OPTIMIZING THE CONDITIONS AND PROCESSES FOR THE PRODUCTION OF PROTEIN NUTRIENT FROM *Parkia biglobosa* SEEDS

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DECLARATION

I Ojewumi Modupe Elizabeth hereby declare that this research work was carried out by me under the supervision of Prof. J.A. Omoleye (Main supervisor) of the Chemical Engineering Department in Covenant University, College of Engineering, Ota Ogun State and Prof. (Mrs.) A. A. Ajayi (Co-supervisor) of the Department of Biological Sciences, College of Applied Sciences in Covenant University Ota.

This research work has never been submitted elsewhere in a previous application for award of a degree to the best of my knowledge. The information used in the writing of this research has been properly acknowledged.

Ojewumi, Modupe Elizabeth

CERTIFICATION

The research work titled **OPTIMIZING THE CONDITIONS AND PROCESSES FOR THE PRODUCTION OF PROTEIN NUTRIENT FROM *Parkia biglobosa* SEEDS** was carried out by Ojewumi Modupe Elizabeth (CUGP100227) under the supervision of Prof. A.J. Omoleye and Prof. A.A. Ajayi meets the regulation governing the award of the degree of Doctor of Philosophy (Ph.D) in Chemical Engineering Department of the Covenant University, Ota Ogun State, Nigeria. We hereby certify that it has not been submitted for the degree of Ph.D here or elsewhere for any other award. This work has been approved for its contribution to knowledge and the society at large.

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Prof. Abiodun J. Omoleye — Date
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Prof. Abiola J. Kehinde — Date
External Examiner

DEDICATION

This research work is dedicated to God the Father, the Son and the Holy Spirit who makes all things beautiful in HIS own time.

Also to my amiable and loving family.

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TO GOD ALONE BE ALL THE GLORY.

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ABBREVIATIONS

AAS	Atomic absorption spectroscopy
B. subtilis	*Bacillus subtilis*
B/S	*Bacillus subtilis*
BFALB	*B. subtilis* fermented African locust bean
BINO 0.0025 (g broth/g seed)	*B. subtilis* fermented African locust bean with Inoculum concentration of 0.0025 (g broth/g seed)
BINO 0.005 (g broth/g seed)	*B. subtilis* fermented African locust bean Inoculum concentration of 0.005 (g broth/g seed)
BINO 0.0075 (g broth/g seed)	*B. subtilis* fermented African locust bean Inoculum concentration of 0.0075 (g broth/g seed)
BINO 0.01 (g broth/g seed)	*B. subtilis* fermented African locust bean Inoculum concentration of 0.01 (g broth/g seed)
ANERO	Anaerobic fermented African locust bean
CFALB	Commercial fermented African locust bean
Ca	Calcium
Cd	Cadmium
CO_2	Carbon dioxide
Copper	Cu
FAO	Food and Agricultural Organization
FDA	Food and Drug administration
FFA	Free fatty acid
FP	Flame photometer
INO	Inoculum
INO. CONC.	Inoculum concentration

IR	Infrared Spectroscopy
Iron	Fe
LAB	Lactic Acid Bacteria
Lead	Pb
MFALB	Mixture of Inoculum fermented African locust Bean
Mn	Manganese
Mg	Magnesium
Mix	Mixture
Nat	Natural
Na	Sodium
NFALB	Naturally fermented African locust bean
ND	Not detected
P.biglobosa	*Parkia biglobosa*
K	Potassium
POV	Peroxide value
Rm T°C	Room temperature
S.cer	*Saccharomyces cerevisiae*
SEM	Scanning Electron Microscope
SFALB	*S.cerevisiae* fermented African locust bean
S/C	*Saccharomyces cerevisiae*
SMF	Submerge Fermentation
SSF	Solid State Fermentation
TA	Titratable acidity
Temp.	Temperature
UFALB	Unfermented African locust bean
WHO	World health organization
Zinc	Zn

ABSTRACT

African Locust Bean (*Parkia biglobosa*) seeds known as 'Iyere' in Yoruba language were fermented aerobically and anaerobically at 40, 50, 60 and 70 °C for five days (120 hours) with the aid of two starter cultures - *Bacillus Subtilis* and *Saccharomyces Cerevisiae* to a vegetable protein based food condiment known as 'Iru'. The Fermenter was fabricated with galvanized iron, thus getting rid of the unhygienic methods of producing it locally. Samples were taken every day (24 hours) and stored in the deep freezer for further analysis. Fermentation increased the percent protein content from 32 to 40 % and to 52 % for anaerobic and aerobic fermentation processes respectively after 3 days (72 hours). Other parameters like % crude fibre, % ash content and % carbohydrate in both aerobic and anaerobic fermentation processes decreased with number of fermentation days from 9.58 to 5.29, 4.05 to 3.30, 36 to 23 and 9.58 to 3.824, 4.05 to 0.832, 36 to 19.89 respectively. The biological changes such as physiological (odour, colour and appearance) and sensory (taste and texture) were studied. From experimental data generated by the design of experiment (DOE), using MINITAB 17 software, optimum conditions were obtained at 40 ^{0}C with inoculum (*Bacillus subtilis*) concentration of 0.005 (g broth/g seed) at fermentation duration of 3.25 days. The product of the fermentation process 'Iru' was analyzed physiologically and organoleptically. The products of anaerobic fermentation were not acceptable organoleptically due to their repulsive odour, although was mild at lower temperature. The organoleptic test showed that the sample fermented with *Bacillus subtilis* was generally acceptable for all parameters tested. The mineral compositions of the fermentation product at different temperatures were analyzed and Magnesium, Phosphorus, Potassium, Sodium, Manganese, Iron, Copper and Zinc were detected. The Scanning Electron Microscope (SEM) was used to study the effect of fermentation and temperature on the morphological structures of both fermented and unfermented samples. The deterioration rate of the fermented samples were studied to estimate the shelf life of the condiment with no additives. Dried condiment was stored for 6 weeks. The stored sample lasted for about 10 months.

CHAPTER ONE
INTRODUCTION

1.1 Background of the Study

In Africa many species of trees serve as sources of food and for medicinal purposes to indigenous people. Some of these trees provide ecological services including microclimate amelioration and soil fertility. They serve as source of income for many poor people in the rural areas; one of these trees is *Parkia biglobosa* (African locust bean tree) (Igba in Yoruba language). Farmers manage and protect this tree for their nuts and fruits. The tree has been used both locally and internationally in drug manufacturing and cosmetics production. Despite the important uses, the populations of this tree is reducing and it remain semi- or undomesticated (Teklehaimanot, 2004).

African locust bean tree was named *Parkia biglobosa* by Robert Brown, a Scottish botanist in 1826 after Mongo Park, a Scottish surgeon who explored West Africa in 1790's. Mongo Park gave this tree a local name 'nitta' (Uaboi-Egbenni *et al*., 2009). In 1995, research indicated that there were about 77 more species. African locust been tree was described by Robert Brown, as a genus of flowering plants in the legume family, Fabaceae, which belongs to the sub-family *Mimosoideae* and *Leguminosae* with the genus *Parkia* and botanical name *Parkia biglobosa* (Abdoulaye, 2012). *Parkia bicolor, Parkia filicoidea, Parkia clappertoiana* and *Parkia biglobosa* are other species of the genus of *Parkia biglobosa* which can also be fermented to produce food condiments for flavouring which also for adding good aroma to food. It was reported that fermented African locust bean seeds is a leguminous plant with an outstanding protein quality. The protein and amino acid composition have been reported by several researchers (Cook *et al*., 2000).

Parkia biglobosa tree have been known to be a native of Africa and is an important multipurpose tree of West African Savannah land (Olorunmaiye *et al*., 2011), which is primarily grown for its pods that contain both a sweet pulp and valuable seeds. Various part of the African locust bean tree are used for medicinal purposes and have high value commercially.

Parkia biglobosa seed is known as Iyere in Yoruba land while the fermented seed as Iru. Iru is one of the major sources of plant protein in African diet which is known as fermented vegetable protein (Ademola *et al.,* 2011). Iru is consumed in many African countries, especially Nigeria. Iru known by different names in different countries - kinda in Sierra Leone, in Nigeria and Ghana it I called *dawadawa* or *Iru* (Azokpota *et al.,* 2005; Odunfa, 1981a), in Benin Republic afintin and *sonru*; *nététu* in Senegal and Burkina Faso as *soumbala*; Japan as *natto;* and *kinema* in Nepal (Azokpota *et al*., 2006). These are all produced by either natural fermentation or inoculated fermention of African Locust bean seeds.

Apart from fermented *Parkia biglobosa* seeds (Iru) serving as a rich source of plant protein to man with low cost. It also serves as good source of protein for animal feeds, chick and fish (Livestock) (Ademola *et al.,* 2011; Campbell-Patt, 1980; Uaboi-Egbenni, 2009). Iru serves as dietary protein in many rural areas in developing countries since some of them cannot afford animal protein because they are either too expensive or simply unavailable. This situation has made many people to depend mainly on carbohydrate diets; comprising of grains or starchy roots and tuber crops with low protein level or content, thus leading to high level of malnutrition. In the quest of rural dwellers to increase the protein level of their food, many wild fruits have been found to be good alternative. Consumption of mainly cereal grains or starchy roots and tuber crops leads to various health problems associated with protein and vitamin / mineral deficiencies. In the search for plant protein and vitamin substitutes, the African locust bean *(Parkia biglobosa)* has found very popular use especially in the form of fermented 'Iru', which is a product of its seeds, (Gernal *et al*., 2007).

Apart from the nutritional values, fermented African locust bean seeds provide dietary fiber, energy, minerals and vitamins (Vitamin B, riboflavin and Vitamin A), it also improve sensory properties of foods which includes the organoleptic characteristics (appearance, aroma and flavor) (Kolapo *et al.,* 2007). One of the major benefits of fermentation is the conversion of sugars and other carbohydrates to usable end products. Other reasons were developed as time goes by, such as the removal of anti-nutritional components, increasing shelf life or storage, reduction in cooking time, detoxification, decrease in the need for refrigeration or other form of food preservation technology, enhancement of nutritional and organoleptic value of the food (Aroma, appearance and flavor), elimination of beany flavours, improvement in digestibility and improved safety (absence of toxins). All these benefits were brought about by microorganisms which perform the greatest role in different parts of West Africa. There are thousands microorganisms both inside and outside of our bodies (William and Akiko*, 2004).*

There seems to be a general agreement on the spore-forming *Bacillus* species as the main fermentation organisms (Sanni, 1993). This was supported by Odunfa, 1981, 1985b, 1998; Omafuvbe *et al.,* 2002; Ouoba *et al.,* 2002, 2003, 2004. During the fermentation of African Locust bean seeds, thorough and systematic investigation showed that *Bacillus subtillis* is the most dominant bacterium responsible for the fermentation (Odunfa 1985b; Sanni *et al.,* 1993; Antai and Ibrahim, 1986). Literature revealed that some species of *bacillus* such as *Bacillus lichenioformis, Bacillus megaterium, L. mesenteriodes* and *Staphylococcus* are also found in the fermented condiment (Iru).

It was reported by various authors that *Staphylococcus, Micrococcus, Leuconostac* and *Enterobacteriaceae* are microorganisms which contributes to individual fermentation based on the sources of their materials (Enujiugba, 2005). They get into the fermenting medium via the container or water been used. However, the indigenous fermentations are mostly achieved via natural inoculations. Depending on the species, *Bacillus* acts either as diseases causing

organisms (pathogenic) or spoilage causing organisms in a fermentation process or any medium the species found themselves. Aside microorganisms, local or traditional starter culture can also be employed in the fermentation of African locust bean seed, such as *Iku-iru* and *yanyanku*. They are made from dried malvacene beans (*Hibiscus sabdariffa*). Yanyanku in Bariba ethnic group language means "Product having aptitude to degrade something". These local starter cultures accelerates the fermentation of African locust beans to produce condiments.

Virtually all the genus of *Bacillus* are regarded as safe by the US Food Agency. *Bacillus subtilis, Bacillus licheniformis, Bacillus amyloliquefaciens, Bacillus firmus, Bacillus pumilus and Bacillus megaterium* are all considered safe, but *B. anthracis, B. cereus, B. mycoides and B. thuringiensis* are termed virulent (extremely harmful) if consumed. *B. cereus* causes various kinds of infections and sometimes food poisoning. This work looked into the best starter culture for the fermentation of this proteineous seed by using two different microorganisms; *B. subtillis* and *Saccharomyces cerevisiae* which are both harmless and even increases the nutritive values of fermented food. The finished product which is the Protein condiment was analyzed for microbial growth to confirm the type of microorganism present before consumption. The short shelf life, objectionable packaging materials, stickiness and the characteristic putrid odour has led to instability in the commercial status of this traditional condiment (Arogba *et al.*, 1995), hence the reason for studying how to prolong its shelf life.

The most valuable part of the locust bean tree is the seed which is high in protein, carbohydrate and is a good source of fat and calcium for rural dwellers (Ntui *et al*., 2012). The seed is first cooked to remove the seed coat and then fermented to produce the desired result depending on the region of the producer and the purpose of production. In a study conducted on the fermentation of Iru, it was found that *Gmelina arborea* as well as banana leaves accelerated

fermentation of seeds, while also bringing "an increase in protein and crude fat contents with corresponding decrease in carbohydrate (Gernah *et al*., 2007).

Sonru and Afitin can also be produced traditionally alongside with 'Iru', this is produced majorly in Benin, Nigeria. The technology and principles used to produce these three condiments varys depending on localities or region of the producers each locality has its own specific know – how.

1.2 Statement of the Research Problem

There have always been traditional methods of fermenting African Locust bean seeds to 'Iru' (dawadawa) which has remained a traditional family art practiced in homes on a small scale basis. This is characterized by the use of chance or natural inocula, unregulated conditions, sensory fluctuations, poor durability and unattractive packing of the processed products with non-sterile utensils such as calabash and leaves (Banana leaves). The traditional method is seen as unhygienic which needed to be improved. This research was based on the fabrication of a fermenter which operated under hygienic condition. This research work studied the method of fermentation with different variables such as temperature, inoculum variation and concentration. The chemical, biochemical and physicochemical analysis of the final product will be carried out. The method of preservation suitable for the condiments was also considered. The final product (condiment) is a vegetable protein which serves as spice for stew and soup. The result was compared with the spices available in the market.

1.3 Aim and Objectives of Research

The project work aims at evaluating the traditional methods of processing 'Iru', a vegetable food protein and upgrading it, the problems associated with the process, the possible ways of

overcoming known challeges and improving the quality of the product in order to bring this health friendly seed into limelight for large scale production.

The objectives of this study are to:

(i) Use MINITAB 17 statistical software package to design an experiment (DOE) for the research study in order to determine the optimum fermentation conditions for the processes involved in the conversion of 'Iru' a protein based condiment.

(ii) Fabrication of a fermenter (bioreactor) for the fermentation processes of African Locust bean seeds (*Parkia biglobosa*).

(iii) Physicochemical, organoleptical and morphological analysis of both the fermented and raw *Parkia biglobosa* seeds.

(iv) Determination of protein content of the condiment.

(v) Determination of Shelf life for processed condiment through moisture content analysis.

At the end of this research, the nutritive values of the fermented African locust bean seeds would have been well enhanced and optimum conditions developed to annul the challenges in its consistency and organoleptic properties. The shelf life would have also been determined to know how long it can stay on the shelf before deteriorating.

1.4 Justification for the Study

In Nigeria protein malnutrition is a major challenge. It has been reported that the diet of Nigerians is lacking in fairly large amount of protein due to the high cost of accessible protein sources e.g. meat. Essien, (1983) reported that approximately, Nigeria spends $120 million on importation of food flavors with a 15 % annual increase. The Food and Agricultural Organization (FAO) in 2003 reported that about a third of the Nigerian populace is malnourished.

Existing literature revealed that there has been no detailed scientific and engineering study of standard methods for the production of African locust bean putting hygiene into consideration. Nothing has ever been done in the study of the structures, variation of microorganism for the fermentation, determination of basic organic functional groups and using the percentage of moisture removed to monitor the storage life. There should be a deeper research into all the unutilized or underutilized crops or seeds in West Africa, Nigeria in particular. The raw unfermented seeds *of Parkia biglobosa* are inedible but fermentation improves the nutritional quality and digestibility. Traditionally, the production process of 'Iru' is tedious, wasteful, requires a large volume of water and also takes a long time to get the end product. The use of starter cultures in the right proportion would guarantee consistency, increase product safety and enhance product quality. Iru can serve as a substitute for meat for low-income earners and can reduce protein-calorie malnutrition and essential fatty acid deficiencies (Oguntoyinbo and Sanni, 2007). The use of fermented local seeds such as *Parkia biglobosa* as condiments are healthier than the mono-sodium glutamate (MSG) rich condiments that are being presently imported.

This study would come up with a refined commercial method for the processing of this useful traditional condiment and bring out the optimum conditions for the fermentation of seeds. The researched product is expected to be of higher quality than the locally produced ones.

1.5 Scope of Research

The scope of this study was limited to the following areas;

(i) Only African locust bean seeds was used for the fermentation.

(ii) Two microorganisms were used as starter cultures – *Bacillus Subtilis* and *Saccharomyces cerevisiae.*

(iii) Fermentation was carried out at various temperatures of 40, 50, 60, 70 °C for both aerobic and anaerobic fermtation.

1.6 Limitations of Locally Produced Condiment to be upgraded

(i) Techniques involved are traditional which uses non-sterile materials and equipment which lead to contamination.

(ii) The natural inocula (chance) being used are under an uncontrolled fermentation condition, this leads to sensory fluctuation.

(iii) The packaging is unattractive and not presentable.

(iv) Preservation problem – shelf life of the condiments, needs to be established.

(v) Even though locally fermented foods have health-promoting benefits; their global consumption is declining as traditional food systems give way to the influence of western diet and fast foods.

(vi) Inadequate raw material grading and cleaning contributing to the presence of foreign matters (such as insects, stones) in the final product.

CHAPTER TWO

LITERATURE REVIEW

2.1 Legumes

Legumes are plants in the family Fabaceae (or Leguminosae) which are grown for their food grain seeds e.g. pulse.

$N_2 + 8H^+ + 8e^- \longrightarrow 2NH_3 + H_2$ ..... (1)

Ammonia is then converted to another form, ammonium (NH_4^+), usable by plants by the following reaction:

$NH_3 + H^+ \longrightarrow NH_4^+$ ..... (2)

All proteins contain nitrogenous amino acids. In the production of proteins nitrogen is a necessary ingredient . They are sometimes referred to as "green manure".

2.2 African Locust Bean Tree (*Parkia biglobosa*)

The African locust bean tree with the botanical name *Parkia biglobosa* is a perennial deciduous tree that is fairly widely distributed all over the natural grassland of Northern Nigeria (Sobande, 2013, Ojewumi *et al*., 2016). The tree starts to bear fruits from five to seven years after planting. It is planted mainly for the food value of its fruit. It grows in the savannah region of West Africa. The tree has a height ranging from 7 to 20 m; in some exceptional cases some might reach heights of up to 30 m, with a wide spreading umbrella-shaped crown (Teklehaimanot, 2004, Ojewumi *et al*., 2016). Its performs an essential function ecologically in cycling of nutrients from deep soils, and in holding the soil particles with the aid of the roots to prevent soil erosion (Alabi *et al.*, 2005). The tree also provides shade for man.

The tree requires an altitude of about 300 metres with an average rainfall of 400 - 700 millimetres per year and an average mean annual temperature of 28 ^oC. It prefers well-drained, deep, cultivated soils, but can also be found on shallow, skeletal soils and thick laterites

(Database.prota.org, 2014). The African locust bean seeds are contained in branches of pods that make up the most valuable part of the plant. The pods are flat and large/irregular cluster of up to 30 seeds (Omafuvbe *et al*., 2004). It is a tree that has spreading branches with a fat bole. It has long tap roots and wide lateral roots up to 10 m spreading from the bole. It has a thick dark grey-brown and a narrow opening bark from which, amber gum comes out gradually in drops when the bark is wounded. It has dark green leaves interchange repeatedly and regularly with one another, they are bipinnate with pinnae of up to 8-16. The tree can also grow on rocky slopes, stony ridges or sandstone hills. *Parkia biglobosa* occurs in a diversity of zones, ranging from tropical forests with high and well-distributed rainfall to arid zones where mean annual rainfall may be less than 400 mm (Agroforestry database, 2014). Due to its deep taproot system and an ability to restrict transpiration it has a capacity to withstand drought conditions. *Pakia biglobosa* can be seen in the savannah region of Nigeria, although they are not cultivated normally. The tree can be found mainly in Africa, especially in the following countries; Benin, Burkina Faso, Cameroon, Chad, Cote d'Ivoire, Democratic Republic of Congo, Gambia, Ghana, Guinea, Guinea-Bissau, Mali, Niger, Senegal, Sierra Leone, Sudan, Togo, and Uganda (Campbell-Platt, 1980).

Pakia biglobosa is beneficial to the soil situated beneath, which is made useful and valuable by the dung and urine of animals that shelter under the tree's shade. It can also be used as timber for making pestles, mortars, bows, and seats (Ntui *et al*., 2012). All trees of the *Parkia* species are carefully and usually kept safe by the people in the particular area where they grow because of their valuable sources as a reliable source of food, especially the seeds which serves as source of useful ingredients for consumption. The pods and husks have also been discovered to be good food for livestock. The various importance of *Parkia biglobosa* made it an ideal plant to promote across Africa, as it combines in a single species two of Africa's greatest needs which are; food security and tree cover alongside the health benefits.

2.3 Usefulness of the African Locust bean Tree

All the parts of *Parkia* are useful, this has made the tree to be called a multipurpose tree. The most important use of African locust bean is found in its seed, which has both food and non - food uses. The seed mainly serves as a source of useful ingredients for consumption (Campbell-platt, 1980). African locust bean is a multipurpose tree that is as highly valued as Shea butter tree (*Vitellaria paradoxa C.F.Gaertn*.). Fermented seeds ('soumbala', 'dawadawa', 'netetu' 'Kula', 'Iru') serve primarily as condiments for seasoning sauces and soups served in various Nigerian meals among the various tribes of Nigeria. The leaves are sometimes eaten as vegetable, usually after boiling and mixed with other foods such as cereal flour. Young flower buds are added to mixed salads. Roasted seeds are used as a coffee substitute known as 'Sudan coffee' or 'café nègre'. Ground seeds are mixed with *Moringa oleifera* Lam leaves to prepare a sauce, and are also used to make doughnuts. The tree enriches soil due to the presence of special nodules on the tree's root that perform nitrogen fixation while simultaneously preventing soil erosion. Shade-tolerant crops such as sesame, yams, beans, peppers and other vegetable are often grown under the shade of the trees. Leaf litter also returns nutrients to the soil and improves soil structure as leaves are rich in nitrogen (Robert, 2006).

The leaves of the tree can used as food both in its natural state and its fermented state (where it is mixed with flour, cooked and sold as a snack). The roasted seeds can be used as coffee substitute known as "Sudan coffee". The leaves also have medicinal purposes in which they are used to treat burns and hemorrhoids. The leaves may also be pounded and used as a local soap. The tree is also very important in apiculture, being a good source of nectar and suitable for placement of hives. Boiled pods are used to dye pottery black; the ash is applied as a mordant. The bark is rich in tannins and may be used for tanning hides, but the resulting leather is often of moderate quality especially with regard to colour, which is often reddish, uneven, and darkens when exposed to light. The pods make up 39 % by weight of the fruits while the

mealy yellowish pulp and seeds make up 61 %. Seeds contain fat and essential proteins high in amino acids such as methionine and cystine. Fermented seeds are also used as condiments and spices in sauces and soups. Dawadawa, one of such condiments is prepared by lightly roasting the seeds then manually removing their seed coats by grinding. It is also important in apiculture, being a good source of nectar and suitable for the placement of hives. It may serve as a decorative avenue tree/motor ways. (Adama and Jimoh, 2011). Feed to livestock and a source of raw material for medicine and fodder, soil amendment, charcoal and fire wood (Campbell-platt, 1980).

The uses of the tree as medicine are especially useful in rural areas where there is no access to modern medicine. In West Africa the bark, roots, leaves, flowers, fruits and seeds are commonly used in traditional medicine to treat a wide diversity of complaints, both internally and externally, sometimes in combination with other medicinal plants. The bark is most important for medicinal uses, followed by the leaves. Medicinal applications include the treatment of parasitic infections, circulatory system disorders, such as arterial hypertension, and disorders of the respiratory system, digestive system and skin. The list of diseases treated by the African Locust Been tree include serious colic, diarrhea, sterility, bronchitis, pneumonia, leprosy, venereal diseases, bad teeth, skin irritations, guinea worm and swellings. The bark leaves and pod husks are rich in tannins, which in general have anti-diarrhea properties. The tannin obtained from the bark may also be used in the production of dye and local snuff.

In veterinary medicine, a root decoction is used to treat coccidiosis in poultry. In fishing, green pods from the tree are crushed and added to rivers to kill fish while leaving the nutritional value of the fish unaffected so long as they are cooked or dried. The pulp of the tree is prepared as flour used to thicken soups and broth. The fresh fruit pulp is sweet (it is made up of about 60 % sugar) and very high in carbohydrates. The fruit pulp may be fermented and processed into an energy drink or made into cakes. The wood from the tree is relatively hard and solid, but

not very durable. It is whitish to pale yellow in appearance and may be used as light structural timber for vehicle bodies, crates, boxes and barrels. Reports made by Alabi *et al*., (2005), showed that the tree have found its usefulness, not only for human consumption purposes, but also as timber for making pestles, mortar, bows, hoe-handles and seats. The husks and pods are reported to be good for feeding livestock (Douglas, 1976). In addition, the fruit pulp and seed residues are also used in making animal feeds (Douglas, 1996; Obioza and Atii (1991). In the arid and semi-arid regions of Africa, *Parkia biglobosa* is very important for food security particularly during food shortage and drought periods.

Figure 2.1 African Locust Bean Tree **(***Parkia Biglobosa*) (Igba tree). (Sobande, 2013 and Ojewumi *et al*., 2016)

In traditional fermented food preparation, microbes are used to prepare and preserve food products, adding to their nutritive value, the flavour and other qualities associated with edibility. The introduction of foreign high technology products especially processed ones, radically changed the Nigerian food culture into a mixed grill of both foreign and local dishes, sold at relatively high prices. These imported items command respect from the fact that their refinement had benefited from decades of research and development in their countries of origin.

Almost in contrast, many of the local indigenous fermented foods lack this appeal. Many people prefer the imported and exotic food items because of the attractive form, long shelf life, ease of transportation and other forms of utility which consumers associate with them (Sobande 2013).

2.4 African Locust Bean Seed *(Parkia biglobosa)*

The unfermented seed is known as *karwa* in Hausa; *Iyere* in Yoruba; *Soumbala* in Burkina Faso, Mali, Cotdeivoire and Guinea. They are traditionally used as food condiment and are known to be rich in protein and contain easily digestible calcium, they also contain 20% edible oil. In the third world countries where the need for protein supplementation is high for both adult and infants, 'Iru' is very important (Emujiugha, 2005). African locust bean (*Parkia biglobosa*) seed grows on a common perennial leguminous tree known as African Locust bean Tree which belongs to the sub-family mimosoideae and family leguminosae (now family fabaceae), (Abdoulaye, 2012). They grow in the savannah region of West Africa up to the southern edge of the Sahel zone 13 °C, (Campbell-Platt, 1980). The plant produces brownish seeds, which are arranged in pods. When processed, the seeds constitute an important condiment that adds taste and flavor to soup. The processed cake, known as 'Iru' is used widely in the south-western and middle-belt zones of Nigeria. The pods, which are slightly flattened and slender, turn from pink-brown to dark brown when mature. These pods are about 45 cm long and 2 cm wide and may contain up to 18 seeds embedded in a yellowish fleshy endocarp. The seeds have a hard testa (averagely weighing about 0.26 g/seed) with large cotyledons forming about 70 % of their weight. Locust bean fruit is normally processed into food condiment, which is popularly taken in the western part of Africa and it is used as a spice that gives an African meal a pleasant flavor. Since the last 10 years they are considered as the most

important food condiments consumed everywhere by rural poor people, (Azokpota *et al*., 2005).

African locust bean (*Parkia biglobosa*) can be processed to produce afitin, iru and sonru, three different types of condiment (Azokpota *et al.,* 2006).). Locust bean is generously added to soups as low-cost meat substitute by low-income families in parts of Nigeria, (Omafuvbe *et al.,* 2004). The technique used in the harvesting of locust bean is by the use of a hooked light pole. The farmer climbs up the tree and leans on bigger branches and stretches out the hooked pole to reach every bunch.

2.5 Reasons for Fermenting African Locust Bean Seeds

The physical, chemical and nutritional characteristics of the African Locust Bean seeds changes immediately after fermentation since the raw African locust beans are nutritionally deficient and unpalatable (Amoa-Awu *et al*., 2005). Nigeria has a variety of people and culture that it is difficult to pick one national dish. Each area has its own regional favorite food that depends on customs, tradition and religion (Abdel and Dadir, 2009; Adebayo *et al*., 2010). The fermentation processes for these foods constitute a vital body of indigenous knowledge used for food preservation, acquired by observations and experience, and passed on from generation to generation (Aworh, 2008; Chelule *et al*., 2010). The fermentation techniques are often on a small scale and household basis, characterized by the use of simple non-sterile equipment, chance or natural inoculums, unregulated conditions, sensory fluctuations, poor durability and unattractive packaging of the processed products resulting in food of unpredictable quality and variation (Olanrewaju *et al*., 2009).

2.7 Previous Study on African Locust Beans Seeds (*Parkia biglobosa)*

Omafuvbe Bridget, Falade Olumuyiwa S. and Adewusi Steve R. (2004), of the Department of Microbiology, Department of Chemistry, Obafemi Awolowo University, Ile-Ife reported the following from results obtained from the proximate analysis and pH after investigating the chemical and biochemical changes during fermentation in African locust beans. They observed that there was an increase in the moisture content of fermented African locust bean and concluded that fermentation seemed to have decreased the ash content of African locust bean seed which agreed with the observation of Eka (1980) of about 30 % decrease in ash after fermentation They concluded that boiling, soaking in water and dehulling of African locust beans led to a loss of 41 % ash. A decrease in crude fibre content of the African locust bean seed during the last 24 hours of fermentation was also observed, this was said to be probably due to the production of extracellular enzymes.They also noticed that the viscosity of the boiling water at the end of the boiling process was more than it was at the beginning of the process which is an indication of the presence of mucilaginous materials in the water, which would explain in part the reduction of the crude fibre content of the African locust bean seed on boiling. They concluded that ether extract of raw African locust bean seed increased by boiling and fermentation in agreement with earlier findings (Eka, 1980). They also agreed that fermentation also led to increase in crude protein for the African locust bean which also correlate with the findings of Eka (1980). The increase in ether extract and crude protein they said, was probably due to the reduction in the content of ash, crude fibre and carbohydrate. An increase in pH was observed for the fermentation as the day increased as reported by Odunfa (1981).

Oladunmoye (2007) reported the proximate composition of raw, naturally fermented and inoculated fermented seeds as shown on Table 2.1. The moisture content was found to be higher

in the fermented samples than the unfermented. Natural and inoculated fermentation gave an increase in the crude protein value over the unfermented sample this might be due to reduction in crude fiber and carbohydrate in the unfermented material. There was significant reduction in the crude fibre, fat and carbohydrate as against ash contents.

The mineral compositions of fermented, naturally fermented and inoculated fermented locust beans are presented in Table 2.2. Ascorbic acid value was shown to have reduced in the inoculated fermented sample than the unfermented one. This may result from the ability of the starter culture to metabolize the ascorbic acid thereby leading to its reduction. Phosphorus and magnesium were also found to be significantly reduced in both types of fermentation. Calcium and iron were found to increase in natural fermentation and in that with inoculated culture.

Table 2.1 Proximate Analysis of Unfermented, Naturally fermented and Inoculated African locust beans.

Nutrient	Unfermented	Naturally fermented	Inoculated fermented
Moisture (%)	12.00	42.65	45.00
Protein (mg/100 g)	30.15	37.32	38.00
Ash (mg/100 g)	4.47	4.31	4.91
Crude fibre (mg/100 g)	13.00	8.3	6.20
Fat (mg/100 g)	21.02	10.10	18.00
Carbohydrate (mg/100 g)	19.30	17.09	15.00

Source: Oladunmoye, (2007)

Table 2.2 Mineral Composition of Unfermented, Naturally fermented and Inoculated African locust beans (mg/100 g)

Mineral	Unfermented	Naturally fermented	Inoculated fermented
Ascorbic acid	2.86	3.56	1.32
Calcium	10.82	12.68	13.64
Potassium	210.40	250.40	300.40
Phosphorus	86.25	80.12	82.61
Magnesium	51.20	48.46	56.50
Iron	2.68	5.69	7.20

Source Oladunmoye, (2007)

Omodara T.R and Aderibigbe E.Y. (2013) of the Department of Microbiology, Faculty of Science, Ekiti State University, and Ado-Ekiti, Nigeria, reported the following from result obtained on the proximate analysis and pH after investigating the effects of the use of starter-culture on the quality of fermented seeds. 'Iru' was prepared using fourteen (14) strains of *Bacillus subtilis* isolated from commercial samples of 'iru' as starter cultures. They observed that the pH values of all the samples increased from 7.01 to 7.58 while that of the unfermented sample was nearly neutral or basic. The liberation of ammonia during fermentation of protein foods is a phenomenon observed by Odunfa (1983), during fermentation of 'iru'. They observed an increase in the moisture contents of the fermented samples over the unfermented samples which they attributed might have been due to the addition of water during soaking, boiling and dehulling. Their result conformed to that of Omafuvbe *et al*. (2004) who carried out similar research on African locust bean. They deduced that increase in protein content of starter culture-fermented sample might be due to the structural proteins that are integral part of the microbial cells (Teng, 2004). The apparent increase in growth and microbial proliferation of microorganisms in form of single cell protein of the starter culture and normal flora may account for the observed trend in the crude protein (Holzapfel, 2002).

They also concluded that the observed decrease in carbohydrate content of the starter culture-fermented samples may be attributed to the leaching of the soluble carbohydrates like sugar into the cooking water and as a result of utilization of some of the sugars by fermenting organisms for growth and metabolic activities. Their result agrees with reports of Omafuvbe *et al*., 2000. There was a significant reduction in crude fiber of the starter culture-fermented products as a result of fermentation. They deduced that the reduction may be attributed to the fermenting micro flora to hydrolyze and metabolize them as carbon source (substrate) in order to synthesize cell biomass. They observed a decrease in ash content of the sample during fermentation. They accredited the ash loss to the microorganisms which use these salts during fermentation for their metabolic processes. They accredited increase in fat concentration as a result of fermentation. They attributed this to the boiling of the sample which might have led

to the cleavage of the protein – lipid as carbohydrate – lipid linkages thereby, facilitating the easy extraction of the oil by the extracting solvent (Madigan and Martinko, 2005).

Eka (1980) studied the effect of fermentation on the nutrient content of locust bean, and reported that protein and fat increased when fermented, whereas the quantity of carbohydrates decreased. Increased levels of the amino acids were also reported except for arginine, leucine and phenylalanine. Similar results were reported for other seed legumes (Sarkar *et al.*, 1995). Achi (2005), reported that food condiments made from vegetable proteins may be a good source of certain B vitamins, but are deficient in ascorbate and some fat-soluble vitamins, which are lost during fermentation. It is evident that fermented food condiments are good sources of nutrients and could be used to produce complementary food supplements. Macronutrients in fermented legumes contribute to enhanced food quality (Achi, 2005).

Plate 1: A - The tree *Parkia biglobosa*, B - The tree with pod, C – The pod with the fruit, D – Fermented processed molded seeds, E – Fermented dried seeds. (Ekum 2013).

2.8 Fermentation

Fermentation is one of the oldest methods of food preservation known to man (Omafuvbe *et al*., 2004) and the oldest preservation technology in the world (Mike and Sue, 1998). This has been known and practiced by the human race since prehistoric times, long before the scientific underlying principles were understood. Fermentation of food (natural or controlled) is a process that has been in existence for a longtime and was traditionally carried out by our ancestors. The transformation of some food material into fermented food was a mystery and a miracle in the early society, for they had no idea what caused the usually sudden, dramatic and welcomed transformation. Some tribes attributed it to divine intervention by their gods while the Egyptians attributed it to '*Osiris*' for the brewing of beer, 'Bacchus' the common wine was seen as the god of wine. Likewise the Japanese miso and shoyu breweries, a small shrine was built at the central place in the town where they bow daily. The first fermentation was probably discovered accidentally when salt was added to food and selected certain microorganisms that allowed the fermentation of the particular food and at the end the product was nutritious and acceptable by people. The process was taken further by the early Chinese who lived then, by inoculating some certain foods with molds, which created enzymes; these aided salt tolerant yeast to act on the food material and ferment them e.g. soy foods such as miso, soy sauce, soy nuggets and fermented tofu (Wiliam and Akiko, 2004).

Fermentation comes from the Latin word *fermentare*, meaning "to leaven." To make bread rise, you use a leavening agent to "wake up" dry yeast by mixing it with water. The yeast then starts "eating" the sugar in the dough and off-gassing alcohol: that's fermentation. (Vocabulary.com).

