PRODUCTION AND EVALUATION OF SAUCES MADE FROM *Treculia africana,* Decne AND *Parkia biglobosa,(*Jacq.) Benth

A THESIS PRESENTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE (M.Sc) DEGREE IN FOOD SCIENCE AND TECHNOLOGY

BY

ADEGEDE, OJONE GRACE

(PG/M.Sc/06/40894)

DEPARTMENT OF

FOOD SCIENCE AND TECHNOLOGY

UNIVERSITY OF NIGERIA, NSUKKA

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CERTIFICATION

Adegede, Grace Ojone, a postgraduate student in the Department of Food Science and Technology, Faculty of Agriculture, University of Nigeria, Nsukka, has satisfactorily completed the requirements for the degree of Master of Science (M.Sc) in Food Science and Technology. The work embodied in this thesis is original and to the best of my knowledge has not been submitted in part or full for the award of any degree or certificate of this or other university.

Dr. (Mrs.) J.C. Ani ______________________ ____________________

(Supervisor) Signature Date

Dr. (Mrs.) J.C. Ani ______________________ ____________________

(Head of Department) Signature Date

DEDICATION

This work is dedicated to Almighty God.

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First and foremost, I appreciate my supervisor and Head of Department; Dr. (Mrs) J.C. Ani for her immense support and motherly advice which has brought me this far.

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ABSTRACT

African locust bean sauce and African breadfruit sauce were produced from African locust bean kernel and African breadfruit seed kernel respectively. Dehulled Seeds of African locust bean and African breadfruit were parboiled and mixed with steamed wheat flour. The mash was inoculated with *Aspergillus oryzae* and on the third day *Zygosaccharomyces rouxii* and *Candida versatilis* were added and the mash was left to ferment in 17% brine for 4 more days at 29^{o}C. The sauce samples were subjected to physico-chemical, microbiological and sensory quality analyses, using soy sauce as control. Monitoring changes during fermentation showed consistent decreases in pH, titratable acidity (as citric acid), glucose, total soluble solids and the minerals especially calcium and potassium as fermentation progressed. The result showed that protein content ranged from 2.62 to 3.50%, ash from 5.15 to 6.12% and fat ranged from 0.15 to 1.15% in African breadfruit and African locust bean sauces, respectively. Profile of essential free amino acid such as methionine, leucine, isoleucine, phenylalanine, histidine and valine was more significant ($p<0.05$) in African locust bean and African breadfruit sauces than in soy sauce (Control). Cystine, glutamic and proline occurred in high concentration in African breadfruit sauce than was found in African locust bean sauce, but lower than the concentration in soy sauce. Sensory evaluation showed that there was no significant difference ($p>0.05$) in color among the sauces. African breadfruit sauce was significantly different in taste and overall acceptability from African locust bean sauce and soy sauce. There was no significant difference ($p>0.05$) between soy sauce and African locust bean sauce in flavor and mouth feel. However, the panelist showed preference for soy sauce to African locust bean sauce and African breadfruit sauce.

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CHAPTER 1

1.1 INTRODUCTION

Sauce is a liquid or sometimes semi-solid food served on or used in preparing other foods. They are not consumed alone; they add flavor, moisture and visual appeal to other dishes. The term “Sauce” is a French word taken from the Latin word salsus, meaning salted. Sauces need a liquid component, but some sauces (e.g. salsa or chutney) may contain more solid component than liquid (Peterson, 1998). Sauces are essential elements in cuisines all over the world. A condiment is a prepared edible substance or mixture of substances, often preserved or fermented. It is added most often at the table in variable quantities to make food palatable to the consumer. Some condiments are dry such as mixtures of herbs and others are seasonings ‘dawadawa’, however many are preserved sauces in bottles, jars, and other containers. For convenience, some condiments are provided in single-serving packets. The terms ‘condiments and sauces’ have been used interchangeably, though they do not mean exactly the same. The basic difference is in their processing and usage. A sauce is a flavorful relish or topping served as an accompaniment to food. It is a preparation, usually liquid or semi liquid, which could be eaten as gravy. Whereas a condiment is a substance used to flavor or complement food. Some condiments may be used during cooking to add flavor or texture to the food e.g. soy sauce which can be used to enhance the taste of a variety of foods. Condiments and sauces can be used for the same purposes, such as addition of flavor and visual appeal to foods (McGee, 2004).

The conventional substrates for condiment production are diverse and each is usually produced from more than one raw material. Quite often, seeds that are used in the production of condiments are inedible in their raw unfermented or uncooked state (Achi, 2005). Legume condiments rich in protein can serve as tasty compliments to sauces and soups and can substitute for fish or meat. The food flavoring condiments are prepared by traditional methods of uncontrolled solid substrate fermentation resulting in extensive hydrolysis of the protein and carbohydrate components.

The use of hydrolyzed vegetable proteins in seasoning has long been recognized. In South Eastern Asia, soy sauce, a fermented soybean product is a notable product among others. In Northern Nigeria 'dawadawa' is extensively used as seasoning in soups. In Nigeria and most African countries, condiments such as fermented locust beans and fermented melon seed or castor oil bean, are widely used to give flavor and taste to food. Food condiments in Nigeria, other countries of West Africa and Central Africa are popular strong-smelling fermented food products that give pleasant aroma to soups, sauces and other prepared dishes. They also have great potential as key protein, fatty acid and energy sources. Therefore, condiments are basic ingredients for food supplementation. Their socio-economic importance cannot be over emphasized especially in Africa, and Asia, where protein calorie malnutrition is a major problem (Iheanyi *et al*., 2006).

Fermented locust bean 'dawadawa', as it is known in the Hausa speaking part of Nigeria, and 'iru' as called by Yorubas, is a food condiment that has been extensively used in Nigerian homes. Besides serving as a seasoning in soups, it is also used in poor rural families as a low cost meat substitute, as it contributes to their protein and calorie intake (Odunfa, 1985). Other protein-rich seeds like soy bean (*Glycine max),* castor oil bean (*Ricinus communis*) and African oil bean (*Pentaclethra macrophylla*) have been used to make food condiments. Among these legumes used in processing condiments, soybean is lowest in starch and has the most complete amino acid mix, relative to reference protein. Soybean seed has been used as a good substitute for African locust bean seed in condiment processing. Fermentation transforms these legume seeds, which are practically inedible in their raw state into condiments that are rich in fat, lysine and other amino acids. It has been reported (Eka, 1980) that approximately 60% of African locust bean fat is unsaturated, the major fatty acid being linoleic acid, which is nutritionally useful, while about 7% of the protein is lysine, similar to that in whole egg. African breadfruit is one of the seeds that have also recently gained attention as a result of its potential as a protein supplement. The seed has been reported (Akubor *et al.*, 2000) to contain about 17-23% protein, 11% crude fat and other essential vitamins and minerals. The biological value of its protein exceeds that of soybean, hexane extract of the seeds has been shown to contain a stearic solid fat fraction, resembling that of palm kernel oil and an oleic fraction with a composition that is similar to that of cotton seed oil (Ihekoronye and Ngoddy, 1985). Legumes rich in protein are often used in sauce production. The fermentation process which the seeds are subjected to help in the breakdown of protein, production of glutamic acid and the release of ammonia, which is an important factor in sauce production. The high protein content of the seeds used in this study make them attractive alternatives to soy bean in the production of sauce, hence the reason for their use in this study.

In many parts of Nigeria today, condiments are still prepared by traditional methods. Starter cultures are not normally used in the traditional methods, and therefore variations in the quality and stability of the products are often observed. Although fermented food condiments have constituted a significant proportion of the diet of many people, there has been a diminishing popularity of these condiments because dried soups and bouillon cubes have been highly promoted in the urban markets.

1.2 JUSTIFICATION

African locust bean (*Parkia biglobosa*) and African breadfruit (*Treculia africana*) seed kernels contain 39-47% and 17-23% protein, respectively. They can serve as good protein supplements in foods, especially in the diets of the people in the region where these trees grow naturally. Presently they are used separately to produce condiments (Locust bean), eaten as snacks and used as soup thickeners; their use can be further diversified if they can be used to produce a more readily available, palatable and widely acceptable product similar to soy sauce. Since the African locust bean and African breadfruit seeds compare favorably with soy bean seed in terms of composition (high protein content and rich in most essential amino acids) and they belong to the same family *leguminosae*, these seeds can also be used as possible raw materials for sauce production just as soy bean is used for soy sauce. It is envisaged that this work will give rise to cottage industries where protein sauce from *Parkia biglobosa* or *Treculia africana* can be produced to diversify the utility of these seeds and generate additional income for the farmer. The diversified use of these tree crops will affect positively their cultivation and discourage their destruction.

1.3 OBJECTIVES

The broad objective of this study therefore, is to evaluate the performance of African breadfruit (*Treculia africana)* and African locust bean (*Parkia biglobosa)* in sauce production.

The specific objectives are:

i. To produce sauces from African breadfruit (*Treculia africana)* seed and African locust bean seed (*Parkia biglobosa)* kernels, thus diversifying their uses;
ii. To evaluate the composition and selected characteristics of the sauces produced in comparison with soy sauce as control;
iii. To assess the acceptability of the products.

