7.58% moisture, 7.03% protein, 14.3% fat, 1.55% ash, 1.26% crude fibre as well as 76.19% carbohydrate content (Uzo-Peters *et. al.*, 2008).

2.5.1 Approaches to improving nutritional value of *kokoro*

Improving the nutritional value of *kokoro* is essential being a widely consumed snack food. Addition of vegetable protein such as textured vegetable protein could be one way of raising the nutritional value of the product (Rosa *et al.*, 2003). Adedokun *et al.* (2004) reported that the supplementation of maize flour with soybean in the production of *kokoro* increased its crude protein, crude fat and total ash content while the carbohydrate content decreased as the quantity of soybean substitution increased and that 10% soybean substitution was preferred in terms of overall acceptability and in comparison to that of 100% maize flour. Uzo-Peters *et al.* (2008) also reported that the substitution of maize flour with soybean/groundnut increased the protein and fat content of *kokoro* while the carbohydrate content decreased and that the product made from 1:1:9 soybean/groundnut: maize substitution were well accepted and compared favourably with whole maize product.

In addition to the approach of improving the products' quality by enriching with leguminous crops employed by earlier researchers, effect of optimization of the frying process on the product quality need evaluation, which was incorporated in this study. Major heat processing involved in the production of *Kokoro* is frying. Frying is one of the factors responsible for variable sensory qualities of this maize-based snack. Even though frying is an old process of manufacturing food product worldwide, optimization of the processing parameters will have effect on the oil content, crispness, appearance, and nutritional value of the final product (Liu-ping *et al.* 2005). Optimisation of frying will suggest optimum conditions for obtaining products with consistent sensory qualities. Frying temperature and time affect the crispness, appearance, flavor (taste and aroma) and oil absorption of food products, (Garayo and Moreira, 2002; Liu-ping *et al.*, 2005) and these processing parameters will be considered in the optimisation procedures designed for this study to affect the quality of *Kokoro*. In addition, use of better quality maize and enrichment with African yam bean is expected to improve the quality of the maize snack which could significantly enhance nutritional status of its consumers, a large proportion of which are growing children.

2.6 Deep Fat Frying

Frying is considered to be one of the world oldest cooking methods in existence (Varela, 1988). For decades, consumers have highly valued the unique flavor, texture combination of deep-fat fried products which also include a smooth mouth feel that improve their overall acceptability. The immersion frying process also referred to as deep-fat frying, is commonly on the increase in the snack food industry. It has been reported that there is an upward trend in the production of snacks especially in the developed world (Mudambi *et al.*, 2006; Tettweiler, 1991).

Deep fat frying is defined as a process of cooking and drying using hot oil and it involves heat and mass transfer. Deep fat frying has been used for many centuries for cooking meat, fish and vegetables (Vitrac *et al.*, 2000). The quality of fried product depends on frying conditions as well as food and oil types used.

For fried snack, surface appearance and texture are significant factors for acceptability. The speed of cooking has to be regulated during frying so that by the time the surface of the food has turned the required colour, enough heat must have been conducted to the centre of the food to cook it and make it pleasant and safe to eat. Despite the fact that various types of foods are produced by this method, frying is still considered by many to be more of an art than a science and technology.

Understanding the complex processes taking place during frying is necessary to control the quality of the final fried product. It is necessary to understand the rate of frying process, oil penetration into the food, oil-food interactions, oil degradation, and texture development during frying, frying time and chemical changes in food that borders on nutrient depletion.

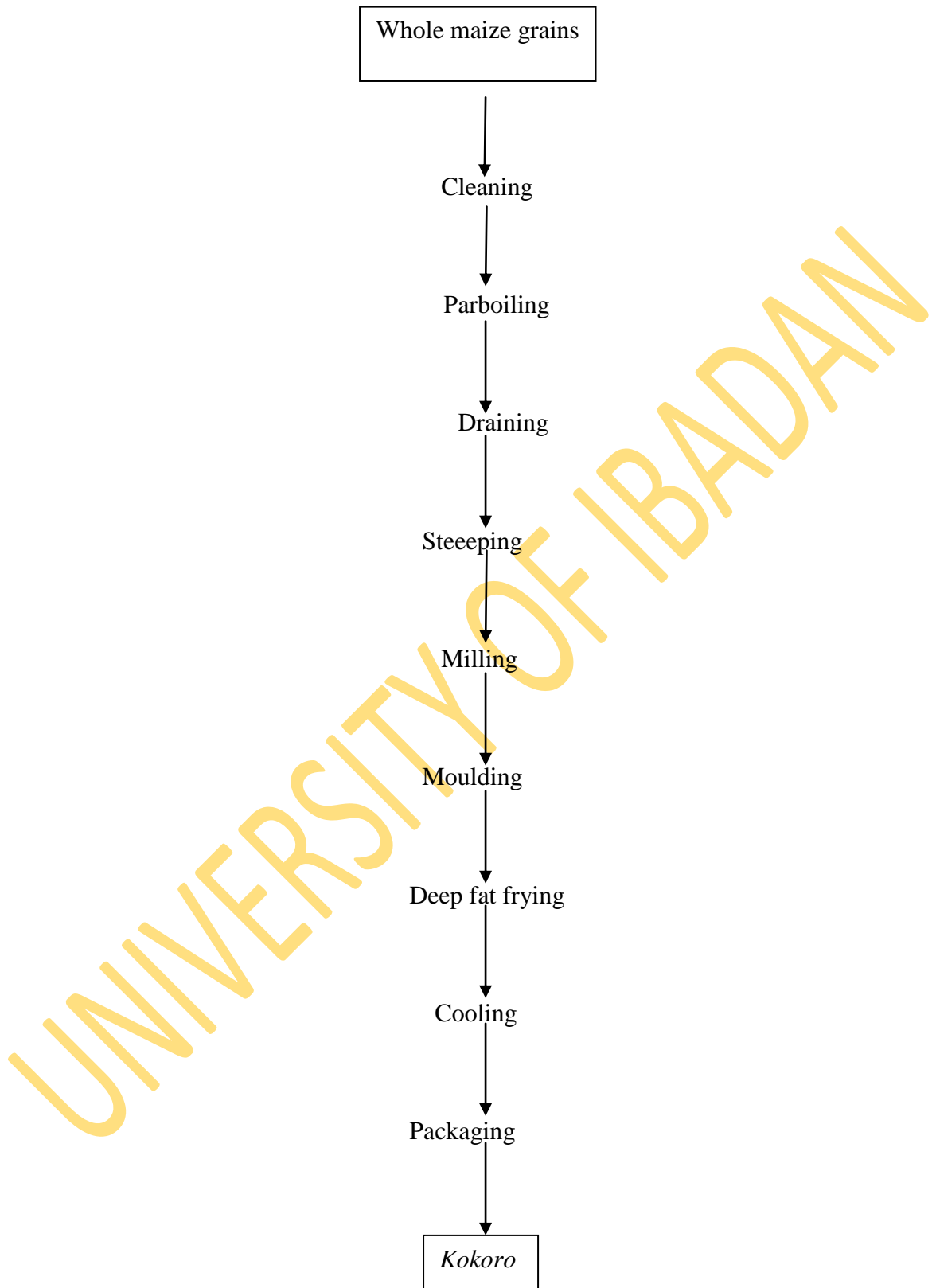


Figure 2.1: Traditional *Kokoro* production (Henshaw and Craig, 1998)

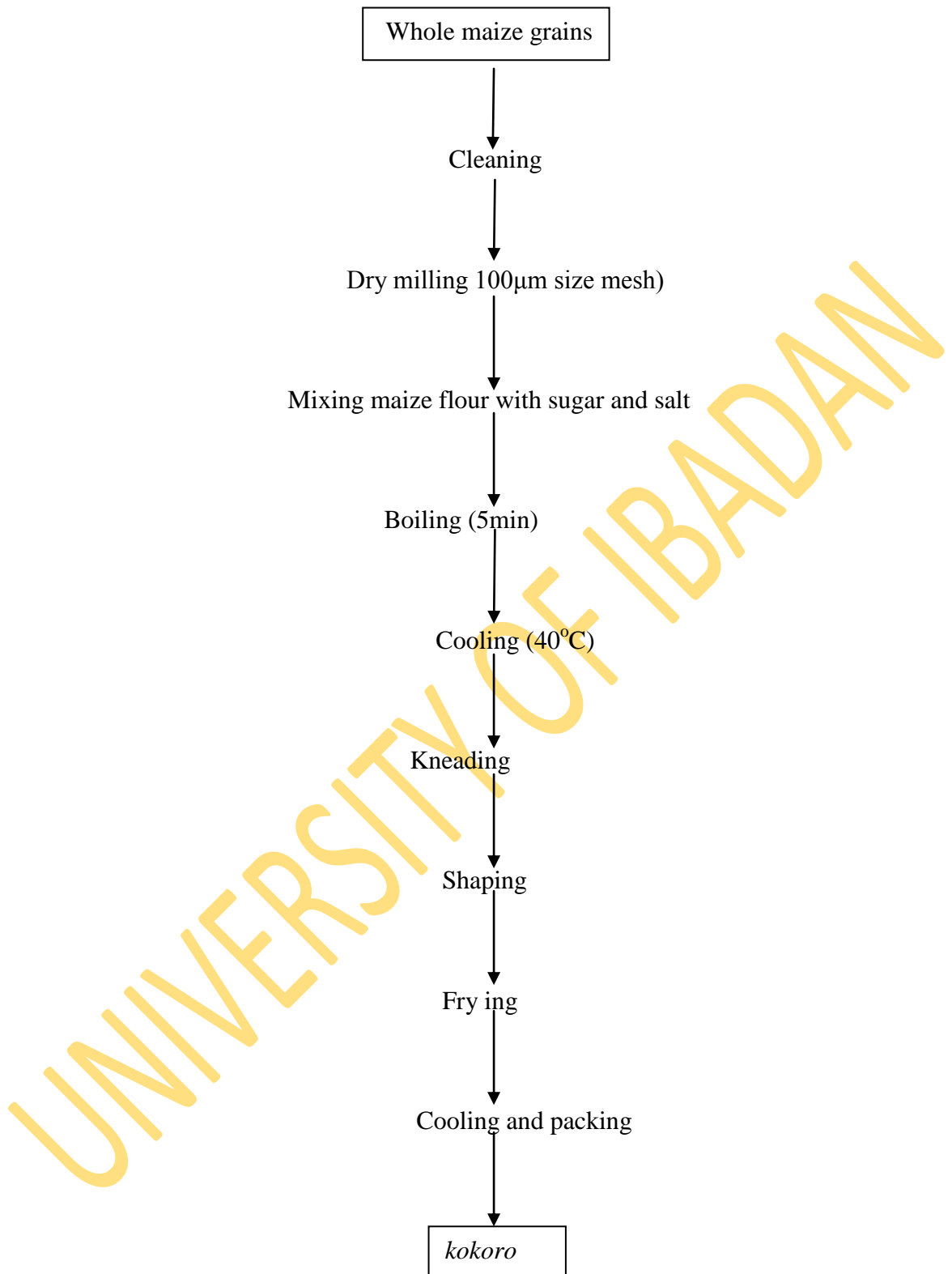


Figure 2.2: Flow chart of *kokoro* production (Uzo-Peters *et. al.*, 2008).

2.6.1 Heat and mass transfer during deep- fat frying

Singh (1995) described two modes of heat transfer taking place during frying as conduction and convection. Conductive heat transfer, under unsteady- state conditions, occurs within a solid food i.e. from the surface to the center of the food. The rate of heat transfer is controlled by the thermal properties of the food, including thermal diffusivity, thermal conductivity, specific heat, and density. The magnitude of these properties changes during frying process (Buhri and Singh, 1994).

Convective heat transfer on the other hand occurs between a solid food and the surrounding oil. As heat is transferred between the food and the oil, there is also moisture migration taking place from the core of the food to the surface. The water bubbles escaping from the surface of the food cause considerable turbulence in the oil (Gertz, 2004). The amount of water vapour bubble escaping from a food material reduces with longer frying time as a result of the reduction in the remaining moisture in the material (Fellows, 2000).

2.6.2 Changes that occur during frying

Significant physical and chemical changes occur during deep-fat frying both in the food material being fried and the oil itself. Fritsh (1991) and Blumenthal (1991) provided a preview of information on these changes that occur in oils as a result of elevated temperature, oxidation, and interactions between oil and water and other food components. Some of these changes in oil include an increase in viscosity of corn oil as reported by Gomes da Silva and Singh (1995) and also a decrease in surface tension with increasing degradation time.

Changes in food as a result of heat transfer during frying include gelatinisation of starch, protein denaturation, water vaporisation, and crust formation (colour and flavour development). Crust formation influences oil uptake. Oil penetration was reported not to go beyond 1mm in fried foods (Smith, 1987). And the amount of oil uptake is a function of food composition like soy supplemented products is known to have lower oil uptake than those with no soybean (Martin and Davis, 1986; Lawhon *et al*, 1975).

Frying oil becomes contaminated with components of food material leaching into oil, water vapour condensing in oil, thermal breakdown of oil, and oxygen absorbed at the oil- air

interface. Some of these contaminants act as surfactant reducing the surface tension of oil (Gertz, 2004). The resultant effect is the increase in wetting of the food surface by the hot oil and influence on mass and heat transfer. Rate of breakdown of oils is potentially increased by higher surfactant levels as a result of increased wetting of heated surfaces. The surfactants entering the food with the oil are suspected to influence moisture pick up by the food during subsequent storage, hence reducing its shelf life.

2.7 Protein Energy Malnutrition (PEM)

The World Health Organization (WHO) defines malnutrition as "the cellular imbalance between the supply of nutrients and energy and the body's demand for them to ensure growth, maintenance, and specific functions". The most common form of malnutrition in Africa is protein energy malnutrition affecting over 100 million people; especially 30-50 million children under 5 years of age and almost additional 200million are at risk (Maziya-Dixon *et al.*, 2006; Maletnlema, 1992). The term protein-energy malnutrition (PEM) applies to a group of related disorders that include marasmus, kwashiorkor, and intermediate states of marasmus-kwashiorkor; which develops in children and adults whose consumption of protein and energy (measured by calories) is insufficient to satisfy the body's nutritional needs (Onis *et al.*, 1993). PEM may also occur in persons who are unable to absorb vital nutrients or convert them to energy essential for healthy tissue formation and organ function (Maureen, 2002). Up till now, protein energy malnutrition (PEM), a known sequel of food insufficiency and poor socio-economic conditions continues to be a major public health problem and a source of major concern in Nigeria (Maziya-Dixon *et al.*, 2006, Dulger *et al.*, 2002).

PEM is grouped into two types; primary and secondary. Primary PEM results from a diet that lacks sufficient sources of protein and/or energy. The major manifestations of this type of PEM are kwashiorkor, marasmus and marasmus-kwashiorkor (Iken *et al.*, 2007; Anthony, 1997). Kwashiorkor, also called wet protein-energy malnutrition, is a form of malnutrition characterised primarily by protein deficiency. This condition usually appears at the age of about 12 months when breastfeeding is discontinued, but it can develop at any time during a child's formative years. It causes fluid retention (oedema); dry, peeling skin; and hair discoloration. The term marasmus is derived from the Greek word *marasmos*,

which means withering or wasting. Marasmus, primarily caused by energy deficiency, is characterised by stunted growth and wasting of muscle and tissue. It usually develops between the ages of six months and one year in children who have been weaned from breast milk or who suffer from weakening conditions like chronic diarrhoea. Marasmic-kwashiorkor is a malnutrition disease, primarily of children, resulting from the deficiency of both calories and protein. The condition is characterised by severe tissue wasting, dehydration, loss of subcutaneous fat, lethargy, and growth retardation

Secondary PEM occurs as a complication of AIDS, cancer, chronic kidney failure, inflammatory bowel disease, and other illnesses that impair the body's ability to absorb or use nutrients or to compensate for nutrient losses (Bennett and Fred, 1996). It can also develop gradually in a patient who has a chronic illness or experiences chronic semi-starvation. It may appear suddenly in a patient who has an acute illness (Anthony, 1997). Secondary PEM symptoms range from mild to severe, and can alter the form or function of almost every organ in the body. The type and intensity of symptoms depend on the patient's prior nutritional status and on the nature of the underlying disease and the speed at which it is progressing. Mild, moderate, and severe classifications have not been precisely defined, but patients who lose 10-20% of their body weight without dying are usually said to have moderate PEM. This condition is also characterised by a weakened grip and inability to perform high-energy tasks (Tavarela, 2004). Losing 20% of body weight or more is generally classified as severe PEM. People with this condition can't eat normal-sized meals. They have slow heart rates and low blood pressure and body temperatures. Other symptoms of severe secondary PEM include wrinkled skin, constipation, drying, thin, brittle hair; lethargy, pressure sores and other skin lesions (Tavarela, 2004).

Food based interventions which involve the establishment of horticultural and home garden projects, whereby support is given to strategic target groups to grow certain crops which could alleviate their dietary deficiency has been used as a strategy to combat PEM (Adewale and Dumet, 2011, Attig *et. al.*, 1993, Smitasiri, 1991;). Nutritional education aimed at getting people to improve their eating habits has also demonstrated positive results in selected situations (Soekirman and Jalal, 1991; Devadas, 1987). Development or promotion of superior plant varieties in terms of their micronutrient content as well as the identification

of processing technologies which maximize vitamin retention are also important food based strategies (Bouis, 1995; FAO, 1992).

2.7.1 Food enrichment

Food enrichment is the process of increasing in a food, the intake level of specific nutrients previously identified as inadequate by the use of another food rich in that specific nutrient (FAO, 2002). This is usually done to prevent micronutrient malnutrition in the developing countries (FAO, 2002). The levels of food enrichment depend on the nutritional needs of the consumers and on both estimated consumption of the enriched food as well as availability of the food product to be used for the enrichment and on the regulations in the country. The enrichment of staple cereal-based foods with legumes shows that there is higher protein content and protein quality and the enriched cereal-based foods showed nutritional value similar to animal protein based products (Umerie *et al*, 2009; Gupta and Kapon, 1980).

Other terminology exists for the addition of nutrients to foods; this includes food fortification, restoration and supplementation (FAO/WHO, 1994). Food fortification has been defined as the addition of one or more essential nutrients to a food, whether or not it is normally contained in the food, for the purpose of preventing or correcting a demonstrated deficiency of one or more nutrients in the population or specific population groups (FAO/WHO, 1994). Enrichment means the addition to a food of essential nutrients which are lost during the course of Good Manufacturing Process (GMP), or during normal storage and handling procedures, in amounts which will result in the presence in the food of the levels of the nutrients present in the edible portion of the food before processing, storage or handling (Probart, 2003). Enrichment on the other hand, has been used interchangeably with fortification, but elsewhere it has been defined as the restoration of vitamin and minerals lost during processing (Hoffpauer and Wright, 1994).

2.7.2. Guidelines to food enrichment

According to the general principles for the addition of essential nutrients to foods within the FAO/WHO food standards programme, the codex alimentarius commission stated that essential nutrients may be added to food in order to achieve any of the following: restoration

of nutrients lost during processing; nutritional equivalence of substitute foods; fortification; and ensuring the appropriate nutrient composition for a special purpose food.

The basic principles for the addition of essential nutrients to foods, as stated by the codex alimentarius commission are (FAO/WHO, 1994):

- The essential nutrient should be present at a level which will not result in either an excessive or an insignificant intake of the added essential nutrient considering amounts from other sources in the diet;
- The addition of an essential nutrient to a food should not result in an adverse effect on the metabolism of any other nutrient;
- The essential nutrient should be sufficiently stable in the food under customary conditions of packaging, storage, distribution and use;
- The essential nutrient should be biologically available from the food;
- The essential nutrient should not impart undesirable characteristics to the food and should not unduly shorten the food shelf life;
- Technology and processing facilities should be available to permit the addition of the essential nutrient in a satisfactory manner;
- Addition of essential nutrients to foods should not be used to mislead or deceive the consumer as to the nutritional merit of the food;
- The additional cost should be reasonable for the intended consumer;
- Methods of measuring, controlling and/or enforcing the levels of added essential nutrients in the foods should be available;
- When provision is made in food standards, regulations or guidelines, for the addition of essential nutrients to foods, specific provisions should be included identifying the essential nutrients which are to be considered or be required and the levels at which they should be present in the food to achieve their intended purposes.

2.8 Micro Nutrient Deficiency

African is not just suffering from the problem of food insecurity but also that of nutrition insecurity. The latter arose as a result of non-availability or insufficient nutrients in the food intakes of the populace. One important nutrition insecurity issue in Africa is micronutrient deficiency in the diet of the people. Micronutrients are those vitamins and minerals needed

in very small amounts that must be present in the diet to stimulate cellular growth and metabolism (Probart, 2003). Kennedy (2003) reported that over 800 million people are not able to meet their daily energy requirements in their diets. More than this number is suffering from micronutrient deficiency. Increasing the amount of bio-available micronutrients such as pro-vitamin A, iron and zinc, in otherwise disease resistant and high yielding hybrids is a potentially more sustainable strategy to improve the micronutrient status for those populations in areas of subsistence farming. Micronutrient-enriched foods could reach larger number of people than nutrient supplements or fortified foods and would be less expensive. In recent years, there has been increasing recognition of the consequences of these micronutrient deficiencies ranging from altered immunity, increased risk of infectious diseases and death, to reduced growth and cognitive development. In Nigeria and other developing countries, emphasis has been on iron, iodine and vitamin A deficiency because most diet is essentially composed of cereals and root crops (Ekop, 2006; Welch and Graham, 1999).

2.8.1 Carotenoids and vitamin A

Carotenoids represent the most ubiquitous group of naturally occurring pigments synthesized only by plants (Mora *et al.*, 1999), whose colour tones range from yellow to orange-red such as tomatoes, carrots and oranges (Mora *et al.*, 1999). Vitamin A activity in animal tissues is in form of retinol. Retinoids are nutritionally active forms of vitamin A. The carotenoids in foods are primarily of plant origin. They include: β -carotene, lycopene, lutein, cryptoxanthin and so on. About 600 carotenoids have been identified, 50 exhibits provitamin A activity, 40 are found to occur in fruits and vegetables, of which only a few are absorbed, metabolized and stocked as liver retinol (Mora *et al.*, 1999).

Vitamin A is a general name for groups of substances that include retinol, retinal, retinoids, carotene and carotenoids (Tolonen, 1990). The active form of vitamin A called performed vitamin A (retinol) are found in animal tissues whereas, the provitamin or precursors forms such as β -carotene are found in yellow, dark green and orange vegetables and fruits, and its content varies with colour intensity of commodities. While the carotene may require bile and fats in the intestine for absorption, the performed vitamin A is not as fat-dependent and

thus it is better absorbed by the body (Kimura and Rodriguez-Amaya, 2003; Robert and Sommer, 1985). Vitamin A is fat-soluble vitamin that is involved in many physiological processes (Takyi, 1999). The best-defined function of vitamin A is vision. It is required for normal functioning of the visual and immune system and for normal growth and tissue repairs (Dijkhuizen *et al.*, 2001).

Vitamin A deficiency (VAD) is a problem in developing countries, it occurs when body stores are depleted and the amount remaining is not sufficient for normal physiological functioning (Mulokozi, 2003). Vitamin A is an essential micronutrient for the normal function of visual system, growth and development, maintenance of epithelial cellular integrity, immune function, reproduction, process of cell differentiation and bone growth (ACC/SCN, 2000). Vitamin A increases resistance to infectious diseases including measles in children (Ross, 1992). Studies have shown that vitamin A is important in maintaining children's health and reducing infant mortality (Rahmethnllah *et al.*, 1990). Vitamin A deficiency is a major public health problem in many areas of the developing world including Nigeria (Akinyele, 1991). The most appropriate and long term approach to VAD prevention is to ensure that diets provide adequate amount of the vitamin (Sungpuag *et al.*, 1999). In most developing countries, the main source of vitamin A for the majority of population is pro-vitamin A from plant sources. At present, even in the developing countries, because of increased scientific evidence linking meat consumption and heart diseases, the focus seem to be on the consumption of more plant materials as substantial sources of many nutrients including micronutrients (Afoakwa, 2007; FAO/WHO, 1988). Plant constituents such as β -carotene, besides having antimutagenic and hypocholesterolemic potentials have the capacity to retard peroxidation and to scavenge dangerous free radicals (Rukmini, 1994). Apart from these advantages, the dietary approach to reducing VAD is being advocated because it is sustainable and provides nutrients other than vitamin A and/or β -carotene and adds variety to the diet (De Pee *et al.*, 1995).

Vitamin A is heat stable with minimal loss from cooking but susceptible to photo-oxidation due to UV rays of sunlight and air, this oxidation can be prevented by vitamin E (Anon., 2000). Several researchers have studied the effects of different methods of cooking. Yellow maize is one of the local dietary sources of β -carotene, to effectively use it for combating

VAD, it is important to obtain accurate analytical data concerning the content and bioavailability of vitamin A in both raw and ready-to-eat form. Thus, there is need to evaluate the effects of processing and cooking procedures on the pro vitamin A content of traditional processed foods (Sungpuag, 1999). Vitamin A activity in food is mainly due to all trans-isomer of retinol, which is the most abundant and biologically active member of the vitamin A group. In diet, β -carotene and other carotenoids provide most of the vitamin A.

Zeaxanthin and lutein are the major carotenoids in maize, with β -carotene and β -cryptoxanthin being present in smaller amounts. β -cryptoxanthin has about one-half of pro vitamin A activity of β -carotene (Kimura and Rodriguez-Amaya, 2003). Lutein and zeaxanthin are vitamin A-inactive, but have important roles in human health in terms of their action against macular degeneration and cataract (Kimura and Rodriguez-Amaya, 2003). Because the absorption of lutein and zeaxanthin will completely mask that of β -carotene, β -carotene is usually separated from lutein, zeaxanthin and β -cryptoxanthin before spectrophotometric measurement. Studies have shown that these carotenoids are susceptible to degradation under certain storage conditions; therefore, appropriate storage conditions are important to the retention of these dietary nutrients in food products.

2.9 Storage Studies

Shelf life is an important property of any food and is of interest to everyone in the food chain from producer to consumer. No single factor may determine the shelf life of a food but the most important factors to be considered in shelf life studies are: microbiological changes, moisture and water vapour transfer, chemical and biochemical changes (Steele, 2004). The standard requires that packed food, with some exceptions, should be date marked and prohibits the sale of packed food after the expiration. A use-by date means the date which signifies the end of the estimated period, if stored in accordance with the stated storage conditions will remain fully marketable and retain the specific quality for which express or implied claims have been made (Man, 2002).

Storage of food at high temperatures can cause changes in food which would not occur at normal ambient temperatures. Also, the normal changes accelerated by high temperatures must be known with acceptable accuracy. Unless a food product has undergone a

commercial sterilization process (e. g. canned foods) or has a water activity which will not permit microbial growth (e.g. sugar, breakfast cereals), the rate of growth of spoilage microorganisms is likely to be the major factor determining shelf life. This rate is determined by a number of factors including: food properties (e. g. pH, total acidity, water activity, presence of preservatives either natural or added); environmental factors (temperature, relative humidity, gaseous atmosphere); any process designed to kill or retard growth of microorganisms (thermal processes, freezing, packaging), the type of micro flora present in the food, and the initial population of microbes in the food (Lillicrap and Cousin, 2010, Adegoke, 2004).

Water activity is a critical factor in determining the quality of food. A gain of water by an initially dry food or loss of water from high moisture content food can lead to a reduction in organoleptic quality and storage stability, hence, the market value and overall acceptability of such food. Most foodstuffs continually regulate their moisture content (mc) value due to their interaction with their surroundings. This phenomenon influences the stability of such food components, but not sufficiently. Some foods are not stable at low moisture content (for example, some fat containing foods like peanut oil) while some are characteristically not stable at high moisture content (for example, some starchy foods).

The amount of water present is not the major concern of food processors, but the amount that is available to support various microbial and chemical reactions that predisposes them to spoilage and deterioration. In studying the availability of water in food, a fundamental property known as water activity (a_w) is measured. This water activity according to Fellows (2000) is the ratio of vapour pressure exerted by the water held by the food to the saturated vapour pressure of water at the same temperature given by this expression:

$$a_w = \frac{P}{P_0}$$

Where P = Water Vapour Pressure exerted by a solution or wet solid.

P_0 = Vapour pressure of pure water at the same temperature

a_w = water activity

Thus, if a food product is in equilibrium with its surrounding atmosphere then the water activity of the food will be numerically equal to the relative humidity (RH) of the atmosphere and the latter will be referred to as equilibrium relative humidity (ERH) of the food. It is expressed by the equation: $a_w = ERH/100$. A plot of moisture content as a function of water activity is known as Sorption isotherm.

2.9.1 Sorption isotherm characteristics

Moisture sorption isotherm are useful thermodynamic tools for determining interaction of water and food substances, and provide information to assess food-processing operations such as drying time, mixing, packaging and storage. Sorption isotherm can also be used to investigate structural features of a food product, such as specific surface area, pore volume, pore size distribution and crystalline property. Such data can be used in selecting appropriate storage conditions, and package system that optimise or maximize retention of aroma, colour, texture, nutrients and biological stability (Lazarides, 1990). Moisture sorption isotherms are graphical representation of food equilibrium moisture content against prevailing relative humidity or water activity at a particular temperature. In most cases, sorption isotherms are non-linear and in the case of food substance the isotherms are of type II and type III of the five types expressed by Bell and Labuza (2000) for adsorption isotherms. Typical isotherm of food is s-shaped or sigmoid shape (Fig.2.3). The curve obtained when a damp food product is placed in dry air and therefore loses its moisture is called the desorption curve; it describes the product's behaviour during a drying process. The curve obtained when a dried product is placed in environment of high RH so that there is weight gain due to water is called adsorption. The adsorption and desorption is expected to superimpose each other, but due to an effect known as Hysteresis, a common phenomenon in most foods, this does not occur.

Food isotherms are often divided into three regions (Fig.2.3) denoted by A, B, and C. These regions describe the way water is held in biological materials (including food). A is known as the monolayer region where water is strongly bound to specific sites on the solid. This water is very stable, unfreezeable and regarded as unavailable as a solvent, hence, does not contribute to microbial or chemical activity. The upper limit of monolayer region referred to as monolayer value is considered to be an optimum water activity, a_w at which food

substance is very stable (Hyun *et al.*, 1991). It is found in the range of 0-0.35 (dry weight basis). Region B is referred to as the multilayer region where water is still bound to solid, but less strongly than in region A. The water here has soluble components. Region C however represents the continued adsorption of additional layer where free water is condensed with the capillary structure of the food. This can easily be removed by drying and it is indicated by the steepness of the curve (Steele, 2004). It is very useful because it gives an idea of food product behaviour during storage.

The relationship between water activity and moisture content of food stuff is important in predicting quality, stability during drying, storage and the selection of appropriate packaging materials for retail purposes (Fellows, 2000; Labuza *et al.*, 1968). Temperature, food composition, RH and pretreatment processes generally influence moisture sorption properties of foods (Falade *et al.*, 2003).

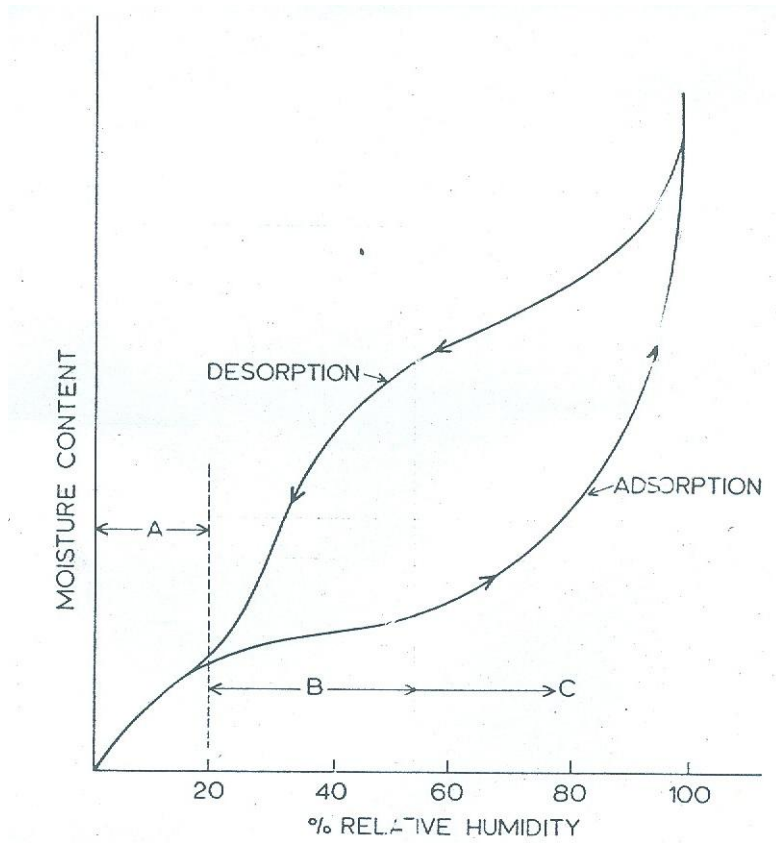


Figure 2.3: A typical Sorption isotherm representation.

Source: Fellows, 2000.

2.9.1.1 Effect of temperature

A change in temperature of any material will result in a definite change in the vapour pressure exerted by the moisture in the product. An increase or decrease in temperature of food at constant moisture content result to an increase or decrease (respectively) in equilibrium relative humidity (ERH) however the magnitude. Also, increase in temperature at constant ERH results in a decrease in moisture content, but for crystalline products, this trend is exhibited till 0.6 a_w where the reverse is the case i.e. increase in ERH brings increase in moisture content. This is accounted for by the dissolution of the hygroscopic crystalline substance in the food (Johnson and Brennan, 2000).

Sorption isotherm of a food material is best described as a plot of the amount of water absorbed as a function of the relative humidity or activity of the vapour space surrounding the food material (Sopade *et al.*, 1995). Under a given vapour pressure of water in the surrounding air, a food product attains an equilibrium moisture content (EMC). A food product is equilibrated at various water activity levels by exposing it to series of atmosphere of constant relative humidity for a period of time. When it reaches constant weight, the water content is measured by gravimetric analysis.

2.9.1.2 Effect of food composition

The equilibrium vapour pressure above a food is determined not only by the temperature but also by the water content of the food, by way in which water is bound in the food, and by the presence of constituents soluble in water. Sorption isotherms vary with different types of foodstuffs; at low a_w , proteineous and starchy food absorb more water than fatty material or crystalline substances. This impact varied the sigmoidal shape of moisture sorption. The sorption isotherm, which is largely dependent on the degree of distribution of the components, on the inner and outer surface of the constitution of a dried foodstuff, gives information about the type of water binding and distribution of the water, whether it is more or less firmly bound, or is easily mobilized and available for certain reactions (Fellows, 2000). The relationship between water content and the relative humidity in the characteristic pattern of the sorption isotherm, contributes to the enzymatic processes in decomposition. Therefore, foods whose water content lies in the monomolecular absorption area are to a

large extent, protected from enzymatic decomposition (Falade *et al.*, 2003; Ihekoronye and Ngoddy, 1985). Enzymatic changes occur only in significant amount when the water content is above this area on the sorption isotherm. The lipolytic processes are an exception and occur when the substrate is present in the form of a liquid aggregate.