2.8.1 The Process of Fermentation

Micro-organisms are capable of yielding a large amount of fermentation through different types of fermentation. However, despite those numerous methods, there are still some unifying

concepts that are true of all fermentations. The first step in fermentation is glycolysis, where glucose molecules are converted into pyruvate.

$C_6H_{12}O_6 + 2\ NAD^+ + 2\ ADP + 2\ Pi \rightarrow 2\ CH_3COCOO^- + 2\ NADH + 2\ ATP + 2\ H_2O + 2H^+$

The two ADP molecules and two phosphate molecules are then converted to two adenosine triphosphate (ATP) and two water molecules through substrate-level phosphorylation. Two molecules of nicotinamide adenine dinucleotide (NAD^+) are also reduced to NADH.

Fermentation is one of the classic methods to preserve food. Lactic acid bacteria (LAB) and yeasts are responsible for most of the fermentation (Klein, 2006). Human beings are known to have made fermented foods since Neolithic times (William and Akiko, 2004). and has been documented dating from 7000–6600 BCE in Jiahu, China (McGovern *et al*., 2004), 6000 BCE in Georgia (Vouillamoz *et al*., 2006), 3150 BCE in ancient Egypt (Cavalieri *et al*., 2003), 3000 BCE in Babylon (FAO, 2007), 2000 BCE in pre-Hispanic Mexico (FAO, 2007) and 1500 BC in Sudan (Dirar, 1993). It was reported by Seven-thousand-year-old jars containing the remains of wine, now on display at the University of Pennsylvania, were excavated in the Zagros Mountains in Iran. There is strong evidence that people were fermenting beverages in Babylon circa 3000 BC (FAO, 2007), ancient Egypt circa 3150 BC (Cavalieri *et al*., 2003), pre-Hispanic Mexico circa 2000 BC (FAO, 2007) and Sudan circa 1500 BC (Dirar, 1993).

A French chemist called Louis Pasteur was the first known zymologist, when in 1856 he connected yeast to fermentation. Pasteur originally defined fermentation as "respiration without air". Pasteur performed careful research and concluded with this quote:

“I am of the opinion that alcoholic fermentation never occurs without simultaneous organization, development, and multiplication of cells. If asked, in what consists the chemical act whereby the sugar is decomposed? I am completely ignorant of it”.

Louis Pasteur (1822–1895), during the 1850s and 1860s, showed that fermentation is initiated by living organisms in a series of investigation. In 1857, Pasteur showed that lactic acid fermentation is caused by living organisms (Lois, 2009). In 1860, he demonstrated that bacteria cause souring in milk, a process formerly thought to be merely a chemical change, and his work in identifying the role of microorganisms in food spoilage led to the process of pasteurization (Lois, 2009). In 1877, working to improve the French brewing industry, Pasteur published his famous paper on fermentation, "*Etudes sur la Bière*", which was translated into English in 1879 as "Studies on fermentation"(Loiu, 2005). He defined fermentation (incorrectly) as "Life without air" (Faulkner and Bobb., 1879) but correctly showed that specific types of microorganisms cause specific types of fermentations and specific end-products. Fermented foods have a religious significance in Judaism and Christianity. The Baltic god Rugutis was worshiped as the agent of fermentation. Beers, wine and the leavened bread were the first to make, which were done by yeast and cheese by bacteria and molds. The Eastern Asian followed with the production of fermented foods like yogurt and fermented milk products, pickles vinegar, butter and lots of traditional alcoholic drinks.

With the help of developing technology, mold has been used industrially to make vitamins like B-2 which is known as Riboflavin and B-12. Some antibiotics are also been produced by fermentation e.g. penicillin. Modern industrial fermentation has also brought about the use of bacteria to make amino acids lysine and glutamic acid. Fermentation in food processing typically is the conversion of carbohydrates to alcohols and carbon dioxide or organic acids using yeasts, bacteria, or a combination thereof, under anaerobic conditions. Fermentation in simple terms is the chemical conversion of sugars into ethanol. The science of fermentation is also known as zymology or zymurgy (Tanuja, 2006).

Fermentation is defined by vocabulary.com as follows:

(i) A process in which an agent causes an organic substance to break down or split into simpler substances; especially, the anaerobic breakdown of sugar into alcohol.

(ii) A state of agitation or turbulent change of development.

(iii) The process by which substance breaks down into a simpler substance usually with the help of microorganisms like yeast and bacteria which plays a vital role in the fermentation process like the beer, wine, bread, kimchi, yogurt and other foods fermented making processes.

(iv) The chemical breakdown of a substance by bacteria, yeasts, or other microorganisms, typically involves effervescence and the giving off of heat.

Fermentation was also defined by Akiko and William (2004) as the chemical transformation of organic substances into smaller compounds by the action of enzymes, complex organic catalysts, which are produced by microorganisms such as molds, yeasts, or bacteria. Enzymes works by hydrolysis which is a process of chemical decomposition in which a compound is split into others by reacting with water from a smaller compound. Protein molecule is being broken down by enzyme protease into polypeptides and peptides, and then into numerous amino acids, which are easily digested by the body. Carbohydrates by enzyme amylase which reduces starches and complex sugars to simple sugars. Fat molecules are hydrolyzed by the enzyme lipase into simpler free fatty acids. These three are the most important enzymes associated with human digestion. In a broader sense, it is a process in which microorganisms produce chemical changes in organic substrates through the action of enzymes produced by these organisms (Dirar, 1993).

Fermentation could be:

(i) Bottom fermentation is a slow kind of alcoholic fermentation at a temperature low enough that the yeast cells can sink to the bottom of the fermenting liquid; used in the production of lager.

(ii) Top fermentation which is a violent kind of alcoholic fermentation at a temperature high enough to carry the yeast cells to the top of the fermenting liquid; used in the production of ale

(iii) Vinification the process whereby fermentation changes grape juice into wine.

Fermentation is also defined by Walker *et al*., 2004 as the "slow decomposition process of organic substances induced by micro-organisms, or by complex nitrogenous substances (enzymes) of plant or animal origin". It can be described as a biochemical change, which is brought about by the anaerobic or partially anaerobic oxidation of carbohydrates by either micro-organisms or enzymes. This is distinct from putrefaction, which is the degradation of protein materials, (FAO, 2003).

2.9 Limiting Factors for Fermentation Processes

Fermentation is affected by various factors, such as substrate constituent, temperature, dissolved oxygen, pH, dissolved carbon dioxide, functional system (continuous or batch), the shear rates and mixing of the fermenter. Variations in these components may affect: the rate of fermentation, product yield, finished product organoleptic properties such as the odour, appearance, flavour and texture), generation of toxins, nutritious quality and other physicochemical properties.

2.10 Importance of Fermentation

Fermented foods often have a large indefinite advantages over the raw materials from which they are produced (i.e. the end product which are made digestible by fermentation), (William and Akiko, 2004). The conventional fermentation of foods serves several functions which incorporates;

(i) The synthesizes of vitamins like B-12, which is difficult to get in vegetarian diets, William and Akiko, 2004

(ii) Eliminate or conceal undesirable beany flavors

(iii) Fermentation reduces or takes off carbohydrates which are believed to cause flatulence in unfermented food.

(iv) Preservation of substantial amounts of food through lactic acid, alcohol, acetic acid and alkaline fermentations. The lowering of the pH to below 4 through acid production inhibits the growth of pathogenic organisms which cause food spoilage, food poisoning and disease and by doing this, the shelf life of fermented food is prolonged (Abdel and Darkar, 2009; Olukoya *et al*., 2011). It makes food safe for consumers in terms of stability, transportation and storage (Chelule *et al.,* 2010).

(v) Biological enrichment of food substrates with protein, essential amino acids, essential fatty acids, and vitamins.

(vi) Elimination of anti-nutrients (Tanuja, 2006). Many unfermented foods contain naturally occuring toxins and anti-nutritional compounds. These can be removed or detoxified by the action of micro- organism during fermentation for instance, the fermentation process that produces the Sudanese product, kawal, removes the toxins from the leaves of Cassia obtusitfolia. Fermentation also removes cyanide from Cassava which contains naturally occurring chemicals, cyanogenic glycoside. When

eaten raw or improperly processed, this substances releases cyanide into the body, which can be fatal. Correct processing removes these chemicals. Anti-nutrient factors (ANFs) such as phytic acid and tannins in food decline during fermentation leading to increased bioavailability of minerals such as calcium, phosphorus, zinc, iron, amino acids and simple sugars (Soetan and Oyewole., 2009; Murwan and Ali, 2011).

(vii) A decrease in cooking times and fuel requirements.

(viii) Transform agricultural wastes e.g. okara into tasty and nutritious human foods like okara tempeh (William and Akiko, 2004).

(ix) Renews intestinal micro flora (like miso or acidophilus soymilk) (William and Akiko, 2004).

(x) Enrichment of the diet through development of a diversity of flavors, aromas, and textures in food substrates. These organoleptic properties make fermented food more popular than the unfermented one in terms of consumer acceptance (Osungbaro, 2009).

(xi) Fermentation makes foods more edible by changing chemical compounds, or predigesting, the foods for human to consume.

(xii) It reduces the amount of supplements the body needs because its production is budget friendly – Incorporating fermented foods into the diet is very economical. Different fermented foods can be made at home.

(xiii) Detoxification during food fermentation: Food and feeds are often contaminated with a number of toxins like fumonisins, ocratoxin A, zearalenone and aflatoxins, (mycotoxins) either naturally or through infestation by microorganisms such as molds, yeast, bacteria and viruses (Ari *et al*., 2012). Using Lactic acid bacteria in fermentation detoxified toxins and is more advantageous, in that it is a milder method which preserves the nutritive value and flavor of foods (Chelule *et al*., 2010). In addition to

this, fermentation irreversibly degrades mycotoxins without adversely affecting the nutritional value of the food (Ari *et al*., 2012) and without leaving any toxic residues.

(xiv) As a probiotics: Eating fermented foods and drinking fermented drinks will introduce beneficial bacteria into your digestive system and help the balance of bacteria in your digestive system. Probiotics have also been shown to help slow or reverse some diseases, improve bowel health, aid digestion, and improve immunity.

2.11 Food Condiments or Spices

The term condiment comes from the Latin word *condimentum*, meaning "spice, seasoning and sauce" are from the Latin *condere*, meaning "preserve, pickle, season" (Nealon, 2014).

Food condiments or spices are strong smelling, sharp tasting substances usually used to improve or enhance the flavour of food (Odebunmi *et al.,* 2009). Other researchers defined spice as dried seeds, fruits, root, bark, leaves or vegetative substances used in small quantities as food additives for the purposes of flavor, colour, or as preservatives. They are usually of vegetable source, e.g. mustard, ginger, garlic, coriander, locust bean etc. FAO, (2004) reported that spices in food minimizes the rate of rancidity, improve colour and flavour intensity of food and food products. Many condiments are either packed in packets or containers e.g. ketchup particularly when served with take-out food meals. Others are used during cooking to add flavor or texture to the food e.g. Iru, barbecue sauce etc.

Condiments were known in some parts of the world like Ancient Rome, Ancient China, Ancient Greece and Ancient India to improve the taste of spoiling food; before food preservation methods discovery, pungent strong smelling spices and condiments were used to make the food more acceptable to the mind or feelings.

2.12 Fermented Foods

Fermented foods are foods produced or preserved by the action of microorganisms.

Fermentation is a process in which food is exposed to bacteria and yeasts, either via inoculation or naturally through contact with air on containing vessels. The results are interesting flavors, textures, and smells. Before refrigeration, curing (meats), pickling (vegetables), and clabbering (milk) were the only way to extend the life of perishables.

Campbell-Platt, (1980) defined fermented foods as those foods which have been subjected to the action of micro-organisms or enzymes so that desirable biochemical changes cause significant modification to the food. However, to the microbiologist, the term 'fermentation' describes a form of energy-yielding microbial metabolism in which an organic substrate, usually a carbohydrate, is incompletely oxidized, and an organic carbohydrate acts as the electron acceptor (Adama and Jimoh, 2011).

Anthropologists made it known that it was the production of alcohol that encouraged primitive people to settle down and become agriculturists. There is reliable information that fermented drinks were being produced over 7,000 years ago in Babylon (now Iraq), 5,000 years ago in Egypt, 4,000 years ago in Mexico and 3,500 years ago in Sudan (Battock and Azam-Ali, 1998). The farmers consumed fresh fruits but at times of scarcity ate rotten and fermented fruits. Repeated consumption led to the development of the taste for fermented fruits.

Mercola reported on his site (www.mercola.com) that Fermented foods helps to reduce high cholesterol levels in the human blood, strengthens and supports digestive and immune systems, thereby helping the body to fight off and prevent diseases, like cancer. Dr. Marcola concluded through his researching this topic that fermentation is an inconsistent process, and some consider it more of an art than a science.

Fermentation is a widespread custom in Asia (Battock and Azam-Ali, 1998). Fermented foods give the body the required nutritional values like protein, minerals and other nutrients that add variety and nutritional fortification to otherwise starchy, bland diets. Soy sauce is an essential ingredient in diets from Indonesia to Japan and throughout the whole world. In Africa fermented cassava like *fufu, elubo and gari* are major component of the diet of more than 800 million people and in some areas these products constitute over 50 % of the diet (Battock and Azam-Ali, 1998). Living organisms cover the surface of the earth, microbes which are responsible for fermentation covers the surface of the organisms. Wild yeasts are found living on grapes (Chamberlain *et al*. 1997), and bacteria line the human digestive tract (Elemo *et al*., 2011).

2.13 Development of Fermented Foods in Nigeria

Nigeria is gifted with an extensive variety of fermentable indigenous staple foods that serve as crude materials for agro-allied cottage industries. These industries use small-scale equipment while adding value to such local produce (Latunde-Dada, 2000). Although fermented food condiments have constituted a significant proportion of the diet of many people, Nigerians have exhibited an ambivalent attitude in terms of consumers' tastes and preferences for such foods. Fermentation contributes significantly to food technological processes in developing countries such as Nigeria. Fermented foods add a variety of flavors, and enhances the nutritional value of processed food with an increase shelf life.

The traditionally fermented foods in Nigeria are sub-divided into:

(i) Roots and tubers (garri, lafun and fufu)

(ii) Cereals (ogi)

(iii) Legumes (dawadawa and iru)

(iv) Milk (local cheeses)

(v) Beverages (palm wine and pito)

Simple and non-sterile materials are used, and there is great reliance on the use of natural inoculation under controlled fermentation conditions. Other contamination, varied sensory characteristics, unattractive packaging and presentation, and short shelf life of the products (Klein, 2006).The industrial development of locally fermented foods can be divided into the following areas;

Table 2.3 Fermented foods of Nigeria and Microorganisms Associated with them

Product	**Substrate**	**Microorganism involved**	**Nature of food**
Iru/Dawadawa	African locust beans (*Parkia biglobosa*)	*Bacillus licheniformis* *Bacillus subtilis* *Lactobacillus* sp. *Rhizopus stolonifer* *Streptococcus* sp. *Aspergillus fumigatus* *Pediococcus* sp. *Triscelophorus monosporus* *Coryneform bacteria*	Condiment
Ogiri/Egusi	Melon seed (*Citrulus vulgaris*)	*Bacillus subtilis* *Escherichia spp.* *Pediococcus spp*. *B. megateruim,* *B. firmus* *Proteus*	Condiment
Fufu	Cassava	*Leucouostoc spp.* *Lactobacillus spp.* *Corynebacterium spp.* *Candida tropicalis* *Stretococcus* spp.	Staple food
Okpehe	Mesquite	*Bacillus subtilis* *B.lichemformis* *B.pumilus*	Condiment

Garri	Cassava	*Leuconostoc sp* *Geotrichum candidum* *Pseudomonas sp.* *Scolecotrichum graminis* *Bacteriodes sp.* *Tallospora aspera* *Actinomyces sp.* *Passalora bacilligera* *Corynebacterium sp.*	Staple food
Elubo-isu	Yam	*Streptococcus sp.* *Articulospora inflate* *Lactobacillus sp.* *Aspergillus niger* *Listeria sp.* *Aspergillus rapen*	Staple food
Ogiri-igbo	Castor oil seeds (*Ricinus communis*)	*Bacillus* sp. *Pseudomonas* sp. *Micrococcuss* sp.	Condiment
Ogiri ugu	Fluted pumpkin seeds (*Telferia ocidentalis*)	*Bacillus* sp. *E. coli,* *Staphylococcus* sp. *Pseudomonas*	Condiment

Source: Omafuvbe, 2004.

2.14 Microorganism Involved in Fermented Food Production

Microorganisms are living creatures that are microscopic in size and are heterogeneous organisms that can be in form of plant or animal such as algae, fungi, (mold and yeasts) and bacteria. The multiplication of microorganisms in food is greatly influenced by the inherent (intrinsic factors) and environmental characteristics of the food (Onyenekwe *et al*., 2012). The yeasts are mainly of the species *Saccharomyces, Candida, Kiluyromyces* and *Debaryomyces* (Chelule *et al.,* 2010). Molds have been used mainly in milk and cheese fermentation and these include penicllium, mucor, Geotrichium, and Rhizopus species (William and Akiko, 2004). Most pathogenic microorganisms found in food cannot survive the low pH, hence, lactic acid fermentation of food has been found to reduce the risk of having pathogenic microorganism's growth in the food (Abdel and Dardir, 2009). Alkaline fermentations cause the hydrolysis of

protein to amino acids and peptide and releasing ammonia, which increases the alkalinity by the *bacillus* species such as *Bacillus subtillis* (dominant species), *B.licheniformis* and *B.pumilius* (Emujiugha, 2005). This indigenous natural fermentation takes place in a mixed colony of microorganisms such as molds, bacteria and yeasts (William and Akiko, 2004). These bacteria are not harmful to the consumers and have enzymes such as proteases, amylase and lipases that hydrolyze food complexes into simple nontoxic products with desirable textures, aroma that makes them palatable for consumption (Nwachukwu *et al*., 2010).

2.14.1 *Bacillus Subtilis*

Bacillus subtilis, is also known as hay *bacillus* or *grass bacillus*. This is a Gram-positive, catalase-positive bacterium. *B. subtilis* is rod-shaped, and has the ability to form a tough, protective endospore, allowing the organism to tolerate extreme environmental conditions. Bacteria are a group of single-celled or multi-cellular organisms, which are often parasitic in nature, without distinct nuclei or organized cell structures. Although this species is commonly found in soil, more evidence suggests that *B. subtilis* is a normal gut commensal in humans. The number of spores found in the human gut is too high to be attributed solely to consumption through food contamination. *B. subtilis* is only known to cause disease in severely immunocompromised patients, and can conversely be used as a probiotic in healthy individuals. It rarely causes food poisoning. *Subtilis* spores can survive the extreme heat during cooking. Some *B. subtilis* strains are responsible for causing ropiness — a sticky, stringy consistency caused by bacterial production of long-chain polysaccharides. For a long time, bread ropiness was associated uniquely with *B. subtilis* species by biochemical tests. Non-toxigenic and non-pathogenic strains of *B. subtilis* are widely available and have been safely used in a variety of food applications, including the documented consumption of *B. subtilis* in the Japanese fermented soy bean, natto, which is commonly consumed in Japan.

Various bacteria species are responsible for decay, fermentation, nitrogen fixation, and many plant and animal diseases. There are three main groups of bacteria namely LAB (lactic acid bacteria), Acetic acid bacteria, and bacteria of alkaline fermentations; which includes *Bacillus* specie. *Bacillus* which belongs to the family *Bacillaceae*, are rod-shaped, gram positive, aerobic or facultative anaerobic spore-forming bacteria. When young, *Bacillus* cultures are gram-positive but as they age, they become gram-negative. There are many different species of *Bacillus*. The largest species are about $2x10^{-6}$ m across by $7x10^{-6}$ m long, and frequently occur in chains, (Omafuvbe *et al*., 2000).

Generally, aerobic spore-formers are chemoheterotrophic in nature, capable of respiration using a variety of simple organic compounds (sugars, amino acids, organic acids). A few species, such as *Bacillus megaterium*, require no organic growth factors; others may require amino acids, B-vitamins, or both. A large number of *Bacillus* species are mesophiles; with optimal temperature between 30 and 45 ^{0}C, while some are thermopiles with optimal temperature as high as 65 ^{0}C. Others are psychrophilic in nature, able to grow and sporulate at 0 ^{0}C. *Bacillus* species are found to grow in environment with pH range between 2 and 11. In the laboratory, under optimal conditions of growth, *Bacillus* species exhibit generation times of about 25 minutes.

The most dominant specie is *Bacillus subtilis*, which leads to the hydrolyzation of protein to peptides and amino while releasing ammonia. This leads to an increase in the alkalinity of substrate and the growth of spoilage organisms. Ferdinand Cohn a German botanist in 1877 described two forms of *B. subtilis*; namely the heat-resistant spores called Endospores, and the other that could be killed upon heat exposure. These dormant forms can be converted to a vegetative or actively growing state. Most *bacillus* species form dormant spores under adverse environmental conditions.

Some types of *Bacillus* species are harmful to humans, plants, or other organisms. For example, *Bacillus cereus* sometimes causes spoilage in canned foods and food poisoning. *B. subtilis* is a common contaminant of laboratory cultures and is often found on human skin. Most *Bacillus stains* are not pathogenic for humans but as soil organisms, may infect humans. Although, *Bacillus anthracis,* has been discovered to cause anthrax in humans and domestic animals. A toxin called BT toxin produced by *Bacillus thuringiensis* has been discovered to cause disease in insects.

B. subtilis and *B. polymyxa* produce useful antibiotics. Also, strains of *B. amyloliquefaciens*, are known to produce various antibiotic substances, including bacillaene, macrolactin, and difficidin in certain plants. These substances serve to protect the host plant from infection by fungi or other bacteria and are being studied for their usefulness as biological pest-control agents. *B. amyloliquefaciens* which contains a gene that encodes an enzyme called barnase, is used in the development of genetically modified plants. This enzyme kills fungal infected plant cells thereby reducing the spread of the disease (Sobande, 2013).

2.14.2 *Saccharomyces cerevisiae*

Saccharomyces cerevisiae is a species of yeast. It is the most valued yeast due to its numerous applications like in baking, brewing and wine production, since time immemorial. *Saccharomyces cerevisiae* is a eukaryotic microbe. More specifically, it is globular-shaped, yellow-green yeast belonging to the Fungi kingdom, which includes multicellular organisms such as mushrooms and molds. It can be found in palm wine, fresh fruits and vegetables especially those with fermentable sugars. Baker's yeast which has a lot of uses in the food industry is yeast in its deactivated form (Tamang *et al*., 2005).

S. cerevisiae is not considered a pathogenic microorganism, but has been reported rarely as a cause of opportunistic infections. A low concern for the pathogenicity *of S. cerevisiae* is also

illustrated by a series of surveys conducted at hospitals over the last several years. *S. cerevisiae* accounted for less than 1 % of all yeast infecti*ons isolated at a cancer hospital and in most of the cases the organism was isolated from the respiratory system (Klein *et al*., 2006). At Yale New Haven Hospital over the past five years, there have been 50 isolates of *S. cerevisiae* recovered from patients; however, most of the isolates were considered contaminants.

2.15 Limitations of Traditionally Fermented Foods

1. Influence of western diet and fast foods.
2. Lack of durability (shelf life)
3. Unattractive presentation
4. Socio-economics of the consumer base:

   Consumer base of traditionally fermented foods in most developing countries is very poor. Price, rather than food safety or nutritional security and quality that make a substantial contribution to their food, is therefore a major preoccupation of this group when purchasing a food (FAO, 2013).
5. Inadequte raw materials grading and cleaning, contributing to the presence of foreign matter such as insects and stones in the final product.
6. Crude handling of traditionally fermented foods: Inadequate raw material grading and cleaning contributing to the presence of foreign matter (such as insects, stones) in final product, crude handling and processing techniques employed, lack of durability (shelf life), lack of homogeneity and unattractive presentation.
7. Health defects:

   A research found that fermented food contains a carcinogenic by-product, ethyl carbamate (urethane). Another research carried out discovered that fermenting under

anaerobic conditions in air-tight plastic containers is dangerous because bolitinum bacteria which causes bolutilism a paralytic illness thrive under those conditions.

2.16 Significance of Locally Fermented Foods to National Economy of Nigeria

Locally fermented Foods have a role to play in developing economies like Nigeria (Anukam and Reid, 2009). Some of the significance of fermented foods are:

1. **Provision of employment opportunities**: By generating employment opportunities in the rural areas, small scale food industries reduce rural-urban migration and the associated social problems (Aworh, 2008).
2. **Reduction in mortality rate:** It prevents the enlargement of pancreas. Eating an enzyme-rich diet decreases the load on pancreas, preserving the body's own natural enzyme potential, thereby reducing the risk of chronic diseases.
3. **Industrialization**: Likewise the locally fermented foods play a unique role in promoting industrial development in Nigeria through employment generation, value-added processing and training of skilled manpower. Their impact is felt greatly in the urban areas (Aworh, 2008). Small-scale food industries that involve lower capital investment and that rely on traditional food processing technologies are crucial to rural development in Nigeria (Aworh, 2008).
4. **Poverty alleviation**: It provides a source of income and means of poverty alleviation contributing to food security of Nigeria (FAO, 1997), e.g. kunun is in many homes in the rural communities and more recently in the urban areas where commercial production due to support from the government through the poverty alleviation scheme, has helped to alleviate poverty among the people (Essien *et al*., 2011).
5. **Food supplement**: The locally fermented foods serve as food supplement like the use of ogi as a weaning food in Southern Nigeria to supplement breastfeeding (Fetuga *al*.,

1973). Traditional fermented protein-rich foods offer excellent opportunities for improving the diets of people in tropical countries providing rich source of starch, vitamins, proteins and minerals (Oladejo and Adetunji, 2012).

2.17 Factors Affecting Fermentation Processes

Fermentation process is controlled by various factors, which comprises of substrate constituent and nature, temperature, dissolved oxygen, pH, dissolved carbon dioxide, functional system (continuous or batch), fermenter shear rates and mixing. Differences in these components may affect: fermentation rate; product range and yield; products organoleptic properties (odour, appearance, flavour and texture); toxins generation; nutritious quality and other physicochemical properties. Fermentation medium preparation influences the yield, rate and product profile. The medium must make available the essential measures of carbon, nitrogen, trace elements and micronutrients.

2.18 Fermentation of *Parkia biglobosa*

Fermented *Parkia biglobosa* seeds is an essential food condiment used in Nigeria as well as numerous nations of Central and West Africa. Iru is used the same way bouillon cubes are utilized in Western nations as nutritious seasonings alongside cereal sauce. It also appreciably contributes to the intake of energy, vitamins particularly riboflavin, and protein in numerous Central and West Africa nations. The production of condiments is largely done on a traditional small-scale household basis under highly variable conditions (Odunfa, 1985).

Fermentation process of *Parkia biglobosa* seeds is exothermic in nature. It is usually carried out in a moist solid state involving contact with appropriate inocula of assorted microorganisms aided by the temperatures of the tropics. The desired state of the 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 process (Omafuvbe *et al.* 2000). The characteristic ammoniacal odour and flavour of condiments enhance the taste of food in which they are used.

Microbial fermentation of *Iru* has been found to involve only bacteria since fungal influence have been regarded as incidental and does not play any notable role in the fermentation process (Ikenebomeh *et al*., 1986). Most bacteria implicated are facultative anaerobes, while approximately 10 % are aerobic. The household or small-scale industry preparation of '*Iru'* involves the use of non-sterile materials and simple practices. This fermentation is spontaneous and depends on natural inocula, which leads to shorter shelf-life and discrepancies in the quality of products (Latunde-Dada, 2000). Nevertheless, in modern industrial processing, the techniques are carefully controlled and monitored with direct inoculation with isolated and purified microbes. For this situation, longer shelf-life is achieved for the product.

During fermentation, the microbes utilize the seeds nutritional compositions, transforming them to products which add to the condiment's chemical constitute as well as flavour. The research of Antai & Ibrahim (1986) and Odunfa (1985) discovered a few microorganisms associated with *Iru* and stated that *Bacillus* specie was the major agent of fermentation after 72 hours of which the spore-forming species *Bacillus subtilis* and *Bacillus lichenformis* were detected to be the key bacteria present. *Leuconostoc mesenteroides and* two varieties of *Staphylococcus sp*. were also detected. Campbell-Platt (1980) also mentioned lactic acid bacteria especially species of *Lactobacillus* and *Pediococcus* as partakers in the fermentation of *Iru*. Proteolytic and salt-tolerant microorganisms have been detected in appreciable numbers (from 10^4-10^6 cfu/g) in samples of fermenting *Parkia* seeds after 36 hours (Odunfa, 1981).

The fermentation of *Parkia biglobosa* by *Bacillus* spp. to produce *Iru* via alkaline fermentation has been discussed by several workers in West Africa (Odunfa, 1986). Isolated *Bacillus* spp.

from different sources was stated to be proteolytic and capable of breaking down oils (Ouoba *et al*., 2003; Forgarty & Griffin, 1973; Frazier, 1967). Odunfa (1985) and Campbell- Platt (1980) noted a high level of proteolytic activity during *Iru* fermentation, which culminated in the formation of peptides and amino acids. Owen *et al.,* 1997 study the flavor/aroma of *Iru,* fermented by *Bacillus* spp. and observed the presence of active aldehydes, ketones and acids. He also observed that active microbial metabolism is required in order to bring about the changes observed in locust beans during fermentation.Odunfa and Oyewole (1998) noted that during fermentation, the pH of the substrate increased from approximately neutral to about 8.0 while moisture increased from 43 to 56 %. The studies on mechanisms and principles of flavor production during the fermentation of *Iru* is being carried out by international food manufacturers. It has also been utilized as a foundation for the development of flavors for incorporation in bouillon cubes.

During fermentation process soluble amino acids, viz. glutamic acid is released. This amino acid salt, monosodium glutamate is commonly used as an additive to improve flavor (Odunfa, 1985). *Iru* is also an essential source of Vitamin B as riboflavin which is usually deficient in most African diet. *Iru* contains the highest riboflavin content when compared to 33 common plant foods i.e. about 0.80 mg per 100 g, (Campbell-platt, 1980). It is a good source of protein as it contains high amount of protein.

2.19 Risk Associated with Fermented Foods

Fermented foods do have their place and can be consumed in moderation by many people**,** who don’t experience too many health issues. But it is not advisable for anyone to depend on fermented foods on a daily basis.

Alaska has witnessed a steady increase of cases of botulism since 1985. It has more cases of botulism than any other state in the United States of America. This is caused by the

traditional Eskimo practice of allowing animal products such as whole fish, fish heads, walrus, sea lion, and whale flippers, beaver tails, seal oil, birds, etc., to ferment for an extended period of time before being consumed. The risk is exacerbated when a plastic container is used for this purpose instead of the old-fashioned, traditional method, a grass-lined hole, as the botulinum bacteria thrive in the anaerobic conditions created by the air-tight enclosure in plastic, (Nout, 1994).

The World Health Organization has classified pickled foods as a possible carcinogen, based on epidemiological studies. Other research is found that the fermented food contains a carcinogenic by-product, ethyl carbamate (urethane). "A 2009 review of the existing studies conducted across Asia concluded that regularly eaten pickled vegetables roughly doubles a person's risk for esophageal squamous cell carcinoma (WHO 2012).

Aldehydes in Fermented Foods: Aldehydes are a type of organic chemical compound that are produced by some fermenting organisms. The human body possesses enzymes that convert it to a less-harmful substance and therefore is protected from small exposures. However, acetaldehyde at toxic levels can make its way into the brain from sources such as alcohol consumption, *Candida* (yeast) overgrowth, as well as breathing air contaminated with acetaldehyde from cigarette and other smoke, smog, vehicle and factory exhaust, synthetic fragrances and many commercially manufactured materials (Nout, 1994). In foods, aldehydes are produced mainly by the action of yeasts, molds and fungi. Aldehydes are not lethal toxins but they definitely affect the body and damage one’s health. Often levels are high in fermented foods such as kombucha tea, some pickles, wine and beer.

Candida Overgrowth and Fermented Foods: Fermented foods are high in lactic acid. Lactic acid results from anaerobic metabolism, the same metabolic process which allows yeast to promulgate. Pathogenic yeast thrive in an anaerobic environment. Some of the most important

things people with candidiasis should eliminate are sugars and most foods that break down quickly into sugars and fermented foods.

2.20 Fermentation Pathways

Fermentation can be carried out either in the absence or in the presence of air. When air introduced into the fermentation vessel, the type of fermentation which occurs is called aerobic fermentation. In aerobic fermentation, free oxygen acts as the hydrogen acceptor. Another type of fermentation pathway occurs when little or no air is introduced into the fermentation vessel. This type of fermentation is called anaerobic.

{A} Aerobic Fermentation

Aerobic fermentation uses air containing oxygen as the continuous phase. Since most known microorganisms live aerobically, aerobic solid state fermentation simulates the natural environment of the microorganisms. Most industrial bioreactors used for fermentation are of the aerobic type, having air continually passed into the vessel.

{B} Aerobic Fermentation Microorganisms

Aerobic fermentation is performed by organisms called aerobes which are able to grow and survive in the presence of oxygen. Aerobes may be classified into three different types:

(i) Obligate Aerobes

There is a strict requirement for oxygen for obligate aerobes. They are unable to survive or grow successfully without oxygen. The organisms use the oxygen to convert sugars and fats into energy. Examples of obligate aerobes include *Norcadia asteroids*, *Mycobacterium tuberculosis* and almost all types of algae**.**

(ii) Facultative Aerobes

Facultative anaerobes and facultative aerobes are basically the same thing. This types of anaerobes do not need a strictly anaerobic environment to survive, they are able to grow and reproduce under both aerobic and anaerobic environments. The energy used for growth is obtained by oxidative phosphorylation. Examples of facultative aerobes are Yeast, *Eschericchia coli, Staphylococcus spp* and *Streptococcus spp.*

2.21 Anaerobic Fermentation

Many industrial fermentation are carried out under aerobic conditions where a few processes such as ethanol production by yeast require strictly anaerobic environments. Anaerobic fermentation has the advantage of being both energy-saving and environmentally friendly. Anaerobic solid-state fermentation can be used to produce bio-based energy, chemicals, traditional food and agricultural feedstock.

Anaerobes are organisms that do not require oxygen for growth. Most anaerobes are bacteria while a few are actinomycetes, mycoplasma or fungi. With recent developments in anaerobic culture techniques, some new aerobes are continually being found.

Anaerobic microorganism, or anaerobes, may be divided into two types: facultative anaerobes and obligate anaerobes.

{A} Facultative Anaerobes

This type of anaerobes do not need a strictly anaerobic environment to survive. They are able to grow and reproduce under both aerobic and anaerobic environments. The energy used for growth is obtained by oxidative phosphorylation. Yeast and Escherichia coli are types of facultative anaerobes which are used in the production of alcohol and in biological engineering research respectively. Facultative anaerobes in the growth phase will reproduce faster in the

presence of oxygen while after the growth phase, oxygen is no longer needed and may even be detrimental to the fermentation process. An example of such is in the production of alcohol using yeast, where only a small amount of air is needed for the cells to multiply and thereafter no air is needed and presence of air will lead to the oxidation of the substrate. Therefore, control of dissolved oxygen is in two parts: initially supplying high amounts of oxygen for cell growth, thereafter controlling the dissolved oxygen content during the actual anaerobic fermentation.

{B} Obligate Anaerobes

Unlike facultative anaerobes, obligate anaerobes can only survive in a purely anaerobic environment. Due to physiological characteristics such as the lack of intracellular respiratory enzyme system, superoxide dismutase, catalase, and cytochrome oxidase, obligate anaerobes show much higher sensitivity to the presence of oxygen. Therefore, they can only survive in anaerobic conditions with low oxidation reduction potential.

{C} Aerotolerant Anaerobes

Aerotolerant anaerobes do not utilize oxygen for either growth or survival, but are also not harmed by the presence of oxygen. The reason why they are not harmed by oxygen is because of the presence of oxygen tolerance enzymes such as catalase and superoxide dismutase. Examples of aero tolerant anaerobes are Clostridium intestinale.

2.22 Comparism between Aerobic and Anaerobic Fermentation

The main difference between aerobic and anaerobic fermentation is that the electron acceptor in aerobic growth of microbes is oxygen, whereas it is the organic substrate itself in anaerobic growth. Thus, anaerobic fermentations will produce a higher degree of reduction of the primary metabolites, which is useful in the production of alternate biofuels, but will also produce a

lower product yield than aerobic fermentations. In addition, anaerobic fermentations produce food with a richer flavor than aerobic fermentation. This makes it the unrivaled methodology in some food fermentation. To combine profitability with production of well-flavored fermented foods, a mixture of both aerobic and anaerobic fermentation is often carried out.