CHAPTER 2

LITERATURE REVIEW

2.1 Sauces and Condiments

Traditional diets in West Africa often consist of large quantities of the staple food (cassava, yam, maize) with supplements of plantain, cocoyam, rice and beans, depending on availability and season. Soups eaten with the staples are an essential component of the diet and may contain a variety of seeds, nuts, pulses and leaves (Achi, 2005). Soups are the main sources of proteins and minerals as the staple foods provide the calories but are poor in other nutrients. Therefore, one of the ways to improve the diet has been to improve the nutrient content of soups. Seed kernels of legumes may account up to 80% dietary protein and may be the only source of protein for some groups. Their cooked forms are eaten as meals and are commonly used in fermented form as condiments to enhance the flavors of foods (Aidoo, 1986). With high contents of protein, legume condiments can serve as a tasty complement to sauces and soups, substituting for fish or meat. Initially, spices and condiments were herbs, leaves and/or fermented seeds, until the discovery of monosodium glutamate by Kikunae Ikeda (1908) and its subsequent commercial production in 1908. In Nigeria and most African countries, condiments such as fermented locust beans "iru" and fermented melon seed kernel "ogiri" are widely used to give flavor and taste to food. The proximate composition indicated that these two condiments could contribute to the protein, lipid and mineral daily intake when used liberally as done in several homes, where the expensive animal product is a luxury (Odunfa, 1981; Omafuvbe *et al.,* 2000). Protein rich seeds are often used to make food condiments. In South East Asia, soybean is fermented to a variety of products notably "shoyu" (soy sauce), "natto", "miso" and "tempeh". In Africa, many other proteinaceous seeds are fermented to make food condiments. The variety of names by which the products are known in different parts of Nigeria maybe based on the oil seed or legume used. The Yorubas, of the southwestern Nigeria locally call fermented condiments 'iru', while the Hausas who inhabit most of the northern part of the country call it 'dadawa'. 'Ogiri' is the name used by the Igbos of the southeastern Nigeria. 'Owoh' on the other hand, is the popular name among the Urhobos and

Itshekiris in the Niger Delta region. Similarly, ‘okpiye’ is popular among the Igalas and Idoma people of the middle belt region (Achi, 2005)

The production of fermented vegetable proteins for use as food condiments is largely craft-based and on a traditional small-scale, household basis under highly variable conditions. The success of the process depends upon observance of good manufacturing practices and control of environmental conditions during the manufacturing phase. In addition, fermentation is usually carried out in a moist solid state, involving contact with appropriate inocula of assorted micro-organisms and is accomplished by the natural temperatures of the tropics. Starter cultures are not normally used and, therefore, variations in the quality and stability of the products are often noticed. The desired state of fermentation of the condiments is indicated by the formation of mucilage and overtones of ammonia produced as a result of the breakdown of amino acids during the fermentation (Omafuvbe, 2006). The characteristic ammoniacal odor and flavor of condiments enhance the taste of traditional soups and sauces especially the various soups used as accompaniment to the starchy root and tuber diets. The conventional substrates for condiment production are diverse and each can be produced from more than one raw material. Almost any edible plant material can be subjected to fermentation. Quite often, seeds that are used for fermentation are inedible in their raw unfermented and uncooked state (Achi, 2005). It is unfortunate that the culinary trend in developing countries like Nigeria today is towards the use of cheap and ubiquitous monosodium glutamate based seasoning salts. The application of these seasoning salts, to the detriment of local condiments, in public and private cooking is partly due to a lack of nutrition information and the high cost of animal proteins (Achi, 2005).

2.2 Soy Sauce

Soy sauce is one of the world’s oldest condiments and has been used in China for more than 2,500years. It is a dark brown salty liquid with a peculiar aroma and a meaty taste. It is the chief savory seasoning agent in Oriental cooking, but it is becoming increasingly popular in many other regions of the world. It was once a homemade staple, but now it has become an important industrial product. It is made from fermenting a mixture of mashed soybeans, salt and enzymes. It is also made artificially through a chemical process known as acid hydrolysis. Industrialization has altered the production process, changed the raw materials used, standardized the products and modified their characteristics. The main ingredients of soy sauce are soybeans (or defatted soybean meal), wheat, salt and water. The heart of the manufacturing process is a complex fermentation whereby the carbohydrates are fermented to alcohol and lactic acid and the proteins are broken down to peptides and amino acids. Chemical reactions between the original components and the fermentation products create the color, consistency and aroma of soy sauce (Fukushima, 1981)

The process of manufacturing soy sauce conventionally, can be divided into three stages: preparation of the ‘koji’, fermentation in brine and product refining. Figure1 shows the basic outline of the manufacturing process of ‘koichuchi’ sauce, a representative type of Japanese soy sauce. ‘Koji’ derived from the Chinese character meaning moldy grains, serves as a source of a variety of enzymes which catalyze the degradation of solid raw materials to soluble products providing fermentable substrate for yeast and bacteria in the subsequent fermentation stage. It is not the end product of a traditional process, but the end of a first stage in a process usually involving a second fermentation. It is analogous to the use of malt in alcoholic fermentation of grain (Yong and Wood, 1974).

Realizing that sodium chloride has a strong inhibitory effect on protease activity, a new method of short term soy sauce making on the industrial experimental scale has been reported (Wood, B.J.B, 1998) in Japan, where the time of the “moromi” stage was divided into 2 functionally independent processes, namely: autolysis of “koji” and brine fermentation. According to Maramatsu In Wood, B.J.B, (1998) in this process, soybean “koji” is auto-digested at various temperatures in the absence of sodium chloride (NaCl), the optimal temperature being 55ºC. The auto digested “koji” is then subjected to two-step brine fermentation. The first step is started by inoculation of *Tetragenococcus halophilus* and *Candida versatilis*. After 16 days, an equal amount of freshly prepared “koji” autolysate is added and *Saccharomyces rouxii* is inoculated to induce major alcohol fermentation. In 2 months, the alcohol fermentation is completed. The “moromi” is then left to age making the entire fermentation process complete in 3 months, yielding good quality soy sauce as compared to that obtained using the conventional method. As practiced in a few factories in Thailand, protein digestibility is increased by preincubation of “koji” with small amount of water for 2 hours prior to the addition of brine to make up the desired salt concentration. An industrial scale experiment showed that the preincubation period could be extended to 5 to 6 hours and that the process resulted in shortening of fermentation time from 36 to 21 days. Soy sauce can also be produced by boiling soybeans in hydrochloric acid. This yields a product with harsh taste and rather undesirable (Aeaungkoon In Wood, B.J.B, 1998). The characteristics of the many varieties of soy sauce produced depend on the kinds and ratio of raw materials used, the kind of microbes employed and the conditions of preparation.

Rose (1982) stated that an important role of salt and acid is that their combined action lowers the activity of the enzymes responsible for the breakdown of the plant tissues, while at the same time inhibiting certain oxidative changes in the vegetable. To achieve a palatable taste for soy sauce, about one half of its nitrogenous compounds must be free amino acids and more than 10% of the nitrogenous compounds must be free glutamic acid. The proteins in wheat kernels are good sources of glutamic acid, which gives an important taste in soy sauce (Rose, 1982).

2.3 Raw materials

- **Preservatives and other additives**

Sodium benzoate or benzoic acid is added to help inhibit microbial growth in finished soy sauce. Sodium chloride also acts as a preservative and exerts a selective action on the microorganisms which grow in the fermentation.

- **Fermenting agents**

The wheat-soy mixture is exposed to specific strains of mold called *Aspergillus oryzae* or *A.sojae*, which breaks down the proteins in the mash. Further fermentation occurs through addition of specific bacteria *(Lactobacillus)* and yeasts which enzymatically react with the protein residues to produce a number of amino acids and peptides. These include glutamic and aspartic acid, lysine, alanine, glycine, and tryptophane, and all contribute flavor to the end product.

- **Salt**

Salt or sodium chloride, is added at the beginning of fermentation at approximately 12-18% of the finished product weight. The salt is not just added for flavor, it also helps establish the proper chemical environment for the lactic acid bacteria and yeast to ferment properly. The high salt concentration is also necessary to help protect the finished product from spoilage. If salt were not present, a dangerous anaerobic bacteria fermentation would occur in the traditional process, and one might expect such organisms as *Botulinus* to grow.

- **Wheat**

Wheat is widely cultivated as a cash crop because it produces a good yield per unit area, grows well in a temperate climate even with a moderately short growing season, and yields versatile, high-quality flour that is widely used in baking. The popularity of foods made from wheat flour creates a large demand for the grain, even in economies with significant food surpluses. Wheat contains about 51.8% carbohydrate, 9.72% Fat and 23.15% protein. In many traditional brewed recipes, wheat is blended in equal parts with the soybeans.Pulverized wheat is made part of the mash primarily to modify the flavor of the product. The proteins in wheat kernels are good sources of glutamic acid, which is an important taste ingredient in soy sauce. The roles of wheat in soy sauce manufacture includes: (1) to make the moisture content of the material to be cultured with mold just adequate for mold growth. (2) To assist in obtaining the highest proteolytic activity from the 'koji'. (3) To serve as the major source of carbohydrates as the precursor of sugars, alcohol and organic acids. (4) To serve as the source of lignin and glycosides, the precursors of vanillic flavor of "shoyu". (5) To serve as a rich source of glutamic acid.

- **Soybean**

Soybean *(Glycine max)* also called soyabeans, sojabeans, Chinese peas, soy peas and Manchurian beans, have been referred to as the 'King of legumes' because of their valuable nutritive properties. The soybean is an annual plant commercially grown primarily for oil and protein production. Though morphological diversity exists, the soybean is generally a plant which grows 90-120cm in height with the first leaves simple and opposite, and all other leaves alternate and trifoliate. Soybeans are short, hairy pods containing two or three seeds which may be small and round or larger and more elongated. Their color varies from yellow to brown, green and black. The developmental morphology of the soybean has been extensively described by Carlson (1973).

Of all beans, soybean is lowest in starch and has the most complete and best protein mix. Its protein content is about 35-40%, hence, the seed is the richest in food value of all plant foods consumed in the world. They are also high in minerals, particularly calcium and magnesium, and in vitamin B. It is the only source that contains all the essential amino acids (Ihekoronye and Ngoddy, 1985). Soybean protein is of high nutritional quality. Since soybean is a rich source of both oil and protein, it has become the raw material for diversity of uses; agricultural and industrial. The utilization of soybean began in the Orient where both medicinal and food values were assigned to this legume. Various types of foods were prepared from it, including beverages, pastes, curds and fermented flavorants or condiments, some resembling milk, cheese and meat. It is used in fresh, fermented or dried form (Norman, 1978). Soybean oil is a major component of the edible oil market and is processed into a variety of products for human consumption. Primarily, shortening and margarine where it encounters competition from other vegetable oils sources e.g. sunflower, palm, peanut, cottonseed, rapeseed, coconut and olives. There are slight differences in the chemical composition of the beans from different countries. Its fat content is about 16 – 21%. Soybean meal is in great demand as a protein source for incorporation into animal and poultry feeds. It finds its primary use as a protein supplement to animal and poultry feeds. The average yield of meal from bulk soybean is 79% (Norman, 1978). The presence of oligosaccharides in soybeans presents a real problem as these compounds are not digested in, nor absorbed from the upper parts of the human gastro intestinal tract. Fermentation is an effective way to reduce or eliminate these oligosaccharides.