2.9.2 Lipid oxidation

Fat and oil also play an important role in the flavor, aroma, texture and nutritional quality of foods, pet food and feeds. Predicting fat and oil quality is an important component of developing and manufacturing high quality products. As soon as a food, feed or ingredient is manufactured, it begins to undergo a variety of chemical and physical changes. One of which is oxidation of lipid, an undesirable chemical change that imparts flavor, aroma, nutritional quality and even texture of product (Mudambi *et al.*, 2006; Anon, 2000). Oxidation results in the replacement of an oxygen ion for a hydrogen ion in the fatty acid molecules; this destabilizes the molecule and makes it possible for all chemical fragments to find a place along the chain leading to an unpleasant change in the flavor of food called rancidity. Unsaturated fats are more susceptible to oxidation than saturated fats.

Factors that accelerate fat oxidation include presence of trace metals (iron, zinc etc) salt, heat, light, water, bacteria, and moulds. Fat oxidation can be retarded by use of anti-oxidants (such as BHT, BHA, vitamin E), or spices such as sage and rosemary and the use of light and/or air tight wrapping (Foskett *et al.*, 2003; Anon., 2000).

The chemicals produced from oxidation of lipids are responsible for rancid flavours and aromas. Vitamin and other nutrients may be partially or entirely destroyed by highly reactive intermediates in the lipid oxidation process. Oxidized fats can interact with proteins and carbohydrates to cause changes in texture (Anon., 2000). Of course, not all lipid oxidation is undesirable. Enzymes, for example, promote oxidation of lipid membrane during ripening of fruit. For most products, predicting and understanding oxidation of lipids is necessary to minimize objectionable flavours and aromas, arising from fat rancidity.

One of the major problems in the storage of any food material is the prevention of deleterious changes resulting in the production of off flavours, colour defects and down

grading of the products. Two principal types of rancidity are: hydrolytic rancidity and oxidative rancidity.

Hydrolytic rancidity involves the breakdown of lipids into smaller units, fatty acids. The ester linkages of lipids are subject to hydrolysis and may result in off-flavour. Lipolytic enzymes are widely distributed in plants, animals and microorganisms. Lipases hydrolyse triglycerides in a stepwise fashion. Fatty acids produced from the hydrolytic action of triglycerides can lead to off-flavours. Titration of the free fatty acids formed by the action of lipases has been the most widely used procedure in determination of free fatty acid.

Oxidative rancidity occurs in all fats and fat containing foods which contain some unsaturated fatty acids, hence, are potentially susceptible to oxidative rancidity (Anon., 2000). Types of oxidation include;

- Auto oxidation-autocatalytic process involving oxidation by molecular oxygen.
- Photo oxidation-autocatalytic process involving molecular oxygen but one in which the initial stages are catalyzed by light.
- Enzymatic oxidation- initial stages are characterised by an enzyme, lipoxidase.

Auto-oxidation involves the oxidation of the unsaturated and polyunsaturated fats and fatty acids (PUFA). The major parts of oxidation proceed through free radical chain reactions (Anon., 2000).

The major consequence is that when a free radical is produced, the high reactivity of the radical with oxygen causes rapid conversion to peroxide or hydroperoxide, this initiates a chain reaction and is responsible for rancid off-flavour produced and myriad of other reactions which reduce shelf life and nutritional value (Drummond and Brefere, 2007; Ihekoronye and Ngoddy, 1985).

2.9.2.1 Lipid oxidation test

The quality of fried food depends greatly on the quality of the frying oil that is absorbed. In deep fat frying, thermo oxidative and hydrolytic reaction takes place resulting in quality

deterioration of the frying oil. Degradation of the frying oil which is absorbed in the fried product, depend on the presence of unsaturated fatty acids (Pangloli *et al.*, 2002). There are various methods used in determining /analyzing foods. Foods which contain high concentration of unsaturated lipids are highly susceptible to lipid oxidation. Lipid oxidation is a form of spoilage in foods because it leads to production of off flavor and potential toxic compounds. It involves numerous reactions and their intermediates. It gives rise to a variety of changes which could be chemical or physical changes. The primary products of the reaction include; peroxides and conjugated diene and the primary products go to secondary products which include; ketones, aldehydes, alcohols and hydrocarbons (Mudambi *et al.*, 2006; Anon., 2000).

Since peroxides are primary products formed in the initial stages of lipid oxidation, concentration of peroxide value can give indication of progress in lipid deterioration. The common approach is to determine peroxide value, utilizing the ability of peroxide to liberate iodine in presence of excess potassium iodide (KI). The higher the peroxide value, the higher the number of peroxides formed. Peroxide value gives an idea of the onset of lipid oxidation (Mudambi *et al.*, 2006; Ihekoronye and Ngoddy, 1985). Peroxide values are not static and care must be taken in handling and testing samples. It is difficult to provide a specific guideline relating peroxide value to rancidity. High peroxide values are a definite indication of a rancid fat, but moderate values may be as a result of depletion of peroxides after reaching high concentrations.

Free fatty acid however, is an indication of hydrolytic rancidity, but other lipid oxidation processes can also produce acids. It may also be useful to know the composition of the free fatty acids present in a sample to identify their source and understand the cause of their formation. Free fatty acids in a fat (or fat extracted from a sample) can be determined by titration. The FFA value is then expressed as % of a fatty acid common to the product being tested. Values are expressed as % oleic acid for tallows or soybean oils or as % lauric acid for coconut oils and other oils that contain high levels of shorter chain fatty acids (Anon., 2000, Pearson, 1976). Due to the limitations of peroxide value highlighted above, FFA value will be more preferable as an indication of rancidity in the products.

2.10 Microbiological Analysis

Bacteria, yeast and moulds attack virtually all constituents of food. Flour is a dried product and the removals of water prevent microbial growth. There is always an optimum water activity for maximum growth. As the water activity decreases, microbial growth decreases until it gets to a stage where it ceases. Micro-organisms vary in water activity required for growth; bacteria are the most sensitive, followed by mould and yeast. Bacteria do not grow at water activity less than 0.9, yeasts are inhibited by water activity less 0.88 while mould will not grow at water activity less than 0.88 (Adegoke, 2004; Frazier and Westhoff, 1988).

Microbial contamination may occur during processing of the indigenous snack foods. The water activity at which the products is stored will also influence the viability of micro organisms (Adegoke, 2004). The processor, processing equipment, processing techniques and the processing environment can be a source of microbial contamination; therefore, it is important to ensure good sanitation of the processor, processing equipment and the processing environment.

2.11 Sensory Evaluation

Three fundamental types of sensory tests include; preference/acceptance test, discriminatory test and descriptive test. Preference /acceptance tests are effective tests based on a measure of preference or a measure from which relative preference can be determined. The personal feeling of a panelist towards the product directs his response. Discriminatory tests are used to determine whether a difference exists between samples. The panelist does not allow his personal likes or dislikes to influence his response. Preference test was used in this research work. Preference test includes; the paired comparison test, the hedonic scale and ranking (Iwe, 2002).

CHAPTER THREE

3. MATERIALS AND METHODS

3.1 Materials

Samples of white maize-TZL Comp 4C2 (usually used by local producers) and improved variety of yellow maize grains (BR 9928-DMR-SR-Y) were obtained from the International Institute of Tropical Agriculture (IITA), Ibadan, Oyo State, Nigeria. African yam bean seeds (AYBS) (*Sphenostylis stenocarpa*) (Tss-9 and Tss-30) were obtained from local market in Umuahia, Abia state, Nigeria. Commercial *kokoro* used for the comparative sensory evaluation with “formulated” *kokoro* was obtained from the cottage industry in Abeokuta. Other ingredients such as frying medium (vegetable oil), seasonings, salt, and onions were obtained from a retail market.

3.2 Analysis of Maize and AYBS Varieties for Processing Suitability

Functional properties analysis of the two maize varieties shown in Plate 3.1 were carried out using standard procedures. Using AOAC (2005) methods, chemical composition (moisture content, fat, ash, crude protein and carbohydrate content), beta-carotene content and physical properties (average seed-weight, length to diameter ratio, bulk density) were analyzed. The variety with superior processing suitability, highest nutrient density in terms of crude protein, fat, ash and beta-carotene content, then AYB seed with better ease of dehulling were selected as suitable for processing.

3.2.1. Dehulling Test for AYBS varieties

Dehulling test was carried out on two selected accessions of AYB (Plate 3.3) using seed/water ratio of 1:10 and the rate of water absorption was reported as increase in weight. The variety with the highest ease of dehulling was identified manually by peeling with fingers tip.



Plate 3.1: Maize cultivars used

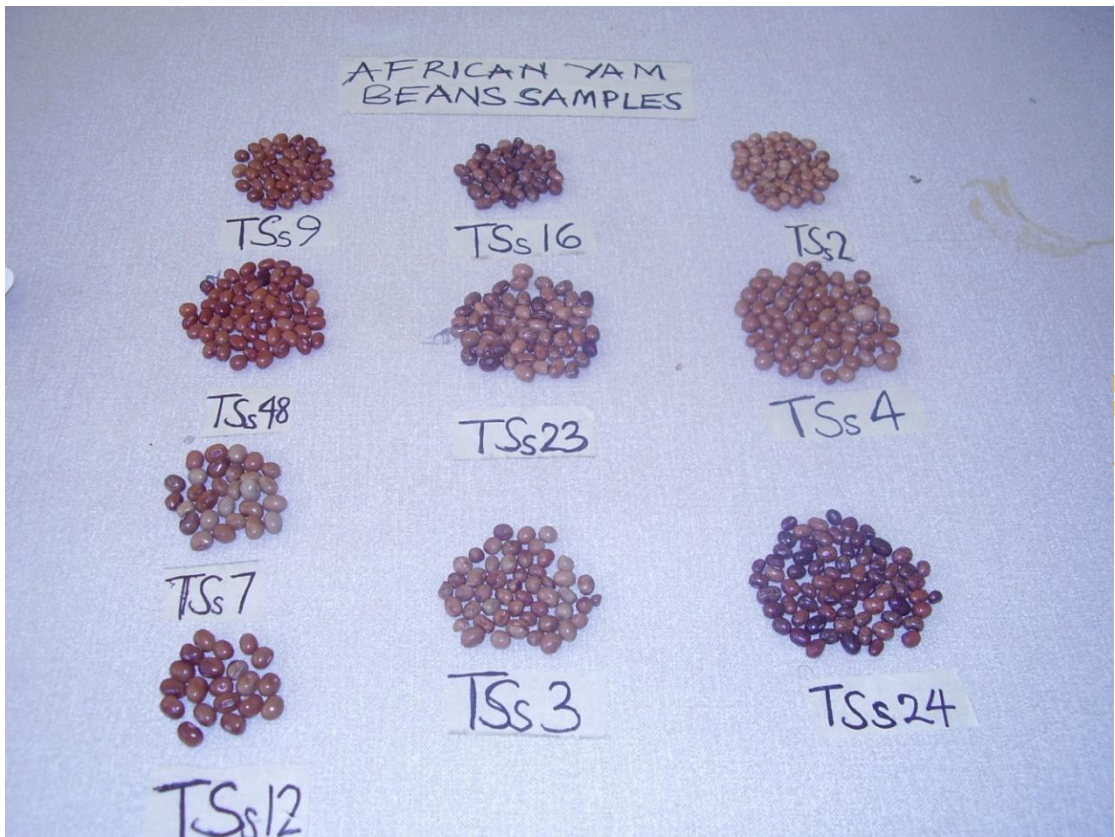


Plate 3.2 Some African yam bean accessions

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Plate 3.3 African yam bean accessions selected for dehulling test

UNIVERSITY

3.3. Analytical Procedures

Physical, functional, physico-chemical and chemical characteristics of the maize grains were carried out using standard procedures.

3.3.1. Physical properties of maize cultivars

Length, breadth and thickness of grains of each variety were measured using vernier calipers (with accuracy 0.001 m). Thousand grains were manually counted and weight was recorded (Punita, 2006). Volume of thousand grains was assessed by water displacement method. Bulk density, B was determined using the formula given below.

$$B (g/ml) = \frac{\text{thousand grain weight (g)}}{\text{thousand grain volume (ml)}} \dots\dots\dots 3.1$$

3.3.2 Functional properties of the maize cultivars

3.3.2.1 Bulk density of maize flours

This was determined using the method of Narayana and Narasinga Rao (1984). A calibrated centrifuge tube was weighed and filled with samples to 5 ml by constant tapping until there is no further change in volume. The tube and contents was weighed and the weight of samples was determined by difference.

$$\text{Bulk density } \left(\frac{g}{ml}\right) = \frac{\text{weight of sample}}{\text{volume occupied}} \dots\dots\dots 3.2$$

3.3.2.2 Water absorption capacity (WAC)

This was determined according to Solsulski (1962). Each of the flour blend (1 g) was weighed and 15 ml distilled water was added in a weighed 50 ml centrifuge tube. The tube was agitated on a vortex mixer for 2min and centrifuge at 4000 rpm for 20 min. The clear supernatant was decanted and the volume was measured and discarded. The adhering drops of water were removed and the tube reweighed. Water absorption capacity was expressed as the weight of water bound by 100 g dry flour.

3.3.2.3 Oil absorption capacity (OAC)

Oil absorption capacity (OAC) was determined according to the method of Solsulski *et al.*, (1976). To 1 g of the flour, 10 ml of refined corn oil was added in a weighed 50 ml centrifuge tube and agitated on a vortex mixer for 2 min and centrifuged at 4000 rpm for

20 min. The volume of the free oil was recorded and discarded. The tube was weighed with the content. Oil absorption capacity was expressed as ml oil bound by 100 g dry flour.

3.3.2.4 Least gelation concentration (LGC)

The method of Coffman and Gracia (1977) was used in the determination of LGC. Appropriate sample suspensions were weighed into 5 ml distilled water each to make 2-20% (w/v) suspension. The test tubes containing these suspensions were heated for 1 h in boiling water (bath) followed by rapid cooling under running tap water. The test tubes were further cooled for an hour under running water and the Least Gelation Concentrations (LGC) was determined as the concentration when the sample from the inverted test tube did not fall or slip.

3.3.2.5 Swelling capacity

Swelling capacity was determined by the modified method of Riley *et al.* (2006). Milled sample was dried to a constant weight, dried sample (1g) was weighed into 50ml centrifuge tube and 15ml of distilled water was added and shaken for 5 min at low speed. The slurry was heated in a thermostatic water bath (THELCO model 83, USA) at 80° C for 40 min. During heating, the slurry was stirred gently to prevent dumping of the starch. The content was transferred into a pre weighed centrifuge tube and 7.5 ml distilled water was added. The tubes containing the paste were centrifuged at 2,200 rpm for 20 min using SORVALL GLC-1 centrifuge (model 06470, USA). The supernatant was carefully decanted into a pre-weighed can and dried at 100°C to constant weight. Then cooled in a desiccator and weighed. The weight of the precipitate and the centrifuge tube was also recorded.

1. Wt of empty can = A
2. Wt of can + dried supernatant = B
3. Wt of soluble = A-B
4. Wt of empty centrifuge tube = D
5. Wt of centrifuge + sediment = E
6. Wt of sediment = E-D

$$\text{Swelling power} = \frac{\text{wt of sediment}}{\text{sample wt} - \text{wt of soluble}} \dots\dots\dots 3.3$$

$$\% \text{ Soluble} = \frac{\text{wt of soluble}}{\text{wt of sample}} \times 100 \dots\dots\dots 3.4$$

3.3.2.6 Amylose content

Amylose content was determined according to the method described by Williams *et al.*, (1958). About 0.1 g of flour sample was weighed into a test tube. To this, 1 ml of 95% ethanol and 9 ml 1M NaOH were carefully added and vortexed with the mouth of the test tube covered. The samples were then heated for 10 min in a boiling water bath to gelatinize the starch, and then allowed to cool to room temperature. Ten (10) times dilution of the extract was made by taking 1 ml of the extract and made up to 10 ml with distilled water. An aliquot of 0.5 ml from the diluents was taken for analysis. Acetic acid solution (0.1 ml) and 0.2 ml of iodine solution were added. The volume was made up to 10 ml with distilled water. The test mixture was left for 20 min for color development after which it was vortexed and the absorbance read at 620 nm.

Calculation:

$$\% \text{ Amylose of sample} = \frac{\% \text{ Amylose of standard} \times \text{absorbance of sample}}{\text{Absorbance of standard}} \dots\dots\dots 3.5$$

Note: The amylopectin content of the flour samples was recorded as 100-% Amylose content.

3.3.2.7 Pasting properties

The pasting characteristics of the flour samples was determined using a Rapid Visco Analyzer (Model RVA-Super 4, Newport Scientific Perten Instruments AB, Huddinge, Sweden) interfaced with a personal computer equipped with the Thermo cline software supplied by same manufacturer. About 3 g of flour samples (moisture content already determined to be less than 12%) were weighed into a canister and made into slurry by adding 25 ml of distil water. This canister (covered with a stirrer) was inserted into the RVA. The heating and cooling cycles were automatically programmed in the following manner. The temperature was kept within 60 °C to 99 °C while maintaining a rotation speed of 160 rpm. The whole cycle was completed within 13 min. The viscosity was expressed in Centipoises (cp). The following parameters were determined automatically by the instrument: peak viscosity (the maximum viscosity during pasting), breakdown viscosity

(the difference between the peak viscosity and the minimum viscosity during pasting), setback viscosity (the difference between the maximum viscosity during cooling and the minimum viscosity during pasting), final viscosity (the viscosity at the end of the RVA run), pasting temperature (°C) (the temperature at which there is a sharp increase in viscosity of flour suspension after the commencement of heating) and peak time (min) (time taken for the paste to reach the peak viscosity) .

3.3.3 Chemical composition of the maize cultivars

3.3.3.1 Moisture content

Moisture content was determined using the method of AOAC (2005). Flour sample (3 g) was weighed into a pre-weighed clean dried dish, after which the dish was placed in a well-ventilated oven (draft air Fisher Scientific Isotemp R Oven model 655F) maintained at 103 ± 2°C for 24 h. The loss in weight was recorded as moisture.

$$\% \text{ Moisture content} = \frac{M_1 - M_2}{M_1 - M_0} \times 100\% \quad \dots\dots\dots 3.6$$

Where M_0 = Weight in g of dish

M_1 = Weight in g of dish and sample before drying

M_2 = Weight in g of dish and sample after drying

Note that $M_1 - M_0$ = weight of sample prepared for drying

3.3.3.2 Total ash content

This was determined by the method of AOAC (2005). It involves burning off all organic constituents at 550 °C for 6 h in a furnace (VULCAN™ furnace model 3-1750). Crucibles were washed, dried and allowed to cool in the dessicators. Each sample (3 g) was weighed into weighed crucibles. The weight of the residue after incineration was recorded as the total ash content.

$$\text{Ash content} = \frac{W_3 - W_1}{W_2} \times 100 \quad \dots\dots\dots 3.7$$

W_3 = Wt. of crucible+ ash

W_2 = Wt of sample only

W_1 = Wt. of crucible

3.3.3.3 Crude protein content

Crude protein was determined using Kjeldahl method (AACC 2005, Method 46-12.01). Exactly 0.2 g of sample was weighed into digestion tube and one tablet of Kjeldahl catalyst (copper) and 4ml of conc. H₂SO₄ were added. This was transferred into a fume cupboard and 4ml of H₂O₂ was added, fuming was allowed to stop. The mixture was placed on Tecator digestion block pre-set at 420° C and digested for 1h; at the end of which all organically-bound nitrogen was converted to Ammonium Hydrogen Sulphate. With the addition of a strong alkali (NaOH, 40 %) and the application of heat, ammonia NH₃ was distilled out, and collected in 1 % boric acid receiver solution containing Bromocresol green/methyl red mix indicator. Blanks were prepared and treated similarly. Rack of digestion tubes was removed from the block and allowed to cool to room temperature.

The tube containing the blank sample was placed in the distillation unit of the system, and the weight of the sample to be analyzed was entered using the key board on the system and the system was programmed to automatically perform the distillation and titration of the sample. Likewise, in turns, the tubes containing the samples' digest were placed in the distilling unit of the system. The system was also programmed to automatically perform the distillation and titration. Results were displayed automatically at the end of each analysis according to equation 3.8

Calculation:

$$\% \text{ g Nitrogen} = \frac{(\text{sample titre} - \text{blank titre}) \times M \times 14.007 \times 100}{\text{Sample weight (g)}} \quad \dots\dots\dots 3.8$$

$$\% \text{ Protein (crude)} = \% \text{ g Nitrogen} \times \text{Conversion factor.} \quad \dots\dots\dots 3.9)$$

M = Molarity of the acid.

3.3.3.4 Crude fat content

An automated method (Soxtec System HT2; AACC, 2005) was used to determine crude fat. About 3 g of sample was weighed; tranfered into a clean thimble plugged with cotton wool and inserted into the Soxtec HT apparatus. Clean pre-weighed extraction cup containing 50

ml n-hexane was placed on the heating mantle of the apparatus previously heated up to 120° C; and then the thimble containing the sample was lowered into it. This set up was left in this boiling position for 15 min. After the extraction, the thimble (i.e. sample) was lifted up and left in the rinsing position for 45min. Thereafter, air knob was turned on and the hexane was allowed to evaporate for some 10min. Extraction cup was further dried in hot-air oven for 20-30 min at 105° C to rid it of residual hexane. This was cooled in the dessicator and weighed. Fat content was calculated as follows:

$$\%Fat = \frac{(Wt\ of\ flask\ +\ fat) - (Wt\ of\ sample\ after\ drying)}{Wt.\ of\ sample} \times 100 \dots\dots\dots 3.10$$

3.3.3.5 Starch and sugar content

This was carried out according to the method described by AOAC, (2005). Finely ground sample (0.02g) was weighed into centrifuge tubes and 1ml of 95% ethanol was added, followed by 2ml of distilled water and 10ml hot ethanol. The mixture was vortexed and centrifuged at 2000 rpm for 10 min. The supernatant was collected and used for free sugar analysis, while the residue was used for starch analysis.

To the residue 7.5ml of concentrated perchloric acid was added and allowed to hydrolyze for 1h. It was then diluted to 25ml with distilled water and filtered through whatman No. 2 filter papers. From the filtrate 0.05ml was taken, made up to 1ml with distilled water, vortexed and the color was developed by adding 0.5ml phenol followed by 2.5ml of conc. H₂SO₄. This was vortexed, allowed to cool to room temperature and the absorbance was read on a spectrophotometer (Milton Roy Company, USA, Model Spectronic 601) at 490nm. To the supernatant made up to 20ml with distilled water, an aliquot of 0.2ml was taken, 0.5ml (5%) phenol and 2.5ml conc. H₂SO₄ was added. This was allowed to cool and the absorbance read at 490nm.

The glucose standard solution was prepared by weighing 0.01g of D-glucose into a 100ml volumetric flask. This was dissolved and made up to 100ml mark with distilled water. 0.1, 0.2, 0.3, 0.4 and 0.5ml of the stock (100µg/ml glucose) solution was dispensed into test

tubes and each was made up to 1.0ml with distilled water. This corresponds to 10, 20, 30, 40 and 50µg glucose per ml. This was then followed by the addition of 0.5ml of 5% phenols and 2.5ml of H₂SO₄, vortexed, cooled and the absorbance read at 490nm.

Then a graph (standard glucose curve) of Absorbance against Concentration was plotted to determine the slope and intercept.

$$\%Sugar = Abs - Intercept \times \frac{Dilution\ factor \times volume}{weight\ of\ sample \times slope \times 10,000} \dots\dots\dots 3.11$$

Where:

Abs. = Absorbance; Dilution factor = 5; Volume = 20ml

$$\%Starch = Abs - intercept \times \frac{Dilution\ factor \times Volume \times 0.9}{weight\ of\ sample \times slope \times 10,000} \dots\dots\dots 3.12$$

Where:

Abs. = Absorbance; Dilution factor = 20; Volume = 25ml.

Note: The slope and intercept used for the calculations was from standard glucose curve.

3.3.3.6 Mineral analysis

Mineral analysis was done by dry ashing (AOAC, 2005). About 0.5 g of the sample was weighed into a clean ceramic crucible and the weight was recorded to the nearest 0.001 g. One empty crucible was included for a blank and all placed in a cool muffle and ramp temperature to 500° C and heated up for over a period of 2 h. It was allowed to remain at 500 °C for an additional 2 h, to obtain grey appearance after which the sample was allowed to cool down in the oven. The sample was removed from the oven making sure that the environment was breeze-free. The ashed sample was poured into already labeled 50 ml centrifuge tubes and the crucible was rinsed with 5 ml distilled water into the centrifuge tube. The crucible was rinsed again with 5 ml of aqua regia. This was repeated two more

times to make a total volume of 20 ml. The sample was vortexed for proper mixing and then centrifuged for 10 min at 3000 rpm. The supernatant was decanted into clean vials for minerals determination using atomic absorption spectrophotometer.

Note: Aqua regia solution was prepared thus: in a 2 L volumetric flask, about 1.2 L of distilled water was added while 400 ml conc. HCl and 133 ml of 70% Nitric acid were carefully added, the 2 L volumetric flask was made up by distilled water.

3.3.3.7 Crude fibre determination

This was determined using method, 962.09E of AOAC (2005). Sample (1 g) was weighed into 500 ml flask and 100 ml of TCA digestion reagent was added. This was allowed to boil and reflux for exactly 40 min counting from the time boiling started. The flask was removed from the heater, cooled a little bit and filtered through no. 4 Whatman filter paper of known weight. The residue was washed six times with hot water and once with industrial spirit. The filter paper was folded and put in previously ignited porcelain dish of known weight and dried overnight at 105 °C in the oven. This was removed, cooled in the dessicator for 45 min and its weight was recorded. The sample and filter paper was burnt on hot plate for about one hour before transferring to muffle furnace at 600 °C for 5 h. After ashing, the dish was cooled in the dessicator and weighed.

% Crude fibre= Difference in weight x100

3.3.4 Determination of colour parameters

Colour parameters (L^* , a^* and b^*) were measured with a colorimeter (Color-Tec-PCM TM, Omega Engineering Inc., Stanford, CT) (Appendix 1). The instrument was standardised each time with a white paper and a black ceramic plate. Samples were scanned at five different locations to determine the L^* , a^* and b^* values as the average of the five determinations (Liu-ping et al, 2005).

3.3.5 Beta carotene content determination

Carotenoid analysis was done using the method of Julie and Sherry (2006). The extraction of carotenoid from dried maize (0.6 g) was done by adding ethanol (6 ml) containing 0.1% BHT and mixed by vortex. The mixture was subjected to ethanol precipitation for 5 min in the water bath at 85°C.

Potassium hydroxide (500 µl, 80% w/v) was added to the mixture to saponify the interfering oil. Samples were vortexed and placed in a water bath (85°C) for 5 min, vortexed again and returned to the water bath for additional 5 min. Upon removal, the samples were immediately placed in an ice bath where 3mls of cold deionized water was added. Carotenoids were separated 3 times with addition of 3 ml of Hexane, vortexed and then centrifuged (1200 rpm) for 5 min. The combined hexane fractions were washed with deionized water 4 times, vortexed and centrifuged for 5 min at 1200 rpm. The Hexane fractions were dried down in a concentrator under Nitrogen gas. Samples were reconstituted in Methanol/Dichloroethane (1ml, 50:50v/v) and 100µl of the samples were injected into the HPLC.

A Waters HPLC system (Waters corporation, Milford, MA) consisting of a guard- column, C30 YMC Carotenoid column (4.6x250mm, 3µl), Waters 626 binary HPLC pump, 717 auto-sampler and a 2996 photoiodode array detector was used for Carotenoids quantification.

Solvent A: consisted of Methanol/Water (92:8v/v) with 10 mM ammonium.

Solvent B: consisted of 100% Methyl tert-butylether.

Gradient elution was performed at 1ml/min.

3.4 Experimental Design

Response surface methodology, Box Behnken rotatable design was used to study the effects of the independent variables on the quality attributes of *kokoro*. The three independent variables chosen were: frying temperature (X_1), frying time (X_2) and % AYBF in the flour blend (X_3). Three levels of each of the three independent variables (the central value and

interval between the levels) were chosen according to pretest studies (Myers and Montgomery, 1995; Box and Behnken, 1960). Seventeen experimental runs were performed according to the experimental design configured for three factors. The coded and the actual values are shown in Table 3.1.

The statistical analysis of the data was conducted using SAS 9.2 version (SAS, 2003), significance expressed at $p=0.05$ level. For the optimisation procedure, differences in quality attributes of samples for the different conditions were studied by analysis of variance, using Tukey's test with a 95% confidence intervals for the comparison test means. Data were analyzed by multiple linear regressions using the method of least squares to fit second order polynomial model for dependent variables (Eq. 3.13).

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 \dots 3.13$$

Where Y is dependent variable (response), $X_1 - X_3$ are coded independent variables and $\beta_0, \beta_1 - \beta_3, \beta_{11} - \beta_{33}$ and $\beta_{12} - \beta_{23}$ are the equation regression coefficients for intercept, linear, quadratic and interaction effects respectively. The significance of the equation parameters for each dependent variable was assessed by F-test. Six dependent variables (responses) were considered to evaluate the effect of frying temperature, frying time and maize: AYB substitution level. These are protein (Y_1), oil (Y_2), and moisture contents (Y_3), appearance (Y_4), overall acceptability (Y_5) breaking force/crispness/textural quality (Y_6).

3.4.1 Optimisation process

In order to deduce workable optimum conditions, the graphical optimisation technique was adopted by minimizing responses with undesirable features and maximizing responses of desirable features. Protein content, crispness and general acceptability were maximized while fat and moisture contents were minimized.

Table 3.1. Custom Response Surface Box Behnken Experimental Design

| Exp.run | Coded variables | | | Actual Values | Responses | | | | | | | |
|---------|-----------------|----------------|----------------|---------------|---------------------|----------------------|--------------------|----------------|----------------|----------------|----------------|----------------|
| | X ₁ | X ₂ | X ₃ | | X ₁ (°C) | X ₂ (min) | X ₃ (%) | Y ₁ | Y ₂ | Y ₃ | Y ₄ | Y ₅ |
| 1 | -1 | -1 | 0 | 150 | 8 | 30 | | | | | | |
| 2 | -1 | 1 | 0 | 150 | 12 | 30 | | | | | | |
| 3 | 0 | 0 | 0 | 160 | 10 | 30 | | | | | | |
| 4 | -1 | 0 | 1 | 150 | 10 | 40 | | | | | | |
| 5 | 0 | -1 | 1 | 160 | 8 | 40 | | | | | | |
| 6 | 1 | 0 | 1 | 170 | 10 | 40 | | | | | | |
| 7 | 1 | 0 | -1 | 170 | 10 | 20 | | | | | | |
| 8 | 0 | 1 | -1 | 160 | 12 | 20 | | | | | | |
| 9 | 0 | -1 | -1 | 160 | 8 | 20 | | | | | | |
| 10 | 0 | 0 | 0 | 160 | 10 | 30 | | | | | | |
| 11 | 0 | 0 | 0 | 160 | 10 | 30 | | | | | | |
| 12 | 0 | 0 | 0 | 160 | 10 | 30 | | | | | | |
| 13 | 0 | 1 | 1 | 160 | 12 | 40 | | | | | | |
| 14 | 1 | 1 | 0 | 170 | 12 | 30 | | | | | | |
| 15 | -1 | 0 | -1 | 150 | 10 | 20 | | | | | | |
| 16 | 1 | -1 | 0 | 170 | 8 | 30 | | | | | | |
| 17 | 0 | 0 | 0 | 160 | 10 | 30 | | | | | | |

Exp.runs=experimental runs

Where X₁ =frying temperature (°C), X₂= frying time (min) and X₃=% African yam bean flour inclusion in the flour blend. Y₁=protein content, Y₂=fat content, Y₃=moisture content, Y₄=appearance, Y₅=overall acceptability and Y₆= breaking force (crispness).

3.4.2 Modelling and Optimisation of processing conditions

3.4.2.1 Modelling

Mathematical equations were developed based on the empirical data to predict the effects of % African yam bean seed flour inclusion, frying temperature and frying time on protein, moisture, fat, appearance, crispness and sensory perception (overall acceptability) of the product. A design expert version 6.0 software packages was used to generate equations.

Using the software package, protein content, moisture content, fat content, appearance, crispness and sensory perception were individually entered as dependent variables (responses) while %AYB flour inclusion (A), frying temperature (B), frying duration (C), were input as independent variables. The adequacy of the models was authenticated by using coefficient of determination (R^2) test and residual analysis criteria.

3.5 Production of Maize-based Snacks (*Kokoro*)

3.5.1. Maize flour production

The maize grains were sorted by hand to remove stones, chaff, and damaged grains. The cleaned maize was dry-milled in an attrition mill and sieved to obtain a particle size of $<750\mu\text{m}$ (Fig. 3.1) according to the method of Awoyale *et al.* (2011).

3.5.2. African yam bean (AYB) flour production.

The beans were hand-sorted to remove unwholesome grains and foreign materials (Umerie *et al.*, 2009) The wholesome grains were dehulled manually after soaking in water (1:5w/v) for 3-4 h at $29\pm 2^\circ\text{C}$, dried at 60°C and milled into flour ($<400\mu\text{m}$) as shown in Fig. 3.2 (Obasi *et al.*, 2012).