Table 2.5 Comparison between Aerobic and Anaerobic Fermentation

	Aerobic fermentation	Anaerobic fermentation
Fermentation conditions	Area is well-ventilated with oxygen; temperature and humidity of supply gas is strictly controlled	Without ventilation, but requires larger doses of inoculation.
Fermentation characteristics	The rate of microbial growth is fast while fermentation period may be relativity short	The rate of microbial growth is quite long, with longer periods of fermentation that may lead to significantly better taste of products.
Fermentation Microorganisms	Most aerobic bacteria	Anaerobes; either facultative or obligate.
Application	Production of antibiotics, bread, beer, cheese.	Production of liquor, biogas and fuel ethanol

Odunfa (1981)

2.23 *Bacillus* in Aerobic and Anaerobic Environments

Bacillus bacteria were previously thought to be strictly aerobic (obligate aerobes). However more recent studies have shown that *Bacillus* can actually grow and survive under anaerobic conditions, which make them facultative anaerobes. This was confirmed in this reaserch work. The bacteria can make ATP by fermentation on sugars, peptides and other substrates, under anaerobic conditions. Fermentation may also yield butanediol as a product. Nitrogen may be obtained from nitrate by ammonification (also called assimilatory denitrification).

For anaerobic respiration, *Bacillus subtilis* can use nitrite or nitrate as a terminal electron acceptor. *Bacillus subtilis* contains two unique nitrate reductases. One is used for nitrate nitrogen assimilation and the other is used for nitrate respiration. However, there is only one nitrite reductase that serves both purposes. Nitrate reductase reduces nitrate to nitrite in nitrate respiration, which is then reduced to ammonia by nitrite reductase. During fermentation, the regeneration of NAD+ is chiefly mediated by lactate dehydrogenase, which is found in the cytoplasm.

2.24 Proximate Analysis

This is a set of methods used in getting information about the nutritional value of food/feed. It is a chemical scheme of food analysis designed to measure only its basic six components namely, moisture content, crude protein, ether extract (fat), crude fibre, total ash and Nitrogen free extracts. This gives the nutritional value of the feed. This is done before and after fermentation so as to determine the changes, whether they increase, decrease or no significant change due to deliberate fermentation with the micro-organisms.

The moisture content is obtained by drying to constant weight at 105 ^{0}C, Crude protein by Kjeldahl's determination of nitrogen, crude fibre as the organic fraction remaining after acid and alkaline hydrolysis and crude fat as the fraction extracted with petroleum ether. These analyses are easy to conduct and are cheap. The demerit of these analyses is that the nutritional value of the carbohydrate fraction is not a good determination. In the past, the remaining fraction of feed was considered to be starch which showed to be untrue. Sometimes sugar and starch are also analyzed to get a better nutritional value of the feed.

Proximate analysis include six constituents:

(i) Ash

(ii) Moisture

(iii) Proteins

(iv) Fat

(v) Crude fibre

(vi) Carbohydrates

Analytically, four out of the six constituents are obtained via chemical reactions and experiments. Carbohydrates is carried out by differnce. Although proximate do not give the entire nutritional assay, they are an inexpensive way to track deviations from the quality of foods.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Statistical Design of Experiments {DOE}

Design of experiments (DOE) can be defined as the systematical method of determining the relationship between factors affecting a process and the output of that process. This investigates the effects of input variables (factors) on output variable (response) simultaneously. It is majorly used to find the cause-and-effect relationships, which is needed to manage process inputs in order to optimize the experimental outputs. In an experiment, one or more process factors or variables are deliberately changed in order to observe the effect the changes have on one or more response variables. The (statistical) design of experiments (DOE) is an efficient procedure for planning experiments so that the data obtained can be analyzed to yield valid and objective conclusions.

An experimental design is the laying out of a detailed experimental plan in advance of doing the experiment. Well chosen experimental designs reduce the amount of information that can be obtained for a given amount of experimental effort. The use of design of experiments is an innovative approach to optimization and screening of experimental parameters. Simple experimental design and statistical tools for data analysis can provide much information about the system under investigation after only a few experiments. Such information can be key in decision-making for further experiments and can enable the development of robust and reliable protocols for chemical synthesis, analytical methods or biological assays. Coupling of design of experiments with modern high-throughput automation systems has the potential to maximise the capabilities of these systems and give increased productivity for many drug discovery applications.

MINITAB 17 (PA USA) was used for the design of experiments, plotting of response surfaces and optimization of % protein composition in fermented African locust bean (*Parkia*

biglobosa) seeds. The method of Box-Behnken design of experiment was used with the response surface method (RSM) for the establishment of optimum conditions for the fermentation of *Parkia biglobosa* seeds. To obtain optimum conditions for the fermentation of African locust bean seeds, three operating factors with one response variable were considered.

Table 3.1 Box-Behnken factorial design for % Protein composition in *P. biglobosa* seeds.

Factors: 3 Replicates: 1

Base runs: 15 Total runs: 15

Base blocks: 1 Total blocks: 1

Factors	Variables	Minimum	Maximum
Fermentation duration (days)	X_1	1	5
Inoculum concentration (g broth/g seed)	X_2	10	40
Temperature (°C)	X_3	40	60

Three operating factors viz. X_1 (fermentation time), X_2 (inoculum concentration) and X_3 (temperature) were taken into consideration, to yield 15 runs. This design was based on the experimental results obtained.

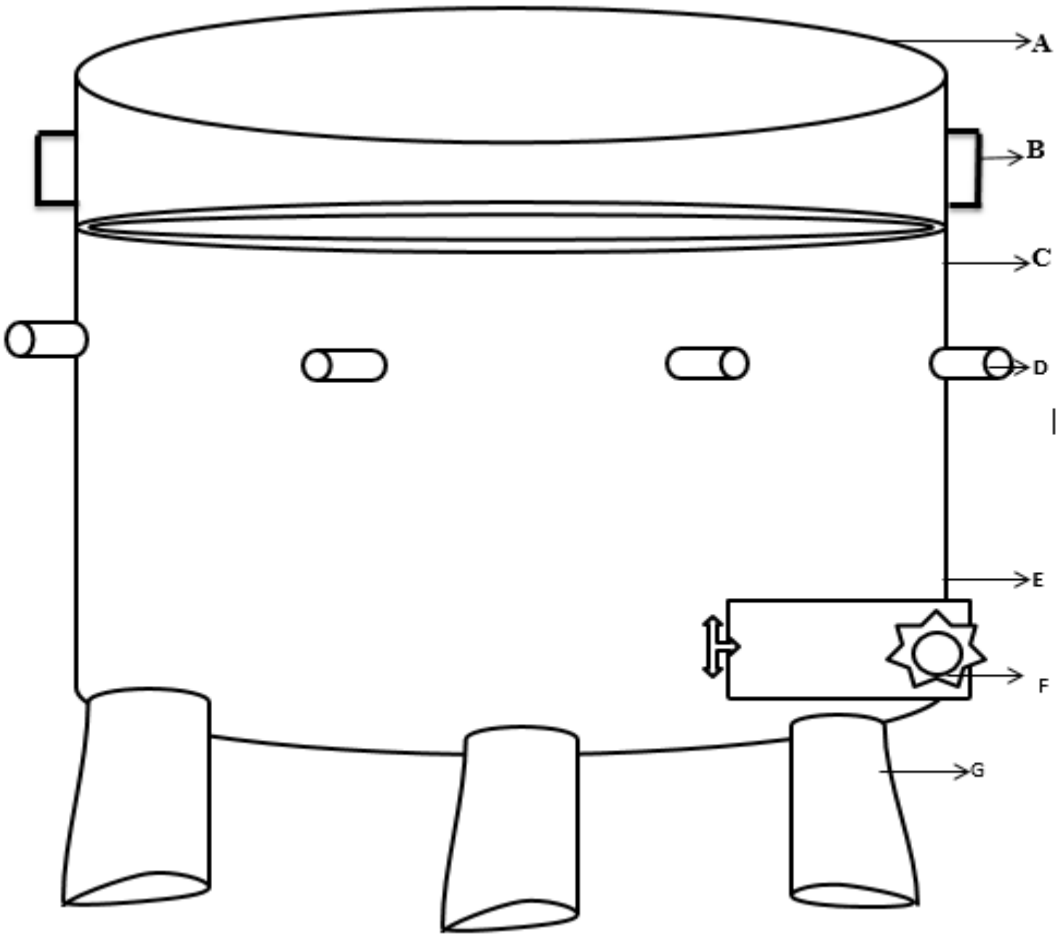


Fig. 3.1 Schematic Diagram of the Fabricated Fermenter

3.2 Legends on the schematic representation diagram:

A = Cover of the fermenter

B = Handle to open and close the fermenter

C = Fermenter

D = Hose passage

E = Housing to the source of heat

F = Thermostat (Thermocouple)

G = Stand

3.3 Specification for Fabrication

Shape : Cylindrical

Material : Galvanized Iron

Material thicknes

Voltage : 220 volt

An Insulator

Height of the fermenter : 450 mm

Diameter of the fermenter : 450 mm

Width of the Fermenter : 150 mm

Width of the Cover of the Fermenter : 150 mm

Height of the cover : 50 mm

Height of clearance from the ground (stands) : 100 mm

Diameter of the stand : 100 mm

Thickness of the fermenter : 1 mm

Depth : 350 mm

Thermostat range : 30 – 110 ^{0}C

Diameter of the hose : 6 mm

3.4 Description of the Fermenter

A 1mm thick galvanized iron was used for the fabrication. The cover was made air tight with rubber gasket to prevent heat loss and oxidation. The fermenter body was also insulated to prevent heat loss. The fermenter was divided into 2 chambers by 12 mm diameter metal with a net placed on the upper apart to prevent samples from dropping into the lower part that houses the source of heat. The lower part contained the heat generating device with the thermostat range from 30 to 110 ^{0}C. The evolved gas was directed into a vessel containing barium hydroxide through rubber hose at the top of the fermenter. The equipment is suspended on a 5 cm iron to give it a good clearance from the ground. A 13 Amp. Voltage socket was attached to connect to electricity.

Figure 3.2 Pictorial Representation of the Fabricated Fermenter

3.5 Raw Materials Procurement

The African Locust bean seeds (*parkia biglobosa*) were purchased from Itapaji, a small village in Ekiti state, Nigeria. All the chemicals used during the experiment were of analytical grade. The Nutrinet broth used was manufactured by BIOTECH Laboratory Ltd. Lanwades Business Park, Kentford (UK). The *Bacillus subtillis* used for the inoculation was prepared in the Microbiology Laboratory of Covenant University Ota.

The Equipment/instrument used for the various laboratory analyses and test include: A fermenter (Bioreactor) which was fabricated, Jenway pH monitoring devices, Binatone laboratory blender, Auto clave (Tuttnauer Model 2340E, USA), HITACH Hot plate, Busch R5 De-vacuuming pump, titration apparatus, Grieve Hendry Industrial Oven (Model L325), 500 ml round botton flask, Measuring cylinder, Thermocool freezer, Dessicator, Fume cupboard, funnels, soxhlet extraction unit.

Scanning Electron Microscope (SEM) (JEOL USA Model) was obtained from Ahmadu Bello University, central Laboratory, Zaria. Atomic Absorption Spectrometer (AAS) (Thermo Fisher Scientific-iCE 3300 Model), Flame Photometer (pfp7 jenway Model), Kjeldahl Apparatus (MQ Model), Rotary Evaporator (Buchi Rotavapor R-200), Mettler Toledo Weighing balance and Agilent Cary 5000 UV Spectrometer were all obtained from the Central Instrumentation Laboratory, Covenant University, Ota. Fourier Transfrom Infrared Spectroscopy (JASCO) was obtained from University of Lagos, Department of Chemistry, Akoka, Lagos.

Iru was produced in the Laboratory using the fabricated bioreactor. The commercial Iru was purhasced aseptically from the ota market and transported to the laboratory in a sterile air tight container.

3.6 Laboratory Preparation of Iru from *Parkia biglobosa* seeds

The *Parkia biglobosa* purchased from the market were manually sorted and washed to remove spoilt seeds, dirt and debris that were packed with the seeds during harvesting and processing. 700 g of seeds were boiled under pressure for 6 hours in other to soften the cotyledon. This was followed by cooling down at room temperature. Rubbing in between palms was employed to remove the seeds from the cotyledons. The cotyledons were washed thoroughly and severally in water. A locally made basket sieve was used to remove the shaft from the seeds. The washed clean seeds were then re-boiled for another 3 hours and drained. The drained re-boiled seeds were grinded using a blender in order to increase the surface area for fermentation process.

3.7. Preparation of Starter Culture

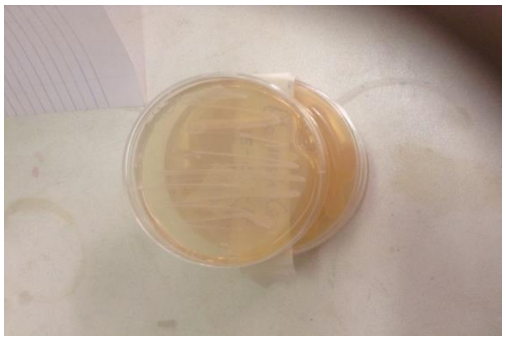
Materials required: Nutrient Broth No. 2, Peptones dextrose agar, Water bath, Conical Flask, Measuring cylinder, Weighing balance, Distilled water, Foil paper, cotton wool and MacCartney bottles.

3.7.1 Preparation of *Bacillus subtilis*

Procedure: *Bacillus subtilis* was prepared according to the directions specified by manufacturer (BIOTECH laboratory Ltd. Lanwades Business Park, Kentford).

6.25 grams of nutrient broth was weighed and dispersed in 250 litre of distilled water in a sterilized 500 litre conical flask. It was allowed to homogenize (dissolve) completely for 40 – 45 minutes in a water bath; a clear yellow solution was formed. The homogenized clear solution was sterilized in the autoclave for 15 minutes at 121 ^{0}C to eliminate all the micro-organisms present. The resulting solution in the flask was clear. The conical flask was removed and allowed to cool. A pure previously isolated *Bacillus subtilis* was obtained in an inactive slant form from Microbiology laboratory, Covenant University, Ota and fed in the homogenized solution. This was then put in the incubator for 24 hours at 37 ˚C, which turned turbid to confirm activation. This was then removed and 10 ml was portion into McCartney bottles as required.

a. Homogenization of Nutrient Broth in the water bath



b. *Bacillus subtillis* plate

c. Sub-culturing of *B.Subtillis*

Figure 3.3 Preparation of *Bacillus subtilis*

3.7.2 Preparation of *Saccharomyces cerevisiae*

Procedure: *Saccharomyces cerevisiae* was prepared according to the directions specified on the nutrient broth container [BIOTECH] or peptose dextrose agar by manufacturer. To produce 200 ml of *Saccharomyces cerevisiae*, 5 g of Nutrient broth or peptose dextrose agar powder was added to distilled or deionized water in a 250 ml conical flask. This was put in a water bath for 45 minutes to cause the broth powder to homogenize or dissolve in the distilled water. The homogenized product was sterilized in the autoclave to kill micro-organisms present in the homogenized solution for 15 minutes at 121 ^{0}C. The resulting solution in the flask was clear. The conical flask was removed and allowed to cool. A pure previously cultured *saccharomyces*

cerevisiae was taken and fed in the homogenized solution and left at room temperature for 24 hours. 10 ml were portion into Marcartney bottles as required.

3.8 Inoculation of Seeds

A. Aerobic Fermentation

100 grams of the processed seeds were put into 200 ml conical flasks. The inoculation was done using 0.005 g broth/g seed of *Bacillus subtilis* per 100 grams of the seed sample. Five flasks were prepared and labeled Day 1, Day 2, Day 3, Day 4 and Day 5. The initial (tare) weight was taken to know the final weight of the substrate. All the 5 flasks were placed in the fabricated fermenter (bioreactor) at the same time and the thermostat was set to the required temperature. Holes of 6 mm diameter were bored into the corks used to close the flask and hose were passed through it into the flasks filled with 90 ml of $Ba(OH)_2$ which traps the Carbon dioxide released by the fermentation reaction as a by-product. This was used to monitor the rate of production of Carbon dioxide. This was repeated for temperatures 40, 50, 60 and 70 °C respectively. Samples were taken daily (24 hours) and kept in the freezer for further analysis. Fermentation was carried out for 120 hours (5 days).

Figure 3.4 The Fabricated Fermenter with samples inside (Aerobic)

B. Anaerobic Fermentation

100 grams with 0.005 g broth/g seed of starter culture were put into 500 litre flasks, corked tightly with a bored rubber cork to avoid air entering after devacuuming. The initial (tare) weight was taken to know the final weight. A valve was put in between hose to control the vacuuming. The flasks were de-vacuumed for 40 minutes with a vacuum pump after which the valve is closed immediately to avoid air entering back into the flask. They were all arranged into the fermenter at the same time and the thermostat was set at the required temperature. The flasks were then connected through a pipe to another flask containing 90 ml of 0.1 M $Ba(OH)_2$. Samples were allowed to ferment for 120 hours (5 days). Fermentation was carried out at temperatures 40, 50, 60, and 70 ^{0}C. Samples were collected at 24 hours (daily) intervals for analysis and kept in the freezer for further analysis.

Figure 3.5 Devacuuming of samples before anaerobic fermentation

C. *Saccharomyces cerevisiae* Fermentation

100 grams of the processed seeds were put into 200 ml conical flasks. The initial (tare) weight was taken to know the final weight. It was tightly corked and inoculation was done using 10 ml of Saccharomyces cerevisiae. Five flasks were prepared and labelled Day 1, Day 2, Day 3, Day 4 and Day 5. The thermostat was set at the required temperature for the fermentation. Fermentation was carried out for five (5) days. Samples were taken daily (24 hours) and kept in the deep freezer for further analysis.

D. Mixture of *Bacillus subtillis* and *Saccharomyces cerevisiae* Fermentation

100 grams of the processed seeds were put into 200 ml conical flasks. The initial (tare) weight was taken to know the final weight. The inoculation was done using 5 ml of Bacillus Subtilis and 5 ml *Saccharomyces cerevisiae* (equal volume).The flask was tightly covered by a rubber cork and a tape to reduce the entering of air into the fermentation system. Five flasks were prepared and labelled Day 1, Day 2, Day 3, Day 4 and Day 5. Fermentation was carried out for five (5) days. Samples were taken daily (24 hours) and kept in the deep freezer for further analysis.

E. Variation of Concentration

100 g of ground de-hulled seeds were weighed and kept inside well labeled 200 ml conical flask. Five flasks were labeled Day 1, Day 2, Day 3, Day 4 and Day 5. Inoculum of the following concentrations 0.0025, 0.005, 0.0075, 0.01 g broth/g seed of *Bacillus subtillis* was added to the samples. These were all carried out in batch by experiments, starting with 0.0025 g broth/g seed *Bacillus subtillis* with 5 days fermentation at the required temperature to 0.01 g broth/g seed *Bacillus subtillis* with 5 days fermentation at the required temperature also. The conical flasks containing samples were then properly closed and covered using corks and tapes to ensure an air tight environment for the fermentation process to properly occur. The samples were then placed in a fermenter which was set at the required temperature. The control fermentation was carried out by fermenting de-hulled beans with no starter culture (Natural fermentation). This procedure was repeated for the various temperatures. At the end of fermentation the physiological analysis was carried out on each sample. Samples were kept in an air tight container and kept in the freezer for further analysis.

3.9 Fermentation Rate monitoring

Three methods were used for monitoring the fermentation rate, namely:

- Weight Loss Method
- pH determination Method
- Carbon dioxide release Monitoring

3.9.1 Weight Loss Method

During fermentation, several changes occur in the seeds of the African Locust bean. The difference in the weight loss (initial and final weight of the fermenting system) was used to

monitor the rate of fermentation of the African Locust bean (*Parkia biglobosa*) seeds to vegetable protein called 'Iru'.

3.9.2 pH determination Method

Fermentation of African locust bean seeds to 'Iru' is an alkaline fermentation, which was confirmed by this work. 5 g of fermented condiments was dissolved into 20 ml of distilled water and readings were taken using the Jenway pH meter (USA).

3.9.3 Carbon dioxide release Monitoring

As means of monitoring the rate of fermentation, the evolution of CO_2 was monitired. A 6 mm hose was passed from the inoculated African locust bean seed in a conical flask into another flask that has 90 ml $Ba(OH)_2$. The two flask were tightly covered with a cork and a tape to avoid the escape of the gas. This was done for the 5 conical flasks and monitored for the 5 days of fermentation. Initially the solution was clear but latter turned white at contact with the process. White precipitate of $BaCO_3$ was formed underneath the flasks. The $BaCO_3$ was filtered and the filtrate titrated with Hydrochloric acid. The volume of the Carbon dioxide released was calculated.

3.10 Deterioration study

Samples were taken after the third day of fermentation and dried to different moisture contents percentage for 2, 4, 6, 8, 10, 12 hours until constant weight was obtained at 0 % moisture. Samples were allowed to cool in a desiccator and stored in an air tight container for 30 days. Samples were taken every week (5 days interval) for analysis. Samples were further stored for about 10 months.

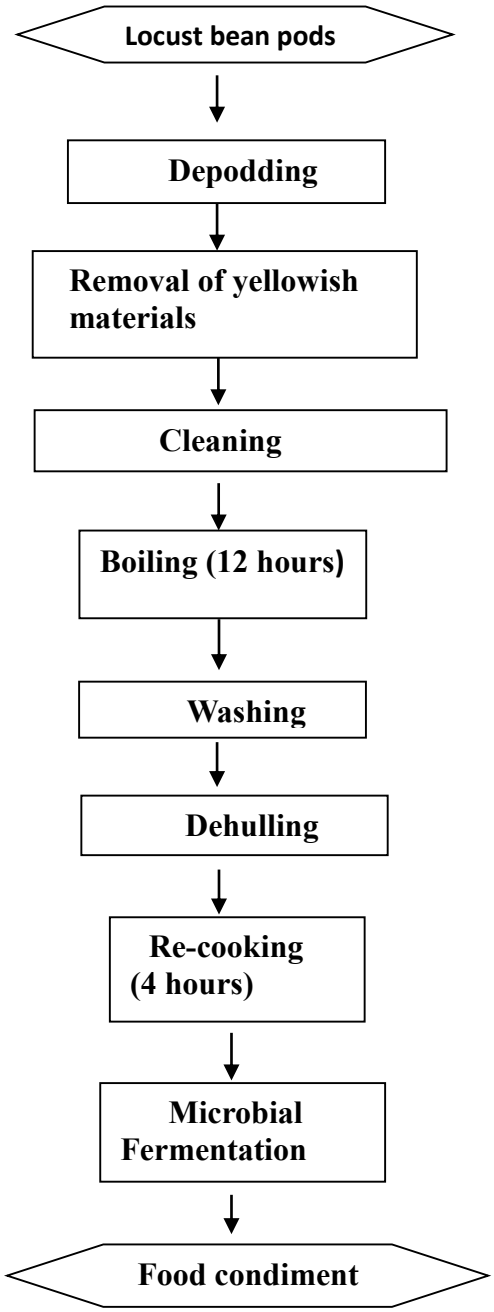


Figure 3.6 Flow Diagram for the Processing of African Locust Beans Seeds to Food Condiment (Iru)

3.11 Proximate Analysis of Fermented African Locust Bean Seeds

Proximate Analysis is the partitioning of compounds in a feed into six groups based on the chemical properties of the compounds. The six categories are:

(i) Moisture

(ii) Ash

(iii) Crude protein (or Kjeldahl protein)

(iv) Crude lipid

(v) Crude fibre

(vi) Nitrogen-free extracts (digestible carbohydrates)

All the analysis above were carried out using satandard Methods of Analysis by the Association of Analytical Chemists (AOAC, 2000).

3.11.1. Determination of Percentage Moisture Content

Moisture content determination is an important factor that critical in food quality, preservation, and resistance to deterioration. Moisture content of foods can be determined by a variety of methods.

Procedure

Apparatus and Instrument needed

- Analytical balance
- Desiccator
- Crucible with lid
- Thermosetting Oven
- Tongs

Washed crucibles were oven dried at 105 ^{0}C for an hour to ensure total dryness. They were then transferred into the desiccator to cool for about 30 minutes. The crucibles were weighed on an electronic balance and the weight recorded as (W_1). 5 g of seed sample were weighed into the dried preweighed crucible (W_2). The crucibles and the content were oven-dried at 105 ^{0}C for 4 hours. The samples were removed from the oven and dried until a constant weight was obtained. After drying, the crucible was transferred into the desiccator to cool for about 45 minutes and weighed (W_3). This analysis was carried out in triplicate and the average value was recorded as moisture content.

Calculations

$$(\%)\ \text{Moisture content} = \frac{\text{Loss in weight due to drying}}{\text{Weight of sample before drying}}$$

$$(\%)\ \text{Moisture content} = \frac{W2-W3}{W2-W1} * 100$$

Where,

W_1 = Weight of empty Crucible

W_2 = Weight of empty crucible + sample before drying

W_3 = Weight of crucible+ sample after drying (constant weight).

$$\%\ \text{Total Solid} = \frac{W3-W1}{W2-W1} * 100$$

Or % Total solid = (100 - % Moisture content)

Totals Solid is the part that is not water.

3.11.2 Determination of Total Percentage Ash Content

Ash is the inorganic residue after the water and organic matter have been removed from a substance by heating in the presence of certain oxidizing agents. The ash is not usually the same as the inorganic matter present in the original material since there may be losses due to

the volatilization or chemical interaction between the constituents. The importance of Ash content is that it gives an idea of amount of mineral elements present and the content of organic matter in the sample. The organic matter account for quantitative constituents of protein, lipids or fat, carbohydrate, plus nucleic acid. The measure of ash content provides a measure of the total amount of minerals within a food. The principle of this method is based on the fact that minerals in a food substance are not destroyed on heating and also have low volatility compared to other components of the food. Analysis of ash content can be done either by dry ashing, wet ashing or plasma dry ashing. The method that is chosen for the analysis depends on the reason for the analysis, the type of food analyzed, and also the equipment available. Ashing is also a preparation step in analysis of specific minerals either by atomic spectroscopy or other traditional methods. Dry ashing for the majority of the samples,

Dry ashing is incineration at high temperature (525 °C or higher). It is accomplished in a muffle furnace. There are many different types of crucibles made of different materials such as quartz, porcelain, steel or platinum crucibles. The crucible selection is based on cost, reactivity of the crucible to the sample and resistance of the crucible to very high temperatures Dry ashing has the advantages of not needing external reagents and requiring little attention.

Apparatus

Crucible or porcelain or metal dishes can be used

Muffled furnace, Desiccator, Tong

Procedure

A clean crucible was pre-dried in an oven for 30 minutes at 100 ^{0}C to assure total dryness of the crucible. It was then transferred into the desiccator to cool for 30 minutes and weighed on an electronic weighing balance as W_1. 5 g of sample was weighed into it and weighed as W_2. It was placed in a muffle furnace for 4 hours and the temperature was slowly increased to 450 ˚C to avoid incomplete ashing. Samples were ash until it becomes whitish in colour. It was

removed into the desiccator with a tong and cooled to room temperature for an hour. Sample was reweighed as W_3. The percentage ash was calculated as followed and average taken:

Calculation: =

$$\% \text{ Ash content} = \frac{weight\ of\ ash}{weight\ of\ sample\ (after\ drying)} * 100$$

$$\% \text{ Ash content} = \frac{W3-W1}{W2-W1} * 100$$

% Organic matter = 100 - % Ash

3.11.3. Determination of Percentage Crude Fat Content

Fats are defined as mixtures of various glyceride of fatty acids, which are sparingly soluble in water and some certain organic solvents e.g ethyl ether, petroleum ether, acetone, ethanol, methanol, benzene. Extraction is carried out with Soxhlet apparatus with ether or petroleum ether. The fat content may be determined by either the Soxhlet method, the Mojonnier method or the Soxtherm automated method. The most conventional of these three is the Soxhlet method. The usual procedure is to continuously extract the fat content using 40/60 petroleum ether in a Soxhlet extractor. The ether extraction method is based on the principle that non-polar components of sample are easily extracted into organic solvents.

Apparatus

- Thimble
- Reflux condenser
- Round bottomed flask
- Heating mantle
- Appropriate solvent

Procedure

Previously dried, fat free thimble was weighed as W_1. 5 g of sample was weighed into the thimble and weighed as W_2. The thimble and the sample was carefully wrapped and tied. Washed and dried 500 ml round bottom flask was weighed as W_3. The flask was half filled with 40/60 petroleum ether and the sample was dropped into the sample holder of the soxhlet extraction apparatus. The Soxhlet extractor was fitted with reflux condenser as shown figure 3.7. The flask was then placed on a heating mantle and the heat source was adjusted to allow it to boil gently at 34 ^{0}C. It was allowed to siphon over 5 hours. The condenser was detached and the thimble removed. Petroleum ether was distilled from the flask. The distilling flask containing the oil was air dried at 100 ^{0}C for exactly 5 minutes to remove the solvent residues in oil. This was put inside a desiccator to cool and the weight was taken as W_4. The percentage fat contained was determined thus:

$$\% \text{ Crude fat} = \frac{Weight \text{ of Flask + oil – Weight of empty flask}}{Initial\ Weight\ of\ Sample} * 100$$

$$(\%) \text{ Crude Fat} = \frac{W4-W3}{W2-W1} * 100$$

Figure 3.7 A set up of Soxhlet apparatus for the extraction of crude fat

3.11.4 Determination of Percentage Crude Fibre

Crude fibre is the portion of the plant material which is not ash. Fibre was originally thought to be bad and the indigestible portion of foodstuffs. However, the benefits of a diet high in fibre have been discovered. Foods with high fibre content can absorb cholesterol and toxic agents in food. It also raises the excretion of bile and sterols. It is known however that fibre consists of cellulose which can be digested to a considerable extent by both ruminants and non-ruminants. The interest in fibre in food and feed has increased, based on the noticed number of serious illness associated with diet low in fibre. Fibre swells and form gelatinous mass with high water retention capacity with the digestive system. Diseases such as constipation (the intestine absorbs water and volume of the feaces will decrease), appendicitis, Hemorrhoid and Diabetes mellitus have been related to low-fibre diet in humans.

Procedure

The starch and the protein part of food were dissolved by boiling with acid and then with a very strong base (NaOH). The residue, which comprises of cellulose and lignin was washed and dried and weighed. The residue is ashed and the weight is subtracted from the weight of the residue.

3 g of defatted sample was weighed (W_1) into 250 ml beaker containing 200 ml of 0.125 M or 1.25 % tetraoxosulphate (iv) acid (Sulphuric acid). The mixture was heated in a steam bath at 70 – 90 ^{0}C for 2 hours, it was then allowed to cool. The cooled mixture was filtered using a muslin cloth over a Buckner funnels. The residue was washed three times with hot distilled water to remove the acid and then put in a beaker containing 200 ml of potassium hydroxide. The mixture was heated as before over a steam bath for 2 hours. The solution was filtered and the residue washed three times with hot distilled water, then with petroleum ether and water. The final residue obtained was put in clean preweighed (W_2) crucible and dried at 120 ^{0}C to a constant weight. The crucible with the oven dried sample as put in a muffle furnace and ashed at 550 ^{0}C for 30 minutes such that the sample became ash white. The crucible and its contents were removed from the furnace, cooled in a desiccator and reweighed (W_3). Percentage fibre was calculated as followed:

$$\% \text{ Crude fibre} = \frac{\text{weight of oven dried sample} - \text{weight of ash X } 100}{\text{Initial weight of sample}}$$

$$\% \text{ Crude fibre} = \frac{W2-W3}{W1} * 100$$

3.11.5. Determination of Percentage Crude Protein

Proteins are necessary nutrients for the human body. They are one of the building blocks of body tissue, and also serve as a fuel source. Protein is a nutrient that is important for growth and maintenance of the body. Crude protein is calculated on the basis that protein is the only food composition that has Nitrogen. Average Nitrogen content of protein is about 16 %, the amount of nitrogen is multiplied by 6.25 to calculate the crude protein, and this is used only for Kjedahl method of analysis of protein.

The analysis of Crude protein was determined using Kjeldahl method. This process involved 3 different stages namely; digestion, distillation and titration.

A chemical mixture of 150 g of K_2SO_4 and 10 g of $CuSO_4$ was made. 1 g of sample and 10 g of chemical mixture was weighed into a 250 ml digesting tube**.** 12 ml of concentrated H_2SO_4 was carefully added to the mixture. The digesting tubes containing samples were kept in a rack and digested for 30 minutes at 420 ^{0}C in a fume cupboard. After digestion, the samples were allowed to cool to room temperature for about 1 hr. 80 ml of distilled water was then added to the digested samples. 25 ml of diluted digested sample as well as 25 ml of NaOH were measured into a distillation tube. Distillation was carried out using 5 ml of Boric Acid and methyl red indicator. This process was stopped when the conical flask containing boric acid-indicator solution reached 100 ml mark. The distillate was titrated using HCl until end point was reached.

At this stage, the purple colour obtained during distillation changed to dark yellow. The formula used for protein calculation is:

$$\% \text{ Nitrogen} = \frac{(V2/V1) \times C \times 0.0140 \times (S-B)}{Z} * 100$$

% Protein = % Nitrogen x 6.25

3.11.6 Carbohydrate Content Determination

The carbohydrate content of the sample was obtained by difference, that is, as the difference between the total summations of percentage moisture, fat, fibre, protein and ash.

% Carbohydrate= 100 – (% moisture + % fat + % protein + % fibre + % ash)

3.12 Determination of Titratable Acidity (TA)

5 g of sample was weighed into a 50 ml beaker and 20 ml distilled water was added to it. This formed a suspension which was filtered using Whatman No. 2V, 18 cm diameter filter paper. 2 to 3 drops of phenolphthalein indicator was added to the filtrate and the filtrate was titrated with 0.1 N NaOH until the pale pink endpoint, which persisted for 30 seconds. Titratable acidity (TA) was determined twice for each sample, and the average values were calculated.

$$TA\ (g\ lactic\ acid/L) = \{(N\ NaOH) \times (mls\ NaOH) \times 75 \times 1.2\}/\ mls\ of\ sample$$

3.13 Peroxide Value Determination (POV)

Rancidity is brought about by the action of air oxidative or by microorganisms in food substances especially oil. In oxidative rancidity oxygen is taken by the oil or fat with the formation of peroxide. Peroxides value is a measure of the peroxide contained in the oil. The peroxides present are determine by titration against thiosuphate in the presence of Potassium iodide using starch indicator.

Reagents and solution

1. Acetic Acid - chloroform solution
2. Saturated Potassium Iodide solution. Store in the dark.
3. Sodium thiosulfate solution, 0.1 N

4. 1 % Starch solution.

5. Distilled or deionized water.

Procedure

A blank determination of the reagents was conducted first.

2.00 g of sample was weighed into a 100 ml glass stoppered Erlenmeyer flask. 12 ml of the acetic acid - chloroform solution was added into the flask with a measuring cylinder. The flask was swirled until the sample was completely dissolved and was careful warmed on a hot plate.

1 ml Mohr pipette was used to add 0.2 ml of saturated potassium iodide solution. The flask was stooped and the contents swirled for exactly one minute.

12 ml of either distilled or deionized water was added immediately, it was stopped and shook vigorously to liberate the iodine from the chloroform layer. The burette was filled with 0.1N sodium thiosulfate. Titration was carried out slowly with mixing until the color lightens.

A dispensing device was used to add 2 ml of starch solution as indicator. Titration continued until the blue gray color disappears in the aqueous (upper layer).

The mls of titrant used was accurately recorded to two decimal places.

Calculations:

S = titration of sample

B = titration of blank

$$\text{Peroxide value} = \frac{(S-B)*N\ \text{thiosulphate}*1000}{\text{Weight of sample (g)}}$$

3.14 Determination of Shelf life of I*ru* using Moisture Content

Iru was produced using *Bacillus subtilis* as starter culture. The shelf life of *Iru* was determined at room temperature after the 72 hours fermentation period (3 days). It was dried at 80 ^{0}C at 2 hours interval until zero moisture content was reached. The samples were stored in a clean labelled air tight containers at room temperature for 5 weeks. Samples were taken every week for analysis. The analyses carried out on the samples were pH, Peroxide value, percentage protein composition and titratable acidity. Physiological test were also carried out for texture, aroma, taste, colour and acceptability.

3.15 Mineral Composition of African Locust Bean (*Parkia biglobosa*).

Atomic absorption spectroscopy was used to identify the mineral composition present in both unfermented and fermented African locust bean seeds. The U.S. FDA (Food and Drug Administration) manual section 4.1 (version 1.1 September 2010) was used for the preparation of samples. Potassium and Sodium composition were determined by Flame Photometry (Jenway Limited, Donmow Essex, UK). Calcium, Copper, Magnesium, Iron, Cadmium, Zinc, Lead and Manganese using Atomic Absorption Spectrophotometer (Thermo Fisher Scientific-iCE 3300 Model).

CHAPTER FOUR

ANALYSIS RESULTS AND DISCUSSION

4.1 Fermentation Rate Monitoring

The fermentation rate was monitored using the following methods: difference in weight, pH and Carbon dioxide release during fermentation.

4.1.1 Weight Loss Monitoring

Figures 4.1 to 4.5 show the rate at which fermentation process reduced the weight of the substrate at various fermentation temperatures and duration.

Under aerobic fermentation condition, it was observed that lower temperature favoured the percentage substrate weight loss with respect to the duration of fermentation (days). At 70 °C the trend observed at lower temperatures was not followed rather an irregular behaviour not only in weight loss but in all other analysis carried out was observed in this work. It is speculated that the reason for the observed behaviour at 70 ºC was because the temperature was too high for the inoculum or starter culture to operate optimally, which led to the formation of a tough, protective endospore.

