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EFFECTS OF PROCESSING TECHNIQUES ON CHEMICAL, FUNCTIONAL AND MICROBIAL PROPERTIES OF TWO VARIETIES OF LIMA BEAN (*PHASEOLUS LUNATUS*) AND MAIZE (*ZEA MAYS*) FLOURS AND SENSORY PROPERTIES OF THEIR PRODUCTS

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DEPARTMENT OF HOME SCIENCE,
NUTRITION AND DIETETICS
UNIVERSITY OF NIGERIA, NSUKKA

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A THESIS SUBMITTED IN FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF DEGREE OF DOCTOR OF PHILOSOPHY (Ph.D) IN HUMAN NUTRITION

TO THE

DEPARTMENT OF HOME SCIENCE, NUTRITION AND DIETETICS
FACULTY OF AGRICULTURE
UNIVERSITY OF NIGERIA, NSUKKA

BY

OBIAKOR, PHILOMENA NGOZI
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DECEMBER, 2009

APPROVAL PAGE

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DEDICATION

To Almighty God with whom all things are possible and
in memory of my beloved father, Sir J. N. Obiakor.

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My profound gratitude goes to Almighty God for making it possible for me to complete this Doctorate programme. I express my sincere thanks to my Supervisor, Prof. I.C. Obizoba and his wife, Mrs. M. Obizoba. Their guidance, advice, encouragement and constant support were prime movers of my zeal in the completion of this programme.

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Philomena Ngozi Obiakor

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Abstract

This study evaluated the effects of different domestic food processing techniques on the nutrients, antinutrients, food toxicants composition, functional properties, microbial types and sensory properties of food products based on two varieties of lima bean (*Phaseolus lunatus*). Black and dark red varieties of lima bean were purchased, cleaned, cooked, soaked, dehulled, germinated, fermented and milled into fine flours. Both the unprocessed and processed flours

were analysed for microflora composition, chemical, functional and sensory properties using standard methods. The 48h fermented maize and lima bean flours that had traces of hydrogen cyanide (HCN) were selected because they had the best nutrient profiles in a ratio of 70:30 (protein basis) to formulate composite flours for moi-moi and akara production. Two local dishes (*oshoto* and *ikpaki*) were prepared from whole unprocessed lima bean and maize. The data were statistically analyzed using percentages, means and standard deviation. Duncan's new multiple range test was adopted to separate and compare means. The 3 and 9h soaking as well as 48h fermentation increased protein in black lima bean (26.07, 26.68 and 32.67%, respectively). On the other hand, dehulling and cooking, 6 and 9h soaking, 72, 96 and 120h germination as well as 48h fermentation increased protein in dark red lima bean. These treatments equally decreased fat, ash and fibre in both varieties. Carbohydrate was increased in both varieties. Dehulling, cooking and 24h fermentation increased zinc in black and decreased it in dark red variety. Processing decreased copper, iron and phosphorus in black lima bean. Dark red lima bean whose cooking water was drained and replaced had decreases in copper relative to the control. Iron and phosphorus decreased regardless of treatments. Treatments slightly increased folate in dark red lima bean flours except for 72h germinated and 48h fermented samples. Phytate, hydrogen cyanide (HCN), trypsin inhibitor and saponin were reduced in both varieties. Tannins and haemagglutinin levels increased in germinated samples. Foam and oil absorption capacities decreased in both varieties due to treatment. Water absorption capacity increased in both varieties except for the black variety fermented for 24h and 120h germinated dark red variety. Emulsion capacity decreased in black variety except for 9h soaked, 72 and 120h germinated flours relative to the control. Emulsion capacity increased in red variety except for the 48h fermented sample. Protein decreased in both composites relative to their controls, except for the 48h fermented black lima bean and maize blend. Fat increased in black lima bean and maize flour blends, except for equal value for the cooked composite relative to the control (1.60%). Ash and fibre decreased in composites of both varieties. The decrease was much more in dark red variety. Carbohydrate values for the composites increased regardless of varietal differences. Mineral levels in composite flours differed ($P < 0.05$). Zinc and copper increased in both varieties relative to their controls except for Cu of 96h germinated and 48h fermented black lima bean composites. Iron increased in the composites of both varieties except for the cooked and 72h fermented black lima bean composite. Phosphorus increased in black composites except for the cooked and 72h fermented composites. Both moi-moi and akara from 48 and 72h fermented varieties and 48h fermented maize blends had increased protein relative to the control. Fat, fibre, ash and carbohydrate of moi-moi and akara from both varieties were slightly higher than their controls. Calcium, magnesium, potassium, phosphorus and iron of both products had increases, except for a few products. Cooking and dehulling of both varieties caused drastic decrease in pathogenic microflora than the other methods. Organoleptic attributes and general acceptability of *moi-moi* and *akara* from both varieties improved due to processing. The protein, fat, ash, fibre and carbohydrate content of traditional dishes (*oshoto*) prepared with cooked whole lima bean and maize were higher than those cooked without maize (*ikpaki*) regardless of varietal differences. Calcium, magnesium, potassium, phosphorus and iron values for traditional dishes containing maize were higher than in the dishes prepared without maize. Organoleptic attributes of dishes from blends of lima bean and maize were slightly lower than the dishes prepared without maize. Domestic food processing techniques, especially cooking and fermentation increased macro and micronutrients, decreased antinutrients and food toxicants in lima bean. Organoleptic attributes of both dishes and products were highly acceptable. Processed lima bean flours and products have diversified food use to reduce food insecurity.

CHAPTER ONE
INTRODUCTION

1.1 BACKGROUND OF STUDY

In Nigeria, a lot of under exploited plant food crops with promising nutritional potentials exist. Lima bean is one of the under exploited food crops with nutritional and food industry processing potentials. Lima bean is among the lesser known legumes in the southern part of Nigeria. The food crop is one of the traditional staples that in the most recent decades was neglected. However, the neglect is attributed to difficulties in production, processing, preparation, utilization and negative image attached to it as "poor man's food". Lima bean has longer cooking time, especially as compared with other legumes. This longer cooking time phenomenon is a particular drawback for its cultivation and food use. It is imperative to investigate the positive attributes of this important food legume mostly its nutrient potentials.

Legumes as protein-rich crops are important source of protein for human food and animal feed. In 1972-1974, legumes contributed 7% to the total protein supply worldwide (FAO, 1982). Generally, legumes contain 17-25% protein (about double that in most cereals) except soybean which contains about 40% protein (Enwere, 1998). Legumes are usually consumed in combination with cereals such as rice, maize, millet, and sorghum. They are sometimes combined with roots and tubers such as yam, cassava, potatoes, and cocoyam or consumed with plantains and bananas. Studies have shown that legume proteins complement cereal and roots to provide an ideal source of dietary protein of vegetable origin for human beings (Latham, 1997). This is because cereal protein which is deficient in lysine and rich in methionine and cystine complement legume proteins which are rich in lysine and poor in methionine and cystine (Bressani, 1975; Segal and Fawcelt, 1976).

Despite the high nutrient content of legume seeds, their utilization is impaired by some inherent constraints such as the presence of several antinutrients and food toxicants. The type and concentration of these antinutrients vary in different legumes (Leiner, 1980). Lima bean has the highest content of hydrogen cyanide 10-312mg/100g) depending on the variety. The hydrogen cyanide content of other major legumes range from 0.5mg/100g to 2.3mg/100g (Ologhobo et al; 1984).

It is obvious that plant foods contain a lot of antinutrients that affect availability and utilization of minerals. Tannins, phytates and food toxicants (anthocyanins and haemagglutinin) impart bitter and unacceptable taste to the legumes. They prevent protein digestibility and decrease the absorption of divalent metal ions in the intestine (Elegbede,

1998). Simple processing techniques can significantly reduce the levels of these antinutrients and toxicants in plant foods. These processing techniques include soaking, cooking, dehulling, germination (sprouting) and fermentation. Traditional method of processing plant foods lower the level of antinutrients and make nutrients much more available (Obizoba and Atii, 1994).

Despite the large number of existing grain legumes in Nigeria, their consumption as staple foods have centred mainly on cowpea and groundnut. Several other locally available species like the lima bean, pigeon pea and jack bean, which have remarkable adaptation to tropical conditions are less commonly used.

Lima bean is a lesser popular legume. This is because many communities are not aware of its nutritional potentials. Lima bean could easily be utilized in many food preparations. It is usually consumed in combination with cereals such as rice, maize, sorghum or with roots and tubers such as yam, cocoyam, or with plantain. It is a good source of B-complex vitamins, protein, fibre, iron, zinc, potassium and magnesium (Apata and Ologhobo, 1994).

In developing countries, especially in Nigeria widespread food shortages, hunger and malnutrition have persisted, particularly among the low-income groups. In order to improve this situation, there is need for promotion of local food crops of high nutritional importance. The promotion of under exploited food crops with high nutritional potentials cannot be over-emphasized. This is because they make substantial contributions to the nutritional needs of the population, especially of the low-income groups.

1.2 STATEMENT OF PROBLEM

Despite the efforts to combat hunger and malnutrition in Nigeria, they are still widespread in both rural and urban communities. The low-income groups suffer most because they lack resources to procure, prepare and utilize adequate diets. Strict economic measures, inadequate production, processing and preservation of foods militate against provision of protein and micronutrient rich foods. Under-exploitation and neglect of our indigenous food crops are some of the problems. Lima bean is a lesser known legume, however, it is a locally available food crops which when adequately processed could be valuable tool to fight hunger and malnutrition.

1.3 OBJECTIVE OF THE STUDY

General objective: The thrust of this work was to identify the commonly consumed varieties of lima bean and determine the effect of various domestic food processing

techniques on the chemical, functional and sensory properties of their flours and blends with maize.

Specific objectives were to:

i. identify the commonly consumed varieties of lima bean in Arondizuogu, Ideato North L.G.A., Imo State, Nigeria;

ii. determine the effects of food processing methods on the nutrients, antinutrients and food toxicants composition of lima bean and maize flours;

iii. determine the functional properties of the flours;

iv. select the flours (lima bean and maize) that had high nutrient profiles, formulate their composites and determine their nutrient composition;

v. evaluate the organoleptic attributes of foods and snacks based on the composite flours; and

vi. prepare and assess the nutrient content of two traditional dishes (*oshoto* and *ikpaki*) based on whole lima bean seeds and maize grains.

1.4 SIGNIFICANCE OF THE STUDY

The results of this study would increase awareness in both rural and urban communities on the nutrient potentials of lima beans. The result would provide basic information to food policy makers for justification for:

a. instituting policies to promote this traditional food crop.

b. diversifying its use to improve food security.

c. food industries to use its flours to produce much cheaper and nutritious food substitutes.

CHAPTER TWO
LITERATURE REVIEW

2.0 LEGUMES AND CEREALS

In Nigeria, particularly among the low-income groups, starchy foods are the most frequently consumed foods. However, legume and cereal seeds provide the greatest

contribution of protein and other nutrients. They are consumed in various forms by more than one half of the population and make a larger contribution to the energy and protein available to the population than any other foods (McWatters and Brantley, 1982). Experiments have shown that cereals are higher in sulphur containing amino acids (cystine and methionine), which are inadequate in legumes. Legumes have higher protein content than cereals and they are rich in lysine and tryptophan which are the limiting amino acids in cereals. If consumed in mixed diet, they will compensate for these limiting amino acids in each other thus increasing their quality and utilization. Eka (1979) noted that the greatest advantage of the cereal and legume protein supplementation are the availability, consumption of nutritious and natural foods based on commonly consumed foods instead of the addition of expensive synthetic amino acid in the diet.

2.1 LEGUMES

The term "legume" is derived from a Latin word, "legumen" meaning seeds harvested in pods. Legumes belong to the family leguminosae. Elegbede (1998) reported that legumes are grouped into two, viz, food legumes and grain legumes. Food legumes known as pulses are those plants characterized by true pods which enclose small, edible seeds of peas, beans and lentils. They include plants that are used as food in the form of unripe pods, green grains and dry seeds directly or indirectly. However, grain legumes, exclude unripe pods or grains and include all dry grains of legumes.

Legumes rank second to the grasses as food for man. They serve man in a greater variety of ways than do the grasses (Enwere, 1998). Legumes as protein-rich crops are becoming increasingly important sources of proteins for human food and animal feed. In 1972-1974, legumes contributed 7% to the total protein supply world wide (FAO, 1982). Legumes have the ability to fix nitrogen (N) from the atmosphere and a much higher efficiency to produce protein per unit land area than animals. They hold a promise of meeting protein needs of an increasing world population. Davidson *et al*. (1975) indicated that there is no part of the world where legumes are not cultivated such that only few people do not enjoy regular contribution of legumes in their diets. However, legume proteins are of lower quality than animal protein due to the limiting SAA, poor digestibility and the presence of antinutrients. The low levels of SAA in legumes are usually corrected by supplementation with methionine, as in animal feed or by complementation with cereals as practised by various human populations (Latham, 1997). Literature showed that legume proteins are higher in lysine and lower in SAA such as methionine and cystine.

The legume family leguminosae is a large one which includes pulses, oil seeds, peas or beans (Enwere, 1998). Legumes (oilseed and pulses) belong to the Febaceae family. Legume seeds used as food for man include cowpeas, broad beans, kidney beans, soy-bean, pigeon peas, bambara groundnuts, african yam beans, baobab, groundnut and many others.

Generally, legumes contain 17-25% protein (about double that in most cereals) except soy-bean which contains about 40% protein and 26.3% carbohydrate (Temple and Bassa, 1991). Legume seeds are good sources of minerals such as phosphorus and iron (Elegbede, 1998). Legume seeds are generally low in fats and oils except for the oil seeds e.g. soy-bean and groundnut which contain 18% and 48% oil, respectively (Muller and Tobin, 1980). Legume seeds as protein sources compete poorly with animal protein sources. However, legume proteins are cheaper. In fact, legumes were at one time regarded as the poor man's meat in different parts of the world. This is because of the poorer class in the society who could not afford meat, fish, milk and egg as protein sources could afford to buy beans to satisfy their protein needs (Elegbede, 1998).

Legumes are usually consumed in combination with cereals such as rice, maize, millet and sorghum. Studies have shown that legume proteins complement cereal proteins to provide an ideal source of dietary protein of vegetable origin for human beings. This is because cereal proteins which are deficient in lysine and rich in methionine and cystine complement legume proteins which are rich in lysine and poor in methionine and cystine (Elegbede, 1998). Legume flour was processed and used in many other food preparations such as baby foods and baked products (Uwaegbute, 1990; Nnam, 1994).

Lima beans appear to be one of the less popular legumes in recent times. Many people are not aware of its nutrient potentials and utilization methods. However, it could easily be utilized in many food preparations. Its hard to cook phenomenon and its toxic component (cyanogenic glucoside) are the main constraints in its wide use.

Legumes contain antinutrients which reduce their protein digestibility and nutritive value. They also contain natural food toxicants (Onigbinde and Akinyele, 1983; Okoli and Ugochukwu, 1988; Egbe and Akinyele, 1990). The type and concentration of these antinutrients vary in different legumes (Leiner, 1980). Protease inhibitors, amylase inhibitors, hemagglutinin, saponins, goitrogens, cyanogenic glycosides, and vitamin factors, metal binding constituents, estrogenic factors, flatulence factors, toxic amino acids, urease, lathyrogens and favism causing agents are found in legumes (Leiner, 1980; Osho, 1989). Studies have shown that all legumes contain trypsin inhibitors to varying degrees and these

inhibit the trypsin and chymotrypsin in humans. It reduces protein digestibility, thereby reducing the nutritive value (Osho, 1989) Raffinose, stachyose and verbascose are the oligosaccharides implicated as the causative factors of flatulence (Leiner, 1980). Literature showed that some legumes, especially lima beans contain cyanogenic glycosides from which hydrogen cyanide (HCN) may be released by hydrolysis (Dibofori *et al*., 1994).

2.1.1 LIMA BEAN (*Phaseolus lunatus*)

2.1.2 ORIGIN OF LIMA BEAN

Lima bean is an important food though of limited local interest in Nigeria. It has a high yield and nutrient potential. Lima beans are one of the most widely cultivated pulse crops both in temperate and subtropical regions. It is a perennial or annual herb. It shows considerable variation in the form of vines, pods and seeds. As a food legume, it is utilized both as a dry pulse and green vegetable in many parts of the world.

Lima bean originated in Peru and has been cultivated there for more than 7,000 years ago. Lima bean got its name from the place where it was first discovered, ("Lima") the capital of the South American country of Peru by early European explorers (Kee *et al*; 1998). The Portuguese explorers introduced lima beans into Africa. This was the beans that can withstand humid tropical weather better than most beans. Lima beans have become an important crop in areas of Africa and Asia.

Ensminger and Ensminger (1986), reported that Lima beans were introduced into the United States in the 19th century, The majority of domestic commercial production centered in California. Today, lima bean is the predominant bean throughout much of the American tropics. These beans were commonly intercropped by native Americans with corn, this led to the origin of "Succotash" a side dish that combines lima beans and corn.

There are many varieties of lima bean, each with feature colour characteristics such as black, dark red, muttle black, white, brown, cream, muttle brown and dark brown. The pod of lima bean is flat, oblong and slightly curved, averaging about three inches in length. Within the pod sides, are four to six flat kidney-shaped seeds that are generally referred to as lima beans.

In Nigeria, lima bean is one of the legumes cultivated in the eastern part of the country. However, the hard to cook phenomenon experienced with lima bean, especially after long storage period and the toxic component cyanogenic glycosides) are the major constraints to the cultivation and utilization of this legume. The cooking water must be discarded and replaced at intervals during cooking to reduce its toxic component. Another main deterrent to

the greater use of this legume is the general ignorance of their food and nutrient potential, range of adaptation and of the practical aspects of their cultivation and use.

A lot of varieties are cultivated in different areas of the south eastern part of Nigeria. The most commonly cultivars are the black and the dark red varieties.

2.1.3 CULTIVATION OF LIMA BEAN

Lima bean (*Phaseolus lunatus*) is the most promising pulse crop for the subhumid to humid tropics. This grain legume appears to have a high yielding potential and a low incidence of pest and diseases. It can be used for mixed, relay and sole-cropping. It is consumed as both dry and green shelled beans.

It is usually planted during rainy season. Lima bean tolerates more rain during the growing season than other grain legumes. Owing to its darkly penetrating taproot, it is relatively resistant to drought once the crop is fully established. Climbing lima beans are usually indeterminate in growth and need to be trained during their growth cycle.

Lima bean grows best on medium to light, loamy soils that are well drained and well supplied with organic matter and develops vigorous extensive root systems. The seeds are sensitive to damage from cold water imbibition, particularly when the seeds are very dry. To reduce the risk of the cold water imbibition damage, the seeds are conditioned to 12-14% moisture before planting. This may be done by opening seed bags and placing them in a protected area to absorb moisture from the air for several days before planting. Lima beans are usually planted about May to mid-June after soil temperature exceeds 65 to 70°C. Ideal germination is at soil temperature of 75 to 85°C. Soil temperatures below 60°C result in poor stands and poor early growth (Kee *et al*, 1998). The seeds can be planted in mounds and trellised or can be broadcast to produce a thick ground cover. It is usually planted with a spacing of 3-4 inches within row and 22-36 inches between rows. Kee *et al*. (1998), reported that soil pH should be at least 6.5 – 7.00 and in place with no recent history of lima bean production, seeds will need to be properly inoculated. Lima bean pods are produced continually throughout the life of the plant, providing multiple harvest. Dry seeds are ready for picking after 3-5 months. The seeds are easy to collect and can be kept in cool, dry storage for many years. Lima bean is usually harvested from August to mid-September but the prime harvest season is from first of September to mid-September. Average yield of lima bean is approximately 2000 to 3000 lb/acre. Unlike most pulse crops, the pods are nonshattering, that is the seeds do not naturally fall from the dry pod onto the ground. They must be shelled because the pods are thick and tough.

Lima bean produces its first ripe pods about 3 months after planting and is ready for

harvesting from 100 – 180 days or more after planting depending on climatic factors (Majek *et al*; 1998).

2.1.4 NUTRITIVE VALUE OF LIMA BEAN

Lima bean is nutrient dense and their richness generates feelings of satiety. These sweet tasting bean has a rich, starchy, meaty texture and a creamy, distinctive flavour. Infact, lima beans are a good source of B-complex vitamins (vitamins B6, niacin and folate), protein (including the important amino acid lysine), fibre, especially soluble fibre in the form of pectin, iron, potassium and magnesium. They have very little fat. Studies, suggest that intake of lima bean lowers LDL cholesterol levels, probably due to their soluble fibre content. Lima bean contains the phytochemicals coumestrol and saponins, compounds that may impart anticancer benefits (Kee *et al*; 1997). National Academy of Science - NAS (1979) assessed the nutrient value of some lima bean varieties and concluded that protein content of lima bean ranged from 21.4 to 36.1%, fat 1.00 to 2.2%, ash 03.4 to 17.9%, fibre 01.7 to 04.9% and carbohydrate 55.6 to 73.2% depending on the variety. Globulins represent a major class of proteins in lima bean that contains substantial amounts of crude fibre. The cooking quality is affected by the presence of pectic substances (Reddy *et al*; 1984). Dela-Vega and Sotelo (1986) reported the nutritional quality and toxic content of wild and cultivated lima bean varieties. The bean contains protein ranging from 17.9 to 29.0%, the lipid content ranges from 0.9 to 2.8%, whereas carbohydrate ranges from 54.5 to 74.2%. Similar values were reported for lima bean seeds grown in Nigeria (Aletor and Aledetini, 1989; Apata and Ologhobo, 1994).

Osagie *et al.* (1986) reported that the lipid content of lima bean ranges from 0.9 to 2.1% and the lipids contain significant amounts of linoleic acids. Lima bean, like many other legumes constitute a rich source of niacin, thiamin and riboflavin (Sathe *et al*; 1984). Lima bean contains high levels of potassium, phosphorus, calcium and iron. The major form in which phosphorus exists in the bean is in the form of phytate phosphorus (Ologhobo and Fetuga, 1984abc; Osagie *et al*; 1996). Hall and Sayre (1971) examined starches from lima bean and reported that stachyose is the major sugar present in lima bean.

2.1.5 HEALTH BENEFITS OF LIMA BEAN

Lima bean is very rich and a good source of cholesterol-lowering fibre, as are most other legumes. In addition to lowering cholesterol, lima bean has high fibre content that prevents blood sugar levels from rising too rapidly after a meal. This qualifies these beans an

especially good choice for individuals with diabetes, insulin resistance or hypoglycemia (Menotti *et al*; 1999). When combined with whole grains such as rice or wheat, lima bean provides virtually fat free high quality protein (Wood, 1988). It is a good source of protein for vegetarians because its combination with whole cereals provides protein comparable to that of meat or dairy foods without high calories or saturated fat, found in these foods.

i. **Stabilisation of blood glucose**

Lima bean soluble fibre stabilizes blood sugar levels for diabetics by providing steady slow-burning complex carbohydrates. Studies of high fibre diets and blood sugar levels have shown the dramatic benefits provided by these high fibre foods. Mintosh and Miller (2001) compared two groups of people with type II diabetes who were fed different amounts of high fibre foods. One group consumed the standard American diabetic diet which contained 24 grammes of fibre per day, the other group consumed a diet containing 50 grammes of fibre per day. They concluded that those who consumed the diet higher in fibre had lower levels of plasma glucose and insulin. The high fibre group reduced their total cholesterol by nearly 7%, their triglyceride level by 10.2% and their VLDL (very low density lipoprotein) by 12.5%.

ii. **Iron**

Lima bean can increase energy and assist to replenish iron stores. A cup of lima bean contains 24.9% of the daily value for this important mineral (Wood, 1988). Mostly for menstruating women who are more at risk for iron deficiency, adding to their iron stores with lima bean is good idea, because iron is an integral component of heamoglobin and part of key enzyme system for energy production and metabolism. Growing children and adolescents also have increased need for iron.

iii. **Manganese for energy production and antioxidant defense**.

Lima bean is very good source of the trace mineral manganese, which is an essential cofactor in a number of enzymes. It is important in energy production and antioxidant defenses. For example, the key oxidative enzyme which disarms free radicals produced within the mitochondria requires manganese and one cup of lima bean supplies 48.5% of the daily value for this very important trace element (Wood, 1988).

iv. **Contribution to healthy heart**

Consumption of legumes such as lima bean and other high fibre diet reduces risk of heart disease. Lima beans contribution to heart health lies not just in its fibre, but in significant amounts of folate and magnesium this bean supplies. Bazzano *et al;* (2003) observed that folate lowered levels of homocysteine, an amino acid that is an

intermediate product in an important metabolic process known as methylation cycle. Elevated blood levels of homocysteine are an independent risk factor for heart attack, stroke or peripheral vascular disease. These are found in between 20-40% of patients that had heart disease. It is estimated that consumption of 1200% of the daily value of folate would reduce the number of heart attack yearly by 10%. One cup of cooked lima bean provides 39.1% of the daily value for folate.

v. **Lower heart attack risk**

Lima bean is good source of magnesium which has beneficial impact in the control of cardiovascular disease. Magnesium is nature's own calcium channel blocker. Adequate magnesium causes veins and arteries to relax, this lessens resistance and improves flow of blood, oxygen and nutrients throughout the body. Studies have shown that magnesium deficiency is associated with heart attack. Lack of sufficient magnesium promotes free radical injury to the heart. However, with regular consumption of at least a cup of lima bean 20.2% daily value of magnesium is assured.

vi. **Prevention of constipation and diverticulosis**

Lima bean contains insoluble fibre, which increases stool bulk and prevent constipation. However, it prevents digestive disorders like irritable bowel syndrome and diverticulosis (Ensminger and Ensminger, 1986). One cup of lima bean gives 65.8% of the daily value for fibre.

vii. **Lowering of plasma lipids and cholesterol levels in blood**

Saponins in lima bean can be extracted and used in the synthesis of drugs which lower blood cholesterol and provide a good market for the use of lima bean in the manufacture of cholesterol lowering drugs (Oboh and Omofoma, 2008).

Oboh and Omofoma (2008) examined the cholesterol lowering ability of lima bean in diet induced hypercholesterolemic rats and concludes that the saponins in raw and processed (cooked) lima bean significantly reduced serum lipids in the experimental rats. Saponins content of the raw and the heat treated samples were found to be 1.26g/kg and 732.3g/kg, respectively. Oakenfull and Sidhu (1990) reported that saponins form strong insoluble complexes with cholesterol and bile, making them unavailable for absorption. They added that this mixture is removed from the body through the normal elimination process. Increased bile excretion may cause compensation increase in bile acid synthesis from cholesterol in the liver. As the body needs more cholesterol for bile acid production used for digestion, the liver removes cholesterol from the blood stream and lowers serum cholesterol. The role of lima

bean in lowering blood cholesterol, especially the cooked bean without dehulling was also evaluated by Oshodi and Meladun (1993). They reported that lima bean contains a fibre level of 4.3% and suggested that the fibre content of this legume could have a contributory role in the reduction of cholesterol levels. The presence of saponins and fibre in the heat treated lima bean is probably responsible for cholesterol lowering ability of this legume. Research have shown that the consumption of lima bean diminishes the risk of artherogenic dyslipidemia. The hypocholesterolemic effect of lima bean could be due to the combined effect of the saponins and fibre.

2.1.6 FOOD USES OF LIMA BEAN

Lima bean is grown for its edible seeds. The fresh tender seeds may be boiled and consumed together with the pod as vegetable. The mature seeds are very hard to cook and so they are soaked in water for about 30 minutes to soften before cooking. The cooked undehulled seeds are mixed with palm oil, salt, pepper, and other ingredients and prepared into pottage. The pottage may contain yam, cocoyam, plantain, sweet potatoes, tapioca or cereals such as maize, sorghum and rice. Lima bean has a delicate flavour that complements a wide variety of dishes.

The form in which the lima bean seeds are commonly consumed is “succotash” a traditional native American dish that combines this delicious bean with corn or maize. Infact, “succotash” is consumed extensively by many household in Arondizuogu, Imo State, Nigeria, with the name “*Oshoto*”.

Lima bean may be prepared into moi-moi (steamed cowpea paste). The seeds are soaked in water for a long time, dehulled, wet-milled into smooth paste and mixed with palm oil or any other vegetable oil, salt, pepper, tomatoes, onions and other ingredients. The paste is wrapped in banana or “uma” leaves or filled into aluminium containers, covered and steamed to produce moi-moi.

In the traditional set-up, lima bean is usually cooked overnight before consumption. This is because, the mature dry seed is very hard to cook. It has a very strong beany flavour which is objectionable to those who are not familiar with the product.

2.1.7 ANTINUTRITIONAL FACTORS IN LIMA BEAN

Despite the interesting nutritional features, lima bean contains a lot of antinutrients like most legumes. This is a major factor that is limiting the wider food use of this crop. This is because its toxic components can precipitate deleterious effects in man and animal when consumed without adequate processing.

It is well documented that lima bean not only contains the trypsin inhibitor and phytohaemagglutinin, but also phaseolunatina cyanogenic glucoside – which releases HCN on hydrolysis. These factors are generally believed to act in concert to produce the myriad of deleterious effects when raw or improperly processed lima bean is consumed. Ingestion of foods containing these toxic cyanogens could lead to either chronic or acute poisoning (Oshuntokun, 1973). There is now increasing evidence that thiocyanate, the main metabolite of cyanide in animals may lead to the production of nitrosamine in vivo which may enhance carcinogensis (Okoh, 1998). Ologhobo *et al.* (1984) reported that lima bean has the highest content of HCN (10-312mg/100g) depending on the variety when compared with other legumes. However, this high level of HCN can be reduced by different domestic food processing methods. Egbe and Akinyele (1990) observed similar levels (420mg/kg) of cyanogenic glucoside in lima bean seeds. Lima bean contains a considerable amount of saponins which are not completely destroyed during processing (Osagie *et al*; 1996). Oakenful and Sidhu (1989) reported that lima bean contains appreciable amounts of saponins which possess both beneficial (cholesterol lowering) and deleterious (cytotoxic permeabilisation of the intestine) properties and exhibits structure dependent on biological activities. Other antinutrients such as phytate and tannins are found in lima bean. Ologhobo and Fetuga (1983c) reported that urea, bilirubin, transaminases and lactic dehydrogenase levels in blood of rats increased during acute intraperitoneal injection of lima bean haemagglutinin.

2.2 CEREALS

Cereals belong to various tribes of the grass family. They constitute important crops which serve as industrial raw materials and staple foods for the world (Enwere, 1998). Cereals are the most widely cultivated and consumed crops on a global basis. Cereal grains are the staple food of over half the world's population (FAO, 1993). The particular cereals utilized in different areas depend on the geographical location. Climatic conditions determine which kind of cereals can be grown in each area and thus also the consumption (FAO, 1989). Ihekoronye and Ngoddy (1985) reported that cereals provide about 75% total caloric and 67% of total protein intakes of the people in the tropics. World cultivated cereals include wheat, maize, rice, barley, oats, rye, sorghum, millet, acha and wild rice. An important characteristics of cereals is that they have high carbohydrate, low fat and a fair protein content (Enwere, 1998). Cereal proteins are deficient in the amino acids lysine and tryptophan and rich in methionine and cystine (Eggum, 1979). He showed that lysine is

the first limiting essential amino acid in cereal proteins. Cereals commonly consumed are maize, millet, wheat, sorghum, rice, barley and oats (Manay and Sharaswamy, 2008).

In the northern states of Nigeria, cereals such as rice, maize, millet and sorghum are the predominant staples. In the southern states, rice and maize feature prominently. Cereals are used for preparation of different foods at both household and industrial levels. Foods may be prepared from only cereals or in combination with other food crops.

2.2.1 MAIZE (*Zea mays*)

2.2.2 ORIGIN AND UTILIZATION

Maize originated in the western hemisphere, possibly in America. It is now cultivated in many parts of Africa, north, south and central America, Europe and Asia (Obi, 1991). FAO (1993) showed that maize cultivation originated in central America, particularly in Mexico, from whence it spread northwards to Canada and southward to Argentina. The oldest maize, about 7000 years old, was found by archaeologist in Teotihuacan, a valley near Puebla in Mexico. Maize cultivation later spread widely in Africa and in many parts of Asia. Okoh (1998) reported that maize is the cereal most widely cultivated and consumed in Africa, particularly south of the Sahara. It is more widely resistant to drought than rice. Maize cultivation is often best in region with at least 90 days of frost-free rain fall of about 25-300 cm annually (Okoh, 1998). Maize is after wheat and rice, the most important cereal grain in the world. It provides nutrients for humans and animals and serve as a basic raw material for production of starch, oil and protein, alcoholic beverages, food sweetners and more recently, fuel (FAO, 1993).

Purseglove (1992) reported that there are different varieties of maize. These include pop corn, flint maize, dent maize, flour or soft maize, sweet corn and waxy maize. Flint and dent maize are common species in use. Banigo and Muller (1972) identified maize as the predominant cereal in southern Nigeria. Maize is widely grown in tropical humid, subtropical and temperate regions of the world. It is suited to a much wider range of climatic conditions than sorghum. In Nigeria, maize is the second most important cereal crop. It ranks behind sorghum in the number of people it feeds (Obi, 1991). Maize like other cereals is deficient in the essential amino acid (EAA) lysine and tryptophan. Nevertheless maize continues to be a principal part of Nigerian diets.

In many developing countries maize is a vital staple, particularly for the rural poor. This is because it is highly adaptable to a wide range of environments and has many valuable properties. Maize is also an important food crop in the Mediterranean region, the Middle East

and parts of Asia (Enwere, 1998). Averaging 9.5% protein, maize contributes 42 million tons of protein yearly which represents 15% of the world annual production of food crop protein. It represents 19% of the world's food calories (NAS, 1998).

Maize is prepared and consumed in a multitude of ways. It is served in various forms as staples, snacks and beverages (Olatunde, 1989). Like sorghum, maize guel serves as traditional complementary food in Nigeria. Maize can be processed into flour, grits, popcorn and starch, breakfast cereal, complementary food, adult foods and animal feed (Enwere, 1998). Nutrend, cerelac maize, babeena are some complementary foods based on maize. Golden morn and cornflakes are breakfast cereals produced and marketed in Nigeria using maize as one of their ingredients.

Maize is used for three main purposes viz:

i. as a staple human food, particularly in the tropics.
ii. as feed for livestock, particularly in temperate and advanced countries; and
iii. as raw material for many industrial products.

Maize can be pounded. The meal is boiled, baked or fried. In Latin America, maize is made into tortillas (an unleavened pancake like maize bread). The whole grain may be boiled or roasted. It may be fermented maize meal cooked with water to provide a thick mass or dough. This is the commonest method of consuming maize in Africa. In Nigeria, products from maize include "foo-foo", pap, maize bread, popcorn, "agidi," "eko," "hatsi" (complementary food), Cornmeal, moi-moi oka, (maize pudding) and "ayaraya," are some of the maize dishes consumed in Igboland. In Yoruba land, they have such maize dishes as "eko," "igbigbona," "eko (mimu)," "ewa eko tutu," "ososo" and so on. In Hausa, they have maize dishes like "gusasa," "kunu," "jaddi," "telle": and "tuwo" massara.

Maize grain is an outstanding feed for livestock. It is high in energy, low in fibre and easily digestible. It is mainly used for pigs, although cattle, sheep and poultry are also fed with it. Other industrial products got from maize include starch which can be processed to dextrin or sugar, oil from germ, used for soap and glycerin production. It can also be used to produce cooking or salad oil. Maize is used in America for the production of starch, various sweetners, corn oil, alcohol and animal feed. Maize is fermented and distilled to provide industrial products such as ethyl, butyl or propyl alcohol, acet-aldehyde, acetone, glycerol and acetic, citric and lactic acids (Purseglove, 1992).

2.2.3 CHEMICAL COMPOSITION OF MAIZE

The chemical composition of different varieties of maize differs, to some extent, with

respect to their carbohydrate, protein, fat, fibre, ash and vitamins. The different parts of maize grain differ in nutrient composition as observed in yellow dent maize (Watson, 1987). The flint and dent maize have similar composition. There is a high level of oil in the embryo of the kernel and a high starch. However, there is low oil level in the endosperm.

Carbohydrates

The carbohydrates and related compound found in maize are mainly starch in addition to cellulose and pentoglycan in the cell wall. Maize grain contains 0.1 to 0.3% raffinose, 0.9 to 1.9% sucrose, 0.2 to 0.5% glucose, 0.1 to 0.4% fructose and smaller quantities of myo-inositol and glycerol in sound maize kernels (Enwere, 1998). Maltose and other sugars are reported during germination.

Protein

Most of the nitrogen in maize is present as protein. The main protein of maize is zein. It is a prolamine and soluble in dilute alcohol. Zein is found mainly in the endosperm. Germ protein accounts for about 15-25% of the total protein of maize and may contribute 25-40% of the total kernel lysine amino acid. The total protein of maize grain ranges between 6-15% (Purseglove, 1991).

Lipids and fatty acids

The oil content of the maize kernel comes mainly from the germ. Oil content is genetically controlled, the values range from 3 to 18% (FAO, 1992). Maize oil has a low level of saturated fatty acids, on average 11% palmitic and 2% stearic acid. It contains relatively high levels of polyunsaturated fatty acids, mainly linoleic acid.It has an average value of about 24% and small amounts of arachidonic acid. Maize oil is highly regarded because of its fatty acid distribution, mainly oleic and linoleic acids.

Minerals

The concentration of ash in maize kernel is about 1.3% (FAO, 1992). The germ is relatively rich in minerals. It has an average value of 11% as compared with less than 1% in the endosperm. The germ provides about 78% of the whole kernel minerals. According to Enwere (1998), maize contains about 2% ash which consists of many minerals such as sodium, potassium, copper, calcium, phosphorus, magnesium and others. The most abundant mineral is phosphorus, found as phytate of potassium and magnesium. All the phosphorus is found in the embryo. It has values as in common maize of about 0.30% and 0.90% in opaque-

2 maize. As with most cereal grains, maize is low in calcium and trace elements.

Vitamins

Maize is rich in many vitamins. These include, thiamin, riboflavin and niacin. Chlorine, folic acid and pantothenic acid are found in very low concentrations (FAO, 1992).The maize kernel contains two fat-soluble vitamins: Provitamin A, or carotenoids, and vitamin E. Carotenoids are found mainly in yellow maize. Most of the carotenoids are found in the hard endosperm of the kernel and only small amount in the germ. The beta-carotene content is an important source of vitamin A. Recent studies have shown that the conversion of beta-carotene to vitamin A is increased by improving the protein quality of maize (FAO, 1992). Vitamin E is found mainly in the germ. The source of vitamin E is four tocopherols, of which alpha-tocopherol is the most biologically active form.

Dietary fibre

After carbohydrates, proteins and fats, dietary fibre is the chemical component found in the greatest amounts. The complex carbohydrate content of maize kernel comes from the pericarp and the tip cap. It is also provided by the endosperm cell and germ cell walls. Sandstead *et al.* (1978) found that maize bran was composed of 75% hemicellulose, 25% cellulose and 0.1% legnin on a dry-weight basis. Dietary fibre content in dehulled kernels would obviously be lower than that of whole kernel.

2.2.4 NUTRITIVE VALUE OF MAIZE

Nutritionally, maize resemble other cereals and differs in some respects. The yellow variety has high provitamin A or carotenoid content (FAO, 1992). Yellow maize is a good source of vitamin A precursor (beta-carotene). According to FAO, (1992), beta-carotene accounts for about 22% of total carotenoids and cryptoxanthin accounts for 51%. These substances are readily converted into vitamin A in animal body. The vitamin A precursors are concentrated in the pigmented layer of the endosperm underneath the hull and to a lesser extent in the embryo. This relatively high content of vitamin A precursor differentiates yellow maize from all other cereals.

The major protein in maize is zein which is deficient in lysine and tryptophan. Maize protein is of relatively poor quality because it consists mainly of the zein fraction which is of low biological value. Ekpenyong *et al.* (1977) showed that the maize protein is low in lysine, very low in tryptophan and reasonably fair in sulphur containing amino acids, methionine and cystine. There are variability in the amino acid and vitamin contents of different varieties of

maize. These constituents vary in different parts of the maize grain. The germ, embryo and scutellum have higher level of protein, vitamins and minerals. Kent (1975) reported that most of these nutrients are eliminated during processing, for example, milling maize into fine flour and grits, or degerming of maize. The high starch content of maize satisfies the energy requirement of people consuming it. Starch provides up to 72 to 73% of the maize kernel weight and is mostly concentrated at the endosperm. The lipid content which is concentrated in the germ is a good source of energy and fatty acid.

According to Enwere (1998), maize oil is high in unsaturated fatty acids. It contains 56% linoleic acid, 36% oleic acid, 0.7% linolenic acid, some stearic and palmitic acids. Studies have shown that maize oil is known to have a hyper cholesteremic effect in man and other animals. It reduces cholesterol in blood due at least in part to the large amounts of plant sterol such as B-sitosterol in maize oil. The vitamins and minerals which are concentrated in the germ, embryo and scutellum contribute to the maintenance of metabolic processes. The nicotinic acid present in maize is in a bound form and does not become available. The absence of lysine and tryptophan is associated with pellagra (Davidson *et al*; 1975).

2.3 SOME ANTINUTRIENTS AND FOOD TOXICANTS IN PLANT FOODS

Antinutrtional factors in food are those food components that interfere with the digestion, absorption or some other aspect of metabolism of a nutrient or nutrients contained in those or other foods. In a nutshell, antinutritional factors represent a considerable number of different chemical compounds having a wide range of metabolic effects. Some of these factors such as enzyme inhibitors, phytates, lectins, polyphenols and allergenic factors are quite common, while others may be specific to just a few plant species, such as cyanogens in cassava, gossypol in cotton seed and favism in broad beans and other legumes. These compounds generally make up a small fraction of the weight of foods and available methods of removing these compounds from food depend primarily on their physicochemical nature. Plant foods contain a lot of antinutrients which are known to exert a deleterious effect in man and animals when ingested without adequate processing. The type and concentration of these antinutrients vary in different plant foods. The toxic factors (toxicants) may occur to all parts of the plant. The seed is normally the most concentrated source (Osagie, 1998). Some of the antinutrients and food toxicants found in legumes includes protease inhibitors, tannins, phytates, haemagglutinins, cyanogenic glucosides, saponin, oxalates and oligosaccharides.