3.5.3. Formulation of Maize-AYB flour blends

Maize flour and AYB flour were weighed and mixed in ratios as follows: 100%:0%, 80%:20%, 70%:30%, 60%/40% and 0%:100%. The various mixes obtained were thoroughly blended with a laboratory blender, packed, and sealed in high density

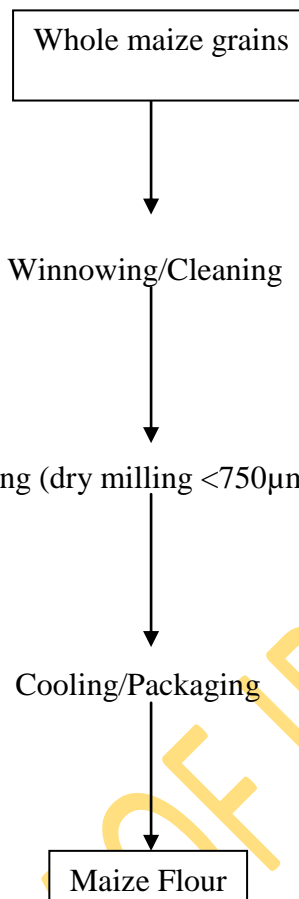


Figure 3.1: Flow chart of maize flour production

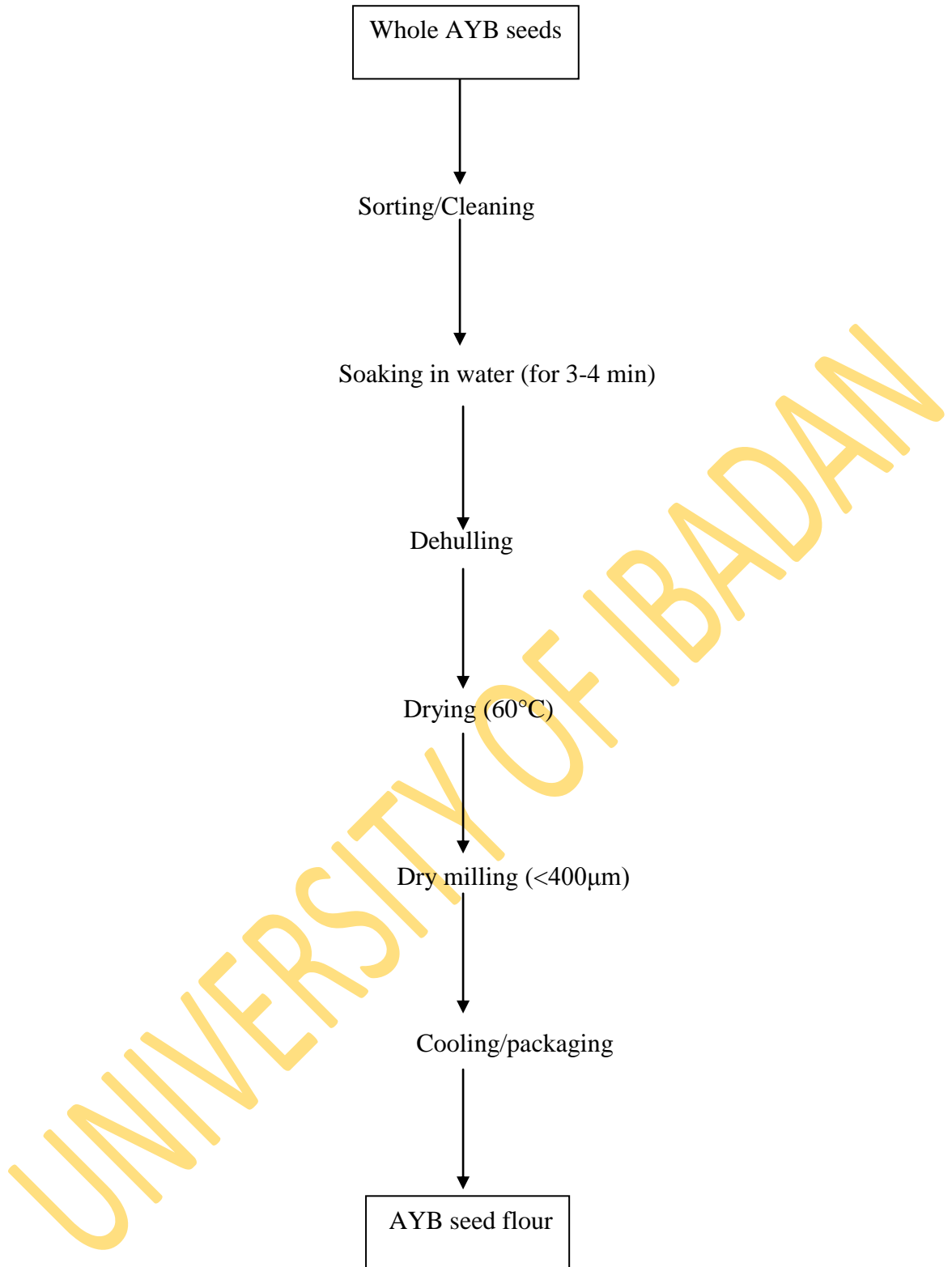


Figure 3.2: Flow chart for production of African yam bean (AYB) flour

polyethylene bags and stored at ambient condition (Temp. $24\pm 2^{\circ}\text{C}$ and RH- $61\pm 3\%$) for about 2 weeks before use.

3.5.4. Recipe formulation of Maize rings

Kokoro-maize rings were produced as described by Uzo-Peters *et al.*, (2008) with a slight modification in the recipe used as onion and salt were used in place of sugar and salt (Fig. 3.3). Half of each blend was mixed and stirred in boiled water to make a paste and the remaining half was first mixed with salt and onion and then added to the paste with continuous stirring for about 3min to form homogenous dough. The dough was allowed to cool to a temperature of 40°C and kneaded by hand on a chopping board. The kneaded dough was put into extruder (Plate 3.4) and extruded into uniform sizes using a locally fabricated extruder and deep-fried in hot vegetable oil, (specific gravity, 0.918) at temperature of 150°C , 160°C and 170°C for 8, 10 and 12min, as indicated in the experimental design (Table 3.1), drained and left to cool. The maize rings were then packed in polyethylene bags ($100\mu\text{m}$) and stored at ambient conditions ($24.2\pm 2^{\circ}\text{C}$, $61\pm 3\%$ relative humidity). The maize rings were produced using a mechanical extruder fabricated solely for this purpose. The extruder diameter is about 55cm and 112cm respectively for small and big stainless steel extruders shown in Plate 3.3 and a die diameter of 15cm for both.

3.5.5 Frying of *kokoro*

A deep fat fryer (Model S-516, Hong Kong, China) with temperature control of $\pm 1^{\circ}\text{C}$ was used. The fryer holds 2.5L of oil and is equipped with a 2kW electric heater. Isothermal conditions was observed during frying by keeping the maize rings-to-oil weight ratio as low as possible (~ 0.0035) (Pedreschi *et al.*, 2005; Krokida *et al.*, 2001) and the frying temperature monitored using a digital multimeter. Frying temperature used were 150°C , 160°C and 170°C . And frying time, 8, 10 and 12min were used. After each frying test, the oil level was checked and replenished; the oil was changed after about 2 h of frying (Blumenthal, 1991).

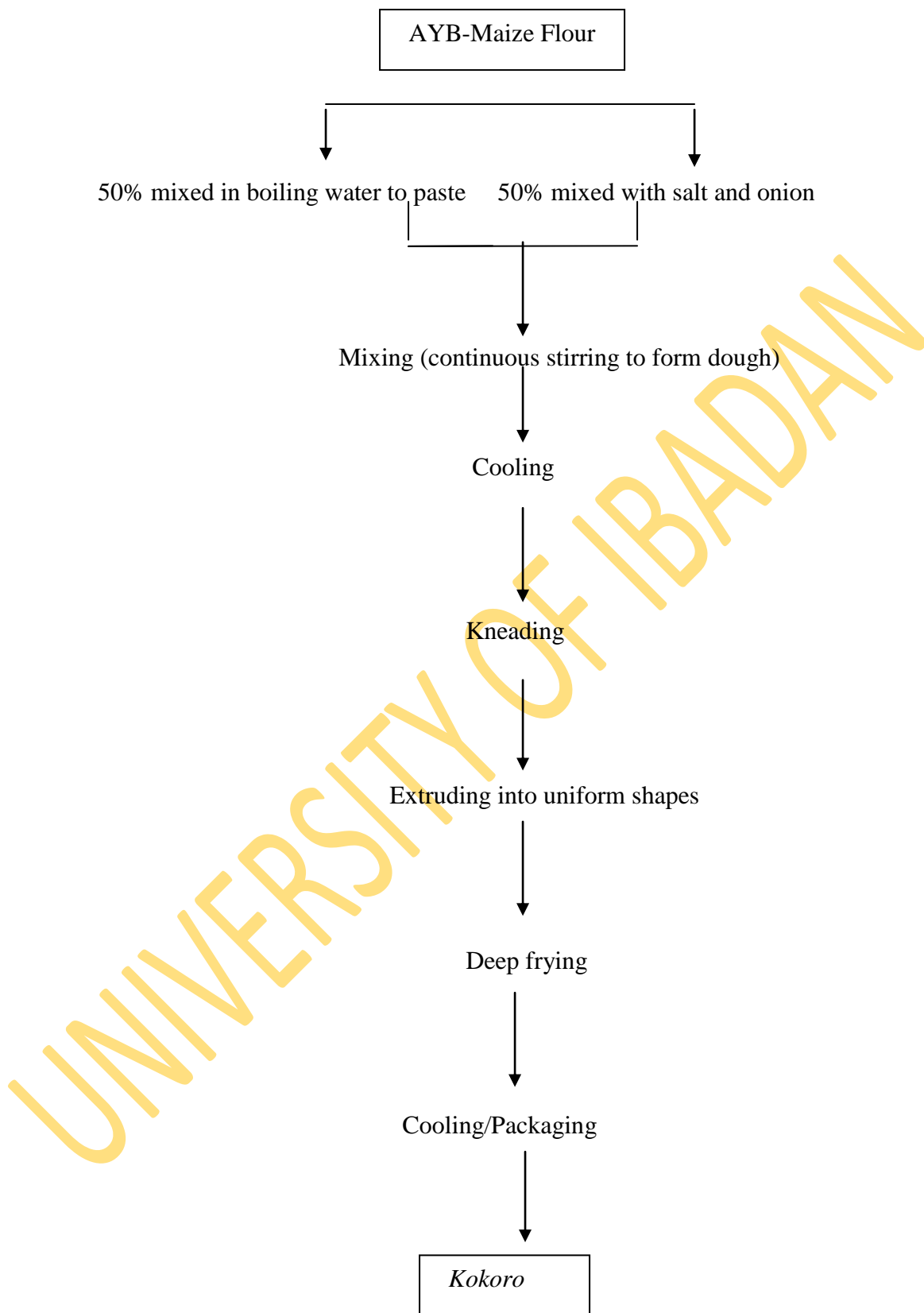


Figure 3.3: Flow chart for Production of “improved” maize rings- *Kokoro*



Plate 3.4. Mechanical extruders

3.6 Analytical procedures for Maize Flour (MF) and African Yam Bean Flour (AYBF) blends

3.6.1. Particle size distribution of maize and AYBS flour blends

Particle size distribution of the flours was done according to the method of (Paulsen and Hill, 1985). About 100g of flour was weighed and sieved through a selected standard sieves for about 10 min using a mechanical sieve shaker (Tyler ro-tap shaker). The weight of the materials retained by each sieve were recorded and expressed as a percentage of the original material's weight.

3.6.2. Functional properties of the maize and AYBS flour blends

The functional properties of the different maize flour (M) and African yam bean seed (AYBS) flour blends were determined as shown in 3.3.2.

3.6.3 Pasting properties of maize flour and AYBS flour blends

The pasting properties of the different maize flour and AYBS flour blends used were determined as described in section 3.3.2.7.

3.6.4 Proximate composition of maize flour and AYBS flour blends and *kokoro*

The proximate composition of flour blends used and *kokoro* were determined as described in section 3.3.3

3.6.5 Determination of colour parameters

Colour parameters were determined as described in subsection 3.3.4.

3.6.6 Determination of beta carotene content

The carotenoid contents of the flour blends of maize and African yam bean seed (AYBS) was determined as described in section 3.3.5.

3.6.7 Determination of Anti-nutritional factor-Trypsin Inhibitor Activity (TIA)

Trypsin Inhibitor activity (TIA) was determined according to a modified method of Kakade *et al* (1974). One gram of already defatted sample (cold extraction) was extracted with 50 ml of 0.01M NaOH. The pH of the suspension was adjusted between 8.4-10.0 using 1M HCl to reduce pH and 1M NaOH to increase the pH to the required level. The sample was left for 3 h, stirring at intervals to maintain the sample in suspension.

The extract (1 ml) was taken into 33ml of distilled water for dilution, from the diluted extract; 2 ml was transferred into 3 test-tube each. To each of the three test tubes, 2ml distilled water was added. The fourth test tube was prepared for Trypsin standard by adding 2 ml of distilled water. 2 ml of Trypsin solution (prepared from 4 mg standard trypsin (bovine pancrease, salt free) in 200ml of 0.001M HCl) was added to the first 2 test-tubes and not added to the 3rd and 4th test-tubes. The solution in each test tube was vortexed and placed in water bath at 37°C for 10 min. BAPA (benzoly-DL-arginine-p.nitroanilidehydrochloride) was prepared by dissolving 0.08 g of BAPA in 2ml of dimethyl sulphoxide and added to the already pre-warmed Tris-Buffer made up of 1.21g of hydroxymethyl amino methane and 0.59 g of CaCl₂.H₂O in 180 ml of distilled water, with pH adjusted to 8.2 and solution finally adjusted to 200 ml with distilled water and pre-warmed in water bath at 37°C for another 1 h.

BAPA solution (5ml) was added to all the test-tubes, vortexed and placed in the water bath at 37°C for 10 min. The reaction was terminated exactly 10 min later by addition of 1ml of 30% glacial acetic acid solution (30 ml of glacial acetic acid was made up to 100 ml with distilled water) to all the test tubes and was vortexed.

Trypsin solution (2 ml) was added to all the 3rd and 4th test tubes that did not contain Trypsin solution initially. Then the samples were filtered and the absorbance was read at 410 nm using a spectrophotometer

Calculations:

$$T.I \frac{mg}{g} \text{ of sample} = \frac{Abs \text{ of standard} - Abs \text{ of sample}}{0.019 \times \text{sample weight}} \times \frac{\text{dilution factor}}{1 \times \text{sample size (ml)}} \dots \dots \dots 3.14$$

$$\text{In 1g sample: } T.I \frac{mg}{g} \text{ of sample} = \frac{(Abs \text{ of standard} - Abs \text{ of sample}) \times \text{dilution factor}}{19}$$

Therefore,

$$T.I \frac{mg}{g} \text{ of sample} = \frac{\text{Abs of standard} - \text{Abs of sample} \times 50 \times 33}{19} \dots\dots\dots 3.15$$

3.6.8 Breaking force determination

A texture analyzer (Model –no 174886, Kiya Seisakusho Ltd. Tokyo Japan.) was used for breaking force determination (Pomeranz *et al.*, 1985). Each *Kokoro* ring was placed over the surface of a stainless plate and pressed with a stainless steel ball flat-end plunger (20mm diameter) at a speed of 2.5mm/min. This was repeated four times to determine the breaking force value as the average of the four determinations and numerical results were expressed in kilograms (kg).

3.6.9 Microbial analysis of the products

The microbial loads, bacteria and fungi were determined as total viable count of microbes using the method described by Pelezar and Chan (1997). The loads were expressed as the number of colony forming units (cfu) per gram of test sample. The spread technique was used. The solutions of the sixth (10^{-6}) and fourth (10^{-4}) dilutions were used for bacterial and fungal analysis, respectively.

For bacteria culture, nutrient agar was used, while potato dextrose agar was used for fungal culture, 0.1cm³ of the required dilution of the test sample was aseptically inoculated on the sterile growth media with the aid of a flamed glass hockey. The petri dishes were incubated in a Gallenkamp incubator at 37° C for 24h for bacteria and at 27°C for fungi. Counting of the colonies was done using Gallenkamp colony counter.

3.6.10. Determination of adsorption isotherm properties

Kokoro produced at the optimum processing condition obtained from the experimental design (AYBS blend 30%, frying temperature 155°C and frying time, 11.5min) was used. The samples were dehydrated in a glass desiccators containing phosphorus pentoxide (P₂O₅) for about 3 days. The adsorption isotherm was determined by the static gravimetric method at 20 °C, 30 °C and 40°C (to simulate the temperatures of marketing and distributing these products in Nigeria). Triplicates of the samples of known weight (2g) were placed above

saturated salt solutions (analytical grade-Merck) Dessicant<0.001, LiCl₂ 0.12, MgCl₂ 0.34, K₂CO₃ 0.49, Mg(NO₃)₂ 0.55, NaNO₂ 0.65, NaCl 0.76, CdCl₂ 0.82, K₂CrO₄ 0.88, KNO₃ 0.94 and Na₂HPO₄ 0.99 (Bell and Labuza, 2000; Labuza,1968) in separate tightly closed glass jars of 12cm diameter and kept in ventilated incubators (Model SG 93/06/369, United Kingdom). Samples were weighed (balance, sensitivity ±0.0001 g) at regular interval until constant weight was reached. Equilibrium was acknowledged when three consecutive weight measurements showed difference less than 0.001 g. The moisture content of each sample was determined by the air oven method by means of triplicate measurements. Samples' weights were determined using a Mettler balance (model AJ150, Greifensee, Switzerland). Formalin (analytical grade) was placed inside the high relative humidity (>65%) dessicators to protect samples from microbial spoilage (Jamali et al., 2006; Moreira et al., 2005). All moisture contents were expressed as a percentage of non-dry weight because fat is known to exhibit no sorption of water below RH 90% (De Jong et al., 1996). The moisture content at which constant weight was reached was recorded as the equilibrium moisture content of the samples. The equilibrium moisture content at each water activity is the mean value of three replications. A graph of equilibrium moisture content (y-axis) was plotted against water activity (x-axis) to give the adsorption isotherm curve.

3.7 Sensory Evaluation

A 9 point Hedonic scale with 1 corresponding to dislike extremely and 9 corresponding to like extremely as described by Iwe (2002) was used to compare the acceptability of *kokoro* produced from flour blends of African yam bean seeds and maize and commercial *kokoro*. Ten semi-trained panelists were selected from the staff and graduate students of IITA, Ibadan, and screened with respect to their interest and ability to differentiate food sensory properties as described by Iwe (2002). Sensory evaluation room is well-illuminated and the booths are well partitioned to avoid distraction or interference by other panelists.

3.8 Nutritional Evaluation of the Products

Twelve wistar rats (male) weighing between 90g and 110g were obtained from the animal breeding centre, Department of Veterinary Physiology and Pharmacology, Faculty of Veterinary Medicine, University of Ibadan, Ibadan. They were randomly distributed into

three groups each consisting of 4 replicates placed in metabolic cages and fed a stabilizing diet consisting of 4% casein for a period of 5 days. After the 5 day period, the animals were reweighed and regrouped for control, basal and experimental diets. Water and food were given *ad libitum*. The diets were fed to the animals for a period of 28 days. This period is nutritionally accepted to be long enough to observe biological and chemical changes in animal tissues (Osundahunsi and Aworh, 2003). Weighed diet was given and unconsumed diet collected and weighed daily, while live weight of the animals were determined and recorded twice a week throughout the experimental period. At the end of test period, the rats were reweighed.

3.8.1 Biological assessment of the product

A basal diet was prepared according to Fanimu (1991) as reported by Osundahunsi and Aworh (2003). The compositions (g/kg) were fermented corn flour (647.5); glucose (50); sucrose (150); non-nutritive cellulose (microgranular cellulose, 50); vegetable oil (100); premix (20); oyster shell (10); bone meal (20) and sodium chloride (2.5). Experimental and control diets were prepared by incorporating fried AYB-maize rings and casein (for control) respectively into the basal diet to achieve an iso-nitrogenous diet at 10% protein level as shown in Table 3.2.

Using the equation;

$$a \times \frac{x}{100} = 10 \times \frac{y}{100} \dots\dots\dots 3.16$$

$$X = \frac{10Y}{a} \dots\dots\dots 3.17$$

Where X is weight of test sample required for the feed mixture

Y is final weight of feed mixture

a is protein content of test sample,

10 is iso nitrogenous protein required

The Feed Efficiency Ratio (FER), Protein Efficiency Ratio (PER), the Net Protein Retention ratio (NPR) Protein Retention Efficiency (PRE) and Feed Consumption Ratio (FCR) were calculated using the formulae given below as reported by Osundahunsi and Aworh (2003).

$$FER = \frac{W \text{ gained}}{\text{food intake (g)}} \dots\dots\dots 3.18$$

$$PER = \frac{W \text{ gained}}{P} \dots\dots\dots 3.19$$

$$NPR = (W \text{ gained} - \text{Average Wloss}) / P \dots\dots\dots 3.20$$

$$PRE = NPR \times 16 \dots\dots\dots 3.21$$

$$FCR = \frac{FC}{W \text{ gained}} \dots\dots\dots 3.22$$

Where W gained is weight gained by the rats after feeding

W loss is weight loss by the rats fed basal diet

P is protein consumed by animals

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Table 3.2: Composition of Iso-nitrogenous Diets at 10% Protein Level

| Group | Sample | Protein Content of the sample as analyzed (%) | Weight of feed Desired (g) | Weight of Basal diet (g) | Weight of sample (g) | Final protein content (%) |
|-------|---------|---|----------------------------|--------------------------|----------------------|---------------------------|
| A | Casein | 87.11 | 3000 | 2655.6 | 344.4 | 10 |
| B | product | 10.12 | 3000 | 35.6 | 2964.4 | 10 |
| C | Basal | 0 | 3000 | 3000 | 0 | 0 |

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3.9 Storage Studies

Samples for storage studies were prepared at the optimum processing condition obtained from the experimental design (AYBSF blend 30%, frying temperature 155°C and frying time, 11.5min). These samples were packaged using polyethylene bags (100µm gauge) and stored at ambient conditions (24.2±2°C, 61±3% relative humidity). During the period of storage for fourteen weeks, the sensory parameters (appearance, taste, crispness and overall acceptability) of the samples were evaluated weekly.

3.9.1 Rancidity test

Measurement of rancidity in the product was carried out by determining free fatty acid (FFA) % in extracted fat of the samples.

3.9.1.1 Determination of free fatty acid (FFA)

Diethyl ether (25 ml) was mixed with 25 ml alcohol and 1 ml phenolphthalein solution (1percent) and carefully neutralized with 0.1 M Sodium Hydroxide. About 1 g of the extracted oil was dissolved in the mixed neutral solvent and titrated with aqueous 0.1 M Sodium Hydroxide shaking constantly until a pink colour that persist for 15 s was obtained.

$$\text{Acid value} = \frac{\text{Titre value (ml)} \times 5.61}{\text{Weight of sample used}} = 2 \times \text{FFA} \dots\dots\dots 3.23$$

FFA is usually calculated as oleic acid. 1ml of NaOH=0.0282g oleic acid.

Progress of lipid deterioration was evaluated by using FFA value for 84 days at regular (7days) interval. This will help to suggest the appropriate shelf life for the products.

3.10 Statistical analysis

The mean of triplicate determinations were reported. The results obtained were subjected to Analysis of Variance (ANOVA) using Statistical Analysis System (SAS version, 9.2 SAS Institute Inc., Cary, NC). Means were separated by using Turkey at $p=0.05$.

CHAPTER FOUR

4. RESULTS AND DISCUSSION

4.1. Selected Properties of Maize and African Yam Bean Seed cultivars for Processing Suitability.

4.1.1 Appearance and physical characteristics of the maize cultivars.

The maize grains showed variation in their physical, chemical and functional properties according to their cultivars. It was observed that the cultivar, TZL-Comp 4C2 possessed ivory cream colour while the BR-9928-DMR-SY grains ranged in colour from orange to burnt-orange. The results of physical characteristics of the maize cultivars are presented in Table 4.1. Measurement of the dimensions of grains revealed variation in size, seed length, diameter and thickness. As shown in Table 4.1, significant difference ($p < 0.05$) was observed only in the grain density with the TZL-Comp 4C2 grains being significantly denser (1.27g/ml) than BR-9928-DMR-SY (1.19g/ml). Similar results were obtained by Iken *et al.*, (2007) during analysis of some newly developed maize varieties in Nigeria.

4.1.2 Functional properties of the maize cultivars

Swelling capacity is the volume of expansion of molecule in response to water uptake which it possessed until a colloidal suspension is achieved or until further expansion and uptake is prevented by intermolecular forces in the swollen particle (Houssou and Ayernor, 2002). The swelling capacity values of the maize cultivars did not show significant difference ($p \geq 0.05$). Houssou and Ayernor (2002) observed that higher carbohydrate or starch content enhance swelling capacity of some flours. This was not the case in this study for maize flour, BR- 9928-DMR-SY which had higher starch content (Table 4.2) than the TZL Comp 4C2. This may be attributed to the fact that chemical composition of maize varieties has been reported to differ from one variety to another (Afoakwa, 2007, Punita, 2006).

Water Absorption Capacity (WAC) is an important functional property in the development of a ready-to-eat cereal grain food, since a high WAC may assure higher yield and product cohesiveness. WAC of the maize cultivars ranged between 130.46% and 136.55%. Similar values were obtained by Ikram *et al.* (2010) for WAC of flours of some maize varieties grown in Pakistan.

Table 4.1: Physical characteristics of the maize cultivars used

| Parameter | TZL-Comp4C2 | BR-9928-DMR-SY |
|---------------------------|--------------|----------------|
| Length (cm) | 1.02±0.08a | 1.01±0.05a |
| Diameter (cm) | 0.86±0.02a | 0.80±0.04a |
| Thickness (cm) | 0.46±0.02a | 0.47±0.01a |
| Thousand seed weight (g) | 278.73±4.03a | 279.80±5.27a |
| Thousand seed volume (ml) | 219.29±9.04a | 235.13±4.22a |
| Density (g/ml) | 1.27±0.03a | 1.19±0.00b |

Values with the same letter along the same row are not significantly ($p \geq 0.05$) different

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Table 4.2 Functional properties of the maize cultivars.

| Parameter | TZL-Comp4C2 | BR-9928-DMR-SY |
|-----------------------------------|---------------|----------------|
| Amylose (%) | 27.18±0.10a | 25.06±0.65b |
| Amylopectin (%) | 72.81±0.10b | 74.94±0.65a |
| Water absorption capacity (%) | 130.46±10.61b | 136.55±11.50a |
| Bulk density (g/cm ³) | 0.83±3.25a | 0.82±2.81a |
| Swelling capacity (%) | 17.80±0.32a | 17.58±0.20a |
| Starch solubility (%) | 9.13±1.22a | 8.79±0.25a |
| Least gelation concentration (%) | 20.04±0.02a | 20.02±0.01a |
| Oil absorption capacity (%) | 86.94±4.98a | 89.52±0.16a |

Values with the same letter along the same row are not significantly ($p \geq 0.05$) different

Oil absorption capacity (OAC) is crucial to assessment of flavour retention. OAC of maize cultivar ranged between 86.94% and 89.52%. Similar values were observed by Punita (2006) during analysis of physico-chemical properties of Quality protein maize. Bulk density (BD) is important with respect to packaging; low bulk density may pose a more serious packaging problem since it will occupy more space. BD of the maize cultivars ranged between 0.82g/cm³ and 0.83g/cm³ which implies that the packaging cost maize cultivars' flours may be low. Least gelation concentration (LGC) is a measure of minimum amount of flour or blends of flour required to form gel in a measured volume of water (Sanni *et al.*, 2006). The LGC of the maize cultivars ranged between 20.02% and 20.04%. This implies that similar amounts of flour will be required for gel formation in the cultivars since there is no distinct difference between the two samples analysed. A similar trend was observed by Awoyale *et al.* (2011) for two different maize varieties analysed.

Solubility of the starch of maize cultivars showed apparent increase between the maize cultivars, however, no significant ($p \geq 0.05$) difference was obtained for the samples. Amylose content of the maize cultivars ranged between 25.06% and 27.18% while the amylopectin content was between 72.81% and 74.94%. Similar results were obtained for starch solubility of maize flour and fermented cassava flour by Awoyale *et al.* (2011) and Shittu *et al.* (2001) respectively.

4.1.3 Pasting properties of the maize cultivars

The result of the pasting properties (Table 4.3) showed useful information on the hot paste viscosity of the starch based food. Values obtained for the final viscosities were 1613 and 1654.5cp. Peak time which is an indication of the cooking time of the maize flour samples ranged between 6.97 and 7min while pasting temperatures were 88.73°C and 82.83°C (Appendix2-3) for TZL-Comp4C2 and BR-9928-DMR-SY respectively. This value is similar to that obtained for traditional unfortified maize dough and samples of dough fortified with raw bambara-nut especially at 10% replacement level (Mbata *et al.*, 2006). Trough which is the minimum viscosity value measures the ability of the paste to withstand breakdown during cooling. This implies that paste formed from maize sample, TZL-Comp4C2 having a higher value (507cp) may withstand breakdown during cooling than that of maize sample, BR-9928-DMR-SY (367cp). Higher value (1654.5cp) obtained for the

final viscosity of the sample, BR9928-DMR-SY implies that it has a higher tendency to form gel after cooking than the maize sample, TZL-Comp4C2 (1613cp). However, higher setback value (1287.5cp) obtained for the former, indicates a higher tendency towards retrogradation than the latter. This implies that there is a higher tendency towards retrogradation of starch in the maize sample, BR9928-DMR-SY which will give resistant starch. This resistant starch is not very digestible, which is of functional value due to its low glycemic index when digested in the body (Smolin and Grosvenor, 2003, Deffenbaugh and Walker, 1989).

4.1.4 Chemical composition of the maize cultivars

The moisture content ranged from 8.11%-8.18% (Table 4.4), this indicates safe moisture level in the maize varieties used for the study as recommended by Afoakwa (1996). The crude protein content varied from 8.67% (TZL-Comp4C2) to 9.64% in BR-9928-DMR-SY where higher values were recorded. This is comparable with the values (8.90%-10.29%) reported by Punita (2006) for maize. Fasasi *et al.* (2007) reported 11.69% protein for maize flour, which was slightly higher compared to the protein content of maize flour in this study. This is possibly due to use of different maize varieties. Studies have shown that the chemical composition of maize differ as a result of agronomic practices, genetic composition and environmental conditions (Punita, 2006; Afokwa, 1996).

The crude fat content of the maize cultivars ranged between 4.73 and 5.71%. Total ash content ranged between 1.26 and 1.53%. The starch content was 59.58% for TZL-Comp4C2 and 64.27% for BR-9928-DMR-SY. While the sugar, moisture, crude fibre and crude fat contents showed no significant ($p \geq 0.05$) difference, significant ($p < 0.05$) differences were observed among the values obtained for crude protein, total ash and starch contents of the maize cultivars. Differences in chemical composition have been attributed to differences in genetic composition as well as environmental factors and agronomic practices (Punita, 2006). According to Anton *et al.* (2009), the effect of cultivar was more relevant on the nutritional, rather than physical properties of corn starch-based extrudates

Table 4.3 Pasting properties of maize cultivars

| Parameter | TZL-Comp4C2 | BR-9928-DMR-SY |
|--------------------------|-------------|----------------|
| Peak 1 (cp) | 519.00a | 479.50b |
| Trough 1 (cp) | 507.00a | 367.00b |
| Break down (cp) | 12.00b | 112.50a |
| Final viscosity (cp) | 1613.00b | 1654.50a |
| Set back (cp) | 1106.00a | 1287.50a |
| Peak time (min) | 6.97a | 7.00a |
| Pasting temperature (°C) | 88.73a | 82.83b |

Values with same letter along the same row are not significantly different at $p \geq 0.05$

cp= centipoises

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Table 4.4 Chemical composition of maize cultivars

| Parameter | TZL-Comp4C2 | BR-9928-DMR-SY |
|---------------------------|-------------|----------------|
| Moisture content (%) | 8.11±0.01a | 8.18±0.67a |
| Crude fat content (%) | 4.73±0.35a | 5.71±0.60a |
| Crude protein content (%) | 8.67±0.05b | 9.64±0.15a |
| Total ash content (%) | 1.26±0.06b | 1.53±0.03a |
| Starch content (%) | 59.58±0.09b | 64.27±0.93a |
| Sugar content (%) | 4.84±0.05a | 5.82±1.43a |
| Crude fibre content (%) | 1.30±0.02a | 1.34±0.01a |

Values with the same letter along the row are not significantly different at $p \geq 0.05$

4.1.5 Colour parameters of the maize cultivars

The result of the colour determination of the samples (Table 4.5) showed that the maize variety, TZL-Comp4C2 had higher values for lightness, L^* than the variety, BR-9928-DMR-SY. BR-9928-DMR-SY showed higher values of a^* (redness) and b^* (yellowness) than TZL-Comp4C2. Colour of some raw materials is likely to affect the final product; the yellow maize may give the final product a better appearance and improve its consumers' appeal. Yellow maize has been reported to be associated with carotenoids. Carotenoids exhibits some pro-vitamin A activity which is partially converted to vitamin A in vivo. It also acts as an antioxidant in the body (Smolin and Grosvenor, 2003). Plant sources of vitamin A (including yellow maize, carrots, cantaloupe, apricots, mangoes and sweet potatoes) contain yellow-orange pigments called carotenoid. Other carotenoids that provide some vitamin A activity that can be found in yellow maize are beta-cryptoxanthin, lutein and zeaxanthin.

4.1.6 Mineral content of the maize cultivars

The mineral composition of the maize cultivars is shown in Table 4.6. Significant difference was obtained for potassium, sodium, manganese, iron and zinc and higher values were obtained for almost all the minerals in the BR-9928-DMR-SY sample. These minerals (iron, zinc, potassium, sodium) are commonly found in Nigerian agricultural crops. However, of the three major cereal grains (wheat, maize, and rice), maize has the lowest concentration of calcium, and niacin (FAO, 2009).

4.1.7 Beta carotene content of the maize cultivars

The result of carotenoid contents is shown in Table 4.7. A high value of β -carotene was obtained in the maize variety, BR-9928-DMR-SY while it was not detected in TZL-Comp4C2 maize variety. This makes the former to be more nutrient-dense in terms of vitamin A. Vitamin A activity in food is mainly due to all trans-isomer of retinol, which is the most abundant and biologically active member of the vitamin A group. Yellow maize can provide substantial amounts of vitamin A, and the maize germ is rich in vitamin E. Furthermore, maize oil contains a high level of polyunsaturated fatty acids and natural antioxidants (Okoruwa, 1996). In diet, β -carotene and other carotenoids provide most of the vitamin A. The food labeling regulations require that vitamin A is calculated as

micrograms of retinol or retinol equivalent (RE) on the basis that 6µg of β-carotene equals 1µg of retinol equivalent (FAO, 2009).

The choice of yellow maize (BR-9928-DMR-SY) became imperative as the appropriate variety for production of *kokoro* in this study as a result of its higher nutrient density (carotenoid, crude protein, total ash, mineral contents and starch contents).

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Table 4.5: Colour parameters of maize cultivars

| Parameter | TZL-Comp-4C2 | BR-9928-DMR-SY |
|-----------------|--------------|----------------|
| L* (lightness) | 84.60±30.61a | 79.17±44.09b |
| A* (redness) | 0.35±2.52b | 5.45±18.18a |
| B* (yellowness) | 22.20±7.09b | 41.00±67.12a |

Values with the same letter along the same row are not significantly different at $p \geq 0.05$.

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Table 4.6: Mineral composition of the maize cultivars

| Minerals | TZL Comp 4C2 | BR 9928-DMR-SY |
|-------------------|--------------|----------------|
| Calcium (%) | 0.49a | 0.49a |
| Magnesium (%) | 0.09a | 0.12a |
| Potassium (%) | 0.46b | 0.56a |
| Sodium (mg/kg) | 10.17a | 8.24b |
| Manganese (mg/kg) | 6.76b | 12.02a |
| Iron (mg/kg) | 19.12b | 31.84a |
| Zinc (mg/kg) | 7.72b | 9.16a |
| Copper (mg/kg) | 1.4a | 0.35b |

Values with the same letter along the same row are not significantly different at $p \geq 0.05$.