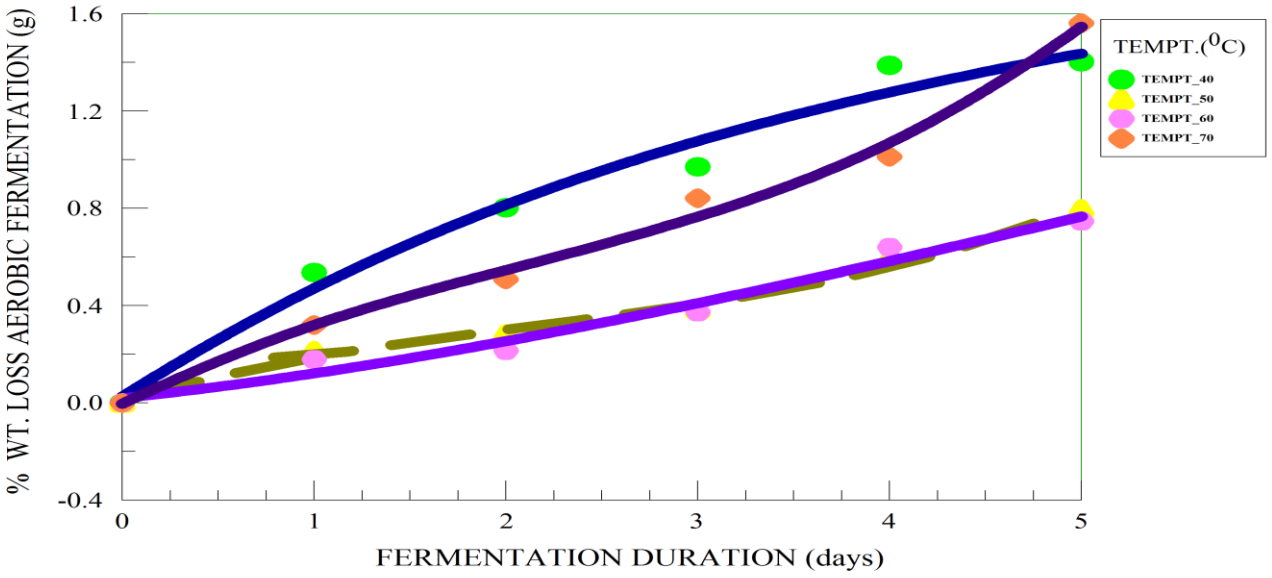


Figure 4.1.1 - Effect of fermentation on Substrate Weight loss (WT.) using *Bacillus subtilis* (Aerobic)

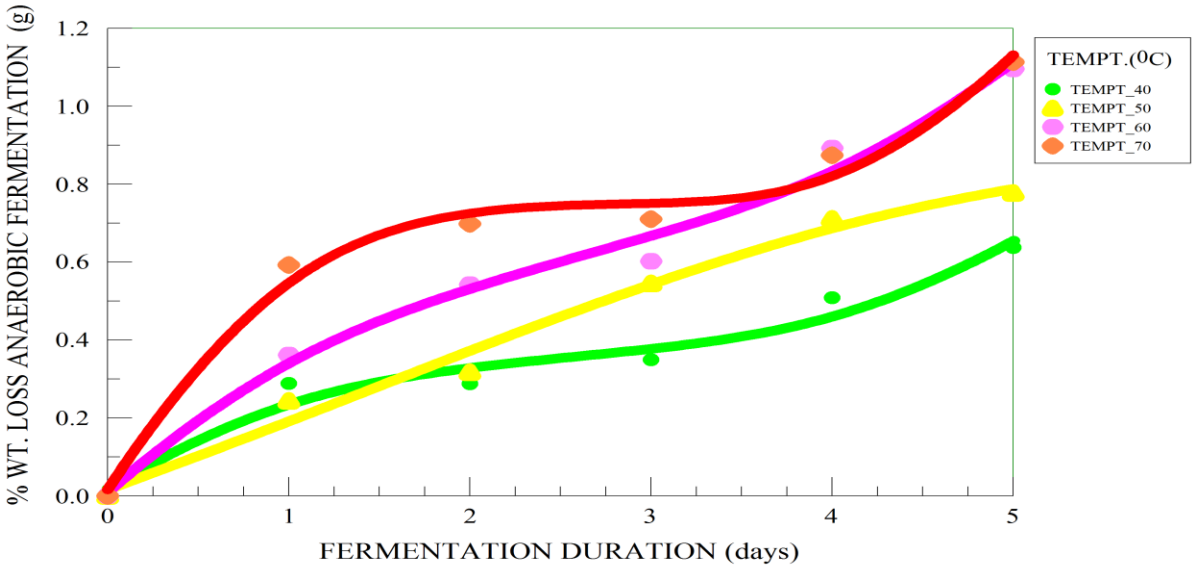


Figure 4.1.2 - Effect of fermentation on Substrate Weight loss (WT.) using *Bacillus subtilis* Anaerobic

The reverse was the case in anaerobic fermentation condition as shown in Figure 4.1.2. The substrate weight loss increased with increasing temperature leading to a lower substrate weight loss at the lower temperatures. This could be as a result of the fermenting organisms (*Bacillus subtilis*) being an obligate aerobic organism that requires oxygen to grow and can also switch to facultative anaerobic organism under a favourable fermentation condition. Highest substrate weight loss was noticed at 70 °C for anaerobic and 40 °C for aerobic fermentation conditions.

Since weight loss and all other parameters monitored for processing at the temperature of 70 °C under anaerobic condition were physiochemically unacceptable, subsequent fermentation parameter evaluations were based only on aerobic fermentation processes at 40, 50 and 60 °C, respectively. The spoilage noticed at 70 °C in anaerobic and aerobic conditions was due to the fact that *Bacillus subtilis* is a mesophilic bacterium that grows best within an optimal temperature range of 25 – 40 °C. Stress and starvation are common in any environment with highly elevated temperature, thus making the organism to develop a strategy to survive under these high temperature conditions by forming stress-resistant endospores. At this stage, the organism is inactive and fermentation is suspended and the substrate is subjected to other non-biochemical reactions like cooking. Maggot could not infest the substrate due to the high temperature. Also *Bacillus subtilis* has the ability to inhibit the growth of other microorganisms in any medium it found itself, therefore fermentation could not continue without any organism at this high temperature condition.

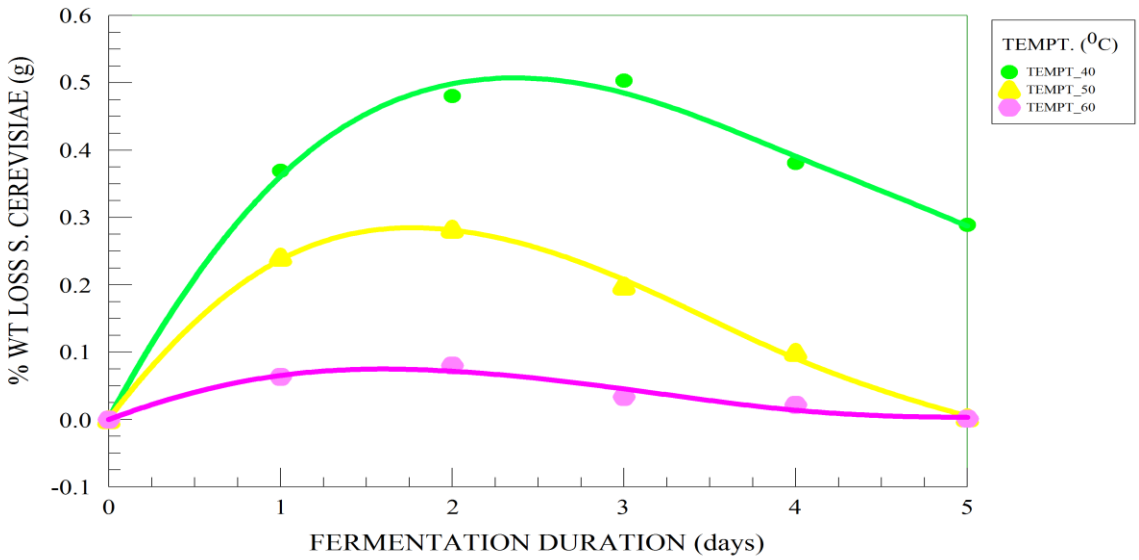


Figure 4.2 - Effect of fermentation on substrate weight loss (WT) using *Saccharomyces cerevisiae* as starter culture

In Figure 4.2, higher percentage substrate weight loss was also steadily favoured at lower temperatures using *saccharomyces cerevisiae* as starter culture under aerobic condition. The results confirmed the fact that the highest optimum fermentation temperature for the growth of S. cerevisiae is 32.3 ºC and a maximum of 45.4 °C for activity (Salvador *et al.*, 2011). Temperature is an important non-living condition (abiotic) that influences the *S. cerevisiae* growth. At high temperatures, *S. cerevisiae* become stressed and their cellular content becomes damaged. Growth rate decreases *as* temperature increases and finally at 50 °C, *S. cerevisiae* die. Walton and John (2005), also reported the use of heat in eliminating *S. cerevisiae* in food at 52 °C. Although several other works have been done on cell damage in yeast and other microorganisms at temperature 12 – 20 °C, but this is of little consequence or no prominence in the present instance since our operating conditions were outside this temperature range.

The results observed in this study support other observations and hypothesis in literatures that the growth rate of *S. cerevisiae* is reduced as it moves away from its optimal growth temperature (Ratkowsky *et al*,.1982), and dies at 50 °C.

Similar result trends were observed for aerobic fermentation in both *Saccharomyces* and *Bacillus* fermented substrate (Figures 4.1 and 4.2) whereby higher weight loss occurred at the lower temperature. On the other hand, *Saccharomyces cerevisiae* performed better under anaerobic fermentation than *Bacillus subtilis* since *Saccharomyces* is a facultative anaerobic fungi and can survive in an oxygen deficient environment for a short period of time. Both end products were not physicochemically acceptable at higher temperatures, probably because of the high production of ammonia which led to a poor characteristic unacceptable aroma. There is a decrease in the functionality of the cell at higher temperatures as a result of increase in the accumulation of ethanol which led to yeast poisoning.

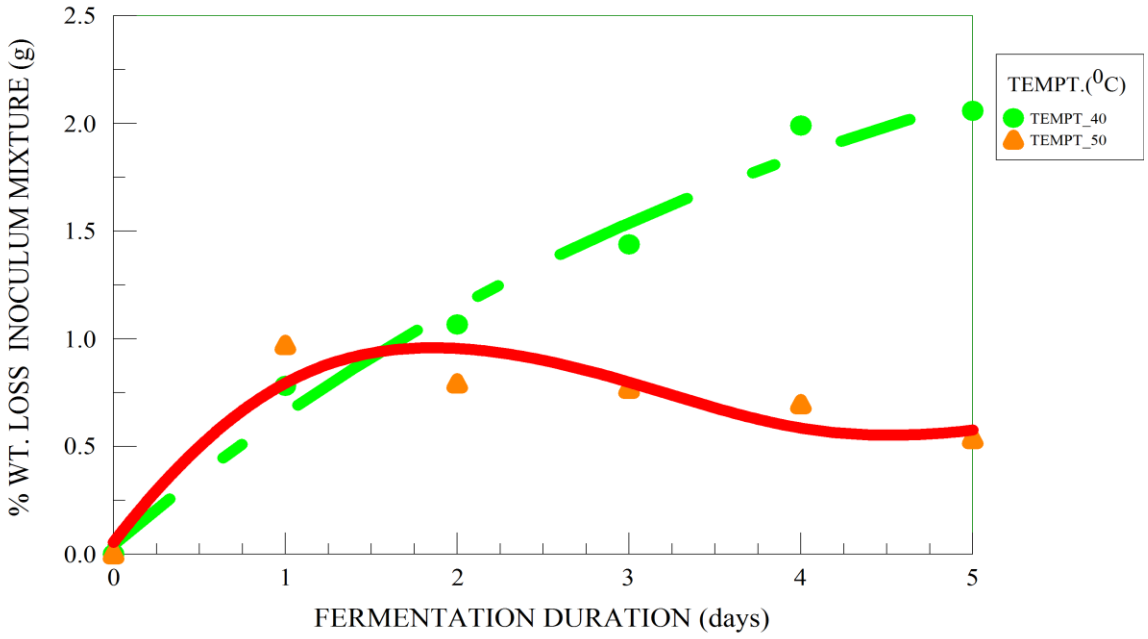


Figure 4.3 - Effect of fermentation on substrate weight Loss (WT) using the mixture of *Saccharomyces Cerevisiae* and *Bacillus subtilis* as starter culture

The effect of mixing *Saccharomyces cerevisiae* and *Bacillus subtilis* was examined at two temperature 40 and 50 ºC. The mixture of *saccharomyces cerevisiae* with *Bacillus subtilis* at 40 °C gave results that have the same trend as those found where *Bacillus subtilis* only was used. This suggested a higher activity by *Bacillus subtilis* than *Saccharomyces cerevisiae* under the same conditions. This is expected since *Bacillus subtilis* tend to dominate in any environment they are found with other organisms. Lower temperature favours substrate weight loss in this mixture as shown in Figure 4.3. The weight loss increased with time for both temperatures during the early period of the fermentation process but as fermentation duration increased, it was observed that the weight loss at 50 °C decreased, probably because the process has gone beyond the maximum operating fermentation conditions for the two organisms.

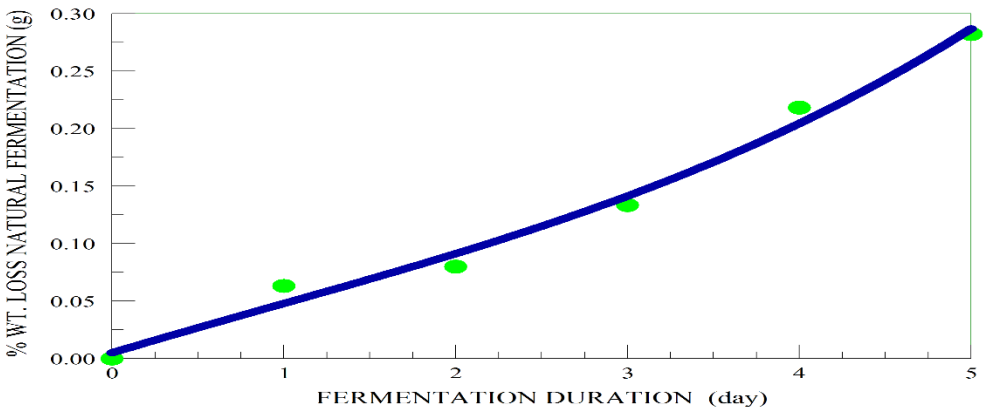


Figure 4.4 - Effect of ambient temperature fermentation on substrate weight Loss

Shown in Figure 4.4 is the effect of natural or spontaneous fermentation at ambient condition on substrate weight loss. This is the process of allowing natural bacteria already present on the processed seeds to start the fermentation. The right environment was created to promote bacterial growth.

Bacillus subtilis probiotic bacteria species which are capable of producing lactic acid; the organism starts the process by consuming sugars present in seeds. With the aid of a thermometer increase in temperature was also noticed during the process, although no attempt was made to prevent heat loss from the system. Changes in temperature have a great role to play in determining which bacteria species will grow fastest and dominate the fermentation process. In spontaneous (natural) fermentation, the sequence in which different microorganisms grow is expedient. A little change in the temperature can change the activity of the microbial process and affect the quality of the final product. An increase in the percentage substrate weight loss was observed to occur with the number of days of fermentation. The increase in pH could have encouraged the growth of *Bacillus spp*, which grows well at pH 7.0 - 8.0.

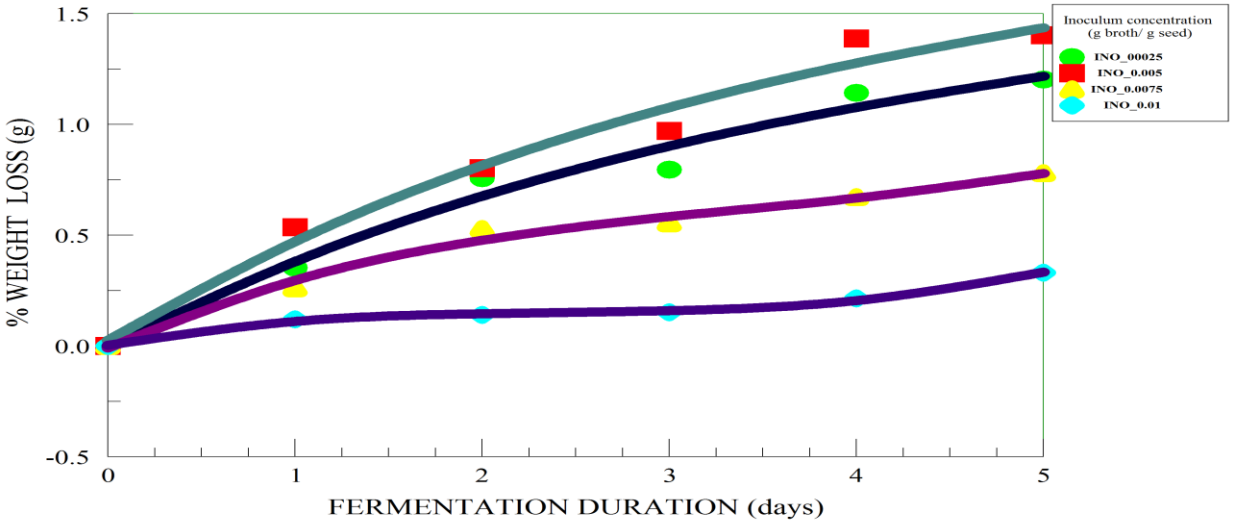


Figure 4.5.1 - Effect of Inoculum concentration on substrate weight loss (WT) using (*Bacillussubtilis*) at 40°C

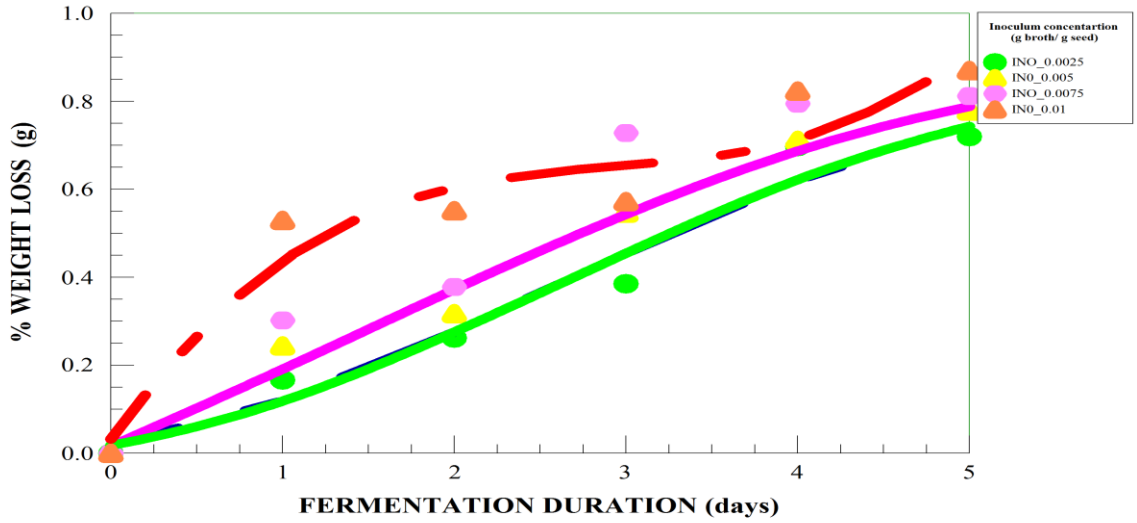


Figure 4.5.2 - Effect of Inoculum concentration on substrate weight loss (WT) using (*Bacillus subtilis*) at 50°C

The effect of inoculum or starter condition on the extent of fermentation was examined at both 40 and 50 ºC. Figure 4.5.1 shows the effect of variation of the concentration of inoculum (starter) culture at 40 °C fermentation temperature. As indicated in the graph, the condition which favours the highest weight loss was the condition involving the use of 0.005 g broth/g seed of *Bacillus subtilis* concentration. On the other hand, the least weight loss resulted when 0.01 g broth/g seed of *Bacillus subtilis* was used. At this concentration a characteristic offensive or poor aroma was noticed. On the basis of this observation, it can be concluded that 0.005 g broth/g seed of *Bacillus subtilis* is the best concentration to be used during fermentation for aerobic fermentation condition.

Figure 4.5.2 shows that the concentration of starter culture at 50 °C favours higher weight loss at highest concentration of inoculum. However, inoculum concentrations above 0.005 g broth/g seed gave an unacceptable end product, probably because the process was carried out outside the optimum operating conditions for the fermentation process. The use of higher concentration of inoculum gave room for deterioration rather than fermentation, more esters and their compounds were probaly produced. The increase in the odour might also be due to the increase in the production of more volatile compounds.

In summary, the changes observed in the weight loss and other parameters analyzed confirmed that microorganisms present were able to degrade and feed on the substrates for growth. The decomposition of some compounds within the substrate and the evolution of gases such as carbon dioxide during the fermentation process can never be over emphasized as being responsible for the loss in substrate weight. Some other volatile compounds were released which are probably responsible for the changes in the aroma at different fermentation temperatures, e.g. Esters and Pyrazines with chemical formulas RCO_2R' and

$C_4H_4N_2$, respectively are perceived at 40 – 50 °C while offensive odours not associated with pyrazine can easily be detected at temperatures above 60 °C for all fermentation conditions. All the volatile compounds identified in this work contributed to the percentage loss in weight of the fermenting substrate. Ouoba *et al*. (2002) in his study identified 5 benzenes, 7 acids, 7 alkanes, 10 esters, 20 aldehydes, 16 pyrazines, 14 alcohol, 12 ketones, 5 phenols, 3 alkenes, 2 amines, 4 pyridines, 4 sulphur, a furan, and 6 other unidentified compounds. Other biochemical changes occurred during the fermentation processes which contributed to the loss in substrate weight, which are proteolysis (a process of breaking down of proteins or peptides into amino acids) and lypolitic (the breaking down of lipids and their hydrolysis into glycerol and free fatty acids). This favours the dominance of *Bacillus spp*. as the fermenting organisms (Kiers *et al*., 2000; Ouoba *et al*., 2003; Achi, 2005).

All these results are in agreement with the works of Odunfa (1981, 1986); Odunfa and Kolawole (1989); Ouoba *et al*., 2003, 2005; Campbell-Platt, 1980; Nwokeleme *et al*., 2014; Popoola *et al.* (2004). In aerobic fermentation, lower temperature favours substrate weight loss, indicating that fermentation is faster and organism is very active at lower temperature (40 – 50 °C). This corresponds with other results analyzed e.g. Percentage Protein increased best at this temperature.

4.1.2 The pH Determination

Earlier studies have confirmed that the fermentation of African locust bean (*Parkia biglobosa)* seed to Iru is an alkaline fermentation which is a fermentation process during which the pH of the substrate increases to alkaline values that may be as high as pH 9 (Omafuvbe *et al*., 2000; Sarkar and Tamang 1995). The effect of temperature on the pH of the fermenting system were

monitored as well as the rate of fermentation. The result obtained confirmed that the fermentation of *Parkia biglobosa* seeds to 'Iru' is an alkaline fermentation.

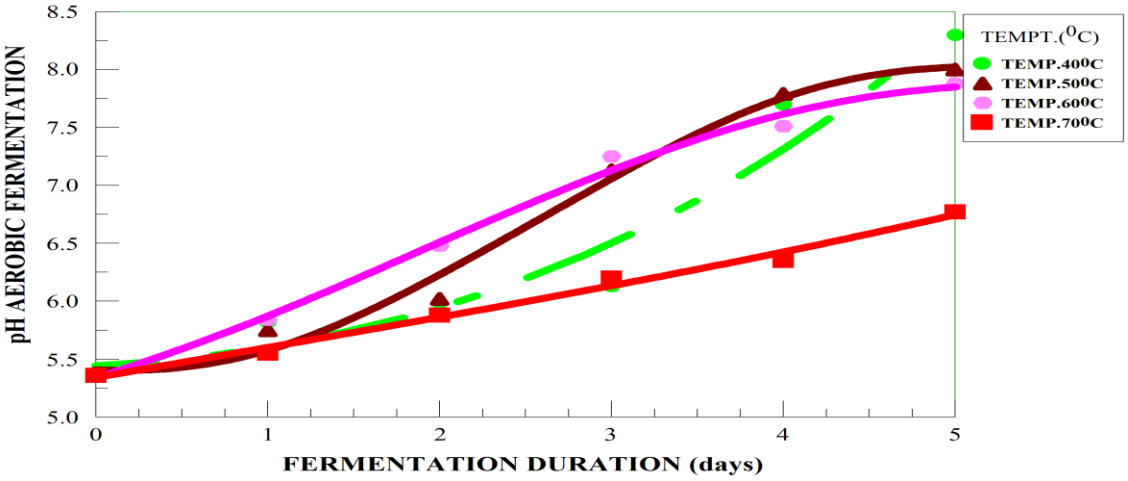


Figure 4.6.1 - Effect of fermentation temperature on the pH value of the substrate using *Bacillus subtilis* (Aerobic)

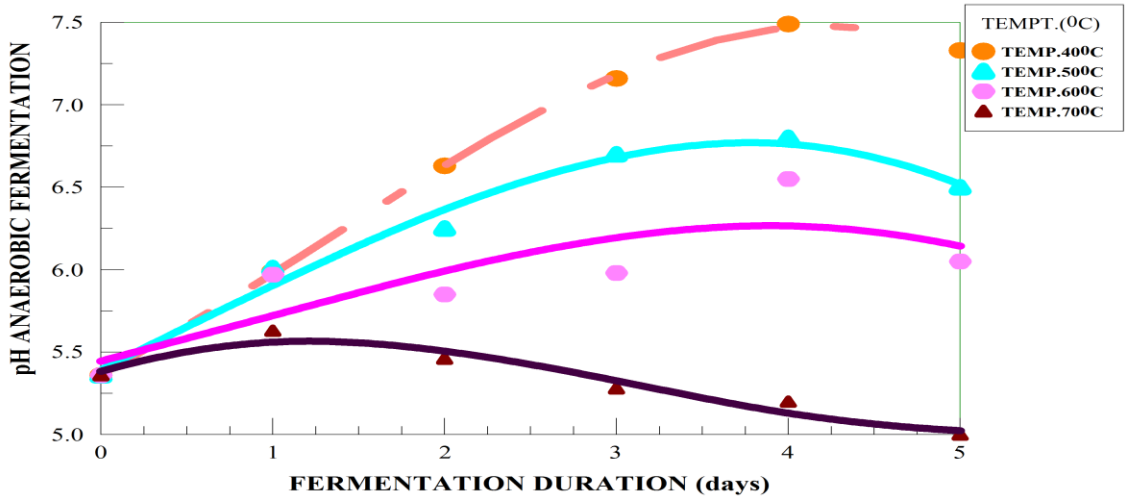


Figure 4.6.2 - Effect of fermentation temperature on the pH value of the substrate using *Bacillus subtilis* (Anaerobic)

Figure 4.6.1 shows that under aerobic fermentation, lower temperature favours increase in pH value towards alkanility during fermentation. This is due to the ability of *B.subtilis* to hydrolyse proteins to produce proteases and obtain amino acids and ammonia as source of carbon and energy for growth, respectively.

Bacillus species dominates bacterial alkaline fermentations. Alkaline conditions provide a suitable environment for the hydrolysis of proteins by *B. subtilis*, resulting in the production of amino acids and the release of ammonia (Odunfa, 1986a). Increase in the alkalinity level of the media is caused by the dissolved ammonia. Increase in the fermentation temperature led to a corresponding increase in pH towards alkinlinity at the initial fermentation stage until the optimum temperature of growth of each starter culture was reached where by any further increase in temperature led to a decrease in pH. This fact is supported by the work of Popoola 2007 in which a pH rise towards alkaline was observed as fermentation progresses.

Achinewhu (1986a) attributed the increase in pH as due to a rapid increase in the soluble nitrogen content during fermentation. This work agrees with the report of Ouoba *et al*. (2003) that the unfermented seed has a pH of 5.3.

It is known that increase or decrease in the pH denatures the microorganism responsible for fermentation thereby decreasing the rate at which reaction takes place while optimal pH favours a faster rate of fermentation depending on the operating fermentation conditions and the organism i.e. the lower the pH the faster the rate of reaction. pH towards acidity affects the microorganism responsible for the breaking down of protein by denaturing them which can lead to low production.

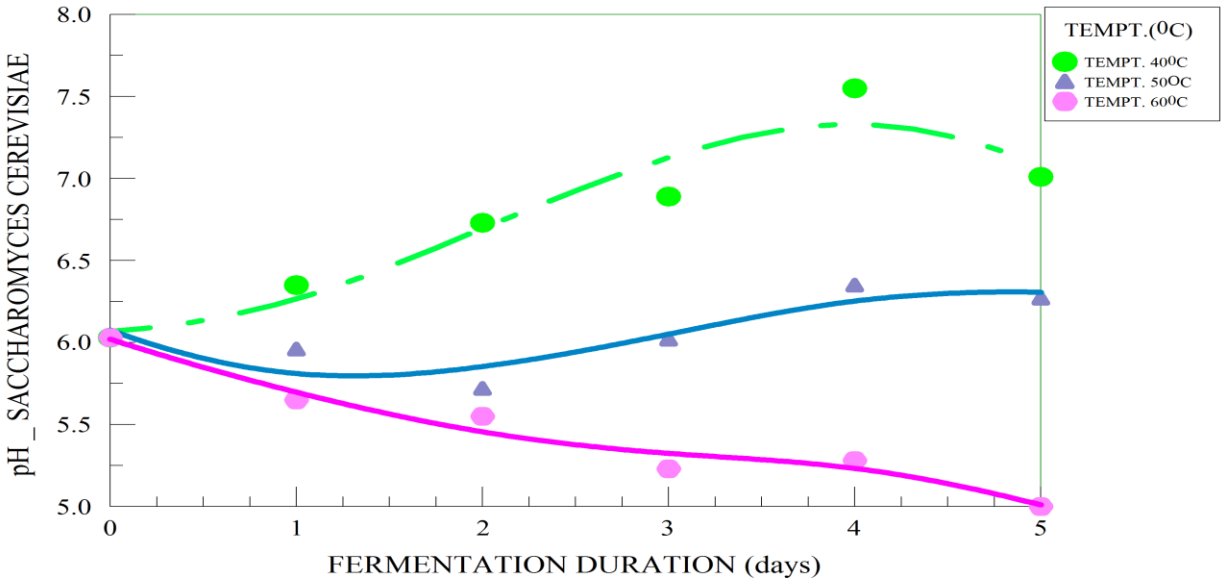


Figure 4.7 - Effect of fermentation temperature on the pH value of the substrate using *Saccharomyces cerevisiae* as starter culture (Aerobic)

Figures 4.6.2 and 4.7 show an entirely different pattern to aerobic fermentation, in that pH increased at the lower fermentation temperatures and later levelled out at the 5th day of fermentation. At all temperatures, pH dropped due to the fact that the fermentation condition has exceeded the optimum temperature by which *B. subtilis* can grow which confirms that *B. subtilis* is a mesophilic bacteria. It cannot thrive in an anaerobic condition as *Saccharomyces cerevisiae* will do.

Environmental factors such as temperature, pH, water activity, especially oxygen levels and concentration of inoculum affect product and significantly affect microbial growth and product formation, this reflects in the weight loss and pH under anaerobic condition.

The increase in pH towards alkalinity is important physiologically for the fermenting microorganism to grow and adapt in the environment.

Several work has been done on the fermentation operating condition of *S. cerevisiae*. Salvado *et al.* (2011), studied the temperature below which growth is no longer observed. He predicted *S. cerevisiae* to be a psychrophilic bacteria which operates at highest optimum temperature of 32.3 °C and maximum of 45.4 °C. Figure 4.7, for the three temperatures studied, high pH values were observed at 40 ^{o}C. Salvado *et al.*, (2011) reported that *S. cerevisiae* performs best within a temperature range of 32 – 45 ^{o}C. Furthermore, the pH values at 40 °C are found to be decreasing after the fourth day. Temperatures 50 and 60 °C are not favourable for the growth as any degree above 45 depresses fermentation with this fungus, hence the unacceptable end product and decline in pH values since more lactic and acetic acids are produced. The temperature of the reaction being too high causes *S. cerevisiae* to be denatured and causing the liquid present in *S. cerevisiae* to diffuse out in order to equalize the concentration outside the cell, this causes the yeast to dehydrate, shrink and eventually die. The results obtained at 50 and 60 ^{o}C are in support of these observations.

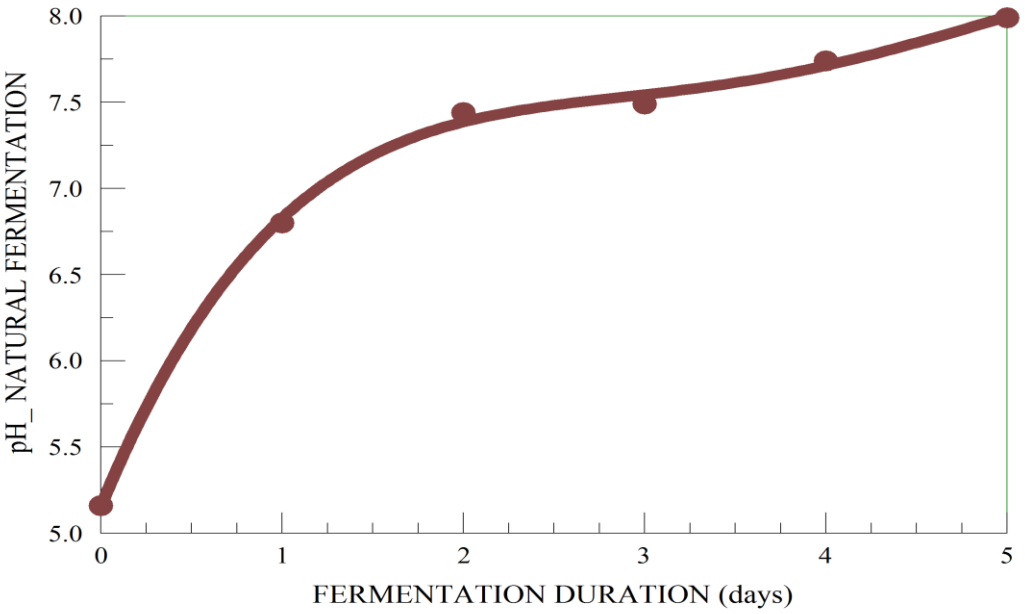


Figure 4.8 - Effect of Natural Fermentation on the pH values of Substrate

Figure 4.8 represents the changes in pH of naturally fermented African locust bean seeds (No inoculum and it was fermented at room temperature). The increase in the pH value with fermentation duration suggests that metabolic activities that occurred may include proteolysis that allows liberation of amino acids and subsequent production of ammonia that leads to an increase in pH. The pH result in Figure 4.8 is supported by similar results obtained by (Odunfa 1985; Ouoba *et al*., 2003a). Odunfa and Oyewole (1986) reported that the pH of African locust bean seeds during fermentation increases from near neutral to approximately 8.0. Other metabolic activities which can also lead to an increase in pH are: Lipolysis and degradation of poly and oligosaccharides (Ouoba *et al*., 2003).

The art of fermenting naturally or spontaneously in an exclusively uncontrollable environmental conditions yields products with variation in quality and organoleptic properties (Sanni, 1993; Ouoba *et al*., 2004). Hence the reason for developing starter culture to initiate fermentation for the production of consistent products with acceptable qualities.

Fermentation of African locust bean and other legumes that involves microorganisms requires little elevation in reaction temperature. An increase in temperature was noticed during the fermentation process at ambient condition (natural fermentation) as shown in Fig. 4.9, the increasing temperature with fermentation duration shows that heat was generated by the process (exothermic) which was probably as a result of the changes in the metabolic activities of the microorganisms. A temperature rise of 20 °C (25 – 45 ^{o}C) was observed between the first and fifth day of fermentation. This corresponds with the work of Hesseltine and Wang (1967) who reported that during the period of fermentation of *Parkia biglobosa seeds to 'Iru',* heat was evolved. The heat generated during the fermentation process probably provided the required temperature conditions for the optimal activity of the proteolytic enzymes, this supported the report of Odunfa, (1985). Odunfa and Adewuyi, (1985), also discovered that the optimum temperature and time required for complete fermentation were between 25 – 45 °C

and less than 72 hour respectively which was very close to the temperature obtained in this work. Results obtained at ambient temperatures may not be accurate since the fermenting vessels might not have been properly insulated, which might result into heat loss through the wall of the fermenting vessels to the environment during the process. Processed African locust bean seed is usually packed into the fermenting vessel immediately after the second boiling, this gives rise to the initial incubation temperature, which favours the growth of the inoculum.