2.4 Legumes

Legume seeds known as beans, pulses or grain legumes are second only to cereals as sources of human and animal foods. COPR (cited in Ofuya and Akhidue, 2005) reported that legumes have a wide range of usage, some are used as fodder green manure, and some are used as silage, while others are extracted for their oil, notably soybeans and groundnut. Such oil contributes a lot to the energy intake of people all over the world. The term ‘pulses’ cover all those grown for their dried seeds. The use of pulses range from their forming a staple diet to their being used as condiments, milk, cheese and snacks (Ofuya and Akhidue, 2005). They play a very important role in human nutrition. Food legumes have exceptional immediate potential for alleviating human malnutrition in tropical Africa by virtue of several inherent advantages. The excellent nutritional values of most food legumes in terms of protein, energy, vitamins and minerals are highly complementary in African diets, which comprise mostly roots and tubers, indigenous herbs and native fruits (Ezedinma, 1988). Legumes are inexpensive sources of edible protein. Many legumes seeds contain 20-40% protein while a few contain 40-60%. The value is about twice that found in cereal and several times that in root tuber (FAO, In Ofuya and Akhidue, 2005), so they can help to improve the protein intake of meals in which cereals, roots and tubers in combination with pulses are eaten. Legumes offer a variety of edible products in addition to the seeds.

Condiments produced from some underutilized legumes in Nigeria were evaluated for their nutritional and sensory qualities. Soybeans *(Glycine max)*, locust bean *(Parkia filicoidae L.)*, Pigeon pea *(Cajanus cajan)* and melon seed *(Citrullus lanatus)* were subjected to solid substrate fermentation and their proximate composition, mineral, antinutrient and organoleptic properties were subsequently determined. According to Oboh (2006) the study revealed that the condiments had high protein, fat, ash and crude fiber. In view of the high nutrient contents, the condiments produced from these underutilized legumes could be used as alternative to the monosodium glutamate based seasoning salts presently in use. The condiments produced from soybeans and locust bean appear to be more promising than those from pigeon pea and melon seed, based on nutrition and sensory evaluation. A source of difficulty with any legume seeds, including the soybean, is the presence of antinutritional factors. These are diverse, and may include proteins to which some people are allergic to phytohaemagglutenins, phytic acid and other naturally present components. Fortunately, most of these factors are destroyed by thorough cooking (Wood, 1998). Legumes undergo one form of processing or the other to produce nutritious products. Such processing methods are cooking, steeping, roasting, fermentation and germination. These processing techniques enhance the nutritional quality of these grains by destroying the antinutrients in the grains.

2.5 African locust bean

Parkia biglobosa is a very small but widely spread member of the *leguminosae* family found in Africa, Brazil, Jara and Suriam. The tree has little susceptibility to pest and diseases with average dry seed production of about 350-500kg per hectare. In the African Sahel and associated savannah, the seeds provide more food consumed directly by humans than any other legumes genus. They are of considerable economic importance in some of these areas. The *Parkia biglobosa* are perennial plants which usually bear large pods as fruits. The contents of these, both pulp and seeds have been exploited to various degrees. The dry, yellow, powdery pulp is rich in sweet carbohydrate and can be mixed with cereal, meat or soup (Fetuga et al., 1974). In West Africa, the seeds are called ‘African locust beans’. They are extremely hard and practically inedible when raw but can be processed and fermented into a palatable product called ‘dadawa’, rich in fat, protein and lysine. Higher protein and B vitamins (thiamine and riboflavin) have also been reported in the fermented samples (Eka, 1980). Other nutritional improvements during fermentation include the extensive hydrolysis of the seed proteins into amino acids, hence making the beans more digestible. Locust bean are not normally used for food in their natural state. The fermentation makes them edible by increasing their digestibility and removing some objectionable flavors. Fermented African locust bean condiment is used in families as a low cost meat substitute. Simmons (1976) reported that the fermented product, 'dadawa', was the single largest source of protein in the average diets of inhabitants of Zaria, northern Nigeria. The locust bean itself is made up of protein (39-47%), oil (31-40%) and carbohydrate (11.7-15.4%) (Campbell-Platt,1980). The seed kernel contains the polysaccharide known as locust bean gum (LBG). The LBG is used to improve the quality of products like ice cream, milkshakes, sauces, sweet beverages etc. It is also used to improve body and viscosity in such production processes (Addy et al., 1995). Locust bean, like most legumes, contain some indigestible oligosaccharide, notably stachyose and raffinose. Anaerobic bacteria in the intestine metabolize them to different products including gases, thereby causing flatulence. Odunfa (1983) reported that the qualities of the flatus-forming oligosaccharides reduced significantly during their fermentation. The reduction was attributed to the activities of α- and β–galactosidase which hydrolyzed the oligosaccharides to simple reducing sugars. The heat generated in the fermenting beans possibly provided the ideal temperatures for the optimal activity of α–galactosidase.

2.6 African breadfruit

The African breadfruit tree (*Treculia africana*) is native to many tropical countries like West Indies, Ghana, Sierra Leone, Nigeria and Jamaica. In Nigeria, it is widely distributed in the southern states, especially Imo, Enugu, Anambra, Edo, Delta, Cross Rivers and Rivers. In the areas, it is either found growing wild or planted in home gardens. *Treculia africana*, commonly known as African Breadfruit is a leguminous plant with large fruit heads. The plant belongs to the mulberry family or the *Moraceae* family. It is evergreen, and grows up to 40m high, producing 30-50 fruits annually. The weight of the fruit varies between 30 and 40kg. Each fruit may yield 5-15kg seeds after processing. Its seed, commonly called 'afon' and 'ukwa' by the Yoruba and Igbos of Nigeria, respectively is popular as a traditional food item. The seeds of *Treculia africana* sub species *africana* (African Breadfruit) have been used in various forms as vegetable crops in south eastern Nigeria. The seeds have traditionally been processed in a variety of ways including boiling the kernels either alone or in combination with maize. The fresh seed kernels are also salted, roasted, dehulled and eaten as snacks or ground and used as thickeners in traditional soups. In these ways, the kernels are consumed to a considerable extent. However, there is a need to diversify the use of the seeds for food, other than the traditional ones (Akubor et al., 2000). The recent increased interest in African Breadfruit (*Treculia africana*) seeds is as a result of their potential as a protein supplement (Nwokolo, 1996). Investigations (Ekpenyong, 1985) have shown that *Treculia africana* seeds (TAS) are rich in amino acids, minerals and fatty acids. The seeds contain about 19-23% protein and 11-19% fat. The seeds have been reported (Edet et al., 1985) as being a good source of edible oil, potassium, phosphorus, magnesium, riboflavin and beta carotene. Makinde et al. (1985) reported that the meal was capable of furnishing most essential amino acids in human diet and the protein was particularly rich in aromatic amino acid. Amino acid profile of African Breadfruit is similar to those of many edible beans and pulses, having very low sulphur amino acid.

2.7 Antinutritional factors

Antinutritional factors (antinutrients) are plant components which interfere with metabolic processes, so that growth and bio-availability of nutrients are negatively influenced. Consumption of foods containing these factors reduces nutrient utilization, feed efficiency and productivity (in animals). At high levels of intake, toxicity ensues and in extreme cases, death. The activity of these compounds can be reduced or removed by dehulling, soaking, cooking, toasting and fermenting (Fasasi et al., 2004). Legumes contain some antinutritional factors that impose problems in their utilization as foods. These antinutritional factors can cause anemia, growth retardation and protein-energy malnutrition in children. Appropriate processing destroys the antiphysiological factors in legumes. A group of phenolic compounds known as 'tannins' have been found in some cereals (especially sorghum) to reduce nutrient value. A few animal studies have indicated that polyphenolic compounds affect nutritional quality (Liener, 1980). Ekpenyong (1985) reported that the deleterious effect of tannin on legumes and cereals could be reduced significantly by cooking. The reduction of tannin with processing was attributed to the destruction of polyphenolic compounds by moist heat. Heat induced the formation of insoluble tannin and protein complex. Ugwu and Oranye (2006) noted that other processing method apart from cooking could be used to reduce tannin. One of such methods was to dehull the seed coat where the color and tannins is concentrated, though some fiber may be lost in the process.

Another method is to soak the legumes for 12-15hours in a litre of water containing a tablespoon of sodium bicarbonate. This method removes up to 60% of the tannins and softens the legume, which then requires half the cooking time needed for beans soaked in plain water.

Giami et al. (2001) reported that boiling proved more effective than roasting for improving protein digestibility and for reducing the levels of trypsin inhibitor, phytic acid and poly phenols in the sample. Results obtained from *Treculia africana* seed kernel (TAS) flour samples processed in different ways showed that the fermented TAS flour sample had the highest protein-energy value. Hence, it would be the flour of choice in any supplementation program involving the use of TAS (Fasasi et al., 2004).

2.8 Fermented foods

Fermentation is a slow decomposition process of organic substance induced by natural or artificial micro-organisms. It is often uncontrolled. It is however, the oldest and remains one of the most economical methods of producing and preserving foods highly acceptable to man (FIIRO, 1984). Fermented foods are usually prepared from plant or animal materials by processes in which micro-organisms play an important role in modifying the substrates physically, nutritionally and sensorily (Aidoo, 1986). In Africa, the art of fermentation is widespread including the processing of fruits and other carbohydrate sources to yield alcoholic and non-alcoholic beverages. Indigenous fermented foods such as cheese and palmwine, have been prepared and consumed for thousands of years and are strongly linked to culture and tradition, especially in rural households and village communities (Achinewhu, 1983). The Food and Agriculture Organization (2004) has estimated that fermented foods contribute to about one third of the diet worldwide. During fermentation processes, microbial growth and metabolism result in the production of a diversity of metabolites. These metabolites include enzymes which are capable of breaking down carbohydrates, proteins and lipids present within the substrate and/or fermentation medium, vitamins, antimicrobial compounds (e.g. bacteriocins and lysozymes), texture forming agents (e.g. xanthan gum), amino acids, glutamic acids, organic acids and flavor compounds(e.g. esters and aldehydes) (Iheanyi et al., 2006). Fermentation being the process of bioconversion of organic substances by micro-organisms and/or enzymes of microbial, plant or animal origin is one of the oldest forms of food preservation which is applied globally. It is applied in the preservation of a range of raw agricultural materials (cereals, roots, tubers, fruits and vegetables, milk, meat, fish, etc). Commercially produced fermented foods which are marketed globally include dairy products, sausages and soy sauce. Fermented foods have a long history in Africa. However, the absence of writing culture in most African countries makes their origin difficult to trace. Foods are fermented for various reasons. Fermentation plays at least five roles in food processing:

1. Enrichment of the human dietary through development of a wide diversity of flavors, aromas and textures in food;
2. Preservation of substantial amounts of food through lactic acid, alcoholic, acetic acid, alkaline fermentations and high salt fermentations;
3. Enrichment of food substances biologically with vitamins, protein, essential amino acids and essential fatty acids;
4. Detoxification during food fermentation processing and
5. A reduction in cooking times and fuel requirements.