2.3.1 PROTEASE INHIBITORS

Protease inhibitors are inherent natural chemical components found in plants and animal tissues which tend to hamper the activities of certain enzymes from performing their function of breaking down complex molecules (Onwuka, 2005). Evidence have shown that trypsin inhibitor in the diet leads to the formation of irreversible condition known as enzyme trypsin inhibitor complex. This causes a decrease in dietary protein digestibility leading to slower animal growth. According to Enwere (1998), all legumes contain trypsin inhibitors to varying degrees and the inhibition of trypsin and chemotrypsin leads to the hypertrophy of pancreas. Trypsin inhibitor in food does not pose a serious health problem because most traditional food processing techniques especially cause significant reduction in trypsin inhibitor activity (Egbe and Akinyele, 1990; Ogun *et al*; 1989)

However, the major toxicity symptoms of protease inhibitor are impaired growth and food utilization and pancreatic hypertrophy. Enwere (1998) revealed that five or more trypsin inhibitors have been reported for beans but only two – "Kunitz and the Bowman-Berk inhibitors," have been purified and studied.

2.3.2 HAEMAGGLUTININS (LECTINS)

Haemagglutinins also known as lectins are proteins. They are characterized by their unique ability to bind specific sugars or glycoproteins (Osagie, 1998). Haemagglutinins are found in beans. Lectins bind to the epithelial cells lining the small intestine. A series of complex events ensue which culminate in severe growth depression and ultimately in the death of the animal. Haemagglutinins have the ability to cause agglutination (clumping) of red blood cells in vitro.

Ologhobo and Fetuga (1983a) noted that values for urea, bilirubin, transaminases and lactic dehydrogenases in blood of rats increased during acute intraperitoneal injection of lima bean and some legume haemagglutinins. Lectin toxicity is due to their production of intestinal lesions and histopathological changes of some organs, particularly in the kidney (Ikegwonu and Bassir, 1977). Lectins cause an increase in tissue invasion by normal innocuous gut bacteria due to reduction of body defences. Among the consistent pathological manifestations of injected toxic lectin are zonal necrosis and fatty infiltration of the liver, oedema, marked distention of capillary vessels and haemorrhages in various tissues.

Aletor and Futega (1985) reported the responses of rats hepatic and serum transaminase to lectins extracted from lima bean. The toxicity of all haemagglutinins is destroyed by heat. Detoxification of haemagglutinins is achieved by traditional methods of household cooking and industrial autoclaving (Onimawo and Akubor, 2005).

Egbe and Fetuga (1990) reported significant reduction in phytohaemagglutinin activity with increased cooking time of lima bean. However, dry heat does not destroy haemagglutinins. Soaking before cooking can completely inactivate them in bean (Noah *et al*; 1980).

2.3.3 CYANOGENIC GLYCOSIDES

Cyanogenic glycosides are group of O-glycosides formed from decarboxylated amino acids. The cyano group arises from the carbon atom and the amino group (Osagie, 1998). The presence of cyanogenic glycosides in most legumes had been established (Okoli and Ugochukwu, 1989). Cyanogenic glycosides yield hydrogen cyanide on treatment with acid and enzymes. According to Onimawo and Akubor (2005), the three major cyanogenic glycosides identified in edible plants are linamarin (acetone cyanosides), amygelalin (benzaldehyde cyanohydrin glycoside) and lotaustralin.

Okoli and Ugochukwu (1989) studied the HCN content of some legume seeds and reported the cyanide content of 381-1093, 285-1223 and 208-953mg/kg dry weight of intact seed, testa and cotyledons, respectively. The testa had 3-5 times higher than cotyledon in some cases. However, they concluded that soaking of seeds in water for 24 hours led to appreciable losses of HCN. Boiling for 3 hours caused drastic reduction (49-95% in HCN). After boiling for 3 hours, innocuous HCN levels were obtained with seeds previously soaked in water for 24 hours and divested of testa. Soaking bean seeds in water and subsequent removal of testa prior to boiling will decrease HCN in bean meals. Egbe and Akinyele (1990) found similar high level (420mg/kg) of cyanogenic glucoside in lima bean. Lima bean has the highest content of HCN (10-312mg/100g) depending on the variety (Ologhobo *et al*, 1984). Lima bean contains a cyanogenic glycoside called Phaseo lunatinin from which HCN is liberated due to enzyme action (Purseglove, 1991). However, this high level of HCN can be reduced by different processing methods.

Evidence have shown that cyanide is widely distributed in nature and that some amount of cyanide is consumed as part of our daily diet (cereals, root crops, legumes and fruits). Cyanide is a normal constituent of blood usually at a very low concentration less than 12(mol) (Osagie, 1998). Aremu (1989) estimated that the per capita daily intake of hydrocyanate in Nigeria was 8mg, almost 90% of which is from gari alone. The consumption of foods containing cyanogens could result in acute or chronic cyanide toxicity (Osuntokun, 1972).

2.3.4 SAPONINS

Saponins are steroid or triterpenoids glycosides, characterized by their bitter or astringent taste, foaming properties and their hemolytic effect on red blood cells (Osagie, 1998). They are widely distributed in plants. Sodipo and Arinze (1985) reported that legumes such as beans contain a considerable amount of saponins (245.0mg/kg).

Saponins possess both beneficial (cholesterol lowering) and deleterious (cytotoxic-permeabilisation of the intestine) properties (Price *et al*; 1987; Oakenful and Sidhu, 1989). Although some saponins can be highly toxic under experimental conditions, acute saponins poisoning is relatively rare both in man and animals (Kee *et al*; 1998). The consumption of saponins is encouraged because of their hypocholesterolemic activity. However, forage saponins cause toxic and anorexic effects in rat and swine (Cheeke *et al*; 1978).

Generally, saponins are not easily destroyed during cooking or processing. Alkaline washing or dry scouring and abrasive dehulling have been suggested as techniques of reducing saponins in legumes (Osagie, 1998).

2.3.5 TANNINS

Tannin is a plant polyphenolic substance with a molecular weight greater than 500 (Osagie, 1998). The two distinctive groups of tannins identified are hydrolysable tannins and condensed tannins. Hydrolyzable tannins are easily hydrolyzed into a mixture of carbohydrate and phenols. Condensed tannins are flavoid polymers. Tannins are found in all plant foods including legumes, cereals, fruits, root and tubers. Egbe and Akinyele (1990) found 0.59(0.07mg/g tannic acid in raw lima bean. The tannic acid content of some wild underutilized crop seed in Nigeria were evaluated (Balogun and Fetuga, 1989). The values ranged between 0.11% in the seed of “Coula edulis” and 1.15% in “Lophira alata”. High tannic acid content were obtained in the seed of baobab (Adansonia digitata), 0.98% and 1.11% in the seed of “Hypaena thebaica”. Nnam and Obiakor (2003) reported tannins values of 0.23, 0.26 and 0.38% in 24, 48 and 72h fermented baobab seeds.

Tannins may decrease protein quality by decreasing digestibility and palatability. Other nutritional effects which have been attributed to tannins include damage to the intestinal tract, toxicity of tannins absorbed from the gut and interference with the absorption of iron and a possible carcinogenic effect (Butler, 1989).

Reddy *et al* (1985) reported that tannins hindered iron absorption by forming tannin-iron complex. A marked decline in the haemoglobin of rats fed 5% tannic acid was reported by Bressani and Elias (1980) due to tannin-iron complex formation. Tannins chelate vitamin

B12 as well as prevent its absorption and bioavailability. However, tannin reduction increases bioavailability of iron in plant foods.

2.3.6 PHYTATE

Phytic acid is a hexaphosphate derivative of inositol which is insoluble and cannot be absorbed in human intestines. It is a storage form of phosphorus in plants Phytic acid has twelve replaceable hydrogen atoms with which it could form insoluble salts with metals such as calcium, iron, zinc and magnesium. The formation of these insoluble salts renders the metals unavailable for absorption into the body. Balogun and Fetuga (1989) concluded that phytate can cause mineral deficiency in population that depends on whole grain and legume based products. The phytate content of plant foods differ and some of the data were overestimated (Osagie, 1998). The studies on levels of oxalic acid and phytic acid in traditional foods of northern Nigeria conducted by Eka (1977) revealed that the quantity of the phytate and oxalate in these foods were below toxic level. However, their roles in prevention of proper utilization of calcium and phosphorus is considerable. Phytate is also associated with increased cooking time in most grain legumes.

2.4 EFFECT OF FOOD PROCESSING

The term food processing is very vast and includes a number of treatment food is subjected to while making it ready for consumption. The primary aim of processing food materials either for direct human consumption or for industrial, commercial or as adjuncts to other food manufacture, is to satisfy the consumer in terms of taste, colour, texture, flavour, convenience of use, availability and quantity, duration and time of availability and storage stability of the final product. In general, processing involves some transformation of the raw materials into products that are different in the physical and chemical forms, thermodynamic and aesthetic appeal from those of the natural raw materials. They are often more convenient and easier to handle because they are less bulky (Roday, 2007). However, processing tries to retain, within limits some of the aesthetic appeal, nutritional quality of the original raw material. Roday (2007) noted that processing often involves one or more distinct unit operations each of which, as an integral part of the whole process, is subject to specified process conditions that ensure that the overall characteristics of the final products are achieved. When food is processed, some loss of nutrients occurs, the loss may be minimal and can be replaced by restoring, enriching or fortifying the food.

2.4.1 BENEFITS OF FOOD PROCESSING

Some of the benefits of food processing are as follows:

i. **Minimize or eliminate antinutritional factors**

Some food materials cannot be eaten ordinarily because of the high content of the naturally occurring toxicants or antinutritional factors that cause flatulence or digestive disorder. For example, oligosaccharides, raffinose, stachyose, verbasose, haemagglutins, trypsin inhibitors, phytic acid, tannins, oxalates, cyanogenic glucosides and sapponins in legumes, cereals, roots and tubers. These must be removed to make the food safe for human consumption. It also facilitates nutrient bioavailability.

ii. **Increase digestibility**

Processing methods such as cooking increase digestibility. Most foods are difficult to digest unless they are cooked. Cooking softens fibre, gelatinizes starch, denatures protein for easier digestion. Exception of fruits, all other foods need some kind of processing to make them more digestible (Roday, 2007).

iii. **Enhance flavour, colour and taste**

The acceptability of food depends to a large extent on its organoleptic or sensory qualities. Processing techniques enhance the appearance and make food more appetizing and tasteful. Examples, cakes and bread, the brown crust formed due to maillard reaction gives baked products their characteristics flavour, aroma and taste.

iv. **Diversification of food use**

The development of appropriate technology for dehulling legumes and cereals and producing ready to use flours as well as formulation of composite flours and useful recipes for preparing many convenience foods, complementary foods and baked products from these composite flours has greatly increased and diversified their utilization and promotion. This is equally applicable to some roots and tubers. Cocoyam is now processed into flour for greater convenience in its use as a traditional soup thickener. Cassava flour is used in baking and the cassava chips is eaten with vegetable as African salad. Gari from cassava is a result of diversification and utilization occasioned by processing (fermentation and frying).

v. **Reduction in post harvest losses and increase in storage stability**

Many of the food crops have relatively short storage life under ambient conditions. This is often a few days after maturity and harvest. Processing increases the shelf life and decreases post harvest losses which can be as high as 40% between harvest and

consumption of perishable.

2.5 PROCESSING TECHNIQUES EMPLOYED IN IMPROVING THE NUTRITIONAL QUALITY OF LEGUMES AND CEREALS

Legumes and cereals are processed to detoxify their toxic component and antinutritional factors, increase palatability and improve bioavailability of nutrients. No matter the palatability rating of any food, once it is ingested, what matters is its nutritive value. Some of the traditional methods for processing of legumes and cereals to improve and upgrade their nutritive value include:

2.5.1 SOAKING

Soaking softens the cotyledon and reduces cooking time in grains. Obizoba and Atti (1991) observed that soaking of legume seeds may be used to moisten and soften the seeds to reduce the duration of cooking or aids in the removal of the seed coat. Enwere (1998) noted that legumes are soaked in water to soften the testa and dissolve the binding gum between the testa and cotyledon. This process facilitates both wet and dry decortication. During paste production, cereal grains and legume seeds are soaked in water to soften the cotyledon and facilitates wet-milling.

Soaking reduces the toxic content and surface contamination during food processing. Some of the antiphysiological substances may be leached out into the soaking water (Uwaegbute and Nnanyelugo, 1989). The soaking of legumes such as soybean in water, aqueous citric acid or alkali such as sodium bicarbonate leaches out colouring matter and substances that impart beany flavour in soymilk and other soy products. Soaking lima bean for 2-6 days caused a significant loss of HCN (Nwokolo, 1996; Ologhobo *et al*. 1984). Soaking in water induces leaching out of water-soluble antinutritional factors such as glycosides, alkaloids, phytates, oligosaccharides and tannins (Sathe and Salunke, 1984). Soaking, cooking as well as other traditional methods caused significant reduction in trypsin inhibitor activity in lima beans (Ogun *et al*. 1989; Egbe and Akinyele, 1990).

Kee *et al.* (1998) revealed that prior to germination, seeds are soaked in water to imbibe sufficient water to act as a solvent, reactant and medium of transport for nutrients during germination process. Unsoaked seeds cannot be germinated. The total content of soluble sugar (reducing and non-reducing) and soluble minerals in legumes decrease during soaking. This is because of leaching losses. In maize, soluble components such as vitamins may be leached out during soaking and washing (FAO, 1993). Plant foods such as cereals, legumes, nuts, roots, tubers, vegetables and fruits are soaked to achieve different purposes

both in industry and home scale food preparation.

2.5.2 DEHULLING

Dehulling involves the removal of the seed coat. According to Enwere (1998), dehulling in cereal and legume processing means the removal of the testa. It is a very important processing operation in legumes because it reduces oligosaccharides such as stchyose, verbascose and raffinose. These are concentrated in the testa and are responsible for flatulence or gas formation in the intestine after consumption of legumes. Dehulling (decortication) assists reduction of fibre and colouring matter in legume which are concentrated in the testa. This results in the production of more refined products (Onoja, 1982). Dehulling improves the texture and colour of products (Osho, 1988). It enhances digestibility of protein (Walker *et al*; 1982). This may be due to removal of antinutritional factors and toxic substances which abound in seed coat.

Akinyele (1989) reported the effect of processing on certain antinutrients in cowpea. He concluded that the traditional methods of processing cowpeas in Nigeria, into ewa, gbegiri, akara and moi-moi, more than compensate for any nutrient losses in cowpeas associated with isolated treatments such as soaking, dehulling and cooking. These processed products ensure a significant reduction in the antinutritional factors and maximum retention of nutrients. Obizoba and Souzey (1989) observed that soaking and dehulling provide African yam bean flours with high quality nutrients. Osagie (1998) noted that abrasive dehulling removed saponins and reduced the total level of indigestible fibre in legumes.

2.5.3 HEAT TREATMENT (COOKING)

Cooking is heat treatment done at 100°C. Heat treatment may have either beneficial or detrimental effects. Heat treatment on foods must be carefully controlled to avoid or minimize damage to nutritive value, functionality and sensory properties which determine acceptability.

Among the major food processing treatments in current use, heat processing is the most encountered. It has a very important effect on various food components and quality. Heating inactivates the enzymes responsible for quality deterioration of foods.

Most proteins of plant origin are nutritionally improved by heat treatment. Antinutritional factors such as trypsin and chymotropsin inhibitors, haemagglutinin, saponins, oligosaccharides, goitrogens, toxic amino acids and others are either inactivated, destroyed, reduced or altered by proper heat treatment, especially when combined with other processes

such as soaking,germination, fermentation, and chemical addition (Leiner, 1985). The traditional methods of household cooking and industrial autoclaving were able to detoxify haemagglutinins using wet-heat. Egbe and Akinyele (1990) examined the effect of cooking on the antinutritional factors of lima bean (Phaseolus lunatus) and concluded that the traditional method of preparing lima bean meals which involves discarding and changing of the cooking water many times, may reduce the level of antinutritional factors, especially the HCN and increase the nutrient digestibility of this legume, at the same time promoting its utilization in the diet. Cooking lowered the zinc, iron and copper content of lima bean (Apata and Ologhobo, 1994).

Heating leads to the gelatinization of starch and the denaturation of protein and other interactions involving other ingredients in food (Enwere, 1998). These changes affect the texture, taste, digestibility and acceptability of the food products. The colour, flavour and aroma development in cooked root and tuber, vegetables, cereals and legume products contribute to their overall acceptability.

However, improper heat treatment can lead to poor quality products. Excessive heating or over cooking can cause a decrease in the palatability and nutritive value due to oxidative amino acids, alterations of natural linkages and charring of the food.

2.5.4 GERMINATION

Germination is a natural process in which dormant viable seeds are induced to start growing into seedlings. Alpha and beta-amylases convert starch into glucose and maltose which are utilized by the germinating seeds. Germination is known to increase vitamin C content of seeds. It increased protein quality of cereals. Obizoba and Egbuna (1992) reported increase in protein content of malted sorghum. Germination resulted in 50% reduction in the phytic acid and tannins levels of some cereals (Gibson *et al*; 1998).

The process of germination is utilized in food industry for various useful purposes such as the conversion of barley into malt used for the production of malt extract (Enwere, 1998). Onofiok and Nnanyelugo (1998) reported that during germination, the insoluble proteins were broken down to soluble components which led to the increase in the level of lysine as well as the B complex and C vitamins.

Germination reduced oligosaccharides content of cowpea after 72h. It increased simple sugars such as glucose, sucrose, galactose and fructose. Protein and energy content were slightly increased while calcium and iron were decreased (Akinyele and AkinsolOtu, 1991).

Ologhobo *et al.* (1984) reported that the longer the germination period the lower the HCN content of lima bean. Dibofori *et al.* (1994) reported increase in protein, ash and moisture content of germinated lima bean (18.8% to 22.9%) on a dry matter basis. This might be due to rapid synthesis of hydrolytic enzymes as well as loss of initial dry matter. They reported a 16% dry matter loss on the 5th day of germination.

There was decrease in HCN content of lima bean as germination period increased (39.1mg/kg – 7.82mg/kg – 4-day germination). This could be attributed to the presence of the hydrolytic enzyme, B-glucosidase which hydrolyzed cyanogenic glucoside linamarin to cyanohydrin and free cyanide which was lost through leaching or translocation to other developing parts such as the roots, shoots or leaves.

2.5.5 FERMENTATION

Fermentation is a processing technique popular in Nigeria as well as many other developing countries. It was identified as an economic processing method that could be used in the homes to improve the nutritional quality of plant foods (Hesseltine and Wang, 1980; Eka, 1980; Obizoba and Egbuna, 1992; Nnam and Obiakor, 2003).

Fermentation has been reported as a possible means of providing nutritionally adequate diets for infants and young children from cereals and legumes (Nout *et al*; 1989) Enwere (1998) observed that fermentation is a process of anaerobic or partially anaerobic oxidation of carbohydrates, especially sugars, by the action of enzymes added to the raw material or produced by microorganisms such as yeast, moulds and bacteria.

Fermentation process may be complete oxidation in which sugars are converted to carbon dioxide and water or it may be partial oxidation in which the sugars are converted to citric acid, acetic acid, lactic acid, butyric acid, alcohols, aldehydes hydrogen and some flavour compounds. Fermentation may impart new colour, flavour, taste, odour and texture to a product (Nnam, 1994). It improves taste of food and reduces contamination with pathogenic bacteria (Tomkins *et al*; 1987). FAO/WHO (1996) defined fermentation as the biochemical modification of primary food products brought about by the action of microorganisms and their enzymes. Fermentation is intentionally carried out to enhance the taste, aroma, shelf-life, texture, nutritive value and other properties of food (Obiakor, 2001).

Fermentation is the basis for the production of many indigenous Nigeria beverages from sugary and starchy substrates (Enwere, 1998). It is used in the production of modern wine from various fruit juices and palm wine from palm sap obtained from palm trees and raffia palm. The modern beer, whisky, brandy and gin are products of controlled

fermentation. Wang *et al.* (1978) acknowledged fermentation for centuries as a natural means of improving the nutritional quality of legume and cereal foods. Fermentation may be used to convert cereals, legumes, roots, tubers, fruits and vegetables to more desirable and storable products under controlled conditions. Where microorganisms capable of hydrolyzing higher carbohydrates such as cellulose, hemicellulose, pectins and starch are allowed to become active, the texture, flavour and acceptability of the product are adversely affected.

In Nigeria, flavouring agents produced through fermentation include "dadawa" produced from soyabean or locust bean seeds, "ogiri" produced from castor oil or melon seeds. These flavouring agents are used to create different flavours in soups, stews, pottage, and vegetables. Fermented indigenous Nigerian foods produced from cassava roots are "gari" "foo-foo" "lafun" and cassava flour. Products from maize, rice, sorghum and millet include pap ("akamu" or "ogi"), "appa" and "agidi".

Fermentation when well controlled leads to significant improvement in the nutritive value, flavour and texture of foods. This increased nutritive value results from increased digestibility of proteins through hydrolysis to amino acids. Carbohydrates such as raffinose, stachyose and verbascose are converted to simple sugars, thus reducing flatulence (Enwere, 1998). Fermentation increases the availability of minerals such as calcium and phosphorus through the hydrolysis of phytate and oxalate. The nutrients are increased through microbial synthesis, especially B-complex vitamins (Aworth, 1993).

Fermentation is known to reduce the antinutritional factors and to increase digestibility of legumes and cereals. Minerals are known to be more available after fermentation (Nnam, 1994). The protein content of maize is increased as a result of fermentation and reduced the level of lysine, methionine and isoleucine (Akobundu and Hoskin, 1987). Fermentation decreases the toxic components of some seeds. Toxicological evaluation of the fermented castor oil bean showed that the toxicity of beans possibly due to toxic proteins, decreased significantly in fermented products (Odunfa, 1985).

According to Reddy *et al*. (1985) fermentation decreased tannins in legumes and increases ionisable iron. Tannins may be important factor responsible for the low absorption of iron. Similarly, tannins and phytate content may be reduced in some cereals, leading to increased absorption of iron. Inedible foods are made edible by fermentation. For instance, some legumes such as African locust bean and oil bean are inedible in their unfermented state. However, during fermentation they are made edible by the extensive hydrolysis of the indigestible components and the removal of the antinutritional factors by the microorganisms (Odunfa, 1983). Without fermentation, the food use of over 200,000 tons of locust bean

produced annually in northern Nigeria (Cobley and Steel, 1976) would be lost.

FAO/WHO (1996) noted that fermentation has various effects on the nutritional quality of food. Microbial fermentation leads to a decrease in the levels of carbohydrates and indigestible oligosaccharides in legumes. A decrease in levels of oligosaccharides reduces abdominal distention and flatulence. Moreover, certain amino acids may be synthesized and the availability of B-complex group of vitamins may be increased by fermentation. Fermentation by lowering pH provides for more optimal conditions for enzymatic degradation of the phytate present in cereals in the form of complexes with protein or polyvalent cations such as iron, zinc, calcium, and magnesium being available. This degradation occurs during the soaking of cereal grains in water for 12-14h. Such a reduction in phytate may increase the quantity of soluble iron, zinc and calcium several folds (Nnam and Obiakor, 2003).

The art of fermentation developed for African foods have achieved: longer keeping quality, variety in flavour, making the foods edible and so on. Fermentation of foods enhanced nutritional values and decreased toxicity. Unfortunately, the art of production of these foods are largely crude and do not allow for increased production to meet increasing demand. In order to improve the fermentation, the processing equipment and the methods of optimizing the fermentation condition should be investigated (Odunfa, 1987). Vander Riet *et al*. (1987) observed increases in protein, calcium, magnesium, iron, sodium, zinc, manganese and phosphorus and decreases in ash, fat, carbohydrate and total fibre as fermentation period increased. There were increases in protein, fat, potassium, copper, iron, zinc, magnesium and phosphorus and decreases in carbohydrate, crude fibre, phytate and hydrocyanate levels in fermented locust bean (Eka, 1980).

Nnam (1999a) reported a significant increase in mineral levels of maize, cowpea and plantain flour blends due to fermentation. Achinewhu and Isichei (1990) found increase in ether extract and slight decrease in crude fibre and carbohydrate content of a six-day fermented fluted pumpkin at 29-32°C. There was no change in total ash and crude protein.

Ene-Obong (1983) observed increases in iron, magnesium, phosphorus, protein, zinc and calcium when African yam-bean was fermented for 12 and 24h when compared with the control and those from other food processing methods. Anosike and Egwuatu (1981) reported decreases in protein, carbohydrate and lipid of fermented castor oil seed. The decreases were attributed to the metabolic activity of microorganisms. Fermentation preserves foods and improves their palatability. It increases the acidity level of the food, thereby preventing pathogenic organisms from multiplying in the food, which keeps it safe and longer shelf-life

(Latham, 1997). Nout and Mortajemi (1997) reported the presence of Escherichia coli and other pathogenic microorganisms in fermented foods. They noted that fermented foods can be contaminated from other sources such as unhygienic practices of food handlers and contaminated water.

In many of the fermented foods, the vitamin B- complex levels, especially riboflavin are increased. There is also improved digestibility, protein efficiency ratio (PER) net protein utilization (NPU) and biological value (BV) when foods are fermented (Obizoba and Egbuna, 1992). The increased digestibility is in part due to the extensive hydrolysis of galacto oligosaccharides to simpler sugars. Minerals in the fermented products are more available. During lactic acid fermentation in "ogi", phosphorus is released from phytate. More calcium and phosphorus were released in "iru" from decreased phytate and oxalate during fermentation (Eka, 1980).

2.5.6 DRYING

The essence of sun-drying is to imitate the traditional technology. Ihekoronye and Ngoddy (1985) described sun-drying as the oldest method of food preservation in developing countries. Fruits and vegetables, cereals, legumes, fish and meat can be preserved when dried. Drying of food reduces moisture content and concentrates nutrients.

2.5.7 DRY-MILLING

This separates the endosperm, germ and the bran from each other. This recovers the maximum amount of endosperm. The products of dry-milling operations have low moisture content in contrast to wet milling techniques (Olatunji *et al*; 1982). Milling reduced phytic acid in cereals because it is localized at the outer aleurone layer. Ranhotra (1983) reported that excessive refinement of cereal grains through milling process to obtain fine flour caused substantial loss of nutrients. Milling processes do much to release nutrients from legumes and cereals by physically rupturing cellulosic and hemicellulosic structures surrounding the endosperm which is rich in digestible carbohydrates and proteins.

2.6 FUNCTIONAL PROPERTIES OF FOODS

Functional property concerns any non-nutritional property of food substances that affects its utilization. They are intrinsic physiochemical characteristics, which affects the behaviour of protein in food systems during processing, manufacturing, storage and preparation. The functional properties of foods are related to the raw food materials, that is the nature, composition and conformation temperature during cooking, drying, pH and the

physical and chemical modification taking place during processing. Onimawo and Akubor (2005) gave the reasons for measuring functionality which include:

i. to determine how the protein has been affected by the processing method employed.
ii. to screen protein and extrapolate functionality test results to performance in the finished product.
iii. to understand why specific proteins function as they do.
iv. to understand how proteins interact with each other and within mixtures or finished products.

2.6.1 WATER ABSORPTION CAPACITY

The interaction of proteins with water is important to properties such as hydration, swelling, solubility and gelation. Water absorption capacity is an index of the ability of protein to absorb and retain water that influences the sensory quality of foods. Water absorption property is a useful indication of whether protein could be incorporated with aqueous food formulations, especially those involving dough funding such as processed cheese, sausages and bread dough. Studies have shown that heat treatment increases the water absorption of seed flours. During heating, major proteins are dissociated into subunits that have more water binding sites than the native or oligomeric proteins. Swelling of crude fibre and gelatinization of carbohydrates take place during heating thereby increasing water absorption. It was observed that fermentation and germination increase water absorption of flours. This might be due to the proteolytic enzyme activity during these processes that cause increase in the number of polar groups, which increase hydrophilicity of the seed proteins.

Water absorption of some seed flours can be improved by the addition of 0.2M of NaCl. When NaCl is added, the chloride ions are bound to the protein, which increase the net charge of the molecules. This unfolds the protein network which in turn increased accessibility to water.

2.6.2 OIL ABSORPTION CAPACITY

The ability of proteins to bind fat is an important property in food formulations. This is because fat acts as flavour retainer and increases the mouthfeel of foods. Oil absorption is attributed mainly to the physical entrapment of oil. However, the mechanisms have not been explained by many workers. Onimawo and Akubor (2005) reported that more hydropholic proteins had superior binding of lipids, implying that non-polar amino acid side chain bind the paraffin chains of fat. Evidence have shown that oil absorption is affected by protein source, particle size, processing and temperature. Heat processing increases the oil absorption

of seed flours. However, this increase might be due to both dissociation of the proteins that occur on heating and denaturation which unmask the non-polar residues from the interior of the protein molecule (Roday, 2007).

2.6.3 FOAM CAPACITY AND FOAM STABILITY

Foam capacity and foam stability are used as indices of formability of protein dispersion. Protein foams are important in many processes in the beverage and food industries. Foam formation and foam stability are dependent on the type of protein, pH, processing methods, viscosity and surface tension. Foam ability is assumed to be dependent on the configuration of the protein molecules. Research showed that flexible protein molecules reduce surface tension to give good foam ability and highly ordered globular molecules are difficult to surface denaturation as such gives poor foamability. Foamability is related to the rate of decrease of surface tension of the air-water interface caused by absorption of protein molecules (Onimawo and Akubor, 2005).

Heat processing decreases the nitrogen solubility of proteins by denaturation and reduces their foaming capacity. This implies that foaming property is dependent on solubilized proteins. Foaming capacity can be improved with the addition of 0.2M NaCl and high concentration of NaCl decrease it. Foams are used to improve texture, consistency and appearance of foods.

2.6.4 PROTEIN SOLUBILITY

Protein solubility has a vital influence on other functional properties which determine the usefulness of the product in food systems. Onimawo and Akubor (2005) noted that among functional properties of proteins. Protein solubility often expressed as nitrogen solubility index (NSI) is the most important. Nitrogen solubility data provide an indication of the inherent solubility of a protein, its potential solubility in food systems and the degree of its denaturation. Protein solubility is dependent on origin, processing conditions, pH and ionic strengths of a given flour. Heat processing decreases nitrogen solubility of flours, possibly due to protein denaturation and subsequent aggregation. Studies have shown that nitrogen solubility increases at low salt concentration and decreases at high salt concentration. The addition of sodium chloride enhances the nitrogen solubility of some flours in the pH range of 4-6 where the flour protein is less soluble in water.

2.6.5 EMULSION ACTIVITY AND EMULSION STABILITY

The emulsion activity and emulsion stability are used to describe the emulsifying properties of proteins in food systems. The emulsion activity measures the maximum oil addition until phase separation occurs. Emulsion stability measures the tendency for the emulsion to remain unchanged (Onimawo and Akubor, 2005). The emulsion activity is related to the ability of the proteins to lower the tension at the interface of water and oil. The capacity of protein to aid the formation and stabilization of emulsion is important in cake batters, milk, mayonnaise and salad dressing. It is known that soluble proteins are surface active and promote oil-in-water emulsions. Increase in protein concentration, enhances emulsion stability. Evidence have shown that the pH of the medium indirectly affects emulsion activity of proteins by influencing protein solubility. However, heat processing decreases emulsion capacity of flours, which might be due to decreases in protein solubility during heating.

2.7 MICROORGANISMS IN FOODS

The food supply is not, nor will it be in the future, free from microorganisms. Microorganisms are ubiquitous in nature, everywhere that food is grown, processed, sold and prepared there are potential sources of microbial contamination. The types or groups of microorganisms most prevalent in a particular food vary depending on their growth, storage and processing environment. Generally, raw food commodities are contaminated with a diverse population of microflora. Sources of microorganisms in foods are numerous. Many raw agricultural products can be contaminated with soil during growth and harvest. Water and soil often contain similar microbial species because of their close relationship during rain and runoff (Randal, 1994). Contaminated drinking water may be a route for certain pathogens to infect live animals. Air is an important source of microbial contamination, especially in the processing environment. For example, a vast abundance of bacteria can be carried on dust particles and vapour droplets into food products. Air is also a transporter of high level of mold spores that can spoil foods. During processing of foods, microbial contamination can occur at numerous points.

According to Arntzen and Ritter (1994), microorganisms signifies a living entity that is smaller than 0.1mm in diameter and cannot be seen without magnification. They include bacteria, molds, yeasts, and protozoa which are the most familiar food borne organisms. Gaman and Sherrington (1990) grouped microorganisms into four, viz:

i. **Pathogens**

These are microorganisms which cause disease. All viruses are pathogenic but only some are pathogenic to man. Certain bacteria also cause disease in man. Some of these diseases can be transmitted by food, for example food poisoning.

ii. **Spoilage organisms**

These microorganisms do not cause disease but they spoil food by growing in the food and producing substances which alter the colour, texture and odour of the food, making it unfit for human consumption. Examples of food spoilage include the souring of milk, the growth of mold on bread and the rotting of fruits and vegetables.

iii. **Beneficial organisms**

Many microorganisms have a beneficial effect and can be used in the service of man. Microorganisms are used at various stages during the manufacture of certain foods. Their activities are essential in the production of foods such as bread, beer, wine, cheese and yoghurt. In the treatment of sewage, microorganisms are used to breakdown complex organic compounds. The complex organic components of dead plants and animals are broken down by microbial acivity into simpler, inorganic compounds which are made available for new plant growth and the whole cycle of life is able to continue.

iv. **Inert organisms**

These organisms are neither harmful nor beneficial to man.

2.7.1 FACTORS AFFECTING THE GROWTH OF MICROORGANISM

All microorganisms require certain environmental conditions for growth and multiplication. There are variations in the growth requirements of different species. Randall (1994) noted that microorganisms require nutrients to grow like all other living organisms and that there are vast differences in the requirements for specific nutrients for specific microorganisms. However, all microorganisms need water, energy, vitamin, minerals and nitrogen to multiply and maintain life. Apart from food nutrients, a lot of other factors influence microbial growth. These include:

a. **Time**

The rate of multiplication of microorganisms varies according to species and the condition of growth. For example, bacterial multiplication assumes a generation time of 20 minutes. In other words, under optimum conditions most bacteria reproduce by binary fission once every 20 minutes. This is why a single cell may produce several

million cells in seven hours, under a favourable condition (Gaman and Sherrington, 1990).

b. **Temperature**

Microorganisms can grow over a wide range of temperatures depending on the specific organisms. It is known that each microorganism has a maximum growth temperature, a minimum growth temperature and an optimum growth temperature. The optimum temperature is that temperature at which it grows best and multiplies most rapidly and closer to the maximum than the minimum temperature.

c. **Oxygen**

The amount of oxygen available affects the growth of microorganisms. This is because some are aerobic while others are anaerobic. For example, moulds are aerobic (require oxygen) and yeasts are either aerobic or anaerobic depending on the conditions.

d. **pH**

Most microorganisms grow best if the pH of the food is between 6.6 and 7.5. Bacteria, particularly pathogens are less acid-tolerant than moulds and yeasts and there are no bacteria which can grow if the pH is below 3.5. The spoilage of high acid foods such as fruits are usually caused by yeasts and mounds. Meat and seasoned foods are more susceptible to bacterial spoilage because the pH of these foods is nearer to 7.0.

2.7.2 CONTROL OF MICROBIAL GROWTH

In order to prevent food spoilage, the growth of microorganisms must be prevented. This can be achieved by removing one or more of the conditions necessary for growth or by providing conditions which interfere with microbial metabolism. Studies have shown that many methods of food preservation rely on more than one method of controlling microbial growth which include:

i. **Removal of moisture**

In order to prevent the growth of microorganisms in a food, the water activity of the food must be reduced to 0.6 or below. Dried foods have very little or no moisture content. Moisture can be removed by sundrying, mechanic dryers, and freeze drying.

ii. **Altering temperature**

Microbial growth may be prevented by either decreasing or increasing the temperature. The lowering of temperature by refrigeration or chilling and freezing can

inactivate or kill some microorganisms. It is possible to destroy most microorganisms by holding the food at a high temperature for a few hours or longer. The complete destruction of microorganisms by heat is known as sterilization. Cooking (heat treatment) is one of the easiest method of reducing microorganisms in foods (Obiakor,2006).

iii. **Exclusion of oxygen and altering the pH**

The exclusion of oxygen inactivates the aerobic microorganisms, because they cannot thrive in the absence of oxygen. Altering the pH also retards the growth of spoilage organisms.

2.8 SENSORY PROPERTIES OF FOOD

Sensory, organoleptic or psychometric analysis of food is a scientific study used to evoke, measure, analyze and interpret reactions to those characteristics of foods and materials, as they are perceived by the sense of sight, smell, taste, touch and hearing. Sensory analysis of food is usually based on hedonic scale, which is a scale upon which degree of liking and disliking is recorded. The hedonic scale indicates likes or dislikes for a specific characteristics of food such as colour, flavour and texture; it does not indicate change in intensity of those characteristics. The organoleptic qualities – flavour, colour and texture - are perhaps the most important characteristics of foods because they are the attributes the consumer can readily assess (Ihekoronye and Ngoddy, 1985).

2.8.1 COLOUR

The first impression of a food is usually visual and a major part of our willingness to accept a food depends on its appearance. Colour can be defined as a psychological interpretation of a physiological response by the eye and brain to the physical stimulus of light radiation at different wavelength (Ihekoronye and Ngoddy, 1985). Colour perception is a phenomenon unique to the individual. Appearance includes optical properties, physical form and mode of presentation. The appearance of the food evokes the initial response. Colour is an important quality of many foods. It is a quality attribute which together with flavour and texture plays a vital role in food acceptability. It is that aspect of visual experience resulting from stimulation of the retina of the eyes by certain intensities and wavelengths of light which initiates photo and electrical chemical activity in the eye optic nerves and cerebral cortex and is interpreted in terms of colour preceptors.

2.8.2 FLAVOUR

Flavour is a complex of sensation that we derive from food, particularly the sensations of taste and smell. Food flavour arises from a subtle interaction of taste and aroma which imparts a pleasing or displeasing sensory experience to a consumer. It is the flavour that ultimately determines the quality and acceptability of foods. No matter how safe, nutritious, inexpensive and colourful a food may be, if the flavour is undesirable, it is rejected. Flavours have insignificant nutritive value but they exert a great influence on food acceptance (Manay and Sharaswamy, 2008). Infact, even hungry and nutritionally deprived populations reject food that does not have the flavour of their choice. The flavour of a given food is determined by the mixture of food ingredients and other component which give foods characteristic aroma. Thus the flavour of a food is quite complex and had not been completely described for most foods. Adding to this complexity is the fact that the same food is often perceived differently by different individuals. This difference is due to cultural and biological differences among people.

2.8.3 TEXTURE

Texture refers to those qualities of food that we can feel either with the fingers, the tongue, the palate or the teeth. According to Roday (2007), texture refers to mechanical properties, of tenderness, hardness, cohesiveness, adhesiveness, fracturability, viscosity, gumminess, springiness and chewiness of food. The range of textures in foods is very great and a deviation from an expected texture is a quality defect. The texture of foods does not remain constant. Water changes play a major role in texture changes. Foods can also change texture on ageing. For example, texture of fresh fruit and vegetables becomes soggy as the cell walls break down and the cells lose water. Sugar and salt affect texture depending on their concentration. Processing also affects the texture of foods. This is because there are correlations between colour and texture in some instances, there are applications where colour may be used as an indication of acceptable texture (Potter and Hotchkiss, 1995).

2.8.4 GENERAL ACCEPTABILITY

This is often dependent on other attributes – colour, flavour and texture of a given food. The consumer's acceptance of food is referred to as acceptability.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1.0 MATERIALS

Two varieties of the identified lima beans (black and dark red) were selected for the study. About 3 kg each of the lima bean varieties as well as maize were purchased from a local market in Arondizuou, Imo State, Nigeria.

3.1.1a IDENTIFICATION OF DIFFERENT LIMA BEAN VARIETIES

Identification of different varieties of lima bean consumed in Arondizuogu was done by experts in Crop Science Department, University of Nigeria, Nsukka..

3.1.1b SELECTION OF SAMPLES

The selection of the lima bean variety for the study was based on the degree of availability. The black and the dark red varieties were selected because they are much more available than other varieties. It was based mainly on colour differentiation, since they are the same specie. The degree of availability for lima bean varieties was ascertained by the local farmers who are producers of lima bean and sellers in the local market.

3.1.2 METHODS

3.1.3 PROCESSING OF LIMA BEAN FLOURS

3.1.4 SOAKING

Two kilogrammes (2kg) of each variety of lima bean were cleaned by sorting out sand, broken seeds and other foreign materials. The cleaned seeds were divided into four equal portions. One portion that served as the control was washed, dried (oven drying), dehulled and milled into fine flour. The remaining three portions were soaked for 3, 6 and 9 hours, respectively in tap water in a ratio of 1:3 (w/v). The soaked seeds were dried, dehulled and milled into fine flour (70mm mesh screen) and stored at room temperature (28-30^{0}C) in polythene bags for analysis (Fig 1).

3.1.5 FERMENTATION

Two kilogrammes (2kg) of each variety of lima bean were cleaned and divided into four portions. One portion served as the control. It was washed, dried, dehulled and milled into fine flour. The flour was kept in an air tight container for analysis. The remaining three portions were subjected to different fermentation periods. The seeds were soaked separately in cold water in a ratio of 1:3 (w/v) and allowed to ferment by the microflora inherent in them for 24, 48 and 72h at 28 ± 2^0C (Fig 2). The fermented seeds were separately dried at 55^0C in a laboratory dryer, dehulled and hammer-milled into fine flours (70mm mesh screen) and stored in polythene bags for analysis (Fig. 2).

3.1.6 GERMINATION

Each lima bean variety was subjected to different germination periods. About 250g of each variety were soaked for 6h and spread on a wet jute bag. Muslin cloth and another wet jute bag were used to cover the seeds. The seeds were allowed to germinate at 28±2^0C for 72, 96 and 120h, tap water was sprinkled on the seeds every 6h until they germinated. At the end of germination, the germinated seeds were washed, dehulled, dried and milled together with the sprouts into fine flours. The ungerminated sample served as the control (Fig. 3).