Table 4.7 Carotenoids contents ($\mu\text{g/g}$) of maize flour (MF) and African yam bean seed flour (AYBF) blend

| Sample ID | lutein | zeaxanthin | β - cryptoxanthin | α - carotene | 13-cis | | | Total β - carotene |
|--------------------|--------|------------|----------------------------|------------------------|--------------|-------|-------|-----------------------------|
| | | | | | Peak area | trans | 9-cis | |
| 20%AYBF/ 80% MF | | | | | | | | |
| | 6.29b | 8.06b | 1.49b | 0.31b | 0.13b | 0.88b | 0.47b | 1.51b |
| 30%AYBF/ 70%MF | | | | | | | | |
| | 5.28c | 6.55c | 1.25c | 0.21c | 0.04c | 0.74c | 0.13d | 1.14c |
| 40%AYBF 60%MF | | | | | | | | |
| | 5.11c | 6.06d | 1.13d | 0.21c | 0.09c | 0.68c | 0.36c | 0.92c |
| 100%YMF | | | | | | | | |
| | 7.2a | 10.36a | 1.83a | 0.36a | 0.16a | 1.12a | 0.59a | 1.84a |
| 100%AYBF | | | | | | | | |
| | ND | ND | ND | ND | ND | ND | ND | ND |
| 100%WMF | | | | | | | | |
| | ND | ND | ND | ND | ND | ND | ND | ND |

Values with different subscript on the same column are significantly different $p < 0.05$

WMF=White maize flour

YMF=yellow maize flour

ND=not detected

4.1.8 Selection of appropriate African yam bean seed cultivar by dehulling test.

The choice of appropriate AYBS cultivar was made by dehulling test. During the dehulling test of the seeds, it was observed that the attachment of the seed coat (testa) to the seed varied from one variety to the other. Some were firmly attached; others were moderately attached while some other ones were loosely attached to the seed coat. Therefore depending on the intended use of the AYB seeds, choice of appropriate cultivars could be made. For instance, in the case of utilisation of the yam bean seeds for fermented sauce (Arisa and Ogbuele, 2007) (which does not require dehulling), firmly attached seeds could be used but in this case of enrichment of maize snack by inclusion of AYB flour (which require dehulling), cultivars of African yam bean seeds that were loosely attached to the seed coat (Tss 30) was chosen to be processed into AYBS flour. Therefore, depending on the intended use, choice of appropriate cultivar for processing suitability could be made.

4.2. Physical, chemical and functional properties of Maize Flour (MF) and African Yam Bean Flour (AYBF) Blends and *kokoro* from Different Ratio of these Flour Blends.

4.2.1 Particle size distribution of the Flours

The particle size distribution of materials used is presented in Table 4.8. AYB flour used in this study was predominantly $<400\mu\text{m}$ while maize flour was predominantly $<750\mu\text{m}$.

Table 4.8: Particle size distribution in maize and AYB flours used for production of *kokoro*.

| Particle size (μm) | Maize flour (%) | AYB flour (%) |
|---------------------------------|-----------------|---------------|
| >850 | 2.82 | - |
| 600-850 | 12.76 | 0.21 |
| 425-600 | 26.34 | 0.77 |
| 200-425 | 36.47 | 16.51 |
| 180-200 | - | 11.41 |
| 150-180 | - | 60.31 |
| 125-150 | - | 7.58 |
| Recovery | 22.18 | 3.16 |

4.2.2 Colour parameters of the Maize Flour (MF) and African Yam Bean Flour (AYBF) blends

The result of the colour determination (Table 4.9) of different samples of flour blends used did not show significant difference ($p \geq 0.05$) for the lightness value (L^*), but the redness (a^*) and yellowness (b^*) values significantly ($p < 0.05$) reduced as the quantity of AYBF increased in the flour blend. Similar results were obtained by Punita (2006) for colour parameters of different maize varieties.

4.2.3 Functional properties of different ratios of Maize Flour (MF) and African Yam Bean flour (AYBF) blends.

The swelling capacity of the blends ranged between 17.95 and 18.71% with no significant ($p > 0.05$) difference among the blends, however, apparent increase was obtained for the flour blends. The 100% AYBSF had the highest while 100% MF had the lowest. This implies that the swelling capacity of the flour will increase with increasing quantity of AYBF in the flour blends as observed in Table 4.10.

Water Absorption Capacity (WAC) is an important functional property in the development of a ready-to-eat cereal grain food, since a high WAC may assure product cohesiveness (Houssou and Ayernor, 2002). WAC of the flour blends ranged from 150% to 155%, with the 100% AYBF having the highest. Protein is mainly responsible for the bulk of the water uptake and to lesser extent the starch and cellulose at room temperature (Afoakwa, 1996). The WAC increased as the amount of AYBF (Table 4.10) increased in the blends. This could be attributed to the protein content of AYB. High WAC obtained with increasing AYBF suggests the usefulness of the flour in bakery products. This implies that the yield of the flour will increase with increasing quantity of AYBF in the flour blends. The results obtained in this study are comparable to the values (271.1%-300%) reported by Fasasi *et al.* (2007) for fermented maize flour-tilapia mix. It is also comparable with the value (280%) reported by Adetuyi *et al.*, (2009) for unmalted-soybean blend.

Table 4.9: Colour parameters of different ratio of maize (M) flour and African yam bean (AYB) flour blends

| Samples | L* | A* | B* |
|-----------------|---------------|-------------|---------------|
| 0% AYBF:100% MF | 75.29±1.41b | 8.05±4.24a | 38.08±2.83a |
| 100% AYBF:0% MF | 79.20.5±0.71a | 4.28±4.24e | 24.69±4.95e |
| 20% AYBF:80% MF | 75.82±25.46b | 7.00±12.02b | 33.60±53.74b |
| 30% AYBF:70% MF | 76.05±1.41b | 6.53±12.02c | 31.84±18.38c |
| 40% AYBF:60% MF | 74.64±241.83b | 6.20±17.68d | 29.57±131.52d |

Values with different subscript along the same column are significantly different at $p < 0.05$.

Oil Absorption Capacity (OAC) is crucial to assessment of flavour retention, which increases the palatability of foods (Nweke, 2010; Shittu *et al.*, 2001, Kinsella, 1976). The OAC of the flour blends ranged between 74.73% and 85.34%. Among the flour blends, the 20%AYBF: 80%MF blend had the highest while the 40% AYBF: 60%MF blend had the least. This is close to the percentage reported by Awoyale *et al.* (2011) during supplementation of maize flour with distillers' spent grain (DSG) containing 85%M: 15%DSG blend having the highest. A similar range was also reported by Adetuyi *et al.* (2009) for malted and unmalted maize.

The 100% MF had the lowest bulk density (0.83g/cm^3), while the 100% AYBF had the highest bulk density (Table 4.10). The bulk density of the blends increased with increasing amount of AYBF in the blend. This is similar to the observation of Iwe and Ngoddy (1998) that reported increase in bulk density of sweet potato with increase in soybean content which was attributed to increase in protein. A similar result was reported by Adetuyi *et al.* (2009) for unmalted maize flour mixed with soybean flour. Low bulk density implies higher volume, therefore, low bulk density food could pose serious packaging problem. The flour blend with lower bulk density implies that the packaging material that will be used for this product will be stronger than the packaging materials used for the other samples.

Apparent increase, but not significant ($p \geq 0.05$) difference was obtained for the gelation concentration of the flour blends. The LGC increases from 20.02% for 100%AYB flour to 20.05% for 40% AYBF: 60%MF blend. The higher the LGC, the more the quantity of flour needed to form a gel (Sanni *et al.*, 2006). This implies that larger quantity of 40%AYBF: 60%MF blend might be needed to form a gel during the production of *kokoro*. The variation in the LGC could be attributed to the relative ratios of different constituent proteins, carbohydrates and lipids in the blends (Edema *et al.*, 2005, Sathe *et al.*, 1982).

Starch granule is made up of straight chain of glucose unit called amylose and branched chains; amylopectin. Significant difference ($p < 0.05$) was obtained for the amylose contents of the flour blends (Table 4.10). The amylose content of the blends reduced significantly from 28.18% (0%AYBF: 100%MF) to 22.40% (100%AYBF: 0%MF) as the quantity of AYB flour increased. Amylose is the starch fraction which retrogrades more rapidly due to the tendency of the linear molecule to associate rapidly but amylopectin retrogrades slowly.

Table 4.10 Functional properties of different ratio of maize flour (MF) and African yam bean flour (AYBF) blends

| Samples | Amylose (%) | Amylopectin (%) | WAC (%) | BD (g/cm ³) | SC (%) | LGC (%) | OAC (%) |
|-----------------|-------------|-----------------|--------------|-------------------------|-------------|-------------|--------------|
| 0% AYBF:100% MF | 28.18±0.16a | 71.82±0.16e | 150.20±2.58a | 0.83±3.98d | 17.95±0.22a | 20.02±0.00a | 85.34±6.18a |
| 100% AYBF:0% MF | 22.40±0.11e | 77.60±0.11a | 155.64±6.05a | 1.02±3.34a | 18.71±0.42a | 20.06±0.01a | 74.73±2.78ab |
| 20% AYBF:80% MF | 26.46±0.16b | 73.54±0.16d | 150.91±4.20a | 0.86±5.48c | 18.49±0.87a | 20.03±0.01a | 82.05±4.63a |
| 30% AYBF:70% MF | 25.47±0.38c | 74.53±0.34c | 152.84±1.84a | 0.88±2.36c | 18.49±0.30a | 20.04±0.01a | 81.30±2.74a |
| 40% AYBF:60% MF | 24.83±0.32c | 75.17±0.32b | 154.28±2.05a | 0.96±0.63b | 18.57±0.23a | 20.05±0.01a | 77.08±6.00ab |

Values with different letters along the column are significantly different at $p < 0.05$

This implies that paste produced from the 20%AYBF: 80% MF blend with higher amylose content might retrograde faster compared to that 40%AYBF: 60%MF with low amylose content. Starch retrogradation is now desirable in food due to its resultant production of resistant starch which is not very digestible. This will result in production of *kokoro* with low glycemic index which prevent colon cancer (Smolin and Grosvenor, 2003).

4.2.4: Pasting properties of the different ratios of maize flour (MF) and African yam bean flour (AYBF) blends

The commonest parameter used to estimate the pasting properties of starch-based products is the amylographic-pasting properties (Shittu *et al.*, 2001, Ruales *et al.*, 1993). Although starch granules are insoluble in cold water, they can become slightly hydrated leading to a larger swollen granule in hot water. There were significant differences ($p<0.05$) in all the parameters measured except for the peak temperature and, some of the peak time.

The final viscosity among the blends ranged between 962cp and 1564.5cp. Final viscosity is the most commonly used parameter to determine a particular starch-based sample's quality as it indicates the ability of the material to form gel after cooking and solidify or retrogrades during cooling. This implies that 100% MF might form gel faster after cooking than 40%AYBF: 60%MF blend. As the quantity of AYBF increased in the blends, the final viscosity reduced (Table 4.11). This implies that the ability of the flour blends to form a thick paste after gelatinization reduced as the proportion of AYBF increased. This is similar to the report obtained for traditional unfortified maize dough and samples of dough fortified with raw bambara-nut especially at 10% replacement level (Mbata *et al.*, 2006). Setback value is the difference between final viscosity and hot paste viscosity or trough and the same as tendency towards retrogradation (Sanni *et al.*, 2006). The phase of the pasting curve commonly referred to as the setback region, is the phase where after cooling of the mixture a re-association between starch molecules occurs to a greater or lesser degree. It therefore affects retrogradation or reordering of the starch molecules (Sanni *et al.*, 2006).

Table 4.11: Pasting properties of different ratios of maize flour (MF) and African yam bean seed (AYBS) flour blends

| Samples | Peak 1 (cp) | Trough1 (cp) | Breakdown (cp) | Final | | Peak time (min) | Pasting Temperature (°C) |
|---------------|----------------|-----------------|-------------------|-------------------|------------------|-----------------------|--------------------------------|
| | | | | Viscosity (cp) | Set back (cp) | | |
| 100%AYBF:0%MF | 816.50a | 720.50a | 96.00e | 962.0d | 241.50e | 5.45b | 81.10c |
| 0%AYBF:100%MF | 479.50d | 367.00d | 112.50a | 1654.50a | 1287.50a | 7.00a | 89.80a |
| 20%AYBF:80%MF | 481.00d | 456.50c | 104.51b | 1363.50b | 850.00b | 7.00a | 89.20a |
| 30%AYBF:70%MF | 516.00c | 469.00c | 101.62c | 1060.50c | 604.00c | 7.00a | 83.83b |
| 40%AYBF:60%MF | 580.00b | 511.00b | 99.81d | 1052.00c | 583.00d | 7.00a | 82.10b |

Values with the same letter along the column are not significantly different at $p \geq 0.05$ cp=centipoises

The set back values for the flour blends ranged from 583cp to 850 cp. But generally among the samples, 100% MF had the highest setback, while 40%AYBF: 60%MF blend had the lowest. Lower setback value has been reported to indicate high stability (Edema *et al.*, 2005, Shittu *et al.*, 2001), therefore 40%AYBF: 60%MF blend might be more stable after cooking compared to the 100% MF, which might retrograde after some time. This was not easily noticed since the paste was fried to *kokoro* almost immediately. Setback also has serious implication on the digestibility of starch pastes when consumed. However, higher setback values might result in reduced *kokoro* digestibility. This will reduce the rate at which the blood glucose levels rise (glycemic index); which is of health benefits, preventing the risk of colon cancer, diabetes and other disease conditions associated with high glycemic index (Smolin and Grosvenor, 2003)..

Peak time is the time required to reach the peak viscosity, and it ranged from 5.45 to 7.00 min. Pasting temperature on the other hand, provides an indication of the minimum temperature required for cooking the various mixes, this has implication on the stability of other components in the mixes and also indicate energy costs (Fasasi *et al.*, 2007). It was observed that all the flour blends might be cooked at temperature around 89°C.

4.2.5 Proximate composition of different ratios of maize flour (MF) and African yam bean seed flour (AYBF) blends and *kokoro* samples

The result of proximate composition of different ratios of maize flour (MF) and African yam bean flour (AYBF) blends and *kokoro* products are shown in Tables 4.12 and 4.13 respectively. Ash content is a reflection of the mineral status, although contamination can indicate a high concentration in a sample (Baah *et al.*, 2009). The total ash content of the flour blends ranged between 1.52 and 2.85% (Table 4.12). The 100% AYBF had the highest, followed by 40% AYBF: 60%MF and 100% MF had the lowest. The result indicates that mixing maize flour with AYBF increases the total ash content of the flour blends. This might be attributed to high total ash content of AYB (2.37%). Comparable ash content (1.39%-3.20%) of blends was reported for cowpea-maize flour and their *ogi* (*akamu*) blends (Zanna and Milala, 2004). Total ash content of *kokoro* ranged from 1.87% to 2.42% (Table 4.13). The *kokoro* (AYB-maize rings) sample C17010 (60%M: 40%AYB) had the highest total ash content while the A17010 (20%AYBF: 80%MF blend) had the

lowest. Total ash content of the *kokoro* samples (1.87 to 2.42%) was higher than that of their respective flour blends (1.52% and 2.85%). This might be attributed to other recipes mixed with the flour blends during *kokoro* production. The total ash contents of the products are similar to the values reported by Uzo-Peters *et al.* (2008); Omueti and Morton (1996) for extruded Soya-maize *kokoro*. Significant difference ($p < 0.05$) were observed among the flour blends (Table 4.12) and AYB-maize *kokoro* (Table 4.13). Processing AYBF-MF to *kokoro* generally increased its total ash contents.

The moisture content of the flour blends was between 5.98 to 7.09 % (Table 4.12). Lower initial moisture content of a product to be stored, improves its storage stability. This implies that all the flour blends might be stored for a long period of time before being utilised for *kokoro* production without spoilage. The results obtained for the blends are in accordance with the values (5.90%-11.69%) reported by Fasasi *et al.*, (2007) for maize flour-tilapia mix as well as the values of 4.00%-9.31% for cowpea-millet blend reported by Zanna and Milala (2004). The moisture content of the AYB-maize *kokoro* ranged between 1.40% and 2.69%. The sample C1608 (40%AYBF: 60%MF blend) had the highest; while A15010 (20%AYBF: 80%MF blend) had the lowest. The results obtained for the AYBF-MF blends are comparable with the values of 5.79-11.24% reported by Uzo-Peters *et al.*, (2008) for defatted groundnut cake-maize flour but higher than the values of 3.40-7.80%) for soybean-maize flour reported by same authors. There were significant differences ($p < 0.05$) among the flour blends and the products. Processing flour blends into *kokoro* reduced the moisture contents significantly. The frying temperature (150 °C -170 °C) allows most of the water present in *kokoro* to be removed during frying which made its moisture content to be lower than that of the flour blend.

Crude fat content of the flour blends ranged between 2.36% and 4.96%. The 100% MF had the highest crude fat content while the 100% AYBF had the lowest. The crude fat content reduced as the proportion of AYBF increased in the blend from 20% to 40%. Similar values (2.90%-5.69%) with the flour blends were reported by Ishiwu and Onyeji (2004) for instant gruel based on blends of maize and African yam bean. But lower values (2.11-3.24%) were reported by Zanna and Milala (2004) on cowpea-millet mixes. Crude fat content of the *kokoro* ranged from 27.35 to 35.01%. The sample, C15010 (40%AYBF: 60%MF) had the highest and that of A15010 (20% AYBF: 80%MF blend) had the lowest. It was also

observed that crude fat content of the products increased significantly compared to their respective flour blends. This may be attributed to the oil-protein binding existing in high protein fried snack. A similar trend was observed by Falade *et al.* (2003) during frying of akara-Ogbomoso made from soybean substitution in cowpea flour. The values obtained for crude fat contents of the AYB-maize rings (*kokoro*) are in accordance with the values (13.00-34.06%) for defatted groundnut cake-maize *kokoro* reported by Uzo-Peters *et al.* (2008). This is different from the values (12.70%-26.80%) for defatted soybean-maize *kokoro* by the same authors as well as the values of 9.95%-18.71% reported by Adelakun *et al.* (2004) for soybean-maize flour blends. There were significant differences ($p < 0.05$) in the *kokoro* samples (Table 4.13) while for the flour blends; significant ($p < 0.05$) decrease was observed with increase in the quantity of AYBF (Table 4.12).

Sugar content of the flour blends ranged from 4.53 to 6.26%. The 100% AYBF had the highest and 100%MF blend had the lowest. This implies that as the proportion of AYBF increased in the blends, the sugar content increased. This could be attributed to the high sugar content of AYBF (6.26%). The sugar content of 4.53% obtained for 100% maize flour (Table 4.12) was higher than the values (1%-3%) reported by Boyer and Shannon (1987) for sugar content of maize. However, similar values were observed by Nweke (2010). The sugar content of AYB-maize *kokoro* ranged from 4.15% to 4.60%. The result obtained shows some correlation with the result obtained from the flour blend. Significant difference ($p < 0.05$) was observed in both the flour blends (Table 4.12) and the products (Table 4.13).

Starch contents of the flour blends ranged from 51.15 to 66.66%. Among the blends, 20% AYBF: 80% MF had the highest; 40% AYBF: 60%MF blend had the lowest (Table 4.12). There was slight decrease in the starch content of the blends as the proportion of the AYBF increased. This might be due to the fact that starch content of AYBF is lower than that of MF, so increasing the quantity of AYBF will decrease starch in the final blend (Table 4.12). The starch content of the 100% maize flour investigated was close to the range of values (65.42-66.88/5) reported for maize by Punita (2006). Starch content of the *kokoro* samples ranged from 39.29% to 56.41% (Table 4.13). Significant difference ($p < 0.05$) was observed among the starch contents of the flour blends (Table 4.12) and among the products (Table 4.13).

Protein is essential in the human diet for growth and repair of worn-out tissue (Baah *et al.*, 2009). The protein content of the flour blends increased from 10.52% (100% MF) to 15.65% (40% AYBF: 60% MF blend) and the protein content of the flour blend increased as the percentage of AYBF increased. Similar values (9.10%-19.96%) were reported by Zanna and Milala (2004) for the protein content of cowpea-maize flour blend. Protein content of AYB-maize rings ranged from 10.88% (C1608) to 14.15% (A17010) revealing the adequacy of the formulation to meet protein requirement of complementary snacks. The protein content of the fried snacks was not significantly ($p \geq 0.05$) affected by the frying conditions but there were significant ($p < 0.05$) difference among fried samples (Table 4.13). The *kokoro* made from 40% AYBF: 60% MF blend had the highest protein content and that of 20% AYBF: 80% MF had the lowest, showing a correlation between the result obtained from the flour blends and that of the products. Significant differences ($p < 0.05$) were observed among the flour blends (Table 4.12) and among the AYB-maize rings (Table 4.13).

Crude fibre content of flour samples ranged from 1.34-5.81%. The 100% AYBF had the highest crude fibre content while the 100% MF had the lowest. The crude fibre content of the flour blends and *kokoro* (Tables 4.12 and 4.13) increased as the proportion of AYBF increased in the blend. A similar report was obtained by Alozie *et al.* (2009) for cakes made from wheat and African yam bean seed flour blends. The high fibre content of AYBF further supports the fact that the crop has a high nutritional content.

4.2.6 Trypsin inhibitor Activity (TIA) of different ratios of MF and AYBSF blends and *kokoro* samples

Trypsin inhibitor is an anti-nutritional factor commonly found in tropical legumes. While TIA was not detected in 100% maize flour, about 11.09% was obtained in 100% AYBSF (Table 4.14). This is higher than the value (22.09TUI/g) reported for raw whole seeds of African yam bean by Nwosu (2013). But comparable to the value (11.64TUI/g) reported by the same author for the 96 hours malted sample of the African yam bean. TIA increased in the flour blends as the percentage of the AYBF increased but significant reduction was observed during the processing of the flour blends into *kokoro* products. Heat treatment is

Table 4.12: Chemical composition (%) of different ratio of maize (M) flour and African yam bean (AYBS) flour blends.

| Samples | Crude Fat | Crude Protein | Moisture Content | Total ash | Sugar | Starch | Crude fibre |
|--------------------|-------------|---------------|------------------|------------|------------|-------------|-------------|
| 0%AYBF/ 100% MF | 4.96±0.20a | 10.52±0.41e | 8.69±0.04a | 1.52±0.01e | 4.53±0.04d | 66.66±0.65a | 1.34±0.04e |
| 100%AYBF/ 0%MF | 2.36±0.57c | 23.69±0.12a | 4.07±0.71a | 2.85±0.02a | 6.26±0.13a | 51.15±0.90e | 5.81±0.01a |
| 20%AYBF/ 80%MF | 4.54±0.08ab | 13.09±0.12d | 7.09±0.11a | 1.93±0.01d | 4.88±0.01d | 63.51±0.02b | 2.23±0.01d |
| 30%AYBF/ 70% MF | 4.18±0.07ab | 14.64±0.29c | 6.28±0.10a | 2.08±0.01c | 5.05±0.05c | 61.97±0.42c | 2.68±0.01c |
| 40%AYBF/ 60% MF | 3.92±0.11b | 15.65±0.04b | 5.98±0.15a | 2.22±0.03b | 5.22±0.09b | 60.42±0.38d | 3.10±0.01b |

Values with different subscripts along the column are significantly different at $p < 0.05$

Table 4.13: Chemical compositions (%) of *kokoro* produced from blends of maize and African yam bean seed (AYBS) flours.

| Sample | Total ash | moisture | Crude fibre | Crude protein | Crude fat | starch | sugar |
|---------|---------------|------------|-------------|---------------|----------------|-------------|------------|
| A15010 | 2.02±0.18def | 1.40±0.08g | 2.43±0.17c | 11.15±0.08fg | 27.35±3.35f | 50.20±0.04b | 4.45±0.03b |
| A16012 | 1.99±0.05ef | 1.84±0.05f | 2.36±0.21c | 11.91±0.05ef | 31.04±0.19bcd | 46.51±0.06c | 4.38±0.03c |
| A1608 | 1.93±0.04fg | 2.42±0.07c | 2.38±0.47c | 12.46±0.05cde | 31.53±0.21bcd | 45.71±0.69d | 4.15±0.01e |
| A17010 | 1.87±0.05g | 2.13±0.1d | 2.42±0.16c | 10.88±1.82g | 34.25±0.41ab | 44.31±0.70e | 4.15±0.05e |
| B15012 | 2.10±0.01bcde | 2.0±0.02e | 2.67±0.50bc | 12.93±0.12bcd | 27.61±3.06ef | 44.44±0.08e | 4.25±0.01d |
| B1508 | 2.07±0.02cde | 2.69±0.03b | 2.65±0.12bc | 12.63±0.02cde | 31.04±1.04bcd | 44.65±0.04e | 4.22±0.04d |
| B16010 | 2.13±0.01bc | 1.76±0.07f | 2.66±0.02bc | 13.04±0.1bc | 34.01±1.33ab | 43.61±0.41f | 4.18±0.05d |
| B17012 | 2.17±0.02bc | 1.72±0.02f | 2.65±0.21bc | 12.06±0.2def | 29.96±0.24cdef | 43.17±0.42f | 4.28±0.05d |
| B1708 | 2.11±0.01bcd | 2.41±0.05c | 2.66±0.16b | 10.90±0.91g | 33.33±1.82abc | 42.29±0.09f | 4.11±0.11e |
| C15010 | 2.17±0.02bc | 2.32±0.02c | 3.10±0.03a | 13.69±0.25ab | 35.01±1.19a | 39.29±0.07h | 4.28±0.02d |
| C16012 | 2.17±0.01bc | 1.78±0.03f | 3.12±0.24a | 13.09±0.1bc | 32.71±0.51abcd | 41.79±0.07g | 4.15±0.06e |
| C1608 | 2.21±0.01b | 2.42±0.05c | 3.11±0.14a | 14.15±0.37a | 30.94±0.18bcde | 40.69±0.10h | 4.20±0.07d |
| C17010 | 2.42±0.24a | 1.97±0.09e | 3.13±0.91ab | 13.11±0.21bc | 29.58±0.76def | 42.15±0.27f | 4.21±0.06d |
| Control | 2.21±0.02b | 3.61±0.08a | 1.35±0.01d | 9.91±0.06h | 21.08±4.34g | 56.41±0.01a | 4.60±0.06a |

Sample code=% flour blend frying temperature frying time

A=80:20 Maize and AYB flour respectively

B=70:30 Maize and AYB flour respectively

C=60:40 Maize and AYB flour respectively

Control=100% maize flour

Values with the different subscript along the column are significantly different at $p < 0.05$.

Table 4.14: Trypsin Inhibitor Activity (%) (TIA) of flour blends and *kokoro* samples.

| Sample | TIA (%) |
|-----------------------------------|---------|
| 0%AYBF (100% maize) | ND |
| 100%AYBF | 11.09a |
| 20%AYBF | 2.95f |
| 30%AYBF | 4.25c |
| 40%AYBF | 6.73b |
| <i>Kokoro</i> 20% AYBF150°C@10min | 2.42g |
| <i>Kokoro</i> 30%AYBF170°C@12 min | 3.60e |
| <i>Kokoro</i> 40%AYBF160°C@12 min | 4.08d |
| <i>Kokoro</i> 40%AYBF150°C@10 min | 4.59c |

Values with the different subscript along column are significantly different at $p < 0.05$

ND= not detectable

reported to reduce trypsin inhibitors in foods. Apata and Ologhobo (1997) reported complete destruction of trypsin inhibitors and haemagglutinin in some tropical legumes by cooking. Iwe (1998) reported decrease in values of extruded sweet potato and soy beans mixtures. Olapade (2010) reported significant reduction during extrusion cooking of acha/cowpea mixtures which implies that heat processing destroys significant proportion of TIA and thus poses no serious problem to the utilisation of the African yam bean seeds.

4.2.7 Mineral contents of flour blends and *kokoro* samples

The mineral contents of the flour blends (Table 4.15) showed that the 100% AYBF had higher values for most of the minerals analysed, this could further buttress the fact that AYB is nutrient dense. Significant ($p < 0.05$) increase for the mineral elements (potassium, iron, sodium, calcium, magnesium, copper and manganese) was obtained in the AYBF-MF blends with increasing quantity of AYBF. Ijarotimi and Bakare (2006) also reported increasing values of mineral content with increasing quantity of fermented African yam bean seed flour in sorghum flour.

4.3. Frying characteristics of the Maize rings

Time of frying significantly affected the frying temperature, feed composition, final moisture content and fat content of the product (Figures 4.1 and 4.2). Temperature of frying is crucial to the frying characteristics of the product. When the temperature was higher than 180°C, it resulted in case hardening whereby the product appeared to be crunchy and dried but still soft inside. This later became very soft rather than remain crunchy (crispy). Frying at too high temperature led to burnt taste in samples, undesirable dark colour and likely destruction of some nutrients. Similar observation was reported by Chen *et al.* (1998). When frying at too low temperature, the *kokoro* absorbed more fat and its surface became less crispy (Liu-Ping *et al.*, 2005, Hayter, 1989). This resulted in products with high oil content, which is not cost-effective for commercialisation; it reduces the consumers' acceptability of the products in addition to the health risk attendant with consumption of too fatty foods. Hence, attainment of optimum frying condition of the product is of importance for consistent sensory quality, economic and health benefits, better storage properties as well as

Table 4.15 Mineral Contents of Maize flour (MF) African yam bean seed flour (AYBSF) and *kokoro* samples.

| Sample | Ca (%) | Mg (%) | K (%) | Na (mg/kg) | Mn (mg/kg) | Fe (mg/kg) | Cu (mg/kg) | Zn (mg/kg) |
|---|--------|--------|-------|------------|------------|------------|------------|------------|
| 0%AYBF | 0.49c | 0.12c | 0.56d | 8.24e | 12.02e | 31.84e | 0.35f | 9.16a |
| 100%AYBF | 0.74a | 0.21a | 1.41a | 144.78b | 21.20a | 47.87a | 2.42a | 8.9c |
| 20%AYBF | 0.55b | 0.15b | 0.84c | 39.23d | 13.86d | 36.64d | 0.76e | 9.10a |
| 30%AYBF | 0.58b | 0.17b | 0.93b | 56.40c | 14.78c | 38.02c | 0.97d | 9.08a |
| 40%AYBF | 0.62b | 0.18b | 0.98b | 62.37c | 15.62b | 41.07b | 1.12c | 9.06b |
| <i>Optimized Kokoro</i> (30% AYBF, 155°C,11.5min) | 0.68a | 0.18b | 0.97b | 397.54a | 15.64b | 41.86b | 1.66b | 9.06b |

AYBF=African yam bean flour

0%AYBF=100% MF

Values with the same letter along the column are not significantly different at $p \geq 0.05$.

better consumers' acceptability. This is crucial and will be of interest to commercial manufacturers who want to know optimum frying conditions for better acceptability. Frying time is crucial to the quality of the fried *kokoro* product. If the frying time is too short, the product appears to be par-fried. At this stage, the moisture content of the product is still high. This also affects the colour. Frying for a longer time on the other hand, makes the product crispier and gives it the desired golden colour. However, if the frying time is exceeded, the product absorbs oil back. As the frying proceeds, the product loses its moisture until it becomes dry and crispy at this point the product no longer bubbles in hot oil as it does at the beginning of the frying up till when the frying process is complete.

The moisture loss during the frying experiments exhibited a classical drying profile, which is usually characterised by three distinct periods. The first is an initial heat-up period during which the wet solid material absorbs heat from the surrounding media. The product is then heated up from its initial temperature to a temperature where moisture begins to evaporate from the food (Garayo and Moreira 2002). This period was observed to be very short as shown in Fig 4.2. It was found to increase with increase in temperature from 150 to 170°C with an initial rapid fall when the *kokoro* rings were fried at higher temperatures of 150 to 170°C. The constant rate period (second stage of drying), where the rate of moisture loss is limited by the rate at which heat is transferred from the drying medium to the product, was not observed distinctly in this work (Fig 4.2). The falling rate period during which the rate of moisture loss decreases occurs when the moisture level in the product is so low that its surface is no longer wet. Hence, controlled by moisture diffusion mechanisms and the water during the period are held in the material by multi molecular and capillary condensation (Ushakumari *et al.*, 2007; Toledo, 1991).

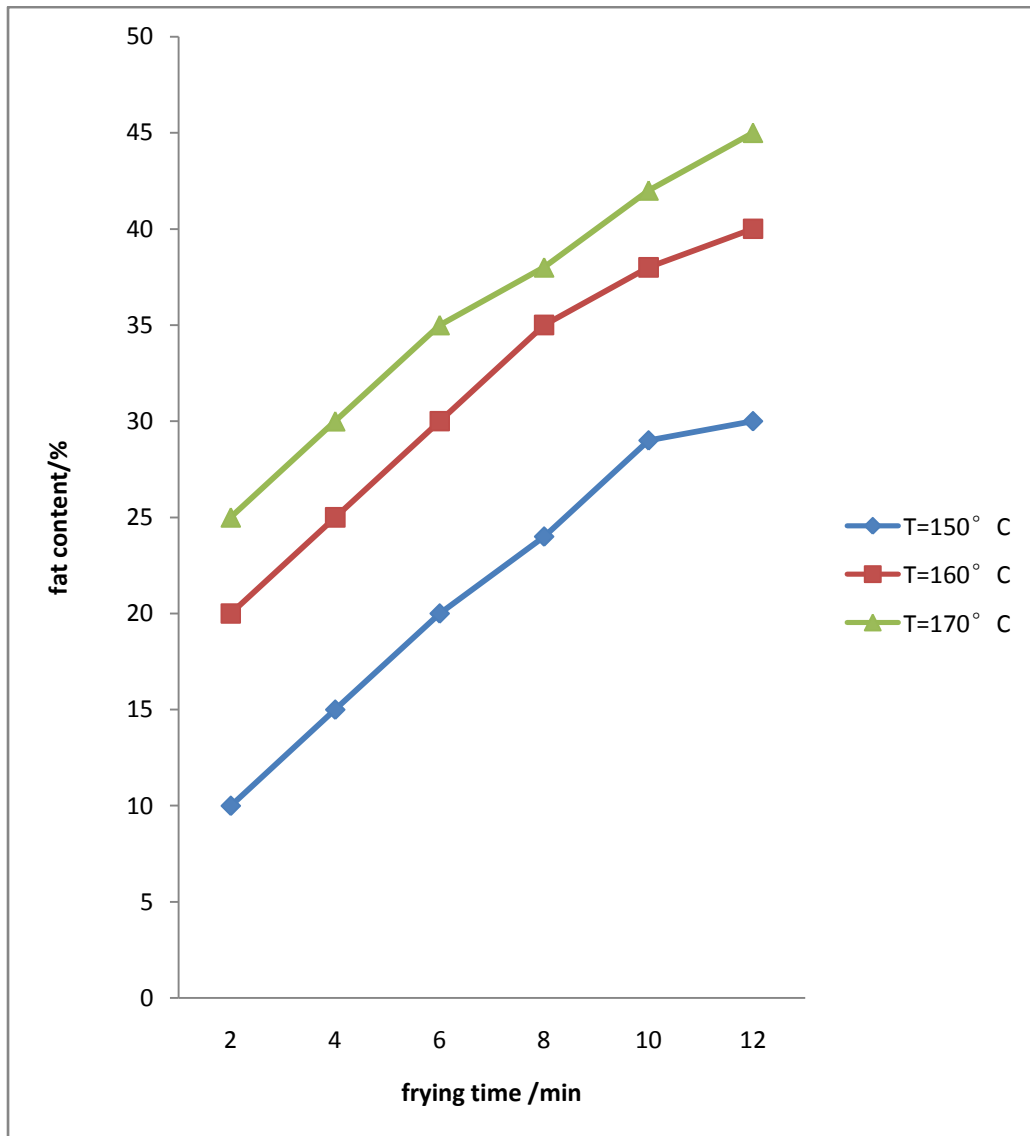


Figure 4.1 Effect of frying time on fat content of fried maize rings

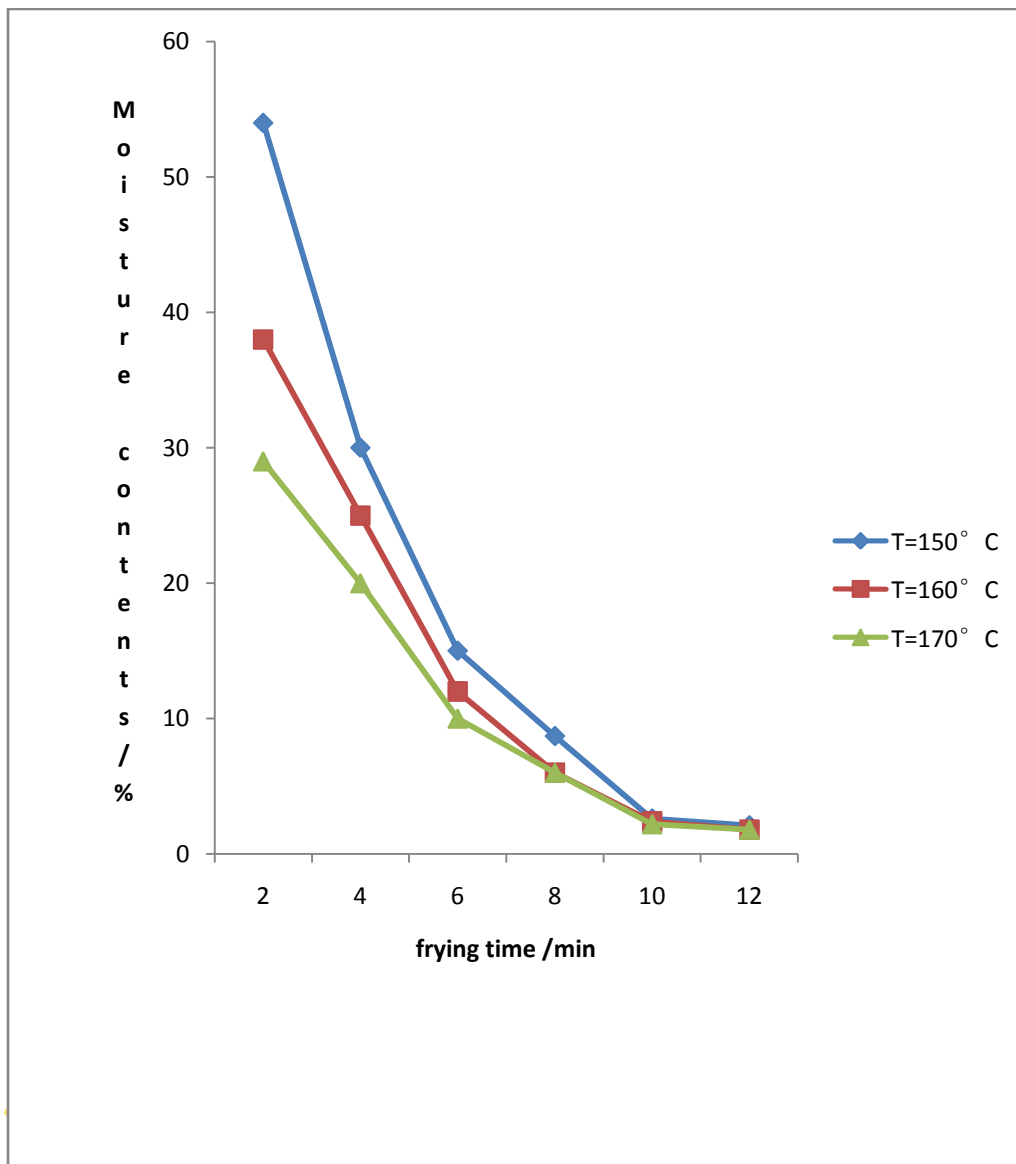


Figure 4.2 Effect of frying time on moisture content of fried maize rings

4.4. Effect of Processing Parameters on Product Quality of Maize Rings (*kokoro*).

The effect of processing variables (frying temperature, frying time and %AYBF inclusion in the flour blend) on each of the dependent variables (fat, moisture and protein contents, texture, overall acceptability and appearance) has been explained below in Figures 4.3- 4.8.