In fermented products of soybeans, the original material has an unpleasant flavor, which must be masked or destroyed to become acceptable for human consumption. Biochemical analysis of 'iru' and some other fermented foods have shown the presence of amino acids (e.g. glutamic acid) which are used as flavoring agents in foods and the presence of which adds to the flavor of these food condiments (Anosike and Egwuatu, 1981).

Oil seeds (African locust bean, castor oil seeds, mesquite bean, melon seed and soybean) are fermented to produce condiments. These African fermented condiments require food processing technologies that will meet the requirement or challenges of human needs. In fermented oil bean seeds, increase in vitamin contents for example, vitamin B and biotin had been reported (Odunfa, 1986). Omafuvbe et al. (2003) reported that fermentation increased the pH, crude protein, reducing sugars and free amino acid values in locust bean. An increase in ether extract of fermented African locust bean was also reported. In the fermentation of African breadfruit seed, Fasasi et al. (2004) stated that there was an increase in mineral composition. They recorded a high rate of fat extraction in the fermented sample.

2.9 Safety of fermented foods

Fermented foods generally have a record of a very good safety even in the developing world where the foods are manufactured by people without training in microbiology or chemistry and under unhygienic, contaminated environments. Application of the principles that lead to the safety of fermented foods could lead to an improvement in the overall quality and the nutritional value of the food supply, reduction of nutritional diseases and greater resistance to intestinal and other diseases in infants (Steinkraus, 2002).

These principles are:

1. Food substances overgrown with desirable, edible micro organisms become resistant to invasion by spoilage, toxic or food poisoning microorganisms. Other, less desirable (possibly disease producing) organisms find it difficult to compete, e.g. Indonesian tempe;
2. Fermentations involving production of lactic acid are generally safe. Lactic acid fermentations include those in which the fermentable sugars are converted to lactic acid by lactic acid organisms, e.g. Yogurts, cheese, “ogi” ;
3. A third principle contributing to food safety is that fermentations involving production of ethanol are generally safe foods and beverages, e.g. wines, beers, leavened bread;
4. Fermentations involving highly alkaline fermentations are generally safe e.g. African condiments like “dawadawa”, Nigerian “ugba”, Sierra Leone “ogiri-saro”, made by fermentation of sesame seed (*Sesamum indicum*). The combination of high pH and free ammonia along with very rapid growth of essential microorganisms at relatively high temperatures-above 40°C make it difficult for other microorganisms that might spoil the product to grow;
5. Addition of salt in ranges from 13% w/v or higher to protein-rich substrates results in a controlled protein hydrolysis that prevents putrefaction, prevents development of food poisonings such as botulism and yields meaty savory, amino acid/profile sauces and pastes that provide very important condiments particularly for those unable to afford much meat in their meats, e.g. soy sauce;
6. Fermentations involving production of acetic acid yield foods/condiments that are generally safe. Acetic acid also is bacteriostatic or bactericidal depending upon the concentration, e.g. vinegar.

CHAPTER 3

MATERIALS AND METHODS

African locust bean (*Parkia biglobosa*) and African breadfruit (*Treculia africana*) seeds were purchased from Orie and Ibagwa markets respectively, in Enugu State. Wheat (*Triticum aestivum*) was bought from Ogige market in Nsukka town. Micro organisms (*Aspergillus oryzae, Zygosaccharomyces rouxii* and *Candida versatilis)* were procured from the United States Department of Agriculture (USDA).

3.1 Preparation of raw materials

Samples were produced using a modification of the method of Fukushima (1981) for soy sauce production (Fig.1). Modification involved reduction of fermentation time at the second stage of fermentation (the brine fermentation process), from 3 months to 4 days (Fig.2). The modification was necessitated by the results from preliminary study carried out on the seeds which revealed that some amino acids and minerals were lost or reduced when fermentation was allowed to go on for a long period.

Dehulled seeds, known as kernels, of African locust bean and African breadfruit were sorted and 500g of each was washed in excess water (5litres), drained and soaked in 3litres of water for 12hours at room temperature (25^{o}C). The water was changed every 4 hours to prevent microbial growth. After soaking, the kernels were parboiled at 98^{o}C for 2hours.

Wheat kernels were sorted and milled into flour using a size 52 Bentall plate mill (model 2000, F. H Bentall, England). The flour (500g) was steamed at 97^{o}C for 5 minutes. The soaked, steamed African locust bean and African breadfruit kernels were each thoroughly mixed with the steamed wheat flour in a 50:50 ratio. Mold starter (*Aspergillus oryzae*) was then added to the flour-coated *P. biglobosa* and *T. africana* kernels. Each mixture was spread out on separate bamboo trays covered with banana leaves for 3days at 29°C to ferment. Even temperature was maintained by mixing. The fermented products referred to as ‘African locust bean “koji” and ‘African breadfruit “koji” were transferred to plastic buckets containing 17% brine. The plastic buckets served as fermenting vats. Yeasts (2% w/w), *Zygosaccharomyces rouxii* and *Candida versatilis* were inoculated into the brined koji and allowed to ferment for 4 days, at 29^{o}C.

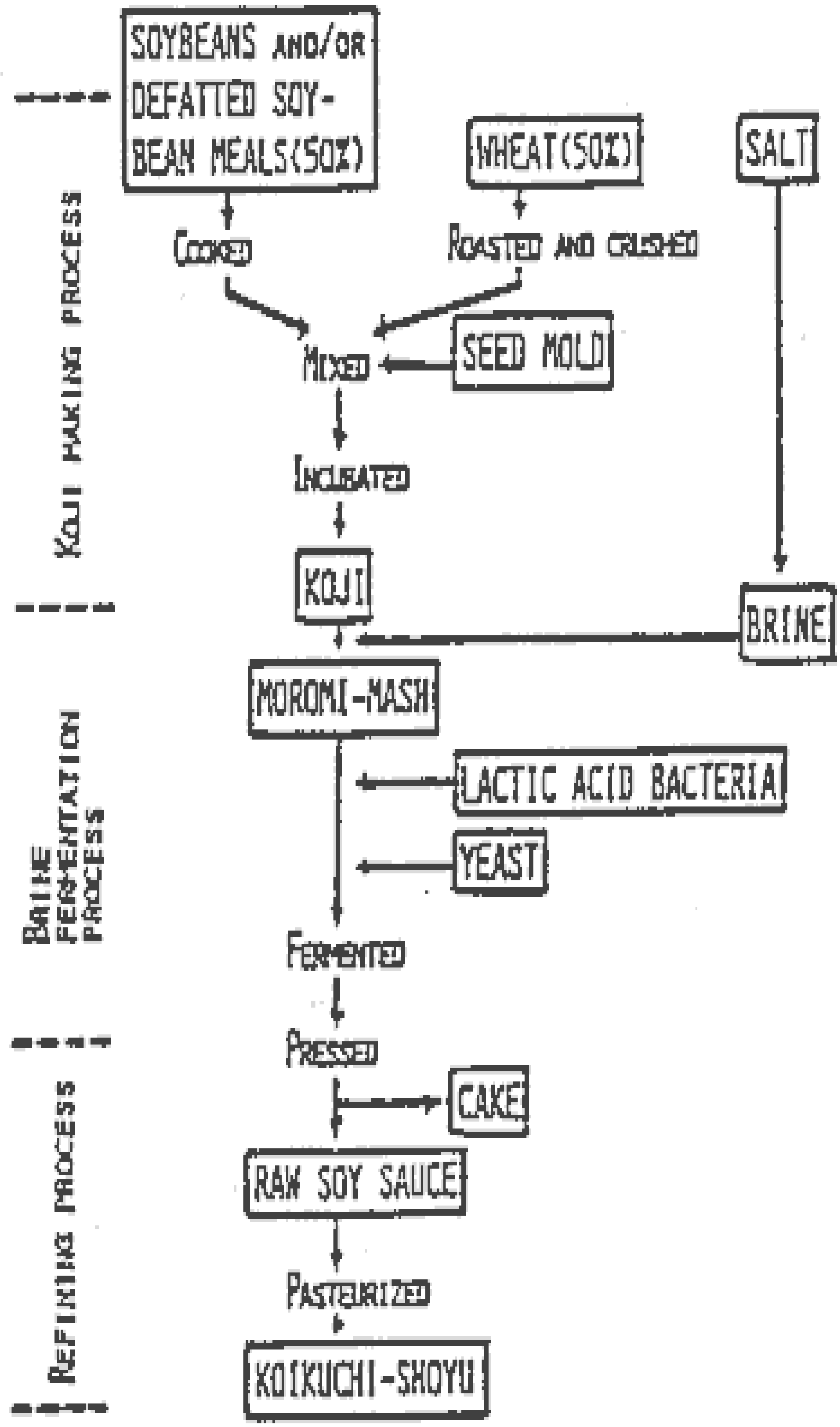
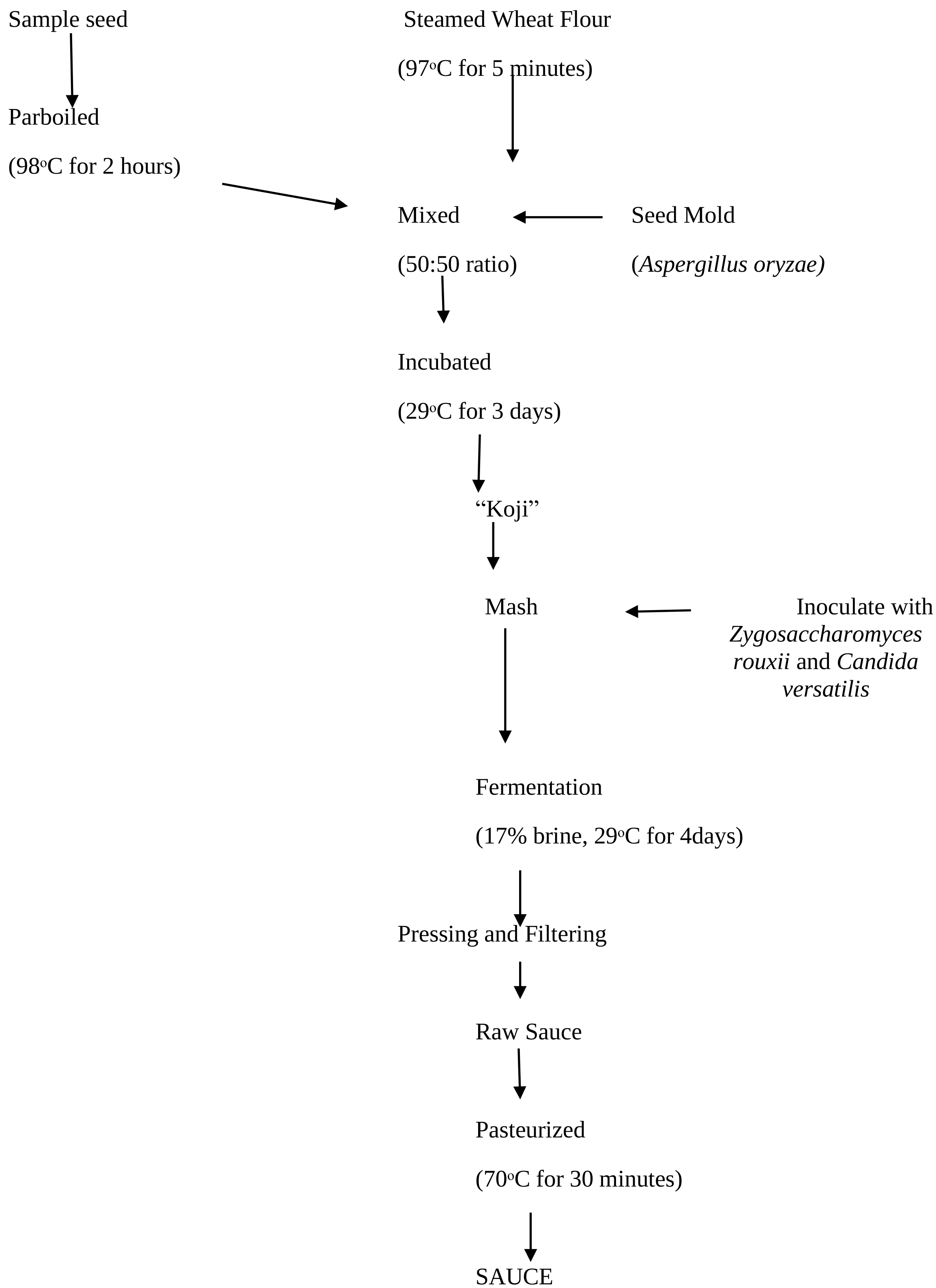