3.1.7 DEHULLING AND COOKING

Two hundred grammes of each lima bean variety were washed, dried, dehulled and cooked with little quantity of water. The cooked beans were dried at 55^0C in an air oven and milled into fine flour (70mm mesh screen). The undehulled cooked sample served as control. It was washed and cooked with little quantity of water until soft enough for human consumption. The undehulled cooked beans were dried at 55^0C in an air oven, milled into fine flour and stored for analysis (Fig 4).

3.1.8 COOKING

About 200g of each lima bean variety were cleaned, washed, soaked for one hour and divided into two portions. Both portions were boiled until soft enough for human consumption. In one portion, the cooking water was discarded at 30 minutes intervals and the other portion was cooked with the initial cooking water. After cooking, the beans were dried separately at 55^0C in a laboratory dryer, milled and sieved into fine flour (70mm mesh screen) (Fig. 5). The uncooked sample served as the control.

3.2 PROCESSING OF FERMENTED MAIZE FLOUR

3.2.1 FERMENTATION

Four kilogrammes of maize grain were cleaned and divided into two equal portions. One portion which served as the control was washed, dried and milled into fine flour (70mm mesh screen). The remaining portion was subjected to 48h fermentation period. The maize grains were allowed to ferment by the microflora inherent in them at temperature of 28 ± 2^0C (Fig. 6). The fermented grains were separately dried at 55^0C in an air circulating oven, hammer milled into fine flour and stored in refrigerator until analyzed.

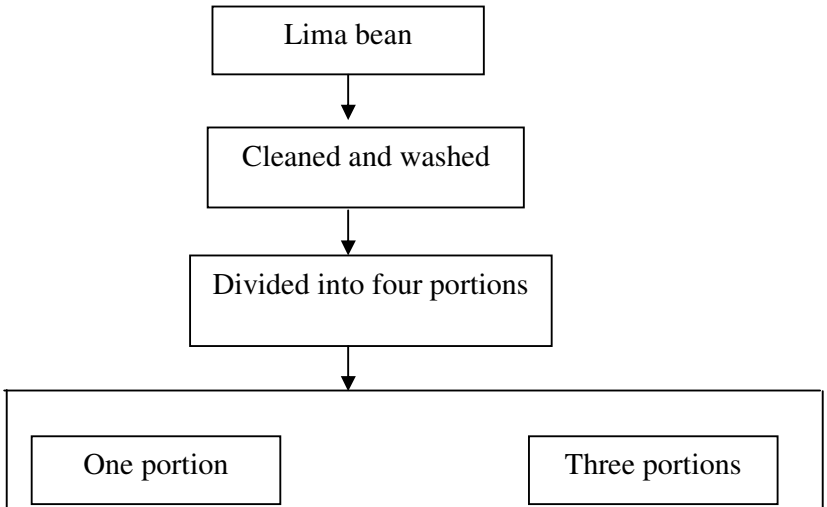

Fig. 1. Processing of soaked and unsoaked lima bean flours.

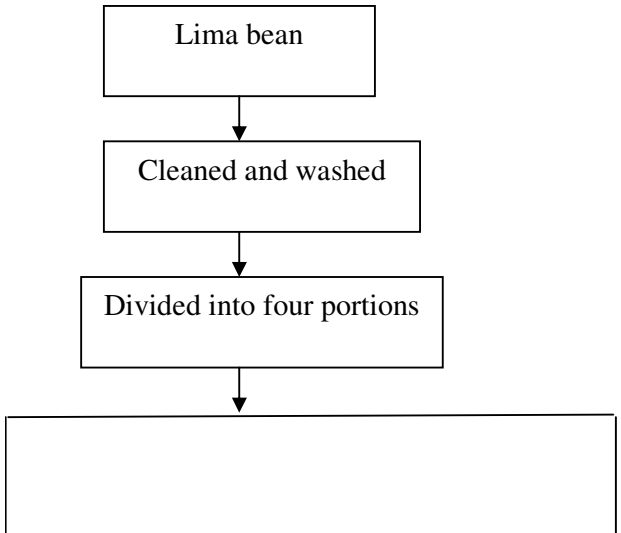

Fig. 2. Processing of fermented and unfermented lima bean flours.

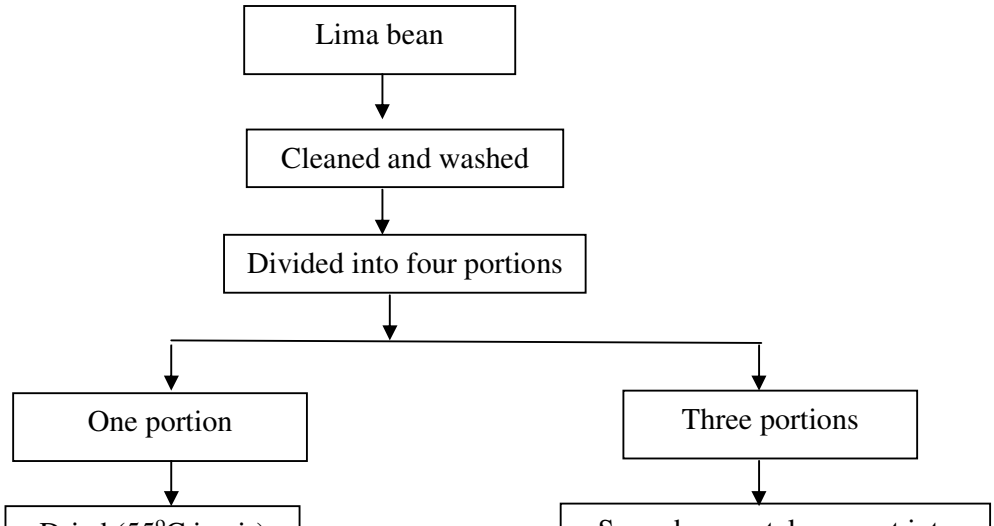

Fig. 3. Processing of germinated and ungerminated lima bean flours.

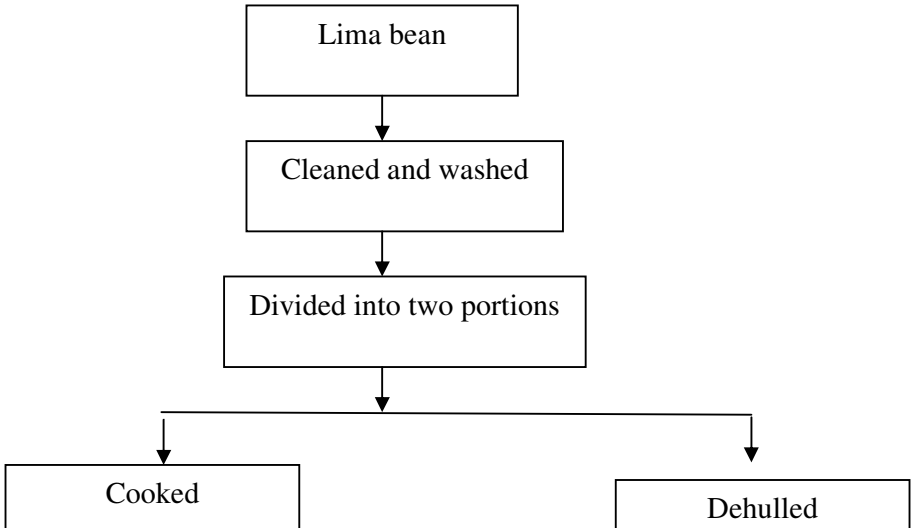

Fig. 4. Processing of undehulled and dehulled cooked lima bean flours.

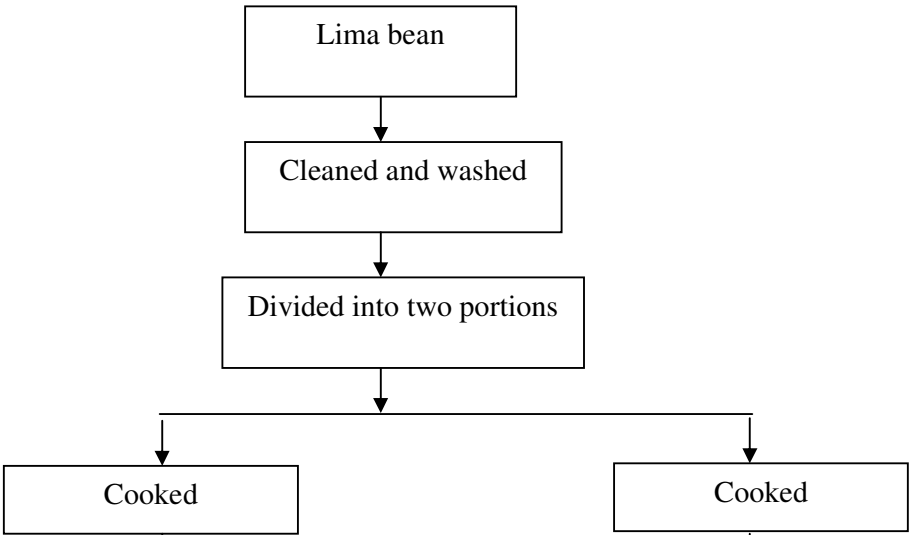

Fig. 5. Processing of cooked lima bean flour

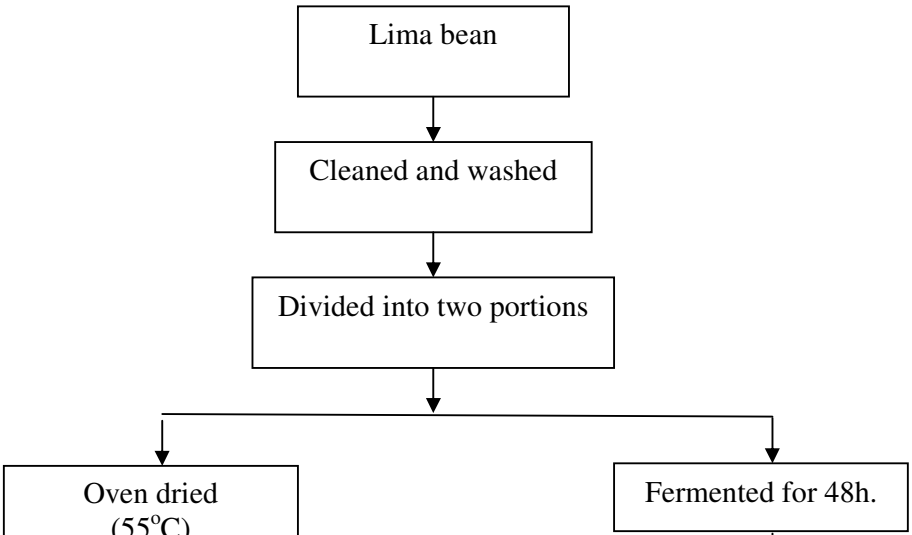

Fig. 6. Processing of fermented maize flour.

Plate 1. Different lima bean varieties.

a. Black variety
b. Muttled black variety
c. Dark brown variety
d. Deep cream variety
e. Dark red variety
f. Golden brown variety
g. Muttled brown variety

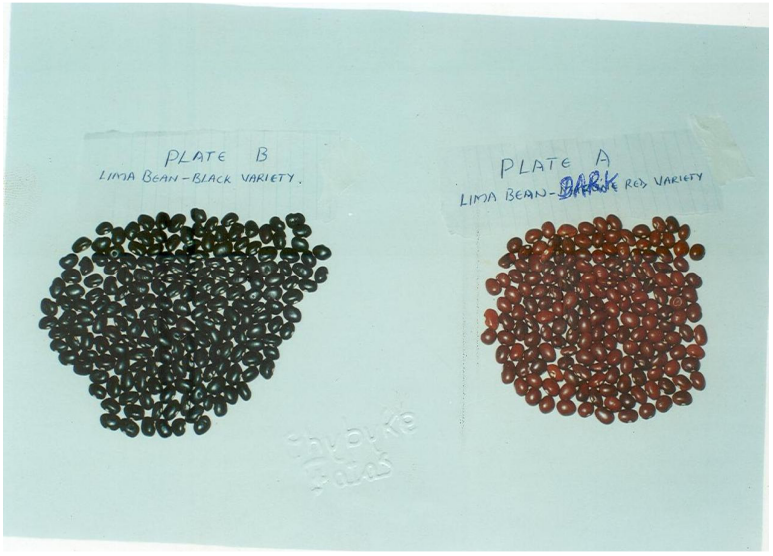

Plate 2. Two varieties of lima bean (Black and dark red) used for the study.

Plate 3: Moi-moi and akara prepared from fermented and unfermented dark red and black lima bean and maize flour.

1. Moi-moi from black lima bean and maize flour fermented for 48 hours.
2. Akara from unfermented dark red lima bean and maize flour
3. Akara from unfermented black lima bean and maize flour
4. Moi-moi from 72h fermented dark red lima bean and 48h fermented maize flour
5. Moi-moi from 72h fermented black lima bean and 48h fermented maize flour
6. Akara from 48h fermented dark red lima bean and maize flour..
7. Moi-moi from 48h fermented dark red lima bean and maize flour.
8. Akara from 48h fermented black lima bean and maize flour.

Plate 4: Moi-moi and akara prepared from fermented and unfermented dark red and black lima bean and maize flour.

GB black lima bean and maize flour fermented for 48h.

GD dark red lima bean and maize flour fermented for 48h.

i. Moi-moi made from 72h fermented black lima bean and 48h fermented maize flour.

ii. Moi-moi made from 48h fermented black lima bean and maize flour

iii. Akara made from unfermented dark lima bean and maize flour.

iv. Akara made from unfermented black lima bean and maize flour.

v. Moi-moi made from 48h fermented dark lima bean and maize flour

vi. Moi-moi made from 72h fermented dark red lima bean and 48h fermented maize flour.

vii Akara made from 48h fermented black lima bean and maize flour

viii Akara made from 48h fermented dark red lima bean and maize flour.

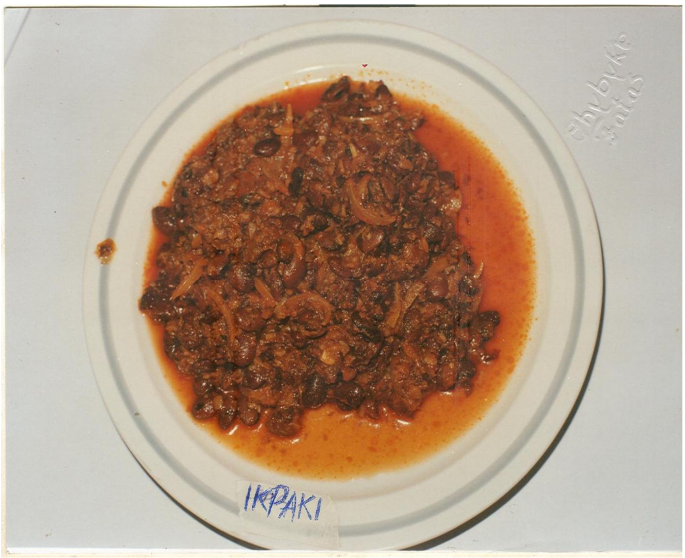

Plate 5: Black lima bean cooked without maize (*Ikpaki*)

Plate 6: Black lima bean cooked with maize (*Oshoto*)

Plate 7: Dark red lima bean cooked without maize (*Ikpaki*)

Plate 8: Dark red lima bean cooked with maize. (*Oshoto*)

3.3 CHEMICAL ANALYSIS

3.3.1 MOISTURE DETERMINATION

The moisture content of the flours was determined using the hot air oven method (AOAC, 1995). Porcelain dishes were washed and dried in a Gallen Kamp oven at 100^0C for one hour and allowed to cool in desiccators. The dishes were weighed. Two grammes of each flour were weighed into the porcelain dishes and placed in an oven and dried at 100^0C for 24h. The dried samples were removed and cooled in desiccators to prevent re-absorption of moisture. After cooling, the dishes were weighed and placed again in the oven, cooled and reweighed. This action was repeated until constant weight was attained. Percentage moisture was calculated as follows:

$$\text{Percentage moisture} = \frac{Moisture\ content}{Weight\ of\ sample} \times \frac{100}{1}$$

3.3.2 CRUDE PROTEIN AND TOTAL NITROGEN DETERMINATION

Crude protein was estimated using micro-Kjeldahl method (AOAC, 1995) Total nitrogen was estimated by extraction of the samples with 10% trichloroacetic acid (TCA) as described by Singh and Jambunathan (1981). The filtrates were analysed for non protein nitrogen (NPN) using the micro-Kjeldahl apparatus. Protein nitrogen was calculated by subtracting NPN from TN. True protein (TP) was calculated by multiplying TN by 6.25 (TN $\times$6.25) (Details in Appendix I)

3.3.3 FAT DETERMINATION

The fat content of the samples were determined by Soxlet extraction method (AOAC, 1995). The extraction flask was washed, dried, allowed to cool and weighed. Two grammes of each flour sample were weighed into filter paper and put into thimble. Petroleum ether was placed in the flask, the apparatus was set up. Extraction was carried out for 6 hours at 120°C. After which the solvent (petroleum ether) was recovered, leaving only the extract in the flask. The extract was dried in the oven for 15 minutes at 100°C to remove any remaining solvent, cooled in the desiccators and weighed (Details in Appendix I)

3.3.4 ASH DETERMINATION

The ash content was determined using AOAC (1995). About 2g of each sample were weighed into already weighed crucibles and heated in a muffle furnace at 600°C until light gray ash was obtained. The crucibles were removed from the furnace, put in desiccators to cool and then weighed to obtain weight of ash (Details in Appendix I)

3.3.5 CARBOHYDRATE DETERMINATION

Carbohydrate content was calculated by difference. The estimated percentages of crude, protein, fat, ash, fibre and moisture were summed up and the value subtracted from 100%.

100% - % of (protein + fat + ash + fibre + moisture).

3.3.6 CRUDE FIBRE DETERMINATION

The crude fibre content of the samples were determined according to the procedure of AOAC (1995) Two grams (2g) of the sample was put in a 250ml beaker, boiled for 30 minutes with 100ml 0.12M H_2SO_4 and filtered through a funnel. The filtrate was washed with boiling water until the washing waterwas no longer acidic. The solution was boiled for

another 30 minutes with 100ml of 0.02M sodium hydroxide solution, filtered with hot water and methylated spirit three times. The residue was transferred into a crucible and dried in the air oven for 1h. The crucible with its content was cooled in a dessicator and then weighed (W_2). This was taken to a furnace for ashing at 6000°C for 1h. The ashed sample was removed from the furnace after the temperature had cooled and put into the dessicator and latter weighed (W_3). The crude fibre content was obtained between the weight before and after incineration. The percentage crude fibre was calculated thus: (Details in Appendix I).

$$\text{Crude fibre} = \frac{W_2 - W_3}{W_1} x \frac{100}{1}$$

3.3.7 MINERAL DETERMINATION

Mineral contents of the flours were estimated by the method of Ranjiham and Gopal (1980). The samples were wet-digested with concentrated nitrate and perchlorate. Iron (Fe), Copper (Cu) and Zinc (Zn) were determined by polarized zeeman atomic absorption spectrophotometer, except phosphorus which was determined spectrophotometrically as yellow phosphor-vanado-molybdate complex (Details in Appendix II)

3.3.8 FOLATE DETERMINATION

The folate content of the samples were estimated using the method of AOAC (2000). About 200mg of the sample were dissolved in potassium phosphate (K_2PO_4), centrifuge at 10,000g for 15 minutes, the extract was read at 256nm.

3.4 ANTINUTRIENTS AND FOOD TOXICANTS ANALYSIS

3.4.1 TANNINS

This was determined by the spectrophotometric method as described by Price and Butler (1977). About 0.5g of each sample was extracted with 3ml methanol. The filtrate was mixed with 50ml water. Another 3ml of 0.1M $FeCl_2$ in 0.1 NHCL and 0.8M$k_2Fe(CN)_6$ were added to 0.1ml of the solution. The extracts were read at 720nm on a spectrophotometer (Details in Appendix III).

3.4.2 PHYTATE

This was determined by a photometric method adapted from the method of Latta and Eskin (1980). About 5g of each sample were extracted with 2.4 HCL, 0.1M NaOH and 0.7M NaCL were added and passed through a resin (200-400 mesh) to elude inorganic phosphorus and other interfering compounds. Modified Wade reagents 0.03% $FeCl_3$ $6H_20$ and 0.3%

saicyclic acid in distilled water) were added and readings were taken at 500nm (Details in Appendix III).

3.4.3 TRYPSIN INHIBITOR

Trypsin inhibitor was determined by the modified method of Hamerstrand *et al*. (1981). The values were estimated by single dilution of each sample extract that inhibit at least 40% but not more than 60% of the trypsin. The values were calculated from differential absorbance readings and reported in absolute units as milligramme of trypsin inhibitor per gramme sample (Details in Appendix III).

3.4.4 SAPONINS

The saponin content of the samples were estimated using the method of Bergmeyer (1974). This was determined by dissolving 0.19 of sample in 50ml distilled water, boiled for 15 minutes and filtered. The filtrate was read at 620nm (Details in Appendix III).

3.4.5 HAEMAGGLUTININ

The haemagglutinating activity was determined according to AOAC (2000). About 29mg of each sample were weighed into a test tube, added 10-20ml H_2O, shaken vigorously and filtered. The extract was stored in the refrigerator at 4^oC. In another four test tubes, 4ml of fresh blood of mice was added together with 4ml of normal saline solution, centrifuged at 7800g for 60 mins. The supernatant was decanted and kept for haemagglutinin activity estimation. About 0.1ml of the blood sample was pipetted into the sample test tube, added 4ml of saline solution and 2 drops of the extract and kept for 16h at a temperature of 4^oC. The turbidity formed was read at 600nm. The turbidity of the extract plus 4ml of saline solution were equally measured at the same wave length as the control.

3.4.6 CYANIDE

The cyanide content of the samples were determined enzymatically using the method of Cooke (1978) Five gram (5g) of sample was introduced into 300ml volumetric flask. 160ml of 0.1M phosphoric acid and homogenized for 15 minutes at low speed and made up to the mark. The solution was centrifuged at 10,000 rpm (revolutions per minute) for 30 minutes. The supernatant was transferred into a screw cap bottle and stored at 4^oC. 5ml aliquot of the extract was transferred into quick fit stoppered test tube containing 0.4ml of 0.2M phosphate buffer pH 7.0. 10ml of diluted linamarase enzyme was added. The tube was incubated at 30^oC for 15 minutes and the reaction was stopped by addition of 0.2M NaOH

(0.6ml). The absorbance of the solution was measured using suitance spectropho9tometer at 450nm against blank. Cyanide is calculated as: (Details in Appendix III).

$$\text{Cynade} = \frac{Ansprnamce \quad xdilutionfactor}{extinction\ coefficient} \; 100\ (mg/100g)$$

Extinction Coefficient ($\sum 1/450$) = 2550

3.4.7 FUNCTIONAL PROPERTIES

These functional properties were determined.

i. Water absorption capacity
ii. Oil absorption capacity
iii. Foam capacity and foam stability
iv. Emulsion activity and emulsion stability
v. Protein solubility.

3.4.8 DETERMINATION OF OIL ABSORPTION CAPACITY

The oil absorption capacity of the flour samples was determined using the method described by Odo and Ishiwu (1999). One gramme of the flour sample was mixed thoroughly with 10ml refined vegetable oil (olive oil) in a graduated centrifuge tube and allowed to stand at ambient temperature for 30 minutes, then centrifuged for 30 minutes at 2000rpm. The volume of oil was read directly from the centrifuge tube and converted to weights (in grammes) by multiplying the volume by the density of the oil (0.902g/ml). Oil absorption capacity was expressed as gramme oil absorbed per grammme flour.

3.4.9 DETERMINATION OF WATER ABSORPTION CAPACITY

The water absorption capacity of the flour samples was determined using the method described by Odo and Ishiwu (1999). One gramme of the flour sample was mixed thoroughly with 10ml distilled water in a graduated centrifuge tube and allowed to stand at ambient temperature for 30 minutes, then centrifuged for another 30 minutes at 2000rpm. The volume of water was read directly from the centrifuge tube and converted to weights (in grammmes) by multiplying the volume by the density of water (1g/1ml). Water absorption capacity was expressed as percent water absorbed per gramme flour.

$$\%\ \text{Water absorption} = \frac{water\ absorbed\ (1ml)}{weight\ of\ sample\ (1g)}\ x\ 100$$

3.4.10 DETERMINATION OF FOAM CAPACITY AND STABILITY

The foam capacity and stability was determined using the method of Onimawo and Akubor (2005). Two grammes of flour sample were added to 50ml distilled water at 30±2°C in a 100ml graduated cylinder. The suspension was mixed and shaken for 5 minutes to foam. The volume of foam at 30 seconds after whipping was expressed as the foam capacity using the formula:

$$\text{Foam capacity:} \quad Vi = \frac{Va - Vb}{Vb} x\,100$$

Where Vi = Volume increase (%)

Va = Volume after whipping (ml)

Vb = Volume before whipping (ml)

The volume of foam was recorded one hour after whipping to determine foam stability as percent of the initial foam volume.

3.4.11 DETERMINATION OF EMULSION CAPACITY

The emulsion capacity of the flours were estimated with the method described by Odo and Ishiwu (1999). The emulsion (1g flour, 10ml distilled water and 10ml refined vegetable oil) was prepared in a calibrated tube. The emulsion was centrifuged at 2000rpm for 5 minutes. The ratio of the height of the emulsion layer to the total height of the mixture was calculated as the emulsion activity expressed in percentage. The emulsion stability was estimated after heating the emulsion contained in a calibrated centrifuge tube at 80°C for 30 minutes in a water bath, cooling for 15 minutes under running tap water and centrifuging at 2000rpm for another 15 minutes. The emulsion stability, expressed as a percentage was calculated as the ratio of the height of the emulsified layer to the total height of the mixture.

$$\text{Emulsion capacity} = \frac{\textit{Height of the emulsified layer}}{\textit{Height of the whole mixture}}$$

3.4.12 DETERMINATION OF NITROGEN SOLUBILITY (PROTEIN SOLUBILITY)

Protein solubility which is the percentage of the total protein that is retained in the supernatant after centrifugation of a protein dispersion at a specified speed and period of time, was determined, using the method described by Onimawo and Akubor (2005). One gram of flour sample was dispersed in 60ml distilled water and the suspension was homogenized for 2 hours at 30±2°C. The suspension (with or without pH adjustment) was then centrifuged at 200rpm for 20 minutes. The nitrogen in the supernatant (5ml) was

determined by Micro-kjeldahl method (A.O.A.C., 1995). The nitrogen extracted was expressed as the percentage nitrogen of the flour

3.5 MICROBIAL ANALYSIS

Viable count method was used to determine the total microbial load. Plate count of microflora of duplicate samples was made after serial dilution in phosphate buffered saline as described by Cruickshank *et al*. (1975). A 0.01ml of sample was spread on a nutrient agar and MacConkey agar plates with sterile bend glass rod and incubated for 24h at 37°C. Dextrose Sabourad agar plates were inoculated with 0.01ml sample and incubated at room temperature (28±2°C) for seven days for microfloral differentiation. Characterization, identification and isolation were carried out as outlined in Bergys Manual of Determinative Bacteriology (Holt *et al*, 1993). (Details in Appendix IV)

3.6 ORGANOLEPTIC STUDY

3.6.1 FORMULATION OF COMPOSITE FLOUR

The processed flours from both varieties of lima bean that have high protein content and least antinutrients values, especially HCN was used together with 48hr fermented maize to formulate composite in a ratio of 70:30 (protein basis).

3.6.2 PREPARATION OF PRODUCTS AND DISHES

The products developed from lima bean and maize flour blends were moi-moi and akara. Two dishes (*Oshoto* and *Ikpaki)* were also prepared from whole lima bean and maize grains.

3.6.3 MOI-MOI

The fermented composite flours were used to prepare moi-moi. Two hundred and fifty grammes (250g) of flour were mixed with warm meat broth in a bowl. Food ingredients (onions, crayfish, tartarshi, maggi, vegetable oil and salt) were blended into the mixture and stirred thoroughly. The mixtures were dispensed into wrapping foil and steamed for 60 minutes. The moi-moi were kept in a food flask for evaluation.

3.6.4 AKARA

Akara balls were prepared from the fermented composite flour. One hundred and fifty grammes of flour were mixed with 50ml of water in a bowl and allowed to absorb water. The paste was whipped with mortar and pestle until fluffy. Salt was added to taste. The paste were moulded into small balls and fried in a hot oil (vegetable oil) at a temperature of 60°C until

golden brown in colour. The akara balls were removed from the oil and put in a colander to drain the excess oil and stored in a food flask for evaluation.

3.6.5 DISHES

3.6.6 *OSHOTO* (SUCCOTASH)

Two hundred grammes (200g) of each lima bean variety were separately cleaned and soaked for 30 minutes. The soaked beans were placed in separate pots, added cold water and boiled at a temperature of 100°C. The cooking water was drained and replaced at intervals, upto four times during cooking, until the bean is soft for human consumption. Equal amount (200g) of fresh or dried maize were also cleaned and cooked until soft for consumption. The cooked maize was drained and added to the already cooked lima bean separately. Food ingredients (palmoil, African oil bean (ugba), maggi cube, salt, crayfish, fresh sliced okro, dried fish, and onions) were blended to the mixture. Little quantity of green vegetable (spinach or fluted pumpkin leaves) were also added and stirred thoroughly and simmer for 10 minutes and the food was ready to serve (Appendix V).

3.6.7 *IKPAKI*

About 300g of each lima bean variety were cleaned, soaked for 30 minutes and put in a clean pot with tap water. The beans were cooked at boiling point 100°C until soft for consumption. During cooking, the cooking water was discarded and replaced upto four times at intervals. When the beans were properly cooked, water was drained, food ingredients (palmoil, salt, crayfish, maggi, onions and fresh tomatoes, fresh or dried fish) were added and mixed thoroughly, simmer for about 10 minutes, and served. The dish *Ikpaki* is prepared without maize.(recipe for products and dishes are shown in Appendix V).

3.7 SENSORY EVALUATION

The organoleptic attributes (colour, texture, flavour and general acceptability) of the products were evaluated using a nine-point hedonic scale. Thirty subjects were selected on the basis that they had participated in sensory evaluation of food products. Each was given a glass of water to rinse their mouth before tasting each product. They assessed the product for texture, flavour, colour and general acceptability.

3.8 STATISTICAL ANALYSIS

The data collcted was analyzed using means, standard deviation and standard error of the means. One way analysis of variance (Anova) and Duncan's New Multiple Range Test (Steel and Torrie, 1960) were used to separate and compare differences between means.

CHAPTER FOUR

RESULTS

Table 1: Different lima bean varieties consumed in Arondizuogu, Ideato North Local Government Area, Imo State, Nigeria.

	Lima bean variety (*Phaseolus lunatus*)	Degree of availability
a	Black variety	Commonly available
b	Muttle black variety	Rare
c	Dark brown variety	Available
d	Deep cream variety	Rare
e	Dark red variety	Commonly available
f	Golden brown variety	Available
g	Muttle brown variety	Rare

Table 1 presents the seven lima bean varieties consumed in Arondizuogu, Ideato North Local Government Area, Imo State, Nigeria.

Black and dark red varieties

Black and dark red varieties are commonly available because they have high yield; so they are cultivated by many farmers in the community..

Muttle black, deep cream and muttle brown

The muttle black, deep cream and muttle brown are very rare. Many farmers do not cultivate these varieties of lima bean. Therefore they are usually very rare.

Golden brown and dark brown varieties

The golden brown and dark brown varieties are available but not as the black and dark red varieties.

Table 2 presents the proximate composition of unprocessed and processed black lima bean flour.

Moisture

The moisture content of raw, undehulled cooked and dehulled cooked black lima bean varied. The values ranged from 9.33 to 12.33%. The raw black lima bean had the highest moisture (12.33%). On the other hand, the undehulled cooked sample had the same moisture as that of the dehulled cooked sample (9.33 vs 9.33%). Dehulling and cooking reduced moisture, when their values were compared with the control (9.33 vs 12.33%). The reduction

was 3.00%. The sample ($BCWD_4$) whose cooking water was drained four times and replaced during cooking had higher moisture than the sample ($BCWND_o$) whose cooking water was not drained and replaced.

The graded soaking periods had varied effect on moisture content of the flour samples. Soaking for 6h reduced moisture from 11.17 to 8.33%, while soaking for 9h increased moisture to 11.33 as compared with those of 3 and 6h soaking (11.33, 11.17 and 8.33%).

Germination had varied effect on moisture content of the samples. Germination for 72 and 96h gave comparable moisture values (9.33%). However, germination for 120h increased moisture by 1% when compared with those of 72 and 96h (10.33 vs 9.33%).

Fermentation for 24 to 72h (BVF_{24} – BVF_{72}) had varied effects on moisture. The samples fermented for 24h had least moisture against those of 48 and 72h (12.17 vs 23.33 and 13.33%, respectively). However, the 48h sample had the highest moisture (23.33%) which was different from others ($P < 0.05$).

Protein

The raw lima bean had high protein 25.55%. The undehulled cooked sample had 14.05% and that of dehulled cooked had 7.18%. Dehulling and cooking had no advantage over the raw and undehulled cooked lima bean, (7.18 vs 25.55 and 14.05, respectively). The sample whose cooking water was drained four times and replaced during cooking had higher protein 8.48% when compared with the sample cooked without draining (8.48 vs 7.07%). Soaking had varied effects on protein content of the samples. The 3 and 9h soaked samples had comparable values (26.70 and 26.68%). The sample soaked for 6h had lower protein (25.13%) as compared with those of 3 and 9h (25.13 vs 26.70 and 26.68%).

Germination had effect on protein content of the samples. As the germination period increased, the values increased 24.03 and 25.34% for 96 and 120h periods. The 72h sample had 23.21% protein, which was lower than those of 96 and 120h (23.21 vs 23.03 and 25.34%, respectively).

Fermentation from 24 to 72h had varied effect on protein content of the samples. The 24h sample had lower protein (18.90%) when compared with those of 48 and 72h samples (32.67 and 20.87%). Fermentation of black lima bean for 48h had an advantage over that of 72h (32.67 vs 20.87%).

Table 2: Effect of processing on proximate composition of black lima bean flours (%).

Samples	Moisture	Protein	Fat	Ash	Fibre	Carbohydrate

RBV	12.33±0.26	25.55±0.23	1.60±0.20	6.12±0.17	4.98±0.22	49.42±0.34
BVUDC	9.33±0.29	7.18±0.06	0.51±0.17	5.00±0.29	4.08±0.23	66.39±0.71
BVDC	9.33±0.29	14.05±0.9	0.70[d]±0.17	1.76±0.11	2.83±0.29	79.39±0.18
$BCWD_4$	11.17±0.27	8.48±0.01	0.53±0.07	3.00±0.16	2.96±0.13	73.86±0.22
$BCWND_O$	10.33±0.25	7.07±1.52	0.41±0.07	4.50±0.07	2.90±0.23	74.79±1.55
BVS_3	11.17±0.27	26.70±0.11	0.56±0.11	2.81±0.12	4.43±0.14	54.33±0.45
BVS_6	8.33±0.24	25.13±0.11	0.51±0.06	4.87±0.29	4.15±0.01	57.01±0.37
BVS_9	11.33±0.27	26.68±0.10	1.20±0.13	4.10±0.18	4.51±0.21	62.18±0.17
BVG_{72}	9.33±0.29	23.21±0.05	0.70±0.06	3.86±0.10	3.93±0.06	59.97±0.14
BVG_{96}	9.33±0.29	24.03±0.06	0.88±0.11	4.44±0.07	3.35±0.07	57.77±0.24
BVG_{120}	10.33±0.25	25.34±0.08	1.15±0.07	5.61±0.28	4.09±0.35	53.48±0.11
BVF_{24}	12.17±0.26	18.90±0.17	0.53±0.07	3.98±0.11	3.64±0.23	60.78±0.30
BVF_{48}	23.33±0.18	32.67±2.07	1.35±0.07	5.35±0.11	4.35±0.14	32.95±2.29
BVF_{72}	13.33±0.19	20.87±0.21	0.35±0.12	5.81±0.18	3.19±0.69	56.45±0.93

Mean±SD of three replications.

RBV - Raw lima bean black variety
BVUDC - Black variety undehulled cooked
BVDC - Black variety dehulled cooked
$BCWD_4$ - Black variety cooking water discarded four times
$BCWND_o$ - Black variety cooking water not discarded
BVS_3 - Black variety soaked for 3 hours
BVS_6 - Black variety soaked for 6 hours
BVS_9 - Black variety soaked for 9 hours
BVG_{72} - Black variety germinated for 72 hours
BVG_{96} - Black variety germinated for 96 hours
BVG_{120} - Black variety germinated for 120 hours
BVF_{24} - Black variety fermented for 24 hours
BVF_{48} - Black variety fermented for 48 hours
BVF_{72} - Black variety fermented for 72 hours

Fat

The raw lima bean (RBV) had high fat 1.60%. The fat values for the samples undehulled cooked (BVUDC) and dehulled cooked (BVDC) varied (0.70 and 0.51%,

respectively). Dehulling and cooking caused the highest decrease in fat (0.51%). The sample whose water was not drained and replaced during cooking had lower fat when compared with the sample whose cooking water was drained and replaced during cooking (0.41 vs 0.53%). Soaking had varied effects on the fat content of the samples. The 3 and 6 h soaked samples had comparable values (0.56 vs 0.51%). The sample soaked for 9h had higher fat (1.20%) as compared with those of 3 and 6h soaking. The fat content of the germinated samples varied. It ranged from 0.70 – 1.15%. The values increased as the germination period increased, with the peak at 120h (1.15%).

Fermentation had varied effects on the fat content of the samples. The 72h fermented sample had the least fat value as against those of 24 and 48h. However, the 48h sample had highest fat (1.35%) which was different from others ($P < 0.05$).

Fibre

The fibre content of the samples varied. The raw lima bean (RVB) had the highest fibre (4.98%). The undehulled cooked sample had higher fibre than dehulled cooked sample (4.08 vs 2.83%). Dehulling and cooking drastically reduced the fibre as compared with the control as well as the undehulled and cooked samples (4.98 and 4.08 vs 2.83%). The samples cooked without draining and replacing the cooking water and the one cooked with cooking water drained and replaced at intervals during cooking had comparable fibre (2.90 and 2.96%).

There was slight variation in fibre values for the soaked samples. The values ranged from (4.15 – 4.51%). The sample soaked for 9h had the highest fibre followed by that soaked for 3h. The 6h soaked sample had the least value (4.15%).

Germination had varied effects on the fibre content of the samples. The 120h germinated sample had the highest fibre (4.09%) as compared to the 72 and the 96h that had 3.93 and 3.35%, respectively. The 96h germination caused the highest decrease in fibre (3.35%).

The fermentation periods had varied effects on the fibre content of the samples. The values ranged from (3.19 – 4.35%). Fermentation beyond 48h decreased fibre most (3.19%) as compared with other periods. However, the 48h sample had the highest value (4.35%).

Ash

Ash value was influenced by treatment. The values ranged from 1.76 to 6.12%. Cooking and dehulling decreased ash from 6.12% control to 1.76%. On the other hand, the undehulled cooked sample had comparable value with the control (5.00 vs 6.12%). The

sample whose cooking water was drained four times and replaced during cooking had lower ash than its counterpart (BCWD4 and BCWNDo) (3.00 and 4.50%, respectively). Soaking had varied effects on ash content of black lima bean. The values ranged from 2.81 to 4.87%. The longer the soaking period, the higher the ash value. The 6h soaked sample had higher value (4.87%) than those of the 3 and 9h samples (2.81 vs 4.10%). Germination increased ash. The range was from 3.86 to 5.61%. The highest increase occurred at the 120h (5.61%) followed by the 96h sample (4.44%).The 72h sample had the least value (3.86%).

There appeared to be a trend towards increase in ash as both germination and fermentation periods increased (Table 2). The 24h fermented sample had the least ash (3.98%) as compared with those of the 48 and the 72h (3.98 vs 5.35 and 5.81%).

Carbohydrate

Cooking and dehulling influenced CHO values. The raw black lima bean had 49.42% CHO. The undehulled cooked sample had 66.39%. On the other hand, dehulling and cooking caused highest increase (79.39%) as against (49.42 and 66.39%) for the raw and the undehulled cooked samples, respectively. Whether black lima bean was drained four times and cooking water replaced during cooking or not, the CHO values were comparable (73.86 and 74.79%). Soaking hour was a function of CHO content of black lima bean. The CHO values for 3, 6, and 9h soaking were 54.33, 57.01 and 62.18%, respectively. Soaking for 9h had highest CHO value as against those of the 3 and the 6h (62.18 vs 54.33 and 57.01%).

Germination periods adversely affected CHO content of black lima bean flour. The value ranged from 53.48 – 59.97%. The 120h sample had the least CHO (53.48%) followed by the 96h sample (57.77%). However, the 72h sample had the highest CHO (59.97%). Fermentation had the same effect on CHO content of black lima bean as germination (Table 2). The values decreased as fermentation period increased. The highest decrease occurred during the 48h. Beyond 48h fermentation, there was an increase from 32.95 to 56.45%. However, the increase was lower than that of the 24h (56.45 vs 60.78%) and higher than that of the control (56.45 vs 49.42%).

Table 3 presents micronutrient composition of processed and unprocessed black lima bean flour.

Zinc (Zn)

Both cooking and dehulling were functions of Zn content of black lima bean. Dehulled cooked lima bean had higher Zn than the raw and the undehulled cooked samples (4.23 vs 4.18 and 3.13mg). The undehulled cooked sample had the least 3.13mg. This low

value indicated that undehulling had adverse effect on Zn content of black lima bean as compared with the raw and the dehulled cooked samples (3.13 vs 4.18 and 4.23mg, respectively). However, the raw and the dehulled cooked samples had comparable values (4.18 and 4.23mg, respectively).