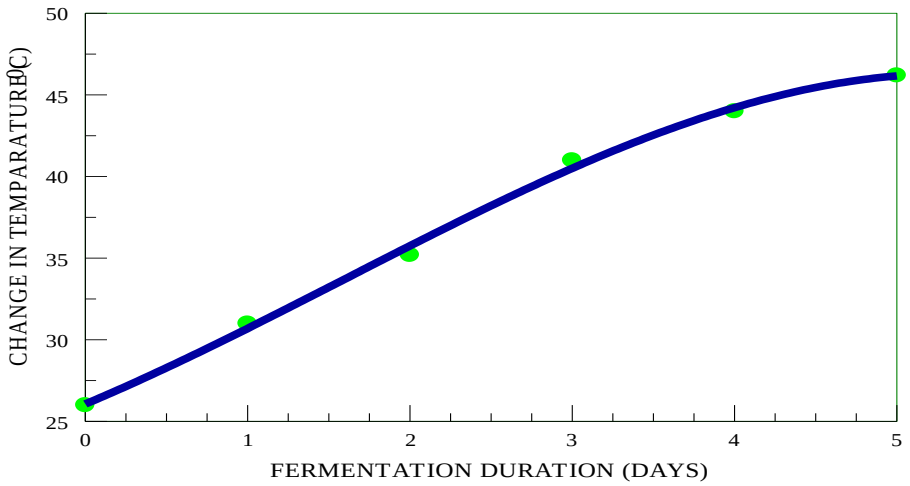


Figure 4.9 - Temperature change during fermentation

4.1.2.1 Effect of Inoculum concentration on pH Values

The concentration of the inoculum used during fermentation process is key to organism's growth and the end product. The effect of inoculum concentration on the resulting fermentation pH is studied in Fig. 4.10 at 40 and 50 ^{o}C. The higher the inoculum concentration, the higher the pH movement towards acidity.

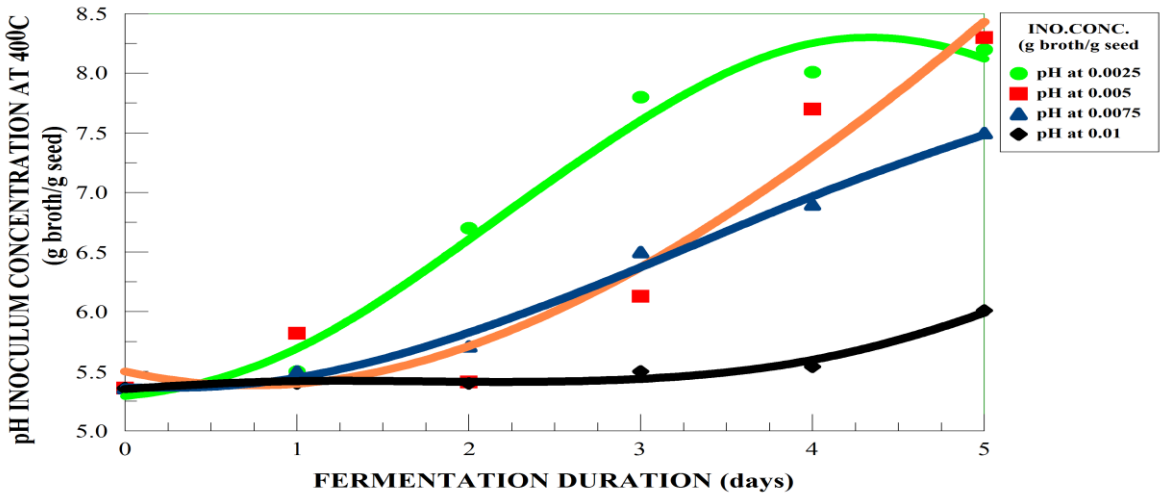


Figure 4.10.1 - Effect of *Bacillus subtilis* concentration on pH of the substrate at 40 °C

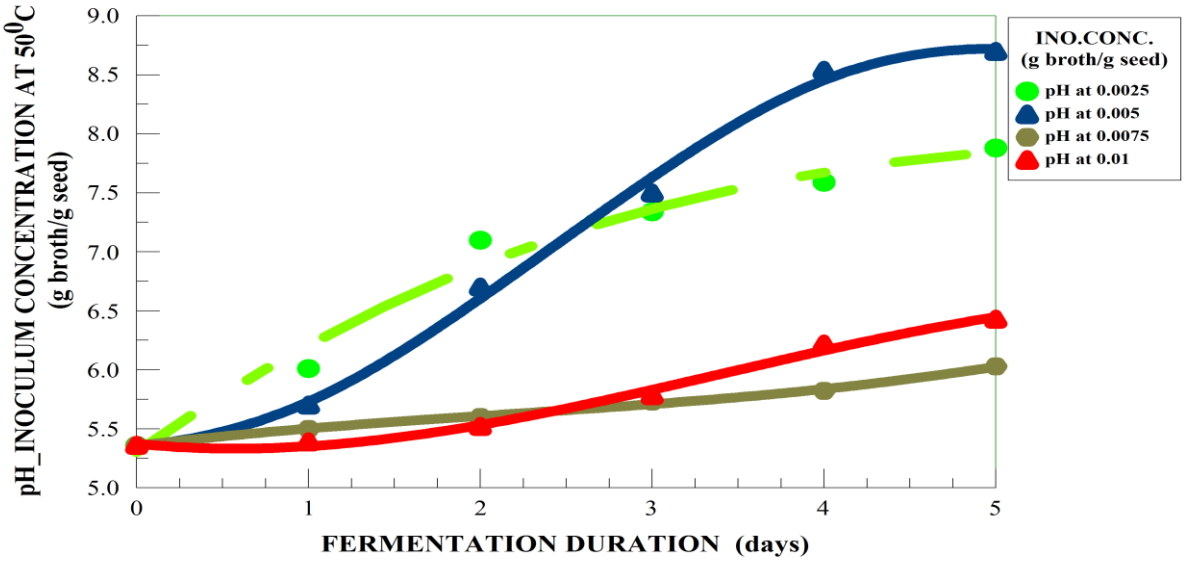


Figure 4.10.2 - Effect of *Bacillus subtilis* concentration on pH of the substrate at 50 °C

All the pH values increased steadily. The 0.01 g broth/g seed quantity has the lowest pH, meaning that the rate of fermentation is very slow, followed by 0.0075 g broth/g seed.

At 50 ºC, a favorable increase or rise in pH occurs at both 0.0025 g broth/g seed and 0.005 g broth/g seed inoculum (Figure 4.10.1). As the duration of fermentation gets closer to the third day, the 0.005 g broth/g seed inoculum led to a much higher rise in pH value a clear evidence that the 0.005 g broth/g seed is the most favorable inoculum concentration. Relative to the results obtained at 40 °C, the lowest pH was obtained at inoculum of 0.0025 g broth/g seed, while 0.01 g broth/g seed of inoculum concentartion yielded the lowest pH values at 40 °C.Lactic acid produced accounted for the rise or increase in pH values. At high pH bacteria slows down and begin to die off, even though their enzymes continue to function.

4.1.3 Carbon Dioxide release Monitoring

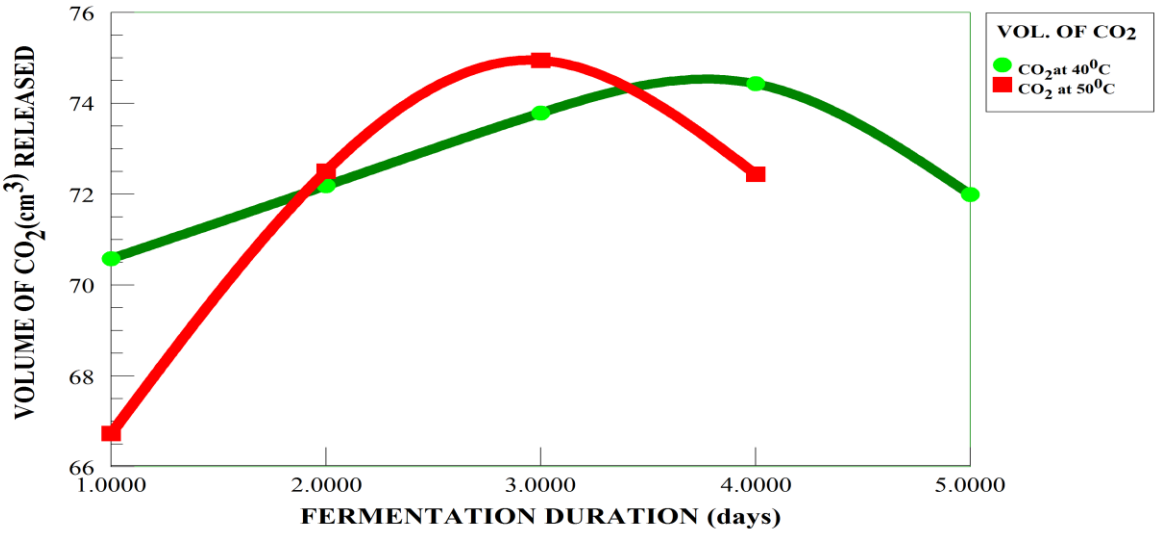


Figure 4.11 - Effect of temperature on the volume of carbon dioxide released (*Bacillus subtilis* Aerobic fermentation)

Figure 4.11 shows the variation of evolved carbon dioxide during fermentation at two different fermentation temperatures. The figure shows that high temperature favours the release of carbon dioxide. On both temperature conditions tested, the evolved carbon dioxide peaked, although at different periods for the temperatures - before the 3rd day for the 50 ^{o}C and the 4th day at 40 ^{o}C.

A decerease in the volume of carbon dioxide released was noticed on the 3rd day of fermentation at 50 ^{o}C fermentation temperature**.**

The fact that evolved carbon dioxide was high at the initial period of fermentation and later decreased after 72 hrs (3 days), suggests that the microorganisms that initiate the fermentation process are most probably the gas-producing *microorganisms*

4.2. Proximate Composition of the African Locust Bean (*Parkia biglobosa*) Seeds

The Proximate composition which is the partitioning of compounds in a feed into categories based on the chemical properties of the compounds was investigated. This is an index to the nutritive value of foods & food ingredients.

This system of food analysis divides the food component into six fractions:

(i) Ash Contents
(ii) Moisture Content
(iii) Crude Protein
(iv) Crude Fibre
(v) Crude Fat or Lipid {Ether extract}
(vi) Carbohydrate

The results for proximate analysis for aerobic and anaerobic fermentation with Inoculum variation under the same fermentation conditions are discussed below.

The values of proximate analysis obtained from this work were compared with those found in the literature. As would be expected, some results were the same while some were slightly lower or higher due to the variation in fermentation conditions, inoculum used and processes.

4.2.1 Aerobic and Anaerobic Fermentation

4.2.1.1 Percentage Ash Content at Various Temperatures

Figure 4.12.1 shows that ash content decreases with numbers of days of fermentation and increases with fermentation temperature. The ash content varies between 2.6 – 4.0 %.

The decrease in percentage ash content was as a result of long hours of boiling, soaking in water and dehulling. A 30 - 40 % reduction was noticed, which implies that the total mineral content of the seeds resides in the hull of the seeds which are leached during processing, Odebunmi *et al*. (2009), Omafuvbe *et al*. (2004). The reduction in percentage ash content may also be due to the utilization of some essential salts during fermentation by microorganisms for their metabolic activities. The loss in Ash may also be due to leaching of soluble inorganic salts into the processing water during soaking and the long hours of boiling of seeds, Esenwah and Ikenebomeh, (2008).

Figure 4.12.2 shows a different pattern in the Ash content. It also supported the decrease but only that the decrease was not steady as in aerobic indicating the behaviour of the inoculum. There was no particular trend for the value of ash of the fermented products. This is similar to the observations made by Omodara and Aderibigbe, (2013).

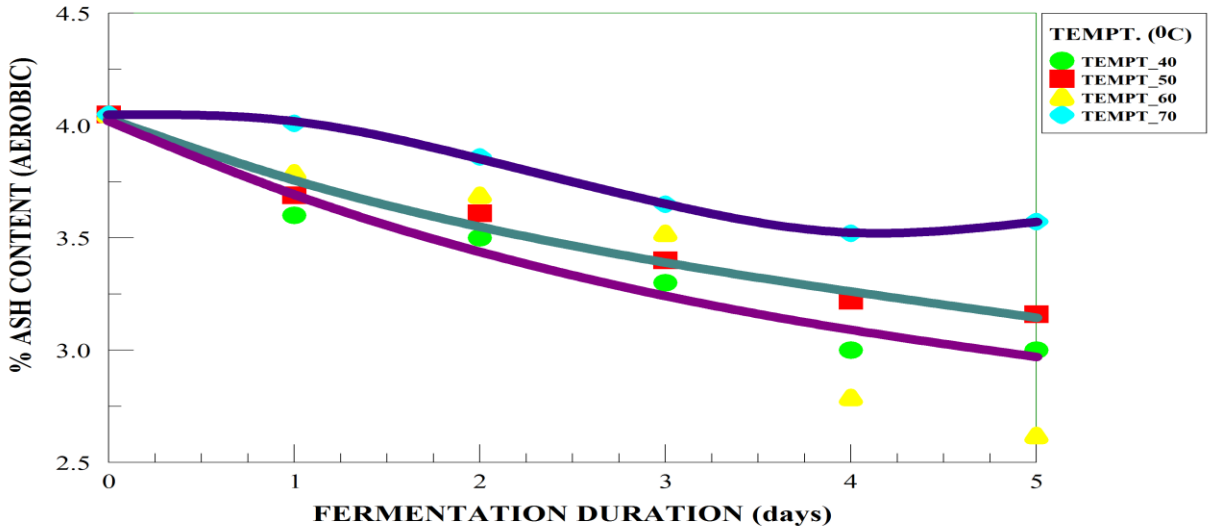


Figure 4.12.1 - Effect of fermentation temperature on percentage Ash content (Aerobic)

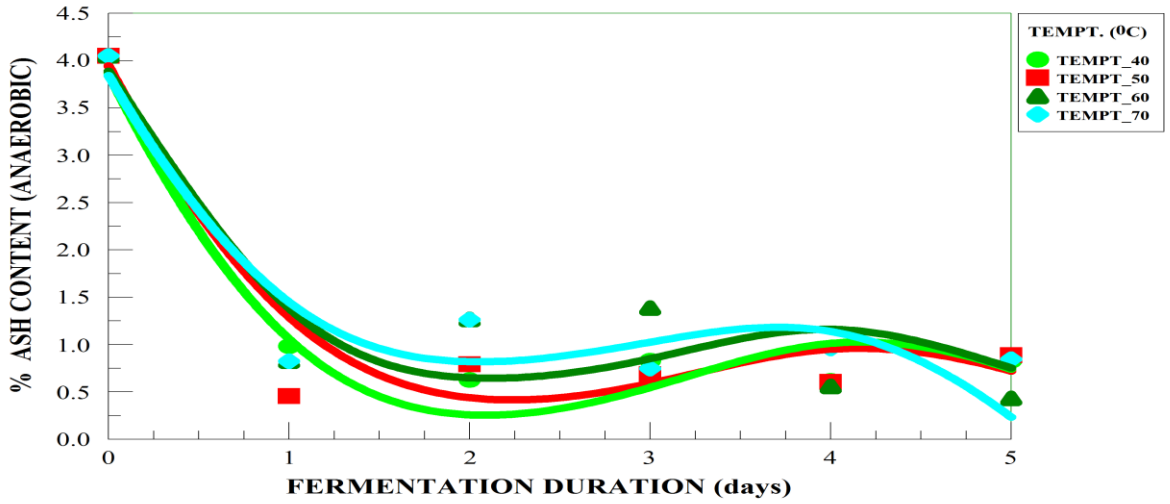


Figure 4.12.2 - Effect of fermentation temperature on percentage Ash content Anaerobic

4.2.1.2 Percentage (%) Moisture Content at various Temperatures

This work confirmed that moisture content increases with respect to fermentation duration. Analysis showed that the initial moisture was 68 % which later increased to 78 % with fermentation duration. The raw unfermented seeds had 9.62 %, while raw cooked unfermented seeds had 56.33 % moisture content. Figure 4.13.1 shows that moisture content increased with fermentation duration. The higher the fermentation temperature, the higher the moisture content. The increase was probably due to the water used in subsequent processing such as soaking, dehulling and boiling of the raw seeds prior to fermentation processes. Omodara and Aderibigbe (2013) and Omafuvbe *et al*. (2004) reported that the metabolic activities of some microorganisms during fermentation time gives out moisture as one of their end products.

Increase could also be due to the activities of the inoculum on the fermented sample as a result of extracellular enzymes production (Omafuvbe *et al*., 2004).

In Anaerobic fermentation (Figure 4.12.2, the results shows that increase in temperature does not correlate to increasing moisture content unlike in Aerobic condition. It is observed that the moisture content was reduced as temperature increases. The moisture contents at temperatures 50 and 70 are very close to each other. *Bacillus* functions well at a minimum of 18 and a maximum of 45 °C temperature.

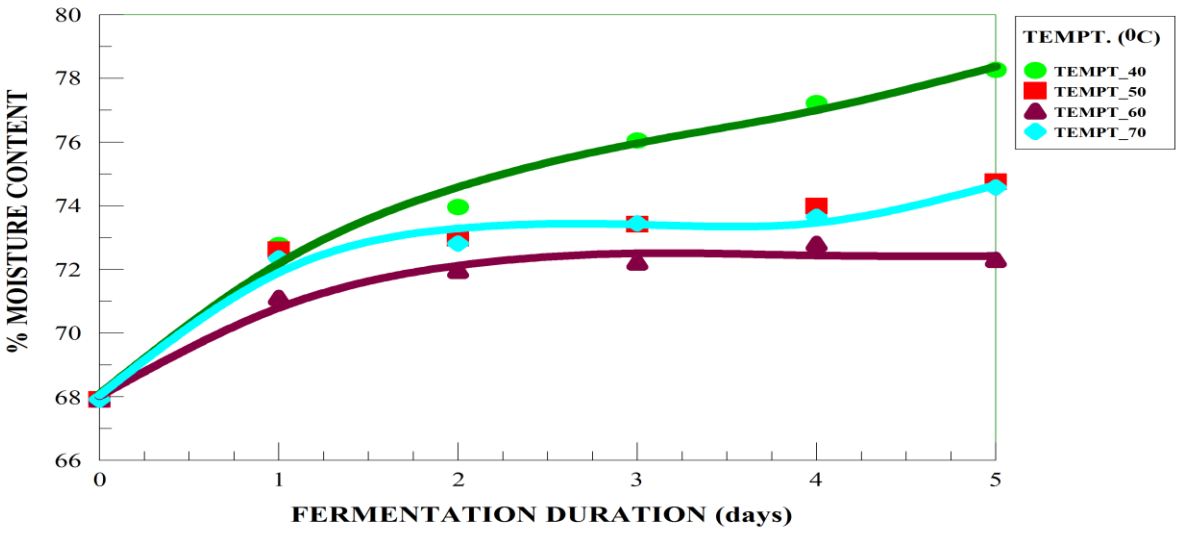


Figure 4.13.1 - Effect of fermentation temperature on percentage Moisture content (Aerobic)

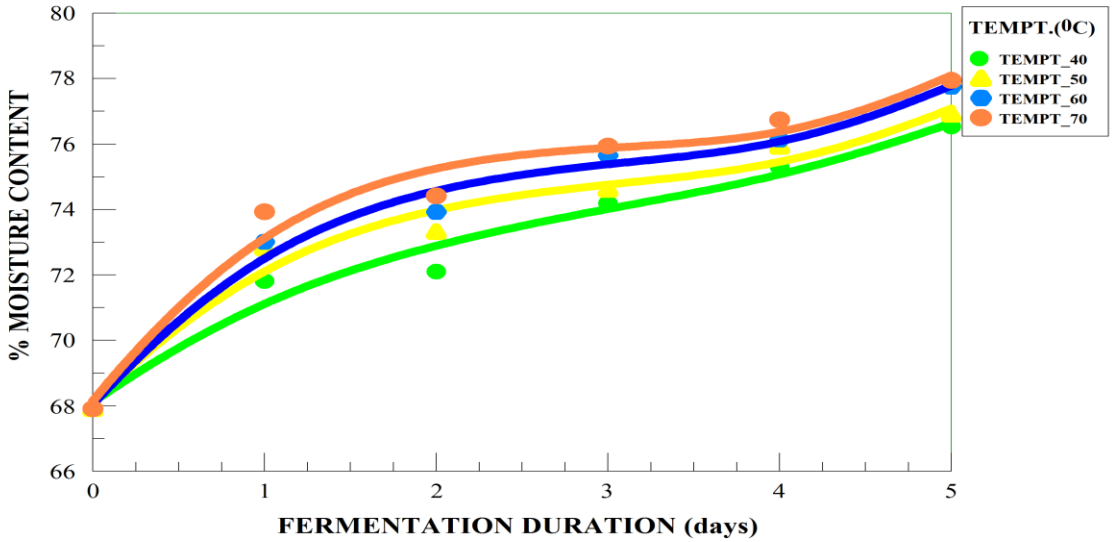


Figure 4.13.2 - Effect of fermentation temrerature on percentage Moisture content (Anaerobic)

4.2.1.3 Percentage (%) Crude Protein Content at various Temperatures

Literature revealed that fermentation of African locust bean seeds increases the protein content which is the major reason while the fermented seed is being consumed by man. Other reason is the aroma it gives to food as a condiment in soup. The results from this work at 40 ^{o}C and 50 ^{o}C Figure 4.13.1 revealed that fermentation increased protein content of African locust bean seeds with respect to duration. Protein content increased from the first day and started decreasing at the third day showing that fermentation should be stopped on the third day. As discussed earlier *bacillus subtilis* functions well at lower temperature (40 – 50 °C).

Achi (2005) and Campbell-Platt (1987) reported that African locust bean does not come out well if fermentation is allowed to continue for more that 3 days, this encourages the fermented media to be suitable for the growth of yeast. Over-fermentation generates unacceptable levels of volatile fatty acids. This explains why all the samples examined after the third day of fermentation were not physiologically acceptable.

Higher temperature denatures both the enzymes responsible for the fermentation while the protein structure coagulates. At 60 – 70 ^{o}C the protein content did not show any appreciable increase with fermentation duration. The higher percentage of protein recorded in the fermented African locust bean seeds may be as a result of reduction in total carbohydrate content during fermentation. This is in agreement with the result of Ene- Obong and Obizoba, (1996). The increase in percentage protein can also be due to the reduction in percentage ash and crude fibre contents. Since *Bacillus substilis* have been reported to be proteolytic in nature, this contributed to high protein content in 'Iru'. The increase could also be attributed to microbial growth of cells by multiplication of parts in forms of single cell protein, Oboh (2006). The anaerobic fermentation of "iru" is shown in Figure 4.14.2. The best results were obtained at between 40 and 50 °C and this correlates with other work done on this particular seeds. Although some resercher had reported *bacillus* to be strictly aerobic (obligate aerobes) meaning

that they will require oxygen for their growth, however, recent reports showed that they can actually grow in anerobic environment if the condition is favourable making them to be facultative aerobic organisms, Nakano *et al.,* (1998) reported *bacillus* to have 18 and 43 °C as the required minimum and maximum temparature for optimal growth. Other reserchers reported 5 - 15 °C and 40 – 50 °C as the minimum and maximum temparature for their growth. Datas in figure 4.14.2 confirmed that *Bacillus subtilis* is a facultative organism which is expected to thrive a little between 40 -50 °C and thus yielding very low percentage protein content that decreased with both fermentation days and temperature. Due to the low yield in protein (major concern in this work) anaerobic fermentation is not encouraged to be used in the production of 'Iru'.

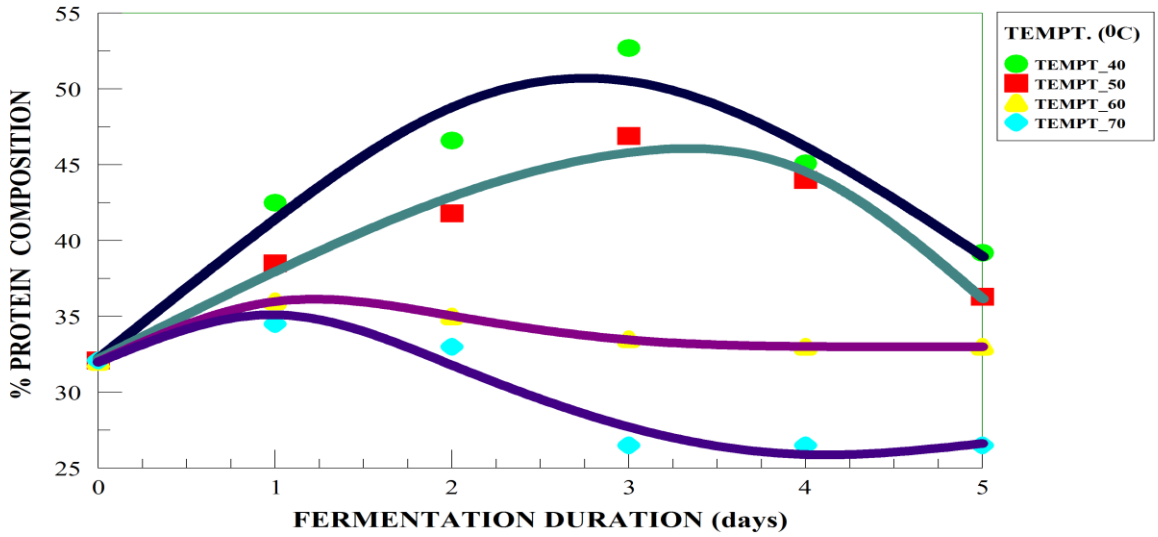


Figure 4.14.1 - Effect of fermentation temperature on percentage Crude Protein content (Aerobic)

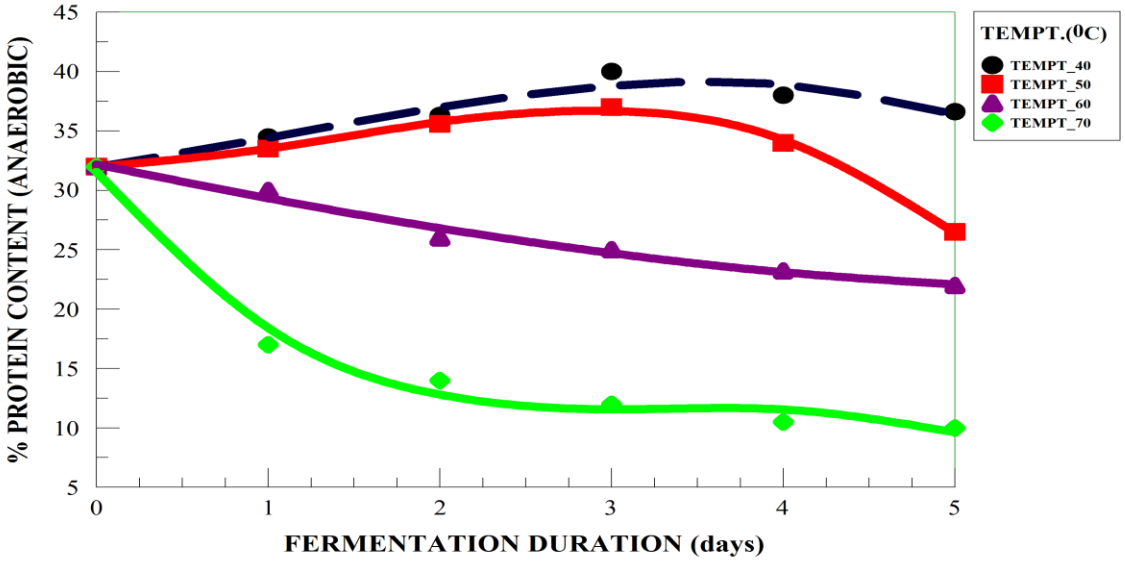


Figure 4.14.2 - Effect of fermentation temperature on percentage Crude Protein content (Anaerobic)

4.2.1.4 Percentage (%) Crude Fibre Content at various Temperatures

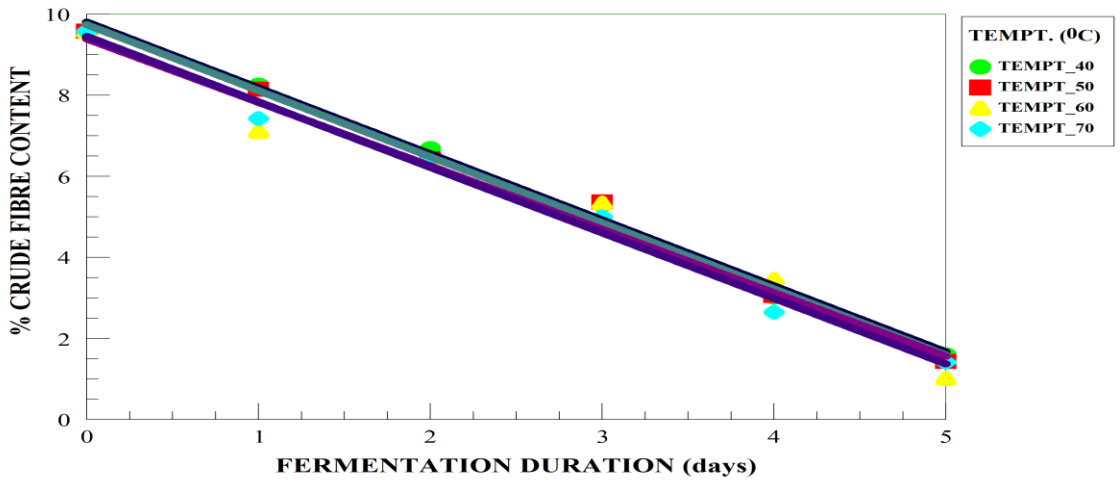


Figure 4.15.1 - Effect of fermentation temperature on percentage Crude Fibre content (Aerobic)

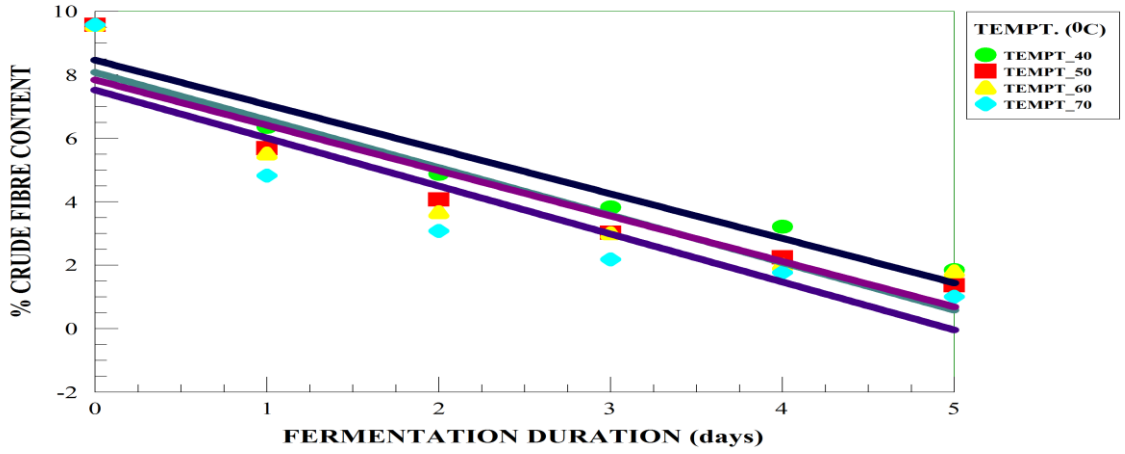


Figure 4.15.2 - Effect of fermentation temperature on percentage Crude Fibre content (Anaerobic)

Figure 4.15.1 shows that fermentation reduced the percentage crude fibre of the seeds with respect to fermentation duration. This is in concordance with the general claims in literature. Temperature seems to have minimal or no impact in the Crude fibre of African locust bean seeds during fermentation. The pattern reduces progressively, showing that there is a consistent decrease in the percentage crude fibre content. Increase in temperature leads to the decrease in the percentage crude fibre.

Figure 4.15.2 for anaerobic fermentation shows a lower percentage crude fibre content resulting from the fermentation process. This suggests that fermentation reduces crude fibre irrespective of the fermentation condition. There was also a steady decrease in the percentage crude fibre during aerobic fermentation as seen in Figure 4.15.2 with respect to time. The percentage crude fibre content reduces with an increase in temperature.

4.2.1.5 Percentage (%) Crude Fat Content at Various Temperatures

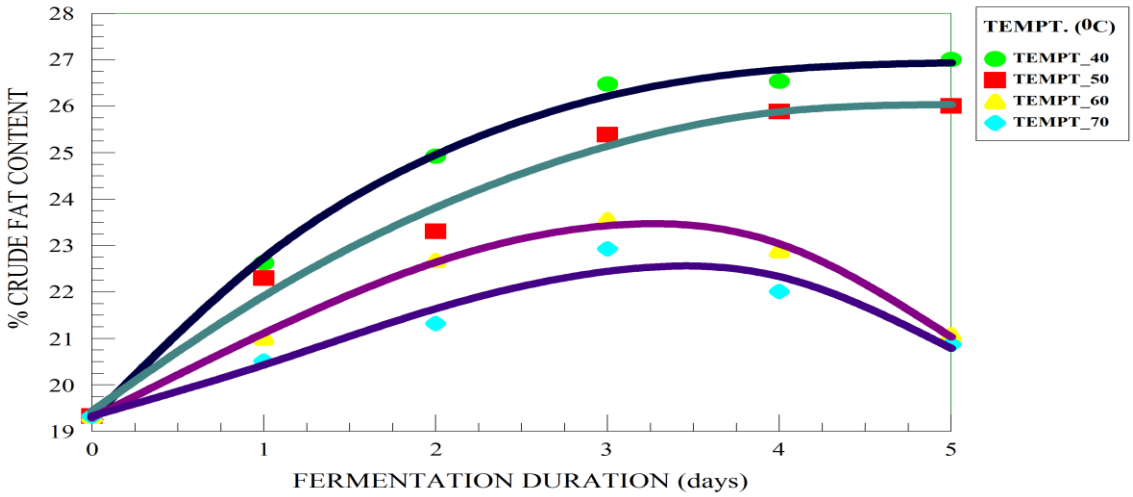


Figure 4.16.1 - Effect of fermentation temperature on percentage Crude fat content (Aerobic)

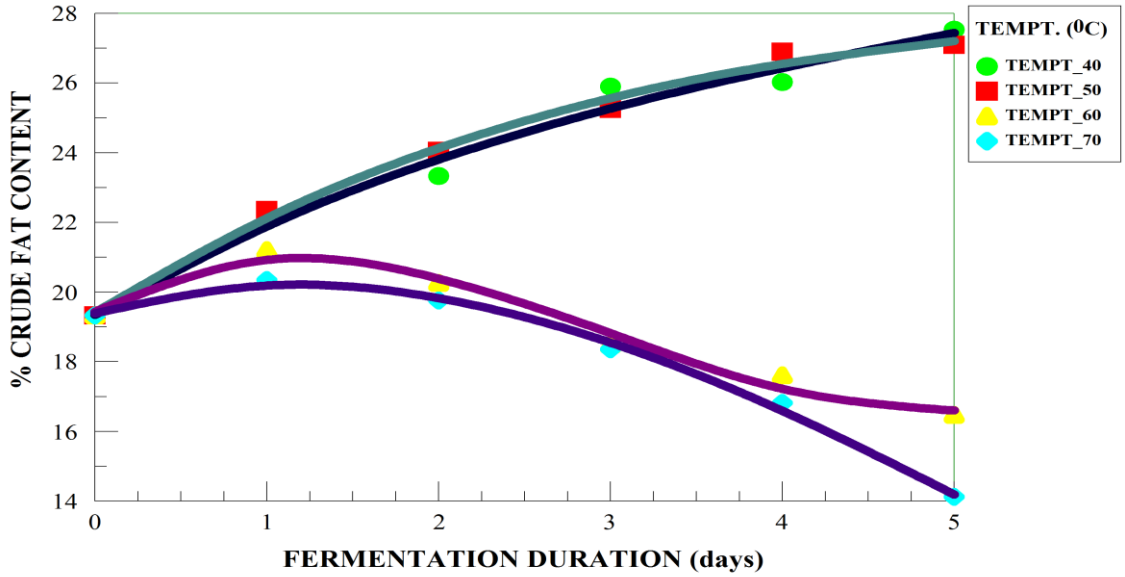


Figure 4.16.2 - Effect of fermentation temperature on percentage Crude fat content (Anearobic)

Oil was noticed at the walls of the vessels used for the cooking of African locust bean seeds and on top of the boiling water during processing, which show that the seeds contain fat or oil. This infers that long hours of cooking brings out the oil in seeds through the fractionating intact part of the oil bodies and rupturing of cellular structure. This work reported 17.33, 22.63, and about 26.45 % of crude fat for the raw, cooked and optimized fermented samples respectively at the optimum temperature of 40 °C, this was in agreement with the report of Alabi *et al*., 2005; Omodara and Aderibigbe, 2003 and Omafuvbe *et al.,* 2004. The low fat content at higher temperature would have been a good advantage if the end products had been acceptable because the fermented samples would have been able to stay for longer periods without spoilage through rancidity, which is one of the characteristics of many legumes. The increase in the percentage fat content was due to some organic constituents of the fermenting seeds which becomes soluble as a result of heat treatment involved in the processing into "Iru".