Figure 1: Production of "koikuchi" Soysauce

Source: Fukushima (1981)



Key: (Sample seeds- African locust bean and African breadfruit).

Figure 2: Flow chart for the production of sauce from African locust bean and African breadfruit seed kernels.

The African locust bean and African breadfruit seed kernel samples were allowed to ferment at 29°C for 4 days. The fermenting mixture was stirred at 12 hour intervals, to ensure even fermentation and organic activity. After the 4^{th} day of fermentation, the mixture was pressed and filtered through a 52 pore sized sieve and the filtrate pasteurized at 70ºC for 30 minutes. The pasteurized liquid was cooled and allowed to stand overnight. The liquid obtained was called African locust bean sauce from African locust bean seed kernels and African breadfruit sauce from African breadfruit seed kernels, respectively. Samples of the products were subjected to analysis for physico-chemical, microbial and sensory characteristics.

3.2 Physico- chemical analyses

3.2.1 pH determination

The pH of samples was determined using the pH meter (Hanna Instruments, 8520). The pH meter was standardized with a buffer solution (pH 4.0 and 9.0.) prior to use. Approximately 2g of the fermenting sample was homogenized with 8ml of distilled water and the pH of the slurry measured with a pH meter. The pH of the fermenting samples was measured at 1^{st}, 3^{rd}, 5^{th} and 7^{th} days of fermentation.

3.2.2 Proximate composition

Each sauce sample was analysed for moisture, crude protein, fat ash according to AOAC (1995).Carbohydrate was determined by difference. Approximately 10ml of each sample sauce was measured into Petri dishes using a pipette and left to dry over a hot water bath (100^{o}C), (model Ex 200). The dried sample was then used for the analyses.

3.2.3 Viscosity determination

Viscosity of the samples was measured with a Brookfield Viscometer; model RVT, using No 6 spindle at a rotating speed of 100rpm. Viscosity was evaluated using 400ml of each sauce sample in a beaker placed in a Brookfield constant temperature water bath at 27ºC; (model Ex 200). The viscometer guard leg and spindle were then lowered into the sample and the speed of rotation set at 100rpm. The digital reading of the viscometer was converted to centipoises, by multiplying with a factor of 100-given for each pair of spindle and 100rpm (speed of rotation). Results were reported as average of triplicate readings.

3.2.4 Determination of reducing sugar

The reducing sugar content of the sample was determined using the phenol-sulfuric method Dubois *et al*. (1956). Using a pipette, 2ml of each glucose working standard (1mg/l, 2mg/l, 3mg/l, 4mg/l, and 5mg/l) was measured into test tubes. About 2ml of 5% phenol was added, mixed gently and 2ml concentrated H_2SO_4 was also added from a fast flowing dispenser. The mixture was mixed with a Votex mixer and allowed to stand for 10minutes. Distilled water was added to make it up to 100ml, this was cooled and the absorbance was read at 470nm and plotted against concentration to get the standard curve.

The sauce sample was prepared by mixing 2mls of the sample with equal volume of 5% phenol. This was mixed gently then 2ml of concentrated H_2SO_4 was added and left for 10 minutes, after which it was made up to 100ml and the absorbance was measured at 470nm.

3.2.5 Determination of total sugar

The phenol-sulphuric method (Dubois *et al*. 1956) was used to determine the total sugar content of the sauces. About 0.5cm^3 of sample (1:4 dilutions) was measured into a 250ml conical flask. A 20ml volume of 25% (w/w) HCl was added to the sample before boiling for 1hour in a water bath. After boiling, the sample was allowed to cool and 5.5-mL aliquot of 40% (w/v) NaOH was added and the content made up to 25cm^3 with distilled water. It was centrifuged at 10rpm and the clear supernatant was then recovered. Using a pipette, 5ml of the supernatant was measured into a test tube to which 1ml of 5% phenol solution was added followed by vigorous mixing. From a fast flowing burette, 5ml of concentrated sulphuric acid was also added and allowed to stand for 15minutes after which it was immersed in a cold water bath for 20minutes. Absorbance reading was taken at 490nm wavelength using a spectrophotometer (Spectrolab 21, India), and plotted. The glucose concentration of each test sample was determined by comparing the absorbance of the test sample of a plot to the absorbances of the glucose standards.

3.2.6 Titratable acidity (as citric acid)

This was carried out by the method described by Pearson (1984) at different fermentation regimes (1^{st}, 3^{rd}, 5^{th} and 7^{th} days). Approximately 2g of the fermenting sample was homogenized with 8ml of distilled water, while the sauce sample ($10cm^3$) was measured into a 250ml beaker and 100ml of distilled water was added in each case. The solution was then titrated with 0.1M sodium hydroxide solution using phenolphthalein as the indicator and the end point was reached when a pink coloration was observed. The volume of sodium hydroxide used up was recorded and the total acidity (as citric acid) was calculated in percentage, using the formula below:

$$\%\ Total\ acid = \frac{1 \times Equivalent\, Weight\ of\ \ Acid \times Normality\ of\ \ NaOH\ \ and\ Titre}{10 \times Weight\ of\ \ Sample}$$

3.2.7 Sodium chloride determination

Volthard (Titrimetric) method described by Pearson (1984) was used in determining sodium chloride content of the sauce samples. Prepared sample ($2cm^3$) was measured into a clean conical flask and 25ml of 0.1M silver nitrate, 15ml of concentrated nitric acid were added and heated for 15minutes. A small quantity of potassium permanganate (oxidizing agent) was also added and the mixture was again heated for 10minutes, then left to cool. Twenty five milliliters of diethyl ether, 2drops of 10% ferric ammonium sulphate and 20ml of distilled water were then added to the cooled mixture. The mixture was titrated with 0.1M potassium thiocyanate and a titre value obtained. The end point of the titration was reached when the solution turned brick red.

The % NaCl content for each sample was calculated as shown:

% Sodium chloride = 0.585 (J- K)

Where, J = Volume of 0.1M silver nitrate used (ml)

K = Titre value (ml)

0.585 = conversion factor

3.2.8 Mineral determination

Sample preparation.

Approximately 5g of oven dried sauce sample was weighed into a crucible and left to ash in a furnace at 600ºc for 4 hours. The ash obtained was dissolved with 10ml of 90% HCl and filtered through a Whatman No1 filter paper. The washings from the residue was filtered into a 100ml volumetric flask, until the flask was full. The solution was stored for determination of Calcium and Magnesium by titration, Sodium and Potassium as described by AOAC (1995).

Calcium

Using a pipette, 10ml of each sample was measured into a 100ml conical flask to which 5ml of 1M NaOH was added to make the sample alkaline, and a drop of murexide (ammonium acid purpurate), which served as the indicator. This was titrated against ethylenediaminetetraacetic acid (EDTA) solution to an end point of purple color. The concentration of the Calcium in the test samples was calculated as follows:

$$\%Calcium = \frac{EDTA\ Vol.\ used \times Mol.\ EDTA \times weight\ of\ Ca \times 100}{1000 \times weight\ of\ sample\ used}$$

Magnesium

Using a pipette 10ml of sample was measured into a 100ml conical flask and 2.5ml of NaOH was added. Eriochrome black T indicator was added (2 drops) and titrated against EDTA immediately to avoid degradation, until color changed to blue (end point). The concentration of magnesium in the test samples was calculated as follows:

$$\%Magnesium = \frac{EDTA\ Vol.\ used \times Mol.\ EDTA \times weight\ of\ Mg \times 100}{1000 \times weight\ of\ sample\ used}$$

Sodium and Potassium

These were determined using a flame photometer according to AOAC (1995).