4.4.1 Predictive model for fat content

Model equation for predicting the fat content present in the AYB-maize rings (*kokoro*) samples is shown in equation 4.1. While Figures 4.3a-c showed how each of the independent variables (two variables at a time while keeping the third variable constant at centre point, $x_n=0$) affected the fat content of the product

$$\text{Fat} = +18.10089 + 3.21697A + 0.079853B - 9.64451C - 0.010506A^2 - 1.33218 \times 10^{-3}B^2 - 0.35158C^2 - 0.018018AB + 0.028327AC + 0.099858BC \dots \dots \dots 4.1$$

$$(R^2=61.73\%, p\text{-level}=0.3911)$$

Where A=%AYB flour inclusion, B=frying temperature, C=frying duration (time)

The predictive model equation for fat content showed that percentage AYBF inclusion (A), frying temperature (B), interaction between AYBF inclusion and frying time (AC), and interaction between frying temperature and frying time (BC) carry positive sign. That is, increase in any of these, increases the fat content of the product. Whereas, frying time (C), 2nd order of frying temperature (B²), 2nd order of AYBF inclusion (A²), 2nd order of frying time (C²) and interaction between AYBF and frying temperature (AB) are negative indicating fat content reduces when each of these variable increase in value. This is similar to the report of Sobukola *et al.* (2008) that oil uptake of fried yam chips increased with increasing time and temperature.

Equation 4.1 shows that the coefficient of determination R^2 is low (61.73%) at 60.89% confidence level while probability of prediction (p -level) was 0.3911, no significant model

term since the model stated that any result with p-level >0.05 and confidence level <95% indicate model terms are not significant.

4.4.2 Predictive Model for moisture content (MC)

Moisture content, MC can be predicted using model equation 4.2 culled from result generated by the statistical analysis of response surface experimental design.

$$MC = +35.18746 + 0.41202A - 0.38119B - 1.65244C + 5.35 \times 10^{-4}A^2 + 1.43585 \times 10^{-3}B^2 + 0.075425C^2 - 2.68 \times 10^{-3}AB - 6.56 \times 10^{-4}AC + 4.33 \times 10^{-6}BC \dots \dots \dots 4.2$$

$$(R^2=89.74\%, p\text{-level}=0.0096)$$

Where A=% AYBF inclusion, B=frying temperature, C=frying duration (time)

As reflected in the equation, the three independent variables, second order derivatives and interaction between them are significant (Figs.4.4a-c).

The coefficient of determination, R^2 is high (89.74%) at 99.04% confidence level, while the probability of prediction (p-level) was 0.0096. This indicates that the model terms are significant since p-level is less than 0.05 and confidence level >95%.

Percentage AYBF inclusion (A), 2nd order of AYBF inclusion (A^2), 2nd order of frying temperature (B^2), 2nd order of frying time (C^2) and interaction between frying temperature and frying time (BC) are positive indicating that increasing any of these parameters will lead to increased moisture content. The negative sign of frying temperature (B), frying time (C), AYBF inclusion -frying temperature interaction (AB) and AYBF inclusion -frying time interaction (AC) show that increase in value of any of these will decrease moisture content. This is similar to the report of Garayo and Moreira (2002) during vacuum frying of potato chips. Therefore, products with lower moisture content can be obtained from frying at a high temperature for a longer time.

DESIGN-EXPERT Plot

fat
X = A: blend
Y = B: f.temp

Actual Factor
C: f.time = 10.00

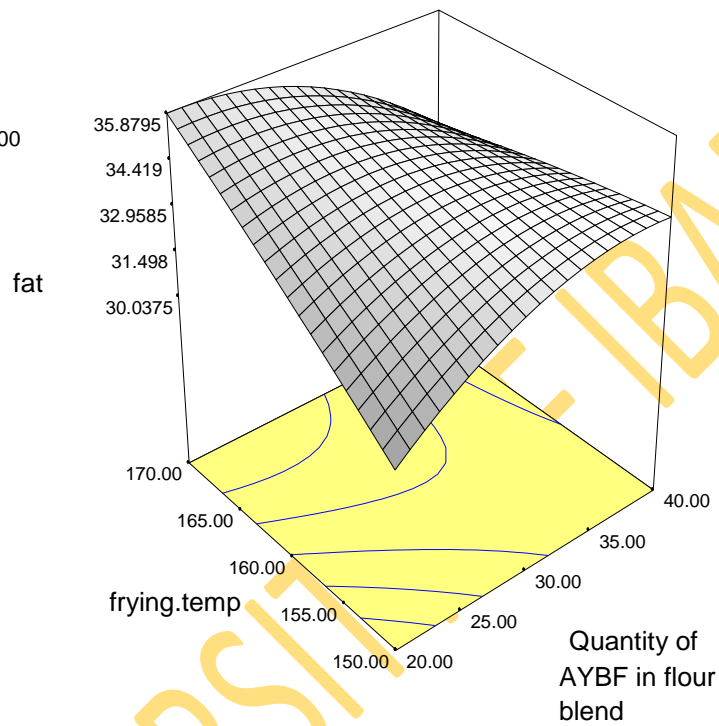


Figure 4.3a Effect of flour blend (x_1) and frying temperature (x_2) on fat content.

DESIGN-EXPERT Plot

fat
X = B: f.temp
Y = C: f.time

Actual Factor
A: blend = 30.00

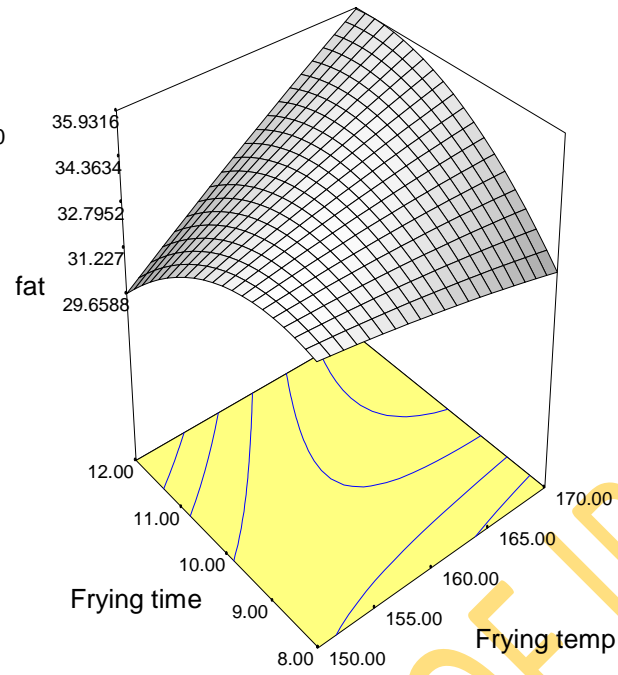


Figure4.3b Effect of frying temperature (x_2) and frying time (x_3) on fat content.

DESIGN-EXPERT Plot

fat
X = A: blend
Y = C: f.time

Actual Factor
B: f.temp = 160.00

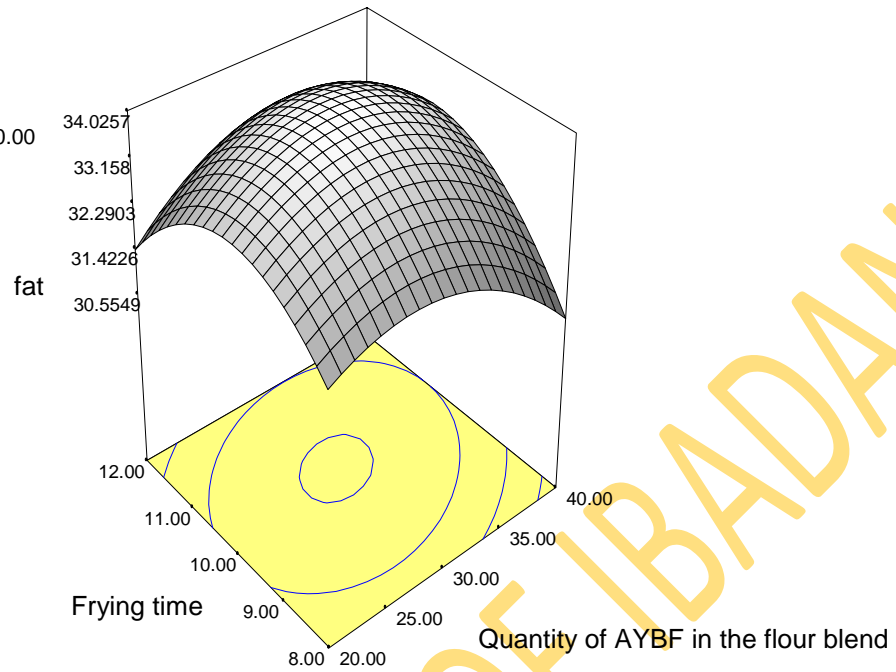


Figure 4.3c. Effect of flour blend (x_1) and frying time (x_3) on fat content.

DESIGN-EXPERT Plot

moisture
X = A: blend
Y = B: f.temp

Actual Factor
C: f.time = 10.00

moisture

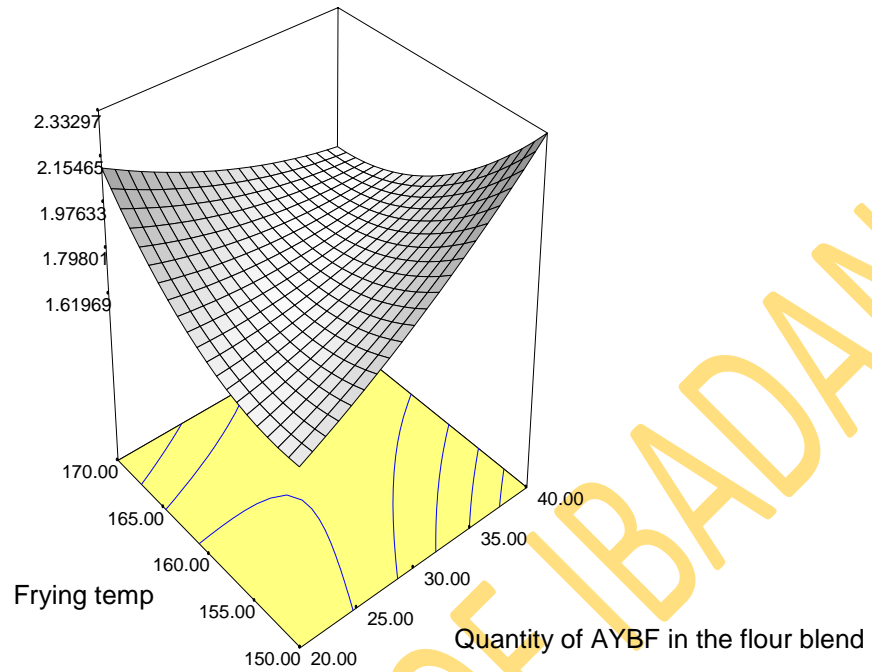


Figure 4.4a Effect of flour blend (x_1) and frying temperature (x_2) on moisture content

DESIGN-EXPERT Plot

moisture
X = B: f.temp
Y = C: f.time

Actual Factor
A: blend = 30.00

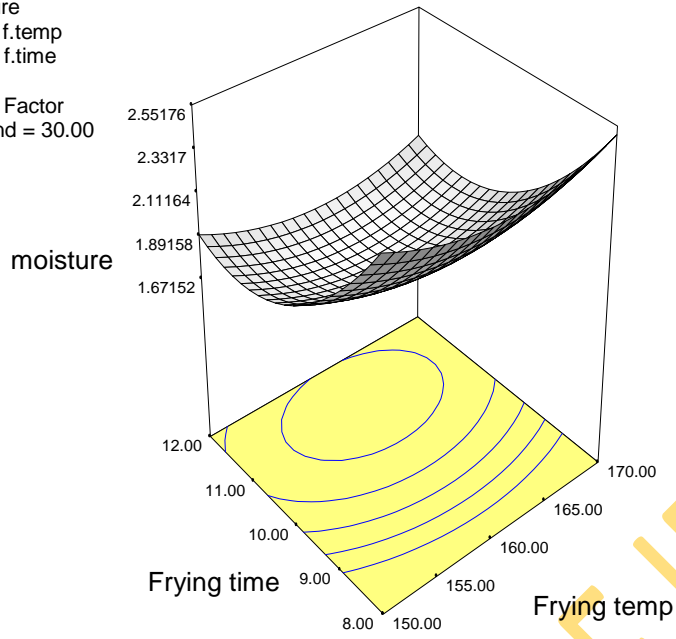


Figure 4.4b. Effect of frying temperature (x_2) and frying time (x_3) on moisture content

DESIGN-EXPERT Plot

moisture
X = A: blend
Y = C: f.time

Actual Factor
B: f.temp = 160.00

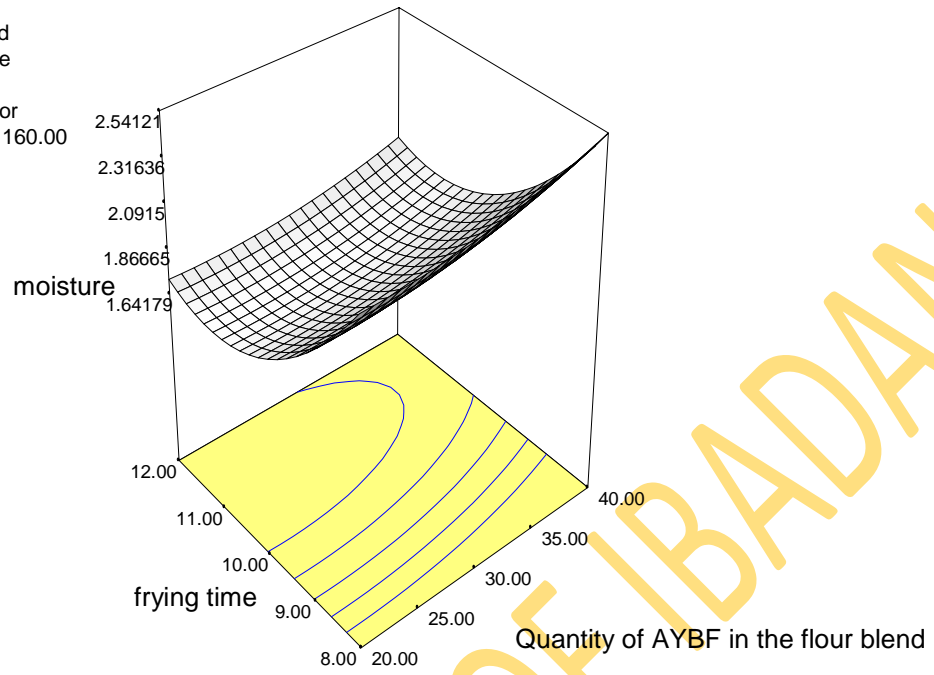


Figure 4.4c. Effect of flour blend (x_1) and frying time (x_3) on moisture content

4.4.3 Textural properties of the fried maize-AYB rings, *kokoro*.

The samples of fried products are shown in Plate 4.1. The textural property of the products has been shown in Table 4.16. The textural properties have important roles to play in determining quality and acceptability of the final products (Yagci and Gogus, 2008). Deep fat frying is a cooking/dehydration process that results in textural changes in the product. The textural property may reflect the crispness of the product. Breaking force (N) has been reported to be an indicator of texture (crispness) of fried products with lower breaking force corresponding to higher crispness (Liu-Ping *et al.*, 2005), hence increased acceptability. From Table 4.16, the breaking force was significantly ($p < 0.05$) affected by the independent variables (frying temperature, frying time and % AYBF in the blend). The value ranged from 1.30 to 2.45 kg force. The breaking force was 1.3kg when the frying temperature was 170°C and frying time was 10min with 30% AYBF: 70% MF.

4.4.3.1 Predictive model for texture

Model equation for texture, T of AYB-maize ring (*kokoro*) is presented in equation 4.3 and the interaction between the independent variables and texture is shown in Figures 4.5a-c

$$T = -128.9625 + 0.5875A + 1.51875B + 1.19375C - 5.25 \times 10^{-3}A^2 - 4.5 \times 10^{-3}B^2 - 0.025C^2 - 2.25 \times 10^{-3}AB + 3.75AC - 2.5 \times 10^{-3}BC \dots \dots \dots 4.3$$

$$(R^2=88.56\%. P=0.0136)$$

Where T=texture, A=%AYBF inclusion, B=frying temperature, C=frying time



Plate 4.1: Samples of fried maize rings- *kokoro* produced from blends of African yam bean and maize flours.

Table 4.16 Colour and Textural parameters of snacks from blends of maize and AYB flours

| %AYBF | F.Temp (°C)/Ftime (min) | l* | a* | b* | Breaking force(kgforce) |
|-------|-------------------------------|----------------|---------------|----------------|----------------------------|
| 20 | 150/10 | 44.36±20.51bc | 14.33±31.11a | 31.87±1.41ab | 2.45±0.71ab |
| 20 | 160/12 | 45.33±3.54bc | 12.00±4.24d | 32.80±16.26ab | 1.50±0.14de |
| 20 | 160/8 | 45.96±0.71bc | 13.38±4.24bc | 33.74±5.66a | 1.80±0.28cde |
| 20 | 170/10 | 42.90±425.68bc | 12.08±12.73d | 28.42±631.45bc | 1.90±0.42bcd |
| 30 | 150/12 | 43.80±21.21bc | 9.79±12.73f | 32.27±17.68ab | 2.10±0.14ab |
| 30 | 150/8 | 46.20±791.25a | 11.00±33.94h | 8.20±330.93e | 1.50±0.42de |
| 30 | 160/10 | 44.53±232.94bc | 12.49±52.99cd | 32.38±266.34ab | 1.80±0.01cde |
| 30 | 170/12 | 46.84±9.19b | 13.44±36.77ab | 32.14±54.45ab | 1.50±0.72de |
| 30 | 170/8 | 47.76±12.73b | 13.53±7.07ab | 34.69±35.36a | 1.45±0.21de |
| 40 | 150/10 | 45.18±86.27bc | 12.71±4.95bcd | 32.73±91.92ab | 2.10±0.14abc |
| 40 | 160/12 | 45.18±86.27bc | 12.71±4.95bcd | 32.73±91.92ab | 1.55±0.49cde |
| 40 | 160/8 | 43.69±89.80bc | 10.79±113.14e | 28.46±131.52bc | 1.80±0.57cde |
| 40 | 170/10 | 41.44±74.25c | 11.06±24.04e | 24.70±34.65c | 1.30±0.14e |
| 0 | Control | 47.70±122.33b | 7.59±37.48g | 16.47±28.99d | 2.50±0.14a |

Values with the different subscript along column are significantly different at $p < 0.05$

0% AYBF=100% MF

20% AYBF=20% AYBF/80%MF

30% AYBF=30% AYBF /70% MF

40% AYBF=40% AYBF /60%MF

F.time=frying time

F.Temp=frying temperature

DESIGN-EXPERT Plot

crispiness
X = A: blend
Y = B: f.temp

Actual Factor
C: f.time = 10.00

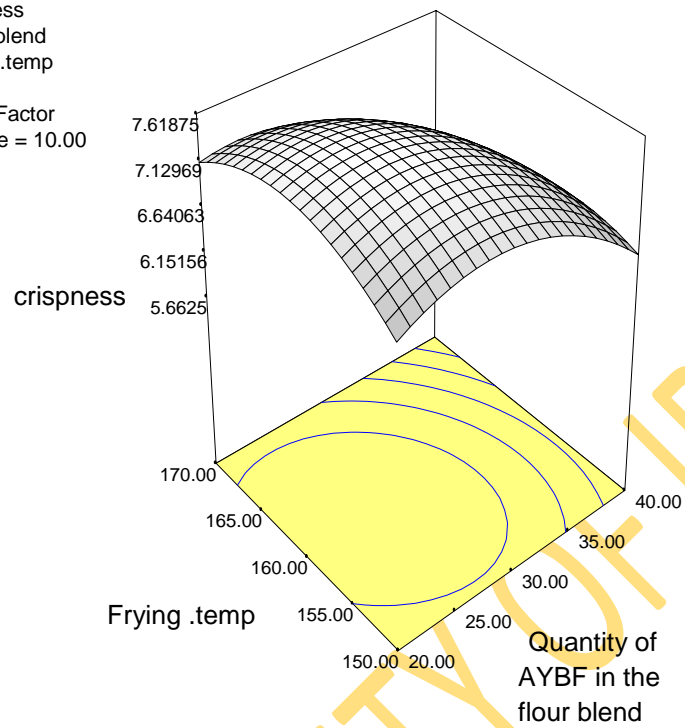


Figure 4.5a Effect of flour blend (x_1) and frying temperature (x_2) on texture.

DESIGN-EXPERT Plot

crispness
X = B: f.temp
Y = C: f.time

Actual Factor
A: blend = 30.00

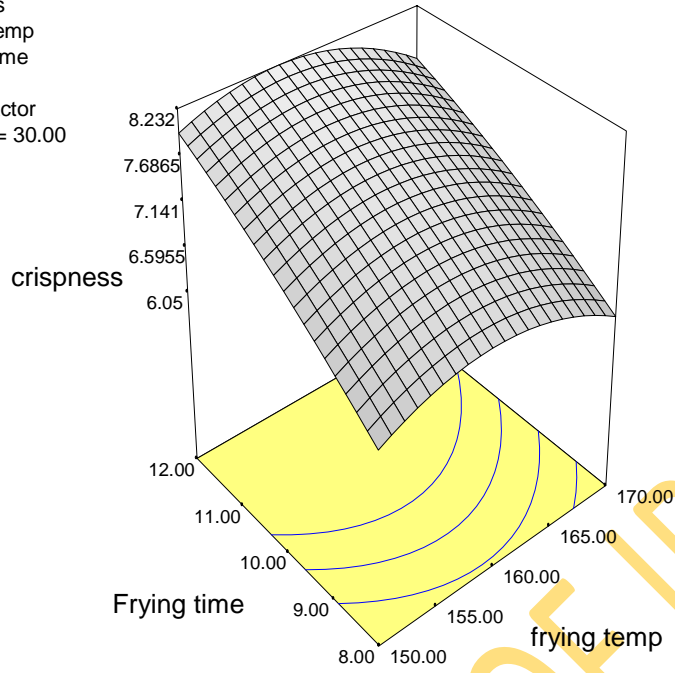


Figure4.5b Effect of frying temperature (x_2) and frying time (x_3) on texture.

DESIGN-EXPERT Plot

crispiness
X = A: blend
Y = C: f.time

Actual Factor
B: f.temp = 160.00

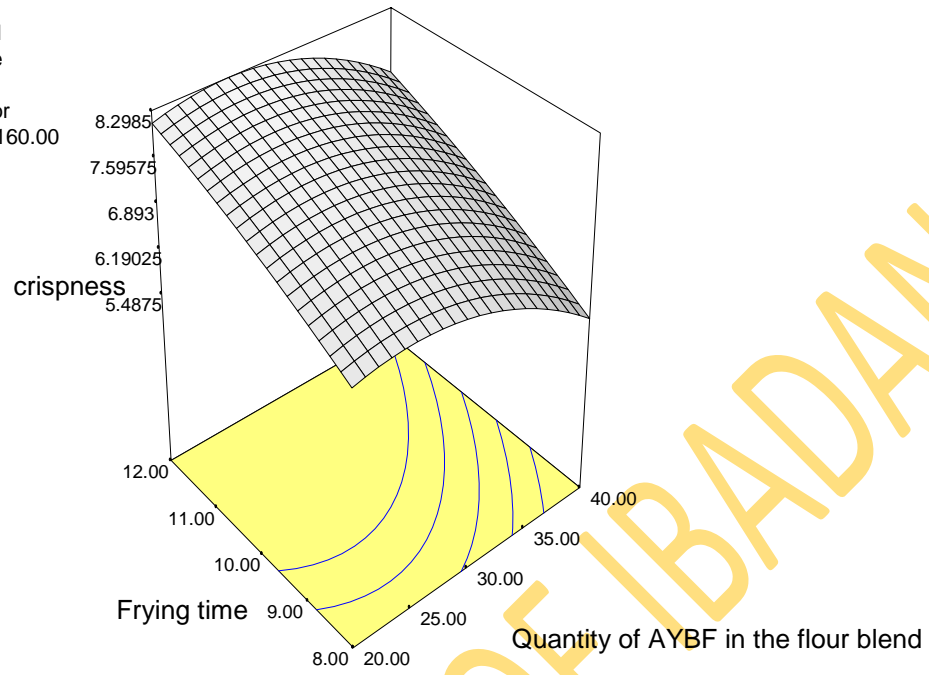


Figure 4.5c Effect of flour blend (x_1) and frying time (x_3) on texture.

As reflected in the equation, the interaction between the three independent variables is quadratic showing second order derivatives and interaction between the variables. The coefficient of determination, R^2 is high (88.56%) while the probability of prediction ($p=0.0136$) is less than 0.05 which indicate that model terms are significant. The %AYBF inclusion (A), frying temperature (B), frying time (C) and interaction between the AYBF inclusion and frying time (AC) carry positive sign which implies that the texture of the product increased with increase in AYBF inclusion in the blend, frying temperature and frying time and interaction A and C. While the second order derivatives of AYBF inclusion (A^2), frying temperature (B^2) and frying time (C^2), interaction between AYBF inclusion and frying temperature (AB), interaction between frying temperature and frying time (BC) carry a negative sign, it implies that texture of the maize rings increase with reduction in values of these parameters.

4.4.4 Colour parameters of the products

Colour is an important parameter to be controlled during processing, colour of fried product is as a result of maillard reaction that depends on the content of reducing sugars and amino acids or proteins at the surface, and the temperature and time of frying (Marquez and Anon, 1986). From Table 4.9, colour parameter, L^* (lightness) increased with increasing %AYBF from 75.82 to 76.05 (from 20%-30%AYBF) and later decreased from 76.05 to 74.65 (from 30%-40%AYBF). While a^* (redness) and b^* (yellowness) reduced consistently from 7.00 to 6.20 and 33.60 to 29.57 respectively as the percentage AYBF increased from 20%-40%. Processing from flour to *kokoro* significantly increased a^* (redness) value while L^* (lightness) was significantly reduced (Table 4.16), but b^* (yellowness) was not consistent. This is similar to the report of Sobukola *et al.*, (2008) that colour parameters (L^* and a^*) were significantly correlated with frying temperature and frying time. Colour parameter L^* , a^* and b^* of *kokoro* samples were affected by the processing variables, redness increased as frying temperature increased while lightness decreased with increasing frying temperature, although with close interaction with the frying time, some products had similar values at different frying temperature (e.g. 170°C for 10min and 160°C for 12min). An increase in a^* (redness) was also obtained with increase in temperature and time of frying. The product tends to get darker as the frying proceeds due to surface non-enzymatic browning reactions. The higher the temperature the darker the product gets since non enzymatic browning

reactions are highly temperature dependent (Liu-Ping *et al.*, 2005, Marquez and Anon., 1986). Hence, to obtain fried products of acceptable colour parameters (higher b*, moderate l* and lower a*), optimisation process (Table 4.19) suggested the optimum processing conditions.

4.4.4.2 Predictive model for appearance

Mathematical expression for predicting the effect of % AYBF inclusion, frying temperature and frying time on the product appearance of fried AYB-maize rings (*kokoro*) is shown below in equation 4.4.

$$Co = 14.71250 + 0.25375A + 0.22375B + 0.17500C - 7.5 \times 10^{-4}A^2 + 7.5 \times 10^{-4}B^2 - 0.0625C^2 - 1.5 \times 10^{-3}AB + 2.5 \times 10^{-3}AC + 6.25 \times 10^{-3}BC \dots\dots\dots 4.4$$

(R²=90.81, p-level=0.0068)

Where A=%AYBF inclusion, B=frying temperature, C=frying time

The three parameters, their second order derivatives and interactions between the variables are significant (Fig. 4.6.). Both the coefficient of determination, R² and probability of prediction are high (Equation 4.4). R² is 90.81% while p value is 0.0068. Based on these criteria, the adequacy of the model is established.

Percentage AYBF inclusion in the flour blend (A), frying temperature (B), frying time (C), interaction between AYBF inclusion and frying time (AC), interaction between frying time and frying temperature (BC) and 2nd order derivatives of frying temperature (B²) are positive indicating the panelists' rating for product appearance increased with increase in all of these parameters. This is consistent with the report of Samatcha *et al.*, (2009) that drying /frying time significantly affected appearance of popped rice. While 2nd order derivative of AYBF inclusion (A²), 2nd order derivative of frying time (C²) and interaction between blend and frying temperature (AB) being negative showed that appearance rating by panelists reduced in value with increase in value of each of the parameters.

DESIGN-EXPERT Plot

appearance
X = A: blend
Y = B: f.temp

Actual Factor
C: f.time = 10.00

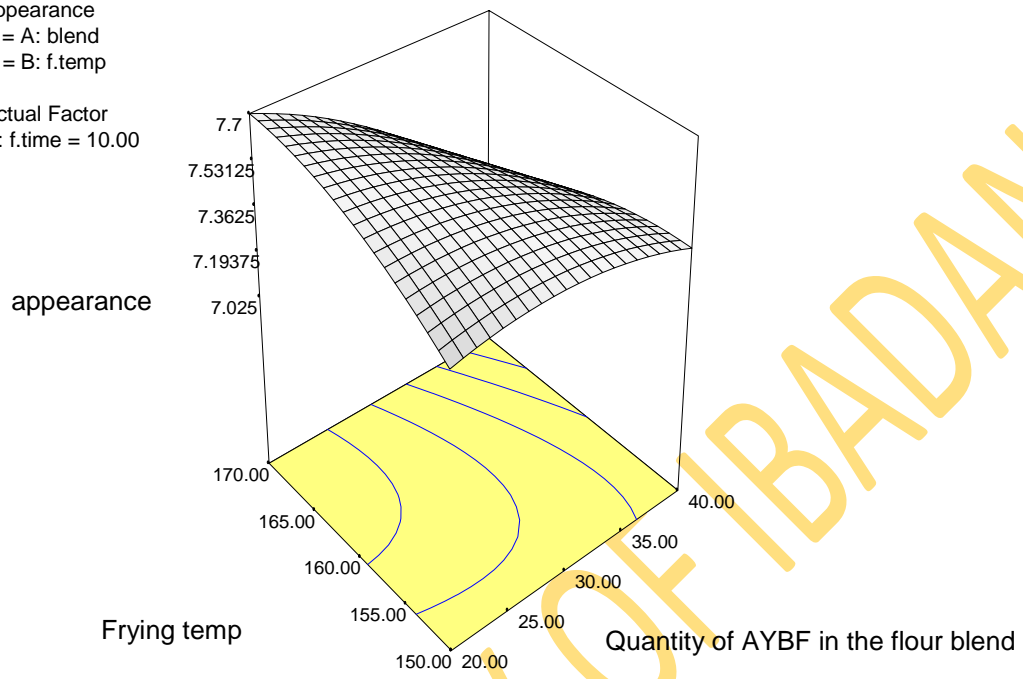


Figure 4.6a Effect of flour blend (x_1) and frying temperature (x_2) on product appearance.