The increase in fat content of the fermented African locust bean seeds can also be attributed to the increase in the activities of lipolytic enzymes, which hydrolyses fat to glycerol and fatty acid, Obizob and Atii (1991) intimated a similar work.

Figure 4.16.1 shows that high fermentation temperature does not favour the percentage fat content, hence temperature should not exceed 40 – 50 °C for optimum yield.

Figure 4.16.2 shows that lower temperature favours fermentation of fat production while higher temperature does not. Two distinct clearance was seen in between the first two lower temperature 40 and 50 °C and the last two higher temperature. This confirmed that *Bacillus subtilis* actually formed stress resistant endospores. The percentage fat content decreases progressively with fermentation duration and temperature. High temperatures caused changes in unsaturated fatty acids, this accounts for the low pH value with an increase in temperature indicating that samples are turning towards acidic medium. This work concluded that further

increase in fermentation temperature led to a gradual denaturation of the enzyme, which invariably led to decrease in microbial activities.

4.2.1.6 Total Carbohydrate Content at various Temperatures

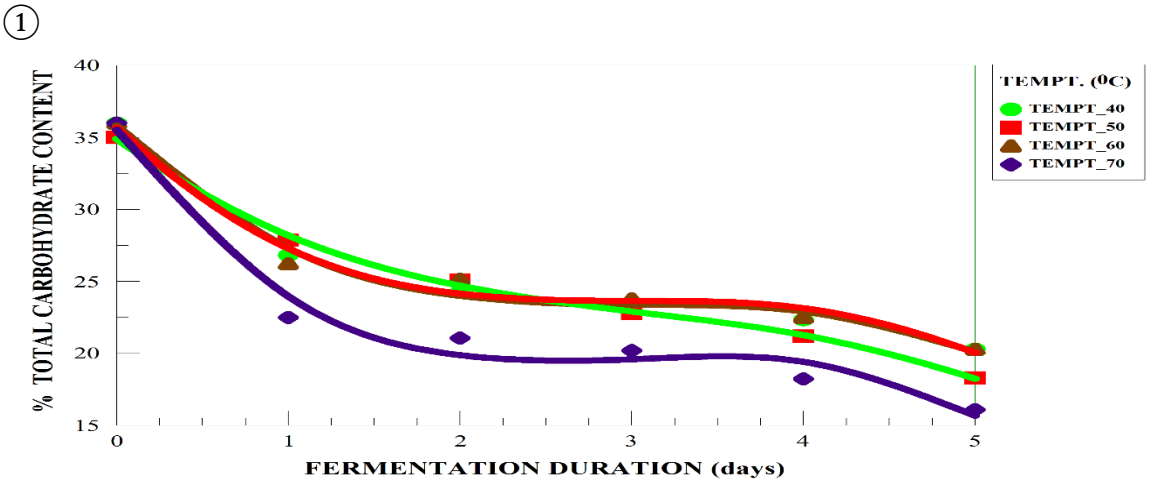


Figure 4.17.1 - The effect of fermentation temperature on total carbohydrate content (Aerobic)

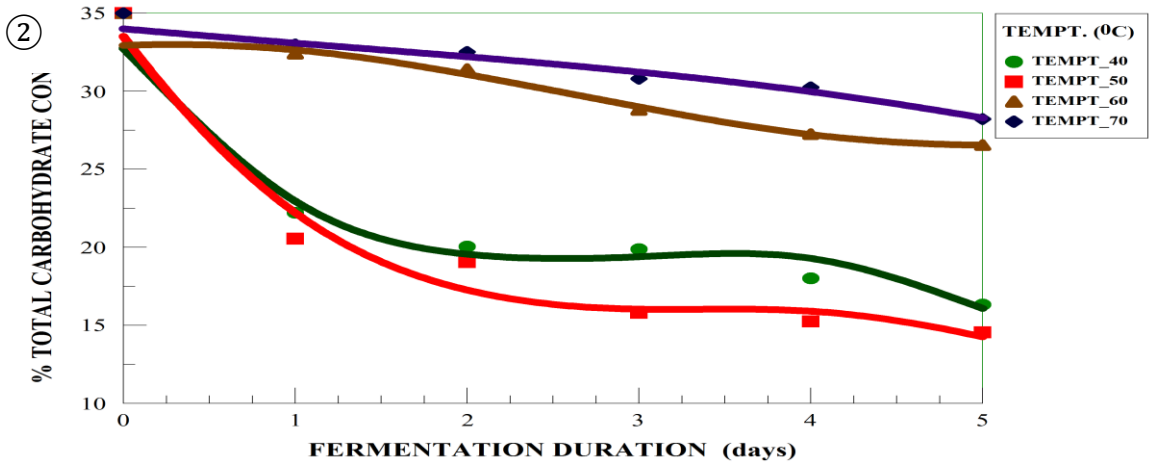


Figure 4.17.2 - The effect of fermentation temperature on total carbohydrate content (Anaerobic)

Since carbohydrate is the main component in the substrate that is fermented, it is been consumed by the fermenting organism as source of energy and growth, hence decrease is expected. Processing of seeds decreased the total carbohydrate content significantly, during boiling and dehulling which further reduced with respect to fermentation duration.

The loss in carbohydrate during soaking and boiling is attributed to the leaching of soluble carbohydrates like sugars into the cooking water and soaking, slippery water was noticed after boiling as a result of this. Loss in carbohydrate during fermentation may also be as a result of the fermenting organisms utilizing some of the sugar for growth and metabolic activities, (Esenwah and Ikenebomeh, 2008).

The remarkable reduction in the total carbohydrate content can also be related to the hydrolysis of starch to simple sugars during the long cooking period which lasted for 10 hours, the longer the boiling time the lower the carbohydrates and every other nutrient which can easily be leached into the boiling water or soaking. Hydrophilic molecules in carbohydrate takes up moisture in proportion to the relative humidity of the environment, this also accounted for the high moisture content in both boiled and fermented seeds of African locust bean (Ihekoronye and Ngoddy, 1985). This characteristic behaviour encouraged moisture uptake and apparent reduction in percentage of carbohydrate, (Akinoso *et al.* 2011a).

Figure 4.17.2 also followed similar pattern as in figure 4.17.1, except for a higher total carbohydrate value, this may be as a result of decrease in all other parameters monitored, hence little conversion of sugar by the microorganisms responsible for the fermentation activities was noticed. The higher the temperature the lower the conversion of carbohydrate to sugar. The value obtained at the end was getting close to the value reported for the raw sample of African locust bean seeds, since it has passed the maximum operating condition (below 50 ^{o}C), indicating that little or no conversion was going on, hence reason for poor unacceptable end products at elevated temperatures. Nevertheless, fermentation was noticed.

4.2.2 The Proximate Analysis of the Inoculum Concentration (*Bacillus subtilis*)

Varying (0.0025 – 0.01 g broth/ g seed) concentrations of *Bacillus subtilis* were used for the fermentation of African locust bean seeds to 'Iru' for 5 days at various temperatures between 40 – 70 ^{o}C

4.2.2.1 The Percentage (%) Ash Content

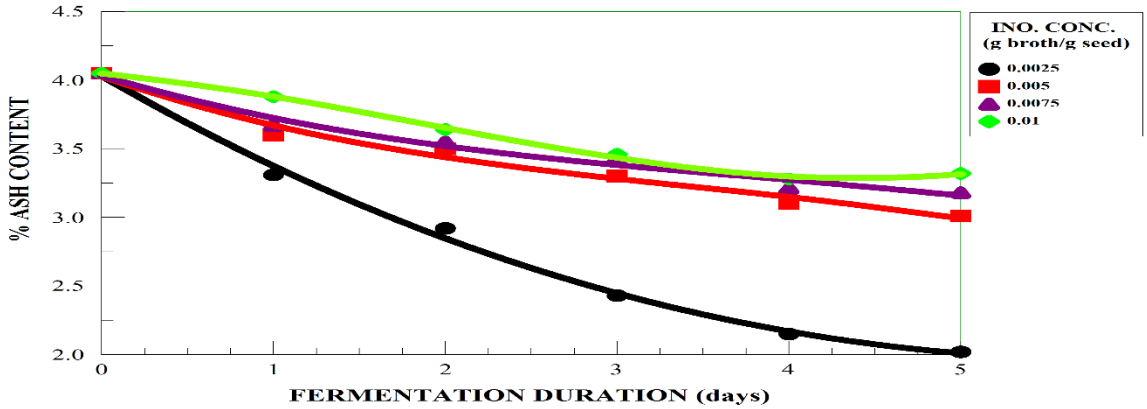


Figure 4.18.1 - Effect of Inoculum concentration on percentage Ash Content at 40 °C

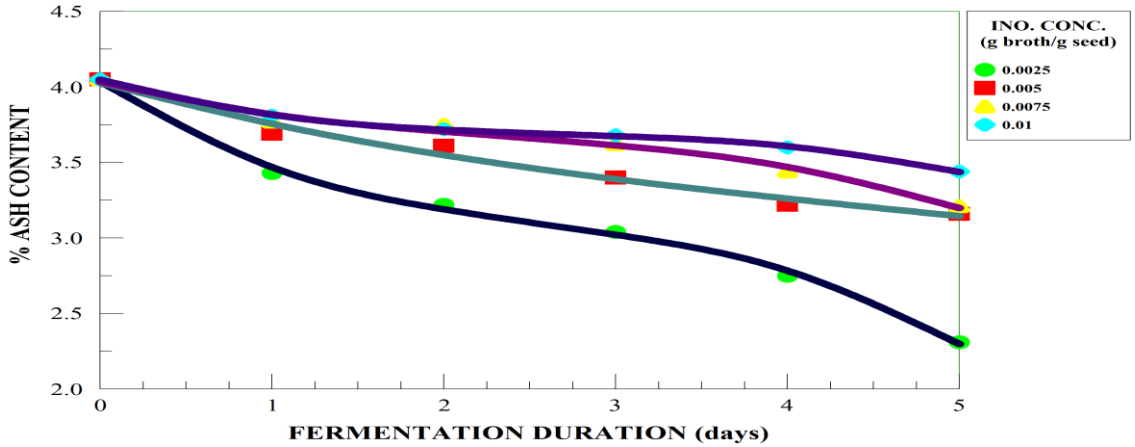


Figure 4.18.2 - Effect of Inoculum concentration on percentage Ash Content at 50°C

The ash content using different inoculum concentration decreases with respect to fermentation days at temperatures 40 and 50 °C. However the result obtained for concentrations 0.005 g broth/g seed at both temperature 40 and 50 °C fell within the range reported by Omodara and Aderibigbe, 2013 and Soetan, 2014, which is 4.81 - 2.22.

4.2.2.2 The Percentage (%) Moisture Content

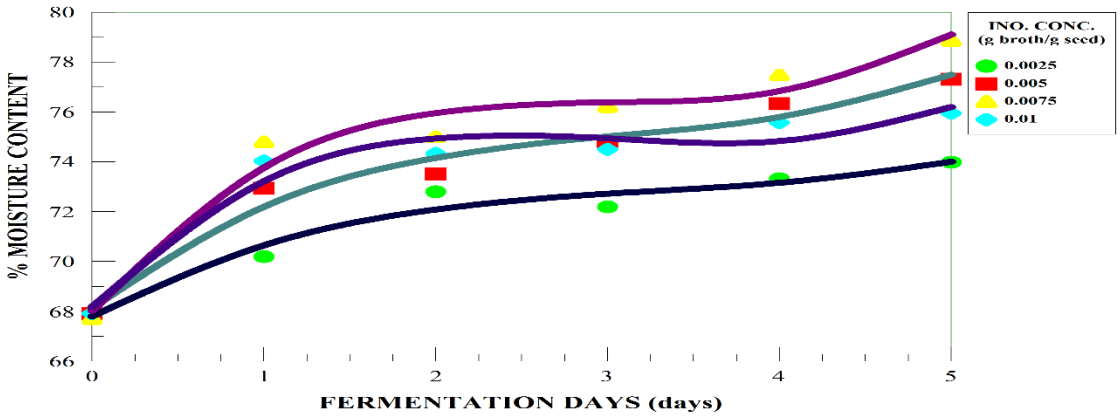


Figure 4.19.1 - Effect of Inoculum concentration on percentage Moisture Content at 40 °C

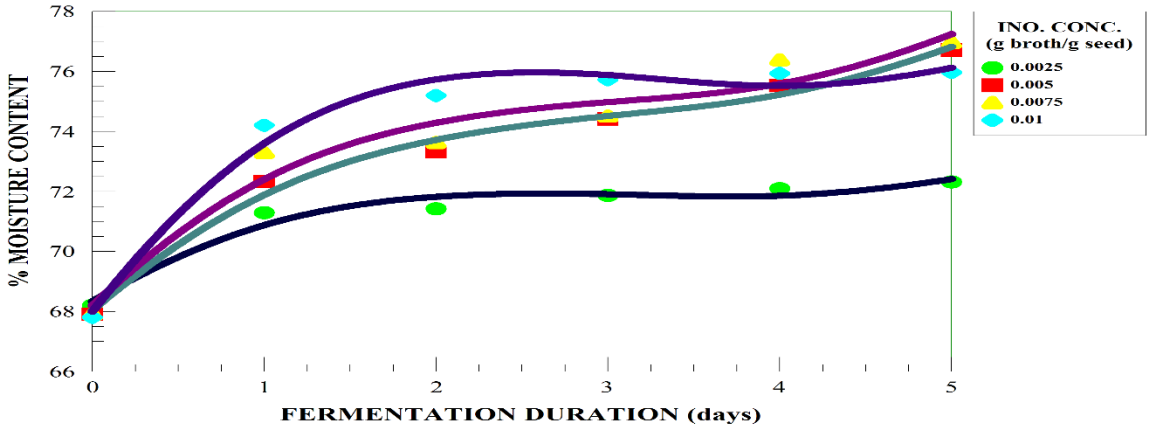


Figure 4.19.2 - Effect of Inoculum concentration on percentage Moisture Content at 50°C

This has been discussed earlier. Figure 4.19.1 corresponded with all the research work carried out on moisture content and confirmed that moisture content increased with respect to fermentation days. The figures above shows that moisture content increased with fermentation duration although not too consistent, especially for inoculum concentration 0.01 g broth/g seed for both 40 and 50 °C fermentation condition. The higher the temperature the lower the moisture content, but moisture content increases with fermentation duration. It has been explained earlier that the increase was due to the water used in subsequent soaking, dehulling and boiling in water of the raw seeds prior to fermentation processes which was supported by Omodara and Aderibigbe, 2013 and Omafuvbe *et al*., 2004 who added that the metabolic activities of some microorganisms during fermentation time gives out moisture as one of their end products.

4.2.2.3 The Percentage Crude Protein Content

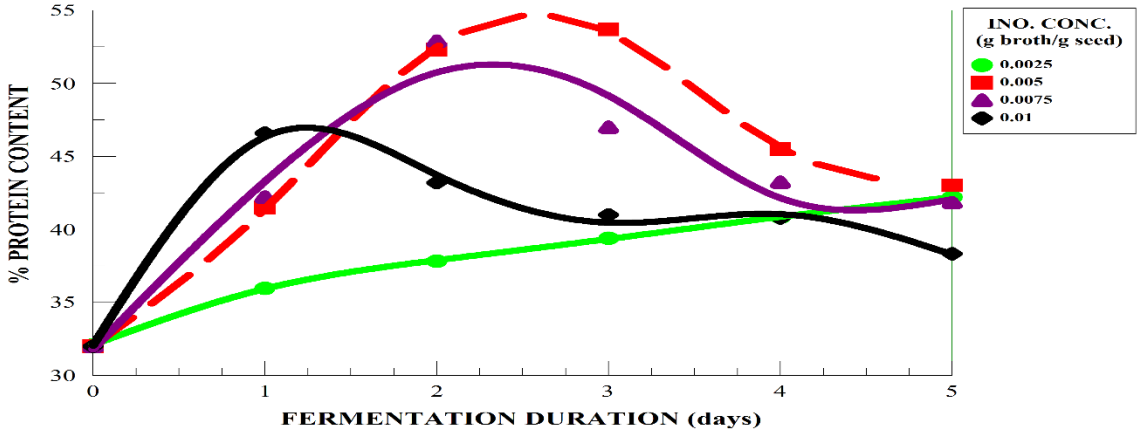


Figure 4.20.1 - The effect of Inoculum concentration on percentage crude Protein Content at 40 °C

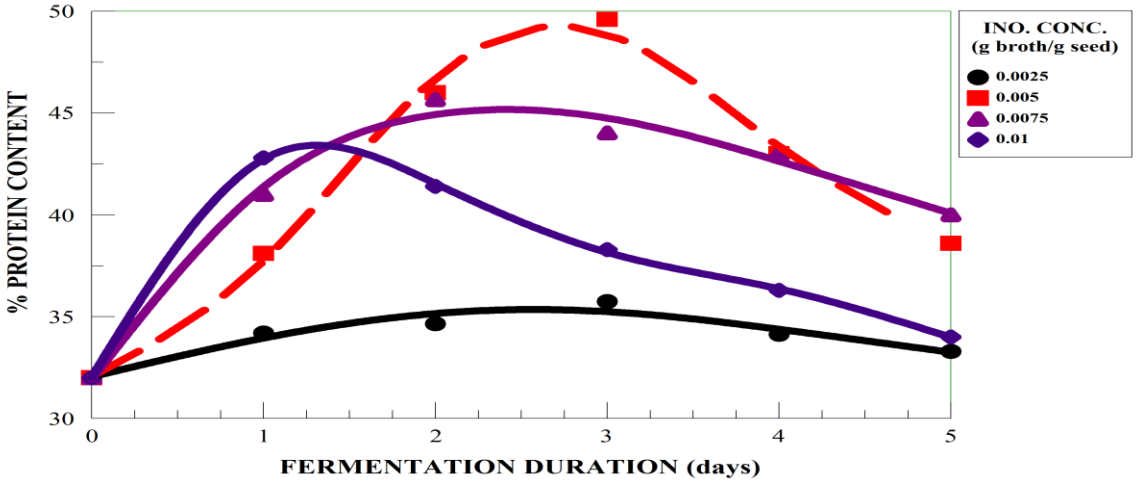


Figure 4.20.2 - The effect of Inoculum concentration on percentage crude Protein Content at 50 °C

Figures 4.20.1 and 4.20.2 show an increase in the percentage protein content with respect to fermentation duration. However, the increase in protein content reached a maximum and started to decline. The fermentation duration at which maximum protein content was achieved varied with organism concentration. The decline in protein content was noticed to occur after the third day for concentrations of 0.005 and 0.0075 g broth/g seed, while such maximum occurred after the first day for the 0.01 g broth/g seed concentration. Based on this, it is suggested that fermentation should not exceed the third for optimal production of "Iru". Highest yield of protein was discovered at concentration 0.005, followed by 0.0075, 0.01 and lastly 0.0025 g broth/g seed. The 0.01 and 0.0075 g broth/g seed, concentration end product were not physiologically and organoleptically acceptable due to the offensive odour and bad colour. This confirmed the report of Achi (2005) and Campbell-Platt (1980) that over fermentation exposes the fermenting medium to yeast growth and generates unacceptable levels of volatile fatty acids. As reported earlier, higher temperature denatures the protein structure by coagulating them.

They all reached their peaks at different days, 0.01 g broth/g seed concentration of *B. subtilis* had the highest protein production at the first day, 0.0075 g broth/g seed at second day with some hours, 0.005 g broth/g seed, which gave the highest yield of protein at the third day while 0.0025 g broth/g seed had no peak, but increased steadily. They all later decreased at the fourth and fifth days showing that the organism responsible for the fermentation had reached it maximum operating conditions and they are already been stressed which led to them forming a stress – resistant endospores.

The role and survival of *Bacillus subtilis* during the fermentation of African locust bean seeds to protein condiment – Iru was explained by their proteolytic and thermotolerant properties.

Conclusively, optimal fermentation condition for the production of this seeds in order to get the highest protein content required is 72 hours (3 days) and at 40 °C with 0.005 g broth/g seed.

4.2.2.4 The Total Carbohydrate Content

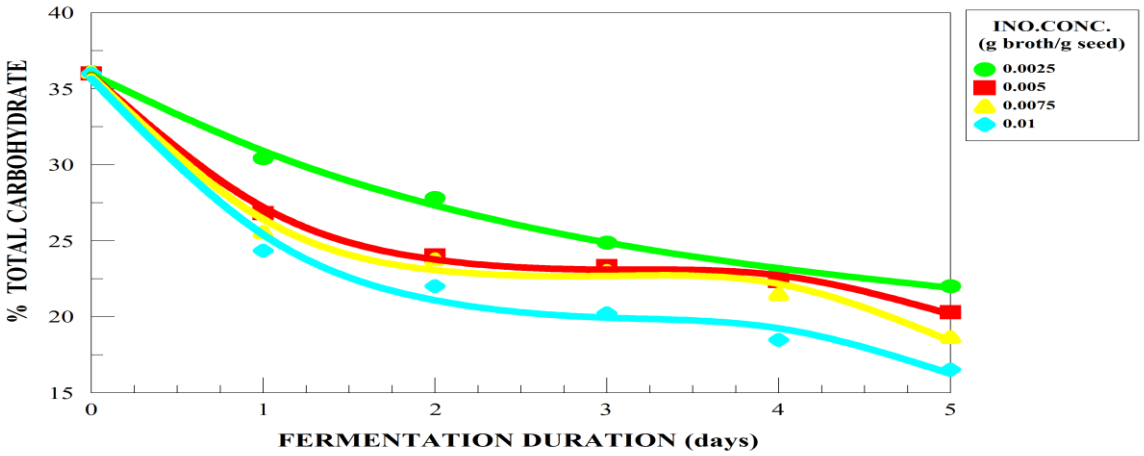


Figure 4.21.1 - The effect of Inoculum concentration on % Total Carbohydrate Content at 40 °C

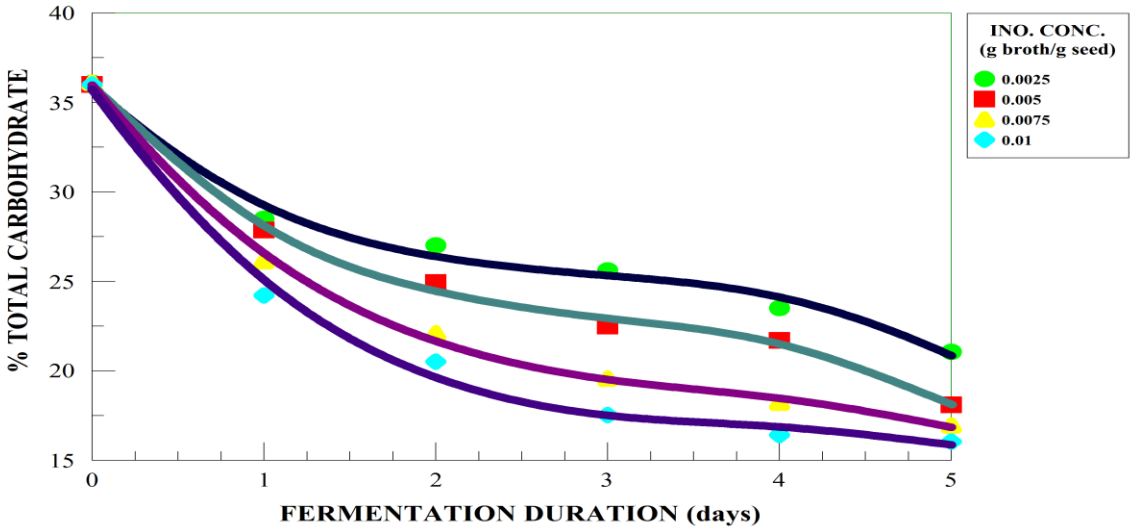


Figure 4.21.2 - The effect of Inoculum concentration on % Total Carbohydrate Content at 50°C

Figures 4.21.1 and 4.21.2 show a decrease in carbohydrate with increase in inoculum concentration. The decrease shows that the rate of conversion into sugar is slow but steady. The reason for the decrease had been discussed earlier. The quantity of the organism added was not enough for the conversion, thereby slowing down the rate.

4.2.2.5 The Percentage Crude Fibre Content

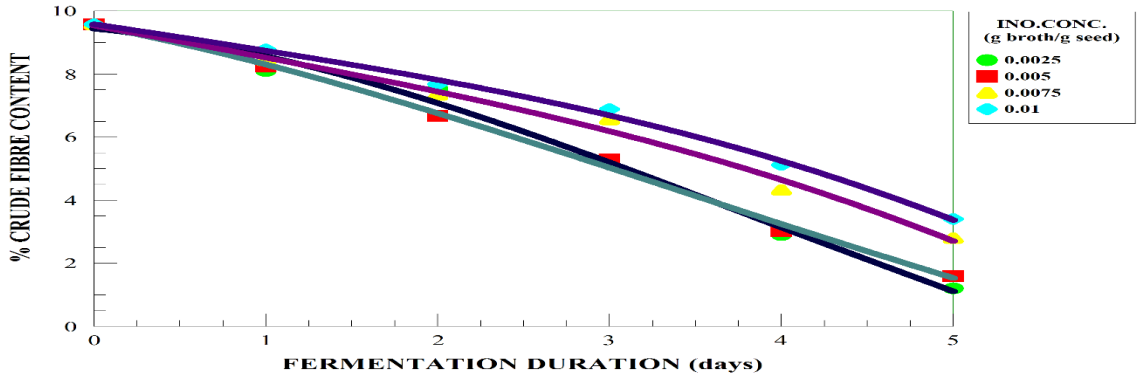


Figure 4.22.1 - The effect of Inoculum concentration on percentage Crude fibre Content at 40 °C

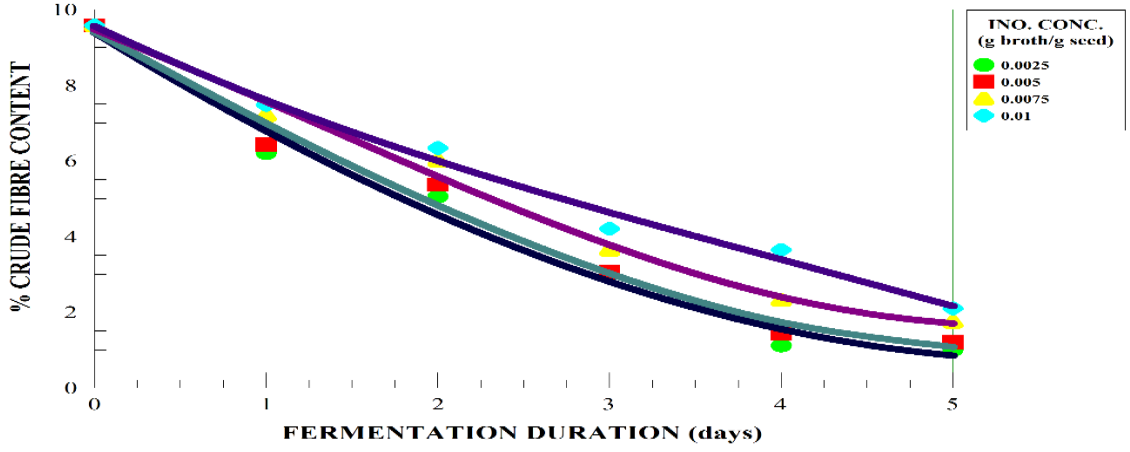


Figure 4.22.2 - The effect of Inoculum concentration on percentage Crude fibre Content at 50°C

Fermentation decreases the percentage crude fibre of the substrate, this has been discussed earlier.The more the concentration of the inoculum, the higher the percentage crude fibre with respect to fermentation days.

4.2.2.6 The Percentage (%) Crude Fat Content

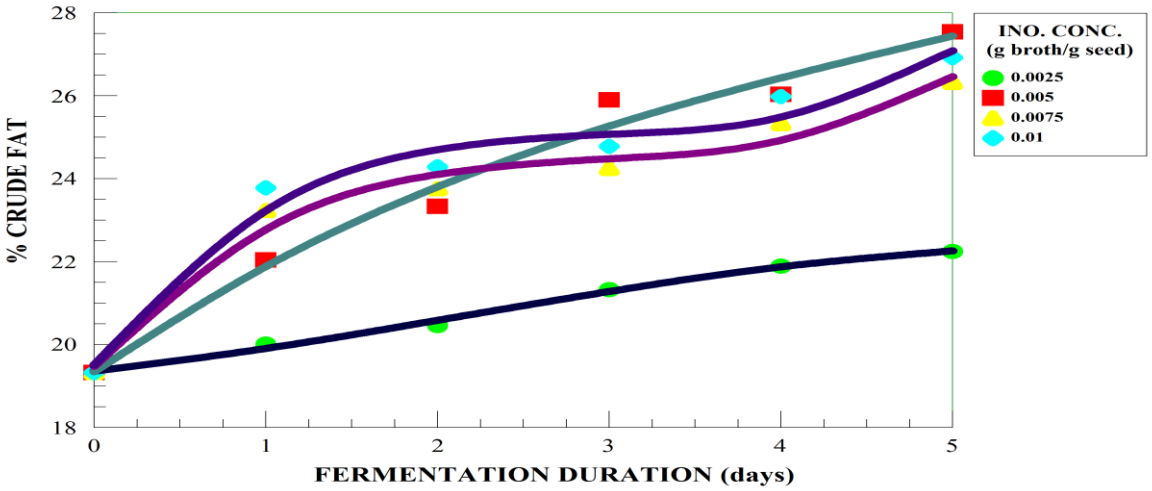


Figure 4.23.1 - The effect of Inoculum concentration variation on percentage Fat Content at 40 °C

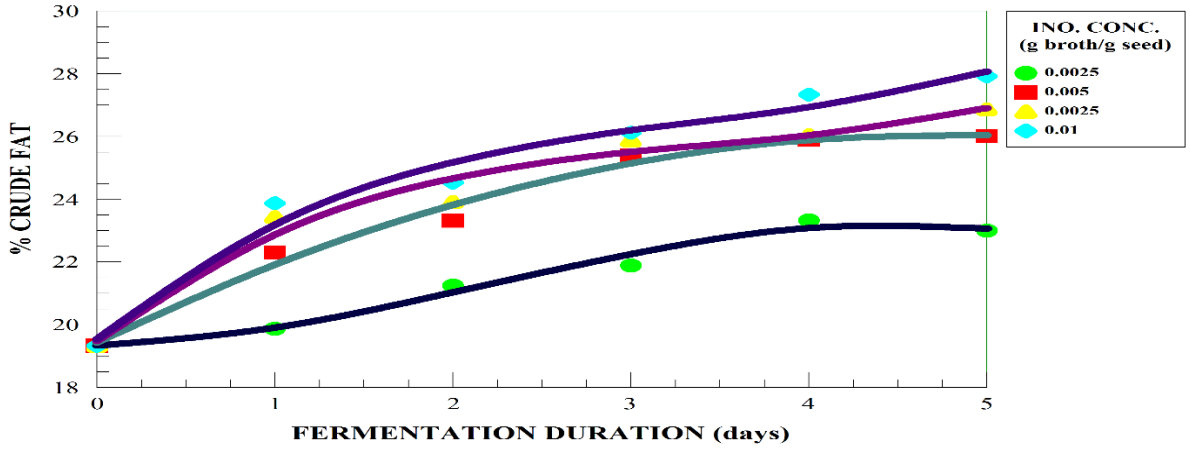


Figure 4.23.2 - The effect of Inoculum concentration variation on percentage Fat Content at 50 °C

Figures 4.23.1 and 4.23.2 show that fermentation increase the fat content in the fermenting substrate which is in concordance with the earlier reports. The addition of inoculum further increases it at higher concentrations. Concentration followed similar trend.

4.2.3 The Proximate Analysis of Inoculum Variation

(Natural, *Saccharomyces cerevisiae*, *Bacillus subtilis* and the mixture)

Two different microorganisms and the mixture were used for the fermentation of African locust bean seeds for 5 days. The results obtained were compared with the commercial (purchased) samples.

The optimum fermentation conditions have been established earlier, therefore temperature 40 ^{0}C only was used and the products analysed.

The proximate analysis of *Bacillus subtilis* have been discussed earlier under aerobic fermentation condition. The figure below shows the result obtained for the proximate analysis using *S. cerevisiae* at temperature 40 and 50 °C since its maximum operating condition is within this range. The mixture of the two inocula was also considered and reported. These two temperature were considered since *B. subtilis* has an optimum temperature between 40 and 50 °C.

4.2.3.1 Percentage (%) Protein Content

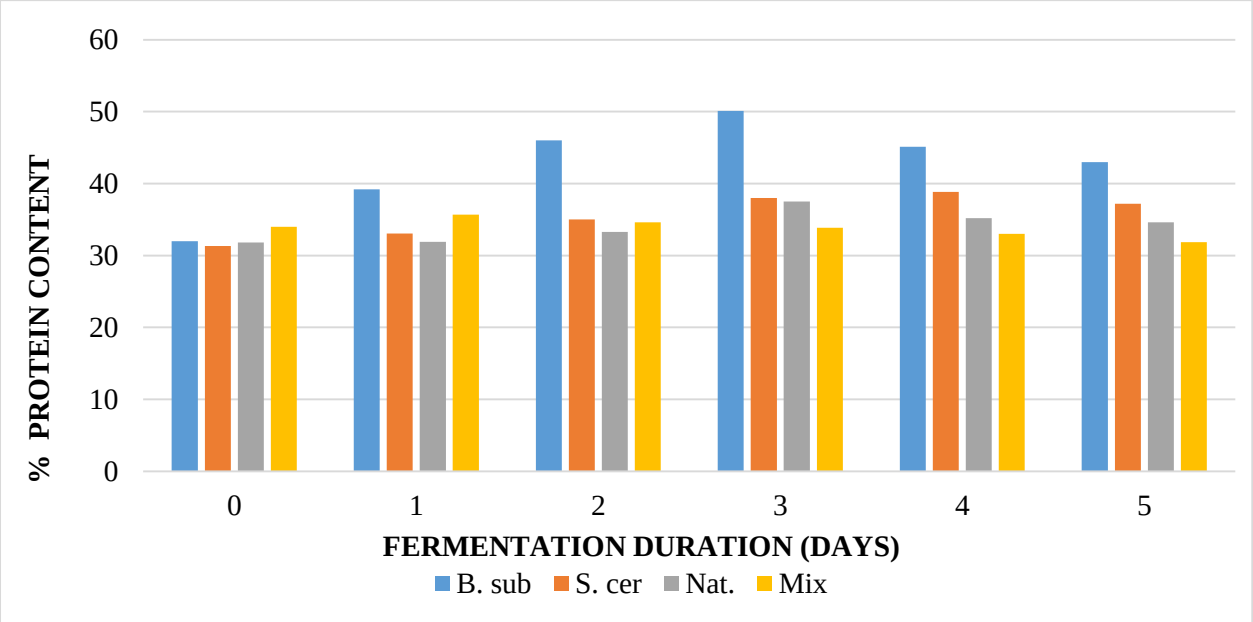


Figure 4.24.1 - Effect of Inoculum variation on percentage crude Protein Content at 40°C

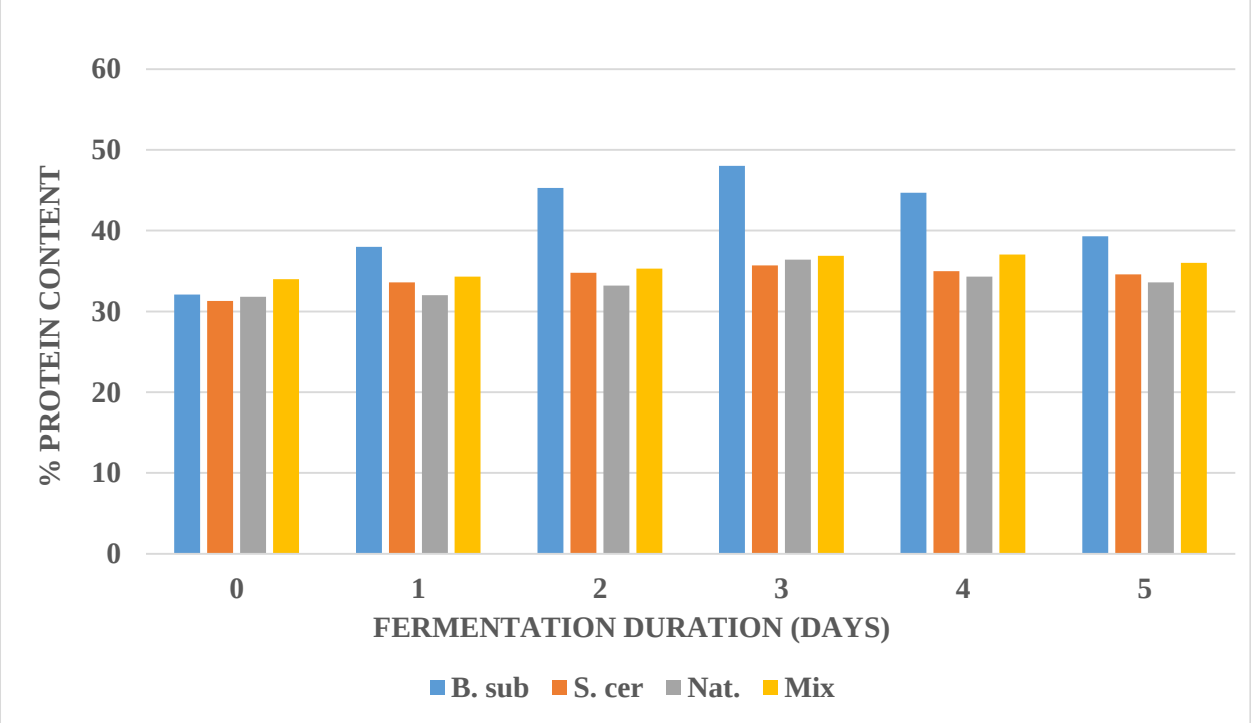


Figure 4.24.2 - Effect of Inoculum variation on percentage crude Protein Content at 50°C

Figures 4.24.1 and 4.24.2 are in agreement with previous work done on the selection of starter culture for the fermentation of African locust bean (Omafuvbe *et al*. 1999; Ouaba *et al*. 2004, 2002, 2003; Diawara *et al*., 1992). Various research work had been carried out on the isolation of the organism responsible for the fermentation of African locust bean seeds and *B. subtilis* had been discovered to be the dominant Microorganism responsible for the fermentation, Odunfa (1981a); Antai and Ibrahim (1986); Popoola and Akueshi (1985); Ogbadu and Okagbue (1988); Omafuvbe *et al*., 2003; Achi, 2005; Sanni *et al*., 2006; Odunfa, 1998, 1985; Sanni *et al*. 1998, 1999; Omafuvbe *et al*. 1999; Ouaba *et al*. 2004, 2002, 2003; Ojewumi *et al*. 2016a; Diawara *et al*., 1992; Odunfa and Adewuyi 1985.