About 5ml of sample was carefully measured into a 250ml conical flask to which 150ml distilled water was added. After mixing, the samples were swirled, poured into a 200ml volumetric flask and made up to 200ml with distilled water and mixed thoroughly. A 1/50 dilution was made, out of which 2ml was measured into a 100ml volumetric flask and made up to 100ml with distilled water.

The flame photometer was set up according to the manufacturer's instruction which relies on the principle that an alkali metal salt drawn into a non-luminous flame will ionize, absorb energy from the flame and then emit light of a characteristic wavelength as the excited atoms decay to the unexcited ground state. The intensity of emission is proportional to the concentration of the element in the solution. Since Na^+ and K^+ emit light of different wavelengths (colours), by using appropriate colored filters the emission due to Na^+ and K^+ (and hence their concentrations) can be specifically measured in the same sample. The instrument readout was calibrated using the standard solution. Meter reading was set at 100%E (Transmittance), while aspirating the most concentrated standard solution. The Percentage emission was recorded for all the solutions and the standard curve was plotted on a linear graph. Emission reading at 0 and 100% were checked with the blank and most concentrated standard solution, after every sample determination. Then the concentration of the element in the test sample solution was read from the standard curve.
Calculation was made using the formular:

$\% = ppm \times 100 \times Df \div 1 \text{ million}$

Where:

ppm = Part per million

Df = Dilution factor (ml).

3.2.9 Total soluble solids

This was done according to the method of AOAC (1995). About 2g of fermenting sample was weighed into a test tube and 10ml of distilled water at 25ºC was gradually added, this was followed by vigorous shaking of the mixture after which 2.5ml of distilled water was again added. The mixture was filtered rapidly and 2ml of the clear filtrate was pipetted into a weighed dish, evaporated to dryness on a steam bath and dried in an air oven at 100ºC for 30minutes to a constant weight. Percentage soluble solid was calculated using the expression:

$$\frac{W_2 - W_1}{W_3} \times 100$$

Where:

W_1= Weight of empty flask

W_2= Weight of flask and extracted solids after drying in the oven.

W_3 = Weight of extracted solid before drying.

3.2.10 Determination of amino acids

The amino acid profile of the test samples (Soy sauce-*Amoy* brand, African locust bean sauce and African breadfruit sauce) was determined using the method of Spackman *et al*. (1958). About 100ml of the prepared sample was measured into a flat aluminum plate and placed in a hot air oven set at 40ºC. This was left until it was dried to constant weighed. The dried sample was then hydrolyzed and evaporated in a rotary evaporator then loaded into the Technicon sequential Multi-sample Amino Acid Analyzer (TSM).

Nitrogen determination

A small amount (50mg) of each of the dried samples was weighed and wrapped in Whatman filter paper (No.1) then put in a Kjedahl digestion flask. Concentrated sulphuric acid (10ml) was added and catalyst mixture (0.5g) containing sodium sulphate (Na_2SO_4), copper sulphate ($CuSO_4$) and selenium oxide (SeO_2) in the ratio of 10:5:1 was also added into the flask to facilitate digestion. Four pieces of anti-bumping granules were again added.

The sample was digested for 3 hours until the liquid turned light green. The digested sample was cooled and diluted with distilled water to 100ml in standard volumetric flask.

Aliquot (10ml) of the diluted sample was mixed with 10ml of 45% sodium hydroxide and distilled into 10ml of 2% boric acid containing 4 drops of bromocresol green/methyl red indicator until about 70ml of distillate was collected.

The distillate was then titrated with standardized 0.01N hydrochloric acid to grey color end point. The percentage nitrogen in the original sample was calculated using the formula:

$$\% \; Nitrogen = \frac{(a-b) \times 0.01 \times 14 \times V \times 100}{W \times C}$$

Where:

a = Titre value of the digested sample

b = Titre value of blank sample

V = Volume after dilution (100ml)

W = Weight of dried sample (mg)

C = Aliquot of the sample used (10ml)

14mg = Nitrogen constant in mg

100 = Conversion factor to percentage

Hydrolysis of the sample

Five grams of each of the test samples was weighed into glass ampoule. About 7ml of 6N HCl was added and oxygen was expelled by passing nitrogen into the ampoule (this is to avoid possible oxidation of some amino acids during hydrolysis e.g. methionine and cystine). The glass ampoule was then sealed and heated at $105^{o} \pm 5^{o}C$ for 22 minutes after which the content was filtered. The filtrate was evaporated to dryness at $40^{o}C$ under vacuum in a rotary evaporator. The residue was dissolved with 5ml of acetate buffer (pH 2.0), stored in plastic specimen bottles and kept in the freezer, until used for analysis.

Loading of the hydrolysate into the TSM Analyzer

About 10 microlitre of the hydrolysate was loaded into the analyzer. This was dispensed into the cartridge of the analyzer and allowed for the analysis and resolution of the amino acid into free acidic, neutral and basic amino acids of the hydrolysate. The resolved amino acids were eluted and the elution process was charted as peaks by the recorder, from where the concentrations of the amino acids were calculated.

The net height of each peak produced by the chart recorder of TSM (each representing an amino acid) was measured. The half-height of the peak on the chart was found and width of the peak at half-height was accurately measured and recorded. Approximate area of each peak was then obtained by multiplying the height with the width at half-height.

The norleucine equivalent (NE) for each amino acid in the standard mixture was calculated using the formula:

$$\text{NE} = \frac{Area\ of\ \ Norleucine\ Peak}{Area\ of\ \ Each\ A\min o\ Acid}$$

A constant S was calculated for each amino acid in the standard mixture:

$S_{std} = NE_{std} \times mol.\ Weight \times uMAAS_{std}$

Where:

$$NE_{std} = \frac{Area\ of\ \ Norleucine\ Peak}{Area\ of\ \ Each\ A\min o\ Acid}$$

$\text{uMAAS}_{\text{std}}$ = A constant (0.025)

The amount of each amino acid present in the sample was calculated in g/16N or g/100g protein using the following formula:

$Concentration\ (g/100g\ protein) = NH \times W\ @\ NH/2 \times S_{std} \times C$

$Where\ C = \underline{Dilution \times 16}\ -\ NH \times W\ (nleu)$

Sample wt (g) × N%10 × vol. Loaded

Where: NH = Net weight

W = Width @ half height

Nleu = Norleucine

3.2.11 Microbial analysis (Harrigan and McCance, 1976)

Total viable count and mould count were determined on the sauce samples using the MacConkey agar and Potato Dextrose Agar (PDA), respectively. For each sample, serial dilutions (using 1ml of sample) was made in 9ml sterile distilled water in test tubes. This process was repeated for a set of nine test tubes until a 10^{-10} dilution was achieved. One milliliter of alternate numbered dilutions was pour plated in duplicate on MacConkey agar and Potato Dextrose agar (PDA) in sterile Petri dishes. This was incubated at 37ºC for 24 hours. After incubation, counts of visible colonies were made and expressed as colony forming unit (cfu/ml) per milliliter of sample. Dishes having more than 300 colonies were discarded.

3.3 Sensory evaluation

Jollof rice was prepared using each of the sauce samples as condiment. This was presented before a panel of 10 judges, selected on the basis of interest, availability and ability to express opinion. The products were evaluated organoleptically by the panelists for color, flavor, mouth feel, taste and overall acceptability, using a 9-point Hedonic scale where 1 represent the least score (dislike extremely) and 9 the highest score (like extremely).

3.4 Statistical analysis

All experiments were conducted in triplicate and means determined. Analysis of variance (ANOVA) was performed on the data to determine differences, while the least significant difference test was used to detect differences among means at 5% level of probability (Ihekoronye and Ngoddy, 1985).

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Proximate composition of soybean, african locust bean and african breadfruit seed kernels

The proximate composition of *Treculia africana*, *Parkia biglobosa* and Soy bean seed kernels is shown in Table 1. All the kernels had protein values that ranged from 19.71 to 45.60%, with African breadfruit having the lowest value. The seed of African breadfruit has been noted for its excellent polyvalent dietetic value; the biological value of its proteins exceeds even that of soybeans (Katende, 1995). Fat/oil content was relatively high, ranging from 13 to 19%, the highest value was found in soy bean. Crude fiber and ash contents of African breadfruit were very low, compared to that found in the other seeds analyzed. This may have contributed to the observed higher carbohydrate value in African breadfruit (53.61%). These results agree with previous works (Edet *et al.*, 1985; Alabi *et al.*, 2005; Uzeh *et al.*, 2006). It was observed that African locust bean and soybean seeds had comparable ash and carbohydrate contents, while there were significant differences in the crude fiber and fat contents. The moisture content of African locust bean and African breadfruit was within a close range 8.70 to 9.60% but much higher than that of Soy bean seed (5.38%). The similarity in the composition of these seeds is the reason for their use in this work.

Table 1: **Proximate composition (%) of soybean, African locust bean and African breadfruit seeds.**

Components	SBS	ALB	ABF	LSD
Moisture	$5.38^{a}\pm0.40$	$8.70^{b}\pm0.00$	$9.60^{c}\pm1.63$	0.258
Protein	$45.60^{c}\pm0.33$	$39.10^{b}\pm0.12$	$19.71^{a}\pm1.00$	0.231
Fat	$19.45^{c}\pm0.82$	$17.03^{b}\pm3.47$	$13.70^{a}\pm0.16$	0.201
Crude fiber	$4.30^{b}\pm0.26$	$5.07^{c}\pm0.43$	$0.88^{a}\pm0.12$	0.026
Ash	$4.05^{b}\pm0.02$	$4.40^{b}\pm0.08$	$2.50^{a}\pm0.82$	0.153
Carbohydrate*	$21.22^{a}\pm0.05$	$25.70^{a}\pm0.37$	$53.61^{b}\pm0.72$	0.165

Values are means ± SD of triplicate determinations.

* Calculated by diference

Means bearing the same superscript are not significantly different ($p\geq0.05$) across a column.

Key: SBS= Soybean seed kernel, ALB= African Locust bean seed kernel, ABF= African Breadfruit seed kernel.