DESIGN-EXPERT Plot

appearance
X = B: f.temp
Y = C: f.time

Actual Factor
A: blend = 30.00

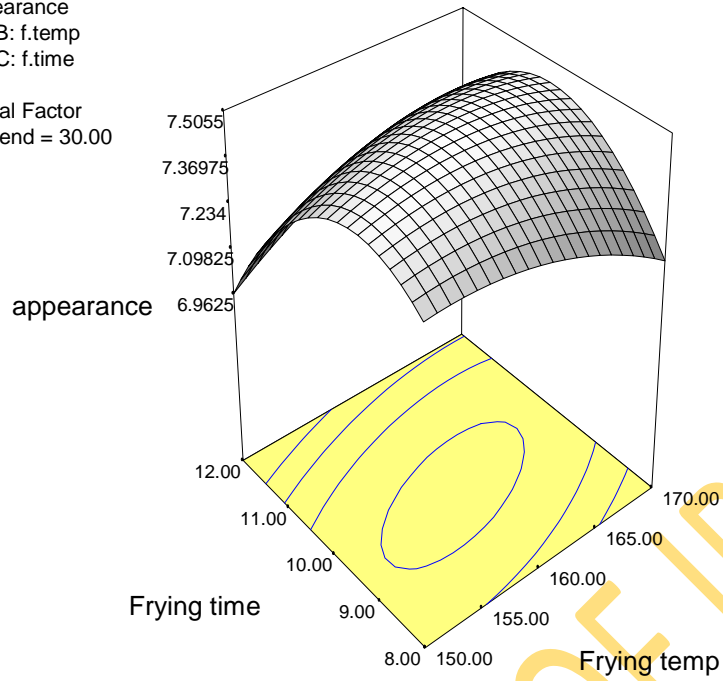


Figure 4.6b. Effect of frying temperature (x_2) and frying time (x_3) on product appearance.

DESIGN-EXPERT Plot

appearance
X = A: blend
Y = C: f.time

Actual Factor
B: f.temp = 160.00

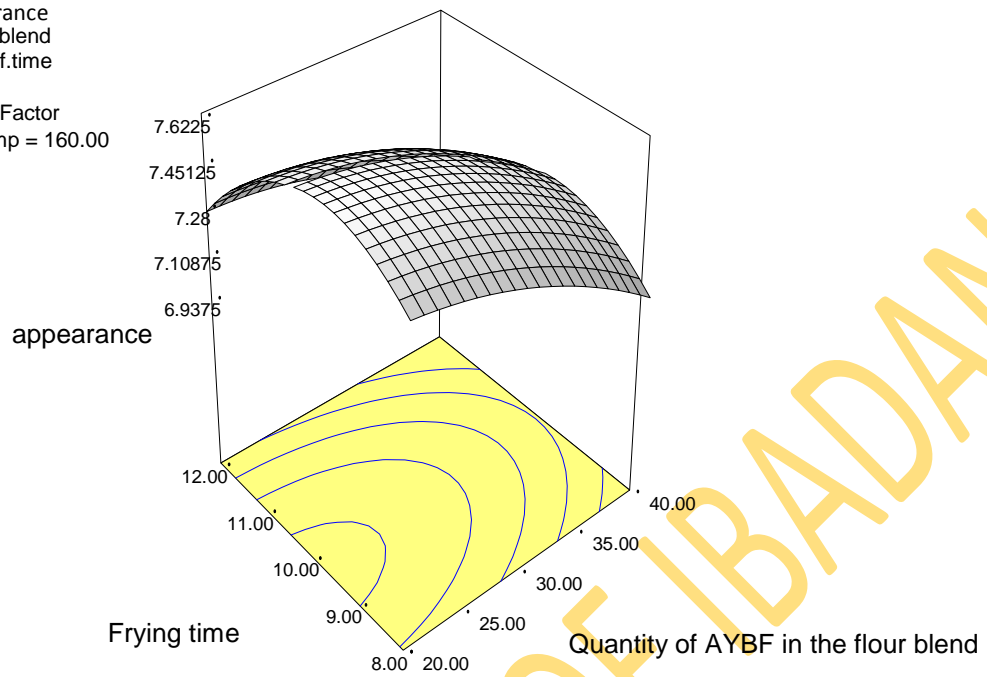


Figure 4.6c. Effect of flour blend (x_1) and frying time (x_3) on product appearance.

4.4.5 Sensory properties of the product

The results of sensory attributes of the fried product are presented in Table 4.17 and Appendix 11. The Hedonic score for the sensory quality of products ranged from 5.1 (neither like nor dislike) to 7.9 (like very much) and compared favourably with the commercial snack in all attributes. The sensory perception scores of the samples vary significantly among the sensory parameters measured especially for crispness (texture) and overall acceptability. Samples with 30%-40% AYB were generally scored higher than that of 20% AYB. Appearance of the sample ranged from 5.3 to 7.5. Values for crispness (texture) were between 5.1 and 7.9. And the overall acceptability was in the range of 6.1-7.6. Sample with 30%-40% AYBF fried at temperature between 150-160°C and frying time, 10-12min had higher score for overall acceptability. Samples C150/10, C160/8, A160/12, A160/8, B170/12 and B170/8 were observed to be significantly better than others in terms of aroma perception within the samples.

4.4.5.1 Predictive model for overall acceptability

Model equation for prediction of overall acceptability, S of AYB-maize snack (*kokoro*) is stated in equation 4.5 and the interaction between its independent variables is shown Figures 4.7a-c.

$$S = -80.225 + 0.63375A + 0.8825B + 1.50625C - 2.75 \times 10^{-3}A^2 + 2.5 \times 10^{-3}B^2 - 0.08125C^2 - 3.25 \times 10^{-3}AB + 2.5 \times 10^{-3}AC + 1.25 \times 10^{-3}BC \dots \dots \dots 4.5$$

$$(R^2=88.95\%, p\text{-level}=0.01)$$

Where A=% AYBF inclusion, B=frying temperature, C=frying time

As reflected in the equation, the three independent variables, second order derivatives and interactions between the variables are significant. The coefficient of determination, R^2 is high (88.95%) and p value is 0.01. Percentage of AYBF inclusion in the flour blend (A), frying temperature (B), frying time (C), interaction between blend and frying time (AC), interaction between frying temperature and frying time (BC) being positive indicate that a higher (better) overall acceptability of the products is expected with increase in value of each of the parameters. While 2nd order derivative of AYBF (A^2), 2nd order derivative of

Table 4.17 Sensory attributes of *kokoro* produced from flour blends of maize and African yam bean.

| Sample | Appearance | Taste | Texture | Aroma | Overall acceptability |
|---------|------------|-------|---------|-------|-----------------------|
| B1508 | 5.3e | 6.1c | 7.1c | 6.3d | 6.1d |
| B15012 | 7.4b | 7.2a | 5.9e | 6.5c | 6.6c |
| B16010 | 7.1c | 6.7b | 7.7ab | 6.8ab | 7.1b |
| C15010 | 7.0c | 7.2a | 7.5bc | 7.0a | 7.6a |
| C1608 | 7.5ab | 7.0ab | 6.4d | 7.2a | 7.3ab |
| C17010 | 6.7d | 6.7b | 5.8e | 6.7b | 6.4c |
| A17010 | 7.5ab | 6.6bc | 5.1f | 6.9ab | 6.2cd |
| A16012 | 7.7a | 7.3a | 7.1c | 7.2a | 7.5a |
| A1608 | 7.2c | 7.0ab | 7.6ab | 7.1a | 7.5a |
| B16010 | 7.1c | 6.7b | 7.7ab | 6.8ab | 7.1b |
| B16010 | 7.1c | 6.7b | 7.7ab | 6.8ab | 7.1b |
| B16010 | 7.1c | 6.7b | 7.7ab | 6.8ab | 7.1b |
| C16012 | 7.5ab | 6.8b | 6.4d | 6.9ab | 6.9bc |
| B17012 | 7.5ab | 6.6bc | 7.5bc | 7.0a | 7.2ab |
| A15010 | 7.2c | 6.8b | 7.9a | 6.9ab | 7.5a |
| B1708 | 7.8a | 6.9ab | 7.5bc | 7.0a | 7.3ab |
| Control | 7.4b | 6.6bc | 6.3d | 6.7b | 6.9bc |

Values with the same letter along the column are not significantly different at $p \geq 0.05$

A=20% AYBF/80% MF

B=30% AYBF/70% MF

C=40% AYBF/60% MF

frying temperature (B^2), 2nd order derivative of frying time (C^2) and interaction between blend and frying temperature (AB) carry a negative value which implies that increase in any of the variables show a reduced overall acceptability on the products.

4.4.6 Protein content of the AYB-maize rings

Maize rings (*kokoro*) produced from flour blend C (40%AYBF: 60%MF) had the highest protein content (Table 4.12) compared to those produced from flour blends containing lower %AYBF inclusion. Effect of frying temperature and other processing parameters used on protein content of maize rings is shown in Figures 4.8a-c

4.4.6.1 Predictive model for protein content

Model equation for predicting protein content is stated in equation 4.6 and Figures 4.9a-c showed the relationship between the variables.

$$P = +10.99239 + 0.099562A - 0.02518B - 0.02375C \dots\dots\dots 4.6$$

($R^2=86.90\%$, $p\text{-level}=0.001$)

Where P=protein content

A=%AYBF inclusion in the flour blend

B=frying temperature

C=frying time

As reflected in the equation, the interaction between the three independent variables is linear as there is no second order derivatives and interaction between the variables. The equation 4.6 is similar to this, $Y = C + mx_1 + mx_2 + mx_3 \dots\dots\dots + mx_n$.

The coefficient of determination, R^2 is high (86.90%) at 99.99% confidence level, while the probability of prediction ($p\text{-level}$) is 0.0010 indicating that the model terms are significant since $p\text{-level} < 0.05$. The percentage AYBF inclusion is positive which implies that the protein content of the product increased as quantity of AYBF increased. While the frying temperature and frying time carry a negative sign indicating that protein increase with reduction in each value. This is similar to the finding of Awoyale *et al.* (2011) who reported

DESIGN-EXPERT Plot

Overall acceptability.

X = A: blend

Y = B: f.temp

Actual Factor

C: f.time = 10.00

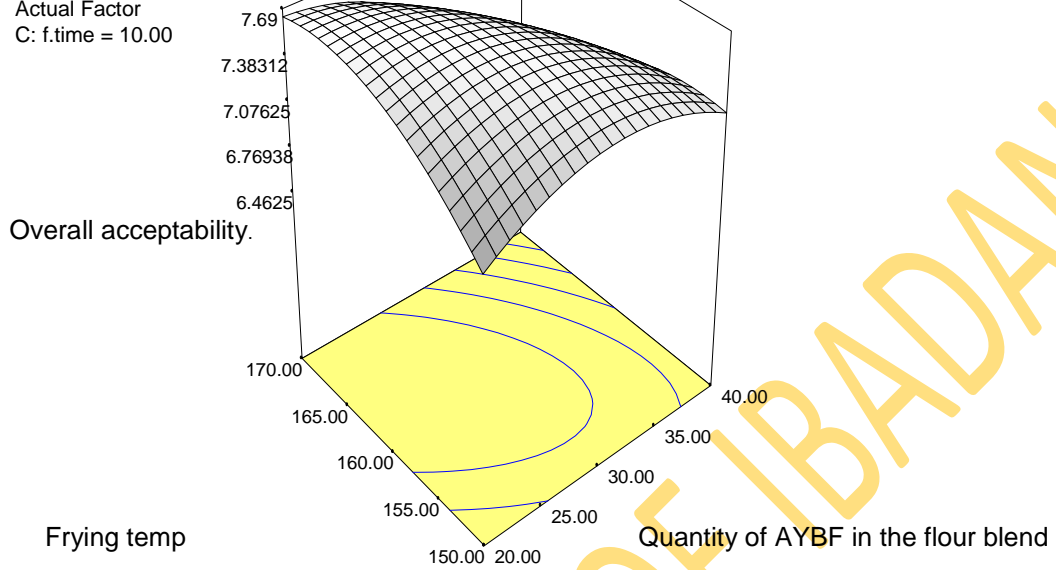


Figure 4.7a Effect of flour blend (x_1) and frying temperature (x_2) on overall acceptability.

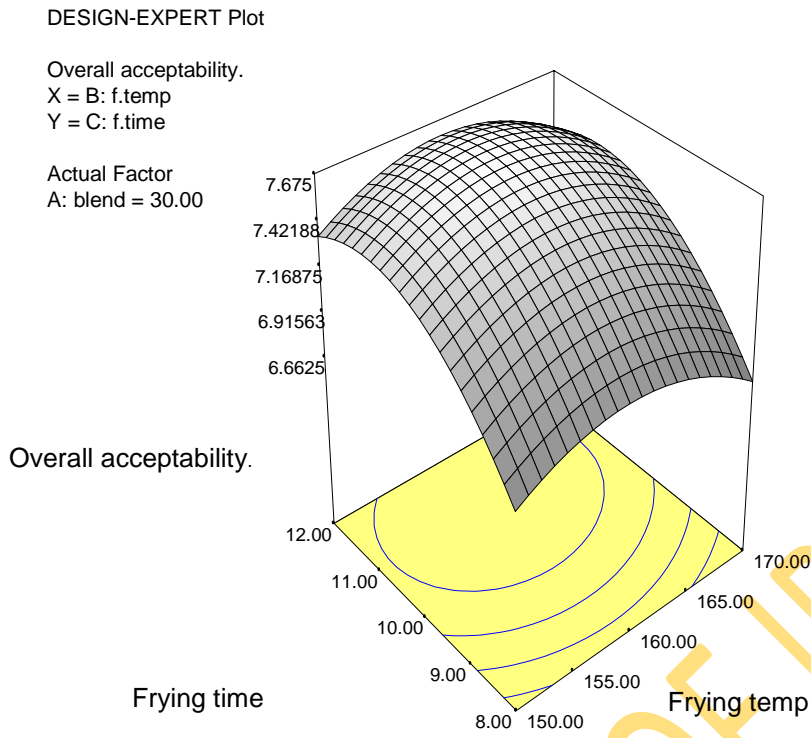


Figure 4.7b Effect of frying temperature (x_2) and frying time (x_3) on overall acceptability.

DESIGN-EXPERT Plot

Overall acceptability.
X = A: blend
Y = C: f.time

Actual Factor
B: f.temp = 160.00

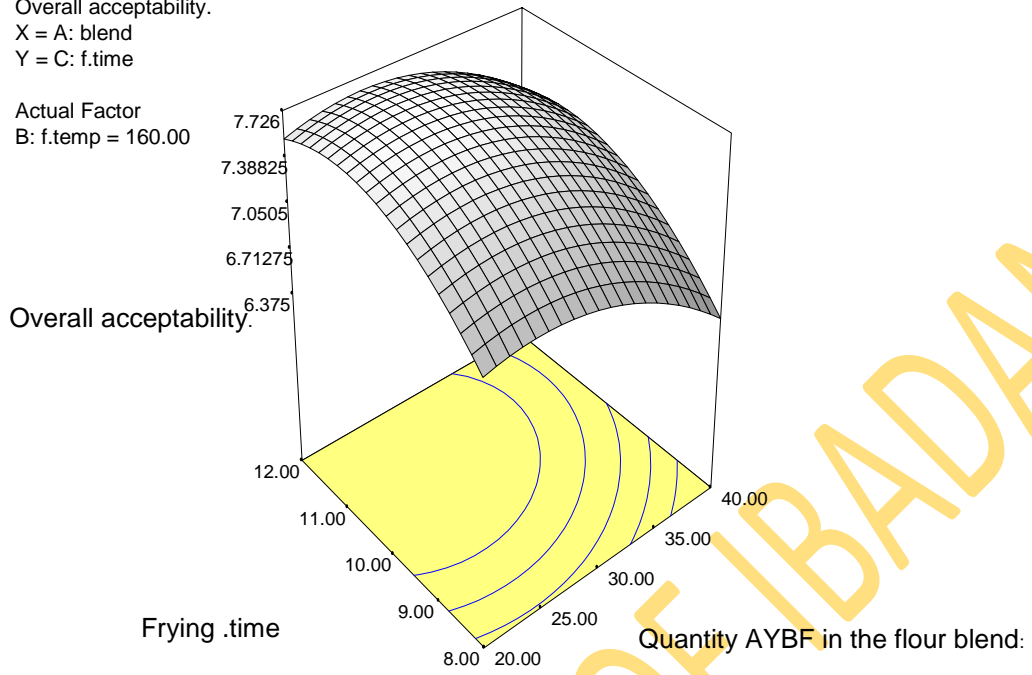


Figure 4.7c Effect of flour blend (x_1) and frying time (x_3) on overall acceptability.

DESIGN-EXPERT Plot

protein
X = A: blend
Y = B: f.temp

Actual Factor
C: f.time = 10.00

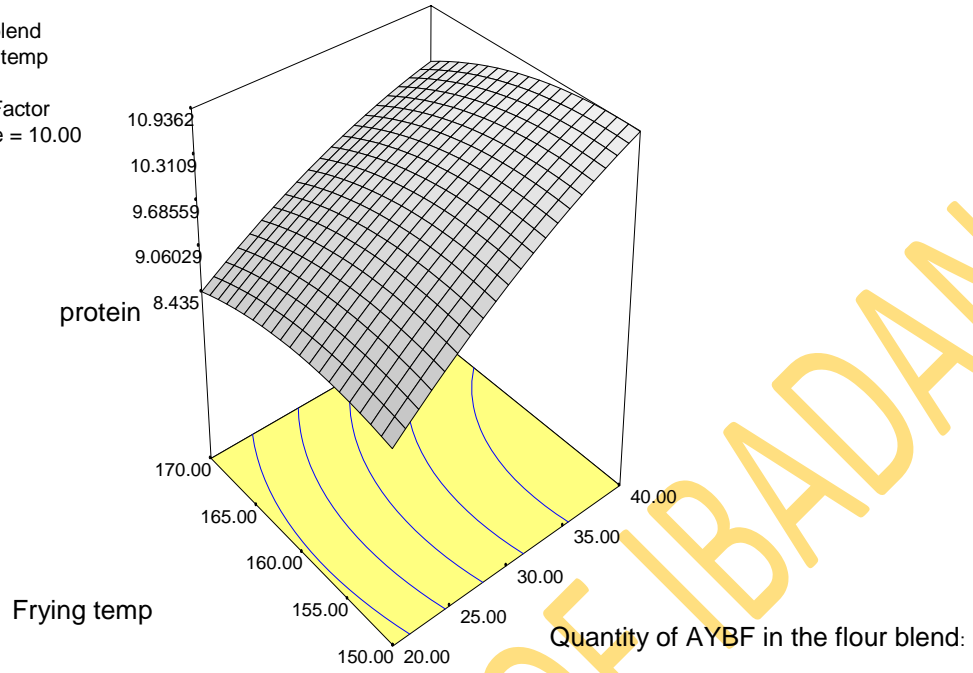


Figure 4.8a Effect of frying temperature (x_2) and flour blend (x_1) on protein content

DESIGN-EXPERT Plot

protein
X = B: f.temp
Y = C: f.time

Actual Factor
A: blend = 30.00

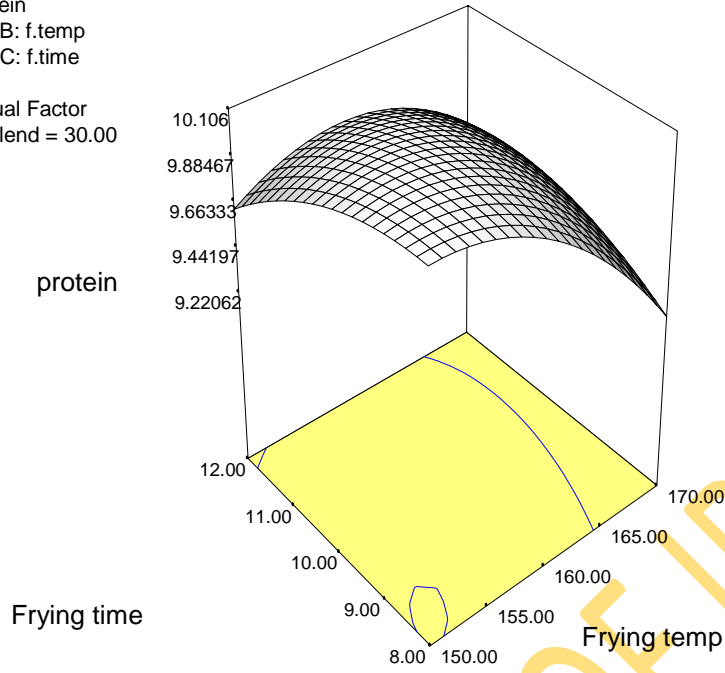


Figure 4.8b Effect of frying temperature (x_2) and frying time (x_3) on protein content

DESIGN-EXPERT Plot

protein
X = A: blend
Y = C: f.time

Actual Factor
B: f.temp = 160.00

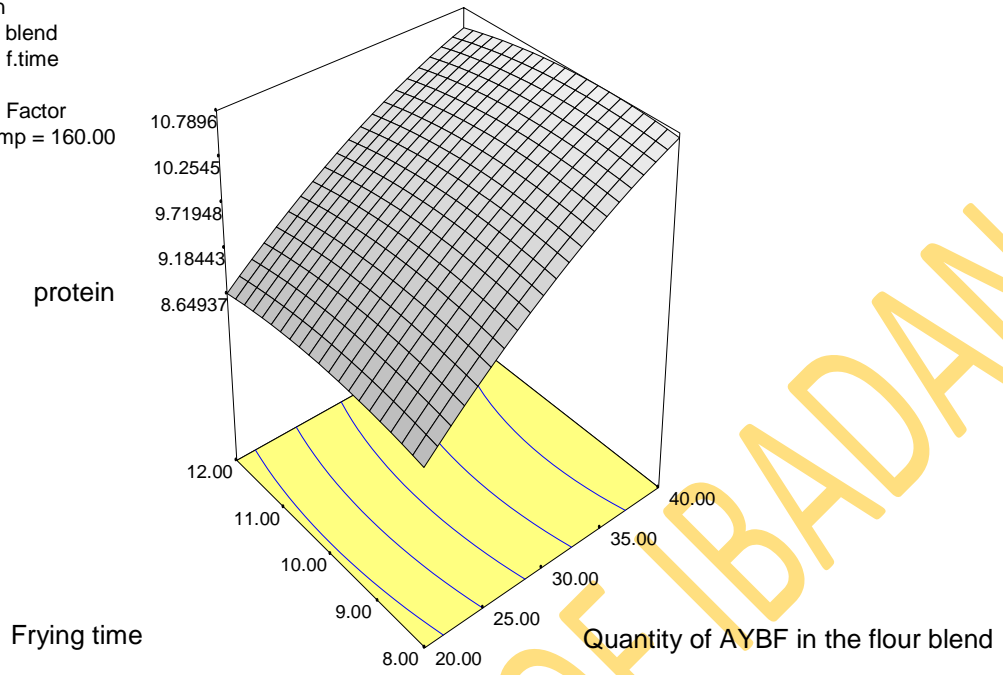


Figure 4.8c Effect of frying time (x_3) and flour blend (x_1) on protein content

that the reduced frying temperature and time increased protein content of *kokoro* supplemented with Distillers' spent grain (DSG-maize *kokoro*).

4.5 Optimisation process

Each of the independent variables used in the study were put back into the experimental design generated by the software with their corresponding responses as shown in Table 4.18. Desirable features (protein content, crispness, appearance and overall acceptability) of the products were maximized, while fat and moisture contents were minimized. In order to deduce workable optimum conditions, the graphical optimisation technique was adopted by fixing a variable per time as shown in Figures 4.3-4.8. It provided comprehensive and informative insight of the system, which led to optimisation of the process. A set of values were generated for all the parameters for the optimum processing condition (Table 4.19). AYBF inclusion of 30%, frying temperature, 155°C and frying time of 11.5min could be considered as optimum conditions for the variables studied for the AYB-maize (*kokoro*) of suitable quality characteristics.

The suitability of the model developed for predicting the optimum response values was tested using the recommended optimum conditions of the variables to validate the predicted values of the responses. The products were prepared at these optimum conditions for the validation and the difference between the actual values and predicted values were subjected to analysis and the standard error was determined as shown in Table 4.20. Other analyses done on samples produced under this optimum condition were rat studies, comparative sensory analysis with the commercial sample, mineral analysis, microbial analysis and rancidity test.

4.6 Microbial analysis of the fried product.

The microbial contents of the flour blend, gelatinized flour blend and fried snack prepared at recommended optimum conditions were obtained. Result (Table 4.21) showed that the microbial load decreased with higher degree of heat intensity in the processing steps. The fried products were free of bacteria, moulds and yeasts.

Table 4.18: Response surface analysis result of processing variables and responses

| Exp.runs | A:blend | B:f.temp | C:f.time | Protein% | Fat% | MC% | Appearance | Overall | |
|----------|---------|----------|----------|----------|-------|------|------------|---------------|---------|
| | | | | | | | | acceptability | Texture |
| 1 | 30 | 150 | 8 | 9.94 | 31.04 | 2.69 | 7.4 | 6.6 | 5.9 |
| 2 | 30 | 150 | 12 | 9.55 | 27.61 | 1.99 | 6.9 | 7.1 | 7.7 |
| 3 | 30 | 160 | 10 | 9.98 | 34.01 | 1.76 | 7.5 | 7.6 | 7.5 |
| 4 | 40 | 150 | 10 | 10.83 | 35.01 | 2.32 | 7.4 | 7.3 | 6.4 |
| 5 | 40 | 160 | 8 | 11.00 | 30.94 | 2.42 | 6.9 | 6.4 | 5.8 |
| 6 | 40 | 170 | 10 | 9.81 | 29.58 | 1.97 | 7.1 | 6.2 | 5.1 |
| 7 | 20 | 170 | 10 | 8.54 | 34.25 | 2.13 | 7.6 | 7.5 | 7.1 |
| 8 | 20 | 160 | 12 | 8.39 | 31.04 | 1.84 | 7.4 | 7.5 | 7.8 |
| 9 | 20 | 160 | 8 | 8.48 | 31.53 | 2.42 | 7.5 | 6.9 | 6.4 |
| 10 | 30 | 160 | 10 | 9.98 | 34.01 | 1.76 | 7.5 | 7.6 | 7.5 |
| 11 | 30 | 160 | 10 | 9.98 | 34.01 | 1.76 | 7.5 | 7.6 | 7.5 |
| 12 | 30 | 160 | 10 | 9.98 | 34.01 | 1.76 | 7.5 | 7.6 | 7.5 |
| 13 | 40 | 160 | 12 | 10.72 | 32.71 | 1.78 | 6.9 | 7.2 | 7.5 |
| 14 | 30 | 170 | 12 | 9.67 | 37.89 | 1.72 | 7.2 | 7.5 | 7.9 |
| 15 | 20 | 150 | 10 | 8.99 | 32.46 | 1.40 | 7.3 | 7.3 | 7.5 |
| 16 | 30 | 170 | 8 | 9.30 | 33.33 | 2.41 | 7.2 | 6.9 | 6.3 |
| 17 | 30 | 160 | 10 | 9.98 | 34.01 | 1.76 | 7.5 | 7.6 | 7.5 |

Exp.runs=Experimental runs, f.temp=frying temperature, f.time=frying time

Table 4.19: Optimum Processing parameters obtained from RSM.

| Solution | %AYB | Frying Temperature | Frying time | Protein | Fat | Moisture | Apperance | Overall acceptability | Crispness | Desirability |
|-----------------|-------------|---------------------------|--------------------|----------------|------------|-----------------|------------------|------------------------------|------------------|---------------------|
| 1 | 32.66 | 155.16 | 11.52 | 10.12 | 32.35 | 1.78 | 7.19 | 7.55 | 7.91 | 0.75 |

Table 4.20: Validation of Predicted Optimum condition

| Parameter | Predicted | Actual | % deviation |
|-----------------------|-----------|--------|-------------|
| Protein (%) | 10.12 | 9.97 | 0.01 |
| Fat (%) | 32.35 | 28.21 | 0.13 |
| Moisture (%) | 1.79 | 1.87 | 0.04 |
| Appearance | 7.20 | 8.00 | 0.11 |
| Overall acceptability | 7.56 | 8.10 | 0.07 |
| Crispness | 7.90 | 7.20 | 0.09 |

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Table 4.21: Microbial Quality of fried AYB-maize snack

| Sample | Bacteria (cfu/g) | Moulds (cfu/g) | Yeasts (cfu/g) |
|--------------------|---------------------|-------------------|-------------------|
| Flour | 4.0×10^2 | 1.1×10^2 | 4.1×10^2 |
| Gelatinized sample | 2.0×10^2 | 8.0×10 | 2.0×10 |
| Fried sample | ND | ND | ND |

Mean values of duplicates.

ND=not detected

cfu=colony forming unit

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4.7 Biological Assessment of the Fried product (*kokoro*)

The result of biological evaluation of protein quality of *kokoro* produced from blends of maize and AYB, basal and casein diet (control) are presented in Table 4.22 and Plate 4.2. Feed efficiency ratios were 0.32, 0.26 and 0.08 for casein, *kokoro* and basal diets respectively. Osundahunsi and Aworh (2003) reported similar results for maize-based complementary foods enriched with soya beans and cowpea Tempe. There were significant differences ($p < 0.05$) in feed efficiency ratio (FER), protein efficiency ratio (PER), net protein ratio (NPR), protein retention efficiency (PRE) and feed conversion ratio (FCR). The *kokoro* had lower values than the casein control diet but higher values than the basal diet. The result is similar to that obtained by Olapade (2010) for complementary snack from cowpea and acha blends. Results of weight of organs of animals fed with the different diets are presented in the Table 4.23. There were no significant differences ($p \geq 0.05$) among weight of liver, kidney, spleen and pancreas of rat fed on casein and the “formulated” *kokoro*. However, there was a significant difference ($p < 0.05$) among these organs compared to those fed on basal diet and those of formulated *kokoro*. Those fed on the latter had higher values than those fed on basal diet.

4.8 Sensory Quality of “formulated” Maize Rings and Commercial Maize Rings (*kokoro*)

The result of sensory evaluation of “formulated” maize rings samples produced at the optimum condition was compared to that of commercial maize rings samples. The score for the sensory quality of the “formulated” maize rings (Table 4.24) ranged from 7.7 (like very much) to 8.1 (like very much), while that of the commercial sample ranged from 5.0 (neither like nor dislike) to 7.3 (like moderately). This shows that the “formulated” maize rings have a better sensory evaluation than the commercial maize rings.

Table 4.22: Biological evaluation of experimental diet compared

| Parameter | Feed Efficiency Ratio (FER) | Protein Efficiency Ratio (PER) | Net protein Retention Ratio (NPR) | Protein Retention Ratio (PRE) | Feed Conversion Ratio (FCR) |
|-------------------|-----------------------------|--------------------------------|-----------------------------------|-------------------------------|-----------------------------|
| Experimental diet | 0.26b | 1.93b | 2.38b | 35.78b | 3.71a |
| Basal Diet | 0.08c | 0.61c | 0.01c | 0.02c | 0.01c |
| Casein Diet | 0.32a | 2.50a | 2.77a | 44.38a | 3.16b |

Values with the same letter along the same column are not significantly different at $p \geq 0.05$.

Table 4.23: Weight of the Organs of Rats fed on the different diets (g/100g weight)

| | Liver | Kidney | Spleen | Pancreas |
|-------------------|-------|--------|--------|----------|
| Experimental diet | 4.67a | 0.64b | 0.56a | 0.58a |
| Basal diet | 3.29b | 0.41c | 0.19b | 0.26b |
| Casein diet | 5.18a | 0.82a | 0.45a | 0.73a |

Values with the same subscripts along the same column are not significantly different ($p \geq 0.05$)



a. Basal diet fed rats



b. Casein diet fed rats



c. Experimental (AYB-maize rings) diet fed rats

Plate 4.2: Biological Evaluation of fried snack from blend of African yam bean flour and Maize flour

Table 4.24: Sensory Quality of the maize-based Rings.

| Parameter | "Formulated" maize | |
|-----------------------|--------------------|------------------------|
| | rings | Commercial maize rings |
| Taste | 7.7a | 6.5b |
| Crispness (texture) | 7.2a | 7.3a |
| Appearance | 8.0a | 5.0b |
| Aroma | 7.8a | 6.4b |
| Overall acceptability | 8.1a | 6.5b |

Values with the same letter along the same row are not significantly different ($p \geq 0.05$).

4.9 Storage Properties of Maize Rings

4.9.1 Adsorption isotherm characteristics of the *kokoro*

The adsorption isotherm of *kokoro* samples produced showed a sigmoid type II, according to Brunauer-Emmet-Teller (BET) classification. The adsorption isotherm result suggested that the equilibrium moisture content of the sample decreased with increase in storage temperature (20, 30 and 40°C) at any given water activity as shown in Figure 4.9. Similar trends were reported for Akara Ogbomoso made from blends of cowpea and soybean (Falade *et al.*, 2003). Monolayer moisture content for “formulated” *kokoro* ranged between 2.05-3.25% (dry basis).

4.9.2 Sensory evaluation of the Stored Snacks

The result of the sensory qualities of snack produced at the optimum conditions and stored at (temperature, 24°C and relative humidity, 61% (Table 4.25) showed that quality attributes of the snack decreased with increase in the period of storage. There were no significant differences ($p \geq 0.05$) among fresh sample and stored samples in terms of appearance, taste, crispness, aroma and overall acceptability until after 3 weeks of storage, after which all the sensory attributes tested scored above the acceptable Hedonic score-5 up to the 10th week. This implies that the products could be stored for a period of twelve weeks under ambient storage condition (temperature-24°C and relative humidity-61%) without unacceptable changes in the sensory qualities.

4.9.3 Effect of storage on free fatty acid (FFA) value of “formulated” *kokoro* snack from blends of Maize and African yam bean seed flour

The results of the free fatty acid (FFA) values of the snacks stored at ambient conditions (temperature-24°C and relative humidity-61%) are presented in Figure 4.11. It showed that the FFA contents increased with increase in the period of storage. The FFA obtained during the storage period did not exceed the 1.2-2.1% limit, which was reported by Pearson (1976) and Idowu *et al.*, (2010) to be the minimum limit for odour to be acceptable. This implies that the snack will maintain a good quality for a storage period of 12 weeks.