Figures 4.24.1 and 4.24.2 confirmed that *Bacillus subtilis* is the best microorganism for the fermentation of African locust beans seeds to Iru a condiment being consumed for its high protein content.

B. subtilis gave the highest percentage of protein followed by the *S. cerevisiae* and naturally fermented sample while the mixture gave the lowest. Asides from the protein content and other parameters been low in the mixture of both *S. cerevisiae* and *B. subtillis*, the end product were not organoleptically acceptable, this work supported the work of Ojewumi *et al.*, (2016a).

At temperature 50 °C, the protein content reduced for all the variation except for *B.subtilis*. This has been explained earlier that *S.cerevisiae* optimum temperature is below 50 °C. Temperature above this denatures the organism and probably kills it through dehydration. At this temperature *Bacillus subtilis* thrives better, therefore suppressing *S. cerevisiae*. However, an increase in percentage protein content was noticed for all the fermentation conditions. This confirmed the work reported earlier that fermentation increases protein content in the fermented substrate (Ojewumi *et al*., 2016b).

4.2.3.2 Percentage (%) Fat Content

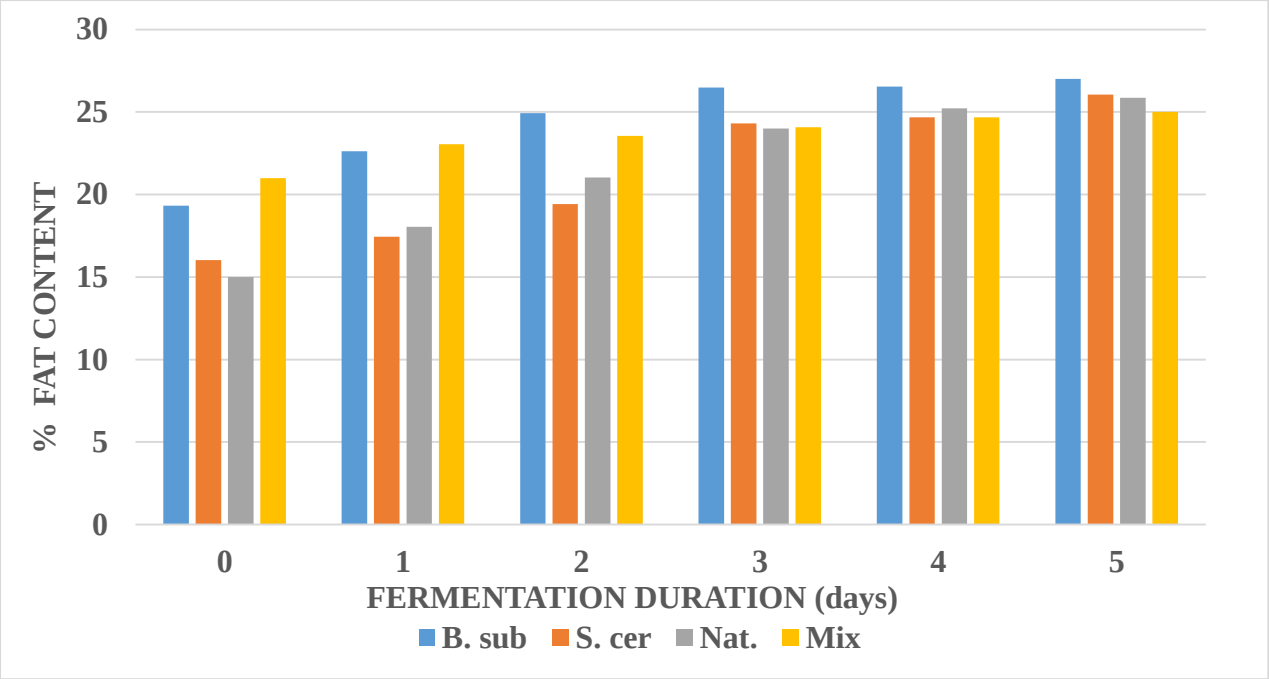


Figure 4.25.1 - The effect of Inoculum vatiation on percentage fat content at 40 °C

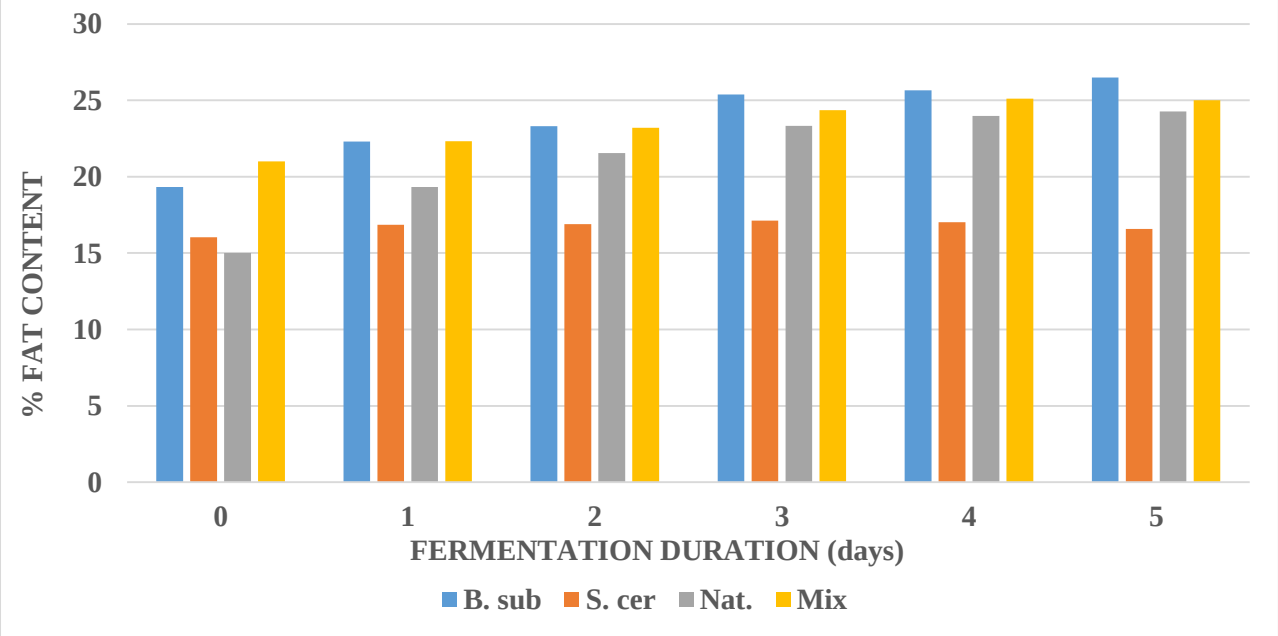


Figure 4.25.2 - The effect of Inoculum vatiation on percentage fat content at 50°C

The sample fermented with *B. subtillis* also had the greatest fat content at temperature 40 °C. Followed by the mixture of *B. subtilis* and *S. cerevisiae*, this was possible since *B. subtilis* dominate any environment it's found. Although an increase was noticed in all the inoculum used. Figure 4.25.2 shows that high temperature does not favour the fat content in all the inoculum but low temperature does. Percentage fat increases with fermentation duration. The percentage fat content was almost the same for the four inoculum variation in the third day of fermentation except for the sample with *Bacillus subtilis*. At the last considered fermentation days decrease in the percentage fat content was noticed at 40 °C through the trend of the graph, showing reduction in activities of all the organisms involved.

4.2.3.3 Percentage (%) Crude Fibre Content

At 50 °C *B. subtilis* still had the highest percentage crude fibre content since the operating condition is still within the range of its operations. *S. cerevisiae* had the lowest since the temperature had gone beyond the optimum temperature form its growth.

The fermented mixture of the inoculum had the highest crude fibre only at the first day, as shown in figure 4.26.1 followed by the *B. subtilis* fermented sample. All later decreases progressively with respect to fermentation duration at 40 °C. It was reported earlier that fermentation reduces the crude fibre with respect to time. *S. cerevisiae* followed the same trend as in *B. subtilis*. The Boiling, de-hulling and soaking of the African locust bean seeds during processing was responsible for the reduction in crude fibre, which further decreased by fermentation.

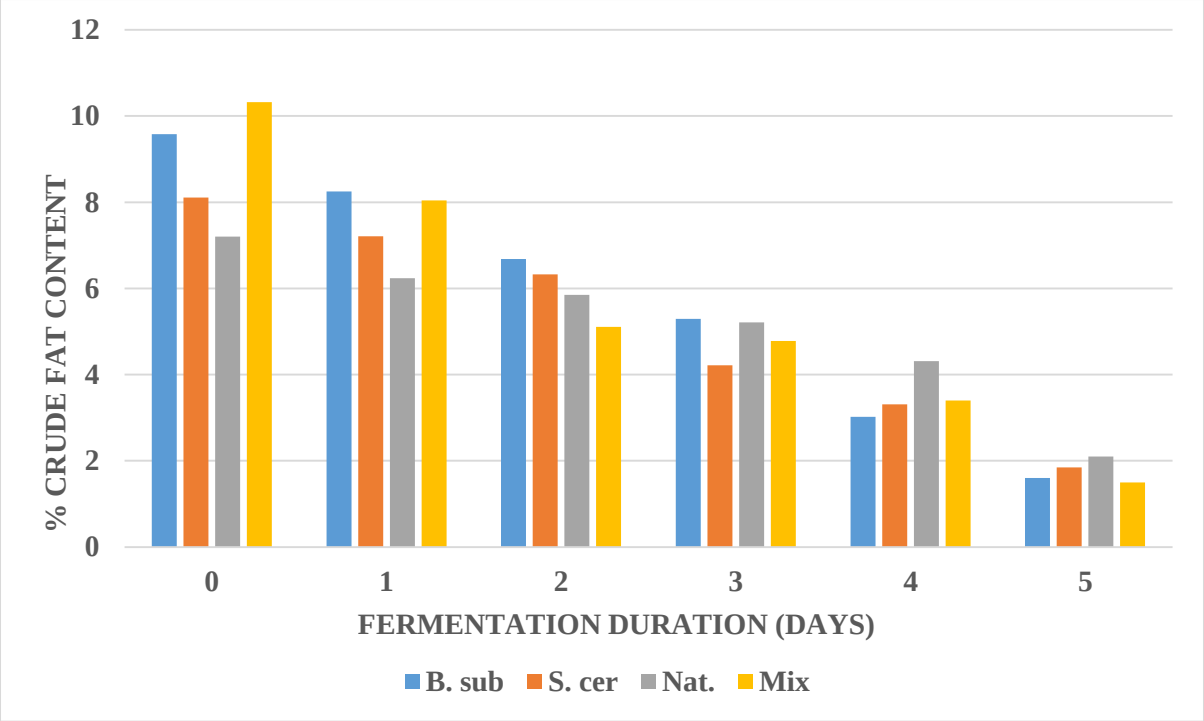


Figure 4.26.1 - Effect of Inoculum variation on percentage Crude fibre Content at 40 °C

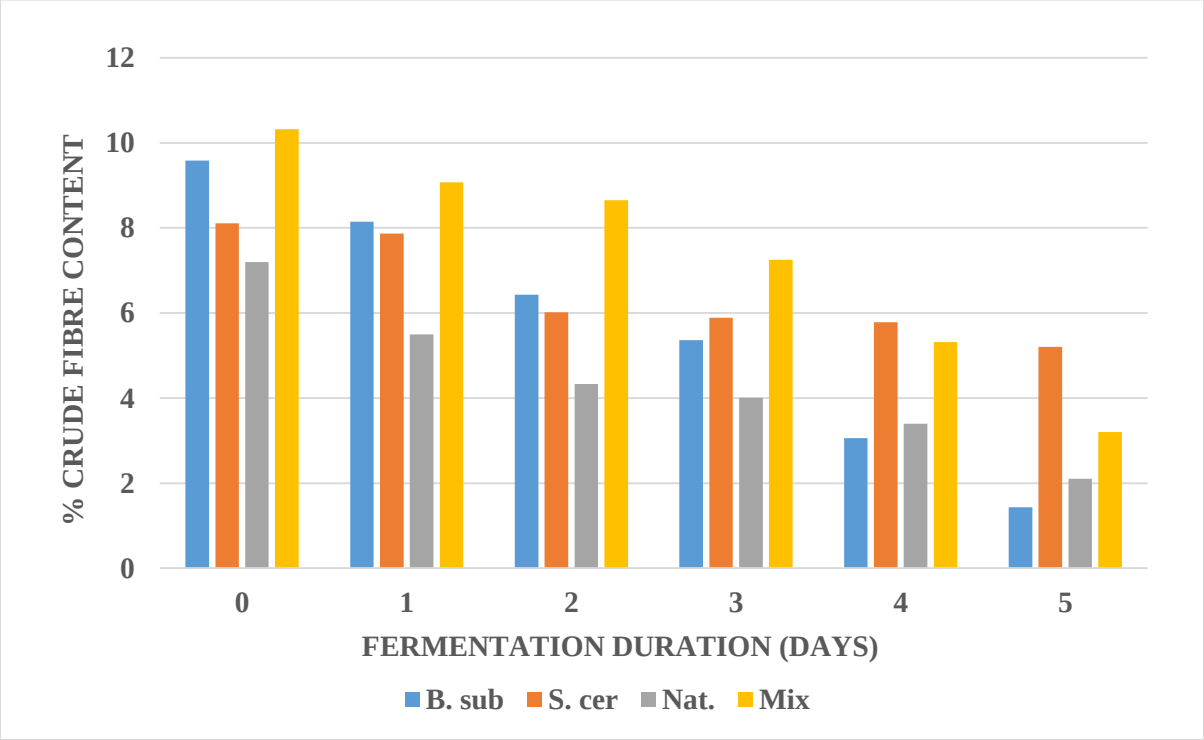


Figure 4.26.2 - Effect of Inoculum variation on percentage Crude fibre Content at 50 °C

4.2.3.4 Total Percentage (%) Carbohydrate Content

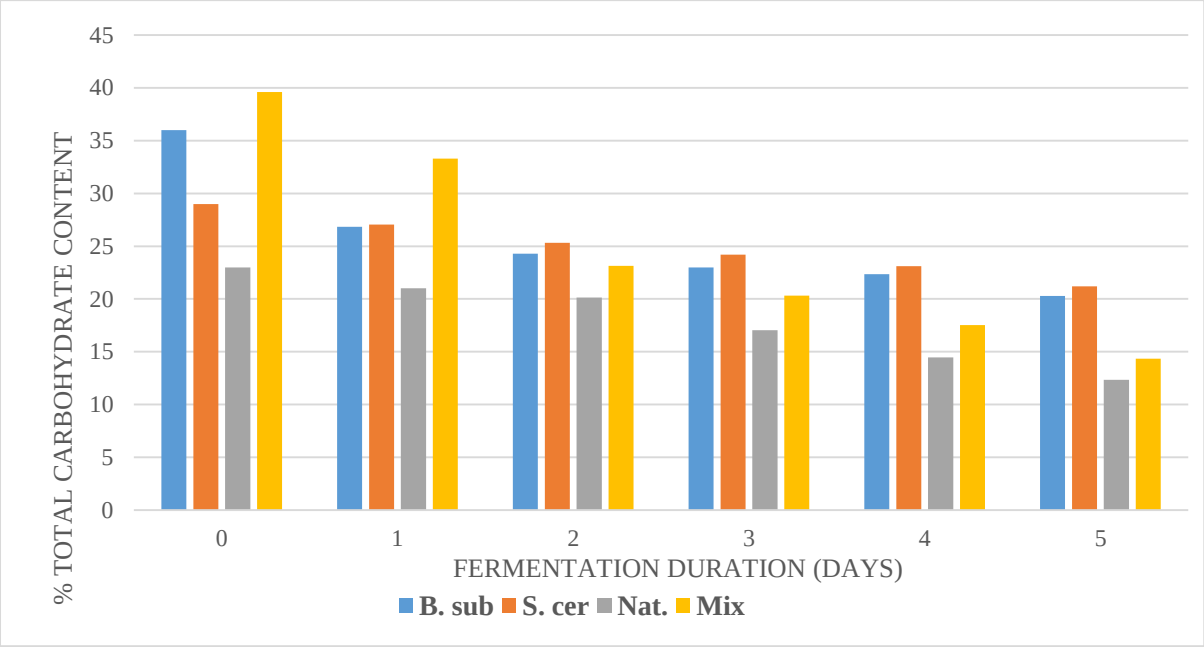


Figure 4.27.1 - Effect of Inoculum variation on Total Carbohydrate Content at 40 °C

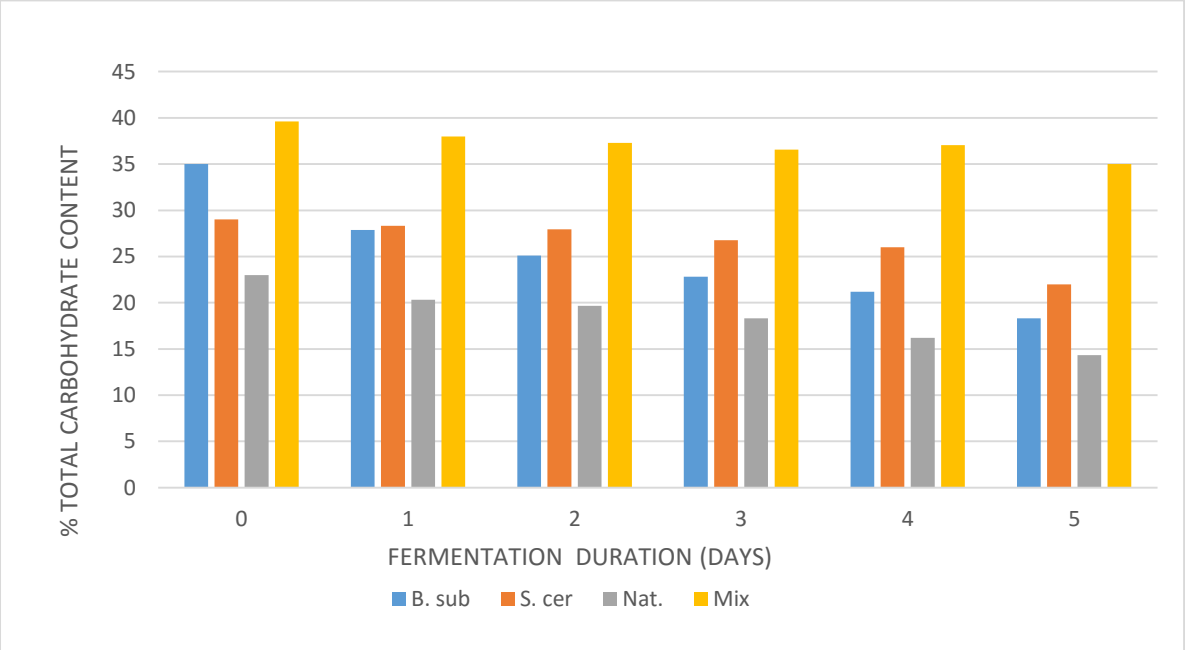


Fig. 4.27.2 - Effect of Inoculum variation on Total Carbohydrate Content at 50 °C

KEY

B.sub - *Bacillus subtilis*

S.cer – *Saccharomyces cerevisiae*

Nat - Natural fermentation (Without inoculum)

Mix - Mixture of *Bacillus subtilis* and *Saccharomyces cerevisiae*

Figure 4.27.1 show a progressive decrease in the total percentage carbohydrate with respect to fermentation duration. Initially, the sample with mixed inoculum had the highest carbohydrate content in the two temperatures considered. This may be due to the presence of two microorganism which worked against themselves to hydrolise starch into simple sugars.

Figure 4.27.1 show similar trend with figure 4.27.2 with a very high crude fibre which had reduced minimally with fermentation duration. Total percentage carbohydrate values are a bit higher at 50 °C than at 40 °C due to different operating fermentation conditionf of various organisms. When the initial percentage carbohydrate is close or same with the final , it is an evidence of either slow activities of enzymes or complete inactive enzymes.

Boateng *et al*. (2014), Nitschke and Pastore (2006) and Rolfe (2000) reported that all the species of *bacillus* produces several enzymes during fermentation which includes amylase, glucosidase and galactanase which are capable of degrading carbohydrates into simple sugars which are been used by the microorganism as an energy source and for metabolic activities. This is also in conjunction with the leaching out of sugars which are water soluble and minerals such as ash during the soaking and boiling of the African locust bean seeds; consequently fermentation further results in the utilization of some sugars by the microbes which led to the reduction (Esenwah and Ikenebomeh, 2008).

4.2.4 Comparison of Optimal Protein Compositions

Since fermented African locust bean seeds is consumed for the high protein content and health benefits embedded in it. This study used the percentage protein composition which is the highest composition discovered in the fermented seed to optimize the number of days and the appropriate fermentation temperature. The microorganism and the concentration to be used was also discovered which confirmed the earlier reports by researchers.

Literature confirmed that raw unfermented African locust bean has about 32 % protein content. This supported by the result of this study and others such as Omodara and Aderibigbe (2013); Ajala *et al.* (2013); Ojewumi *et al*. (2016b); Soetan *et al.* (2014) and Omafuvbe *et al.* (2004).

Figure 4.24.1 shows that at the third day *B.subtilis* gave the highest yield of protein at temperature 40 °C, amongst all. The sample fermented with 0.005 g broth/g seed inoculum also gave a good yield of protein, followed by sample bought from the open market. 0.0075 g broth/g seed fermented sample also had an appreciable amount of protein, but the end product was not organoleptically acceptable. The protein composition decreases with an increase in inoculum concentration.

The naturally fermented sample was almost in the range of the 0.0025 g broth/g seed inoculum fermented sample, meaning that *Bacillus* species or other microorganism was present in the assumed naturally fermented sample which was responsible for the fermentation process similarly with anaerobic fermented sample. The mixture of the two microorganism gave a poor percentage protein yield probably because there was conflict in the action of the two microorganism. The anaerobically fermented sample was able to produce protein because *B.subtilis* can survive both aerobic an anaerobic fermentation at a favourable condition. *S.cerevisiae* can also do this, they survive in an oxygen deficient environment for a period of time depending on the environmental conditions.

This work concluded that African locust bean should be fermented with 0.005 g broth/g seed *B.subtilis* for three days and at temperature 40 °C. This is in aggrement with the report of Odunfa (1981); Antai and Ibrahim (1986) and Ogbadu *et al.* (1988). Achi (2005) reported three days (3 days) within temperature range 28 – 42 °C.

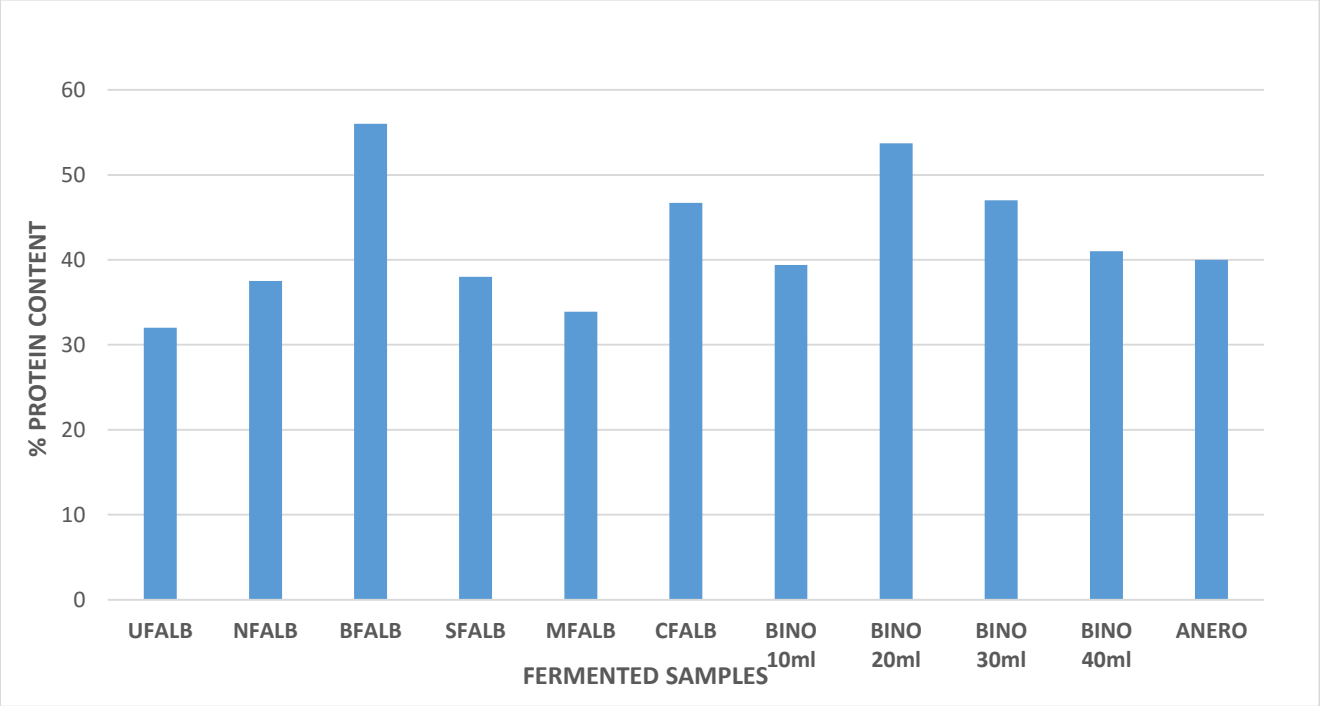


Figure 4.28 - Comparison of the percentage Protein content in different fermented samples

KEY

UFALB – Unfermented African locust bean

NFALB – Naturally fermented African locust bean

BFALB - *B. subtilis* fermented African locust bean

SFALB - *S. cerevisiae* fermented African locust bean

MFALB – Mixture of Inoculum fermented African locust bean

CFALB – Commercial fermented African locust bean (Bought from the Market)

BINO 10 ml – *B. subtilis* fermented African locust bean concentration 0.0025 g broth/g seed

BINO 20 ml - *B. subtilis* fermented African locust bean concentration 0.005 g broth/ g seed

BINO 30 ml - *B. subtilis* fermented African locust bean concentration 0.0075 g broth/g seed

BINO 40 ml - *B. subtilis* fermented African locust bean concentration 0.01 g broth/g seed

ANERO – Anaerobic fermented African locust bean

4.3. Physiological Analysis

The physiological evaluation of the fermenting stages of the bean was done by smell and sight tests of the seeds before and during the fermentation period.

Raw Seed

The processed raw seed had a normal mild smell with a creamy brown colour. It had a slightly coarse texture and tasteless. The smell was acceptable and not offensive or odorous.

The starting sample was a fine creamy brown coarse mass with a beany aroma substrate which translated to a more brown substrate and finally to a dark and darker substrate with respect to fermentation duration. The end product was softer than the starting samples. Ammoniacal flavour was observed in the fermenting samples at higher temperature in form of a choking pungent smell. The samples fermented after the fourth day all produced ammonia smell.

It was discovered in the course of this work that flavour varies as fermentation progresses with days. Flavours are been formed as a result of the breaking down of some organic compounds and the formation of microbial metabolites. Ammoniacal flavour noticed during the fermentation of 'Iru' is believed to have resulted from the alkaline degradation of protein component of the seeds by *B. subtilis* during fermentation, Ogueke *et al.* (2010) and Nwokeleme and Obeta (2015). Other aroma can also come from the breaking down of fat and other available compound since the seed is rich in fat and its compositions increases with fermentation duration.

In natural fermentation, microorganism were not totally absent. They are still involved in the process, only that they were not intentionally added as an inoculum. The presence of other microorganisms may modulate the quality and quality of the product which invariable affects the volatile compounds produced. Brown colouration was noticed which can be attributed to the phenolic compounds present in the fermented samples. Increase in the strength of flavour

was noticed in this work which may be attributed to the presence of volatile organic compounds.

The Appendix G show the results for the physiological analysis of the fermented products from the 1st to the 5th day of fermentation. At day 0 of Aerobic, Anaerobic and singularly inoculated fermented samples, brownish colour was noticed with a mild pleasant aroma which is slightly beany. All the starting samples are not edible since fermented seeds are not edible in their raw forms. Between Days 1 and 3 the colour of the sample changed to a darker brown with a pleasant aroma that is more of ammonia, with an acceptable products. A pleasant but choking smell was noticed as fermentation progresses, the last day gave an offensive roasting flavour. All the product at 70 °C were not acceptable, this is probably due to the high temperature as reported earlier for the fermenting organisms. Fermented sample with *S. cerevisiae* was acceptable till the third day of fermentation with a mild pungent flavour.

The higher the temperature, the stronger the odour. The colour became browner as fermentation progresses except for the higher temperature in anaerobic fermentation with a butter light colour. The pungent smell noticed in this work was not as strong as the one noticed in the locally fermented sample, increase in temperature favours the release of offensive odour. The pungent smell which accompanies 'Iru' was due to the release of ammonia.

4.4 Deterioration Study of Fermented African Locust Bean (*Parkia biglobosa*) seeds

In order to overcome the problems encountered by the processors of 'Iru' in keeping this product for a long time, the deterioration (shelf life) was studied. 'Iru' has a shelf life of 2 - 3 days without additives, which implies that the producer has to dispose of the product within three days if not consumed. The bacteria, yeast and molds which are responsible for deterioration needs moisture for growth which can be removed by total drying. Although some fragile or volatile nutrient can be destroyed by it, such as vitamin C.

African locust bean seeds were fermented using the optimized process and conditions of three days (72 hours) at 40 °C respectively with inoculum concentration of 0.005 g broth/g seed. The Percentage (%) moisture content variation method was used. Condiment were portioned into 5 pre-weighted aluminum foil with equal amount of fermented condiment. They were slowly dried in the oven and each sample was picked at the intervals of 2 hours for 12 hours. The percentage moisture content and the amount of substrate remaining (total solid) were calculated. Results were compared with the locally produced 'Iru' (Purchased from Ota Market in Ogun state). Undried samples were also monitored (samples with moisture).

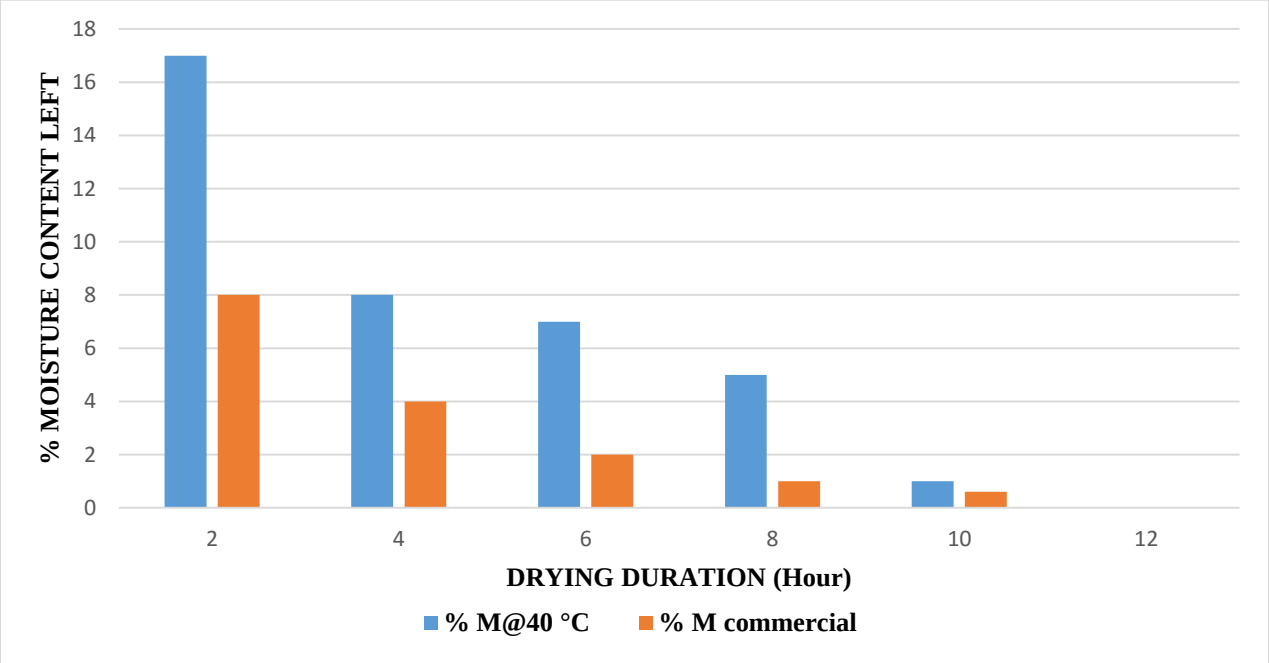


Figure 4.29 - The Percentage Moisture Content Variation

KEY

% M at 40 °C - % Moisture content @ 40 °C

% MCOMM - % Moisture content of commercial sample (Purchased sample)

4.4.1 Evaluation of the Deterioration Study of Fermented African Locust Bean (*Parkia biglobosa*) Seeds

The following functional properties were studied as indices of deterioration of stored 'Iru':

(i) pH

(ii) Titratable Acidity

(iii)Peroxide Value

(iv)% Protein content

(v) Physiological Analysis: Colour, Aroma and Appearance

The condiments were stored in an air tight container at ambient temperature for 30 days after the normal three days of fermentation (72 hours.). At 5 days interval samples were taken and analyzed for the above functional properties to monitor the deterioration.