Draining cooking water four times during cooking of black lima bean had an advantage over its counterpart (4.08 vs 3.16mg). The periods of soaking increased Zn in black lima bean. The values for 3, 6 and 9h soaking were as follows 2.90, 3.16, 3.76mg, respectively. The 9h soaking had the highest value 3.76mg. The 6h soaked sample had higher value 3.16mg than that of the 3h sample (2.90mg). However, the differences among these values were similar ($P > 0.05$).

Germination decreased Zn as the germination periods increased when compared with the raw sample. The values ranged from 3.23 to 4.18mg.The 120h germination had the highest Zn (3.63mg), followed by those of the 96 and the 72h (3.51 and 3.23mg, respectively).

Fermentation increased Zn in 24h sample when compared with the control (4.24 vs 4.18mg). The 48 and the 72h fermented samples had comparable Zn (4.18 and 4.10mg, respectively). However, fermentation for the 48 and the 72h caused decreases in Zn when compared with the 24h sample.

Copper (Cu)

Both dehulling and cooking caused decreases in Cu when compared with the control (RBV).The values ranged from 13.17 to 13.73mg.The raw sample had the highest (13.73mg) followed by the undehulled cooked sample (13.30mg). Dehulling and cooking caused much more decrease in Cu as compared with the other samples (13.17 vs 13.30 and 13.73mg, respectively) (Table 3). Draining cooking water in black lima bean caused slight increase in Cu as compared with its counterpart. BCWNDo (12.50 vs 12.33mg). Soaking for 3, 6 and 9h had varied effects on Zn content of black lima bean. The 6h soaked sample had the least value (11.63mg). The 9h sample had comparable Cu with the control (13.73mg).

Table 3: Effect of processing on micronutrient composition of black bean flours (mg/100g).

Sample	Zinc	Copper	Iron	Phosphorus	Folate
RBV	4.18±0.02	13.73±0.21	87.33±1.53	83.00±3.00	7.23±0.15
BVUDC	3.13±0.06	13.30±0.10	49.00±1.00	48.67±3.06	5.77±0.06
BVDC	4.23±0.03	13.17±0.15	23.67±1.15	16.00±2.00	7.37±0.06
$BCWD_4$	4.08±0.02	12.50±0.10	14.33±1.15	18.00±2.00	7.27±0.06
$BCWND_O$	3.16±0.02	12.33±0.21	33.33±0.60	14.00±1.00	5.77±0.11
BVS_3	2.90±0.02	13.23±0.06	47.00±1.00	43.33±1.53	5.47±0.12
BVS_6	3.16±0.10	11.63±0.06	45.00±1.00	46.00±1.00	5.57±0.42

BVS_9	3.76±0.06	13.73±0.12	41.00±1.00	49.67±2.08	5.57±0.23
BVG_{72}	3.23±0.01	12.30±0.44	43.33±1,53	42.00±2.00	5.50±0.10
BVG_{96}	3.51±0.02	12.70±0.10	44.33±1.53	47.00±1.00	5.50±0.44
BVG_{120}	3.63±1.15	13.20±0.20	44.00±2.00	49.33±1.53	5.40±0.10
BVF_{24}	4.24±0.07	13.33±0.12	45.00±1.00	48.00±2.00	5.63±0.23
BVF_{48}	4.08±0.08	13.57±0.06	41.00±1.00	44.00±2.00	5.60±0.00
BVF_{72}	4.10±0.06	13.20±0.10	21.67±1.53	42.67±1.53	4.37±0.21

Mean±SD of three replications.

RBV -Raw lima bean black variety
BVUDC -Black variety undehulled cooked
BVDC -Black variety dehulled cooked
$BCWD_4$ -Black variety cooking water discarded four times
$BCWND_o$ -Black variety cooking water not discarded
BVS_3 -Black variety soaked for 3 hours
BVS_6 -Black variety soaked for 6 hours
BVS_9 -Black variety soaked for 9 hours
BVG_{72} -Black variety germinated for 72 hours
BVG_{96} Black variety germinated for 96 hours
BVG_{120} -Black variety germinated for 120 hours
BVF_{24} -Black variety fermented for 24 hours
BVF_{48} -Black variety fermented for 48 hours
BVF_{72} -Black variety fermented for 72 hours

Germination had varied effects on Cu. As germination period increased from 0 – 120h, Cu decreased from 13.73 to 12.30mg. However, the 120h sample had higher Cu than those of the 72 and the 96h (13.20 vs 12.30 and 12.70mg, respectively).

Fermentation had varied effects on Cu, the values ranged from 13.20 – 13.57mg. There were differences in Cu, among the three periods. However, the differences were not significant ($P > 0.05$).

Iron (Fe)

Iron values differed. The range was from 23.67 – 87.33mg. The control had highest Fe (87.33mg), followed by the undehulled cooked sample (49.00mg). The dehulled cooked sample had the least 23.67mg. Dehulling and cooking had adverse effect on Fe content of black lima bean. Draining cooking water four times and replacing it in black lima bean caused decrease in Fe than in the undrained sample (14.33 vs 33.33mg).The drained samples had 19mg Cu lower than the undrained sample. Soaking had adverse effects on Fe content of black lima bean when compared with the control (87.33 vs 47.00, 45.00 and 41.00mg). The 9h soaking caused the greatest reduction in iron when compared with the control.

Germination as well as fermentation had adverse effects on Fe levels in black lima bean. The 72h germination and the 72h fermentation caused highest reduction in Cu values when compared with the control. (43.33 and 21.67 vs 87.33mg).

Phosphorus (P)

Dehulling and cooking caused tremendous reduction in phosphorus as against the control as well as the undehulled cooked sample (16.00 vs 48.67 and 83.00mg).The raw black lima bean had much more phosphorus than those of the dehulled cooked and the undehulled cooked samples (83.00 vs 16.00 and 48.67mg, respectively). Draining cooking water four times in black lima bean and replacing it increased phosphorus than not draining cooking water (18.00 vs 14.00mg).

Soaking caused losses in phosphorus as compared with the unsoaked sample (control) (83.00 vs 43.33, 46.00 and 49.67mg). The greatest decrease occurred at the 3h soaking 43.33mg. followed by that of the 6h (46.00mg).

Germination and fermentation had similar effects on phosphorus content of black lima bean when compared with the control. The values ranged from 42.00 – 83.00mg. The raw sample (RVB) had the highest phosphorus (83.00mg) and the 72h germinated sample had the least (42.00mg). The 120h germinated and the 24h fermented samples had higher phosphorus than any other treatments (49.33 and 48.33 vs 42.00 – 47.00mg) (Table 3).

Folate

There were variations in folate values for the samples. Dehulling and cooking increased the folate content of the sample when compared with the control (7.37 vs 7.23mg). The folate level of the undehulled cooked black lima bean was drastically reduced as against the dehulled cooked sample and the control (5.77 vs 7.37 and 7.23mg). Cooking without draining and replacing the water at intervals caused a decrease in folate as against its counterpart whose cooking water was changed at intervals during cooking (5.77 vs 7.27mg).

Soaking caused a slight increase in folate content of the samples. The folate value for the soaked samples ranged from 5.47 – 5.57mg. The 6 and the 9h soaked samples had comparable value 5.57mg, which was higher than that of the 3h soaked sample (5.47mg).

Germination and fermentation caused decreases in folate levels of the samples when compared with the control (7.23 vs 5.40 and 5l.30mg) and (7.23 vs 4.37 and 5.63mg). The 120h germinated sample had the least folate (5.40mg). The 72 and the 96h samples had comparable values 5.50mg. It was observed that as fermentation period increased, the folate content decreased. (5.63 – 4.37mg). There was a marked reduction in folate level at the 72h period.

Table 4 presents the antinutrient and food toxicant composition of processed and unprocessed black lima bean flour.

Phytate

The phytate content of raw, undehulled cooked and dehulled cooked black lima bean varied. The values ranged from 0.28 to 47.47mg. The raw sample (RBV) had the highest phytate (47.47mg), followed by the undehulled cooked sample (4.23mg). On the other hand, dehulled cooked sample had the lowest (0.28mg). Dehulling and cooking synergistically reduced phytate in the samples (BVDC and BVUDC) when their values were compared with the control (0.28 and 4.23 vs 47.47mg). The low phytate value for the $BCWD_4$ and the $BCWND_o$ as against the control were a function of treatments (2.35 – 47.47mg).The sample (BCWD) whose water was drained and replaced at intervals during cooking had lower phytate than the BCWND (2.35 vs 2.72mg).

There were variations in the phytate level of the soaked samples. It ranged from 0.28 to 47.47mg. The sample soaked for 6h had the least phytate followed by the 3h sample (3.10mg). The 9h sample had the highest value among the soaked sample (8.50mg).

Germination for 72 and 96h decreased phytate to traces. However, beyond 96h germination, there was an increase to 2.60mg as compared with the control. Fermentation drastically reduced phytate when compared with the control (47.47 vs 2.80, 2.77 and 0.50mg) (Table 4). The 72h fermentation caused the highest decrease in phytate (0.50mg).

Tannins

The combination of dehulling and cooking reduced tannins to zero when compared with the control (12.70mg). The sample whose cooking water was drained and replaced had comparable value with that of the sample whose cooking water was not drained (0.00mg).

Soaking for 3, 6 and 9h drastically reduced tannins levels from 12.70 to 0.00mg. The control had the highest tannins, followed by the 3 and the 6h samples (12.70, 7.52 and 4.36mg, respectively). The 9h soaking reduced tannins to zero.

Germination for 72h reduced tannins to zero. The 96h germination also caused drastic reduction in tannins, however, it was not as that of the 72h (0.00 vs 1.20mg). Surprisingly, germination beyond 96h increased the value from 12.70 to 47.23mg – a commonly observed phenomenon.

Fermentation decreased tannins. The values ranged from 0.00 to 12.70mg. The 72h sample had the least tannins (0.00), followed by the 48h as well as the 24h values (0.00, 1.33, and 6.06mg, respectively).

Hydrogen cyanide

Dehulling and cooking, draining and replacing cooking water as well as soaking for 3, 6 and 9h significantly reduced hydrogen cyanide (HCN) when compared with the control. (0.00 vs 12.25mg) (Table 4). Dehulling and cooking, drainage of cooking water as well as soaking for 3 and 9h caused the greatest reduction in HCN (0.00mg) as against the control (36.44mg). Dehulling and cooking alone, no changing of cooking water and 6h soaking reduced HCN but not as much as the other treatments when compared with the control value (36.44mg).

Table 4: Effect of processing on antinutrients and food toxicants composition of black lima bean flours (mg/100g).

Sample	Phytate	Tannins	HCN	Trypsin inhibitor	Haemaglutinin	Saponin
RBV	47.47±0.31	12.70±0.10	36.44±0.33	27.73±0.21	2.56±0.11	113.07±0.66
BVUDC	423±0.03	0.00±0.00	0.00±0.00	1.40±0.20	0.64±0.01	0.43±0.01
BVDC	0.28±0.25	0.00±0.00	12.25±0.33	1.50±0.10	0.76±0.02	0.27±0.02
$BCWD_4$	2.35±0.10	0.00±0.00	0.00±0.00	0.67±0.12	0.26±0.03	0.12±0.01
$BCWND_O$	2.72±0.13	0.00±0.00	2.10±0.07	1.33±0.15	0.45±0.03	0.48±0.43
BVS_3	3.10±0.10	7.52±0.10	0.00±0.00	22.73±0.15	1.30±0.03	27.50±0.02
BVS_6	0.28±0.03	4.36±0.42	2.38±0.38	4.27±0.15	0.98±0.02	12.41±0.02
BVS_9	8.50±0.10	0.00±0.00	0.00±0.00	0.00±0.00	0.42±0.04	4.31±0.02
BVG_{72}	0.00±0.00	0.00±0.00	4.58±0.38	6.27±0.15	3.92±0.20	36.22±0.17
BVG_{96}	0.00±0.00	1.20±0.10	4.37±0.16	12.60±0.20	3.58±0.23	44.23±0.12
BVG_{120}	2.60±0.10	4.23±0.06	8.07±0.06	18.67±0.21	3.84±0.07	96.71±0.02
BVF_{24}	2.80±0.10	6.06±0.06	0.00±0.00	2.60±0.20	2.08±0.08	4.86±0.09
BVF_{48}	2.77±0.15	1.33±0.10	0.00±0.00	0.00±0.00	1.48±0.10	0.22±0.02
BVF_{72}	0.50±0.10	0.00±0.00	0.00±0.00	0.00±0.00	0.06±0.02	0.66±0.02

Mean±SD of three replications.

RBV - Raw lima bean black variety
BVUDC - Black variety undehulled cooked
BVDC - Black variety dehulled cooked
$BCWD_4$ - Black variety cooking water discarded four times
$BCWND_o$ - Black variety cooking water not discarded
BVS_3 - Black variety soaked for 3 hours
BVS_6 - Black variety soaked for 6 hours
BVS_9 -Black variety soaked for 9 hours
BVG_{72} -Black variety germinated for 72 hours
BVG_{96} -Black variety germinated for 96 hours
BVG_{120} -Black variety germinated for 120 hours
BVF_{24} -Black variety fermented for 24 hours
BVF_{48} -Black variety fermented for 48 hours
BVF_{72} -Black variety fermented for 72 hours

Germination reduced HCN in black lima bean. The reductions were from 36.44 to 4.37mg. The 72 and the 96h samples had comparable values (4.58 and 4.37mg). On the other hand, the 120h sample had the highest value when compared with those of the 72 and the 96h samples (8.07 vs 4.58 and 4.37mg, respectively).

Fermentation reduced HCN to zero regardless of the period of fermentation.

Trypsin inhibitor

The combination of dehulling and cooking, draining and replacement of cooking water drastically reduced trypsin inhibitor from 27.73 to 0.67mg (Table 4). The changing of cooking water had the highest reduction of trypsin inhibitor when compared with the control (27.73 to 0.67mg) followed by the other treatments (1.33, 1.40 and 1.50 vs 27.73mg).

Soaking for 9h reduced trypsin inhibitor to zero level when compared with the control (27.73 vs 0.00mg).The 3h soaking had the least effect on trypsin inhibitor (22.73 vs 27.73mg). The reduction due to 3h soaking was only 5mg, followed by that of the 6h (4.27mg). Germination from 72 to 120h had varied effects on trypsin inhibitor as against the control (27.73 vs 6.27, 12.60 and 18.67mg, respectively). The 120h germination had least effects on trypsin inhibitor followed by the 96h (18.67 and 12.67, respectively).

Fermentation beyond 24h reduced trypsin inhibitor to zero. On the other hand, the 24h fermentation had least effect on trypsin inhibitor when compared with the control (26.00 vs 27.73mg, respectively).

Haemaglutinin

Dehulling and cooking, changing cooking water and not changing it caused reduction in haemaglutinin. The range of reduction was from 0.26 to 0.76mg as compared with the control (2.56mg) (Table 4). Changing cooking water reduced haemaglutinin much more than any of the treatments (0.26mg vs 0.76, 0.64, and 0.45mg, respectively).

Soaking beyond 6h, reduced haemaglutinin more than those of the 3 and the 6h (0.42 vs 0.98 and 1.30mg, respectively). Germination increased levels of haemaglutinin when compared with the control (2.56 vs 3.92, 3.58, and 3.84mg). This is a commonly observed fact. On the other hand, fermentation caused decreases in haemaglutinin levels. The 72h fermentation had much more effect in reduction of haemaglutinin from 2.56 to 0.06mg. The 24 and the 48h fermentation decreased haemaglutinin but not as that of the 72h (0.06 vs 2.08 and 1.48mg, respectively), when compared with the control (2.56mg).

Saponins

The saponins values varied due to dehulling and changing cooking water or not. The values ranged from 0.12 to 0.48mg when compared with the control (113.07mg). Changing cooking water had much more effect on saponins than dehulling and cooking (0.12 vs 0.27, 0.43 and 0.48mg). The longer the soaking, the lower the saponins levels, when compared with the control (27.50, 12.41 and 4.31mg vs 113.07mg). The 9h soaking had highest effect on saponins, when compared with the control (4.31 vs 113.07mg). The 3 and the 6h soaking reduced saponins but not as much as that of the 9h (27.50, 12.41 vs 4.31mg).

Germination, regardless of the period (72 – 120h) caused least decrease in saponins in black lima bean when compared with the other treatments (cooking, dehulling, changing cooking water and fermentation). However, the 120h germination caused the least reduction of saponins when compared with the control (113.07 vs 96.71mg). The 72h germination had the highest effect on saponins when compared with those of the 96 and the 120h (36.22 vs 44.23 and 96.71mg).

Fermentation had varied effect on saponins. The 48h fermentation caused the highest reduction of saponins as compared with the 24 and the 92h as well as the control (0.22 vs 4.86, 0.66 and 113.07mg) (Table 4).

Table 5 presents the functional properties of processed and unprocessed black lima bean flour.

Foaming capacity and stability

Foaming capacity of black lima bean flours was drastically reduced by all treatments except for the 96 and the 120h germination. The range of reduction was from 0.13 to 3.03 percent when compared with the control (4.20%). Fermentation for 24 and 48h as well as retention of cooking water had highest adverse effect on foaming capacity (0.13 and 0.17, respectively). On the other hand, germination for 120h had value very close to that of the control (3.30 vs 4.20%). The 96h value was an increase, however, the value was much lower than that of the control as well as the 120h samples (2.60 vs 4.20 and 3.30%, respectively).

Table 5: Effect of processing on functional properties of black lima bean flours (%).

Samples	Foaming capacity (FC)	Water absorption capacity (WAC)	Oil absorption capacity (OAC)	Emulsion capacity (EC)	Nitrogen solubility/ protein solubility
RBV	4.20±0.10	5.00±1.00	14.00±1.00	11.00±2.65	5.10±0.10
BVUDC	0.23±0.06	31.00±1.00	5.33±1.53	6.00±1.00	0.98±0.02
BVDC	0.47±0.15	30.00±2.00	6.33±1.53	4.00±1.00	0.00±0.00
$BCWD_4$	0.20±0.10	29.67±3.51	6.00±1.00	4.33±1.53	0.34±0.03
$BCWND_O$	0.17±0.06	30.00±1.00	6.00±1.00	6.33±1.53	2.84±0.08
BVS_3	0.40±0.10	10.00±1.00	15.00±1.00	3.67±0.58	4.83±0.08
BVS_6	0.40±0.10	21.67±2.08	8.00±1.00	8.00±1.00	5.11±0.02
BVS_9	0.30±0.10	25.00±1.00	5.00±1.00	16.00±1.00	4.08±0.17
BVG_{72}	0.57±0.06	23.00±1.00	5.67±2.08	12.00±1.00	4.20±0.11
BVG_{96}	2.60±0.20	31.00±1.00	8.67±3.06	2.00±1.00	10.61±0.40
BVG_{120}	3.30±0.10	9.00±1.00	11.00±1.00	19.67±1.53	12.84±0.19
BVF_{24}	0.17±0.06	4.00±1.00	15.33±2.52	4.00±2.00	3.66±0.19
BVF_{48}	0.13±0.06	30.33±1.53	10.00±1.00	3.00±1.00	2.37±0.04
BVF_{72}	0.30±0.10	3.00±1.00	5.00±1.00	4.00±2.00	1.86±0.03

Mean±SD of three replications.

RBV -Raw lima bean black variety
BVUDC -Black variety undehulled cooked
BVDC -Black variety dehulled cooked
$BCWD_4$ -Black variety cooking water discarded four times
$BCWND_o$-Black variety cooking water not discarded
BVS_3 -Black variety soaked for 3 hours
BVS_6 -Black variety soaked for 6 hours
BVS_9 -Black variety soaked for 9 hours
BVG_{72} -Black variety germinated for 72 hours
BVG_{96} -Black variety germinated for 96 hours
BVG_{120} -Black variety germinated for 120 hours
BVF_{24} -Black variety fermented for 24 hours
BVF_{48} -Black variety fermented for 48 hours
BVF_{72} -Black variety fermented for 72 hours

Water absorption capacity

All the treatments (dehulling, cooking, changing of cooking water, soaking, germination and fermentation) except for the 24 and the 72h increased water absorption capacity as compared with the control (5.00%). The 24 and the 72h fermentation had the most adverse effect on water absorption capacity (WAC) of the flours followed by the 120h germination and the 3h soaking (3.00, 4.00, 9.00 and 10.00%, respectively). The 3h soaking as well as the 120h germination caused the least increase in water absorption capacity (WAC) when compared with the control (10.00, 9.00 vs 5.00%). Undehulled cooked sample as well as the 96h germination had the highest increase in water absorption capacity when compared with the control (31.00 vs 5.00%).

Oil absorption capacity

All the food processing techniques adopted in the study to improve the quality of lima bean flour had positive effect on oil absorption capacity except for the 3h soaking and the 24h fermentation, when compared with the control (19.00 and 15.33 vs 14.00%) (Table 5). The 72h fermentation and the 9h soaking as well as undehulled cooked and the 24h germination had the most positive effect on oil absorption capacity (OAC). The other treatments had positive effects on OAC, however, the effect was not as those of the 72h fermentation, the 9h soaking, the undehulled cooked as well as the 72h germinated samples.

Emulsion capacity and stability

All the treatments adversely affected the emulsion capacity of the flours except for those of the 72h germination, the 9h soaking and the 120h germination as compared with the

control (12.00, 16.00 and 19.00 vs 11.00%). It was observed that the 96h germinated (BVG_{96}) sample, followed by the 48h fermented sample (BVF_{48}) and the 3h soaked sample had lower emulsion capacity. The BVG_{96} had the least value (2.00%) (Table 5). The 24 and the 72h fermented samples as well as the BCWD and the BVDC had comparable values (4.00 to 4.33%) for emulsion capacity.

Nitrogen solubility

The nitrogen solubility of the processed flour samples differed. It ranged from 0.00 to 12.84%. The BVG_{120} and the BVG_{96} had the highest value (12.84% and 10.61%,) followed by the BVS_6 (5.11) when compared with the control (5.10%) and the other samples. Dehulling and cooking drastically reduced nitrogen solubility (soluble protein) to zero. The sample (BCWND) whose cooking water was not changed had higher soluble protein than that of the BCWD in which the cooking water was discarded at intervals. The BVF_{72} and the BVG_{72} samples had the lowest value (1.86 and 4.20) among all the germinated and fermented samples.

Table 6 presents the proximate composition of processed and unprocessed black lima bean and maize composite flours.

Moisture

The moisture content of the seven composite flours differed. The flour based on raw lima bean and maize (UBVM) had highest moisture as well as the $FBV_{72}M_{48}$ and $GBV_{120}M_{48}$ (8.17, 7.27 and 7.33%, respectively). The SBV_9M_{48} and $GBV_{96}M_{48}$ had the least moisture (3.17 and 4.33%). The $CBVM_{48}$ and $FBV_{48}M_{48}$ had comparable moisture (6.33 and 6.17% respectively).

Protein

The protein values for the composites flours varied. The range was from 4.90 to 21.06%. The $CBVM_{48}$ had the least protein when compared with the control (UBVM) (4.90 vs 19.10%). On the other hand, the $FBVM_{48}$ had the highest value as compared with the control and the other composites (21.06 vs 19.10, 14.70, 18.68%) (Table 6).

Ash

The ash values for the composite flours varied. It ranged from 1.11 to 4.46%. The control (UBVM) had the highest value (4.46%), however, the $FBV_{72}M_{48}$, the $GBV_{96}M_{48}$ and the $GBV_{120}M_{48}$ had the least and comparable ash values (1.15, 1.15 and 1.11%, respectively). The second highest value was that of the $CBVM_{48}$ (3.31%) followed by those of the

SBV_9M_{48} and the $FBV_{48}M_{48}$ (2.55 and 2.06%).

Fat

The fat content of the composite flours differed. The SBV_9M_{48} had the highest fat as well as the $FBV_{48}M_{48}$ (3.24 and 2.74%). The UBVM, the $CBVM_{48}$ and the $FBV_{72}M_{48}$ had comparable values (1.60 and 1.69% each). The $GBV_{96}M_{48}$ as well as the $GBV_{12}M_{48}$ had similar values (2.44 and 2.19%).

Table 6: Effect of processing on proximate composition of composite flour from black lima bean and maize (%).

Sample	Moisture	Protein	Ash	Fat	Fibre	Carbohydrate
UBVM	8.17±0.29	19.10±0.08	4.46±0.11	1.60±0.07	5.19±0.07	61.48±0.18
$CBVM_{48}$	6.33±0.26	4.90±0.03	3.31±0.11	1.60±0.11	4.59±0.11	79.27±0.16
SBV_9M_{48}	3.17±0.21	16.29±0.06	2.55±0.15	3.24±0.16	3.30±0.11	71.45±0.16
$GBV_{96}M_{48}$	4.33±0.21	17.41±0.06	1.15±0.06	2.44±0.16	3.28±0.07	63.35±0.25
$GBV_{120}M_{48}$	7.33±0.29	14.70±0.35	1.11±0.10	2.19±0.06	4.71±0.15	68.59±0.39
$FBV_{48}M_{48}$	6.17±0.26	21.06±0.08	2.06±0.06	2.74±0.07	4.62±0.18	71.39±0.05
$FBV_{72}M_{48}$	7.27±0.27	18.63	1.15±0.10	1.69±0.22	2.62±0.16	69.96±0.56

Mean± SD of three replications.

UBVM - Unprocessed black lima bean and maize composite flour
$CBVM_{48}$ - Cooked black lima bean and 48h fermented maize flour
SBV_9M_{48} - Black lima bean soaked for 9h and 48h fermented maize flour
$GBV_{96}M_{48}$ - 96h germinated black lima bean and 48h fermented maize flour
$GBV_{120}M_{48}$ - 120h germinated black lima bean and 48h fermented maize flour
$FBV_{48}M_{48}$ - Black lima bean and maize fermented for 48h
$FBV_{72}M_{48}$ - 72h fermented black lima bean and 48h fermented maize

Fibre

The fibre values differed. The values ranged from 2.62 to 5.19%. The UBVM had the highest fibre (5.19%). The $CBVM_{48}$, the FBV48 and the $GBV_{120}M_{48}$ had comparable values (4.59, 4.62, and 4.71%, respectively). The $FBV_{72}M_{48}$ had the least value (2.62%). However, the SBV_9M_{48} and the $GBV_{96}M_{48}$ had also similar values (3.30 and 3.28%, respectively).

Carbohydrate

The CHO values for the composite flours varied. The variation ranged from 61.48 to 79.27%. The $CBVM_{48}$ had the highest value (79.27%) relative to the control and other processed flour samples. The SBV_9 M_{48} and the $FBV_{48}M_{48}$ had similar values (71.45 and 71.39%) as well as those of the $FBV_{72}M_{48}$ and the $GBV_{120}M_{48}$ (69.96 and 68.59%, respectively). On the other hand, the $GBV_{96}M_{48}$ had the least CHO value (63.35%) relative to other processed composite flours.

Table 7 presents the mineral composition of processed and unprocessed black lima bean and maize flour blends.

Zinc (Zn)

The Zn values for the composite flours varied. The range was from 3.27 to 5.46mg. The $FBV_{72}M_{48}$, the $GBV_{96}M_{48}$ and the $GBV_{120}M_{48}$ had the highest and comparable values (5.46, 5.37 and 5.43mg, respectively). The SBV_9M_{48} had the second highest Zn (4.74mg). The $FBV_{48}M_{48}$ had 4.02mg. On the other hand, the UBVM and the $CBVM_{48}$ had comparable value (3.27 and 3.14mg).

Copper (Cu)

The Cu values differed. It ranged from 12.63 to 14.63mg. The $FBV_{48}M_{48}$ and the GBV12048 had the highest and comparable values (14.60 and 14.63mg). The $GBV_{96}M_{48}$ had the least Cu

(12.63mg). The UBVM, the $CBVM_{48}$, the SBV_9M_{48} and the $FBV_{72}M_{48}$ had similar Cu (13.73, 13.80, 13.45 and 13.80mg, respectively).

Iron (Fe)

There were variations in Fe values. The range was from 31.00 to 38.67mg. The $GBV_{120}M_{48}$ had the highest (38.67mg) which was comparable to those of the SBV_9M_{48} and the $FBV_{48}M_{48}$ (37.33 and 36.00mg). The UBVM and the $GBV_{96}M_{48}$ had comparable Fe as well as the $CBVM_{48}$ and the $FBV_{72}M_{48}$ (31.00 and 31.67mg).

Table 7: Effect of processing on mineral composition of composite flour from black lima bean and maize (mg/100g).

Samples	Zinc	Copper	Iron	Phosphorus
UBVM	3.27±0.01	13.73±0.21	33.00±1.00	33.67±2.08
$CBVM_{48}$	3.41±0.02	13.80±0.10	31.00±1.00	31.67±1.15
SBV_9M_{48}	4.74±0.02	13.43±0.15	37.33±1.15	38.33±1.53
FBV_{48} M_{48}	4.02±0.01	14.60±0.30	36.00±2.00	35.00±1.00
$FBV_{72}M_{48}$	5.46±0.02	13.80±0.10	31.67±0.58	32.00±0.00
$GBV_{96}M_{48}$	5.37±0.01	12.63±0.38	33.00±1.00	38.33±0.58
$GBV_{120}M_{48}$	5.43±0.02	14.63±0.38	38.67±1.15	33.67±1.15

Mean± SD of three replications

UBVM - Unprocessed black lima bean and maize composite flour
$CBVM_{48}$ - Cooked black lima bean and 48h fermented maize flour
SBV_9M_{48} - Black lima bean soaked for 9h and 48h fermented maize flour
$GBV_{96}M_{48}$ - 96h germinated black lima bean and 48h fermented maize flour
$GBV_{120}M_{48}$ - 120h germinated black lima bean and 48h fermented maize flour
$FBV_{48}M_{48}$ - Black lima bean and maize fermented for 48h
$FBV_{72}M_{48}$ - 72h fermented black lima bean and 48h fermented maize

Phosphorus (P)

The phosphorus content of the composites varied. The values ranged from 31.67 to 38.33mg. The SBV_9M_{48} and the $GBV_{96}M_{48}$ had highest and comparable values (38.33mg). The UBVM, the $CBVM_{48}$, the $FBV_{48}M_{48}$, the $FBV_{72}M_{48}$ and the $GBV_{120}M_{48}$ had different and comparable values (31.67 – 35.00mg) (Table 7).

Table 8 presents the proximate composition of processed and unprocessed dark-red lima bean flours.

Moisture

Dehulling and cooking were a function of moisture content of dark red lima bean (Table 8). Whether it was dehulled cooked or undehulled cooked, the moisture values were higher than those of the control (10.17 and 12.33% vs 9.33%). Changing of cooking water and its replacement lowered moisture as compared with the one whose cooking water was neither drained nor replaced (10.17 vs 13.33%). Soaking for 3 and 9h reduced moisture in lima bean as compared with its control (9.17 and 6.17 vs 9.33%, respectively). The 9h soaking reduced moisture more than the 3h soaking (6.17 vs 9.17%). The 6h soaking, however, caused slight increase in moisture when compared with the control (9.50 vs 9.33%). The increase was 0.27%.

Germination, regardless of the period increased moisture when compared with the control (13.33, 15.33 vs 9.33%). The 96 and the 120h periods had similar increase in moisture (15.33%). The 72h germination had caused smaller increase in moisture when compared with those of 96 and 120h (13.33 vs 15.33, respectively).

Fermentation for 24h reduced moisture from 9.33 to 7.33%, when compared with the control. Fermentation for 48h, did not change moisture relative to the control (9.33 vs 9.33%). The 72h fermentation, however, increased moisture from 9.33 – 14.33%. The increase was 5% (Table 8).

Protein

Dehulling and cooking had effect on protein content of dark red lima bean flour. The undehulled cooked (DRVUDC) dark red lima bean had lower protein when compared with the control (RDRV) (6.90 vs 24.65%). On the other hand, dehulling increased protein from 24.65 to 26.73%. Changing and replacing cooking water had much adverse effect on protein (5.96 vs 24.65%) when compared with the control. Cooking dark red lima bean without changing cooking water also reduced protein but not as that of its counterpart (5.96 vs 8.19%).

Soaking for 3 and 6h caused some slight increases in protein when compared with the control (25.57 and 25.93 vs 24.65%). However, soaking for 9h slightly decreased protein in comparison with the control (24.57 vs 24.65%).

Germination, regardless of the period, increased protein when compared with the control (26.12, 26.50 and 25.11 vs 24.65%). The 96h germination caused the highest increase in protein in comparison with the other treatments (26.50 vs 26.12 and 25.11%).

Fermentation for 24 and 72h decreased protein when compared with the control (17.56 and 23.05 vs 24.65%). Meanwhile the 48h fermentation increased protein from 24.65 to 26.34%.

Ash

Dehulling and cooking had varied effect on ash content of dark red lima bean. The control had 6.69% ash. Dehulling caused much more reduction in ash in comparison with both the control and the undehulled cooked (DRVUDC) samples (1.41 vs 6.69 and 5.13%, respectively). The changing and replacement of cooking water ($DRVDC_4$) or otherwise (DRVWNDo) caused decreases in ash (3.38 and 4.62 vs 6.69%, control). The ash was much more reduced in the DRVWD4 than in the DRVWNDo (3.38 vs 4.62%, control).

Soaking reduced ash regardless of the soaking period when compared with the control (5.58, 4.94 and 4.30%, respectively). Soaking for 9h decreased ash much more than 3 and 6h soaking (4.30 vs 5.58 and 4.94%). All germination periods decreased ash. However, the 120h germination caused the least decrease in ash in comparison with the control (6.50 vs 6.69%), the decrease was only 0.19%.

The 96h period caused highest decrease in ash when compared with both the 72h and the control (4.25 vs 5.04 and 6.69%, respectively). Fermentation decreased ash, the values were from 2.88 to 3.86% as against 6.69% for the control. The 72h sample had the least value (2.88%) as compared with 2.91 and 3.86%, for the 24 and 48h respectively). The 48h

fermentation caused the least decrease in ash in comparison with the control (3.86 vs 6.69%).

Table 8: Effect of processing on proximate composition of dark red lima bean flours (%).

Samples	Moisture	Protein	Ash	Fat	Fibre	CHO
RDRV	9.33±0.23	24.65±0.27	6.69±0.14	1.62±0.06	5.00±0.12	52.71±0.10
DRVDC	10.17±0.25	6.90±0.05	5.13±0.12	0.87±0.06	2.89±0.27	55.86±0.11
DRVUDC	12,33±0.27	26.73±0.09	1.41±0.06	0.71±0.07	4.12±0.19	71.88±0.11
$DRVCWD_4$	10.17±0.26	5.96±0.21	3.38±0.06	0.89±0.00	2.86±0.29	76.74±0.16
$DRVCWND_o$	13.33±0.29	8.19±0.08	4.62±0.12	0.65±0.07	2.58±0.06	70.63±0.13
$DRVS_3$	9.17±0.23	25.57±0.12	5.58±0.12	1.65±0.11	4.44±0.18	53.59±0.10
$DRVS_6$	9.50±0.21	25.93±0.92	4.94±0.07	0.70±0.07	3.57±0.11	55.36±0.17
$DRVS_9$	6.17±0.18	24.57±0.11	4.30±0.06	0.57±0.06	3.73±0.27	60.66±0.12
$DRVG_{72}$	13.38±0.29	26.12±0.09	5.04±0.06	1.69±0.07	4.31±0.13	49.51±0.18
$DRVG_{96}$	15.33±0.29	26.60±0.12	4.25±0.11	1.69±0.17	4.41±0.05	47.82±0.18
$DRVG_{120}$	15.33±0.29	25.11±0.07	6.50±0.10	0.59±0.11	5.04±0.18	47.43±0.21
$DRVF_{24}$	7.33±0.18	17.56±0.01	2.91±0.10	0.50±0.06	2.48±0.11	69.22±0.09
$DRVF_{48}$	9.33±0.23	26.34±0.08	3.86±0.12	0.48±0.07	3.93±0.29	56.06±0.13
$DRVF_{72}$	14.33±0.29	23.05±0.06	2.88±0.42	1.48±0.07	4.12±0.23	54.14±0.15

Mean±SD of three replications.

RDRV -Raw dark red lima bean variety
DRVDC-Dark red variety dehulled cooked
DRVUDC-Dark red variety undehulled cooked
$DRVCWD_4$-Dark red variety, cooked and cooking water discarded and replaced four times
DRVCWND-Cooked dark red variety, with cooking water not changed and replaced
$DRVS_3$ -Dark red variety soaked for 3 hours
$DRVS_6$ -Dark red variety soaked for 6 hours
$DRVS_9$ -Dark red variety soaked for 9 hours
$DRVG_{72}$-Dark red variety germinated for 72 hours
$DRVG_{96}$-Dark red variety germinated for 96 hours
$DRVG_{120}$-Dark red variety germinated for 120 hours
$DRVF_{24}$ -Dark red variety fermented for 24 hours
$DRVF_{48}$ -Dark red variety fermented for 48 hours
$DRVF_{72}$ -Dark red variety fermented for 72 hours

Fat

Dehulling and cooking were a function of fat. Dehulling and cooking reduced fat from 1.62 to 0.71% and undehulling caused a 0.97% decrease (1.62 vs 0.87%). The changing and replacing of cooking water ($DRVWD_4$) reduced fat less than its counterpart (DRVWNDo) as well as the control (DRV) (0.89 vs 0.65 and 1.62%, respectively). Equally, soaking periods except for 3h ($DRVS_3$) reduced fat when compared with the control (0.70, 0.57 vs 1.62%). Soaking for the 3h, on the other hand, slightly increased fat from 1.62 to 1.65%.

Germination increased fat except for the 120h sample (0.59%). The 72 and the 96h samples had highest and comparable values when compared with the control (1.69 vs 1.62%, respectively). The increase was only 0.07%.

Fermentation periods caused reduction in fat when compared with the control (0.50, 0.48 and 1.48 vs 1.62%). The 72h fermentation caused the least decrease in fat (1.48 vs 1.62%). The 24h and the 48h had comparable decrease in fat as against the control (0.50 and 0.48 vs 1.62%, respectively).

Fibre

All treatments reduced fibre in dark red lima bean when compared with the control (5.00%) except for the 120h germination (5.04%). The values for all samples except that of the 120h germination ranged from (2.48 – 4.44%). Undehulled cooked sample, changed and replaced cooking water sample and its counterpart ($DRVWND_o$) and the 24h fermented sample had the highest decrease in fibre as against that of the control (2.89, 2.86, 2.58 and 2.48% vs 5.00%) (Table 8). Soaking for 6 and 9h and fermentation for 48h caused the second least reduction in fibre (3.57, 3.73, 3.93%, respectively) as against the control (5.00%).

Carbohydrate

Dehulling and cooking increased CHO as against the control (55.86 and 71.88 vs 52.71%). The highest increase was that of the DRVUDC (71.88%), followed by its counterpart (DRVDC) (56.86%). The changing and replacing of cooking water at intervals increased CHO as compared with the control (76.74 vs 52.71%). The sample whose cooking water was not changed and replaced (DRVWNDo) had increased CHO 70.63%. However, the increase was less than that of the sample whose cooking water was drained and replaced (70.63 vs 76.74%).

Soaking increased carbohydrate as compared with the control (53.59, 55.36, and 60.66% as against the control 52.71%). The 9h soaking caused the highest increase in CHO as compared with those of the 3 and the 6h values (60.66 vs 53.59 and 55.36%, respectively).

The germination periods (72 – 120h) had equal effect on CHO content of dark red lima bean flour. The values were 49.51, 47.82 and 47.43% for the 72, the 96 and the 120h, respectively.

Fermentation increased CHO in dark red lima bean in comparison with the control (69.22, 56.06 and 54.14 vs 52.71%, respectively). The 24h fermented sample had the highest increase as compared with the other periods of fermentation (69.22 vs 56.06, and 54.14%).

Table 9 presents the micronutrient composition of unprocessed and processed dark red lima bean flour.

Zinc (Zn)

The food processing techniques had varied effects on zinc. The 9h soaking, the 72 and the 96h germination and the 72h fermentation caused least reduction in zinc when compared with the control (4.16, 4.06, 4.09 and 4.09 vs 4.17mg). Soaking for 9h reduced Zn from 4.17 to 4.16mg, the reduction was only 0.01mg. The values for the other treatments as compared with that of the control ranged from 3.24 to 3.85mg (Table 9). The highest reduction occurred in the DRVDC and the $DRVS_3$ as well as in the $DRVG_{120}$ when compared with the control (3.24, and 3.26 vs 4.17mg). The DRVDC and the $DRVS_3$ samples had comparable reduction in zinc as compared with the rest of the treatments (3.24mg each).

Copper (Cu)

The treatments had varied effect on Cu composition of dark red lima bean flours. The 3 and the 9h soaking, the 72 and the 96h germination as well as the 48 and the 72h fermentation had the highest and comparable increases in Cu, as compared with the other treatments. Equally, the DRVDC, the DRVUDC, the $DRVS_6$, the $DRVG_{120}$ as well the $DRVF_{24}$ had comparable increases in Cu. It ranged from 12.47 to 12.83mg. However, the $DRVCWD_4$ and the DRVCWNDo had comparable values (12.10 and 12.03mg).

Table 9 Effect of processing on micronutrient content of dark red lima bean flours (mg/100g).

Samples	Zinc	Copper	Iron	Phosphorus	Folate
RDRV	4.17±0.01	12.17±0.15	85.67±3.06	85.00±1.00	5.43±0.38
DRVUDC	3.24±0.02	12.63±0.15	17.00±1.00	17.00±1.00	5.60±0.17
DRVDC	3.85±0.10	12.70±0.10	18.67±0.59	19.33±1.53	5.43±0.12
$DRVCWD_4$	3.35±0.02	12.10±0.10	14.00±1.00	14.00±1.00	5.63±0.31
$DRVCWND_O$	3.51±0.02	12.03±0.25	43.00±1.00	43.00±1.00	5.67±0.21
$DRVS_3$	3.24±0.02	13.67±0.15	34.00±1.00	43.671.53	5.57±0.06
$DRVS_6$	3.55±0.01	12.57±0.15	48.00±1.00	47.67±0.58	5.50±0.20
$DRVS_9$	4.16±0.02	13.67±0.25	68.00±1.00	67.33±4.04	5.77±0.23
$DRVG_{72}$	4.06±0.05	13.63±0.15	49.33±2.52	48.00±1.00	5.27±0.21
$DRVG_{96}$	4.09±0.01	13.33±0.15	46.67±1.53	47.00±1.00	5.50±0.10
$DRVG_{120}$	3.26±0.01	12.47±0.06	46.00±1.00	46.33±0.58	5.53±0.23
$DRVF_{24}$	3.83±0.11	12.83±0.06	46.00±1.00	45.67±2.52	5.47±0.15
$DRVF_{48}$	3.38±0.01	13.30±0.10	46.00±1.00	44.00±2.00	5.40±0.10
$DRVF_{72}$	4.09±0.03	13.23±0.12	48.00±0.02	47.67±3.51	5.44±0.29

Mean±SD of three replications.