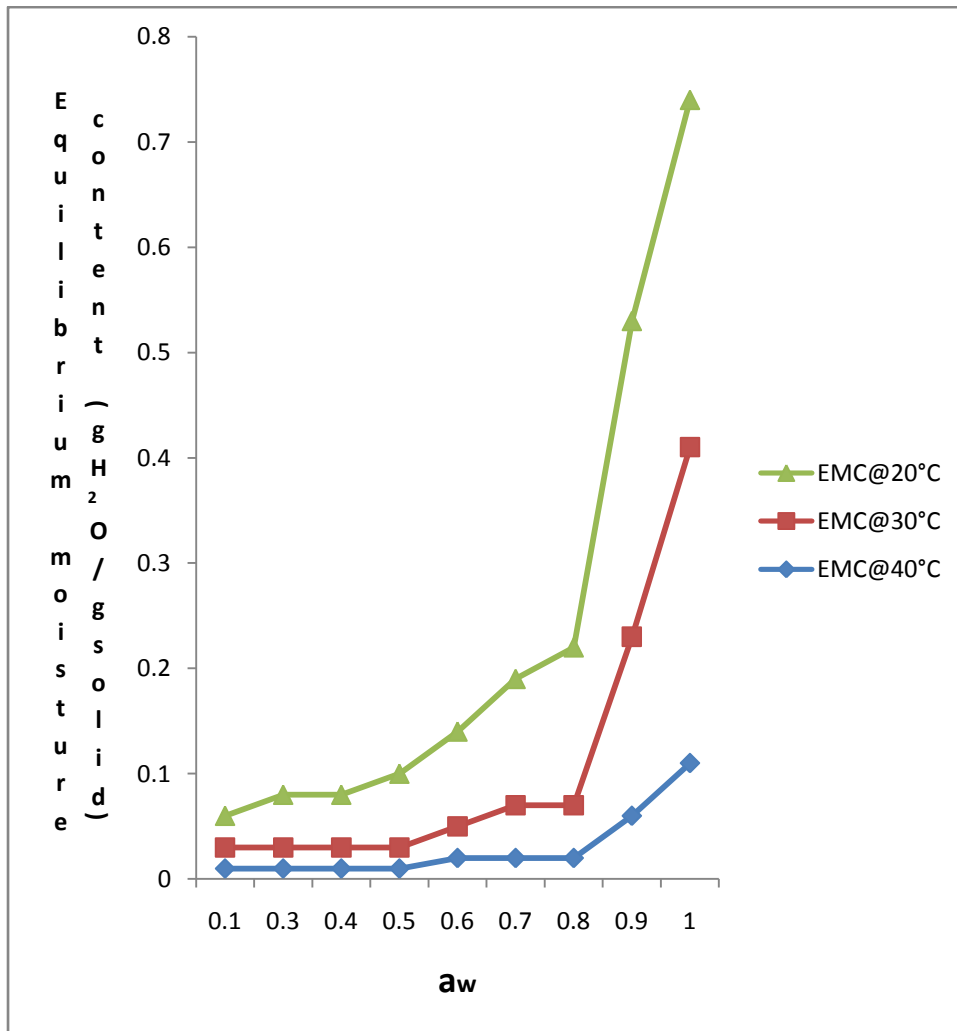


Figure 4.9 Plots of Adsorption Isotherm of AYB-Maize rings

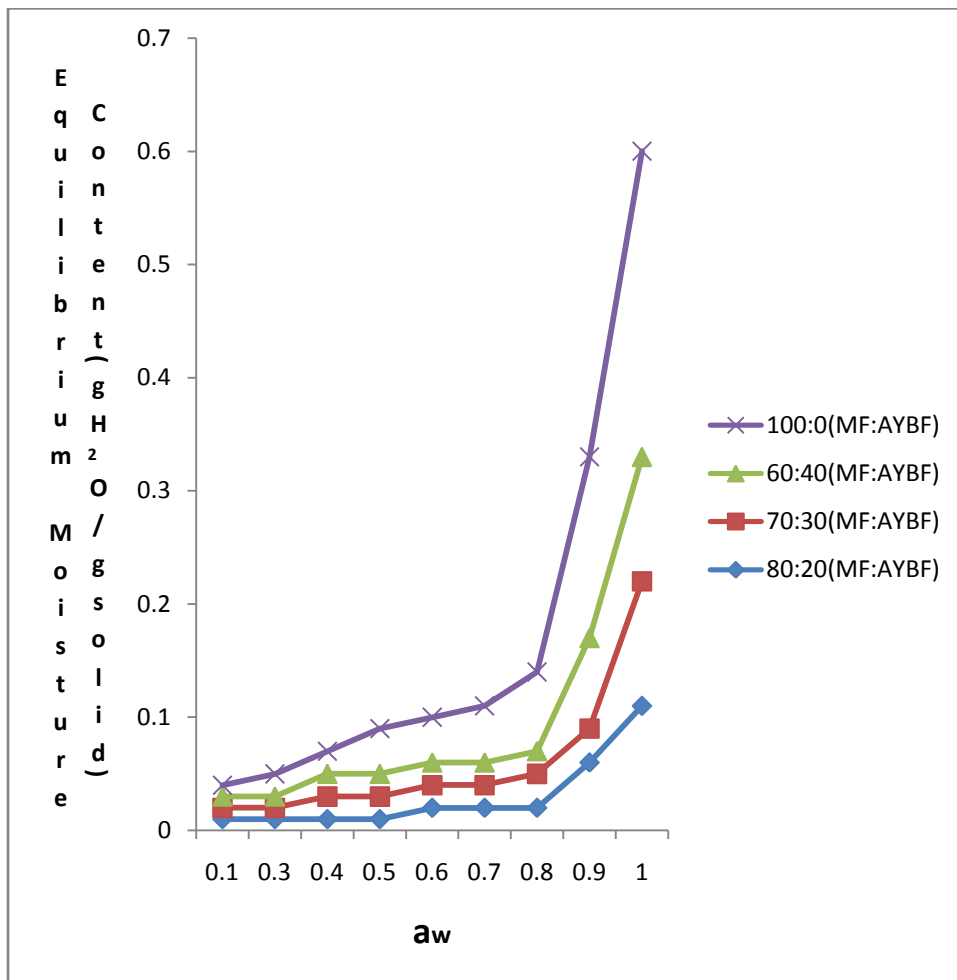


Figure 4.10 Plots of Adsorption isotherm of AYB-maize rings produced at different levels of maize flour: African yam bean flour (MF: AYBF) ratio.

Table 4.25: Sensory Qualities of Stored snack produced from blends of African yam bean flour and maize flour.

| Storage Period (weeks) | Appearance | Taste | Crispness | Aroma | Overall acceptability |
|------------------------|------------|-------|-----------|-------|-----------------------|
| 0 | 7.9a | 7.6a | 7.8a | 7.5a | 8.1a |
| 1 | 7.8a | 7.6a | 7.7a | 7.5a | 8.0a |
| 2 | 7.7a | 7.6a | 7.5a | 7.2a | 7.8a |
| 3 | 7.7a | 7.6a | 7.4ab | 7.1b | 7.7ab |
| 4 | 7.2abc | 7.5a | 7.3ab | 6.9bc | 7.6ab |
| 5 | 7.1abc | 7.3a | 7.3ab | 6.8c | 7.6ab |
| 6 | 7.4ab | 7.2a | 7.2ab | 6.7c | 7.4b |
| 7 | 7.2abc | 7.0ab | 7.2ab | 6.7c | 7.4b |
| 8 | 7.6a | 6.9ab | 7.0ab | 6.7c | 7.3bc |
| 9 | 7.1abc | 6.8ab | 7.0ab | 6.6c | 7.0c |
| 10 | 6.9bc | 6.8ab | 6.6bc | 6.2d | 6.8d |
| 11 | 6.9bc | 6.7ab | 6.5bc | 6.2d | 6.7d |
| 12 | 6.6d | 6.1b | 5.9c | 6.2d | 6.2e |

Values with the same letter along the column are not significantly different at $p \geq 0.05$

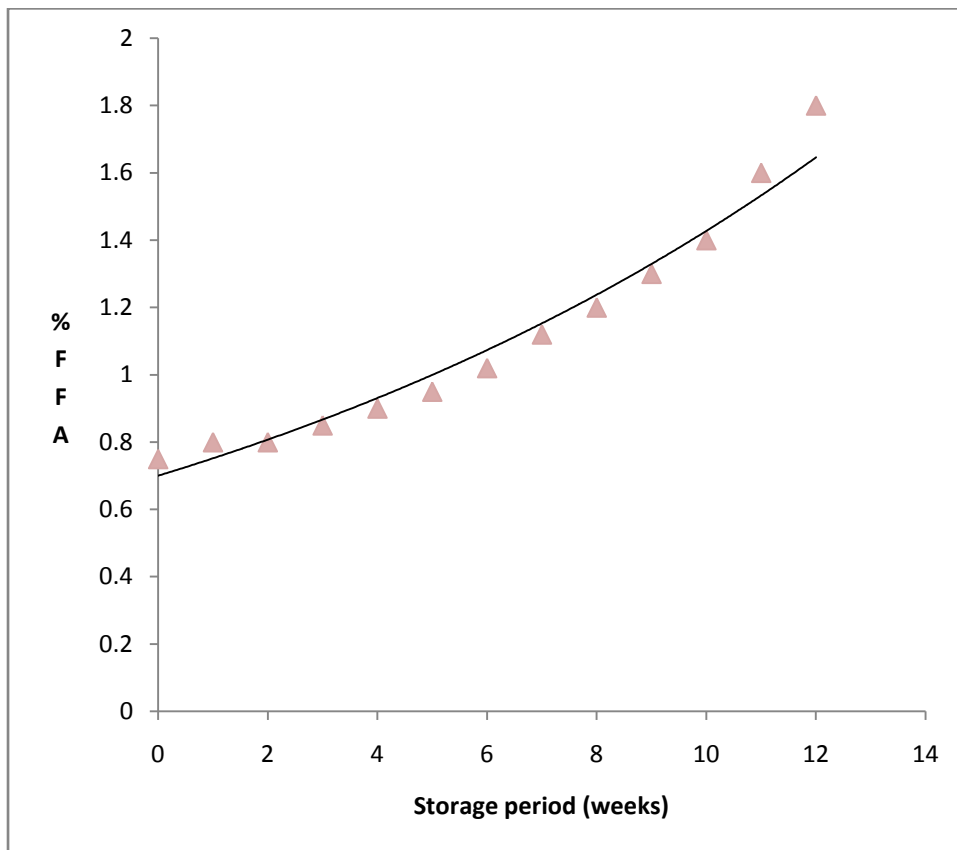


Figure 4.11: Plot of Free Fatty Acid (FFA) value and storage period of *kokoro* produced from blends of maize and African yam bean seed flours.

CHAPTER FIVE

5. CONCLUSION AND RECOMMENDATION

5.1 Conclusion

Acceptable snack was developed by incorporating African yam bean seed (an abundant but underutilised legume in Nigeria) to maize to produce *kokoro*. The study has shown that yellow maize variety is superior to white maize usually used by local producers of *kokoro* in terms of processing suitability and nutrient density. During the evaluation of the raw materials to be used, yellow maize (BR-9928-DMR-SY) was chosen because of its higher nutrient density while African yam bean, Tss-30 was selected because of its ease of dehulling. Crude protein, total ash, crude fibre, sugar contents increased while crude fat, starch, amylose and beta-carotene contents decreased with increased proportion of African Yam Bean Flour (AYBF) in the flour blends. Functional parameters showed no significant change among the flour blends except oil absorption capacity, amylose and bulk density. The addition of AYBF to the maize flour had significant effect ($p < 0.05$) on the pasting characteristics: trough and peak viscosity increased while breakdown, final viscosity, set back and pasting temperature decreased with increase in AYBF in the flour blend. Processing of flour blends into *kokoro* increased the total ash, crude fat, starch and sugar contents, protein content remained the same while the moisture content and TIA decreased significantly. The processing parameters (frying temperature, frying time and % AYBF inclusion in the flour blend) had significant effect ($p < 0.05$) on the product qualities. For colour parameters, lightness values (L^*) reduced while redness value (a^*) increased with higher frying time and temperature. Sensory perception varied significantly ($p < 0.05$) among the products especially for crispness, aroma and appearance while taste did not show significant ($p \geq 0.05$) difference. Breaking force reduced with higher frying time, giving the product better crispness.

Optimum condition for processing of the *kokoro* was 30% AYBSF, frying temperature, 155°C and frying time, 11.5min. The AYB-maize rings compared favourably with commercial maize rings popularly known as *kokoro* in all sensory attributes. The protein quality of the “formulated” *kokoro* is comparable with that of the casein control diet, hence has a potential to be commercialised and used to alleviate the protein-energy malnutrition problem in Nigeria. Each 100g pack of the AYB-maize rings contains 10g of protein which

implies that consumption of two packs of the product will furnish a child consumer with the required RDA of protein and about 6 packs in the case of an adult. The use of yellow maize has improved nutritional value of the product from little or no vitamin A content (associated with white maize products) to 30RE (retinol equivalent) of vitamin A.

Adsorption isotherm showed a sigmoid curve characteristic of a carbohydrate product and its equilibrium moisture content decreased with increase in storage temperature at any given water activity. The monolayer moisture content ranged between 2.05 and 3.25% (dry basis). Level of rancidity of the snack was tolerable up to 12 weeks and the sensory quality of the product was acceptable up to 10 weeks under ambient storage condition (temperature- $24\pm 2^{\circ}\text{C}$, relative humidity- $61\pm 3\%$). Addition of African yam bean seed flour to *kokoro* improved its nutritional content, creating a novel use for African yam bean seed, the result obtained could be recommended to cottage industries as the optimum processing conditions for production of *kokoro* of improved nutritional contents and consistent sensory qualities.

5.2 Recommendation

In view of the outcome of this study, there is need to further enrich the product using vitamin A premix to meet up with Recommended Dietary Allowance (RDA) of Vitamin A. Also investigation into clinical evaluation of the product is recommended for further study.

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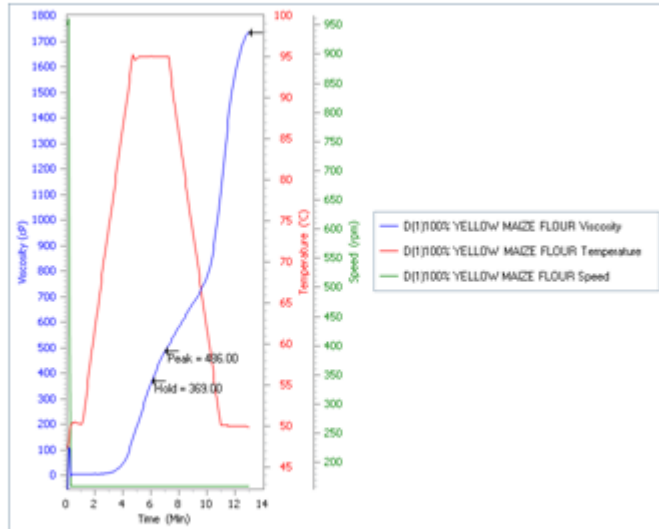
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APPENDIX

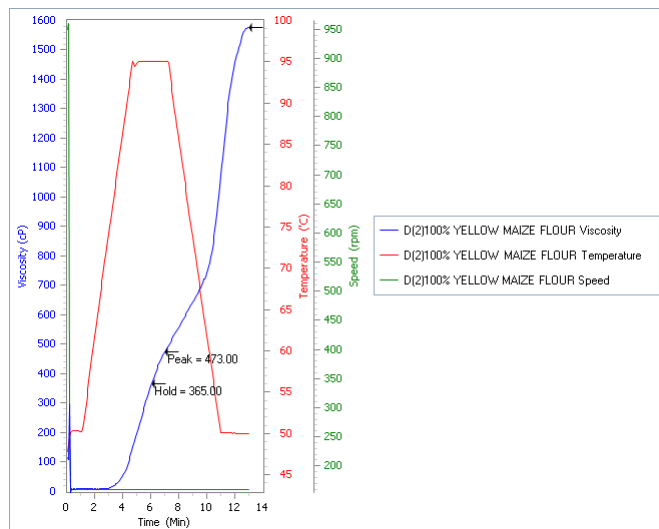
Appendix 1 Experimental facilities/equipment used and location

1. Colourimeter (Color Tec-PCM, TM, USA) International Institute for Tropical Agriculture (IITA), Ibadan, Oyo State.
2. Soxtec System HT2 fat extractor- International Institute for Tropical Agriculture (IITA), Ibadan, Oyo State.
3. Convective hot air oven (draft air Fisher Scientific Isotemp^R Oven model- International Institute for Tropical Agriculture (IITA), Ibadan, Oyo State.
4. Temperature regulated deep-fat fryer (Model S-516, Hong Kong, China)- International Institute for Tropical Agriculture (IITA), Ibadan, Oyo State.
5. Incubator (Gallenkamp, United Kingdom). Food Microbiology laboratory, Department of Food Technology, University of Ibadan, Ibadan, Oyo State.
6. Rapid Visco Analyzer RVA super 4, Newport Scientific, Perten instruments AB, Huddinge, Sweden. Central Multidisciplinary research laboratory, University of Ibadan, Ibadan. Oyo State.
7. Foss KjeltacTM 2300 Auto distillation unit and Foss Tecator scrubber. International Institute for Tropical Agriculture (IITA), Ibadan, Oyo State.
8. Texture analyzer (Model –no 174886, Kiya Seisakusho Ltd. Tokyo Japan. International Institute for Tropical Agriculture (IITA), Ibadan, Oyo State.

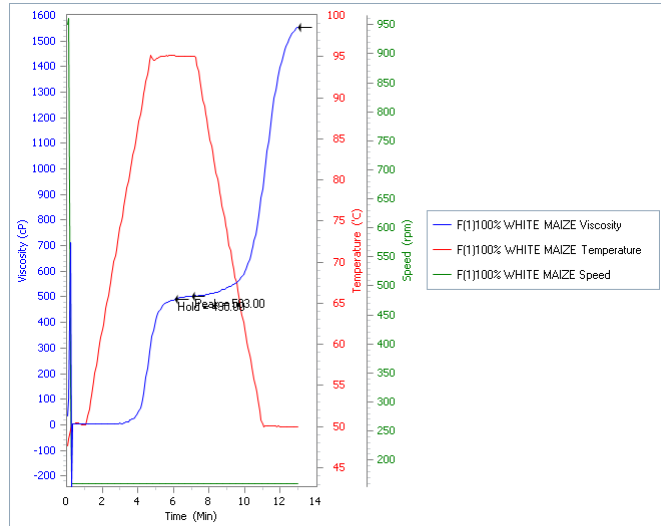
Appendix 2 Pasting characteristics chart for yellow maize (BR-9928-DMR-SY)



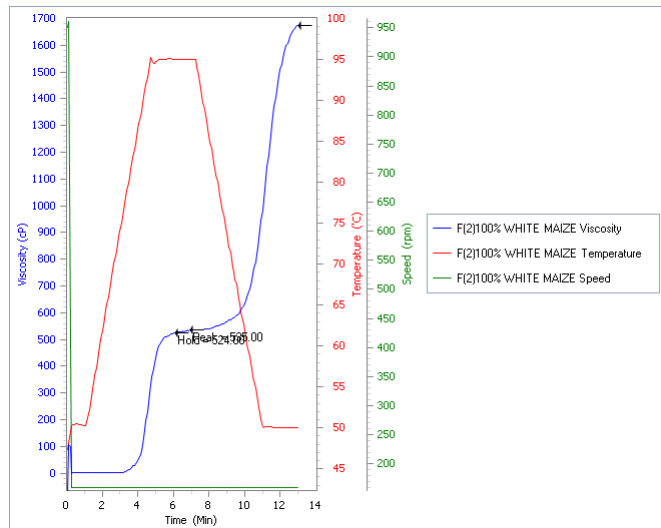
JAN



Appendix 3 Pasting characteristics chart for white maize (TZL comp4C2) cultivar



JAN



Appendix 4 Questionnaire on the sensory properties of maize-based snack *kokoro*

**QUESTIONNAIRE ON THE SENSORY PROPERTIES OF “MAIZE SNACK (KOKORO)”
ENRICHED WITH AFRICAN YAM BEAN (AYB) FLOUR.**

Dear Sir/Ma,

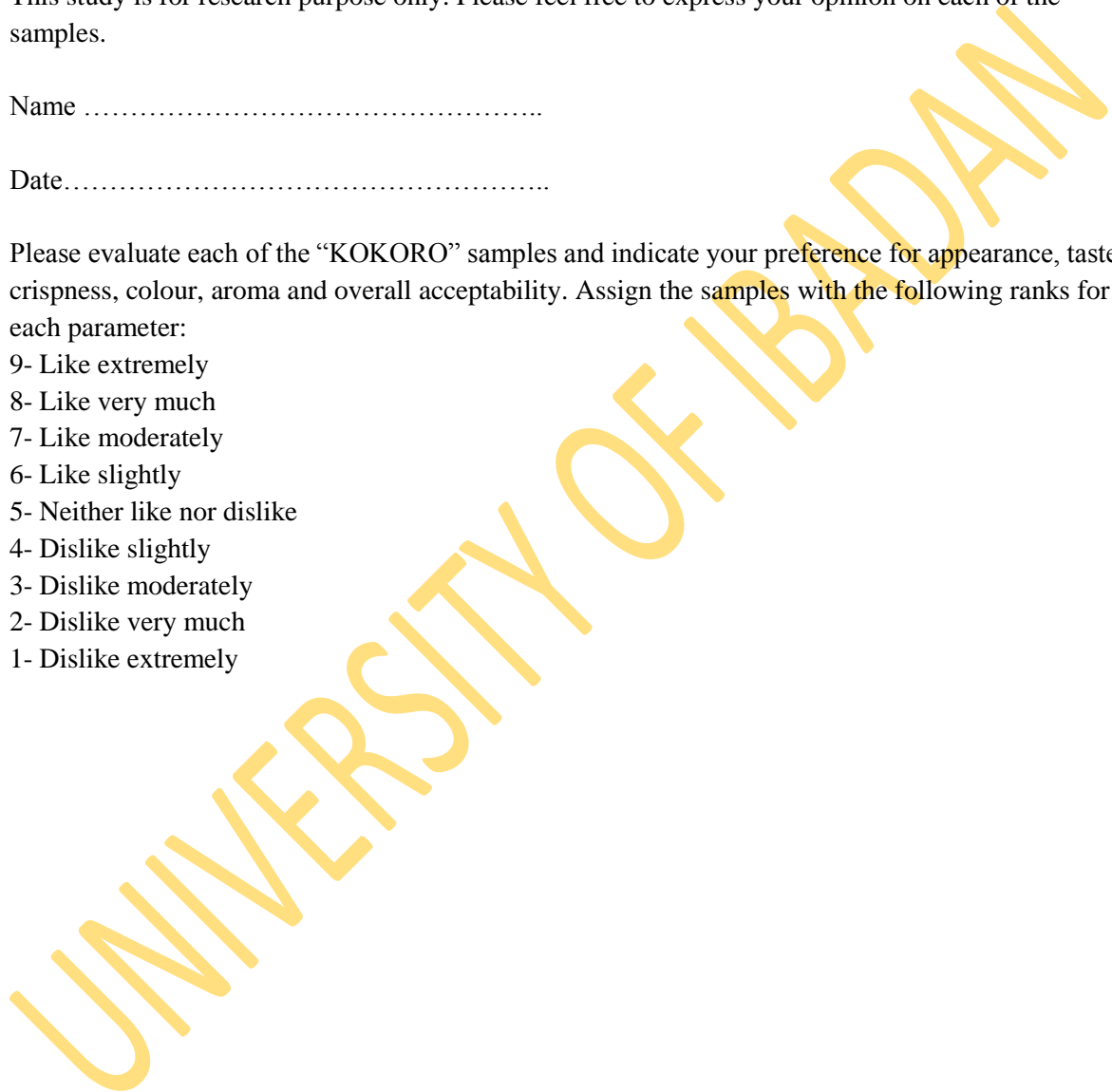
This study is for research purpose only. Please feel free to express your opinion on each of the samples.

Name

Date.....

Please evaluate each of the “KOKORO” samples and indicate your preference for appearance, taste, crispness, colour, aroma and overall acceptability. Assign the samples with the following ranks for each parameter:

- 9- Like extremely
- 8- Like very much
- 7- Like moderately
- 6- Like slightly
- 5- Neither like nor dislike
- 4- Dislike slightly
- 3- Dislike moderately
- 2- Dislike very much
- 1- Dislike extremely



| Sample | Appearance | Taste | Crispness | Colour | Aroma | Overall Acceptability |
|--------|----------------------|----------------------|----------------------|----------------------|----------------------|-----------------------|
| 2001 | <input type="text"/> | <input type="text"/> | <input type="text"/> | <input type="text"/> | <input type="text"/> | <input type="text"/> |
| 2002 | <input type="text"/> | <input type="text"/> | <input type="text"/> | <input type="text"/> | <input type="text"/> | <input type="text"/> |
| 2003 | <input type="text"/> | <input type="text"/> | <input type="text"/> | <input type="text"/> | <input type="text"/> | <input type="text"/> |
| 2004 | <input type="text"/> | <input type="text"/> | <input type="text"/> | <input type="text"/> | <input type="text"/> | <input type="text"/> |
| 2005 | <input type="text"/> | <input type="text"/> | <input type="text"/> | <input type="text"/> | <input type="text"/> | <input type="text"/> |
| 2006 | <input type="text"/> | <input type="text"/> | <input type="text"/> | <input type="text"/> | <input type="text"/> | <input type="text"/> |
| 2007 | <input type="text"/> | <input type="text"/> | <input type="text"/> | <input type="text"/> | <input type="text"/> | <input type="text"/> |
| 2008 | <input type="text"/> | <input type="text"/> | <input type="text"/> | <input type="text"/> | <input type="text"/> | <input type="text"/> |
| 2009 | <input type="text"/> | <input type="text"/> | <input type="text"/> | <input type="text"/> | <input type="text"/> | <input type="text"/> |
| 2010 | <input type="text"/> | <input type="text"/> | <input type="text"/> | <input type="text"/> | <input type="text"/> | <input type="text"/> |
| 2011 | <input type="text"/> | <input type="text"/> | <input type="text"/> | <input type="text"/> | <input type="text"/> | <input type="text"/> |
| 2012 | <input type="text"/> | <input type="text"/> | <input type="text"/> | <input type="text"/> | <input type="text"/> | <input type="text"/> |
| 2013 | <input type="text"/> | <input type="text"/> | <input type="text"/> | <input type="text"/> | <input type="text"/> | <input type="text"/> |
| 2014 | <input type="text"/> | <input type="text"/> | <input type="text"/> | <input type="text"/> | <input type="text"/> | <input type="text"/> |

Comment freely

.....

.....

Appendix 5- Questionnaire on the Comparative Sensory Evaluation of “improved” maize rings and commercial maize rings (*kokoro*).

Dear Sir/Ma,

This study is for research purpose only. Please feel free to express your opinion on each of the samples.

Name

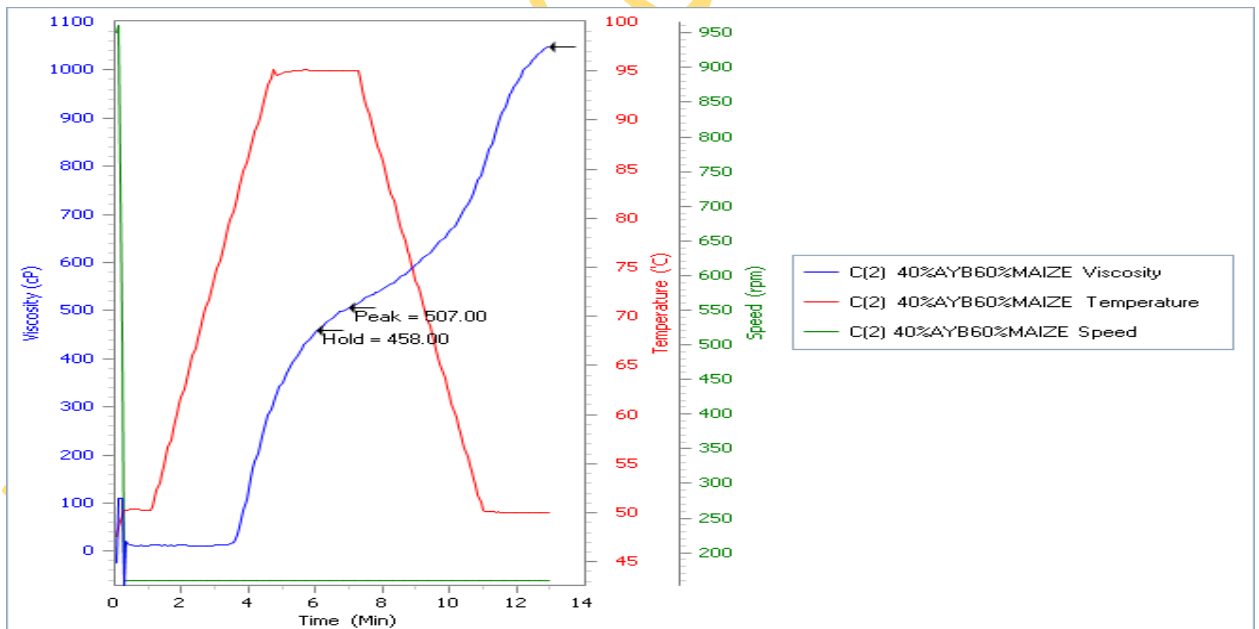
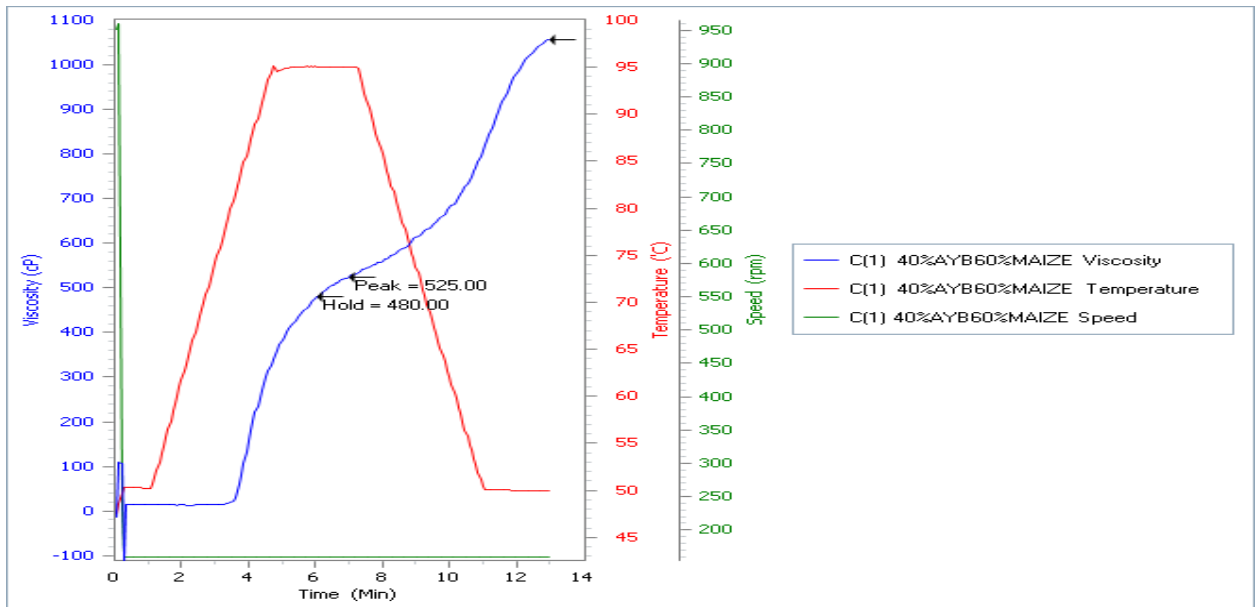
Date.....