4.2 Composition of soy sauce, African locust bean sauce and african beadfruit sauce.

Table 2 shows the proximate composition of commercial Soy sauce (*Amoy* brand), African locust bean (*Parkia biglobosa*) sauce and African breadfruit (*Treculia africana)* sauce. As expected, the moisture content of all the sauces was high, ranging from 71.10 to 83.65%. The ash content of the sauces was relatively high, ranging from 5.15 to 6.12%. This will be a plus to the sauces since high ash level suggests high level of mineral in the food. It has been observed that fermentation increases the ash content of some foods products, and consequently, their mineral levels. A similar effect was observed by Eka (1980) in his study on the effect of fermentation on the nutritional content of locust beans in condiment production.

The fat content of the sauces was quite low, the highest fat content was observed in *Parkia biglobosa* sauce (1.15%), while *Treculia africana* had the lowest. Previous work by Fasasi *et al* (2004) showed that a lower level of fat and crude fiber was found in fermented African breadfruit seed kernel flours than in unfermented ones. Low lipid content of sauces from African locust bean and African breadfruit is desirable since high amounts of unsaturated fatty acids in foods generally could lead to rancidity and also impart an objectionable taste. Odunfa (1985) reported low levels of lipase activity in *Parkia biglobosa* during "dawadawa" production.

Table 2: **Composition of soy sauce, African locust bean and African beadfruit sauces.**

Parameters	SS	LB	BF	LSD
Moisture (%)	$71.10^{a}\pm0.08$	$80.10^{b}\pm0.74$	$83.65^{c}\pm 0.56$	0.12
Protein (%)	$5.20^{c}\pm0.06$	$3.50^{b}\pm0.18$	$2.62^{a}\pm0.16$	0.13
Fat (%)	$0.10^{a}\pm0.17$	$1.15^{b}\pm0.01$	$0.15^{a}\pm0.09$	0.03
Ash(%)	$5.21^{b}\pm1.30$	$6.12^{c}\pm1.20$	$5.15^{a}\pm1.02$	0.03
Carbohydrate* (%)	$17.59^{c}\pm2.10$	$9.13^{b}\pm0.49$	$8.43^{a}\pm0.89$	0.20
Sodium Chloride (%)	16.00±0.80	6.60±0.27	14.49±0.71	0.17
Viscosity (cp)	50.00±2.60	54.67±0.38	30.00±1.10	2.99
Total Sugars (%)	0.06±0.06	0.0023±0.54	0.0035±1.11	0.06

Values are means ± SD of triplicate determinations.

* Calculated by difference

Any two means bearing the same superscript are not significantly different ($p\geq0.05$) across a column.

Key: SS=Soy Sauce; LB=African Locust bean Sauce; BF=African Breadfruit Sauce.

The protein content ranged from 2.62 to 5.20%, with commercial soy sauce having the highest value and breadfruit sauce the lowest value (2.62%). The protein content of *Parkia biglobosa* sauce was quite low; this may be due to the length of fermentation period or the addition of ingredients like salt in the production process. The low protein content observed could be that the organisms involved, being proteolytic, were able to breakdown and use proteins in the mash for growth. The carbohydrate content of the sauces ranged from 8.43 to 17.59%, which could be considered low. This could be due to losses which often accompany heat-processed foods and the activity of fermentative microbes. Fermentation is known to be an effective way of reducing or eliminating oligosaccharides in foods by the action of amylases which hydrolyzes carbohydrates into sugars, which are more readily digestible.Viscosity of African locust bean sauce showed a higher value (54cp) than African breadfruit sauce (30cp). It was reported (Akubor, 2000) that the addition of sodium chloride reduced the viscosity and increased the degree of Non-Newtonian behavior of soy protein. The addition of sodium chloride decreases viscosity by preventing molecular expansion and water uptake. This could also explain what was observed in this study. The total sugar content of both products analyzed was relatively lower than their control.

4.3 Effect of fermentation time on selected quality characteristics of African locust bean and African breadfruit sauces.

pH

Table 3 shows changes in selected quality characteristics such as pH, titratable acidity (as citric acid), total sugars, total soluble solids, sucrose, glucose, viscosity, sodium chloride and amino acid contents during and after fermentation. There were variations in pH values during the fermentation of African breadfruit, ranging from 3.78 to 6.70%. African locust bean sauce ranged from 4.11 to 7.14%. Toxin producing organisms like *Clostridium botulinum* does not grow or produce toxin below pH 4.5. So botulism is not considered a danger in highly acidic foods, such as this. pH value decreased with increase in fermentation period, this could be due to the effect of fermentation on wheat flour that was mixed with the seed kernels. This observation agrees with the report of Skrede *et al*. (2001), that lactic acid fermentation of wheat resulted in low pH. In soy sauce production, it was observed that the growth of lactic acid bacteria in soy mash resulted in the formation of organic acids and made the mash acidic, a condition which is necessary for “ sound fermentation, to remove undesirable flavors, and add indispensable good flavors to the soy sauce”(Yong and Wood, 1977). According to Odunfa and Oyeyiola (1985), the initial increase in pH observed in fermented foods could be attributed to proteolysis and the release of ammonia through deaminase activity following utilization of amino acids as carbon and energy sources. Ammonia production is probably a common feature during fermentation of protein rich plant materials.

Table 3: Effect of fermentation time on selected quality characteristics of African locust bean and African breadfruit sauces.

Sample code	pH	Titratable Acidity (mg/g)	Sucrose (mg/g)	Glucose (mg/g)	Total Soluble Solids (mg/g)
LB0Control	6.70^{a}±0.39	5.92^{a}±2.14	25.45^{a}±2.00	24.06^{a}±0.09	59.8^{a}±0.88
LB0	6.71^{a}±0.51	15.94^{a}±.87	25.33^{a}±1.80	24.06^{a}±0.05	59.8^{a}±0.71
LB1Control	5.93^{a}±0.80	3.01^{b}±2.70	0.73^{a}±2.00	0.69^{a}±0.08	41.44^{a}±0.62
LB1	7.14^{b}±0.37	0.23^{a}±0.19	1.65^{a}±0.90	1.50^{a}±0.03	65.03^{b}±0.54
LB2Control	5.16^{a} ±0.86	1.90^{a}±0.86	4.67^{b}±0.60	4.44^{b}±0.08	23.20^{b}±0.24
LB2	4.12^{a}±0.90	0.85^{a}±0.92	0.33^{a}±0.20	0.31^{a}±0.09	19.12^{a}±0.32
LB3Control	4.93^{a}±0.93	0.94^{a}±0.16	10.26^{b}±0.92	9.75^{b}±0.31	18.01^{a}±0.05
LB3	4.11^{a}±0.11	0.83^{a}±0.93	0.90^{a}±0.94	0.86^{a}±0.80	18.44^{a}±0.23
BF0Control	6.20^{a}±0.70	4.64^{a}±0.12	52.20^{a}±0.01	49.60^{a}±0.03	63.3^{a}±0.90
BF0	6.70^{a}±0.71	4.68^{a}±0.70	52.25^{a}±0.23	49.64^{a}±0.12	63.3^{a}±0.67
BF1Control	3.83^{a}±0.93	1.12^{a}±0.50	0.00^{a}±0.90	0.00^{a}±0.01	52.59^{a}±0.33
BF1	4.50^{b}±0.12	0.43^{a}±0.51	1.78^{b}±0.45	1.69^{b}±0.44	53.34^{a}±0.26
BF2Control	3.51^{a}±0.20	0.98^{a}±0.80	6.10^{b}±0.33	5.80^{b}±0.30	32.30^{b}±0.25
BF2	3.80^{a}±0.83	1.35^{a}±0.30	1.00^{a}±0.75	0.95^{a}±0.59	20.01^{a}±0.00
BF3Control	3.30^{a}±0.78	0.94^{a}±0.35	10.77^{b}±0.44	10.23^{b}±0.30	18.01^{a}±1.78
BF3	3.78^{a}±0.30	0.96^{a}±0.94	1.38^{a}±0.50	1.3^{a}±0.02	19.05^{b}±1.00

Values are means ± SD of triplicate determinations. Any two means bearing the same superscript on same column are not significantly different ($p \geq 0.05$). Key: LB0 = Locust bean sauce on day 0 of fermentation, LB1= Locust bean sauce on 3rd day of fermentation, LB2= Locust bean sauce on 5th day of fermentation, LB3= Locust bean sauce on 7th day of fermentation, BF0 = African breadfruit sauce on day 0 of fermentation, BF1= African breadfruit sauce on 3rd day of fermentation, BF2= African breadfruit sauce on 5th day of fermentation, BF3=African breadfruit sauce on 7th day of fermentation.

Titratable acidity(as citric acid)

Change in Titratable acidity (as citric acid) during fermentation of African locust bean and

African breadfruit seeds was highest on the first day (5.94% and 4.68%) and lowest on the 3rd day (0.23% and 0.43%) respectively. A similar observation was reported by Njoku and Okemadu (1989) during the fermentation of some protein rich plant materials for condiment production. Increases in pH and titrable acid (as citric acid) suggest that both acid and alkaline producing activities occurred in the fermenting system. This could be due to the proteolytic activities which were taking place on the protein components of the kernels, the carbohydrate components were also being hydrolyzed to sugars and organic acid.

Glucose

Changes observed in the glucose level of the samples during fermentation ranged from 0.95 to 49.64% and 0.31 to 24.06% for African breadfruit and African locust bean sauces respectively. A decrease was observed in the glucose level during fermentation of both kernels. A significant decrease in glucose level was observed on the third day of fermentation, with the introduction of *Zygosaccharomyces rouxii* which ferments glucose to glycerol, under aerobic conditions at high saline concentration. The decrease in the level of sugar with fermentation could be related to its utilization by the fermenting microorganisms for their metabolic activities. A similar observation was reported by Omafuvbe (2006) in African locust bean during fermentation to condiments, that there was a fluctuation in the level of sugar as fermentation progressed. Fermentation was reported to cause decreases in sucrose and glucose content in sauce production.

4.4 Effect of Fermentation Period on Some Mineral Composition of African Locust Bean Sauce and African Breadfruit Sauce

Table 4 shows the effect of fermentation period on calcium, magnesium, sodium and potassium. There were significant ($p \leq 0.05$) changes in the level of mineral content of the fermenting samples of African locust bean and African breadfruit. Calcium varied from 400 to 20mg/g in African locust bean, and 120 to 40mg/g in African breadfruit sauces. The highest value of calcium was observed on the 1st day of fermentation in both samples and as fermentation progressed, the level of calcium decreased progressively.