RDRV -Raw dark red lima bean vari
DRVDC-Dark red variety dehulled co
DRVUDC-Dark red variety undehulle
$DRVCWD_4$-Dark red variety, cooked discarded and replaced fo
$DRVCWND_o$-Cooked dark red variety water not changed and repla
$DRVS_3$ -Dark red variety soaked for
$DRVS_6$ -Dark red variety soaked for

Iron (Fe)

Iron values were influenced by the various food processing techniques. The control (DRV) had the highest iron (85.67mg). However, the dehulling and cooking (DRVDC), the undehulling and cooking (DRVUDC) as well as the DRVCWD caused much more decreases in Fe as compared with the other treatments. The range was from 14.00 to 18.67mg. The DRVDC and the DRVUDC had equal effect on Fe levels (17.00 vs 18.67mg). Except for the $DRVS_9$ as well as the $DRVG_{72}$, the other treatments had different comparable values for Fe. These variations ranged from 43.00 to 49.33mg (Table 9). On the other hand, the $DRVS_9$ had the least decrease in Fe in comparison with the control (68.00mg vs 85.67mg).

Phosphorus (P)

Phosphorus levels were a function of treatments. The control DRV had the highest

phosphorus (85.00mg). The DRVCWD, the DRVDC as well as the DRVUDC caused the highest decreases in phosphorus. These decreases ranged from 14.00 to 19.33mg when compared with the control (14.00, 17.00, 19.33 vs 85.00mg, respectively). The $DRVS_9$ caused the least decrease in phosphorus as in iron when compared with the control (67.33 vs 85.00mg). On the other hand, the other treatments had comparable decreases in phosphorus as against the control. These decreases ranged from 43.00 to 48.00mg as against that of the control (85.00mg).

Folate

Folate levels were influenced by treatments. The control sample (RDRV) had 5.43mg folate. The DRVDC, the DRVCWD as well as the DRVCWND and the soaked samples had higher increases in folate when compared with the control. Germination, except for the 72h sample and fermentation except for the 48h sample had increased folate in comparison with the control (5.60, 5.63, 5.67, 5.57, 5.50, 5.77, 5.50, 5.53, 5.47 and 5.44mg vs 5.43mg, respectively).

The $DRVF_{72}$ caused the least increase in folate (5.44 vs 5.43mg). The DRV as well as the DRVDC had comparable values (5.43mg). On the other hand, the $DRVG_{72}$ caused highest decrease in folate when compared with the control and other processed samples (5.27 vs 5.43mg).

Table 10 presents the antinutrients and food toxicant composition of unprocessed and processed dark red lima bean flours.

Phytate

All the processed samples had decreased phytate levels in relation to the control. The values ranged from 0.00 to 4.07mg as against the control (31.33mg). Dehulling and cooking reduced phytate in comparison to the control. (2.47, 2.50, 2.83, and 4.07 vs 31.33mg) (Table 10). The DRVDC had the least phytate level (2.47mg) among the cooked samples.

Soaking caused a decrease in phytate. It was observed that as the soaking hour increased, the phytate content decreased. The least decrease occurred in the 9h soaking.

Germination for 72 and 96h as well as fermentation for 72h reduced phytate content to zero when compared with the control (0.00 vs 31.33mg). The 120h germination period caused positive but lower value relative to the control.

Tannins

Tannins values were drastically reduced by treatments except that of 120h germinated sample. This had more than a four fold increase when compared with the control (47.90 vs 10.84mg).

Dehulling and cooking synergistically reduced phytate to zero level relative to the control (0.00 vs 10.84mg). Soaking reduced tannins in dark red lima bean. Soaking beyond 3h caused a significant decrease in tannins. At the 9h soaking period, tannins level was reduced to zero (Table 10). The 3h soaking caused the least decrease in tannins among all the soaked samples (7.27, 3.07 and 0.00mg), The 72h germinated and fermented samples had zero tannins levels when compared with the control (0.00 – 10.84mg). The 24h fermented sample had the second least reduction of tannins as against the control (5.51 vs 10.84mg). However, 96h germinated sample had comparable value with the 48h fermented sample (1.10 and 1.22mg).

Hydrogen cyanide

Cooking and dehulling, 9h soaking and fermentation reduced hydrogen cyanide (HCN) to zero as against the control. Soaking for 3h and germination for 120h caused least decrease in HCN (7.82 and 8.43 vs 14.85mg). The DRVCWND and the 6h soaking reduced HCN more than the 72 and the 96h germination (2.07 and 2.18 vs 4.63 and 4.,29mg, respectively).

Table 10: Effect of processing on antinutrient and food toxicant content of dark red lima bean flours (mg/100g).

Samples	Phytate	Tannins	HCN	Trypsin inhibitor	Haemagluttinin	Saponin
RDRV	31.33±23.69	10.84±0.75	14.85±20.20	29.77±0.15	2.94±0.03	90.08±0.07
DRVDC	2.47±0.06	0.00±0.00	0.00±0.00	1.77±0.15	0.77±0.03	0.24±0.02
DRVUDC	4.07±0.15	0.00±0.00	0.00±0.00	1.30±0.10	0.66±0.06	0.37±0.03
$DRVCWD_4$	2.50±0.06	0.00±0.00	0.00±0.00	0.77±0.06	0.25±0.05	0.35±0.02
$DRVCWND_O$	2.83±0.17	0.00±0.00	2.07±0.08	1.20±0.10	0.46±0.06	0.29±0.01
$DRVS_3$	3.77±0.15	7.27±0.21	7.82±0.06	23.60±0.20	1.22±0.02	28.60±0.01
$DRVS_6$	3.13±0.15	3.07±0.06	2.18±0.04	5.80±0.10	0.98±0.02	12.84±0.03
$DRVS_9$	1.13±0.32	0.00±0.00	0.00±0.00	0.00±0.00	0.51±0.07	4.87±0.02
$DRVG_{72}$	0.00±0.00	0.00±0.00	4.63±0.08	6.47±0.05	4.11±0.01	33.86±0.02
$DRVG_{96}$	0.00±0.00	1.10±0.10	4.29±0.30	12.80±0.10	3.69±0.02	40.75±0.01
$DRVG_{120}$	2.27±0.15	47.90±0.79	8.43±0.36	19.13±0.12	3.66±0.07	87.64±0.02
$DRVF_{24}$	2.03±0.06	5.51±0.30	0.00±0.00	2.38±0.04	2.13±0.07	3.54±2.29
$DRVF_{48}$	2.90±0.10	1.22±0.06	0.00±0.00	0.00±0.00	1.43±0.03	0.28±0.02
$DRVF_{72}$	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.08±0.02	0.53±0.02

Mean±SD of three replications.

RDRV -Raw dark red lima bean variety
DRVDC -Dark red variety dehulled cooked
DRVUDC-Dark red variety undehulled cooked
DRVCWD-Dark red variety, cooked and cooking water discarded and replaced four times
DRVCWND-Cooked dark red variety, with cooking water not changed and replaced
$DRVS_3$ - Dark red variety soaked for 3 hours

$DRVS_9$ -Dark red variety soaked for 9 hours
$DRVG_{72}$ -Dark red variety germinated for 72 hours
$DRVG_{96}$ -Dark red variety germinated for 96 hours
$DRVG_{120}$-Dark red variety germinated for 120 hours
$DRVF_{24}$ -Dark red variety fermented for 24 hours
$DRVF_{48}$ -Dark red variety fermented for 48 hours
$DRVF_{72}$ -Dark red variety fermented for 72 hours

Trypsin inhibitor (TI)

Treatments reduced trypsin inhibitor (TI) levels, especially the 9h soaking as well as the 48 and the 72h fermentation (0.00mg) in relation to the control (RDRV) which had 29.77mg.

Soaking for 3h and germination for 120h had least effect on TI. On the other hand, the DRVCWD caused the second largest decrease in TI (0.77mg). The DRVDC, the DRVUDC, and the DRVCWND samples had comparable values (1.77, 1.30 and 1.20mg).

Fermentation for 24h (DRVF24) reduced TI from 29.77mg to 2.38mg. The 6h soaking and the 72h germination reduced TI from 29.77mg to 5.80 and 6.47mg, respectively. Germination for 96h reduced TI from 29.77 to 12.80mg (Table 10).

Haemagglutinin

Dehulling, undehulling and cooking, the 6 and the 9h soaking and the 72h fermentation reduced haemagglutinin to less than 1mg as against the control (2.94 vs 0.77, 0.66, 0.25, 0.46, 0.98, 0.57 and 0.08mg, respectively). On the other hand, all periods of

germination (72 – 120h) increased haemagglutinins from 2.94 to 4.11, 3.69 and 3.66mg. The 3h soaking as well as the 48h fermentation had comparable values (1.22 and 1.43mg). The 24h fermentation caused the third least reduction in haemagglutinin levels of the samples (from 2.94 to 2.13mg.)

Saponin

The control sample had 90.08mg saponin. However, dehulling and cooking as well as the 48 and the 72h fermentation reduced saponin to less than 1mg (0.24, 0.37, 0.35, 0.29, 0.28 and 0.53mg, respectively), relative to the control (90.08mg). Surprisingly, germination for 120h had little or no effect on saponin when compared with the control (87.64 vs 90.08mg).

Soaking for 3h, germination for 72 and 96h caused the second least decrease in saponin (28.60, 33.86 and 4.75mg). The 6h soaking caused 12.84mg reduction in saponin as against the control (12.84 vs 90.08mg). On the other hand, soaking for 9h and fermentation for 24h had different saponin values, however, the values were comparable (4.87 and 3.54mg) – a commonly observed phenomenon.

Table 11 presents the functional properties of unprocessed and processed dark red lima bean flours.

Foam capacity (FC)

Treatments had much adverse effect on foaming capacity of the dark red lima bean flours. However, the 3h soaking, all the germination periods caused less decrease in foaming capacity when compared with the control (2.53, 2.07, 2.27 and 4.40 vs 5.40mg). The rest of the treatments reduced foaming capacity to 1mg (Table 11).

Water Absorption Capacity (WAC)

Treatments increased water absorption capacity except for the 120h germinated sample (1.67mg). The 3h soaking caused the least increase in WAC when compared with the other treatments (10.33 vs 23.00 to 31.00mg) as against the control (4.00mg).

Oil Absorption Capacity (OAC)

OAC was reduced by processing as against the control (16.00mg). The 120h germination had little or no effect on oil absorption capacity (15.00 vs 16.00mg). The reduction was only 1mg. The reduction in OAC ranged from 4.67 – 15.00mg. Dehulling and

cooking, the 9h soaking and the 72h germination as well as the 72h fermentation reduced OAC to one third (1/3) its original value (16.00mg). These values ranged from 4.67 to 5.67mg (Table 11). However, the 3h soaking, the 96h germination and the 24h fermentation had comparable reduction of OAC (8.00mg). The 48h fermentation caused the second least reduction of OAC (9.00mg).

Emulsion capacity (EC)

Treatments influenced emulsion capacity. They caused increases except for the 48h fermentation (4.00 vs 5.00). The 3h soaking, the 24h fermentation and the 120h germination caused the highest increase in EC (21.00, 12.00 and 16.33mg) as against the control (5.00mg). The changing of cooking water at intervals (DRVCWD4) and the 72h germination as well as the 6h soaking had comparable EC values (7.00 and 8.00mg). Dehulling and cooking (DRVDC, DRVUDC and DRVCWD, the 9h soaking, the 96h germination and the 72h fermentation) had similar value (6.00mg). The range was from 5.67 to 6.00mg. These treatments caused little or no effect in emulsion capacity in relation to the control (6.00 vs 5.00mg).

Table 11: Effect of processing on functional properties of dark red lima bean flours (mg/100g).

Samples	Foam capacity	Water absorption capacity (WAC)	Oil absorption capacity (OAC)	Emulsion capacity (EC)	Nitrogen solubility (protein solubility)
RDRV	5.40±0.17	4.00±1.00	16.00±2.00	5.00±1.00	4.84±0.15
DRVUDC	0.33±0.06	30.67±3.06	5.00±1.00	6.00^{ef}±1.00	0.00±0.00
DRVDC	0.53±0.06	31.00±1.73	5.00±1.00	5.67±1.53	3.96±0.04
$DRCWD_4$	0.27±0.06	31.00±2.65	5.33±1.53	8.00±1.00	0.64±0.18
$DRVCWND_O$	0.33±0.12	30.33±2.52	4.67±1.53	6.00^{f}±1.00	2.87±0.26
$DRVS_3$	2.53±0.12	10.33±1.53	8.00±1.00	21.00±1.00	4.32±0.01
$DRVS_6$	0.17±0.06	24.00±2.00	3.00±1.00	7.00±1.00	5.03±0.07
$DRVS_9$	0.10±0.00	29.00±1.00	5.67±2.08	6.00±1.00	4.25±0.11
$DRVG_{72}$	2.07±0.06	30.00±4.00	5.67±2.08	8.00±1.00	4.86±0.12
$DRVG_{96}$	2.27±0.06	24.00±2.00	8.00±1.00	6.00±2.00	11.4±0.72
$DRVG_{120}$	4.40±0.20	1.67±0.58	15.00±1.00	12.00±1.00	12.52±0.32
$DRVF_{24}$	0.17±0.06	23.67±2.52	8.00±1.00	16.33±1.53	4.22±0.11
$DRVF_{48}$	0.10±0.00	23.00±1.00	9.00±1.00	4.00±1.00	2.42±0.04
$DRVF_{72}$	0.43±0.40	30.67±2.08	5.00±1.00	6.00±1.00	1.87±0.09

Mean±SD of three replications.

RDRV - Raw dark red lima bean variety
DRVDC-Dark red variety dehulled cooked
DRVUDC-Dark red variety undehulled cooked
$DRVCWD_4$-Dark red variety, cooked and cooking water discarded and replaced four times
$DRVCWND_o$-Cooked dark red variety, with cooking water not changed and replaced
$DRVS_3$ -Dark red variety soaked for 3 hours
$DRVS_6$ -Dark red variety soaked for 6 hours
$DRVS_9$ -Dark red variety soaked for 9 hours
$DRVG_{72}$-Dark red variety germinated for 72 hours
$DRVG_{96}$-Dark red variety germinated for 96 hours
$DRVG_{120}$-Dark red variety germinated for 120 hours
$DRVF_{24}$ -Dark red variety fermented for 24 hours
$DRVF_{48}$ -Dark red variety fermented for 48 hours
$DRVF_{72}$ -Dark red variety fermented for 72 hours

Protein solubility

Combination of dehulling and cooking drastically reduced soluble protein in dark red lima bean to zero when compared with the control (0.00 vs 4.84mg). Undehulling and cooking (DRVUDC) had little or no effect on soluble protein when compared with the control (3.96 vs 4.84mg). Cooking without changing cooking water at intervals had also little effect on soluble protein but not as that of the DRVUDC (2.87 vs 3.96mg). On the other hand, changing water at intervals when cooking reduced soluble protein from 4.84mg to 0.64mg.

Soaking slightly reduced soluble protein relative to the control except for the 6h soaking period (4.25, 4.32, and 5.03 vs 4.84mg, respectively).

Germination increased soluble protein of dark red lima bean as against that of the control (4.86, 11.46 and 12.52mg vs 4.84mg). The 72h germination caused the lowest increase (4.86 vs 4.84mg). The 120h germination and that of the 96h increased the value to a three fold (11.46 and 12.52 vs 4.84mg). All fermentation periods were not beneficial when compared with the control (4.22, 4.42 and 1.87mg) as against the control (4.84mg). The 72h fermentation markedly reduced soluble protein from 4.84 to 1.87mg as well as the 48h fermentation (2.42mg).

Table 12 presents the proximate composition of dark red lima bean and maize composite flours.

Moisture

Treatments had varied effect on moisture content of dark red lima bean and maize flour blends. They caused slight increase in the 96h germinated lima bean and the 48h fermented maize composite $GDRV_{96}M_{48}$ (8.77 vs 8.33%). The blend of the 48h fermented dark red lima bean and the 48h fermented maize ($FDRV_{48}M_{48}$), had lower moisture relative to the control (UDRVM) (5.33 vs 8.33%). The difference in moisture between the two samples was 3% (8.33 – 5.33%). The other treatments caused much more decrease in moisture as against the control. The values ranged from 7.17 – 7.43% (Table 12).

Protein

Treatments had varied effect on protein content of the blends. Cooking dark red lima bean and fermentation of maize for 48h ($CDRVM_{48}$) drastically reduced protein from 19.11 to 7.30%. On the other hand, fermentation of dark red lima bean and maize for 48h ($FDRV_{48}M_{48}$) as well as germination of lima bean for 120h and fermentation of maize for 48h ($GDRV_{120}M_{48}$) had slight decrease in protein when compared with the control (18.90 and 18.02 vs 19.11%, respectively).

Soaking for 9h and germination for 96h ($SDRV_9M_{48}$ and $GDRV_{96}M_{48}$) had comparable reduction in protein (17.00 and 17.29%, respectively) as against that of the control (19.11%). Fermentation of lima bean for 72h and maize for 48h had the second highest reduction in protein, as compared with the other treatments as well as the control (15.29 vs 17.00 to 19.11%) (Table 12).

Ash

All treatments had adverse effect on ash of the dark red lima bean and maize flour combination. Fermentation of both lima bean and maize for 48h ($FDRV_{48}M_{48}$) and germination of lima bean for 96h and fermentation of maize for 48h ($GDRV_{96}M_{48}$) caused highest decrease in ash relative to the control (0.35 and 0.55 vs 2.76%). The other treatments and blends had comparable decreases in ash when compared with the control. The decreases ranged from 1.04 to 1.15%.

Fat

The fat content of the flour blends were influenced by treatments. The control (UDRVM) had 1.75% fat. The blends containing cooked lima bean and 48h fermented maize

($CDRVM_{48}$), the one containing 48h lima and maize ($FDRV_{48}M_{48}$) as well as the blends containing 120h germinated lima bean and 48h fermented maize ($GDRV_{120}M_{48}$) had comparable values (3.27 and 3.35%).. On the other hand, the $SDRV_9M_{48}$ and the $FDRV_{72}M_{48}$) had comparable fat (1.08 and 1.11%). The $GDRV_{96}M_{48}$ had the second highest fat when compared with the control (12.78 vs 1.75%).

Fibre

Cooking, soaking, germination and fermentation had varied effects on fibre levels of the composite flours. The control UDRVM had 4.62% fibre. The $FDRV_{48}M_{48}$, the $GDRV_{96}M_{48}$, and the $GDRV_{120}M_{48}$ blends had comparable fibre relative to the control (4.61, 4.60 and 4.57 vs 4.62%, respectively). The decreases ranged from 0.01 to 0.05% when compared with the control. The $FDRV_{72}M_{48}$ blend had the least fibre when compared with the control (2.63 vs 4.62%). The $CDRVM_{48}$ and the $SDRV_9M_{48}$ had similar value (3.77 and 3.88%).

Table 12: Effect of processing on proximate composition of composite flour from dark red lima bean and maize (%).

Sample	Moisture	Protein	Ash	Fat	Fibre	Carbohydrate
UDRVM	18.33±0.29	19.11±0.04	2.76±0.28	1.75±0.19	4.62±0.14	63.43±0.61
$CDRVM_{48}$	7.17±0.29	7.30±0.07	1.11±0.06	3.27±0.17	3.77±0.12	77.38±0.31
$SDRV_9M_{48}$	7.17±0.29	17.00±0.45	1.04±0.06	1.08±0.11	3.88±0.31	69.73±0.26
$FDRV_{48}M_{48}$	5.33±0.29	18.90±0.47	0.35±0.06	3.27±0.17	4.61±0.31	67.54±0.99
$FDRV_{72}M_{48}$	7.33±0.29	15.29±0.04	1.15±0.12	1.11±0.16	2.63±0.23	72.49±0.10
$GDRV_{96}M_{48}$	8.77±0.11	17.29±0.03	0.55±0.11	2.78±0.07	4.60±0.12	66.01±0.29
$GDRV_{120}M_{48}$	7.43±0.15	18.02±0.06	1.08±0.22	3.35±0.18	4.57±0.06	65.55±0.18

Mean ± SD of three replications.

UDRVM Unprocessed dark red lima bean and maize flour
$CDRVM_{48}$ Cooked dark red lima bean and 48h fermented maize
$SDRV_9M_{48}$ 9h soaked dark red lima bean and 48h fermented maize
$FDRV_{48}M_{48}$ Dark red lima bean and maize fermented for 48h
$FDRV_{72}M_{48}$ 72h fermented dark red lima bean and 48h fermented maize
$GDRV_{96}M_{48}$ 96h germinated dark red lima bean and 48h fermented maize
$GDRV_{120}M_{48}$ 120h germinated dark red lima bean and 48h fermented maize

Carbohydrate

Carbohydrate content of the blends increased when compared with that of the control (63.43%).The $CDRVM_{48}$ and the $FDRV_{72}M_{48}$ blends had the highest CHO as against the control and the other blends (77.38 and 72.49 vs 63.43, 65.55, 66.01, 67.54 and 69.73%, respectively) (Table 12).

Table 13 presents the mineral composition of unprocessed and processed dark red lima bean and maize composite flours.

Zinc (Zn)

The zinc content of the composites varied. The variation was due to treatments. Cooked dark red lima bean and the 48h fermented maize ($CDRVM_{48}$) blends had less Zn (3.25mg) as against the control (4.06mg). The other blends had higher Zn when compared with the 96h germinated lima bean and the 48h fermented maize ($GDRV_{96}M_{48}$), (5.55, 5.47 and 5.56 vs 4.09mg). The rest of the blends had varied and comparable values which ranged from 5.47 to 5.56mg.

Copper (Cu)

The Cu content of the blends varied. The control had 13.37mg Cu. The 9h soaked dark red lima bean and the 48h fermented maize ($SDRV_9M_{48}$), the 48h fermented lima bean and maize ($FDRV_{48}$) and the 96h germinated lima bean and the 48h fermented maize

($GDRV_{96}M_{48}$) had higher and comparable values (14.30, 14.37 and 14.43mg, respectively) ($P > 0.05$). The cooked dark red lima bean and the 48h fermented maize ($FDRV_{72}M_{48}$) as well as the 120h germinated lima bean and the 48h fermented maize ($GDRV_{120}M_{48}$) had higher Cu than the control (13.40, 13.50 and 13.60 vs 13.37mg). The $CDRVM_{48}$ had the least increase in Cu relative to the control (13.40 vs 13.37mg).

Iron (Fe)

All the blends had increases in iron except for the 72h fermented dark red lima bean and the 48h fermented maize (32.33mg).

The blend of the 9h soaked lima bean and the 48h fermented maize had the highest iron (53.67mg) as well as the $CDRVM_{48}$ (44.67mg). On the other hand, the 96 and the 120h germinated lima bean and the 48h fermented maize blends had high and comparable iron values (37.00mg).

Table 13: Effect of processing on mineral composition of composite flour based on dark red lima bean (mg/100g).

Sample	Zinc	Copper	Iron	Phosphorus
UDRVM	4.06±0.02	13.37±0.21	33.00±1.00	34.67±2.08
$CDRVM_{48}$	3.25±0.02	13.40±0.20	44.67±2.08	47.00±1.00
$SDRV_9M_{48}$	5.55±0.01	14.30±0.10	53.67±1.53	38.00±1.00
$FDRV_{48}M_{48}$	5.47±0.01	14.37±0.32	33.67±2.08	34.00±1.00
$FDRV_{72}M_{48}$	5.55±0.03	13.50±0.50	32.33±2.52	36.00±1.00
$GDRV_{96}M_{48}$	4.09±0.03	14.43±0.21	37.00±1.00	32.00±1.00
$GDRV_{120}M_{48}$	5.56±0.02	13.60±0.20	37.00±1.00	37.67±1.53

Mean ± SD of three replications.

UDRVM Unprocessed dark red lima bean and maize flour
$CDRVM_{48}$ Cooked dark red lima bean and 48h fermented maize
$SDRV_9M_{48}$ 9h soaked dark red lima bean and 48h fermented maize
$FDRV_{48}M_{48}$ Dark red lima bean and maize fermented for 48h
$FDRV_{72}M_{48}$ 72h fermented dark red lima bean and 48h fermented maize
$GDRV_{96}M_{48}$ 96h germinated dark red lima bean and 48h fermented maize
$GDRV_{120}M_{48}$ 120h germinated dark red lima bean and 48h fermented maize

Phosphorus (P)

Treatments had varied effect on phosphorus content of the six blends and the control. The control had 34.67mg phosphorus. The 96h germinated lima bean and the 48h fermented maize blends had lower phosphorus in comparison with the control (32.00 vs 34.67mg) as well as that of the 48h fermented lima bean and maize ($FDRV_{48}M_{48}$) (34.00mg vs 34.67mg). The $CDRVM_{48}$ had the highest phosphorus (47.00mg) when compared with both the control and the other blends (47.00 vs 32.00 – 37.67mg) (Table 13). However, the 9h soaked lima bean and the 48h fermented maize ($SDRV_9M_{48}$), the 72h fermented lima bean and the 48h fermented maize ($FDRV_{72}M_{48}$) as well as the120h germinated lima bean and the 48h fermented maize $GDRV_{120}M_{48}$ had comparable values.

Table 14 presents the microflora content of processed and unprocessed black and dark red lima bean flours.

There were five microflora studied (K*lebsiella, aerogenes, Bacillus substilis Proteus vulgaris, Candida albicans* and *Escherichia coli*). However, the microorganism that was found in the flours regardless of treatments and varietal differences was *Escherichia coli*. All the flours of cooked black and dark red (dehulled, cooked or undehulled cooked, cooked with changed or unchanged cooking water) destroyed *Escherichia coli* as compared with the control (0.00×10^1 vs 2.00×10^1). On the other hand, the untreated black and dark red lima bean had Escherichia coli (2.00×10^1).

Soaking, germination and fermentation of both lima bean varieties had varied effect on E. coli population. Soaking for 6-9 hours increased E. coli more in black lima bean than in the dark red variety (1.00×10^1 - 3.00×10^1) as against the dark red variety which decreased as soaking period increased (2.00×10^1 – $1..20 \times 10^1$) (Table 14).

Germination of the black lima bean (72-120h) have no effect on Escherichia coli population relative to the control (2.00×10^1) However, germination increase the microflora population in the dark red lima bean (3.00×10^1 – 5.00×10^1) On the other hand, fermentation of dark red lima bean from 24-72h decreased E. coli population much more than in the black variety (4.00×10^1 – 1.60×10^1 vs 3.00×10^1 – 6.00×10^1).

Table 14: Microflora present in black and dark red unprocessed and processed lima bean flours.

Samples	TVC (Cfu/ml)	Microorganism Present	Samples	TVC (Cfu/ml)	Microorganism Present
RBV	2.00×10^1	*E. coli*	DRV	2.00×10^1	*E. coli*
BVDC	0.00×10^1	-	DRVDC	0.00×10^1	-
BVUDC	0.00×10^1	-	DRVUDC	0.00×10^1	-
BVCWD	0.00×10^1	-	DRVCWD	0.00×10^1	-
BVCWND	0.00×10^1	-	DRVCWND	0.00×10^1	-
BVS_3	1.00×10^1	*E. coli*	$DRVS_3$	2.00×10^1	*E. coli*
BVS_6	3.00×10^1	*E. coli*	$DRVS_6$	2.00×10^1	*E. coli*
BVS_9	3.00×10^1	*E. coli*	$DRVS_9$	1.20×10^1	*E. coli*
GBV_{72}	2.00×10^1	*E. coli*	$GDRV_{72}$	3.00×10^1	*E. coli*
GBV_{96}	2.00×10^1	*E. coli*	$GDRV_{96}$	3.00×10^1	*E. coli*
GBV_{120}	2.00×10^1	*E. coli*	$GDRV_{120}$	5.00×10^1	*E. coli*
BVF_{24}	3.00×10^1	*E. coli*	$DRVF_{24}$	4.00×10^1	*E. coli*
BVF_{48}	5.00×10^1	*E. coli*	$DRVF_{48}$	6.00×10^1	*E. coli*
BVF_{72}	6.00×10^1	*E. coli*	$DRVF_{72}$	1.60×10^1	*E. coli*

E. coli - *Escherichia coli* *Cfu/ml - Colony forming unit per ml
TVC - Total viable count

RBV	-	Raw black lima bean variety	DRV	-	Raw dark red lima bean variety
BVDC	-	Black lima bean dehulled cooked	DRVDC	-	Dark red lima bean dehulled cooked
BVUDC	-	Black lima bean undehulled cooked	DRVUDC	-	Dark red lima bean undehulled cooked
$BVCWD_4$	-	Black lima bean cooking water discarded	$DRVCWD_4$	-	Cooked dark red lima bean, cooking water discarded
$BVCWND_o$	-	Black lima bean cooking water not discarded	$DRVCWND_o$	-	Cooked dark red lima bean, cooking water not discarded
BVS_3	-	3h soaked black lima bean flour	$DRVS_3$	-	3h soaked dark red lima bean
BVS_6	-	6h soaked black lima bean flour	DRV_6	-	6h soaked dark red lima bean
BVS_9	-	9h soaked black lima bean flour	DRV_9	-	9h soaked dark red lima bean
GBV_{72}	-	72h germinated black lima bean	$GDRV_{72}$	-	72h germinated dark red lima bean

		flour			
GBV_{96}	-	96h germinated black lima bean flour	$GDRV_{96}$	-	96h germinated dark red lima bean
GBV_{120}	-	120h germinated black lima bean flour	$GDRV_{120}$	-	120h germinated dark red lima bean
BVF_{24}	-	Black lima bean fermented for 24h	$DRVF_{24}$	-	Dark red lima bean fermented for 24h
BVF_{48}	-	Black lima bean fermented for 48h	$DRVF_{48}$	-	Dark red lima bean fermented for 48h
BVF_{72}	-	Black lima bean fermented for 72h	$DRVF_{72}$	-	Dark red lima bean fermented for 72h.

Table 15. Proximate composition of moi-moi and akara based on black and dark red lima bean and maize flours.

Moisture

The moisture content of moi-moi and akara from both lima bean varieties differed. It ranged from 11.24 to 19.72%. The moi-moi from both black and dark red lima bean and maize flour blends had higher moisture values (17.31 to 19.72%) when compared with akara (11.24 to 16.14%)..

Protein

The moi-moi based on black lima bean fermented for 48 and 72h, and the 48h fermented maize flours (AOO and MCO-$FB_{48}M_{48}$ and $FB_{72}M_{48}$), had higher protein than the moi-moi based on unfermented black lima bean and maize flour (30.47 and 29.15 vs 28.41%, respectively). The increase in protein for akara based on 48h fermented black lima bean and maize blends tends to follow the same trend as that of moi-moi.

The akara based on the 48h fermented black lima bean and maize flour as well as that of 72h fermented black lima bean and the 48h fermented maize composites had higher protein than the akara based on unfermented black lima bean and maize flour blends (31.45, 27.89 vs 25.67%, respectively). The protein content of products (moi-moi and akara) based on fermented flour blends were much higher than those of unfermented composites.

Fat

Fat values for moi-moi and akara regardless of the variety of lima bean had slight increases in some of the blends when compared with their controls. Moi-moi based on black lima bean and maize flour blend fermented for 48h had comparable and higher fat as against

the control (1.55 vs 1.24%). On the other hand, akara based on the same blends had decreased fat in the 72h fermented black lima bean and 48h fermented maize flours (1.46 vs 1.25%). However, the blend based on the 48h fermented lima bean and maize (PNO-$FB_{48}M_{48}$) had an increase in fat as against the control (1.62 vs 1.46%). The moi-moi based on unfermented dark red lima bean and maize flour (JCO) had higher fat (1.73%) as against those of the 48 and the 72h fermented dark red lima bean and 48h fermented maize (MEO and AOB) (1.73 vs 1.19 and 1.52%).

The MEO ($FD_{48}M_{48}$) blend had the least fat (1.19%) as against those of the JCO, the UFDM and the AOB (1.19 vs 1.73 and 1.52%). There was a slight increase in fat of KCC ($FD_{48}M_{48}$) akara as compared with the control (UFDM) (1.28 vs 1.26%). On the other hand, the SCC ($FD_{72}M_{48}$) had 0.05%) decrease in fat when compared the control (1.21 vs 1.26%).

Ash

The moi-moi based on unfermented black lima bean and maize had higher ash than the AOO($FB_{48}M_{48}$) and the MCO($FB_{72}M_{48}$) (6.62 vs 4.97 and 6.35%, respectively). The AOO($FB_{48}M_{48}$) had the least ash (4.97%) followed by the MCO, $FB_{72}M_{48}$) moi-moi that had 6.35 vs 6.62% for the control. There was a little increase in ash of the akara based on $FB_{72}M_{48}$ (VAO) when compared with that of the control (VNO-UFBM) (5.55 vs 5.46%)., The PNO ($FB_{48}M_{48}$) akara had the least ash (4.15%). The JCO (UFDM) moi-moi, had higher ash as against its counterparts (6.15 vs 6.02 and 5.22%, respectively). The akara based on unfermented dark red lima bean and maize TAC (UFDM) (control) had the least ash (5.00%). However, its counterparts the KCC and the SCC had slight edge over it (5.00 vs 5.06 and 5.11%, respectively). The KCC ($FD_{48}M_{48}$) had the least increase in ash as against the control (5.00%).

Fibre

The fibre values for both moi-moi and akara based on black lima bean and maize had comparable values except for those of the MCO ($FD_{72}M_{48}$) and the VAO ($FB_{72}M_{48}$) (7.95 and 6.90 vs 9.24 and 9.28%) (Table 15). The moi-moi based on unfermented dark red lima bean and maize (control) had 6.24% fibre. On the other hand, the moi-moi based on the 48h fermented dark red lima bean and maize MEO($FD_{48}M_{48}$) had lower fibre as against that of the control (5.95 vs 6.24%, each). The AOB ($FD_{72}M_{48}$) had higher fibre (8.74%). This value was higher than those of the JCO and the MEO. There were slight decreases in fibre content of akara based on dark red lima bean and maize. The control TAC (UDFM) had higher fibre than the other two products (6.90 vs 6.74 and 6.39%, respectively). The SCC($FD_{72}M_{48}$) had the least value (6.39%).

Table 15: Proximate composition of products (moi-moi and akara) made from black and dark red lima bean varieties and maize flours (%).

Sample	Moisture	Protein	Fat	Ash	Fibre	CHO
JNO (UFBM)	19.63±0.09	28.41±0.55	1.24±0.04	6.62±0.02	9.25±0.02	34.85±0.11
AOO ($FB_{48}M_{48}$)	18.83±0.09	30.47± 0.22	1.55±0.05	4.97±0.04	9.28±0.02	34.90±0.11
MCO ($FB_{72}M_{48}$)	17.31±0.06	29.15±0.22	1.55±0.05	6.35±0.02	7.95±0.02	38.69±0.12
VNO (UFBM)	14.42±0.04	25.67±0.11	1.46±0.03	5.46±0.02	9.25±0.02	33.74±0.10
PNO ($FB_{48}M_{48}$)	12.12±0.03	31.45±0.55	1.62±0.03	4.15±0.02	9.24±0.05	41.42±0.09
VAO ($FB_{72}M_{48}$)	11.24±0.03	27.89±0.23	1.25±0.02	5.55±0.02	6.90±0.02	57.17±0.06
JCO (UFDM)	18.41±0.09	29.39±0.42	1.73±0.01	6.02±0.03	6.24±0.03	38.08±0.12
MEO ($FD_{48}M_{48}$)	19.72±0.09	30.70±0.14	1.19±0.02	5.22±0.02	5.94±0.02	36.43±0.11
AOB ($FD_{72}M_{48}$)	19.36±0.09	30.70±0.14	1.52±0.02	5.00±0.06	8.74±0.04	34.46±0.10
TAC (UFDM)	12.75±0.07	27.06±0.16	1.26±0.07	5.06±0.03	6.90±0.01	47.03±0.09
KCC ($FD_{48}M_{48}$)	13.11±0.07	29.10±0.21	1.28±0.01	5.11±0.09	6.74±0.02	44.71±0.07
SCC ($FD_{72}M_{48}$)	16.14±0.07	27.35±0.18	1.21±0.03	5.11±0.09	6.39±0.01	33.80±0.11

Mean±SD of three replications.

JNO - Unfermented black lima bean and maize moi-moi

AOO - Moi-moi from black lima bean and maize fermented for 48h

MCO - Moi-moi from 72h fermented black lima bean and 48h fermented maize

VNO - Unfermented black lima bean and maize akara

PNO - Akara from black lima bean and maize fermented for 48h

VAO - Akara from 72h fermented black lima bean and 48h fermented maize

JCO - Unfermented dark red lima bean and maize moi-moi

MEO - Moi-moi from 48h fermented dark red lima bean and maize

AOB - Moi-moi from 72h fermented dark red lima bean and 48h fermented maize

TAC - Unfermented dark red lima bean and maize akara

KCC - Akara from 48h fermented dark red lima bean and maize

SCC - Akara from 72h fermented dark red lima bean and 48h fermented maize

Carbohydrate

The CHO content of the two products (moi-moi and akara) varied. The variation was a function of varietal differences and treatments. The moi-moi and akara based on black lima bean and maize fermented for 48 and 72h AOO-$FB_{48}M_{48}$, PNO – $FB_{48}M_{48}$, MCO – $FB_{72}M_{48}$ and VAO – $FB_{72}M_{48}$ had higher CHO values when compared with their controls (34.90, 38.69 vs 34.85%) and (41.42, 57.17 vs 33.74%). On the other hand, the moi-moi based on MEO ($FD_{48}M_{48}$) and AOB ($FD_{72}M_{48}$) produced the least increase in CHO against the control (36.43 and 34.46 vs 38.08%). There was a decrease in the Carbohydrate content of akara based on 48 and 72h fermented dark red lima bean and maize flour blends as against the control (44.71 and 33.80 vs 47.03%).

Table 16: Mineral composition of moi-moi and akara made from unprocessed and processed black and dark red lima bean and maize flours

Calcium (Ca)

The calcium content of moi-moi based on black lima bean and maize were influenced by treatments. The moi-moi based on unfermented black lima bean and maize had lower Ca (89.51mg). On the other hand, the moi-moi based on black lima bean fermented for 48h and 72h and maize fermented for 48h had the highest calcium AOO ($FB_{48}M_{48}$) and the MCO ($FB_{72}M_{48}$) (122,24 and 117.57mg) – a commonly observed phenomenon. There were significant increases in the calcium content of all the moi-moi and akara when compared with their control except the akara based on the fermented dark red lima bean and maize flour blends..

Magnesium (Mg)

There were increases and decreases in Mg content of moi-moi and akara based on black and dark red lima bean and maize blends (Table 16). The AOO ($FB_{48}M_{48}$, caused increase in magnesium and decrease when fermentation was beyond the 48h (16.19 and 13.44.). The akara based on the 48 and the 72 fermented black lima bean and maize caused decreases (20.34, 13.31 vs 41.23mg). On the other hand, there were increases in magnesium of moi-moi based on the 48 and the 72h fermented dark red lima bean and the 48h fermented maize (MEO-$FD_{48}M_{48}$), and the AOB-$FD_{72}M_{48}$) (16.88 and 16.95 vs 15.77mg).However, akara based on fermented dark red lima bean had slight increase than the control (21.97 vs

20.13mg). There was also decrease in magnesium for the SCC ($FD_{72}M_{48}$) akara, (13.48 vs 212.97mg as well as 20.13mg, respectively).

Potassium (K)

There were increases in potassium values for moi-moi and akara based on fermented black lima bean and maize flour blends. Fermentation of black lima bean beyond 72h had mixed effects on potassium content of black lima bean moi-moi and akara. In moi-moi, it decreased potassium from 362.20 to 310.02m and in akara, the decrease was from 426.40 to 326.70mg.

On the other hand, fermentation decreased potassium in moi-moi based on dark red lima bean from 410.80 to 287.13 and the AOB-$FD_{72}M_{48}$ (312.00mg), respectively. There were increases in potassium in akara based on the 48 and the 72h fermented dark red lima bean and the 48h fermented maize blends (KCC-$FD_{48}M_{48}$, and SCC-$FD_{72}M_{48}$ as against the control TAC-UFDM (266.80 and 337.63 vs 165.93mg, respectively). However, fermentation of dark red lima bean for 72h caused much more increase in potassium when compared to that of the 48h fermentation as well as the control (337.63 vs 266.80 and 165.93mg, respectively).

Phosphorus

The effect of fermentation on moi-moi and akara based on fermented black and dark red lima bean and maize blends varied. Fermentation of black lima bean for 72h had an edge in phosphorus against the 48h (286.80 vs 265.13mg) as well as the unfermented lima bean and maize (209.87mg, respectively). Meanwhile fermentation reduced phosphorus in akara based on black lima and maize and in unfermented lima bean and maize (187.87 and 242.63 vs 244.83mg, respectively). However, the akara based on the 72h fermented black lima bean had the least decrease in phosphorus as against that of the control (242.63 vs 244.83mg). The phosphorus content of moi-moi based on fermented dark red lima bean and maize caused increases as against that of the unfermented blend (248.80 and 213.83 vs 165.87mg).

Fermentation either for 48 or 72h caused decreases in phosphorus content of akara based on dark red lima bean and maize (134.43 and 124.43 vs 214.67mg, respectively). The 72h fermentation of dark red lima bean caused much more decrease in phosphorus content of akara as compared with the 48h blends (SCC-$FD_{72}M_{48}$ vs KCC-$FD_{48}M_{48}$,, 124.43 vs 134.43mg).