Please evaluate each of the “KOKORO” samples and indicate your preference for appearance, taste, crispness, colour, aroma and overall acceptability. Assign the samples with the following ranks for each parameter:

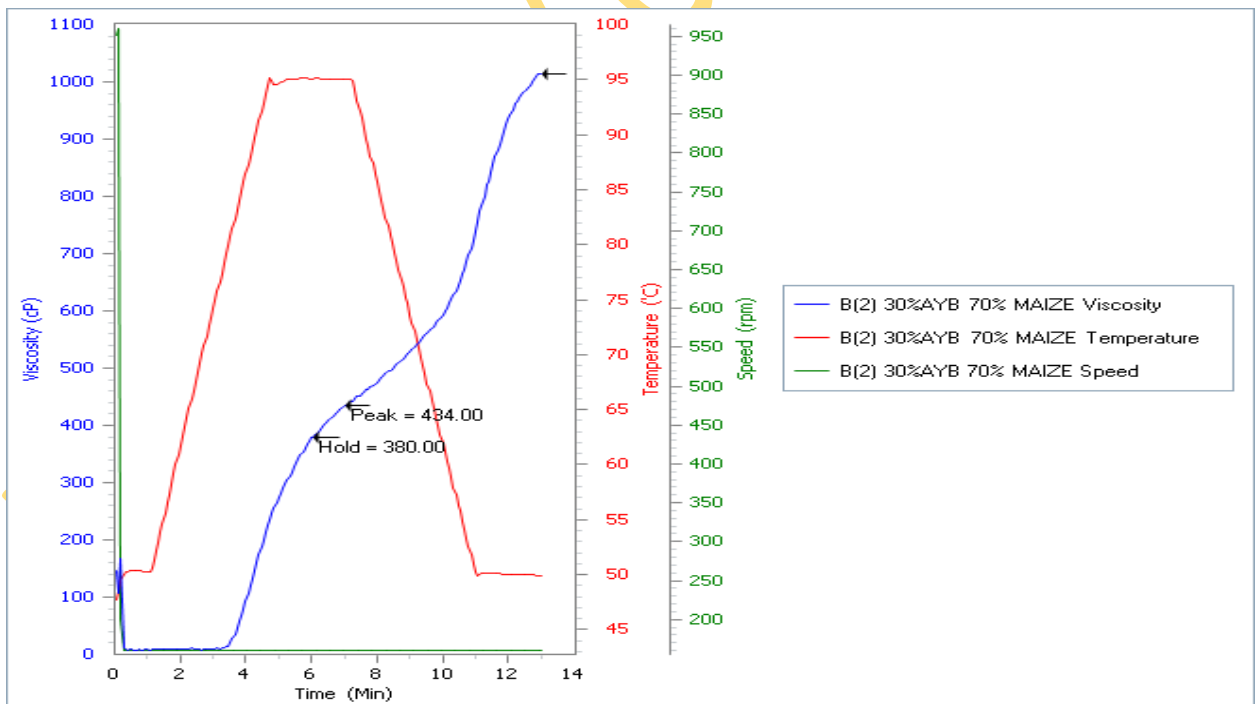
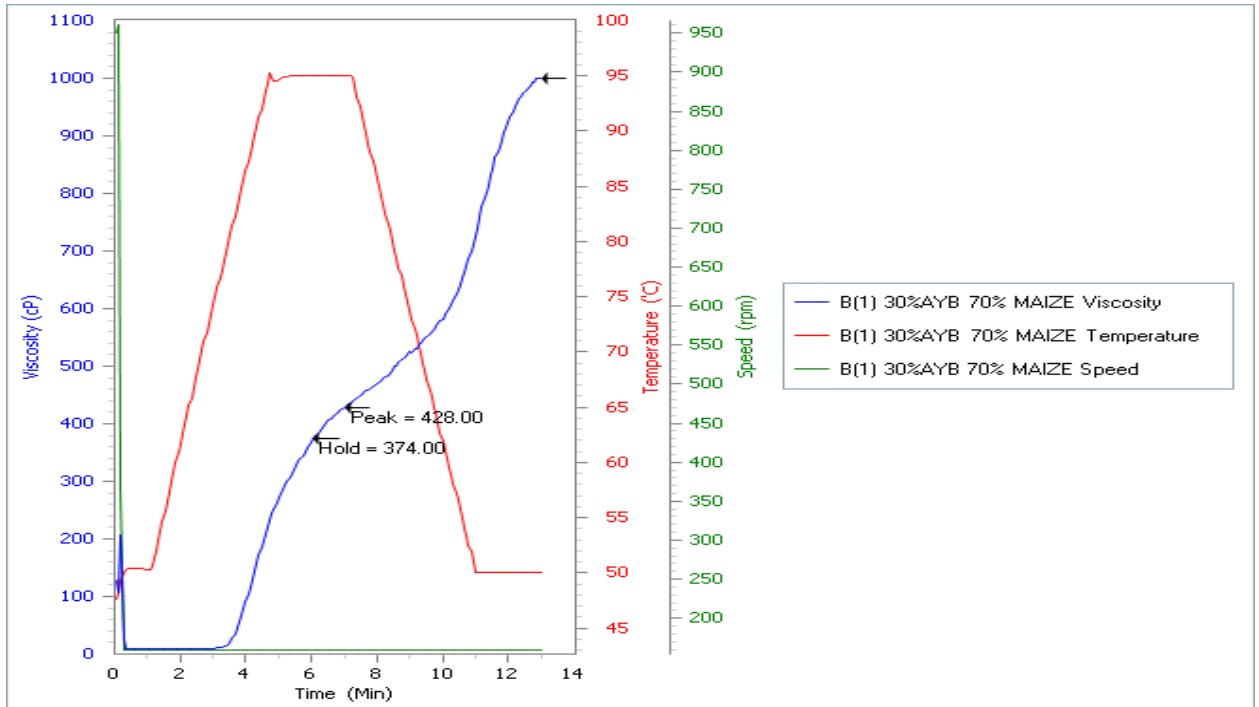
- 9- Like extremely
- 8- Like very much
- 7- Like moderately
- 6- Like slightly
- 5- Neither like nor dislike
- 4- Dislike slightly
- 3- Dislike moderately
- 2- Dislike very much
- 1- Dislike extremely

| Sample | Appearance | Taste | Crispness | Colour | Aroma | Overall Acceptability |
|--------|----------------------|----------------------|----------------------|----------------------|----------------------|-----------------------|
| 2001 | <input type="text"/> | <input type="text"/> | <input type="text"/> | <input type="text"/> | <input type="text"/> | <input type="text"/> |
| 2002 | <input type="text"/> | <input type="text"/> | <input type="text"/> | <input type="text"/> | <input type="text"/> | <input type="text"/> |

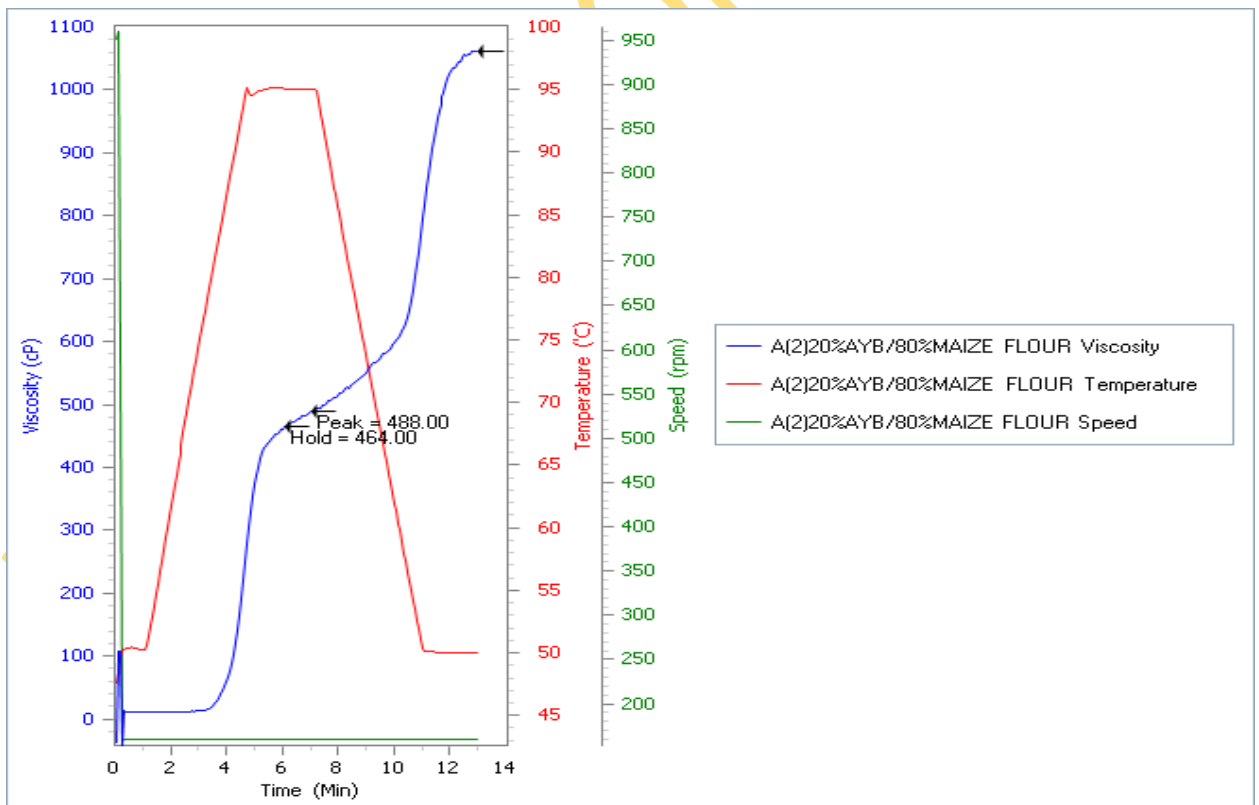
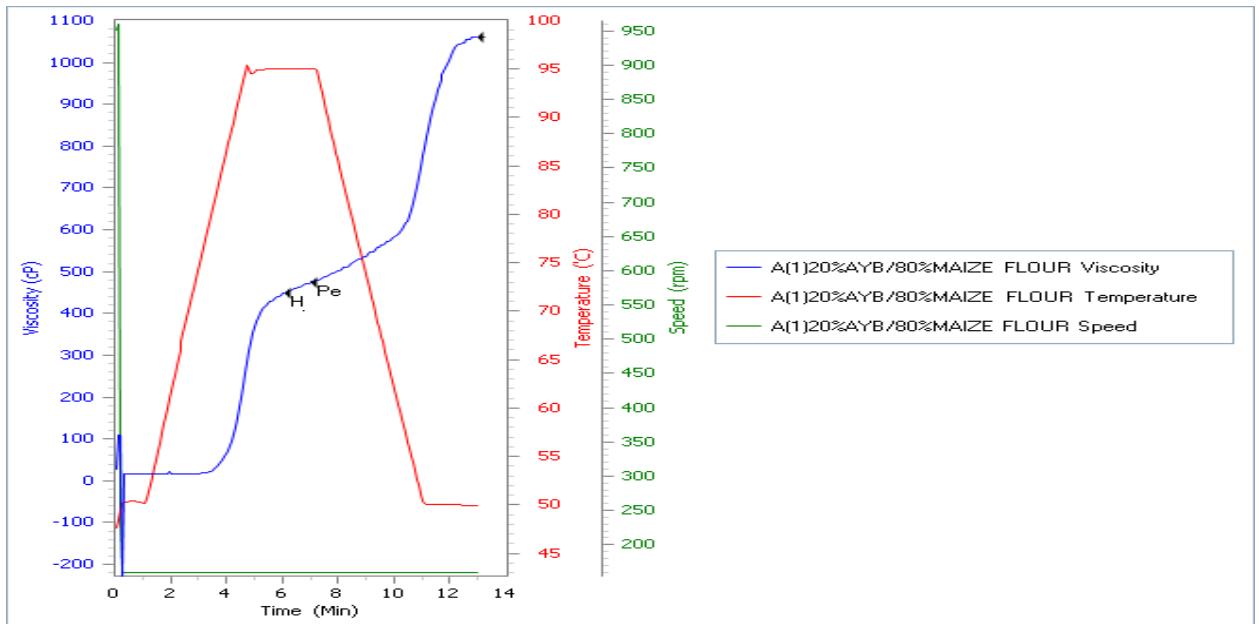
Appendix 6 Pasting characteristics chart for Maize-AYB flour blend C (60%M:40%AYB)



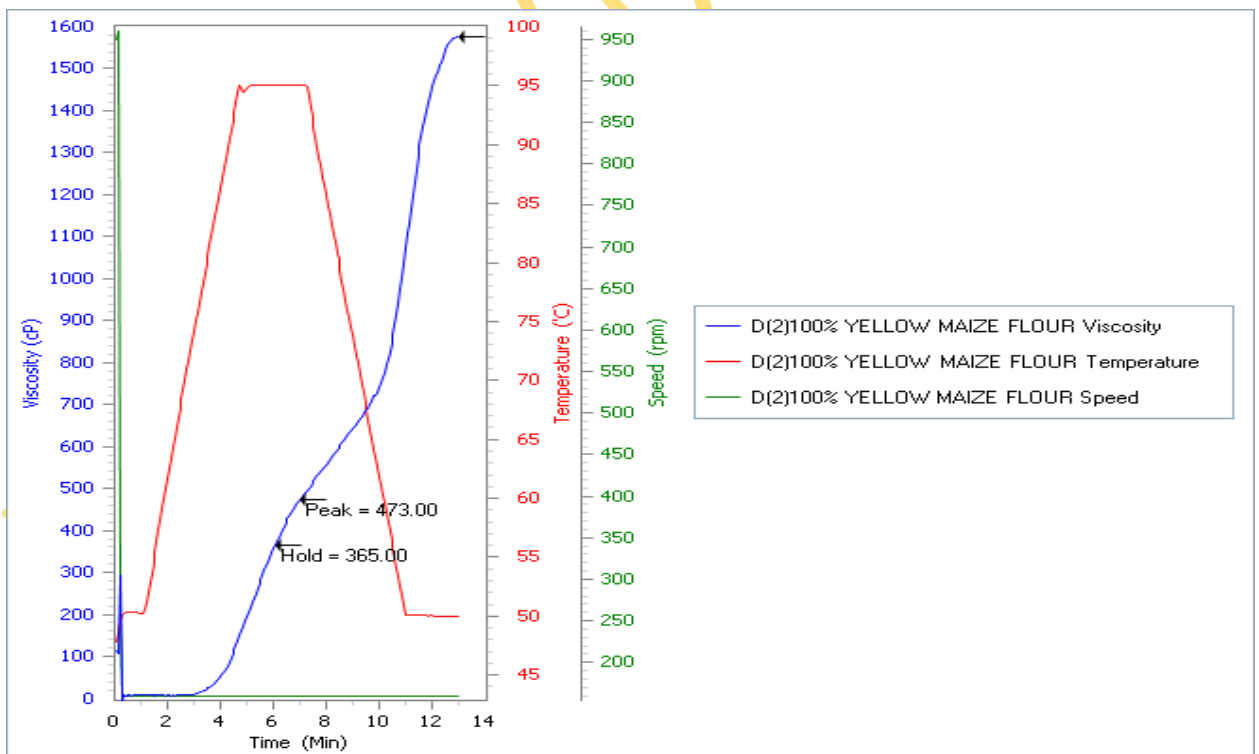
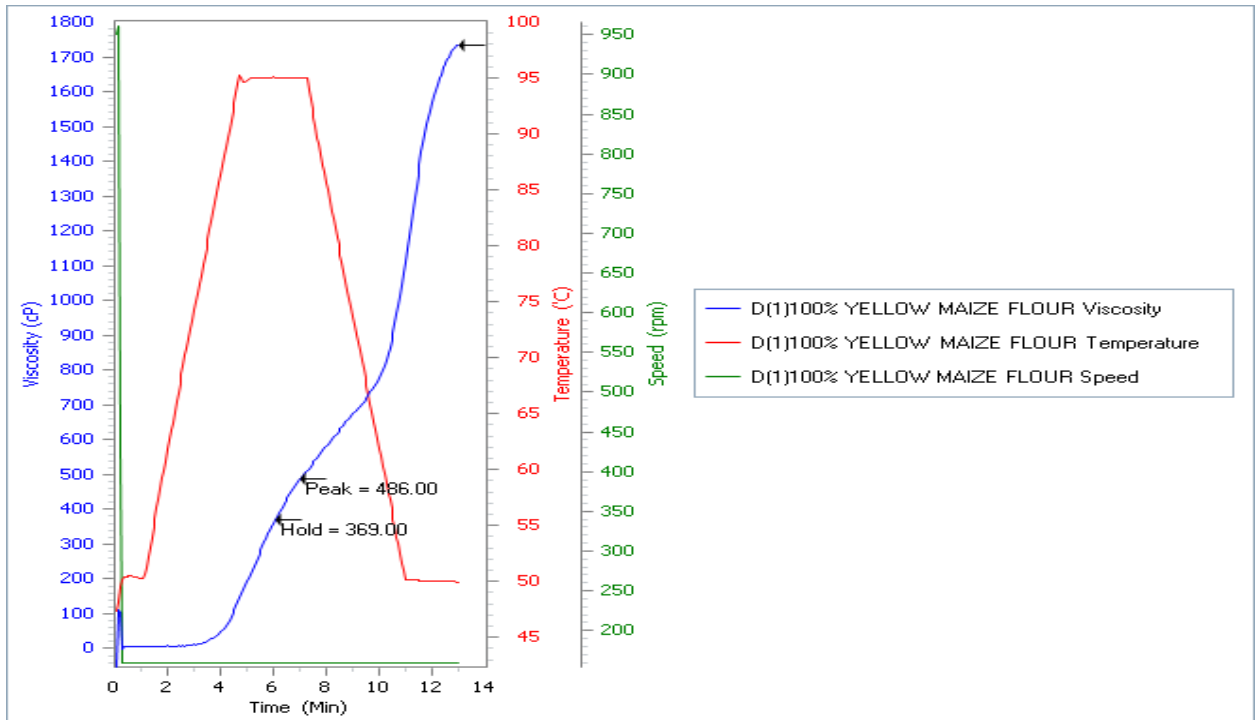
Appendix 7 Pasting characteristics chart for Maize-AYB flour blend B (70%M:30%AYB)



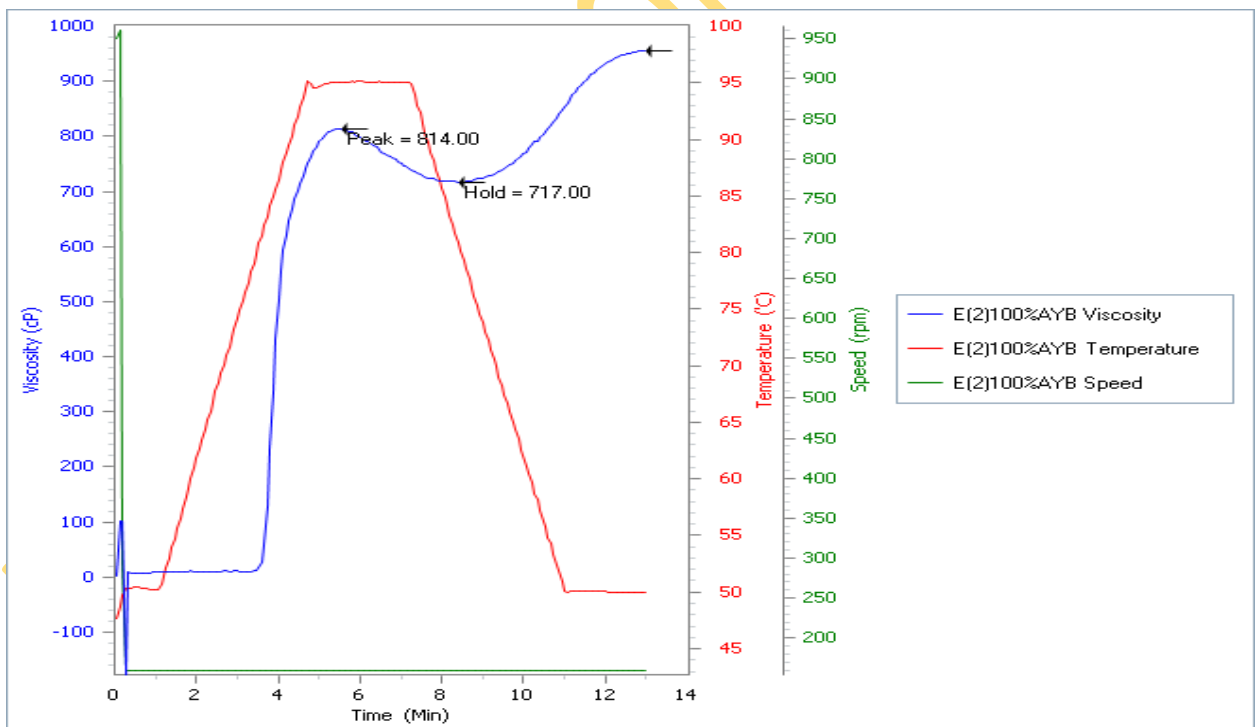
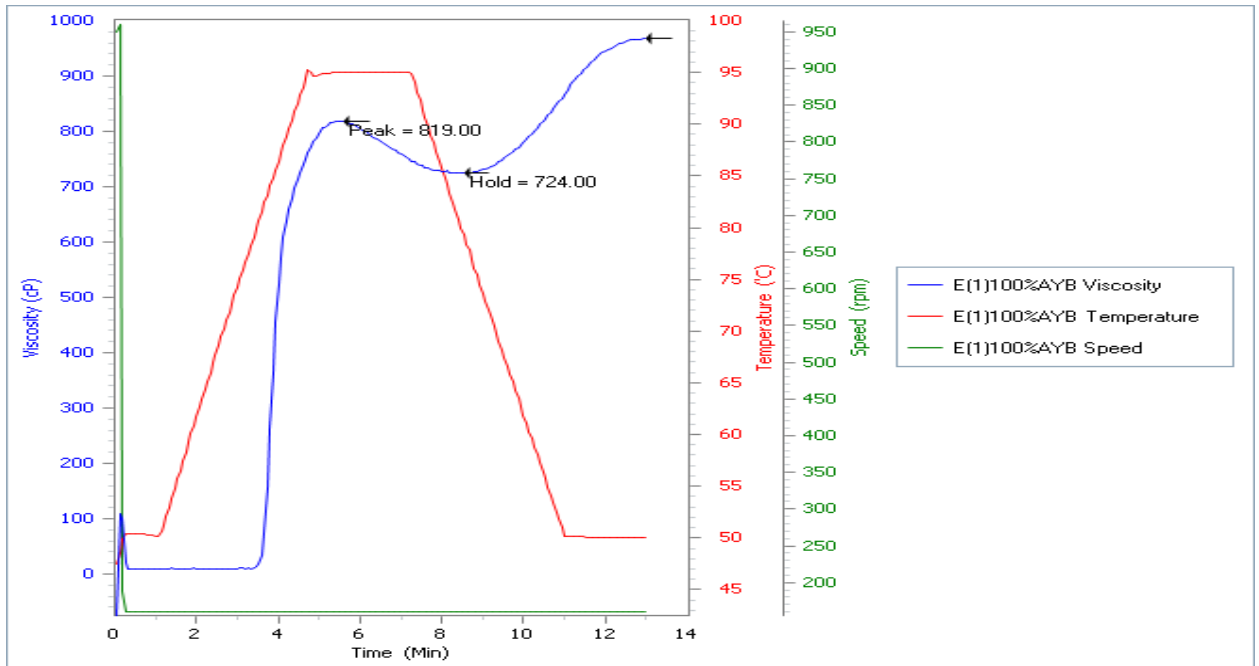
Appendix 8 Pasting characteristics chart for Maize-AYB flour blend A (80%M:20%AYB)



Appendix 9 Pasting characteristics chart for 0%AYB:100% Maize flour (D).



Appendix 10 Pasting characteristics chart for 100%AYB:0% Maize flour (E).



Appendix 11 Anova of sensory properties.

| Level of sample | N | App | | taste | | Crispness | | Colour | |
|-----------------|----|------------|------------|------------|------------|------------|------------|------------|------------|
| | | Mean | Std Dev | Mean | Std Dev | Mean | Std Dev | Mean | Std Dev |
| 1 | 10 | 5.30000000 | 2.05750658 | 6.10000000 | 1.52388393 | 7.10000000 | 2.13177026 | 5.40000000 | 1.64654520 |
| 2 | 10 | 7.40000000 | 0.96609178 | 7.20000000 | 0.78881064 | 5.90000000 | 1.66332999 | 7.40000000 | 0.96609178 |
| 3 | 10 | 7.10000000 | 1.28668394 | 6.70000000 | 1.15950181 | 7.70000000 | 0.82327260 | 6.90000000 | 1.19721900 |
| 4 | 10 | 7.00000000 | 1.49071198 | 7.20000000 | 0.91893658 | 7.50000000 | 1.43372088 | 7.50000000 | 0.84983659 |
| 5 | 10 | 7.50000000 | 0.84983659 | 7.00000000 | 1.15470054 | 6.40000000 | 2.01108042 | 7.40000000 | 0.51639778 |
| 6 | 10 | 6.70000000 | 1.25166556 | 6.70000000 | 1.15950181 | 5.80000000 | 1.31656118 | 6.90000000 | 0.99442893 |
| 7 | 10 | 7.50000000 | 1.26929552 | 6.60000000 | 1.17378779 | 5.10000000 | 1.10050493 | 7.10000000 | 0.99442893 |
| 8 | 10 | 7.70000000 | 1.05934991 | 7.30000000 | 1.05934991 | 7.10000000 | 1.44913767 | 7.60000000 | 0.96609178 |
| 9 | 10 | 7.20000000 | 0.78881064 | 7.00000000 | 1.05409255 | 7.60000000 | 0.96609178 | 7.40000000 | 0.84327404 |
| 10 | 10 | 7.50000000 | 0.52704628 | 6.80000000 | 1.54919334 | 6.40000000 | 1.64654520 | 7.50000000 | 0.97182532 |
| 11 | 10 | 7.50000000 | 0.84983659 | 6.60000000 | 1.17378779 | 7.50000000 | 0.97182532 | 6.90000000 | 1.10050493 |
| 12 | 10 | 7.20000000 | 1.03279556 | 6.80000000 | 1.22927259 | 7.90000000 | 0.73786479 | 7.20000000 | 1.22927259 |
| 13 | 10 | 7.80000000 | 1.03279556 | 6.90000000 | 0.99442893 | 7.50000000 | 0.84983659 | 7.30000000 | 1.15950181 |
| 14 | 10 | 7.40000000 | 0.96609178 | 6.60000000 | 1.17378779 | 6.30000000 | 1.94650684 | 7.20000000 | 0.91893658 |

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| Level of sample | N | Aroma | | overall_acc | |
|-----------------|----|-----------|-----------|-------------|-----------|
| | | Mean | Std Dev | Mean | Std Dev |
| 1 | 10 | 6.3000000 | 1.4181364 | 6.1000000 | 1.5951314 |
| | | 0 | 9 | 0 | 8 |
| 2 | 10 | 6.5000000 | 0.9718253 | 6.6000000 | 1.1737877 |
| | | 0 | 2 | 0 | 9 |
| 3 | 10 | 6.8000000 | 0.7888106 | 7.1000000 | 0.7378647 |
| | | 0 | 4 | 0 | 9 |
| 4 | 10 | 7.0000000 | 0.8164965 | 7.6000000 | 1.0749677 |
| | | 0 | 8 | 0 | 0 |
| 5 | 10 | 7.2000000 | 1.0327955 | 7.3000000 | 1.4944341 |
| | | 0 | 6 | 0 | 2 |
| 6 | 10 | 6.7000000 | 0.6749485 | 6.4000000 | 1.3498971 |
| | | 0 | 6 | 0 | 2 |
| 7 | 10 | 6.9000000 | 0.7378647 | 6.2000000 | 1.4757295 |
| | | 0 | 9 | 0 | 7 |
| 8 | 10 | 7.2000000 | 0.6324555 | 7.5000000 | 1.0801234 |
| | | 0 | 3 | 0 | 5 |
| 9 | 10 | 7.1000000 | 0.7378647 | 7.5000000 | 0.8498365 |
| | | 0 | 9 | 0 | 9 |
| 10 | 10 | 6.9000000 | 1.1005049 | 6.9000000 | 1.1005049 |
| | | 0 | 3 | 0 | 3 |
| 11 | 10 | 7.0000000 | 0.9428090 | 7.2000000 | 0.9189365 |
| | | 0 | 4 | 0 | 8 |
| 12 | 10 | 6.9000000 | 0.7378647 | 7.5000000 | 0.8498365 |
| | | 0 | 9 | 0 | 9 |
| 13 | 10 | 7.0000000 | 0.8164965 | 7.3000000 | 0.9486833 |
| | | 0 | 8 | 0 | 0 |
| 14 | 10 | 6.7000000 | 0.8232726 | 6.9000000 | 1.1005049 |
| | | 0 | 0 | 0 | 3 |

Analysis of variance studying the effect of sample on measured sensory parameters

The GLM Procedure

t Tests (LSD) for app

| Means with the same letter are not significantly different. | | | | |
|---|---|--------|----|--------|
| t Grouping | | Mean | N | sample |
| | A | 7.8000 | 10 | 13 |

| | |
|-------------------------------------|----------|
| Alpha | 0.05 |
| Error Degrees of Freedom | 126 |
| Error Mean Square | 1.339683 |
| Critical Value of t | 1.97897 |
| Least Significant Difference | 1.0244 |

Note This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Analysis of variance studying the effect of sample on measured sensory parameters

The GLM Procedure

t Tests (LSD) for app

| | | | | |
|---|---|------------|----|----|
| | A | | | |
| B | A | 7.700 0 | 10 | 8 |
| B | A | | | |
| B | A | 7.500 0 | 10 | 11 |
| B | A | | | |
| B | A | 7.500 0 | 10 | 10 |
| B | A | | | |
| B | A | 7.500 0 | 10 | 5 |
| B | A | | | |
| B | A | 7.500 0 | 10 | 7 |
| B | A | | | |
| B | A | 7.400 0 | 10 | 14 |
| B | A | | | |
| B | A | 7.400 0 | 10 | 2 |
| B | A | | | |
| B | A | 7.200 0 | 10 | 9 |
| B | A | | | |

Analysis of variance studying the effect of sample on measured sensory parameters

The GLM Procedure

t Tests (LSD) for app

| | | | | |
|---|---|------------|----|----|
| B | A | 7.200 0 | 10 | 12 |
| B | A | | | |
| B | A | 7.100 0 | 10 | 3 |
| B | A | | | |
| B | A | 7.000 0 | 10 | 4 |
| B | | | | |
| B | | 6.700 0 | 10 | 6 |
| | | | | |
| | C | 5.300 0 | 10 | 1 |

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Analysis of variance studying the effect of sample on measured sensory parameters

The GLM Procedure

t Tests (LSD) for taste

Note: This test controls the Type I comparison wise error rate, not the experiment wise error rate.

| | |
|-------------------------------------|--------------|
| Alpha | 0.05 |
| Error Degrees of Freedom | 126 |
| Error Mean Square | 1.36269 8 |
| Critical Value of t | 1.97897 |
| Least Significant Difference | 1.0331 |

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Analysis of variance studying the effect of sample on measured sensory parameters

The GLM Procedure

t Tests (LSD) for taste

| Means with the same letter are not significantly different. | | | | |
|---|---|--------|----|--------|
| t Grouping | | Mean | N | sample |
| | A | 7.3000 | 10 | 8 |
| | A | | | |
| | A | 7.2000 | 10 | 2 |
| | A | | | |
| | A | 7.2000 | 10 | 4 |
| | A | | | |
| B | A | 7.0000 | 10 | 9 |
| B | A | | | |
| B | A | 7.0000 | 10 | 5 |
| B | A | | | |
| B | A | 6.9000 | 10 | 13 |
| B | A | | | |
| B | A | 6.8000 | 10 | 10 |
| B | A | | | |
| B | A | 6.8000 | 10 | 12 |
| B | A | | | |
| B | A | 6.7000 | 10 | 3 |

Analysis of variance studying the effect of sample on measured sensory parameters

The GLM Procedure

t Tests (LSD) for taste

| | | | | |
|---|---|------------|----|----|
| B | A | | | |
| B | A | 6.700 0 | 10 | 6 |
| B | A | | | |
| B | A | 6.600 0 | 10 | 7 |
| B | A | | | |
| B | A | 6.600 0 | 10 | 11 |
| B | A | | | |
| B | A | 6.600 0 | 10 | 14 |
| B | | | | |
| B | | 6.100 0 | 10 | 1 |

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Analysis of variance studying the effect of sample on measured sensory parameters

The GLM Procedure

t Tests (LSD) for crispness

Note: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

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Analysis of variance studying the effect of sample on measured sensory parameters

The GLM Procedure

t Tests (LSD) for crispness

| Means with the same letter are not significantly different. | | | | | | |
|---|---|---|---|--------|----|--------|
| t Grouping | | | | Mean | N | sample |
| | | A | | 7.9000 | 10 | 12 |
| | | A | | | | |
| | | A | | 7.7000 | 10 | 3 |
| | | A | | | | |
| B | | A | | 7.6000 | 10 | 9 |
| B | | A | | | | |
| B | | A | C | 7.5000 | 10 | 4 |
| B | | A | C | | | |
| B | | A | C | 7.5000 | 10 | 13 |
| B | | A | C | | | |
| B | | A | C | 7.5000 | 10 | 11 |
| B | | A | C | | | |
| B | D | A | C | 7.1000 | 10 | 8 |
| B | D | A | C | | | |
| B | D | A | C | 7.1000 | 10 | 1 |
| B | D | | C | | | |
| B | D | E | C | 6.4000 | 10 | 10 |
| B | D | E | C | | | |
| B | D | E | C | 6.4000 | 10 | 5 |
| | D | E | C | | | |
| F | D | E | C | 6.3000 | 10 | 14 |
| F | D | E | | | | |
| F | D | E | | 5.9000 | 10 | 2 |
| F | | E | | | | |
| F | | E | | 5.8000 | 10 | 6 |

Analysis of variance studying the effect of sample on measured sensory parameters

The GLM Procedure

t Tests (LSD) for crispness

| | | | | | | |
|---|--|--|--|--------|----|---|
| F | | | | | | |
| F | | | | 5.1000 | 10 | 7 |

| | |
|-------------------------------------|--------------|
| Alpha | 0.05 |
| Error Degrees of Freedom | 126 |
| Error Mean Square | 2.05555 6 |
| Critical Value of t | 1.97897 |
| Least Significant Difference | 1.2689 |

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Analysis of variance studying the effect of sample on measured sensory parameters

The GLM Procedure

t Tests (LSD) for colour

Note: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

| | |
|-------------------------------------|--------------|
| Alpha | 0.05 |
| Error Degrees of Freedom | 126 |
| Error Mean Square | 1.11031 7 |
| Critical Value of t | 1.97897 |
| Least Significant Difference | 0.9326 |

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Analysis of variance studying the effect of sample on measured sensory parameters

The GLM Procedure

t Tests (LSD) for colour

| Means with the same letter are not significantly different. | | | |
|---|--------|----|--------|
| t Grouping | Mean | N | sample |
| A | 7.6000 | 10 | 8 |
| A | | | |
| A | 7.5000 | 10 | 10 |
| A | | | |
| A | 7.5000 | 10 | 4 |
| A | | | |
| A | 7.4000 | 10 | 9 |
| A | | | |
| A | 7.4000 | 10 | 5 |
| A | | | |
| A | 7.4000 | 10 | 2 |
| A | | | |
| A | 7.3000 | 10 | 13 |
| A | | | |
| A | 7.2000 | 10 | 14 |
| A | | | |
| A | 7.2000 | 10 | 12 |
| A | | | |
| A | 7.1000 | 10 | 7 |
| A | | | |
| A | 6.9000 | 10 | 3 |
| A | | | |
| A | 6.9000 | 10 | 11 |

Analysis of variance studying the effect of sample on measured sensory parameters

The GLM Procedure

t Tests (LSD) for colour

| | | | |
|---|--------|----|---|
| A | | | |
| A | 6.9000 | 10 | 6 |
| | | | |
| B | 5.4000 | 10 | 1 |

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Analysis of variance studying the effect of sample on measured sensory parameters

The GLM Procedure

t Tests (LSD) for aroma

Note: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

| | |
|-------------------------------------|--------------|
| Alpha | 0.05 |
| Error Degrees of Freedom | 126 |
| Error Mean Square | 0.80317 5 |
| Critical Value of t | 1.97897 |
| Least Significant Difference | 0.7932 |

| Means with the same letter are not significantly different. | | | | |
|--|---|-------------|-----------------|----|
| t Grouping | | Mean | N sample | |
| | A | 7.2000 | 10 | 5 |
| | A | | | |
| | A | 7.2000 | 10 | 8 |
| | A | | | |
| | A | 7.1000 | 10 | 9 |
| | A | | | |
| B | A | 7.0000 | 10 | 4 |
| B | A | | | |
| B | A | 7.0000 | 10 | 11 |
| B | A | | | |
| B | A | 7.0000 | 10 | 13 |
| B | A | | | |
| B | A | 6.9000 | 10 | 10 |
| B | A | | | |

Analysis of variance studying the effect of sample on measured sensory parameters

The GLM Procedure

t Tests (LSD) for aroma

| Means with the same letter are not significantly different. | | | | |
|---|---|--------|----------|----|
| t Grouping | | Mean | N sample | |
| B | A | 6.9000 | 10 | 12 |
| B | A | | | |
| B | A | 6.9000 | 10 | 7 |
| B | A | | | |
| B | A | 6.8000 | 10 | 3 |
| B | A | | | |
| B | A | 6.7000 | 10 | 6 |
| B | A | | | |
| B | A | 6.7000 | 10 | 14 |
| B | A | | | |
| B | A | 6.5000 | 10 | 2 |
| B | | | | |
| B | | 6.3000 | 10 | 1 |

Analysis of variance studying the effect of sample on measured sensory parameters

The GLM Procedure

t Tests (LSD) for aroma

Note This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

| | |
|-------------------------------------|--------------|
| Alpha | 0.05 |
| Error Degrees of Freedom | 126 |
| Error Mean Square | 1.33095 2 |
| Critical Value of t | 1.97897 |
| Least Significant Difference | 1.021 |

| Means with the same letter are not significantly different. | | | | | |
|--|---|--|-------------|----------|---------------|
| t Grouping | | | Mean | N | sample |
| | A | | 7.600 0 | 1 0 | 4 |
| | A | | | | |
| | A | | 7.500 0 | 1 0 | 9 |
| | A | | | | |
| | A | | 7.500 0 | 1 0 | 8 |
| | A | | | | |
| | A | | 7.500 0 | 1 0 | 12 |

Analysis of variance studying the effect of sample on measured sensory parameters

The GLM Procedure

t Tests (LSD) for aroma

| Means with the same letter are not significantly different. | | | | | |
|---|---|-----|------------|--------|--------|
| t Grouping | | | Mean | N | sample |
| | | A | | | |
| B | | A | 7.300 0 | 1 0 | 5 |
| B | | A | | | |
| B | | A | 7.300 0 | 1 0 | 13 |
| B | | A | | | |
| B | | A C | 7.200 0 | 1 0 | 11 |
| B | | A C | | | |
| B | D | A C | 7.100 0 | 1 0 | 3 |
| B | D | A C | | | |
| B | D | A C | 6.900 0 | 1 0 | 10 |
| B | D | A C | | | |
| B | D | A C | 6.900 0 | 1 0 | 14 |
| B | D | A C | | | |
| B | D | A C | 6.600 0 | 1 0 | 2 |
| B | D | | | | |
| B | D | | 6.400 0 | 1 0 | 6 |

Analysis of variance studying the effect of sample on measured sensory parameters

The GLM Procedure

t Tests (LSD) for aroma

| Means with the same letter are not significantly different. | | | | | | |
|---|---|---|------------|--------|--------|--|
| t Grouping | | | Mean | N | sample | |
| | D | C | | | | |
| | D | C | 6.200 0 | 1 0 | 7 | |
| | D | | | | | |
| | D | | 6.100 0 | 1 0 | 1 | |

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