Table 4: Effect of Fermentation Period on Some Mineral Composition of African Locust Bean Sauce and African Breadfruit Sauce (mg/g).

Sample code	Calcium	Magnesium	Sodium	Potassium
LB0Control	367.00^{a}±0.67	220.00^{a}±2.02	271.00^{a}±1.72	800.00^{a}±0.80
LB0	400.00^{a} ±0.25	220.00^{a}±0.65	270.00^{a}±2.20	805.00^{a}±0.37
LB1Control	170.00^{a}±0.75	170.00^{a}±0.23	600.00^{a}±1.70	700.00^{a}±0.24
LB1	120.00^{b} ±0.86	140.00^{b}±0.31	681.25^{b}±1.30	100.00^{b}±1.36
LB2Control	130.00^{a}±1.02	24.00^{a}±0.42	160.00^{a}±0.42	270.00^{a}±0.21
LB2	40.00^{b}± 1.96	36.00^{a} ± 0.10	456.25^{b}±0.36	315.00^{b}±0.10
LB3Control	90.00^{a}±1.10	24.00^{a}±0.11	186.00^{a}±0.12	160.00^{a}±1.86
LB3	20.00^{b}±2.03	36.00^{a}±0.91	500.00^{b}±0.15	310.00^{b}±1.50
BF0Control	150.00^{a}±0.69	120.00^{a}±0.64	540.00^{a}±0.80	700.00^{a}±0.63
BF0	120.00^{a}±0.80	170.00^{b}±0.30	540.00^{a}±1.71	690.00^{a}± 0.70
BF1Control	30.00^{a}±0.92	100.00^{a}±0.59	200.00^{a}±0.40	160.00^{a}±2.10
BF1	80.00^{b}±0.74	120.00^{a}±1.30	637.00^{b}±0.67	155.00^{a}± 1.84
BF2Control	24.00^{a}±0.31	33.00^{a}±0.22	120.00^{a}±0.17	101.00^{a}±0.17
BF2	40.00^{a} ±0.11	48.00^{a}±0.10	525.00^{b}±0.24	295.00^{b}± 0.24
BF3Control	40.00^{a}±1.12	33.00^{a}±1.19	231.00^{a}±0.15	70.00^{a}±0.15
BF3	60.00^{a}±0.78	48.00^{a} ±1.18	456.00^{b}±0.18	290.00^{b}± 0.18

Values are means ± SD of triplicate determinations. Any two means bearing the same superscript are not significantly different along the columns ($p \geq 0.05$). Key: LB0 = Locust bean sauce on day 0 of fermentation, LB1= Locust bean sauce on 3rd day of fermentation, LB2= Locust bean sauce on 5th day of fermentation, LB3= Locust bean sauce on 7th day of fermentation, BF0 = African breadfruit sauce on day 0 of fermentation, BF1= African breadfruit sauce on 3rd day of fermentation, BF2= African breadfruit sauce on 5th day of fermentation, BF3=African breadfruit sauce on 7th day of fermentation.

Magnesium

Magnesium content decreased with fermentation (220 to 36mg/g) in African locust bean and varied from 170 to 48mg/g in African breadfruit. The highest value of magnesium in both samples was observed on the first day of fermentation. It was generally noticed that during fermentation significant ($p<0.05$) decreases occurred in the mineral content of all samples analyzed. A similar trend was reported in the processing of 'ugba' for Ca, Mg, K and P (Achinewhu, 1983). There was no significant ($p\geq0.05$) difference in the values obtained in African breadfruit sauce.

Potassium

The highest value of Potassium was observed on the 1st day of fermentation (805 and 690mg/g) in both samples respectively and as fermentation progressed, a fluctuation was observed in the values. There was a consistent decrease in the level of potassium in the control. The initial reduction in potassium was attributed to leaching which could have occurred with the addition of brine on the 3rd day of fermentation. The values differed significantly ($p<0.05$) in African locust bean sauce.

Sodium

There were significant changes in the levels of sodium as fermentation progressed, ranging from 681 to 270 mg/g in African locust bean and 637 to 455 mg/g in African breadfruit sauces. Sodium content increased in both samples on the 3rd day of fermentation, this might be as a result of the initial introduction of brine in the production process. A decrease was observed on the 5th and 7th days of fermentation in both samples which might be as a result of the activity of micro organisms, especially *Zygosaccharomyces rouxii.*

4.5 Amino acid composition of soy sauce, African locust bean kernel sauce and Afr breadfruit kernel sauce.

Amino acid such as methionine, leucine, isoleucine, phenylalamine, histidine and valine were hi in African locust bean and African breadfruit kernel sauces than in soy sauce (Control). As in c legumes, the amino acid profile of African breadfruit seed kernel is characterized by concentration of Sulphur amino acids and high concentration of lysine and other essential amino a this will invariably have an effect on the concentration of these amino acids in the sauce prod from this seed kernel. The high content of lysine in African breadfruit sauce is of particular int because lysine is a limiting amino acid in maize and other cereals (Ouoba., et al 2003). Apart from essential amino acids, other nonessential amino acids found to play a vital role in human nutrition metabolism are arginine and histidine, were observed to occur in reasonable quantity in African lo bean sauce and African breadfruit sauce. African breadfruit kernel sauce had a higher concentratio most of the amino acids analyzed than African locust bean sauce and soy sauce. Cystine, glutamic proline occur in higher concentration in African breadfruit kernel sauce than was found in Afr locust bean kernel sauce, but lower than the concentration in soy sauce. African locust bean sauce the highest concentration of tyrosine, while Soy sauce had the lowest concentration. I fermentation period of legumes had been reported (Achi, 2005) to cause a reduction in some esse amino acids e.g. lysine. This might be responsible for some of the low values obtained in this st African locust bean kernel sauce and African breadfruit kernel sauce were found to be rich in ar acids.

Table 5: Amino acid composition (g/16gN$_2$ sample) of the sauces.

Amino acids	Soysauce	African locust bean kernel sauce	African bread fruit kernel sauce
Threonine	2.09	2.10	2.48
Isoleucine	2.45	2.79	3.04
Leucine	4.14	5.65	6.25
Lysine	2.94	2.16	3.02
Methionine	0.75	0.78	1.07
Cystine	0.91	0.66	0.79
Phenylalanine	2.72	3.08	3.26
Tyrosine	1.88	3.38	3.02
Valine	2.56	3.00	3.87
Arginine	3.57	2.62	3.62
Histidine	1.34	1.77	2.14
Alanine	2.27	3.02	3.57
Aspartic acid	5.55	5.56	6.40
Glutamic acid	12.18	6.65	8.56
Glycine	2.29	3.39	3.75
Proline	3.80	1.33	2.34

4.6 Microbial count of African locust bean kernel sauce and African breadfruit kernel sauce.

Parkia sauce and *Treculia* sauce both showed mould count that ranged from 3 to 1.78 $\times 10^{-4}$ cfu/ml 2.75 to 1.58 $\times 10^{-4}$ cfu/ml, respectively. The total bacteria count ranged from 100 to 1 $\times 10^{-6}$ cfu/n *Treculia* sauce and 100 to 8 $\times 10^{-6}$ cfu/ml in *Parkia* sauce. The sauces can be considered safe since the total viable count was not higher than the standard acceptable microbial count for food sau Standard plate count is <10,000 cfu/ml and Standard yeast count is < 50cfu/ml for food sa (www.yamasausa.com).

Table 6: Microbial count of African locust bean kernel sauce and African breadfruit kernel sauce (cfu/ml).

Sample Code	TVC cfu/ml	Mold count
LB	0.5133	0.0489
BF	0.5022	0.0338

Values expressed as $\log_{10}$ cfu ml^{-1} wet weight

Key: LB=African Locust bean kernel Sauce; BF=African Breadfruit kernel Sauce.

4.7 Sensory evaluation of the sauces.

Results of sensory evaluation of African locust bean sauce, African breadfruit sauce and soy sauce shown in Table 7. It was observed that there was no significant difference ($p \geq 0.05$) in color among sauces. African breadfruit sauce differed significantly ($p<0.05$) in taste and overall acceptability f African locust bean sauce and soy sauce. There was no significant difference ($p \geq 0.05$) between sauce and African locust bean kernel sauce in the entire quality attribute evaluated. However, panelists prefer soy sauce to African locust bean kernel sauce and African breadfruit kernel sauce.

TABLE 7: Sensory scores of the sauces.

	Attributes				
Sample code	**Color**	**Flavor**	**Mouthfeel**	**Taste**	**Overall Acceptability**
ALB	7.4^{a}	6.6^{b}	6.2^{ab}	6.1^{b}	6.5^{b}
ABF	7.1^{a}	4.5^{a}	5.3^{a}	3.4^{a}	4.7^{a}
ASS	6.4^{a}	6.8^{b}	6.5^{b}	6.8^{b}	7.1^{c}
LSD	1.74	3.58	1.22	2.61	2.25

Values in an entire column followed by a common letter superscript are not significantly different

($p \geq 0.05$). Key: Samples ALB, ABF, and ASS are African locust bean, African breadfruit and soy sauces, respectively.

CHAPTER FIVE

CONCLUSION

Sauces produced from fermentation of African locust bean seed kernel and African breadfruit seed kernel (underutilized Nigerian legume seeds) were rich in essential amino acids. These sauces could be used as condiment in the preparation of foods (gravies, soups, stews, jollof and fried rice, pastry fillings, etc.) at homes, restaurants and fast food companies. The commercial production of these sauces could help in the reduction of the pressure on soybeans, currently the major raw material for the production of soy sauce, and increase the utilization of these crops and income of the farmers of African locust bean and African breadfruit.

RECOMMENDATION

Although fermented food condiments have constituted a significant proportion of the diet of many Nigerians, yet they have shown mixed, uncertain feelings in terms of preference for such foods. Fermented condiments have often been considered as food for the poor. Also, the traditional condiments have not attained significant commercial status due to their objectionable packaging material and inconsistent quality. The introduction of improved technology products, with consistent quality as these, could change the Nigerian food culture towards local dishes. As such, the use of unconventional low-cost but high quality protein sauces as these should be encouraged. Though soy sauce was preferred to these sauces organoleptically, a sauce combining *Parkia biglobosa* and *Treculia africana* could give a product comparable in nutrient composition to soy sauce- the percentage inclusion of each (that will give the best result), however, needs to be determined.

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