Iron (Fe)

The iron content of moi-moi and akara based on fermented black and dark red lima bean and maize blends increased except for the VAO-$FB_{72}M_{48}$, when compared with their controls (Table 16). Fermentation makes minerals more available, of which iron is one.

Table 16: Mineral composition of products (moi-moi and akara) made from unprocessed and processed black and dark red lima bean varieties and maize composite flours (mg/100g).

Sample	Calcium (Ca)	Magnesium (Mg)	Potassium (K)	Phosphorus (P)	Iron (Fe)
JNO (UFBM)	89.51±4.62	13.54±0.00	258.20±2.80	209.47±1.62	11.87±0.20
AOO ($FB_{48}M_{48}$)	122.27±2.01	16.19±0.20	362.20±1.64	265.13±1.10	27.57±0.75
MCO ($FB_{72}M_{48}$)	117.57±2.32	13.44±0.02	310.02±1.75	286.80±1.39	19.43±1.62
VNO (UFBM)	89.51±2.31	41.23±0.24	306.27±2.10	244.83±1.19	26.78±0.54
PNO ($FB_{48}M_{48}$)	92.19±6.94	20.34±1.84	426.40±3.30	187.87±1.10	31.05±2.28
VAO ($FB_{72}M_{48}$)	90.85±2.31	13.31±0.28	326.70±7.00	242.63±3.13	19.13±0.83
JCO (UFDM)	88.84±3.06	15.77±0.58	410.80±1.39	165.87±1.62	10.10±0.28
MEO ($FD_{48}M_{48}$)	97.53±4.63	16.88±0.92	287.13±1.27	248.80±1.39	19.50±0.07
AOB ($FD_{72}M_{48}$)	93.52±6.12	16.95±0.08	312.00±6.93	213.83±3.34	12.21±0.03
TAC (UFDM)	86.84±1.16	20.13±1.65	165.93±4.36	214.67±1.15	9.26±0.08
KCC ($FD_{48}M_{48}$)	77.13±0.89	21.97±0.29	266.80±1.38	134.43±7.22	13.01±0.17
SCC ($FD_{72}M_{48}$)	57.46±2.30	13.48±0.03	337.63±8.50	124.43±2.27	11.70±0.02

Mean±SD of three replications.

JNO -Unfermented black lima bean and maize moi-moi
AOO -Moi-moi from black lima bean and maize fermented for 48h
MCO -Moi-moi from 72h fermented black lima bean and 48h fermented maize
VNO -Unfermented black lima bean and maize akara
PNO -Akara from black lima bean and maize fermented for 48h
VAO -Akara from 72h fermented black lima bean and 48h fermented maize
JCO-Unfermented dark red lima bean and maize moi-moi
MEO-Moi-moi from 48h fermented dark red lima bean and maize
AOB-Moi-moi from 72h fermented dark red lima bean and 48h fermented maize
TAC-Unfermented dark red lima bean and maize akara
KCC-Akara from 48h fermented dark red lima bean and maize
SCC-Akara from 72h fermented dark red lima bean and 48h fermented maize.

Table 17 presents the sensory properties of moi-moi and akara based on lima bean (black and dark red varieties) and maize flour blends.

Colour

The colour of the products was influenced by both fermentation and type of composite flour used for production. All the controls (unfermented lima bean and maize) had values lower than one half of the nine point hedonic scale. The JNO, the UFBM and the JCO, the UFDM had 3.77 and 4.07, respectively. The akara based on unfermented dark red lima bean and maize had colour value of 4.80. This value was more than one half of the scale. The AOO ($FB_{48}M_{48}$,), the MCO ($FB_{72}M_{48}$) and the MEO ($FD_{48}M_{48}$,) had highest and comparable values when compared with the other products. The PNO ($FD_{48}M_{48}$) as well as the KCC ($FD_{48}M_{48}$,) had comparable values 6.87 and 6.23. Similarity, exists among the VAO ($FB_{72}M_{48}$), the AOB ($FD_{72}M_{48}$) and the SCC ($FD_{72}M_{48}$) (5.90, 5.47 and 5.10). Equally, the VNO (UFBM) and the TAC (UFDM) also had comparable colour value (4.77 and 4.80).

Flavour

Flavour values varied for all the products. The range was from 3.57 to 7.07 (Table 17). The AOO ($FB_{48}M_{48}$) and the PNO ($FB_{48}M_{48}$,) had the highest value followed by those of the MCO ($FB_{72}M_{48}$) and the MEO ($FD_{48}M_{48}$,) (7.07, 7.03, 6.56 and 6.50, respectively). On the other hand, the VAO ($FB_{72}M_{48}$), the KCC ($FD_{48}M_{48}$,) and the SCC ($FD_{72}M_{48)}$) had comparable flavour as well as the AOB ($FD_{72}M_{48}$) and the TAC (UFDM) (5.37, 5.60, 4.57, 4.70, respectively) (Table 17). All the products based on untreated black or dark red moi-moi had lower flavour when compared with the other products (moi-moi and akara) (3.57 and 3.87, as well as 4.13, respectively).

Table 17: Sensory properties of products (moi-moi and akara) made from unprocessed and processed lima bean (black and dark red varieties) and maize composite flours.

Sample	Colour	Flavour	Texture	General Acceptability
JNO (UFBM)	$3.77^{e}\pm2.24$	$3.57^{f}\pm1.83$	$3.13^{f}\pm1.83$	$2.87^{e}\pm2.18$
AOO ($FB_{48}M_{48}$)	$7.40^{a}\pm1.45$	$7.07^{a}\pm1.34$	$6.23^{a}\pm2.25$	$6.17^{a}\pm2.04$
MCO ($FB_{72}M_{48}$)	$7.00^{a}\pm2.31$	$6.56^{b}\pm1.36$	$6.94^{a}\pm2.05$	$5.81^{b}\pm2.29$
VNO (UFBM)	$4.77^{d}\pm2.06$	$4.13^{d}\pm1.98$	$3.67^{d}\pm2.12$	$4.03^{c}\pm2.20$
PNO ($FB_{48}M_{48}$)	$6.87^{b}\pm1.89$	$7.03^{a}\pm1.27$	$6.10^{a}\pm2.11$	$6.07^{a}\pm2.08$
VAO ($FB_{72}M_{48}$)	$5.90^{c}\pm2.40$	$5.37^{d}\pm2.37$	$5.30^{b}\pm2.39$	$5.23^{b}\pm2.53$
JCO (UFDM)	$4.07^{d}\pm2.03$	$3.87^{e}\pm1.80$	$3.57^{d}\pm1.89$	$3.93^{d}\pm1.72$
MEO ($FD_{48}M_{48}$)	$7.10^{a}\pm1.21$	$6.50^{b}\pm1.68$	$5.83^{b}\pm1.90$	$6.87^{a}\pm1.14$
AOB ($FD_{72}M_{48}$)	$5.47^{c}\pm2.13$	$4.70^{d}\pm1.75$	$4.30^{c}\pm2.17$	$4.33^{c}\pm1.67$
TAC (UFDM)	$4.80^{d}\pm2.37$	$4.57^{d}\pm2.43$	$3.70^{e}\pm2.39$	$4.07^{c}\pm2.00$
KCC ($FD_{48}M_{48}$)	$6.23^{b}\pm1.50$	$5.60^{c}\pm1.54$	$5.43^{b}\pm1.74$	$4.97^{c}\pm1.75$
SCC ($FD_{72}M_{48}$)	$5.10^{c}\pm1.92$	$5.60^{c}\pm1.71$	$4.40^{c}\pm2.04$	$4.87^{c}\pm2.08$

Means within the same column with different superscripts are significantly ($P < 0.05$) different.

JCO -Unfermented dark red lima bean and maize moi-moi

MEO -Moi-moi from 48h fermented dark red lima bean and maize

AOB -Moi-moi from 72h fermented dark red lima bean and 48h fermented maize

TAC -Unfermented dark red lima bean and maize akara

KCC -Akara from 48h fermented dark red lima bean and maize

SCC -Akara from 72h fermented dark red lima bean and 48h fermented maize.

JNO -Unfermented black lima bean and maize moi-moi

AOO -Moi-moi from black lima bean and maize fermented for 48h

MCO -Moi-moi from 72h fermented black lima bean and 48h fermented maize

VNO -Unfermented black lima bean and maize akara

PNO -Akara from black lima bean and maize fermented for 48h

VAO -Akara from 72h fermented black lima bean and 48h fermented maize

Texture

The moi-moi and akara based on unfermented black and dark lima bean and maize had values lower than half of the scale (4.50 vs 3.33, 3.67 and 3.70). The two moi-moi products based on black lima bean variety had higher values (6.23 and 6.94) and 6.10 in comparison with the other products. However, the akara based on black lima bean and maize fermented for 48h had similar value (6.10). The moi-moi and akara based on 48h fermented dark red lima bean and maize flour blends had similar values (5.83 and 5.43, respectively). The moi-moi and akara based on the $FD_{72}M_{48}$ (AOB) as well as that of the $FD_{72}M_{48}$ (SCC) had comparable values 4.30 and 4.40.

General acceptability

The general acceptability of moi-moi and akara based on the two lima bean varieties were very low mostly those from the unfermented flour blends (2.87, 4.03, 3.93 and 4.07, respectively).

The moi-moi and akara based on the 48h fermented black lima bean and maize ($FB_{48}M_{48}$ – AOO and PNO) and moi-moi based on the $FD_{48}M_{48}$ were much more accepted than the other products (6.17, 6.07 and 6.87, respectively). The products that had the second highest and comparable general acceptability were those based on 72h fermented black lima bean and 48h fermented maize flour blends (5.81 and 5.23). The two akara based on the 48 and the 72h fermented dark red lima bean and the 48h fermented maize had equal acceptability (4.97 and 4.87, respectively).

Table 18 presents the proximate and mineral composition of dishes (*Oshoto* and *Ikpaki*) based on blends of whole black and dark red lima bean and maize grains.

Moisture

The moisture content of the dishes varied. It ranged from 18.70 to 21.40%. The moisture content of dishes based on black lima bean varieties were higher than those of the dark red lima bean (20.60, 20.11 and 21.40 vs 18.70, 19.60 and 19.80%, respectively). However, the moisture contents of all the dishes were relatively high.

Protein

Protein values were influenced by the type of lima bean with or without addition of maize, because the added food condiments were the same for the dishes. The black and dark red lima bean dishes (BVCP and BIKP) cooked without addition of food ingredients and maize had low protein values (6.48 and 4.46%, respectively). However, the protein of the dark red lima bean was lower than that of the black (4.46 vs 6.48%). The dish that contained

maize for both varieties (BOSH and DROSHI) had highest and comparable protein (30.11 and 31.37%) as against those cooked without maize (27.63 and 24.62%).

Table 18: Proximate and mineral composition, of dishes (*oshoto* and *ikpaki*) prepared from whole lima bean (black and dark red varieties) and maize grains as consumed (%).

Components	BVCP	BOSH	BIKP	DRVCP	DROSH	DRIKP
Moisture (%)	20.60±0.11	20.11±0.09	21.40±0.08	18.70±0.06	19.60±0.06	19.80±0.09
Protein (%)	6.48±0.06	30.11±0.12	27.63±0.12	4.46±0.03	31.37±0.13	24.62±0.12
Fat (%)	0.53±0.09	1.50±0.02	1.16±0.00	0.89±0.00	1.57±0.00	1.26±0.00
Ash (%)	3.00±0.01	4.45±0.03	4.37±0.01	3.38±0.01	4.70±0.02	4.15±0.02
Fibre (%)	2.96±0.00	9.08±0.06	7.03±0.01	2.86±0.01	9.26±0.03	7.95±0.03
Carbohydrate (%)	67.43±0.16	34.75±0.17	38.41±0.14	69.81±0.19	23.50±0.11	42.22±0.16
Calcium (mg/100g)	98.60±0.06	134±2.32	116.24±4.00	88.67±0.06	129.59±2.31	101.54±2.32
Magnesium (mg/100g)	12.30±0.10	16.10±0.01	13.42±0.27	11.29±0.01	17.89±0.50	16.77±0.84
Potassium (mg/100g)	203±2.07	316.83±23.67	308.33±6.17	276.29±1.22	431.07±2.62	362.27±4.20
Phosphorus (mg/100g)	153.21±0.22	260.73±7.00	184.27±3.59	210.33±0.18	283.10±7.15	234.17±1.59
Iron (mg/100g)	18.67±1.00	34.27±0.42	16.45±0.95	14.34±0.02	22.37±0.06	16.26±0.50

Mean ±SD of three replications.

BVCP - Black lima bean cooked plain
BOSH - Black lima bean cooked with corn and food ingredients
BIKP - Black lima bean cooked with food ingredients but no maize added
DRVCP- Dark red lima bean cooked plain
DROSH- Dark red lima bean cooked with maize and food ingredients
DRIKP- Dark red lima bean cooked without maize but food ingredients added.

Fat

The fat content of the dishes was controlled by the type of lima bean and food condiments. The lima bean cooked without the addition of food ingredients had lower values for fat than their counterparts (0.53 and 0.89%). The addition of maize and ingredients increased fat values as against those without maize (1.50, 1.57 vs 1.16 and 1.26%, respectively). However, the dark red lima bean cooked without maize had slightly higher fat than the black (1.26 vs 1.16%).

Ash

The ash values differed. It ranged from 3.00 to 4.70%. Both black and dark red lima bean cooked without any food ingredients had lower ash than the other dishes (3.00 and 3.38%).

However, both black and dark red lima beans cooked with maize had higher ash as against their counterparts (4.45, 4.47 vs 4.37 and 4.15%). The black lima bean cooked without maize had slightly higher ash than its counterpart (4.37 vs 4.15%) (Table 18).

Fibre

The fibre values varied. The values were again controlled by type of lima bean and the addition of both maize and food ingredients. The black and dark red lima bean cooked without food ingredients had the least fibre (2.96 and 2.86%). However, the dish based on dark red lima bean with only food ingredients (DRIKP) had lower fibre than its counterpart (2.96%). The dishes based on black and dark red lima bean maize had comparable fibre value (9.08 and 9.26%). However, the dish based on dark red lima bean and maize (DROSH) had a slight edge in fibre over the black lima bean cooked with maize and food condiments (9.26 vs 9.08, respectively). Equally, the dark red lima bean cooked without maize (DRIKP) had higher fibre than that of the black (BIKP) (7.95 vs 7.03%).

Carbohydrate

The carbohydrate composition of the dishes was controlled by the addition of food condiments and maize (Table 18). Both black and dark red lima beans cooked without food ingredients and maize had the highest and comparable CHO values (67.43 and 69.81%). Addition of maize to both types of lima bean caused differences in CHO. The black lima bean cooked with maize (BOSH) had higher Carbohydrate than the dark red counterpart

(34.75 vs 23.50% respectively). The CHO value for the dark red lima bean cooked without addition of maize was higher (42.22 vs 38.41%) than that of black.

Calcium (Ca)

The mineral content of the dishes based on cooked black and dark red lima bean with or without addition of maize varied The Ca values for the cooked black and dark red lima bean with maize differed. The Ca value for black was higher than that of the dark red (134.93 vs 129.59mg) (Table 18). Equally, the black lima bean cooked without maize (BIKP) had higher Ca than that of the dark red lima bean (DRIKP) cooked without maize (116.24 vs 101.54mg).

Magnesium (Mg)

The magnesium value for the dishes from the dark red lima bean were higher than those of the black (17.89 and 16.77mg vs 16.10 and 13.42mg) (Table 18). The black lima bean cooked without maize had the least magnesium as against the other dishes. Equally, the black lima bean cooked with maize (BOSH) had lower Mg. than its counterpart (DROSH) (16.10 vs 17.89mg) respectively.

Potassium (K)

The potassium content of the dishes varied. It ranged from 308.33 to 431.07mg. Both dishes based on dark red lima bean had higher potassium in comparison with those from black lima bean (431.07 and 362.27mg vs 308.33 and 316.83mg, respectively). The "*Oshoto*" (cooked lima bean and maize) prepared with dark red lima bean had the highest potassium than the rest of the dishes.

Phosphorus (P)

The phosphorus level of the dishes varied. The variations were due to the types of lima bean and the food ingredients added during preparation. The values ranged from 184.27 to 283.10mg. The black lima bean cooked with maize (BOSH) had lower phosphorus (260.73mg) than that of its counterpart DROSH (283.10mg). The *Ikpaki* black lima bean cooked without maize (BIKP) had the least phosphorus (184.27mg). This value is lower than that of the dark red lima bean (DRIKP) (184.27 vs 234.17mg).

Iron (Fe)

The Fe level for the dishes (*Oshoto* and *Ikpaki*) differed. The *Ikpaki* (cooked lima bean without maize) based on black lima bean variety had higher Fe (16.45mg) than that of

the dark red dish (16.26mg). Equally the black lima bean cooked with maize (*Oshoto*) had higher Fe than that of the dark red dish (34.27 vs 22.27mg).

Table 19 presents the sensory properties of dishes (*oshoto* and *ikpaki*) based on blends of whole black and dark red lima bean and maize grains.

Colour

The colour values for dishes based on both black and dark red lima bean with or without addition of maize differed. However, the differences were not significant (7.60, and 7.43 vs 7.40 and 7.83) ($P > 0.05$). The difference in colour between the black lima bean cooked with maize and that of dark red cooked with maize was 0.20mg (7.60 – 7.40). On the other hand, the dark red lima bean cooked without maize had an edge over that of the black dish (7.83 vs 7.43) (Table 19).

Flavour

The flavour of the dishes differed. The flavour of the dish based on black lima bean and maize was slightly higher (0.17) than that of the dark red dish (7.60 vs 7.77). The dish based on dark red lima bean without maize was slightly rated higher in terms of flavour than that of the black counterpart (6.93 vs 6.37).

Texture

Texture values were influenced by the type of lima bean and with or without addition of maize. The texture of both lima bean cooked with maize were low and comparable (6.47 and 6.33). The texture of the dark red lima bean cooked without maize had an edge over that of the black (8.27 vs 7.17).

General acceptability

There were variations in the general acceptability of the dishes. The type of lima bean influenced the general acceptability. The dishes based on dark red lima bean with or without maize were much more acceptable than those of the black lima bean (7.50 vs 7.00 and 6.73).

Table 19: Sensory properties of dishes (*Oshoto* and *Ikpaki*) prepared from lima bean (black and dark red varieties) and whole maize grain

	Colour	Flavour	Texture	General Acceptability
BVCP	5.63^{e}±1.23	6.00^{d}±1.27	6.94^{d}±1.71	6.56^{d}±1.56
BOSH	7.60^{b}±1.19	7.77^{a}±1.07	6.47^{e}±2.66	7.00^{b}±1.62
BIKP	7.43^{c}±1.96	6.37^{c}±2.40	7.17^{c}±1.26	6.73^{c}±2.08

DRVCP	6.60^{d}±1.12	$6,31^{c}$±1.19	8.00^{b}±0.93	7.00^{b}±1.21
DROSH	7.40^{c}±1.79	7.60^{a}±1.40	6.33^{f}±2.32	7.50^{a}±1.87
DRIKP	7.83^{a}±1.98	6.93^{b}±1.15	8.27^{a}±0.94	7.50^{a}±0.07

Means within the same column with different superscripts are significantly ($P < 0.05$) different.

BVCP - Black lima bean cooked plain
BOSH - Black lima bean cooked with corn and food ingredients
BIKP - Black lima bean cooked with food ingredients but no maize added
DRVCP- Dark red lima bean cooked plain
DROSH- Dark red lima bean cooked with maize and food ingredients
DRIKP- Dark red lima bean cooked without maize but food ingredients added.

CHAPTER FIVE

DISCUSSION

51. DIFFERENT VARIETIES OF LIMA BEAN (*Phaseolus lunatus*)

The seven varieties of lima bean identified were black, dark brown, muttle black, deep cream, dark red, golden brown and muttle brown. These variation in colour of these lima beans were attributed to varietal differences. The muttle black, deep cream and muttle brown were very rare. However, the black and dark red varieties were much more available because they are commonly cultivated and consumed by majority of the household in Arondizuogu. The black variety produce more yield than the dark red and can grow well even when planted in an unfertile land (Table 1).

5.2 EFFECT OF PROCESSING ON PROXIMATE COMPOSITION OF BLACK LIMA BEAN FLOUR

Moisture

The lower moisture for the BVDC and BVUDC (9.33%) showed that dehulling had little or no effect on the moisture content of the flours (Table 2). This lower moisture for the two flours as against the control suggested that they would have longer keeping quality. It is known that the lower the moisture content of a given food, the higher is the keeping quality or shelf life. The lower moisture for the $BCWND_o$ as against the control indicated that cooking without draining water reduces the moisture content of the flour, as such increased the shelf life. Researches have shown that some microorganisms that cause food spoilage do not thrive well in the absence of water (Frizer and Westhoff, 1978; Gaman and Sherrington, 1990).

The lower moisture (8.33%) for the 6h soaked sample indicated that it would keep longer than the control and the other soaked (3 and 9h) flours. Germination of black lima bean for 72 and 96h had equal effect on moisture (9.33%). The lower moisture for the 24h fermented flour indicated that 24h is the best period for reducing moisture in black lima bean.

Protein

The lower protein for the BVDC (7.18%) as compared with the control and the lima bean cooked without dehulling (BVUDC) was not surprising. It could be that some of the protein in the dehulled sample was overcooked and protein leached into the cooking medium. This finding was in line with the report of many workers (Enwere, 1998; Onimawo and Akubor, 2005). They observed protein denaturation during heat processing. Manay and Sharaswamy (2008) reported that longer cooking causes a drop in the nutritive value of pulses due to loss of lysine. Pulses should not be over cooked, to maintain protein quality. The protein level of the raw lima bean was high because it did not loose any protein to the

cooking water as its counterpart (14.05 vs 7.18%). The higher protein for the lima bean whose cooking water was drained four times and replaced during cooking is because it imbibed more water which broke the bond between antinutrients, enzymes and proteins to release more protein than cooking without changing water (8.48 vs 7.07%) (Table 2). This observation was similar to that of Egbe and Akinyele (1990).

The lower protein for the 6h soaked sample might be that it was the time, when protein could easily be leached out into the soaking medium. FAO (1993) reported decrease in nutritional value of cereals and legumes due to leaching losses during soaking. On the other hand, the comparable protein value for the 3 and the 9h soaked flour (26.70 vs 26.68%) showed that these soaking hours had no adverse effect on black lima bean – a commonly observed fact.

The slightly higher protein (25.34%) for the 120h germinated flour indicated that beyond 96h germination, more protein is released from its complex by germinating microflora enzymes. It is known that during germination, inactive enzymes are activated to hydrolyse bonds between food component, protein and enzyme. This releases more protein and precipitates antinutrients (e.g. phytate) into the germination medium to release and concentrate more protein. This increase in protein due to germination was in agreement with results obtained by other workers. Idouraine *et al.* (1989) reported a 12% increase in crude protein content of tepary beans germinated for 14h. King and Puwastein, (1987); Akpapunam and Achinewhu (1985) gave similar reports for winged beans and cowpea, respectively. This increase might be due to rapid synthesis of hydrolytic enzymes as well as loss of initial dry matter.

The higher protein for the 48h fermented flours might be the optimum enzyme activity to release more free amino acids and reduce use of protein for microflora metabolism.

Fat

The lower fat (0.51 to 1.60%) for both the control and the treated flour samples was not a surprise. This was because legumes store energy in form of carbohydrate and not fat. The much lower fat for BVDC (0.51%) might be that the fat content of black lima bean was volatile and lost during dehulling.

The slightly higher fat (0.70%) for the undehulled cooked flour might be that the hull prevented loss of fat. The slightly higher fat (0.53%) for the $BVCWD_4$ as against the

$BVCWND_o$ (0.41%) indicates that changing water during cooking of black lima bean increased fat than without changing cooking water (Table 2).

The lower fat (0.51 and 0.56%) for the 3 and the 6h soaked flours indicated that these flours would have longer keeping quality because there would be least chance for oxidative rancidity. It is known that the higher the fat content of a food, the higher is its rancidity risk. Obizoba and Amaechi (1992) noted that the lower the lipid level of flour, the higher the shelf life and the better the flavour and aroma.

The comparable fat (0.70 and 0.88%) for the 72 and the 96h germinated flour samples indicated that these germination periods are beneficial in reducing fat content of lima bean as against 120h (Table 2). The reduction in fat during germination was probably caused by the breakdown of fat by β-oxidation for energy purposes for embryo development. This finding was in agreement with the report of Idouraine *et al.* (1989) who observed a 20% decrease in fat of tepary beans germinated for 14h.

The lower fat for the 24 and the 72h fermented flour might be attributed to the metabolic activity of microorganisms. Anosike and Egwuatu (1981) reported decrease in lipid content of fermented castor oil. Vander Riet *et al.* (1987) observed decrease in fat as fermentation periods increased.

Ash

The lower ash (1.76%) for the dehulled cooked sample as against its control and undehulled cooked samples (BVDC, BVUDC and RBV) (1.76 vs 5.00 and 6.12%) is simple to explain. Minerals are found in hulls of grains as such, when the grains are dehulled and cooked, the ash might be lost in the hull as well as the cooking water. Magdi (2007) had similar findings in cowpea and lablab bean, respectively.

The lower ash (3.00%) for the flour whose cooking water was changed and replaced four times might be because of vegetative loss of ash as well as in cooking water and vice versa. The higher ash for the 6 and the 9h soaking as against 3h indicated that the longer the soaking hour, the higher the release of ash from black lima bean seeds (4.87, 4.10 vs 2.81%). The slightly higher ash for the 6h soaked sample than that of the 9h (4.87 vs 4.10%) indicated that soaking beyond 6h was not beneficial. The much more ash (5.61%) for the 120h germinated sample indicated that 120h was the optimum germination period to release available ash from black lima bean. The higher ash (5.81 and 5.35%) as against 3.98% for the 48 and 72h fermented flours indicated that the 48 and the 72h fermentation were better

fermentation periods to increase ash content of black lima bean. However, this increase in ash may be due to loss of dry matter (Obizoba and Anyika, 1993).

Fibre

The lower fibre (2.83%) for the black lima bean dehulled and cooked as against the control (2.83 vs 4.98%) indicated that dehulling and cooking adversely reduced fibre needed in foods to induce faster bowel movement, thus prevent constipation and diverticulosis. Dehulling of grain seeds reduces their fibre content (Rao and Deosthale, 1983).

On the other hand, the lower fibre for the BVUDC as against the RBV (4.08 vs 4.98%) clearly showed that undehulling and cooking equally had adverse effect on fibre content of lima bean. The slightly higher fibre for the sample whose cooking water was drained and replaced (BVCWD) had an edge over its counterpart (2.96 vs 2.9%) in fibre availability. The slightly higher fibre for the 9h soaked sample as against the 3h showed that it is a better soaking period to increase fibre in black lima bean (4.51 vs 4.43 and 4.15%). The soaking for 6h had much more adverse effect on fibre content when compared with those of the 3 and the 9h (4.15 vs 4.43 and 4.51%, respectively). The higher fibre for the 120h germination (4.09%) as against those of the 72 and the 96h (4.09 vs 3.93 and 3.35%) indicated that 120h germination is the optimum germination period to increase fibre in black lima bean. However, the 96h had much more adverse effect on fibre values (3.35%). The lower fibre (3.35%) might be that the growing seedling used it for metabolism or loss in germination medium – a commonly observed phenomenon.

The lower fibre for the 72h fermentation is difficult to explain. However, it might be due to increase in microflora number and the use of fibre for metabolism. On the other hand, the higher fibre (4.35%) for the 48h fermented black lima beanmight be that more fibre was produced by microflora enzymes and less used for metabolism as against the fibre of the 72h (4.35 vs 3.19%).

Carbohydrate

The higher carbohydrates for the BVDC than the control was due to loss of moisture (49.42 vs 79.39%). It is known that loss of moisture increases dry matter of which carbohydrate is one. The slightly higher carbohydrate in the $BVCWND_o$ as against that of the $BVCWD_4$ (74.79 vs 73.86%) might be that carbohydrate was not lost because the cooking water was not discarded.

The higher carbohydrate (62.18%) for the 3h soaked sample, might be that at shorter soaking period, more carbohydrate is released from the whole grain as against those of the 6

and the 9h samples (62.18 vs 54.33 and 57.01%). The lower carbohydrate value (53.48%) for the 120h germinated samples as against the 72 and the 96h (53.48 vs 59.97 and 57.77%) is simple to explain. At 120h germination, the seedling might be utilizing carbohydrates for its metabolism. There might be an increase in the number of microflora as compared with the other two periods of germination. Sathe and Salunkhe (1984) reported 91% reduction of oligosaccharides after a 4-day germination of chick pea. The lowest carbohydrate (32.95%) for the 48h fermented sample could be due to: (a) increased microflora activity and their number;

(b) increased dependence of these microfloras on carbohydrate from fermenting lima bean as a source of energy. The slightly lower carbohydrate for the 72h fermented flour (56.45%) as against that of the 24 (60.78%) indicates that the higher the fermentation period beyond 48h, there is decrease in carbohydrate concentration. This use of sugar for metabolic processes by microorganisms during fermentation reduces carbohydrate levels have been reported by many researchers (Eka, 1980; Achinewhu and Isichei, 1990; Obizoba and Egbuna, 1992; Anosike and Egwuatu, 1981).

5.3 EFFECT OF PROCESSING ON MICRONUTRIENT COMPOSITION OF BLACK LIMA BEAN FLOURS

Zinc (Zn)

The lower zinc (3.13mg) for the undehulled cooked black lima bean might be due to treatment (Table 3). Cooking reduced the Zn level in all the cooked flour samples in comparison with the control except for the BVDC. This clearly indicated that raw black lima bean is a good source of zinc. Aletor and Ojo (1989) observed a decrease in the mineral constituents of legume seeds after cooking and it is likely that the decrease was due to leaching of minerals on account of the enhanced permeability of the seed coat by the process of cooking.

The higher zn (3.76mg) in the 9h soaked sample demonstrated that increased soaking improved zinc availability in black lima bean. The higher zn at 120h germination, might be due to increased activity of the activated enzymes due to absorption of more water that releases free zinc for use in metabolism. The lower zinc values for the 48 and the 72h fermented flours (4.08 and 4.10 vs 4.24mg) indicated that fermentation of black lima bean beyond 48h was not beneficial for zinc extraction. This finding was contrary to the report of FAO/WHO (1996).

Copper (Cu)

The lower Cu value for the black lima bean dehulled and cooked (BVDC) as against the raw and the undehulled cooked samples (13.17 vs 13.30 and 13.73mg) could be attributed to Cu loss during dehulling and cooking. Apata and Ologhobo (1994) reported that cooking decreased Cu, Zn and Fe content of lima beans.

The slightly higher Cu between undehulled cooked and dehulled cooked black lima bean (13.30 vs 13.17mg) proved that dehulling and cooking had adverse effect on Cu availability than undehulling and cooking. The similarity in Cu between lima bean whose cooking water was drained and replaced and its counterpart (12.50 and 12.33mg) showed that neither of the domestic food processing technique had an edge over the other. The lower Cu for the flour sample soaked for 6h (11.63mg) indicated that this method has an adverse effect on Cu nutriture. On the other hand, a comparable Cu value (13.23 and 13.73mg) for 3 and 9h soaked sample showed that either of the soaking periods had equal effect on availability of Copper. The equal availability of Cu due to germination or fermentation indicated that these two processing techniques had beneficial effect on Cu availability (12.30 to 13.20mg and 13.20 to 13.57mg, respectively). .This observation confirms the report of Manay and Sharaswamy (2008) that Cu increased in dry seeds after sprouting. They attributed the increase in Cu to decreased phytate that released Cu and made it more available in the dry seeds.

Iron (Fe)

The higher iron (87.33 and 49.00mg) for the raw and the undehulled cooked black lima bean appears to suggest that iron is much more available in unprocessed black lima bean. The lower Fe value 23.67mg for dehulled cooked lima bean might be due to loss during dehulling and cooking. This finding accords that of Ologhobo and Apata (1994). This might be because iron concentration is more in the hull than in the cotyledon. The lower Fe value (14.33mg) for black lima cooked with cooking water discarded and replaced four times, showed that cooking and changing cooking water decreased iron in the bean much more than its counterpart (14.33mg vs 33.33mg). This decrease in iron was not surprising. Excessive heat treatment (over-cooking) may have detrimental effect on nutritive value, functionality and sensory properties which determine acceptability of food products (Manay and Sharaswamy 2008). The higher and comparable iron for the 3 and the 6h soaking (47.00 and 45.00mg) indicated that either of the two periods could be adopted to maintain availability of

Fe. On the other hand, the least Fe value (41.00mg) showed that the 9h soaking period was not as good as those of the 3 and the 6h soaking.

The similarity in iron concentration among the three periods of germination (72-120h) showed that germination had equal effects on iron availability (43.33, 44.33 and 44.00mg, respectively. This observation was in agreement with that of Obizoba (1991). The lower Fe for the 72h fermentation of black lima bean (21.67mg) indicated that fermentation of lima bean beyond 48h is not advisable for availability of Fe in black lima bean. However, the slight differences in iron (45.00 and 41.00mg) in the 24 and the 48h fermentation periods showed that none of the periods had an edge over the other.

Phosphorus (P)

The drastic reduction in phosphorus, particularly in the dehulled cooked black lima bean (16.00mg) versus the control (83.00mg) strongly supports that dehulling had adverse effect on phosphorus availability in black lima bean dehulled cooked as against the raw and the undehulled cooked samples (83.00, 48.67 vs 16.00mg).

The slightly higher P (18.00mg) in the black lima bean whose cooking water was changed and replaced against its counterpart (14.00mg) indicated the slight superiority between the two food processing techniques (18.00 vs 14.00mg). The higher P for the 6 and the 9h soaking periods (46.00 and 49.67mg) suggested that the 6 and the 9h soaking are better periods for increasing availability of phosphorus in black lima bean. Furthermore, soaking for 6h had a difference of 2.57mg when compared with the sample soaked for 3h (46.00 vs 43.33mg).

The lower phosphorus value (42.00mg) for the black lima bean germinated for 72h when compared with those of the 96 and the 120h (47.00 and 49.33mg) showed that the later periods had an edge over the former (72h). This decrease in P for the 72h germinated lima bean was in line with the reports of Yousef *et al* (1987) in faba beans and Akpapunam and Achinewhu (1985) in cowpea. The lower P values (44.00 and 42.67mg) for flour samples fermented for 48 and 72h as against that for 24h (48.00mg) indicates that the 24h fermentation is the optimum period for much more phosphorus availability.

Folate

The lower folate (5.77mg) for the black lima bean undehulled cooked as against the dehulled cooked sample (7.37mg) depicts the superiority of dehulling against undehulling. The comparable folate (7.23 and 7.37mg) for the raw and the dehulled cooked black lima

bean demonstrated that folate could be equally available in the raw and the dehulled cooked sample. This confirms the report of Wood (1988) that lima bean is a rich source of folate. The lower folate (5.77mg) for the black lima bean cooked without discarding and replacing cooking water was an indicative of superiority of cooking and changing cooking water four times (7.27 vs 5.77mg).

Soaking black lima bean for 3 to 9h had comparable effect on folate. However 3h soaking had only 0.10mg folate over the other soaking periods (5.57 vs 5.47mg) (Table 3). Equally, germination and fermentation had comparable effect on folate. However, the 72h fermentation produced a lower folate (4.37mg) as against 5.40 to 5.63mg for both germinated and fermented flours.

The decrease in folate for germinated flours relative to the control (5.40 – 5.50 vs 7.23mg), was in agreement with the report of Manay and Sharaswamy (2008) that folic acid content of pulses greatly decreases during germination.

5.4 EFFECT OF PROCESSING ON ANTINUTRIENTS AND FOOD TOXICANT CONTENT OF BLACK LIMA BEAN FLOURS.

Phytate

Dehulling and cooking drastically reduced phytate from 47.47mg to 0.28mg. On the other hand, cooking alone (BVUDC) had little effect on phytate when compared with that of the raw RBV (42.30mg vs 47.47mg). There was only 0.02mg reduction in phytate due to draining and replacing cooking water. The least value 0.28mg phytate for the 6h soaking showed its edge over the other soaking periods in reduction of phytate level in black lima bean (0.28mg vs 3.10 and 8.50mg). Magdi (2007) attributed the decrease of phytate content of beans by soaking, cooking and dehulling to the leaching out of this compound in water. It is well known that phytate concentration is much in the hull, as such dehulling usually cause a rapid decrease in phytate levels of most legume seeds. The zero phytate value for the 72 and the 96h germination as against the 120h period (2.60mg) demonstrated that the 120h germination period had no beneficial effect on phytate reduction.The drastic decrease in phytate for the 72 and the 96h germination accords the report of Sandberg (1991) that during germination process, activity of the enzyme phytase increases, causing the phytic acid to break down. Fermentation of black lima bean beyond 48h (0.50mg) had the most beneficial effect in the reduction of phytate as against the other periods (0.50 vs 2.77 and 2.8mg). The significant decrease in phytate level of the 72h fermented flour was in agreement with the suggestions of Sandberg (1995). Manay and Sharaswamy (2008) reported that toxic

substances in legumes can be drastically reduced or eliminated through fermentation, especially longer periods. The observed decrease in phytate of the 72h fermented flour as compared to the control was due to the activities of the enzyme phytase. This enzyme activity increased during fermentation (Fardiaz and Markakis, 1981; Sutardi, 1988). It caused degradation of the phytate in legumes which may increase certain metals and decrease phytate after fermentation. Many workers observed similar decrease in phytate of fermented foods (Akpapunam and Achinhewhu, 1985; Obizoba and Atti, 1991; Nnam, 1994).

Tannins

The lower tannins due to dehulling, cooking, changing and replacing cooking water showed that these processing methods can effectively reduce tannins to zero levels when compared with raw lima bean (12.70 to 0.00mg) (Table 4). Osagie (1998) recorded similar findings in his research on effect of processing on the reduction of antinutrients in plant foods. The lower tannins (0.00mg) for the 9h soaked sample suggested that it is much more effective in the reduction of tannins to zero as against the 3 and the 6h soaking as well as the control (0.00 vs 4.36, 7.52 and 12.70mg). The possible reasons, why the 3 and the 6h soaking did not effectively reduce tannins in black lima bean might be that the soaking periods were not long enough. Although both the 3 and the 6h reduced tannins when compared with raw (control) (7.52 and 4.36 vs 12.70mg). These values demonstrated that the longer the soaking period, the lower the tannins levels (7.52, 4.36 and 0.00 vs 12.70mg, respectively). Obizoba and Egbuna (1992) reported that tannins content of legumes could be reduced by soaking and fermentation. Surprisingly, the longer the germination period, the higher is the tannin concentration. The lower tannins for the 72h germination (0.00mg) showed that 72h was the optimum germination period to reduce tannins to zero and germination beyond 72h had higher tannins, particularly the 120h germination period. The possible reason for this increase in tannins as germination periods increased (0.00 to 47.23mg) might be that beyond 72h period, this process hydrolysed and released some non-tannin compounds which was not able to be eliminated. This is possibly because the equipment was not sensitive enough to detect the accompanied compound – a commonly observed phenomenon in germination of legumes and cereals.

The lower tannins values for the 24 to the 72h fermentation against the control (0.00 to 6.06mg vs 12.70mg) was that the longer the fermentation period, the lower the tannin level as shown in the 48 and the 72h fermented sample. The zero value for the 72h fermented flour indicates the optimum fermentation period to reduce tannins in lima bean as well as the 48h

fermentation and vice versa. Tannins is known to affect the availability of both protein and minerals. The hydrolysis of tannins complexes (protein-tannins, tannic acid-starch and tannins-iron complexes) during fermentation was probably the explanation for the decreases in tannins levels (Nnam and Obiakor, 2003).

Hydrogen Cyanide

The lower hydrogen cyanide (HCN) for BVDC, BVUDC, BCWD and BCWND (12.25, 0.00, 2.10mg, respectively) depict the effectiveness of dehulling cooking, changing cooking water at intervals and not changing cooking water on the reduction of hydrogen cyanide in black lima bean when compared with the control (36.44mg). This observation was in accord with the report of Egbe and Akinyele (1990). They reported decrease in hydrogen cyanide in cooked lima bean. The lower hydrogen cyanide for all the three soaking periods (0.00 to 2.38mg) indicates that soaking is one of the best methods used to reduce HCN in lima bean, particularly the 3 and the 9h periods (0.00mg). This observation was in agreement with the reports of Nwokolo, (1996); Sathe and Sahunke (1984).

The low hydrogen cyanide (2.38mg) due to 6h soaking as compared with the control (36.44mg) showed that it drastically reduced HCN. However, it was not as effective as the other two soaking periods.

The reduction in HCN from 36.44mg to 4.58, 4.37 and 8.07mg for the germination periods (72-120h) demonstrated the efficacy of germination to reduce the food toxicant (HCN).The decrease in the HCN content of the germinated flours could be attributed to the presence of the hydrolytic enzyme β-glucosidase, which hydrolysed the parent cyanogenic glucoside (linamarin) to Cyanohydrin and finally to free Cyanide in the presence of the appropriate enzyme (hydroxynitrile lyase). The free cyanide formed is lost either through leaching or translocation to other developing parts such as the root, shoot or leaves of the growing plant for defensive purposes. Dibofori *et al.* (1994) reported a significant reduction in the cyanide content of lima beans during germination, especially on the 3-4 days.

However, the higher HCN for the 120h germination (8.07mg) indicates that 72 and 96h germination periods had advantage over the 120h germination (4.58 and 4.37mg vs 8.07mg, respectively). The zero HCN values for the 24, 48 and 72h fermented flours indicates that any of the periods is good as the other in effective reduction of HCN in black lima bean. This zero HCN level in all the fermented samples was not surprising. Manay and Sharaswamy (2008) noted that the toxic substance in food legumes (pulses) can be eliminated by fermentation. However, all the processed flours had a Cyanide content within safe level.

According to Manay and Sharaswamy (2008) a cyanide content in the range of 10-20mg per 100g of pulse is considered safe. They emphasized that many legumes, except lima bean (*Phaseolus lunatus*) contain Cyanide within this limit.

Trypsin inhibitor

The lower trypsin inhibitor values for cooking and discarding cooking water at intervals, cooking without changing water, undehulling and cooking, and dehulling and cooking (0.67, 1.33, 1.40 and 1.50mg) respectively as against the control (27.73mg) showed that these processes were efficacious in reduction of trypsin inhibitor. However, cooking and changing cooking water at intervals was much more effective (0.67mg) as compared with other processes. This finding accords the report of Manay and Sharaswamy (2008) that cooking destroys the enzyme inhibitors and thus improves the nutritional quality as well as palatability of food pulses. The lower trypsin inhibitor for the 9h soaked sample as against those of the control and other soaking periods (0.00 vs 4.27, 22.73 and 27.73mg) indicated that soaking is very effective and can be regarded as the optimum soaking period in the reduction of trypsin inhibitor level in black lima bean.

The lower trypsin inhibitor for the 6h soaking period (4.27mg) as against that of the 3h, further demonstrated that the longer the soaking period, the lower is the level of trypsin inhibitor (4.27 vs 22.73) (Table 4). This decrease in trypsin inhibitor might be attributed to leaching into the soaking medium (Khokhar and Chauhan, 1986).

The lower trypsin inhibitor for the 72h germination (6.27mg) as compared with the other two germination periods showed that the 72h germination was much more effective than the other periods in reduction of trypsin inhibitor (6.27 vs 12.60 and 18.67mg, respectively).

The zero values (0.00mg) for trypsin inhibitor in the 48 and the 72h fermented flours as against the control and the 24h fermentation (0.00 vs 26.00 and 27.73mg, respectively, proved the efficacy of the 48 and the 72h fermentation periods in the drastic reduction of trypsin inhibitor. The high trypsin inhibitor for the 24h fermentation period relative to the control (26.00 vs 27.73mg) further demonstrated that trypsin inhibitor was reduced with increased fermentation period. The synergistic effect of soaking, cooking, fermentation and germination in the reduction of trypsin inhibitor level in lima bean was not surprising. Ene-Obong (1995) observed that a decrease in trypsin inhibitor, phytate and tannins content of legumes can be achieved through traditional food processing methods.

Haemagglutinin

The less than one miligramme (1mg) level of haemagglutinins in the dehulled cooked, (BVDC), the undehulled cooked (BVUDC), the BVCWD and the BVCWND (0.76, 0.64, 0.26 and 0.45mg) showed that these processes were very effective in the reduction of haemaglutinin in black lima bean as against the control (2.56mg). However, cooking and changing cooking water four times was much more effective for reducing haemaglutinin when compared with the other treatments (0.26 vs 0.45, 0.64, 0.76mg, respectively). Lowgren and Leiner (1986) reported 90% reduction in haemaglutinin level in cooked brown and kidney beans.

The lower haemaglutinin values for soaked samples as against the control (0.42, 0.98 and 1.30 vs 2.56mg) showed that soaking for the 6 and the 9h was more effective in reducing haemaglutinin than the 3h (0.42, 0.98 and 1.30mg, respectively).

The increase in haemaglutinin due to germination as against the control (3.58 to 3.92 vs 2.56mg) was not surprising. It has been shown that some of the food processing techniques instead of reducing antinutrients or food toxicants, increased them. This might be attributed to hydrolysis of other food toxicants along with haemaglutinin by active germination enzymes such as phytase, which were not detected by the method of analysis employed.

The lower haemaglutinin as fermentation period increased might be due to increased activity of microflora enzymes as compared with the control (2.08, 1.48 and 0.06 vs 2.56mg) (Table 4).

Saponin

The lower saponin for the dehulled cooked, the undehulled cooked, the cooking and changing cooking water at intervals and cooking without discarding and replacing cooking water (0.27, 0.43, 0.12 and 0.48) relative to the control (113.07mg) showed that these processing techniques were effective methods to reduce saponin in black lima bean, especially cooking and changing cooking water at intervals (0.12mg) (Table 4). This observation was contrary to the report of Osagie (1998) that saponin are not easily destroyed during cooking. The low saponin values for all the soaked samples as against the control (27.50, 12.41 and 4.31 vs 113.07) showed that soaking was effective in reducing saponin but much lower than cooking (27.50, 12.41, 4.31 vs 0.27, 0.12 and 0.48mg, respectively).

Germination reduced saponin in black lima bean relative to the control (36.22, 44.23, 96.71 vs 113.07mg). However, the 72 and the 96h germination were much more effective in the reduction of saponin in black lima bean than the 120h germination (36.22, 44.96 vs 96.71mg). These values further showed that the longer the germination period, the higher is

the saponin concentration in black lima bean. The low saponin due to fermentation might be due to high activity of microflora as compared with that of the control (4.86, 0.22 and 0.66mg vs 113.07mg). The values indicate that fermentation beyond 48h period was not beneficial as regards saponin reduction (0.22 vs 0.66mg).

5.5 EFFECT OF PROCESSING ON FUNCTIONAL PROPERTIES OF BLACK LIMA BEAN FLOURS.

Foam capacity and foam stability

The lower values for foaming capacity of black lima bean except for the 96 and the 120h germination (2.60 and 3.3%) regardless of treatments showed that any of the domestic food processing techniques adversely affected the foam capacity of black lima bean, relative to the control (0.13 to 3.30 vs 4.20%). Thus decreasing the flour suitability in baking because foam is used to improve texture, consistency and appearance. The low foam capacity of all the cooked flour samples when compared with the control (0.17, 0.20, 0.23, 0.47%% vs 4.20%) indicated that foam stability is reduced in heat processed flours. This is attributed to the denaturation of the proteins on heating that became less soluble. It is well known that foam stability is affected by protein denaturation (Onimawo and Akubor, 2005). Native protein gives higher foam stability than denatured protein. Infact, foam capacity and foam stability are used as indices of formability of protein dispersion.

Water absorption capacity (WAC)

The lower water absorption capacity for the 24 and the 72h fermentation showed that these two periods of fermentation adversely affected water absorption capacity of black lima bean in comparison with the control (4.00, 3.00 vs 5.00%, respectively). These flours had poor ability to absorb and retain water. The increases between two and six folds of the control for all the processed samples except the 23 and 72h fermented flours demonstrated that the48h fermentation and the other treatments are good food processing techniques to increase water absorption capacity in black lima bean flour. It is well known that water absorption capacity is an index of the ability of protein to absorb and retain water which influence the sensory quality of foods. It is assumed that the protein in all the processed flour samples and the 48h fermented sample had high ability to absorb and retain water, hence the much more increase in water absorption capacity relative to the control (9.00 – 31.00).

The higher and comparable values for undehulled cooked and the 96h germinated black lima bean flour (31.00%) showed that they had slight edge over the other food

processing techniques in improving water absorption capacity. It is known that the higher the water absorption capacity of a flour, the higher is its suitability in baking industries. This is because of the interaction of protein with water for properties such as hydration, swelling, solubility and gelation. High water absorption capacity enable bakers to add more water to doughs and so improves handling and characteristics (Onimawo and Akubor, 2005).

The higher water absorption capacity values for all the cooked flours (29.67 to 31.00%) relative to the control (5.00%) was not surprising. These observations were similar to the findings of Oniwawo and Akubor (2005). They observed that heat treatment increased the water absorption of seed flours. They added that during heating, major proteins are dissociated into subunits that have more water binding sites than the native or oligomeric proteins. Gelatinization of carbohydrates and swelling of crude fibre may also occur during heating leading to increased water absorption.

Oil absorption capacity (OAC)

The lower oil absorption capacity except for the 3h soaked and the 24 fermented samples (15.00% and 15.33%) suggested that all the treatments were beneficial for reducing oil absorption capacity. It is known that the lower the oil absorption capacity of a given flour, the lower the risk of spoilage due to rancidity for its products. On the other hand, the higher oil absorption capacity for the 3h soaked and the 24h fermented flours (15.00 and 15.33%) indicated that these flours would have good baking quality because fat improves the texture and flavour of baked products and also gives feeling of satiety (Nnam and Obiakor, 2003). Furthermore, the mechanism of fat absorption is attributed to the physical entrapment of oil and the binding of fat to the polar chains of protein although it has not been fully explained (Kinsella, 1976).

The lower values for oil absorption capacity observed in all the cooked black lima bean (6.33, 5.33, 6.00 and 6.00%) as against the control (14.00%) were contrary to the report of Onimawo and Akubor (2005). They reported that heat processing increases the oil absorption capacity of seed flours. This increase could be due to both dissociation of the proteins that occur on heating and denaturation which unmasked the non-polar residues from the interior of the protein molecule.

Emulsion capacity (EC)

The high emulsion capacity for the 9h soaked, the 72 and the 120h germinated samples (16.00, 12.00 and 19.67%) relative to the control (11.00%) indicated that these treatments improved the emulsion capacity of the flours. Studies have shown that the higher

the emulsion capacity of a flour, the more suitable the flour is for baking products. It has been observed that increase in protein concentration facilitates absorption of protein resulting in enhanced emulsion stability.

The low emulsion capacity value for other treatments (2.00 to 8.00%) suggested that they are not beneficial for enhancement of emulsion capacity in black lima bean. The lower the emulsion capacity of a given flour, the poor is the quality for use in baking products – a commonly observed fact.

The low emulsion capacity for the cooked flour samples, the BVDC, the BVUDC, the BVCWD and the BVCWND relative to the control, was due to long cooking time. Cooking decreases emulsion capacity of flours. This might be attributed to decreases in protein solubility during heating, as protein concentration and solubility affects emulsion capacity. It is observed that as protein solubility decreases, the emulsion capacity decreases and vice versa.

Nitrogen (protein) solubility

The lower values for nitrogen solubility (0.00 to 2.84%) for all the cooked, soaked and fermented flour samples as against the control (5.10%) showed that cooking, soaking and fermentation had adverse effect on nitrogen solubility in black lima bean. The lower protein solubility for the four cooked flour samples in comparison with the soaked, germinated and fermented samples except for the 48 and the 72h fermented flours might be attributed to heat treatment. Heat decreases nitrogen solubility of legume (seed) flours, possibly as a result of protein denaturation and subsequent aggregation. Manay and Sharaswamy (2008) noted that prolonged cooking result in loss of lysine and this reduces the quality of protein.

On the other hand, the high values for the 6h soaking, the 96 and the 120h germination, indicated that they were the better food processing techniques to improve protein solubility in black lima bean flours.

5.6 EFFECT OF PROCESSING ON PROXIMATE COMPOSITION OF BLACK LIMA BEAN AND MAIZE FLOUR BLENDS.

Moisture

The lower moisture values for the SBV_9M_{48} and the GBV_{96} M_{48} (3.17 and 4.33%) indicated that the blends would have better keeping quality when compared with the other blends. The comparable moisture values for the $FBV_{72}M_{48}$ and the $GBV_{120}M_{48}$ (7.27 and 7.33%) suggested that none of these composites had an edge over the other, as well as those of the $CBVM_{48}$ and the $FBV_{48}M_{48}$ (6.33 and 6.17%, respectively).

Protein

The lower protein for the cooked black lima bean and the48h fermented maize flour blend ($CBVM_{48}$) as compared with the other blends was not a surprise. It is known that heating at high temperature denatures protein as such lowers its quality. Manay and Sharaswamy (2008) gave similar report that cooking causes a drop in the nutritive value of pulses as it results in the loss of lysine and this reduces the quality of proteins. The lower protein might also be due to poor mutual supplementation effect between black lima bean and maize flours. The highest protein for the $FBV_{48}M_{48}$ (21.06%) showed that the 48h fermented lima bean and maize flours had much more mutual supplementation effect and vice versa. This higher protein might also be due to microbial synthesis. Some of the dormant proteolytic enzymes were activated during fermentation process which hydrolysed protein to release more free amino acids. This observation was in line with the report of previous works on fermented legumes and cereals (Zamora and Field, 1979a; Hamad and Field, 1979).

Fat

The least fat values for the cooked black lima bean and the 48h fermented maize blends, ($CBVM_{48}$ and the $FBV_{72}M_{48}$ (1.60 and 1.69%) showed that these composite flours would have better shelf life and lower risk of oxidative rancidity (Obiakor, 2001). Furthermore, the low fat content of these flours are beneficial to health, because of the role of low fat diet in cholestrol reduction in human system (Ensminger and Ensminger, 1986). The highest fat value (3.24%) for the 9h soaked black lima bean and the 48h fermented maize blend (SBV_9M_{48}) suggested that it would have a low shelf life and high risk of oxidative rancidity. However, it is known that the higher the fat content of a given flour, the higher is the flavour and improved texture of its baked products. Depending on the products the flour would be used for. In the preparation of akara, for instance, if the composite flour has high fat content, it may absorb more oil and thus increase the fat content of the akara. The increased fat may adversely affect the quality of akara as well as health of the consumers.

Ash

The lower ash for the $FBV_{72}M_{48}$, the $GBV_{96}M_{48}$ and the $GBV_{120}M_{48}$ showed that these combinations when consumed would be a poor source of ash or mineral. On the other

hand, the slightly low ash value for the $CBVM_{48}$ when compared with the control appears to have an edge over the other blends (3.31 vs 4.46%).

Fibre

The slightly lower fibre for all the composite flours (3.28 – 4.71%) except for the $FBV_{72}M_{48}$ (2.62%) relative to the control (5.19%) indicated that these blends from black lima bean and maize would be a good source of fibre. High fibre flours, food and food products are highly recommended for good health. Evidence abound that high fibre prevents development of colon cancer and increases gastrointestinal motility as well as lowering of cholesterol levels (Wood, 1988).

Carbohydrate

The similarity in carbohydrate value for the $GBV_{120}M_{48}$ and the $FBV_{72}M_{48}$ (68.59 vs 69.96%) suggested that treatment had no influence on the carbohydrate content of these black lima bean and maize composite flours. The comparable carbohydrate values (71.45 and 71.39%) for the SBV_9M_{48} and the $FBV_{48}M_{48}$ showed that neither soaking for 9h nor fermentation for 48h influenced carbohydrate concentration. The higher carbohydrate for the $CBVM_{48}$ (79.27%) might be due to mutual supplementary effect between the two flours. This increase in carbohydrate level for the $CBVM_{48}$ suggested that cooking increases carbohydrate content of lima bean flours. This increase in carbohydrate might be due to gelatinization of starch (Roday, 2007).

The slightly higher carbohydrate value (63.35%) for the $GBV_{96}M_{48}$ than the control ($UBV_{12}M_{48}$) (61.48% %) showed that none of the composites had an edge over the other in influencing carbohydrate concentration.

5.7 EFFECT OF PROCESSING ON MINERAL COMPOSITION OF BLACK LIMA BEAN AND MAIZE COMPOSITE FLOUR.

Zinc (Zn)

The increases in Zinc for all the flour blends, except for the $CBVM_{48}$ as against the control (3.27mg) demonstrated that soaking, fermentation and germination had an edge as domestic food processing techniques over cooking (4.02, 5.46, 3.41 vs 3.27mg, respectively). The higher and comparable zinc for the blends containing the 72h fermented, the 96 and the 120h germinated lima bean and the 48h fermented maize (5.46, 5.37 and 5.43mg) suggested that these treatments and these periods were the optimum period to improve Zn availability in

black lima bean and maize flour blends.

Copper (Cu)

The similar Cu values for the UBVM, the $CBVM_{48}$ and the SBV_9M_{48} appears to suggest that cooking and soaking did not affect copper levels when compared with the control (13.80 and 13.45 vs 13.73mg). The comparable higher Cu values for the $FBV_{48}M_{48}$ and the $GBV_{120}M_{48}$ (14.60 and 14.63mg) suggested that these fermentation and germination periods increased copper in black lima bean and maize flour blends. The lower Cu (12.63mg) for the $GBV_{96}M_{48}$ might be attributed to poor mutual supplementary effect of the 96h germinated lima bean and the 48h fermented maize.

Iron (Fe)

The higher and comparable iron for the SBV_9M_{48}, the $FBV_{48}M_{48}$ and the $GBV_{120}M_{48}$ (37.33, 36.00 and 38.67mg) indicated that these composites had higher mutual supplementary effects than the other composites. This finding was in agreement with the report of Osagie (1998) about increased mineral bioavailability after legume and cereal processing.

There were similarity in iron values for the control (UBVM) (33.00mg) and the $GBV_{96}M_{48}$ (33.00mg) as well as the $CBVM_{48}$ and the $FBV_{72}M_{48}$ (31.00 and 31.67mg, respectively) showing that none of these composite is better than the other as regards Fe availability.

Phosphorus (P)

The higher and comparable phosphorus for the SBV_9M_{48}, the $FBV_{48}M_{48}$ and the $GBV_{96}M_{48}$ indicated that these composite flours had an advantage over the other flours (38.33, 35.00 and 38.33mg, respectively) in phosphorus availability. This might be due to the desirable mutual supplementary effect between the flours.

On the other hand, the lower and comparable phosphorus for the $CBVM_{48}$ and the $FBV_{72}M_{48}$ (31.67 and 32.00mg) as well as the UBVM and the $GBV_{120}M_{48}$ (33.67mg) suggested that cooking and fermentation had equal effect on phosphorus content of the composites.

5.8 EFFECT OF PROCESSING ON PROXIMATE COMPOSITION OF DARK RED LIMA BEAN FLOURS .

Moisture

The lower moisture for the DRV, the $DRVS_3$, the $DRVS_6$, the $DRVS_9$, the $DRVF_{24}$ and $DRVF_{48}$ (9.33, 9.17, 9.50, 6.17, 7.33 and 9.33%, respectively) suggested that these flours would have longer shelf life and better keeping quality as against other flour blends.

On the other hand, the high moisture content for the DRVUDC (12.33%), the $DRVWND_o$ (13.33%), the $DRVG_{72}$ (13.33%) and the $DRVG_{96}$ and the 120h with the highest moisture (15.33%) showed that these flours would not have longer shelf life and better keeping quality. It is known that the higher the moisture content of a given flour, the lower is its keeping quality – a commonly observed phenomenon. This increase in moisture for the 96 and the 120h germinated flours was in agreement with the report of Dibofori *et al* (1994). They reported significant increase in moisture content of lima bean on the 5th day of germination (12.7 to 67.9%).

Protein

The lowest protein for the DRVUDC, the DRVCWD and the DRVCWND (6.90, 5.96 and 8.19%) relative to the control (24.65%) could be due to the synergistic effect of dehulling and cooking. It is well known that protein denaturation occurs in prolonged cooking. This may be responsible for the decrease in protein levels of the cooked flour samples. In support of this observation, Manay and Sharaswamy (2008) noted that overcooking reduces the quality of protein in pulses as a result of lysine loss.

The higher protein values for soaked flour except for the 9h, the DRVG96 and the $DRVG_{120}$ as well as the $DRVF_{48}$ (25.57, 25.93, 26.16, 26.60, 25.11 and 26.34%, respectively) as against the control (24.65%) suggested that soaking except for the 9h, germination (72-120h) and fermentation for the 48h had improved protein quality for dark red lima bean more than the other treatments (Table 8). Many research findings attributed increase in protein to hydrolysis of crude protein into free amino acids by proteolytic enzymes. These free amino acids are used for synthesis of new protein and or breakdown of protein-tannins and tannins-enzyme complexes that released more free amino acids (Akobundu and Hoskin, 1987; Zamora and Fields, 1979a; Eka, 1980). Onuoha (2000) observed a similar increase in protein content of 48h fermented plain oxblood lima bean.

Fat

The decreases in fat except for the DRVS3, the DRVG72 and the DRVG96 (1.65, 1.69 and 1.69%) relative to the control (1.62%) (Table 8) demonstrated that dehulling and cooking, soaking, germination for 120h and all fermentation periods had efficacy to lower fat in dark red lima bean. The flours whose fat were reduced below 1%, would have lesser chances of oxidative rancidity, as low fat enhances the keeping quality of flour and food products. This apparent decrease in fat for the fermented dark red lima bean flours could be attributed to hydrolysis of lipids to free fatty acids by lipases. The free fatty acids could be used for synthesis of new lipids. Odunfa (1983) reported that microorganisms might also use

lipid or protein as a source of energy when utilizable carbohydrate is not available. This decrease in fat for all fermented flours accords the report of Anosike and Egwuatu (1981) and Vander-Riet *et al.* (1987).

Ash

The lower ash for all treatments as compared with the control showed that these food processing techniques were not beneficial for ash availability in dark red lima bean. The decreased ash for all the cooked flour especially the dehulled cooked (1.41%) relative to the control (6.69%) was due to prolonged cooking. Dehulling and cooking adversely affected ash in dark red lima bean. It is known that longer cooking results in loss of mineral, vitamins, and other nutrients, as such reducing the nutritional value of foods. On the other hand, germination for 120h had comparable ash as the control (6.50 vs 6.69%).

The observed increase in ash for the 120h germinated flour, accords the report of Dibofori et al (1994) that 5 days germination had significant increase in ash from 3.12 to 5.12% in lima bean. Yousef et al (1987) recorded similar increase in percentage ash in Faba beans germinated for 3 days. This increase in ash due to germination could be attributed to mobilization of organic and inorganic substances to meet the increased metabolic rate of the growing embryo.

Fibre

The comparable fibre for the 120h germinated flour and the control (5.04 vs 5.00%) suggested that germination for 120h slightly increased fibre and had an edge over the other treatments in increasing fibre in dark red lima bean.

The least fibre for the $DRVF_{24,}$ the DRVUDC, the $DRVWD_4$ and the DRVWNDo (2.48, 2.89, 2.86 and 2.58%) showed that these processing techniques were not beneficial for improvement of fibre in dark red lima bean.

However, the slightly lower fibre for the DRVDC than the control (4.12 vs 5.00%) as well as the $DRVS_3$, the $DRVG_{72}$ and the $DRVF_{72}$ (4.44, 4.12 and 4.31%, respectively) demonstrated that these processing techniques would not enhance the fibre content of dark red lima bean. The observed decrease in the $DRVF_{72}$ were similar to the findings of Eka (1980) and Achinewhu and Isichei (1990). They recorded decrease in crude fibre of fermented legume seeds.

Carbohydrates

The variations in carbohydrate content of dark red lima bean flours was attributed to processing techniques. However, dehulling and cooking increased carbohydrate from 52.71 to 55.85%. The higher increase in carbohydrate for the DRVUDC, the $DRVCWD_4$, the $DRVCWND_o$ and the $DRVF_{24}$ as well as the $DRVS_9$ (71.88, 76.74, 70.63, 69.22 and 60.66%, respectively) were due to the synergistic effect of cooking, soaking and fermentation.

On the other hand, the lower carbohydrate for all germinated flours (49.51, 47.82 and 47.43%) might be that the seedlings used the carbohydrate for metabolic processes. This usage of carbohydrate as a source of energy by the growing seedlings decreased the carbohydrate content. Jaya and Venkatarman (1981) observed decrease in total carbohydrate and oligosaccharides. The slightly higher carbohydrate for the $DRVS_3$ the $DRVS_6$, the $DRVF_{48}$ and the $DRVF_{72}$ (53.59, 55.36, 56.06 and 54.14%, respectively) might be that less carbohydrate was leached into soaking water and fermentation medium.

5.9 EFFECT OF PROCESSING ON MICRONUTRIENT COMPOSITION OF DARK RED LIMA BEAN FLOURS.

Zinc (Zn)

The lower zinc for all dehulled, cooked and soaked flour samples (3.24, 3.85, 3.35, 3.51 and 3.55mg, respectively) were due to loss in cooking and soaking media. These findings were in line with the report of Apata and Ologhobo (1994). They reported that cooking and discarding cooking water at intervals reduced zinc, iron and copper content of lima bean.

The slight loss in zinc for the $DRVS_9$, the $DRVG_{72}$ and the $DRVG_{96}$ as well as the $DRVF_{72}$ when compared with the control (4.16, 4.06, 4.09 vs 4.17mg) showed that these processes slightly reduced zinc in comparison with the other processing techniques. The comparable reduction in zinc (3.26, 3.83 and 3.38mg) as against the control (4.17mg) showed that germination beyond 96h and fermentation for 24 and 48h were not beneficial with regards to zinc availability in dark red lima bean (Table 9).

Copper (Cu)

The increases in copper except for those of the DRVCWD and the DRVCWND as against the control (12.10 and 12.03 vs 12.17mg) may be related to genetic origin and treatments. The increase in copper for the fermented flours was in line with the report of the International Life Science Institute (1998) that fermentation improves the bioavailability of minerals such as copper, zinc and iron as a result of phytic acid hydrolysis.

Iron (Fe)

The decreases in Fe were not equal. The DRVWND, the $DRVS_6$ and the $DRVG_{72}$ – DRVG 120h, as well as the $DRVF_{24}$ – 72h (43.00, 48.00, 68.00, 49.33, 46.67, 46.00 and 48.00% vs 85.67% respectively) showed that these processes caused less losses of iron than those of the other processes. The drastic decrease in Fe levels of all the cooked dark red lima bean flours accords the findings of Manay and Sharaswamy (2008) that longer cooking results in loss of some minerals and vitamins, and subsequent loss of nutritional value.

Phosphorus

The losses in phosphorus due to treatments established the same trend as that of iron (Table 9). Except for the $DRVS_9$ (67.33mg), the other processes had more adverse effect on phosphorus as against the control (14.00 to 47.67mg vs 85.00mg). This observation was in agreement with the report of Roday (2007).

Folate

The slightly higher folate except for the $DRVG_{72}$ (5.27mg) and the $DRVF_{48}$ (5.40mg) relative to the control, the other treatments had comparable increases in folate indicating that any of the domestic food processing techniques could be adopted for increase in folate in dark red lima bean.

5.10 EFFECT OF PROCESSING ON ANTINUTRIENTS AND FOOD TOXICANTS CONTENT OF DARK RED LIMA BEAN.

Phytate

The zero phytate value for the $DRVG_{72}$ and the $DRVG_{96}$ as well as the $DRVF_{72}$ (0.00mg) indicated that at these periods of germination and fermentation that phytate is completely reduced as compared with the other treatments and the control (0.00mg vs 1.13 to 31.33mg, respectively). The decreased phytate was due to hydrolysis of phytates from their organic complexes and leaching into either fermentation or germination medium. Osagie (1998) observed that 72h fermentation drastically reduced phytate in both legumes and cereals. However, the higher phytate for the DRVUDC, the $DRVS_3$ and the 6h soaking (4.07, 3.77 and 3.13mg) showed that these processes decreased phytate much less than the other processes This suggests that they are not the best processing methods for complete elimination of phytate in dark red lima bean.

Tannins

The zero tannins for all the cooked flour samples, the 9h soaked sample and the 72h germinated and fermented samples showed that these food processing methods were able to

reduce tannins to zero value as compared with the control (0.00 vs 10.84mg) (Table 10).

The zero tannins for some of the processed flours was not a surprise. Osagie (1998) noted that traditional food processing reduced tannins in legumes and cereals. On the other hand, the higher tannins than for the 120h germinated flour sample against the control (47.90 vs 10.84mg) indicated that germination for 120h was the least effective method of reducing tannins in dark red lima bean. This high value (47.90mg) may not necessarily be all tannins. It has been shown that some of the antinutrients may increase due to processing because they contain other materials which the equipment may not be sensitive enough to defect.

Hydrogen cyanide (HCN)

The zero value for hydrogen cyanide in all the cooked flour samples, the 9h soaked and all fermented flours showed that these treatments were much more effective in reduction of hydrogen cyanide when compared with the control (0.00 vs 14.85mg) (Table 10). Ologhobo *et al* (1984) reported that the longer the soaking, cooking and fermentation periods, the lower the hydrogen cyanide content of lima bean.

The much lower hydrogen cyanide for the DRVCWND and the 6h soaked samples indicated that they were more effective in reduction of hydrogen cyanide than the 3h soaking and all germination periods (2.07 and 2.18 vs 7.82, 4.63, 4.29 and 8.43mg), respectively.

Trypsin inhibitor

The zero trypsin inhibitor for the 9h soaked flour sample as well as the 48 and the 72 fermented flours suggested that these treatments reduced trypsin inhibitor in dark red lima bean than other treatments. The low trypsin inhibitor in processed legumes has been reported by several researchers (Egbe and Akinyele, 1990; Ogun *et al*; 1989). Akinyele, (1989) observed that cooking, soaking and other traditional methods of processing caused significant reduction in trypsin inhibitor activity. The slightly decrease in trypsin inhibitor for the 3h soaked, the 96 and 120h germinated flour samples (23.00, 12.80 and 19.13mg) showed that these treatments (3h soaking, 96 and 120h germination)had the least effect on reduction of trypsin inhibitor in dark red lima bean.

Haemaglutinins

The zero haemagglutinin in all the cooked as well as the 6 and the 9h soaking dark red lima bean flours were not surprising. Egbe and Fetuga (1990) reported decrease in haemagglutinin with increased cooking time of lima beans.

The increases in haemagglutinins in all germinated samples – $DRVG_{72}$, 96 and 120h (4.11, 3.67 and 3.66mg) as against the control (2.94mg) appears to suggest that germination

periods up to 120h rather increased haemagglutinins in dark red lima bean. These increases in haemaglutinins were contrary to some reports in literature. Although, it has been shown that some domestic food processing would rather increase haemaglutinins than reduction. This is because some other compounds might be hydrolysed and released and the equipment for eluding haemaglutinin might not be sensitive enough to detect and eliminate them during estimation. The lowering of haemaglutinins to less than 1mg except for the 3h soaked and the 24 and the 48h fermented flour samples (1.22, 2.13 and 1.43mg) as against the control (2.94mg) demonstrated the efficacy of 3h soaking and 24 and 48h fermentation in haemaglutinin reduction. The least haemaglutinin value for the 72h fermentation (0.08mg) further confirmed that the higher the fermentation period, the lower is the haemaglutinin levels in dark red lima bean.

Saponins

The lower saponins for the dehulled cooked, the undehulled cooked and the cooked flour sample with and without changing cooking water as well as the 48 and the 72h fermentation (0.24, 0.27, 0.35, 0.29, 0.28 and 0.53mg), respectively as against the Control (90.08mg) showed that these processes were much more effective in reduction of saponin in dark red lima bean. However, the higher value for the $DRVG_{120}$ (87.64mg) as against the other treatments clearly suggest that at 120h germination of dark red lima bean, saponin reduction was at its lowest level (90.08 vs 87.64). The second highest values of saponin were 3, 6 and 9h soaking, the 72 and the 96h germination as well as the 24h fermentation (28.60, 12.84, 33.86, 40.75, 4.87, 3.54mg respectively), showed that these processes did reduce saponins but not as efficient as those that reduced saponin to less than 1mg.

5.11 EFFECT OF PROCESSING ON FUNCTIONAL PROPERTIES OF DARK RED LIMA BEAN. FLOUR

Foam capacity

The less than one percent reduction in foam capacity for various treatments except for the 3h soaked and all germinated flour samples indicated that these flours had good baking quality implications. It is known that the higher the foaming capacity of a given flour, the better the baked products from such flours. The higher foaming capacity value for the 120h germination relative to the control (4.40 vs 5.40%) showed that the 120h germinated flour had an edge over other processed flours in terms of improved foam capacity.

Water absorption capacity

The increases in water absorption capacity above the control (4.00%) except for the 120h germinated flour (1.67%) suggested that all the treatment could be applied to improve water absorption capacity in dark red lima bean flours. However, the 3h soaked flour had the least increase in water absorption capacity (10.33%). The increased water absorption capacity for all the germinated and fermented flour samples might be due to proteolytic enzyme activity during these processes which caused increase in the number of polar groups. These increased hydrophilicity of the seed proteins and thus increased water absorption capacity. The flours with high water absorption capacity is good for use in preparation of meat analogs, sausages, breads and cakes (Onimawo and Akubor, 2005).

Oil absorption capacity

The lower values for oil absorption capacity as well as high values (4.67 to 15.00%) in dark red lima bean flours had some nutrition implication. High oil absorption capacity in flours indicated their suitability in baking. This is because oil acts as flavour retainer and increases the palatability of foods. It is known that the formation of continuous gluten system in baking is prevented by oil and makes baked products easier to chew (Ihekoronye and Ngoddy, 1985).

Emulsion capacity

The increase in emulsion capacity of the samples, except for the 48h fermented flour has some desirable nutrition implication. Evidence have shown that the higher the emulsion capacity of a given flour, the better the protein solubility and vice versa. The high emulsion capacity values for the 3h soaked sample, the 120h germinated sample as well as the 24h fermented sample (21.00, 2.00 and 16.33%) indicated that these flours would have much more improved protein solubility than others.

Protein solubility

The higher protein solubility (5.03, 4.80, 11.46 and 12.52%), respectively for the 6h soaking, all periods of germination (72 – 120h) showed that these processes enhanced protein solubility in dark red lima bean. It is known that the higher the protein solubility of a flour the better the quality. This is because protein solubility determines the baking quality of the flours. It influences other functional properties. However, the lower protein solubility values for the flour samples dehulled cooked, the cooking and discarding cooking water as well as the 72h fermentation (0.00, 0.64 and 1.87% respectively), suggested that dark red lima bean flours based on these treatments would have low baking quality. The lower protein solubility

for all the cooked flours might be attributed to protein denaturation during cooking. Ihekoronye and Ngoddy (1985) had similar observations.

5.12 EFFECT OF PROCESSING ON PROXIMATE COMPOSITION OF COMPOSITE FLOURS BASED ON DARK RED LIMA BEAN AND MAIZE

Moisture

The lower moisture for the $FDRV_{48}M_{48}$ (5.33%) as against the control (8.33%) demonstrated that the flour would have higher shelf-life than others. The higher moisture for $GBRV_{96}M_{48}$ (8.77%) showed that the composite flour would have the least shelf life and lower keeping quality as the control (8.77 vs 8.33%). The comparable moisture values for other flour blends (7.17, 7.33 and 7.43%) (Table 12), indicated that none of these has an edge over the other in terms of moisture availability.

Protein

The lowest protein for the $CDRVM_{48}$ might be due to cooking at high temperatures. It is known that protein denatures at a very high temperature. This denaturation seriously affected the protein content of foods. This finding was similar to the observation of many workers (Manay and Sharaswamy, 2008; Enwere, 1998; Onimawo and Akubor, 2005; Ihekoronye and Ngoddy, 1985). The slightly lower protein for the $FDRV_{48}M_{48}$ and the $GDRV_{120}M_{48}$ (18.00 and 18.02%) as against the control (19.11%) indicated that they would be as good as the control when used for baking.

Ash

The lowest ash for the $FDRV_{48}M_{48}$ and the $GDRV_{96}M_{48}$ might be that during germination and fermentation, the microflora in both media used ash for metabolism or the ash leached into fermentation and germination media – a commonly observed fact.

The slightly higher ash (1.11, 1.04, 1.15 and 1.08%) for the $CDRVM_{48}$, the $SDRV_{9}M_{48}$, the $FDRV_{72}M_{48}$ and the $GDRV_{120}M_{48}$, respectively as against those of the $FDRV_{48}M_{48}$ and the $GDRV_{96}M_{48}$ showed that the treatments did not decrease the nutrients as those of the later.

Fat

The variations in fat values was attributed to treatments. The lower fat for the $SDRV_9M_{48}$ and the $FDRV_{72}M_{48}$ (1.08 and 1.11%) indicated that these composite flours would keep longer than others. When flours or food contain less fat, they have longer shelf life. (Nnam and Obiakor, 2003).

On the other hand, the flours that had higher fat than the control (3.27, 2.78, 7.35 vs 1.75%) are much more prone to oxidative rancidity which in turn would adversely affect the baking quality of the flour.

Fibre

The lower fibre for the cooked, the soaked and the 72h fermented flour samples demonstrated that this food processing techniques reduced fibre when compared with the control and other treatments (3.77, 3.88, and 2.63% vs 4.62%). On the other hand, the similarities in fibre (4.62 vs 4.61, 4.62 and 4.7%) suggested that these processing methods improves fibre availability which is essential for health and that these flour blends would contain adequate fibre as that of the control, when used for food products.

Carbohydrate

The variations in carbohydrate values for the composite flours were a function of treatments. The control UDRVM and the $GDRV_{120}M_{48}$ had lower and comparable CHO values when compared with other composite flours (63.43 vs 65.55%). On the other hand, the cooked dark red lima bean and the 48h fermented maize composite ($CDRVM_{48}$) had the highest carbohydrate (77.38%) relative to the control and other composite flours.

5.13: MINERAL COMPOSITION OF COMPOSITE FLOURS BASED ON DARK RED LIMA BEAN AND MAIZE

Zinc (Zn)

The higher Zn for all the composites except for that of the $CDRVM_{48}$ (3.25mg) indicated that the blends had desirable mutual supplementation effect. However, the increase were lower in the $GDRV_{96}M_{48}$ (4.09mg).

Copper

The higher and comparable Cu except for the control (13.37mg) showed that the blends had good mutual supplementation effect. However, the blends containing the 9h soaked, the 48h fermented and the 96h germinated lima bean (14.30, 14.37 and 14.43mg) had an edge over the other blends as sources of Cu in dark red lima bean (Table 13).

Iron (Fe)

The higher Fe for the cooked and the 9h soaked blends as against the control (44.67, 53.67 vs 33.00mg) showed that cooking and soaking are better processing methods, especially the 9h soaking, to increase Fe in blends of dark red lima bean and maize.

The second highest and comparable values (37.00mg) for the 96 and the 120h germinated dark red lima bean, indicated that none of the germination periods had an advantage over the other in fe availability in dark red lima bean.

Phosphorus (P)

The lower P for the 96h germinated blends (32.00mg) and the 48h fermented soaked lima bean and maize (34.00mg) suggested that these blends would be poor sources of P when compared with the control (34.67mg). The higher P (47.00mg) for the $CDRVM_{48}$ suggested that cooking and fermentation had an edge in releasing free P in blends of dark red lima bean and maize.

5.14: MICROBIAL COMPOSITION OF PROCESSED AND UNPROCESSED BLACK AND DARK RED LIMA BEAN FLOURS.

The zero *E. coli* for all the cooked samples for both black and dark red lima bean showed the efficacy of this simple domestic food processing on destruction of *E. coli*. It is known that heat treatment (cooking) at a very high temperature destroys most of the pathogenic and non pathogenic microorganisms in foods. This finding accords the observation of many previous research works (Fraizer and Westhof, 1978; Fox and Cameron, 2007; Randall, 1994; Arntzen and Ritter, 1994).

The lower *E. coli* population in dark red lima bean flour soaked for 3-9h (1.20×10^1 – 2.00×10^1) and that of the black (1.00×10^1 – 3.00×10^1) might be due to varietal differences. It is known that the deeper the colour of food grains, the higher is the antinutrients as well as microflora content.

This observation was similar to that of many workers. (Osagie, 1998; Elegbede, 1998; Okoli and Ugochukwu, 1988; Chikwendu, 2004). The lower *E. coli* in black 120h germinated lima bean flour (2.00×10^1) as against the higher *E. coli* in the 120h germinated dark red lima bean flour (5..00 x 10^1) might be due to varietal differences. It could also be due to efficacy of germination in black lima bean than in the dark red - a commonly observed phenomenon. During germination, the seeds absorbed moisture which facilitates the growth of

microorganisms, as such the increase in the number of the microflora during germination (Gaman and Sherrington, 1990).

On the other hand, the lower *E. coli* in the dark red lima bean fermented for 72h (1.60 x 10^1) as against the black (6.00 x 10^1) showed that 72h fermentation decreased microflora much more in dark red than in black lima bean. Nout and Mortajemi (1997) reported the presence of *E. coli* and other pathogenic microorganisms in fermented foods. The presence of E. coli in both the raw and all the processed flours except the cooked flour samples indicated that the *E. coli* is inherent in the lima bean seeds.

5.15: PROXIMATE COMPOSITION OF PRODUCTS (MOI-MOI AND AKARA) BASED ON BLACK AND DARK RED LIMA BEAN AND MAIZE FLOURS.

The proximate composition of moi-moi and akara were shown as consumed. (Table 15).

Moisture

The higher moisture for moi-moi than akara from both lima bean varieties was attributed to the ingredients and much water added to the mixture during the moi-moi preparation.

The cooking method (steaming) used in moi-moi preparation also contributed to the high moisture value (Obiakor, 2006). The high moisture conent (17-19%) for the moi-moi suggested that they would not have longer shelf-life and better keeping quality. It is known that the higher the moisture content of a food, the lower is its keeping quality – a commonly observed phenomenon.

However, in the preparation of akara, little quantity of water was used to mix the flour and no food ingredients added, hence the low moisture values for all the akara balls.

Protein

The higher protein values for both moi-moi and akara based on blends of the 48h fermented black or dark red lima bean and maize demonstrated that the 48h fermentation for either variety had an edge in protein availability over the other fermentation periods and their controls (30.47, 31.45,30.70 and 29.10% vs 28.41, 25.67, 29.39 and 27.06%, respectively). This observation was similar to that of Akinyele (1989) in products (moi-moi, akara, gbegiri, ewa) from processed cowpea. However, the higher protein might be attributed to lima bean which is a legume. Legumes are known to be rich sources of protein.

Fat

The increases in fat for both moi-moi and akara based on the48h fermented lima bean and maize flour except for the akara (VAO-$FB_{72}M_{48}$) clearly indicated that the 48h fermentation of black lima bean would increase fat content of the products. The higher increase in fat for akara (PNO-$FB_{48}M_{48}$) might be due to the absorption of oil during frying. Fox and Cameron (2007) reported that frying increases the fat content of foods. Hence, the high fat content of fried foods. The decrease in fat for both moi-moi and akara based on dark red lima bean are simple to explain. It is known that when the oil absorption capacity of a given flour or flour blend is low, the lower the oil or fat content of the cooked or fried product because the flour does not absorb more oil, especially when the food is fried.

Ash

The lower ash values for both moi-moi and akara based on blends of the 48h fermented black or dark red lima bean and maize flours(4.97, 4.15, 6.02%) suggested that the 48h fermented black or dark red lima bean flour blended with the 48h fermented maize, were lower sources of ash as compared with the other products based on the 72h period of fermentation (Table 15).

Fibre

The decreases in fibre in both products (moi-moi and akara) based on blends of black and red lima bean and maize except for the AOB ($FB_{72}M_{48}$) suggested that the 72h fermentation had adverse effect on fibre content of the products relative to their control except for that of moi-moi based on dark red lima bean (AOB – 8.74%) The higher fibre for both moi-moi and akara based on black lima bean blends suggested that black lima bean and maize flour blends had higher fibre than dark red lima bean and maize, hence the low fibre content of their products (Table 15).

Carbohydrate

The high carbohydrate content (41.42 and 57.17%) and (44.71 and 47.03%) of akara from both dark red and black lima bean flour blends is simple to explain. Legumes are lower in CHO than cereals. However, when both are combined in a mixture, the cereal that contains more CHO influences the CHO content of the blends – a commonly observed phenomenon.

5.16: MINERAL COMPOSITION OF PRODUCTS (MOI-MOI AND AKARA) BASED ON PROCESSED AND UNPROCESSED DARK RED LIMA BEAN AND MAIZE FLOUR BLENDS

Calcium (Ca)

The higher increases in calcium content for both moi-moi and akara based on the 48h fermented black or dark red lima bean except for the akara based on the 48h fermented dark red lima bean and maize (KCC-$FD_{48}M_{48}$) (122.24, 92.19, 97.53 and 77.13mg, respectively), indicated that fermentation improves the bioavailability of minerals such as Ca, Fe, Zn and Mg (Svanberg, 1995). The much more increase in Ca in moi-moi based on black lima bean (AOO and MCO – 122.24 and 117.57mg) as against the control (89.51mg) might be as a result of phytic acid hydrolysis (ILSI, 1998)

Magnesium (Mg)

The higher Mg in moi-moi and akara based on the 48h fermented lima bean and maize except for the akara (PNO – 20.34mg) vs its control (41.23mg) suggested that the 48h fermentation of both lima bean and maize had advantage over the other periods of fermentation as source of Mg.

Potassium (K)

The much more increases in potassium in both moi-moi and akara based on the 48h fermented black lima bean and maize as against their controls (362.80, 426.42 vs 258.20 and 306.27mg) were due to desirable mutual blending (Table 15).

On the other hand, the decrease in potassium of moi-moi (MEO – $FD_{48}M_{48}$) as against the control (287.13 vs 410.80mg). might be due to poor mutual supplementation effects. The higher potassium (337.63mg) for the SCC($FD_{72}M_{48}$) as against the other treatments and control (337.63 vs 266.80 and 165.93mg, respectively) clearly indicated that the SCC ($FD_{72}M_{48}$) had an edge over both the control and the other fermentation periods.

Phosphorus (P)

The increases in phosphorus in moi-moi based on black lima bean and not akara is difficult to explain. The much more increases in phosphorus for moi-moi based on the 72h fermented black lima bean and the 48h fermented maize suggested that the longer the fermentation period of black lima bean the higher the phosphorus content of moi-moi as against the other treatments and the control (286.80 vs 265.13 and 209.87mg).

The decrease in phosphorus in akara PNO ($FB_{48}M_{48}$) as against the control. (187.87 vs 244.83mg) suggested that PNO was not adequate for improving phosphorus availability as

the VAO ($FB_{72}M_{48}$) (242.63 vs 187.87mg). The decrease also suggested that the higher the fermentation period the higher the P content of akara based on black lima bean.

The P content of moi-moi and akara based on dark red lima bean followed the same trend as that of black lima bean. The increases in phosphorus in moi-moi based on flour blend of dark red lima bean fermented for 48 and 72h and 48h fermented maize (248.80 and 213.83mg) as against the control (165.87mg) indicated that the two fermentation periods could improve P in dark red lima bean moi-moi. However, fermentation for 48h had an edge over the 72h period (248.80 vs 213.83mg).

On the other hand, the decreases in P of akara, based on dark red lima bean might be explained thus: (a) fermentation of dark red lima bean had no beneficial effect on P content of its akara and (b) fermentation of dark red lima bean for 72h the decrease in P when compared with that of the 48h fermentation period and the control (124.43 vs 134.43 and 214.67mg, respectively).

Iron (Fe)

The much more increases in Fe content of moi-moi and akara based on the 48h fermented black and dark red lima bean and maize as against their controls (27.57, 31.05, 19.50 and 13.01mg vs 11.87, 26.78, 10.10, and 13.10mg, respectively) might be that the 48h fermentation is the optimum period for Fe availability in both varieties of lima bean. The decrease in Fe content of akara based on the 72h fermented black lima bean and the 48h fermented maize (VAO-$FB_{72}M_{48}$) as against the control (19.13 vs 26.78mg).indicated that the 72h fermentation hindered Fe bioavailability in black lima bean.

On the other hand, the increase in Fe in both moi-moi and akara based on the 72h fermented dark red lima bean and the 48h fermented maize as against their controls (12.21 and 91.70 vs 10.10 and 9.26mg, respectively) might be attributed to varietal differences. As a buttress to this observation, there was a decrease in Fe content of akara based on black lima bean from 26.76mg to 19.13mg relative to dark red lima bean akara whose Fe content increased from 9.26 to 13.01mg (Table 16).

5.17: SENSORY PROPERTIES OF MOI-MOI AND AKARA BASED ON UNPROCESSED AND PROCESSED BLACK AND DARK RED LIMA BEAN AND MAIZE FLOUR BLENDS.

Colour

The increases in colour of moi-moi and akara based on the 48h fermented black and dark red lima bean and maize against their controls might be associated with improved colour

due to fermentation. Chikwendu (2004) observed that processing, especially fermentation enhanced the colour of moi-moi based on ground bean. The colour scores (7.40 and 7.00) for the moi-moi based on 48 and 72h fermented black lima bean and 48h fermented maize were in agreement with the report of Onuoha (2000).

The decreases in colour of moi-moi and akara based on the 72h fermented black and dark red lima bean and the 48h fermented maize as against the 48h fermented products (7.00, 5.90, 5.47 and 5.10 vs 7.40, 6.87, 7.10 and 6.23, respectively) indicated that fermentation of the two varieties of lima bean beyond 48h decreased colour. Regardless of decreases in colour the values were more than one half of the nine point hedonic scale (Table 17).

Flavour

The flavour of the products (moi-moi and akara) followed the same trend as the colour. The flavour of the products based on the 48h fermented lima bean and maize flour blends increased and those based on the 72h fermented flour decreased (7.07, 7.03, 6.50 and 5.60 vs 6.56, 5.37, 4.70 and 5.60, respectively) as against the 48h fermented flour and their controls (Table 17). It is known that fermentation, especially the 48h period improved the organoleptic attributes as well as the nutritional quality of plant foods (Obiakor, 2001; Lorri, 1993; Onuoha, 2000).

Texture and general acceptability

The texture and general acceptability of the products (moi-moi and akara) followed the same trends as those of colour and flavour (Table 17). There were increases in both values in the two products based on the 48h fermented flours as against their controls. There were also decreases in texture and general acceptability of the products based on the 72h fermented except for the moi-moi based on black lima bean (6.94 vs 6.23) (Table 17).

5.18 PROXIMATE AND MINERAL COMPOSITION OF DISHES (*OSHOTO* AND *IKPAKI*) BASED ON WHOLE BLACK AND DARK RED LIMA BEAN AND MAIZE GRAINS.

Moisture

The higher moisture content of the dishes based on black lima bean variety (20.60, 20.11 and 21.40%) as against those of the dark red variety (18.70, 19.60 and 19.80%) might be due to the varietal difference. It also indicated that the black lima bean had high water absorption capacity than the dark red variety. However, the high moisture content for all the dishes, suggested that, they have low keeping quality or shelf-life. Research have shown that

some microorganism that cause food spoilage thrive well with adequate water (Fraizer and Westhoff, 1978; Gaman and Sherrington, 1990).

Protein

The increases in protein of dishes based on black and dark red lima bean with and without addition of maize when compared with the controls (30.11, 27.63, 31.37 and 24.62 vs 6.48 and 4.46%, respectively) (Table 18) is simple to explain. The increase might be due to added food condiments. The much more increase in protein of dishes with added maize BOSH and DROSH (30.11 and 31.37%) might be due to the additional protein from maize. The higher protein content for dish based on black lima bean without maize (BIKP)as against that of dark red lima bean DRIKP (27.63 vs 24.62%) indicated that black lima bean had an edge in protein over the dark red lima bean in protein availability.

Fat

The increases in fat for both dishes based on the two lima bean varieties as against their controls (1.50, 1.16, 1.57 and 1.26 vs 0.53 and 0.89%) could be due to extra fat from maize as well as added oil and other food ingredients, such as fermented oil bean (oil seed) known to contain fat. Evidence have shown that addition of African oil bean seeds increases the fat content of traditional dishes (Obiakor, 2007).

On the other hand, the lower fat for the dishes based on black lima bean without maize as against that of the dark red lima bean indicated that dark red lima bean contains more fat (1.50 and 1.16 vs 1.57 and 1.26%, respectively).

Ash

The increases in ash for dishes based on the two lima bean varieties with or without addition of maize (4.45, 4.37, 4.70 and 4.15 vs 3.00 and 3.38%, respectively) were attributed to the addition of food ingredients. The much more increase in ash for dishes based on black lima bean as against those of dark red lima bean (4.45 and 4.37 vs 4.70 and 4.15%) might be due to varietal differences.

Fibre

The decreases in fibre in dishes based on black and dark red lima bean without maize (7.03 and 7.95 vs 9.08 and 9.26%) might be attributed to exclusion of maize which has more fibre than lima bean. The lower fibre for the dish based on black lima bean as against that based on dark red lima bean, might be due to varietal differences. The comparable fibre for

the control dishes (2.96 and 2.86%, respectively) indicated that any of the lima bean would supply equal fibre.

Carbohydrate

The higher carbohydrate content of the dish based on dark red lima bean prepared without addition of maize and food condiments (DRVCP) indicated that dark red lima bean had a slight edge in carbohydrate availability over the black lima bean variety (69.81 vs 67.43%, respectively). The higher CHO for dishes based on dark red lima bean without maize as against those of black lima bean (42.22% vs 38.41%, respectively) might be due to the varietal differences of the lima bean, since the same food condiments were added.

Minerals

The high mineral values for dishes based on both lima bean varieties and maize might be attributed to the addition of maize. The lower mineral values (Ca, Mg, P, K and Fe) for the dishes based on black and dark red lima bean cooked without corn might be due to varietal differences and treatments (Table 18). It is known that cooking results in loss of some minerals and vitamins.

5.19 SENSORY PROPERTIES OF DISHES (*OSHOTO* AND *IKPAKI*) BASED ON WHOLE BLACK AND DARK RED LIMA BEAN AND MAIZE GRAINS

Colour

The differences in colour of dishes based on black and red lima bean cooked with or without maize is solely attributed to treatments as well as varietal differences.

Flavour

The higher and comparable flavour for dishes based on black and dark red lima bean cooked with and without maize (7.77, 7.60 and 6.37 and 6.93) indicated that the two lima bean varieties can produce nearly the same flavour in any dish.

Texture

The increases in texture for the two dishes (7.17 and 8.27) without addition of maize indicated that the addition of maize to either of the two lima beans had no influence on the texture of the dishes. The lower texture for the dishes based on black and dark red lima bean with maize (6.47 and 6.33, respectively) might be due to varietal differences.

General acceptability

The lower acceptability of black lima bean dishes cooked without maize could be due to unacceptable appearance of the dishes (Plates V & VI).

On the other hand, the higher acceptability of black lima bean dish cooked with maize (7.00) and those of the dark red lima bean cooked with or without maize (7.50) showed that addition of maize influenced the acceptability of black lima bean dish and not that of dark red lima bean.

Conclusion

This work has provided a baseline information on lima bean availability, consumption, as well as the chemical, functional, microbial and sensory properties of its flour and products. Lima bean is one of the under-exploited food crops that has high nutrient potentials. It is one of the legumes that have been neglected in the recent decades due to difficulties in its production, processing, preparation and utilization. It is clear now that lima bean could substantially improve the nutritional needs of a population. Both urban and rural populations, especially the low income groups would benefit from nutrient potentials of lima bean, particularly at times of seasonal scarcity of staple crops. Lima bean is rich in both macro and micro-nutrients.

Despite its high nutritional quality, lima bean contains various antinutrients and food toxicants that militate against its full nutrient bioavailability. Traditional domestic food processing techniques effectively reduced some antinutrients and food toxicants in black and dark red lima bean to safe levels. Cooking and fermentation were much more effective methods, especially in the reduction of hydrogen cyanide (HCN). The HCN safe levels in legumes range from 10-20mg per 100g. Properly processed and prepared lima bean pose no health problems. Cooking alone reduced E. coli to traces and improved its functional properties.

Moi-moi, akara and traditional dishes (*Oshoto* and *Ikpaki*) based on processed and untreated whole lima bean and maize had high nutrient profile and desirable organoleptic

attributes. It offers opportunity for increased use of neglected and under-exploited legumes in Nigeria ecosystem.

Recommendation

Based on the findings of this research, the following recommendations were made:

(1) There is urgent need to increase awareness of lima bean and its nutrient potentials.

(2) Improved consumption of lima bean should be established and implemented to eradicate malnutrition.

(3) Adequate nutrition education on lima bean be established, especially on its production, processing and contribution to household food security.

(4) Adequate processing of lima bean should be encouraged, as it would widen the food use.

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A P P E N D I C E S

APPENDIX I

PROXIMATE ANALYSIS

CRUDE PROTEIN DETERMINATION

Principle

The sample is digested in sulphuric acid in the presence of a catalyst. The nitrogen from protein and some other constituents is converted to ammonium sulphate. The ammonia is distilled into standard acid after the digest has been made alkaline. The percentage of nitrogen is calculated and the result converted to "crude protein" by multiplication by a factor (usually) 6.25).

Apparatus

1. Kjeldahl flasks, borosilicate glass, 300, 500 or 800ml.
2. Distillation unit.

Reagents

1. Sulphuric acid
2. Catalyst
3. Saturated sodium hydroxide
4. 0.1 N hydrochloric acid
5. 2% boric acid solution
6. Methyl red indicator
7. 0.1 N sodium hydroxide solution

Procedure

Place about 1g of sample, accurately weighed in the Kjeldahl digestion flask. The weight of sample taken should contain about 0.03g of nitrogen. Add 25ml of sulphuric acid and 10g of catalyst and digest in a fume hood, slowly at first to prevent undue frothing. Continue to digest for at least 45mins after the digest has become a clear pale green. Allow to cool and rapidly add 100-200ml water. Mix and transfer to the distillation flask. Add 80-85ml of saturated sodium hydroxide solution from a measuring cylinder so that ammonia is not lost. Distil into 25ml of 0.1 N hydrochloric acid containing a few drops of methyl/red indicator. Distil until the contents of the flask "bump". Titrate the excess acid with 0.1 N NaOH.

Calculation

% nitrogen in sample = 14 x v/100 x 0.1 x w/100

Where v = ml of 0.1N acid added – ml of 0.1N NaOH used to neutralize the ammonia nitrogen.

w = g of sample.

Crude protein = N x 6.25; N is the percentage of nitrogen and 6.25 is a general factor suitable for products in which the proportions of specific proteins is not well defined.

FAT DETERMINATION

Principle

Crude fat can be determined by extracting the dried ground food material with anhydrous ethyl or petroleum ether (BP 40-60°C) in a continuous extraction apparatus of the soxhlet type. The solvent is then removed from the extract by evaporation and the residue is weighed and reported as fat.

Apparatus

1. Soxhlet extraction apparatus
2. Extraction thimble
3. Filter paper
4. Desiccator

5. Evaporating dish
6. Water-bath

Reagents

1. Ethyl ether, anhydrous

Procedure

Accurately weigh 2g of sample into a thimble lined with a circle of filter paper. Place thimble and contents into a 50ml beaker and dry in a mechanical convection oven for 6hrs at 100-102°C for 1-2h at 125°C. Transfer thimble and contents to extraction apparatus. Rinse beaker several times with ethyl ether, adding rinsing to apparatus. Extract the sample contained in the thimble with ethyl ether in a soxhlet extraction apparatus for 6-8h at a condensation rate of at least 3-6 drops per second. After the extraction, transfer the fat extract from the extraction flask into a pre-weighed evaporating dish. Dry the dish and contents in a mechanical convection oven for 30 minutes at 100°C. Remove from the oven, cool in a desiccator and weigh dish plus contents.

Calculation

Crude fat (ether extract)% = $[(W_2-W_1) \times 100]/S$

Where W_1 = weight of empty evaporating dish

W_2 = weight of evaporating dish + contents after drying.

S = sample weight in g.

ASH DETERMINATION

Principle

The ash of a food stuff is the inorganic residue remaining after the food stuff is ignited until it is carbon free, usually at a temperature not exceeding red heat. The ash obtained is not necessarily of exactly the same composition as the mineral matter present in the original food as there may be losses due to volatilization.

Apparatus

1. Porcelain dish
2. Drying oven
3. Muffle furnace

Procedure

Weigh 5g of the sample into a weighed porcelain dish. Dry at 100°C for 3-4h in a convection oven. Remove the porcelain dish from the oven. Do an initial carbonization by placing dish over a Bunsen flame. Heat gently until the contents turn black

Transfer the dish and contents to a muffle furnace and ignite 500-6oo^{o}C until free from carbon. Remove from muffle oven and moisten this first ash with a few drops of water. Re-dry in oven at 100°C for 3-4h and re-ash at 500-600°C for another 1h. Remove from muffle furnace, allow to cool for a moment, place in a desiccator until cool and weigh. Calculate and express results as % ash.

Calculation

Ash %	=	[(B-C) x 100]/A
Where A	=	sample weight in g
B	=	wt in g of dish and contents after drying
C	=	wt in g of empty dish

CRUDE FIBRE DETERMINATION

Principle

Crude fibre is the organic residue left after the defatted material has been treated with boiling dilute sulphuric solution, boiling dilute sodium hydroxide solution, dilute hydrochloric acid, alcohol and ether.

Apparatus

1. 1-litre conical flask
2. Buchner funnel and flask and vacuum pump
3. Hot plate
4. Silica or porcelain crucibles
5. Muffle furnace

Reagents

1. Petroleum ether
2. Sulphuric acid, 0.255N standardized
3. Sodium hydroxide solution
4. Ethanol, 95%
5. Diethyl ether
6. 1% hydrochloric acid

Procedure

Weigh 3g of sample into conical flask or extract the sample on the soxhlet in a thimble or hardened paper that will not contribute fibre. Add 200ml of boiling 0.255N acid and place the flask on a hot plate or over a Bunsen burner so that the solution will boil. Cover with funnel to avoid evaporation. As the liquid boils, control the heating so that gentle ebullition is maintained and continue for 30±2 minutes. Swirl occasionally to remove solids from the sides of the flask. Prepare a Buchner flask and funnel connected to a vacuum pump. Place a what man 11-cm No. 52 paper in the funnel, fill with hot water. After boiling, remove the flask, leave to settle and decant through the Buchner funnel until most of the flask contents is transferred. Pour a measured 200ml of near boiling 0.313N base solution into a wash bottle and use to wash the residue on the paper into the flask. Any particles of fibre in the Buchner funnel must be washed into the flask. Once the bulk of the fibre and solution are transferred, then filter with 1% HCL. Dry in the oven, cool and weigh. Incinerate at 500°C, cool and weigh. The loss in weight represents the fibre content. The fibre residue from the test consists mainly of cellulose with some lignin but not all the cellulose is determined.

Calculation

$$\% \text{ Fibre} = \frac{\textit{Loss in weight from incineration}}{\textit{Weight of sample before defatting}} \; x \; 100$$

MOISTURE DETERMINATION

The moisture content of the flours was determined using the hot air oven method (AOAC, 1995). Porcelain dishes were washed and dried in a Gallen Kamp oven at 100^{0}C for one hour and allowed to cool in desiccators. The dishes were weighed. Two grammes of each flour were weighed into the porcelain dishes and placed in an oven and dried at 100^{0}C for

24h. The dried samples were removed and cooled in desiccators to prevent re-absorption of moisture. After cooling, the dishes were weighed and placed again in the oven, cooled and reweighed. This action was repeated until constant weight was attained. Percentage moisture was calculated as follows:

$$\text{Percentage moisture} = \frac{Moisture\ content}{Weight\ of\ sample} \times \frac{100}{1}$$

APPENDIX II

MINERAL DETERMINATION

MINERALS (CALCIUM, PHOSPHORUS, IRON, ZINC AND COPPER DETERMINATION – DRY ASHING)

1. Weigh 0.5g of finely ground sample in a crucible.
2. Ignite the sample in a muffle furnace for 6-8h or overnight at a temperature of 500°C obtain grey or white ashes.
3. Cool sample and add 5ml 1NHNO_3 solution.
4. Evaporate to dryness on a steam bath.
5. Return sample to the furnace and heat at 400°C for 10-15 minutes until a perfectly white or grayish white is obtained.
6. Cool and add 10ml 1NHCl and filter solution into a 50ml volumetric flask.
7. Wash the crucible and filter paper with additional 10ml portion of 0.1NHCl three times and make up to volume with 0.1NHCl solution.
8. Store the filtrate for minerals determination.

Phosphorus determination by the vanadamolybdate colorimetric method

1. Ash sample
2. Boil ash with 10ml of HCL and wash the solution into the 100ml flask with H_2O.
3. Filter and neutralize by the addition of 0.88 ammonia to 50-60ml volume.
4. Proceed as the standard graph.
5. Make just acid with dilute nitric acid, add 25ml of vanado-molybdate reagent. Dilute to mark and measure the optical density after allowing to stand for 10 minutes.

Preparation of standard curve

1. To a series of 100ml volumetric flasks add 0, 2.5, 5, 10, 20, 30, 40, and 50ml of the standard phosphate solution (3.834g hydrogen phosphate (KH_2PO_4) per litre.
2. Dilute each to 50-60ml with distilled water.
3. Add a few drops of ammonia solution (0.88) and make just acid with nitric acid (1:2).
4. Add 25 ml of the vanadate molybdate reagent (dissolve 20g ammonium molybdate in 400ml warm water (50°C) and cool. Dissolve 1.0g ammonium vanadate in 300ml boiling distilled water and add 140ml conc nitric acid gradually while stirring. Then gradually add the molybdate solution to the acid vanadate solution with stirring and dilute to a litre with water.
5. Dilute to the mark and mix.
6. Allow to stand for 10 minutes and measure the optical density in a 0.25cm cell at 470nm.
7. Plot on a standard curve and read.

EDTA Titration method (Calcium and Potassium)

1. Use stock solution

2.. Dilute 25ml sample to approximately 50ml with H_2O.

3. Add 2ml buffer solution (dissolve 16.9g NH_4Cl in 143ml. NH_4OH, and 1.25g EDTA and dilute to 250ml with H_2O.
4. Add 250mg NaCH (pH 10.0) and 200mg indicator eriochrome black T (mix 0.5g eriochrome black T and 100g NaCl).

5. Titrate with 0.01m EDTA (3.723g $Na_2EDTA_2H_2O$ and dilute to 1 litre with H_2).
6. End point is when solution turns blue from reddish finge.

$[a \times Tca \times v_1 \times 100]/w \times v_2$

a	=	mlEDTA
Tca	=	Titration factor of EDTA
v_1	=	Total volume
v_2	=	Aliquot for determination
w	=	Weight of sample

Volumetric method for Iron determination

1. Dry ash
2. Add H_2SO_4 and heat to dissolve ash
3. Evaporate on steam bath and heat until copious fumes of SO_4 evolve
4. Dissolve in water and let stand on steam bath to cool.
5. Pass H_2S through solution to reduce Fe and ppt any contamination.

Determination of copper by the use of atomic absorption spectrophotometer

Reagents

1. Copper metal
2. HNO_3
3. Stock copper standard.

A stock solution was prepared by dissolving 1.000g copper metal in 50ml of 5m HNO_3. Dilute to 1 litre in a volumetric flask with deionised water. Store in a polythene bottle. 1ml of this stock solution – 1mg = 100µg. The 1ml was further dissolved in litre of deionized water. 0.2ml of the stock solution in 100ml of deionized H_2O = 2µg Cu ml^{-1}. 0.4 ml of the stock solution in 100ml of deionized H_2O = 4µg Cu ml^{-1}. 0.6ml of the stock solution in 100ml of deionized H_2O = 6µg Cu ml^{-1} 0.8ml of the stock solution in 100 ml of deionized H_2O = 10µg Cu ml^{-1}.

Principle

Copper in the residue remaining after the destruction of the organic matter of the plant material is dissolved in HNO_3. The concentration of Cu in the solution is determined using sp g model atomic absorption spectrophotometer at 324.8nm. The absorption is proportional to the concentration of copper.

Methods

The sample solution were placed through the atomic absorption spectrophotometer and reading obtained.

Calculation

A calibration curve was constructed and used to calculate the concentration of Cu in the sample.

$$\frac{\mu g\ of\ the\ curve\ x\ dil.\ factor\ x\ 100\ (\%)\ x\ 1000mg}{Wt.\ of\ sample\ x\ 10^{6}\ \ (g)}$$

= Result in mg/100g sample.

Determination of zinc by the use of atomic absorption spectrophotometer

Reagents

1. Zinc
2. HCl
3. Stock zinc standard

A stock solution was prepared by dissolving 1.00g of zinc in 30ml of 5m HCl. Dilute to 1 litre in a volumetric flask with deionized water. Store in a plastic bottle. 1ml of the stock solution = 1mg = 1000µg.

The 1ml was further dissolved in 1 litre of deionized water. 0.2ml of the stock solution in 100ml of deionized H_2O = 2µg Zn^{n-}

0.4ml of the stock solution in 100ml of deionized water = 4µg Zn ml^{-1}.

0.6ml of the stock solution in 100ml of deionized H_2O = 6µg Zn ml^{-1}.

0.8ml of the stock solution in 100ml of deionized H_2O = 8µg Zn ml^{-1}.

1.0ml of the stock solution in 100ml of deionized H_2O = 10µg Zn ml^{-1}.

Principle

Zinc is the residue remaining after the destruction of the organic matter of plant material obtained.

Calculation

A calibration curve was constructed and used to calculate the concentration of zinc.

$$\frac{\mu g\ of\ the\ curve\ x\ dil.\ factor\ x\ 100\ (\%)\ x\ 1000mg}{Wt.\ of\ sample\ x\ 10^6\ \ (g)}$$

= Result in mg/100g sample.

APPENDIX III

ANTINUTRIENTS AND FOOD TOXICANTS ANALYSIS

PHYTATE DETERMINATION

Reagents

1. 2.4% HCL
2. 0.1m NaCl
3. 0.7m NaCl
4. Wade reagent: This was prepared with 0.03% $FeCl_2.6H_2O$ and 0.3% Sulfosalicylic acid in deionised water.
5. Phytic acid standard: This was prepared by weighing 0.1g Phytic acid in 100ml distilled water. The following serial dilutions were made into test tube: 0.1, 0.2, 0.3, 0.4, 0.5, 0.6. The blank was made with deionized water to zero the Specrophotometer. 1ml of the modified wade reagent was added.

Principle

Determination of phytate has been on its precipitation as insoluble ferric phytate in acid solution. The 0.1m NaCl eludes the inorganic phosphorus and the 0.7m NaCl eludes phytate.

Methods

1. Weigh 0.5g sample into 400ml tall beakers
2. Extract with 100ml 2.4% HCL for 1hr at room temperature and decant.
3. To 10ml of the diluted sample, add 15m of 0.1m sodium chloride to elude in organic phosphorus and 15ml 0.7m sodium chloride to elude phytate.
4. Pipette 3ml of each extract to test tubes and add 1ml of the modified wade reagent (0.03% $FeCl_2.6H_2O$ and 0.3% sulfo-salicylic acid in distilled water).
5. Read at 500nm by using water to zero the spectrophotometer.

TANNIN DETERMINATION

Reagents

1. Methanol
2. 0.1m $FeCl_2$ in 0.1 NHCl
3. 0.008m $K_3Fe(CN)_6$
4. Tannic acid std: A standard tannic acid was prepared by weighing 0.1g tannic acid in 100ml of deionized water. The following dilutions were made into test tube 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.010, 0.011, 0.012. The blank was made with deionised water to zero the spectrophotometer. 3ml of 0.008m $K_3Fe(CN)_6$ were added to the serial dilutions.

Method

1. Weigh 0.6g powdered sample, add 5ml methanol and shake
2. Decant the contents into a test tube and mix filtrate with 50ml water.
3. Add 3 ml 0.1m $FeCl_2$ in 0.1 NHCl 33ml 0.008m $K_3Fe(CN)_6$.
4. Read both the standard and sample solutions with Spectronic 20 at 720nm within 10 minutes.

Calculation

A calibration curve was constructed and used to calculate the concentration of tannin.

TRYPSIN INHIBITOR DETERMINATION

Sample preparation

1.0g of the flour sample was extracted with 50ml of 0.01N NaOH. The pH was adjusted to 9.5 – 9.8. The solution was allowed to stand for 3h with sufficient stirring to keep the sample in suspension. This suspension was diluted so that 2ml of the sample extract inhibit 40-60% of the trypsin used as standard in the analysis.

Procedure

Portions of 0, 0.6, 1.0, 1.4 and 1.8ml of the suspension were pipetted into triplicate sets of test tubes and adjusted to 2.0ml with water. 2.0ml of trypsin solution was added to each test tube, the tubes were placed on a water bath of 37°C. To each test tube, 5ml of BAPA (benzoyl-DL-arqinine-P-nitroanilide hyudrochloride) solution was added (BAPA was dissolved in 1ml dimethyl sulfoxides and diluted to 100ml previously warmed at 37°C). The reaction was terminated at exactly 10 minutes later by adding 1ml of 30% acetic acid. After thorough mixing with vortex mixer, the content of each tube was filtered (Whatman No. 3) and the absorbance of the filtrate was measured against blank at 410nm. The blank was prepared with the same procedure except that trypsin solution was added after the reaction had terminated. The values obtained were subtracted from the trypsin standard. The values were averaged, and the trypsin inhibitor content was determined.

Calculation

$$TI = \frac{differential\ absorbance}{0.019\ x\ 1000g}\ x\ 100\ (mg/100g)$$

SAPONIN DETERMINATION

0.1g of the sample was boiled with 50ml distilled water for 15 minutes and filtered with Whatman No.1, 5ml of the filtrate was pipetted into a test tube and 2ml of olive oil was added. The solution was shaken vigorously for 30 seconds and read at 620nm against a blank.

Calculation

Saponin =

$$\frac{reading\ \ from\ curve\ x\ dilution\ \ factor}{Weight\ of\ \ the\ sample\ x\ 10^6}\ x\ 100\ (mg/100g)$$

CYANIDE DETERMINATION

Five gramme (5g) of flour sample was introduced into 300ml volumetric flask. 160ml of 0.1M Orthophosphoric acid and homogenize for 15 minutes at low speed and made up to the mark. The solution was centrifuged at 10,000 rpm (revolutions per minute) for 30 minutes. The Supernatant was transferred into a screw cap bottle and stored at 4^oC. 5ml aliquot of the extract was transferred into quick fit stoppered test tube containing 0.4ml of 0.2m phosphate buffer pH 7.0. 10ml of diluted linamarase enzyme was added. The tube was incubated at 30^oC for 15 minutes and the reaction was stopped by addition of 0.2M NaOH (0.6ml). The absorbance of the solution was measured using suitable spectrophotometer at 450nm against blank.

Calculation

$$Cyanide = \frac{absorbance\ x\ dilution\ \ factor}{extinction\ coefficient}\ \ x\ \ 100\ (mg/100g)$$

Extinction Coefficient (Σ1/450) = 2550.

APPENDIX IV

MICROBIAL EXAMINATION OF FLOUR SAMPLES

Materials

i. Samples

ii. Glass test tubes

iii. Test tube racks

iv. Cotton wool

v. Glass petridishes

vi. Glass spreaders

vii. Graduated glass pipettes (10ml, 1ml)

Media

Nutrient Agar

Mac-Conkey Agar

Normal Saline (0.85%).

Preparation of Media

28g of Nutrient Agar and 52g of Mac-Conkey Agar powder were weighed and dissolved in 1000ml of distilled water. Each medium was completely dissolved in a pressure cooker before it was autoclaved at 121°C (15lb pressure) for 15 minutes. The autoclave was allowed to cool completely before the medium was brought out. When the temperature of the medium has come down to 50-45°C, about 25ml of the medium was poured into each sterile

glass petridish. The medium was allowed to set at room temperature before they were put in the incubator for 18-24hr for sterility test.

Normal Saline

8.5g of sodium chloride was weighed and dissolved in 1000ml of distilled water and sterilized by autoclaving at 121°C for 15 minutes.

Methods

9ml of 0.85% normal saline was pipetted into each glass test tube. The test tubes were covered with cotton wool and sterilized at 121°C for 15 minutes. The sterile saline contained in the test tubes were arranged in a test tube rack and labeled serially in 10 fold dilution.

Isolation of the Bacteria

Ten fold dilutions were made on the test samples. 1ml of the sample mixture was removed and added to the 2nd tube to give 10^{-2} dilution. This process continued up to 8th tube. The 9th tube was the control and contained only 9ml of normal saline without the test sample.

A set of 9 sterile Nutrient agar and 9 Mac-Conkey agar plates were set up to inoculate the serial dilutions. 0.1ml of each dilution was dropped on the sterile agar plates and spread with sterile glass spreaders. The inoculated agar plates were incubated for 37°C for 18-24hrs aerobically. After 24hrs incubation, the colony growth in each dilution was counted and the results taken.

Identification of the Isolates

The identification of isolates were based on their cultural morphology and biochemical characteristics. The cultural morphology includes colony shape, size, colour, odour, texture and Gram reaction of the bacteria were considered. The biochemical ability of the microorganism to utilize sugars was also considered.

APPENDIX V

RECIPE AND METHODS OF PREPARATION OF PRODUCT AND DISHES

Preparation of moi-moi

Ingredients

250g flour

40g onions

10g crayfish (ground)

2g pepper

3 dessert spoon vegetable oil

200ml water (warm water)

5g Tartashi

2 cubes of magi

Salt to taste

200ml meat broth

Method

Put the flour in a bowl or basin

Add 200ml of metal broth to the flour, stir thoroughly to incorporate air

Add onions, crayfish, meat broth, magi cubes, pepper, salt, tartarshi. Add the water and mix very well.

Wrap the mixture in foil

Put in little quantity of water at boiling point and steam for 45-60 minutes

Preparation of Akara

Ingredients

150g flour

50ml water

Salt

Vegetable oil

Method

Put the flour in a mortar, add 50ml of water and stir thoroughly to incorporate air. The paste was whipped until it becomes fluffy. Salt was added to taste. The paste were moulded into small balls and fried in a hot vegetable oil until golden brown colour. The akara balls were drained oil in a colander.

Preparation of *Oshoto*

Ingredients

200g lima bean (black or dark red)

200g fresh or dried maize

Palm oil 3 dessert spoon

African oil bean 50g

Maggi 2 cubes

Crayfish 50g

Dried fish 50g

Onion 10g

Spinach or fluted pumpkin leaves 30g

Method

Cook the lima bean until soft for human consumption. Drain the water after cooking. Equal amount of cooked maize should be added to the cooked beans. Add Palm oil, African oil bean, magi cube, salt, crayfish, dried fish, onions and mix thoroughly little quantity of green vegetable (spinach or fluted pumpkin leaves were added) The simmer for another 10 minutes and serve.

Preparation of *Ikpaki*

Ingredients

300g lima bean (any variety)

Palm oil. 3 dessert spoon

Crayfish 10g (ground)

Salt (to taste)

Maggi 2 cubes

Onions

Fresh or dried fish

Pepper

Method

Soak the beans for 30 minutes, pour away the soaking water and cook until soft for human consumption. Cooking water will be discarded and replaced up to four times at intervals. After cooking, drain the water and add the food ingredients palmoil, salt, magi, onions, crayfish, fresh fish, pepper, fresh tomatoes. Then simmer for about 10 minutes and serve. This dish Ikpa is prepared without maize.

APPENDIX VI

SENSORY EVALUATION FORM

UNIVERSITY OF NIGERIA, NSUKKA
DEPARTMENT OF HOME SCIENCE, NUTRITION AND DIETETICS

Name: ……

Date: ……..

Products: Moi-moi and Akara

Instruction: Please, taste the coded samples before you and indicate how much you like or dislike each of them. Evaluate these samples for colour, texture, flavour and general acceptability. Check the point on the scale that best describes the products as they appeal to you, by ticking (√)

Hedonic Scale	S	SENSORY ATTRIBUTES											
Description		COLOUR											
	Scores	JNO	AOO	MCO	VNO	PNO	VAO	JCO	MEO	AOB	TAC	KCC	SCC
Like extremely	9												
Like very much	8												
Like moderately	7												
Like slightly	6												
Neither like nor dislike	5												
Dislike slightly	4												
Dislike moderately	3												
Dislike very much	2												

Dislike extremely	1												

SENSORY EVALUATION FORM

Name: ……

Date: ……..

Products: Moi-moi and Akara

Instruction: Please, taste the coded samples before you and indicate how much you like or dislike each of them. Evaluate these samples for colour, texture, flavour and general acceptability. Check the point on the scale that best describes the products as they appeal to you, by ticking (√)

Hedonic Scale	S	SENSORY ATTRIBUTES											
Description		TEXTURE											
	Scores	JNO	AOO	MCO	VNO	PNO	VAO	JCO	MEO	AOB	TAC	KCC	SCC
Like extremely	9												
Like very much	8												
Like moderately	7												
Like slightly	6												
Neither like nor dislike	5												
Dislike slightly	4												
Dislike moderately	3												
Dislike very much	2												
Dislike extremely	1												

SENSORY EVALUATION FORM

Name: ……

Date: ……..

Products: Moi-moi and Akara

Instruction: Please, taste the coded samples before you and indicate how much you like or dislike each of them. Evaluate these samples for colour, texture, flavour and general acceptability. Check the point on the scale that best describes the products as they appeal to you, by ticking (√)

Hedonic Scale	S	SENSORY ATTRIBUTES											
Description		FLAVOUR											
	Scores	JNO	AOO	MCO	VNO	PNO	VAO	JCO	MEO	AOB	TAC	KCC	SCC
Like extremely	9												
Like very much	8												
Like moderately	7												
Like slightly	6												
Neither like nor dislike	5												
Dislike slightly	4												
Dislike moderately	3												
Dislike very much	2												
Dislike extremely	1												

SENSORY EVALUATION FORM

Name: ……

Date: ……..

Products: Moi-moi and Akara

Instruction: Please, taste the coded samples before you and indicate how much you like or dislike each of them. Evaluate these samples for colour, texture, flavour and general acceptability. Check the point on the scale that best describes the products as they appeal to you, by ticking (√)

Hedonic Scale		SENSORY ATTRIBUTES
Description		GENERAL ACCEPTABILITY

	Scores	JNO	AOO	MCO	VNO	PNO	VAO	JCO	MEO	AOB	TAC	KCC	SCC
Like extremely	9												
Like very much	8												
Like moderately	7												
Like slightly	6												
Neither like nor dislike	5												
Dislike slightly	4												
Dislike moderately	3												
Dislike very much	2												
Dislike extremely	1												

SENSORY EVALUATION FORM

Name: ……

Date: ……..

Products: *Oshoto* and *Ikpaki* dishes

Instruction: Please, taste the coded samples before you and indicate how much you like or dislike each of them. Evaluate these samples for colour, texture, flavour and general acceptability. Check the point on the scale that best describes the products as they appeal to you, by ticking (√)

Hedonic Scale		SENSORY ATTRIBUTES					
Description		COLOUR					
	Scores	BVCP	BOSH	BIKP	DRCCP	DROSH	DRIKP
Like extremely	9						
Like very much	8						
Like moderately	7						
Like slightly	6						
Neither like nor dislike	5						
Dislike slightly	4						
Dislike moderately	3						
Dislike very much	2						

Dislike extremely	1						

SENSORY EVALUATION FORM

Name: ……

Date: ……..

Products: *Oshoto* and *Ikpaki* dishes

Instruction: Please, taste the coded samples before you and indicate how much you like or dislike each of them. Evaluate these samples for colour, texture, flavour and general acceptability. Check the point on the scale that best describes the products as they appeal to you, by ticking (√)

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Hedonic Scale		SENSORY ATTRIBUTES					
Description		TEXTURE					
	Scores	BVCP	BOSH	BIKP	DRCCP	DROSH	DRIKP
Like extremely	9						
Like very much	8						
Like moderately	7						
Like slightly	6						
Neither like nor dislike	5						
Dislike slightly	4						
Dislike moderately	3						
Dislike very much	2						
Dislike extremely	1						

SENSORY EVALUATION FORM

Name: ……

Date: ……..

Products: *Oshoto* and *Ikpaki* dishes

Instruction: Please, taste the coded samples before you and indicate how much you like or dislike each of them. Evaluate these samples for colour, texture, flavour and general acceptability. Check the point on the scale that best describes the products as they appeal to you, by ticking (√)

Hedonic Scale		SENSORY ATTRIBUTES					
Description		FLAVOUR					
	Scores	BVCP	BOSH	BIKP	DRCCP	DROSH	DRIKP
Like extremely	9						
Like very much	8						
Like moderately	7						
Like slightly	6						
Neither like nor dislike	5						
Dislike slightly	4						
Dislike moderately	3						
Dislike very much	2						
Dislike extremely	1						

SENSORY EVALUATION FORM

Name: ……

Date: ……..

Products: *Oshoto* and *Ikpaki* dishes

Instruction: Please, taste the coded samples before you and indicate how much you like or dislike each of them. Evaluate these samples for colour, texture, flavour and general acceptability. Check the point on the scale that best describes the products as they appeal to you, by ticking (√)

Hedonic Scale		SENSORY ATTRIBUTES					
Description		GENERAL ACCEPTABILITY					
	Scores	BVCP	BOSH	BIKP	DRCCP	DROSH	DRIKP
Like extremely	9						
Like very much	8						
Like moderately	7						
Like slightly	6						
Neither like nor dislike	5						
Dislike slightly	4						
Dislike moderately	3						
Dislike very much	2						
Dislike extremely	1						