4.4.1.1 Effect of Moisture Level and Storage days on the pH

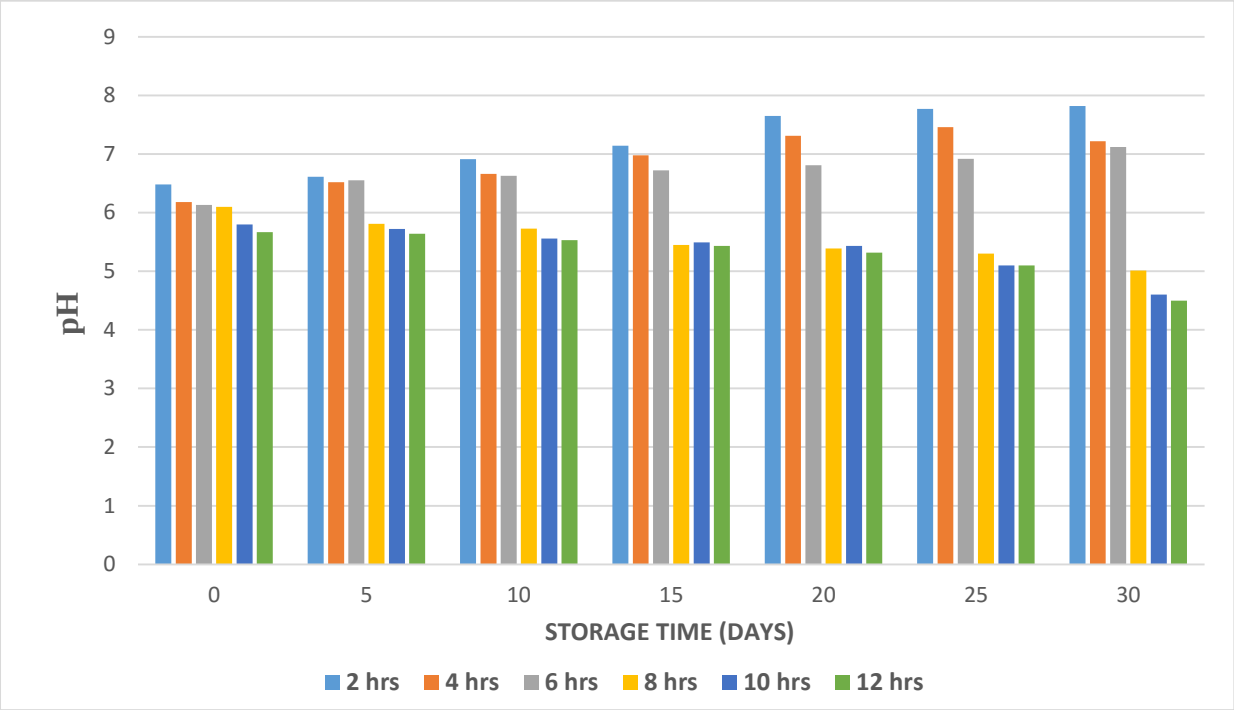


Figure 4.30.1 - Effect of Storage Time on pH of Fermented Dried Condiments

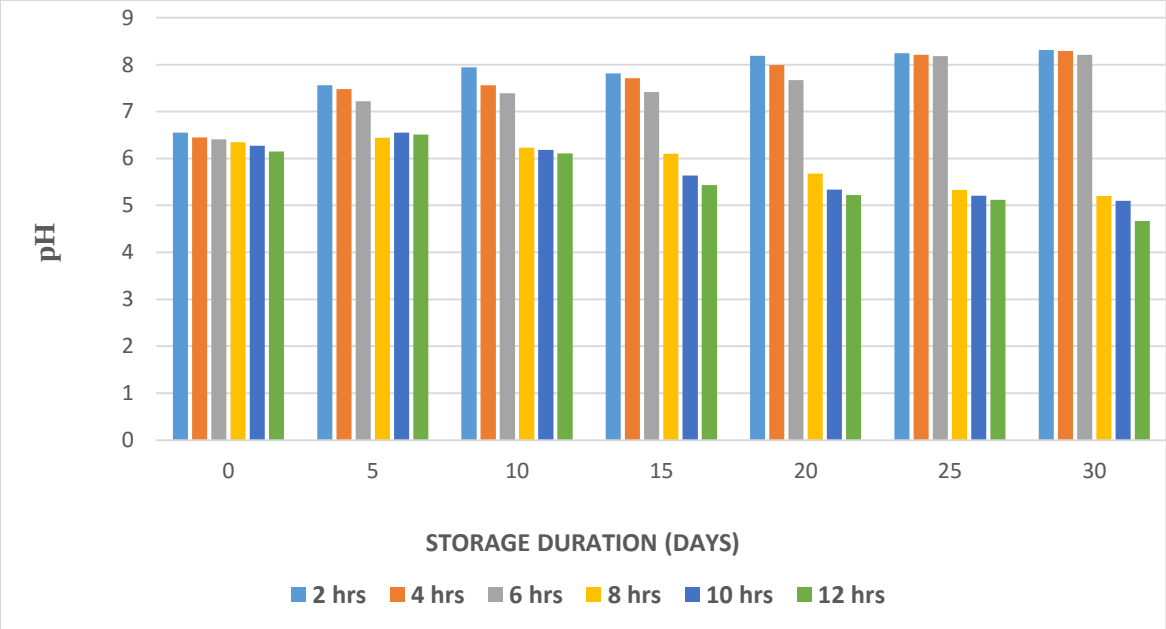


Figure 4.30.2 - Effect of Storage Time on pH of Purchased Sample

Figures 4.30.1 and 4.30.2 shows that the pH decreased with number of storage days, and with reduction in percentage moisture content. The lower the pH the lower the risk and rate of spoilage. Decrease in pH towards the acidic range was recorded for drying durations 8, 10 and 12 hours while increase in pH towards alkaline medium was noticed at 2, 4 and 6 hour, this is responsible for the spoilage, as microorganism grows best in an alkaline medium. Spoilage was noticed at the 15th day of all the samples with increased alkalinity why spoilage was never noticed in the samples towards acidic medium, although the flavour was affected due to the long duration of drying. However in the earlier reports, an increase in pH towards alkaline during fermentation has been related to the proteolytic activities and the production of ammonia by *B.subtilis*, which is a common feature of all legumes that produces vegetable protein via fermentation (Hesseltine, 1967).

The increase in pH observed in this study is an indication that fermentation still continued after the processing period of African locust bean seeds to 'Iru' (post fermentation operation). This confirmed that the organisms responsible for the fermentation are still present at consumption. Furthermore the higher drying duration with lower pH showed that the microorganism's structure had been either altered, from a protective endospore till a favourable condition is reached or die due to the long hour of gentle heat, hence they will have a longer shelf-life.

Figure 4.30.2 (purchased sample) shows that decrease in pH is favoured by longer duration of drying which directly reduced spoilage. The pH trend followed the same pattern as in the fermented sample at 40 °C although with higher values in favour of acidity in the 5, 10 and 12 hours dried sample, and lower values in favour of alkaline as storage time increases.

4.4.1.2 Effect of Moisture Level and Storage Time on the Titratable Acidity

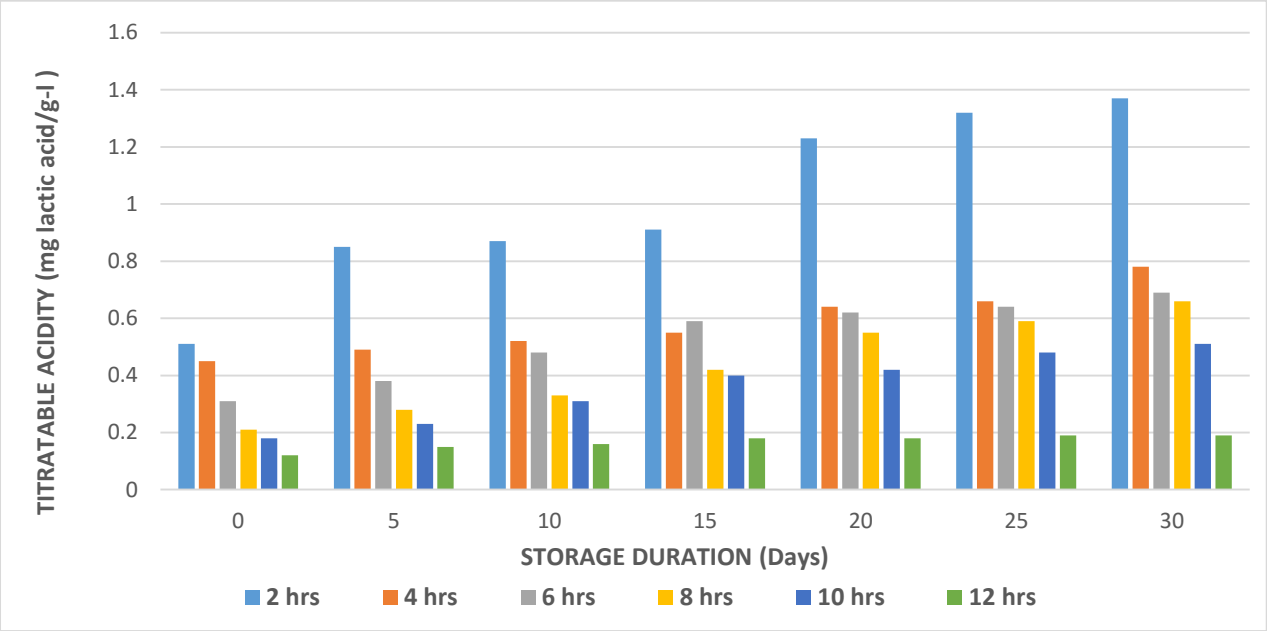


Figure 4.31.1 - Effect of Storage Time on Titratable Acidity (mg lactic acid/g-l) in fermented dried condiment

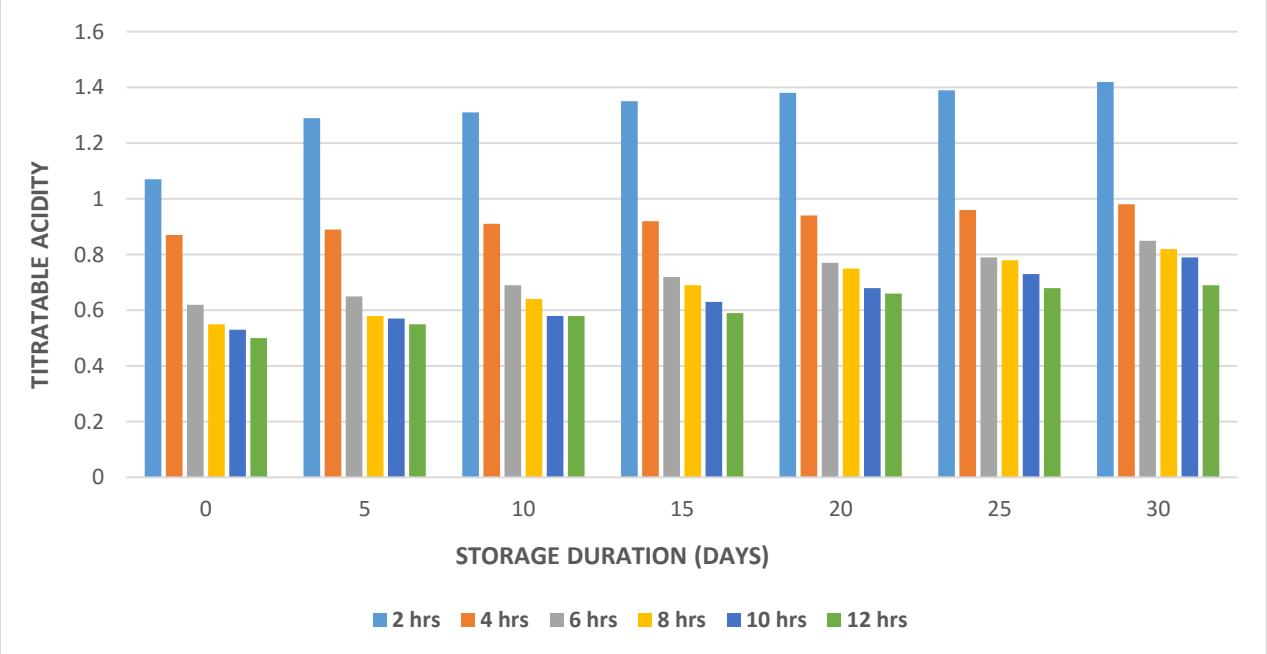


Figure 4.31.2 - Effect of Storage Time on Titratable Acidity (mg lactic acid/g-l) in Purchased Sample.

4.4.1.3 Effect of Moisture Level and Storage Days on the Peroxide Value

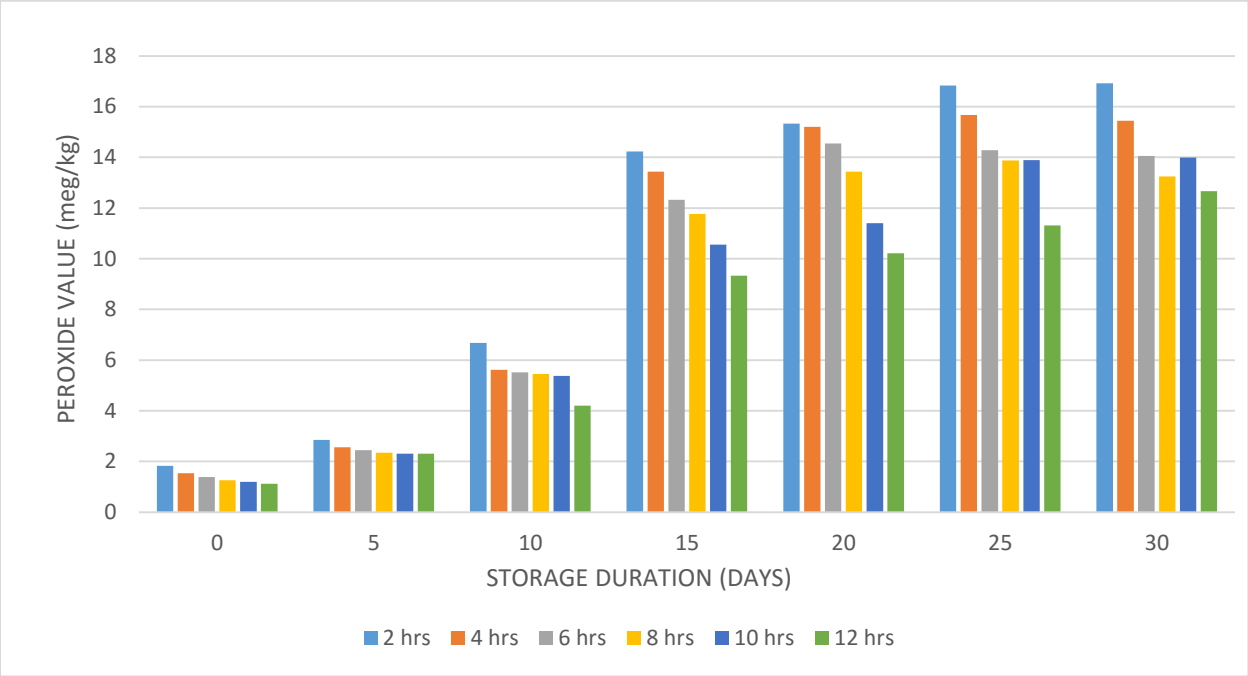


Figure 4.32.1 - Effect of Storage Time on the Peroxide Value in fermented dried condiment

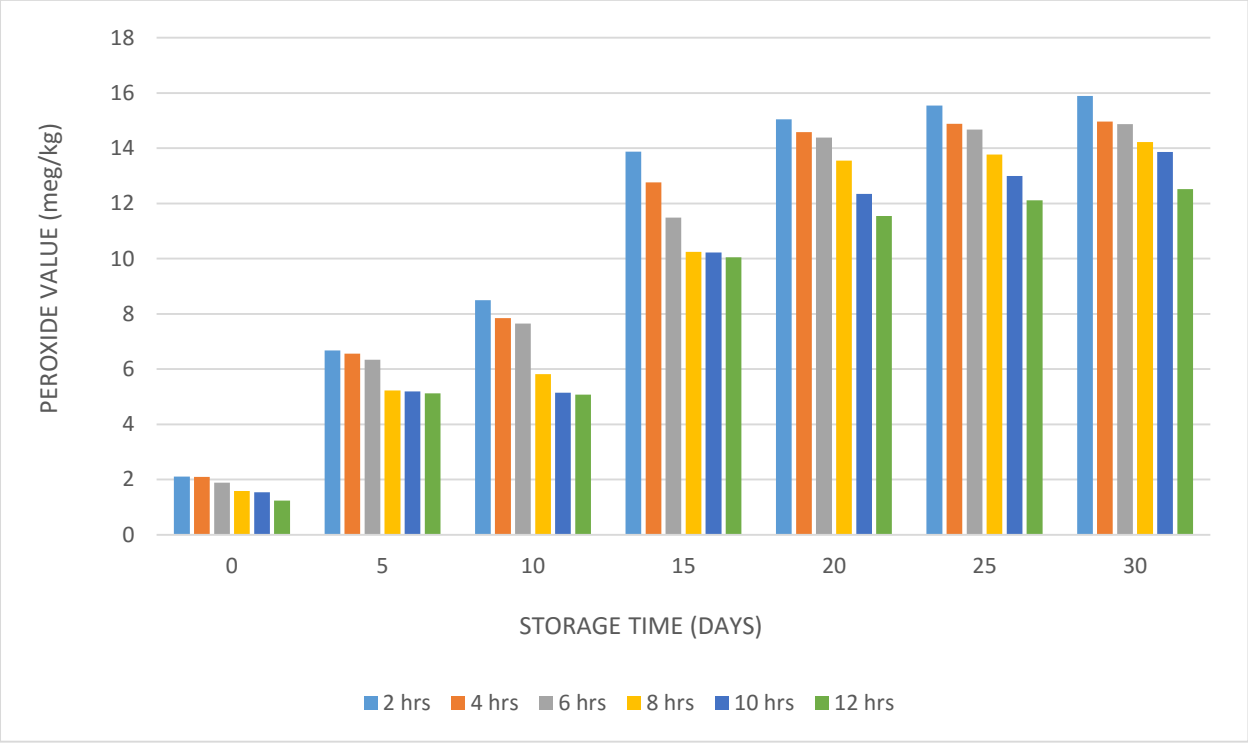


Figure 4.32.2 - Effect of Storage Time on the Peroxide Value in purchased sample**.**

Figures 4.31.1 and 4.31.2 show a significant increase in titratable acidity with storage time, which is as a result of some acid producing processes going on during the deterioration stage. The significance of the increase towards acidity in both pH and titratable acidity in stored 'Iru' is the continuation of fermentation process in storage. The simultaneous increase of the two in 'Iru' have been reported by Ikenebomeh (1986); Wagenknecht *et al.* (1986); Ojewumi *et al.* (2016a) and Popoola *et al.* (2007). The increase is attributed to the activities of proteolytic enzymes which takes place in the degradation of protein and the hydrolysis of carbohydrates components to sugar and organic acids. Similar results were obtained for the purchased sample. This shows continuation of acid producing organism at post fermentation period.

Figure 4.32.1 Peroxide values gives the initial evidence of rancidity in an unsaturated fats and oil, this is an indication of peroxidation in food.

Findings showed that peroxidation of stored 'Iru' increased with storage duration (Kolapo *et al.,* 2007 and Popoola *et al.,* 2007). An increase in peroxide value is an indication of fat deterioration which brings rancidity. This work reported an increase in the peroxide value of stored 'Iru', which is a good predictor that peroxidation occurred during the storage of 'Iru'. The higher the peroxide value, the more susceptible is the condiments to spoilage. Although fat acts as flavour retainer and increase mouth feel of food (Kinsella and Melachouris, 1976), it can also act otherwise if it goes rancid. This work reported an increase in percentage fat content with fermentation days. Literature reavealed that fatty foods with peroxide value ranging from 20 – 40 meg/kg is rancid, peroxide value should be less than 10 milliequivalents/kg.

4.4.1.4 Effect of Moisture Level and Storage Days on the Protein Content

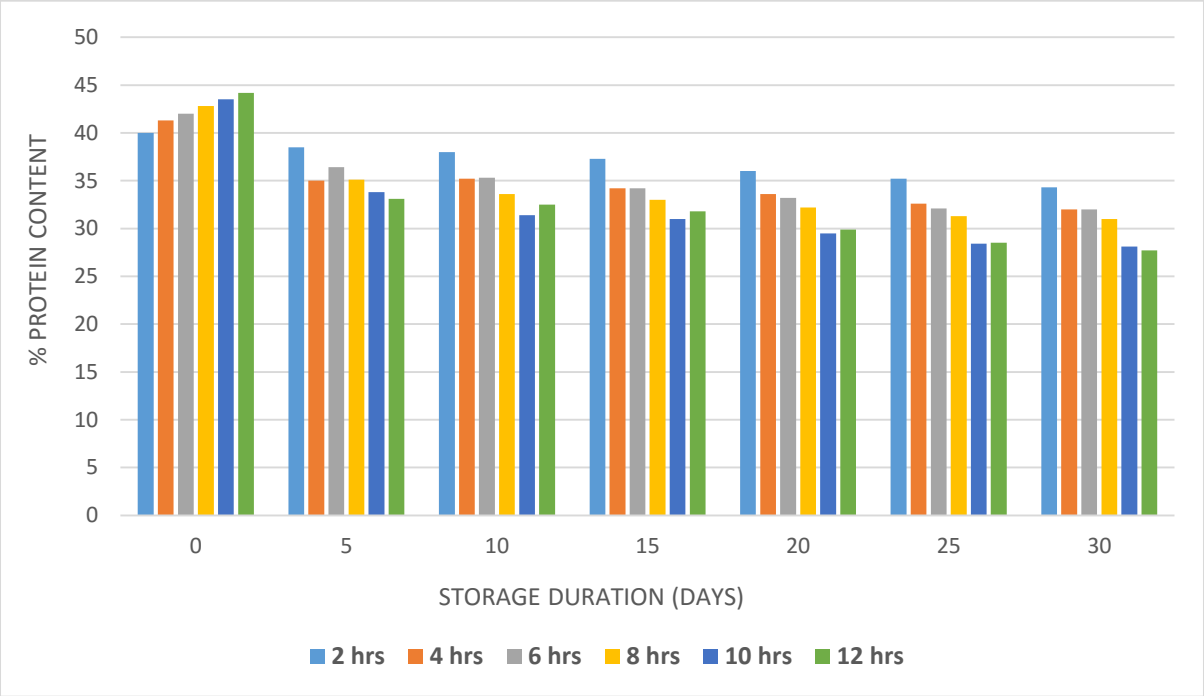


Figure 4.33.1 - Effect of Storage Time on the Protein Content of the fermented dried condiment

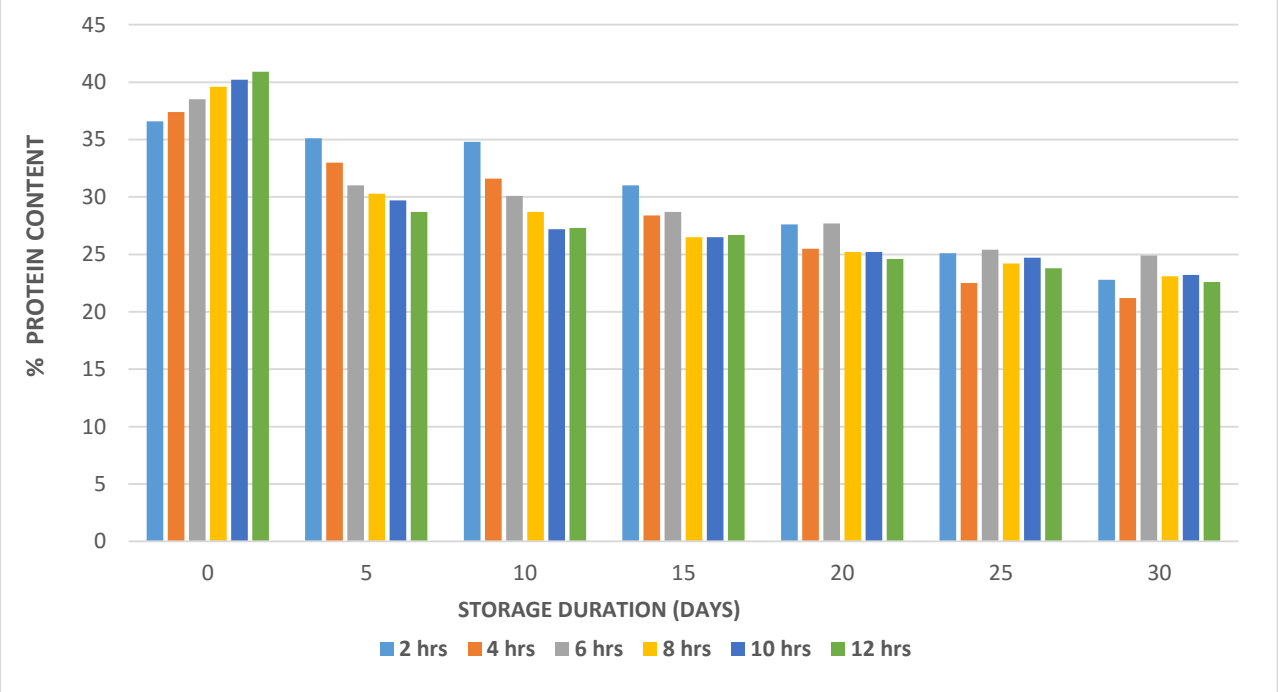


Figure 4.33.2 - Effect of Storage Time on the Protein Content of the purchased sample

Figures 4.33.1 and 4.33.2 explained how the moisture level and storage days increases the pH of the stored condiments towards acidity, this will invariably reduce the protein content since the increase in pH was due to the degradation of proteins. Shapes of protein structure is denatured in an unconducive environment. The protein content increased with drying on the 0 day and later started decreasing with respect to storage days. Storage days denatured protein with the long hour of drying. Spoilage was noticed on the 15th day with an off flavour.

Oxidation is one of the major causes of deterioration in any protein based foods since they are very rich in fat. 'Iru' becomes susceptible to oxidative deterioration due to its high concentrations of unsaturated fat which always manifests in form of discoloration, formation of toxic compounds, poor shelf life, development of off flavour, nutrient losses, respectively with storage duration (Contantini and Bonadonna, 2010; Palmieri and Sblendorio,2007).

4.4.1.5 Deterioration in Fermented 'Iru' at Optimised Condition (Without removing Moisture Content)

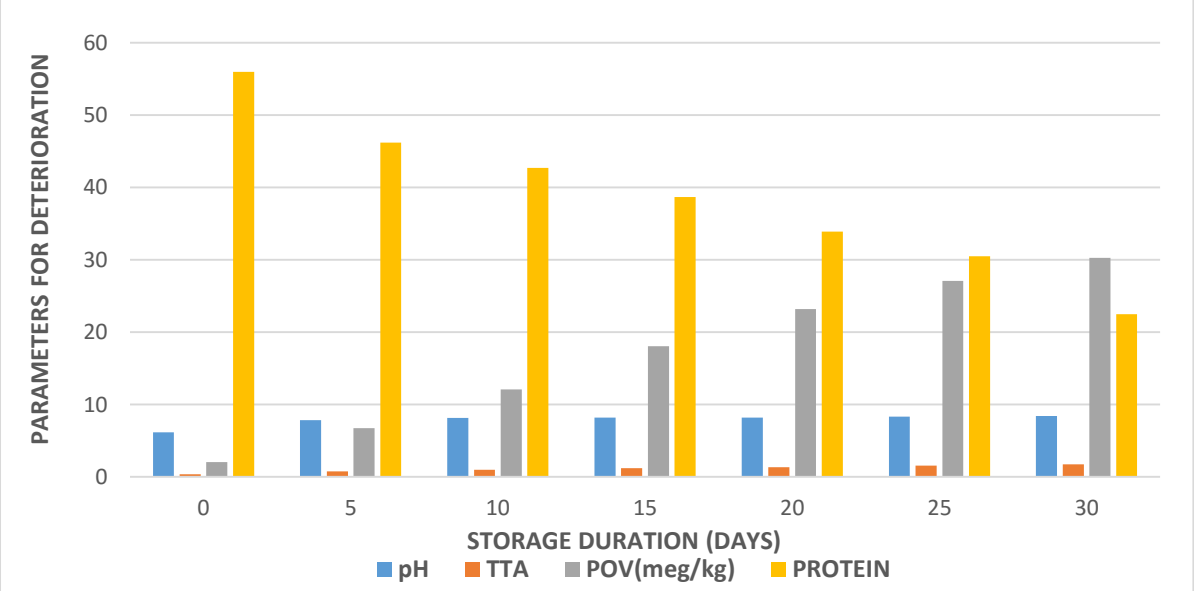


Figure 4.34 - Effect of storage duration on fermented African locust bean seeds using different parameters

Figure 4.34 shows how long fermented African locust bean can stay before deteriorating (shelf life) and the importance of moisture on deterioration. Here the moisture was not removed and deterioration was noticed on the second day of storage with an ammonia flavour which increased as storage progresses. An increase in the pH, titratable acidity and peroxide values were all noticed in stored 'Iru' progressively. The increment in the pH towards the alkaline medium encouraged the growth of spoilage organism like mold and yeast. However mold growth was not noticed until the fourth day. Okorie and Olasupo (2013) also predicted the 3 day spoilage. Since a decrease in protein content was noticed due to spoilage, it shows that nutritional content of stored 'Iru' decreases with storage. Since the fat content in freshly fermented 'Iru' was reported to be high (about 27 %), deterioration is expected to be fast.

The study of the deterioration in stores 'Iru' using moisture difference has never been study by any researcher. However attempt has been made in the past to prolong the shelf life of fermented African locust bean seeds and Soybean seeds with the aids of additives and other preservative means. Ogbulie *et al.* (1998) attempted packaging the processed product inside a high density polyethylene sachets and aluminum foil after treating with 2 % NaCl, this method kept the product for only 8 days before spoilage sets in. Other researchers applied pasteurization at temperature of about 100 °C for 30 minutes, with the assumption that all the microorganism had been eliminated due to high heat, including the organism used for the fermentation, (Mbata *et al.*, 2008), this also lasted for 8 days. Returnable and sterilizable bottles for packing the finished products, this was able to keep the product for 4 weeks. The report in this work is therefore an improvement to all the reports on the preservation of "Iru' and will serve as a good method to local producers.

Most of the biochemical deterioration in monitored functional properties of 'Iru' were noticed on the 15^{th} day of storage for the samples with high pH range towards alkaline medium, 20^{th} day for sample dried for 4 hours , while they were not noticed in the samples with lower pH

towards acidic medium. Although physiological analysis varies, totally dried sample had an improved (longest) shelf life of up to 9 months.

4.4.2 Physiological Analysis for the Deterioration of Fermented Stored Condiment

The deterioration of fermented African locust bean seeds was studied with the following sensory attributes: Colour, flavour, texture and overall appearance. The effect of storage and moisture on the physical quality of the product was reported in this work.

The colour of unfermented processed African locust bean seed is creamy brown with a characteristic flavour of bean. Appendix L showed the colour, flavour, texture and appearance of fermented 'Iru' from Day 0 to 30 days.

Dried 'Iru; samples were dark brown in colour after drying, this was due to browning reactions involving the amino gropus of some amino acids and the non-reducing sugar present in the fermented seeds.

The samples stored at ambient room temperature (28 – 30 ºC) kept for three days before deterioration started, maggot was not noticed until the fifth day of storage.

The dried samples were stored for 30 days with close monitoring. Samples with moisture content of below 4 % moisture content were without any noticeable changes in the colour, taste and aroma. Samples were further stored for about 10 months.

4.5 Organoleptic Properties of Fermented African Locust Bean Seeds

10 assesors were used with the following results:

Colour: The colour of the finished product were acceptable by all the assesors.

5 like extremely

5 like slightly (moderately)

Taste: With regards to flavour, all the sample were acceptable with

8 like extremely

2 like slightly (moderately)

Appearance: 5 like extremely

4 like slightly (moderately)

1 Neither like nor dislike

Texture: Considering how the samples were in between fingers,

5 like extremely

4 like slightly (moderately)

1 Neither like nor dislike

Overall acceptability: The finished product which is the preserved protein based condiment were given general acceptability by all the assesors.

4.6 The Mineral Composition in African Locust Bean (*Parkia biglobosa*) Seeds

Our bodies need vitamins and essential minerals, but if taken in large amounts they can adversely affect our health. Large amounts of many vitamins and minerals can be toxic, Judy, 1981. Minerals (nutrients) in the body can be deficient, adequate or toxic. When the intake is too low to meet body needs for that specific mineral or nutrient it is deficient, when the body gets sufficient quantity, it is adequate and when a person gets an overdose of a specific nutrient it is toxic.

The qualitative and quantitative analysis of the mineral present in *P.biglobosa* was evaluated using Flame Photometer (FP) for Sodium and Potassium and Atomic Absorption Spectrophotometer (AAS) for other element present.

The traditionally fermented and the commercially processed cube were also evaluated with FP and AAS.

The mineral compositions of fermented African locust beans, purchased sample and processed cube (knorr) were shown in Table 4.1. Potassium was found to be the most abundant mineral and decreased significantly during the period of fermentation for all the samples. Magnisum was found to be the second highest mineral which decreases with fermentation days. The amount of calcium increased significantly with fermentation days while Zinc was also found in an appreciable amount.

The amount of manganese, Iron and copper detected in all the samples was moderate.

Table 4.1 The Mineral Composition of African locust bean seeds

SAMPLES	Ca (mg/L)	Na (mg/L)	Cd (mg/L)	Fe (mg/L)	Zn (mg/L)	Mn (mg/L)	Cu (mg/L)	Mg (mg/L)	K (mg/L)
Raw unprocessed sample	2.128	14.342	ND	2.414	0.505	0.453	0.172	12.77	48.55
Day 0	3.457	9.343	ND	0.731	0.398	0.606	0.024	6.570	20.65
Day 3 @ 40 °C (B/S)	3.974	11.572	ND	1.062	0.487	0.770	0.043	8.096	18.54
Day 3 @ 40 °C (S/C)	2.660	11.642	ND	0.772	0.402	0.274	0.080	3.983	12.54
Day 3 @ 40 °C (Mixture)	2.836	12.124	ND	1.190	0.396	0.304	0.065	4.654	17.65
Day 3 @ Rm T°C (Mixture)	3.345	12.0	ND	0.528	0.246	0.623	0.111	5.112	14.45
Day 3 Rm T °C (No inoculum)	3.146	13.75	ND	0.613	0.508	0.575	0.010	6.932	11.56
Day 3 Rm T °C (S/C)	3.234	12.5	ND	0.674	0.243	0.397	0.087	3.432	8.67
Commercial Maggi (Knorr)	7.789	215.6	ND	20.183	0.181	18.68	0.202	15.79	15.60
Traditionally Purchased sample	3.432	25.0	ND	0.826	0.405	0.534	0.117	6.657	4.68

KEY:

B/S	*Bacillus subtilis*
S/C	*Saccharomyces cerevisiae*
Rm T°C	Room temperature
Calcium	Ca
Sodium	Na
Cadmium	Cd
Iron	Fe
Zinc	Zn
Manganese	Mn
Copper	Cu
Magnesium	Mg
Lead	Pb
Potassium	K
ND	Not detected

The analysis of percentage ash content revealed that mineral element are leached into boiling water during processing of African locust bean seeds. This was also revealed in the big difference between the elemental composition of the raw seed to the processed and fermented seeds.

The raw seed is very rich in sodium, this was revealed by a yellow flame that came up during the analysis with AAS. Every other analyzed element had a high composition in the raw form which reduced with respect to fermentation duration except for Calcium. This work discovered that inoculum does not only increase the nutritional values of fermented seeds but also improves the elemental compositions. The commercial monoglutamate based spice had the highest value of composition, this is due to the additives added during processing. The sodium composition is as high as 215.6 mg/L, this explains why it increases the blood pressure. In the same vein ‘Iru’ has a deposition of a compound known as Histamine which causes the blood vessel to dilate and gives a free flow of blood.

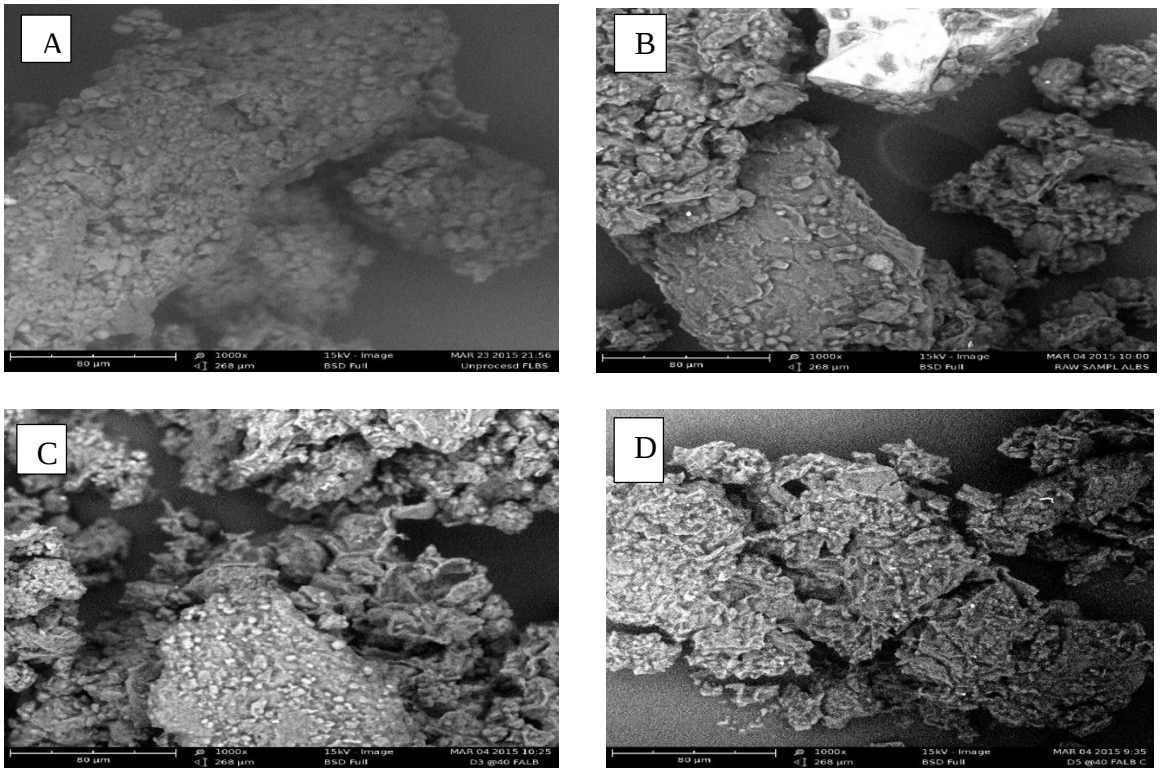
4.7 The Morphological Structures of African Locust Bean Seeds

The Scanning Electron Microscope was used to characterize the morphology of particles of selected samples of African locust bean (*Parkia biglobosa*) seeds. The effect of temperature change, fermentation with respect to the number of days were examined. The raw unprocessed seed and traditionally produced (purchased) samples structures were also considered.

Magnifications 500, 540, 1000, 2000, 3000, 5000, 7000 and 10000 were used for the viewing of all the samples, but 1000, 2000 and 5000 gave the best image using SEM.

Every image was described based on the morphology of the surface pattern.

Figures 4.35 A – I shows the morphological structures of both unfermented and fermented African locust bean seeds.



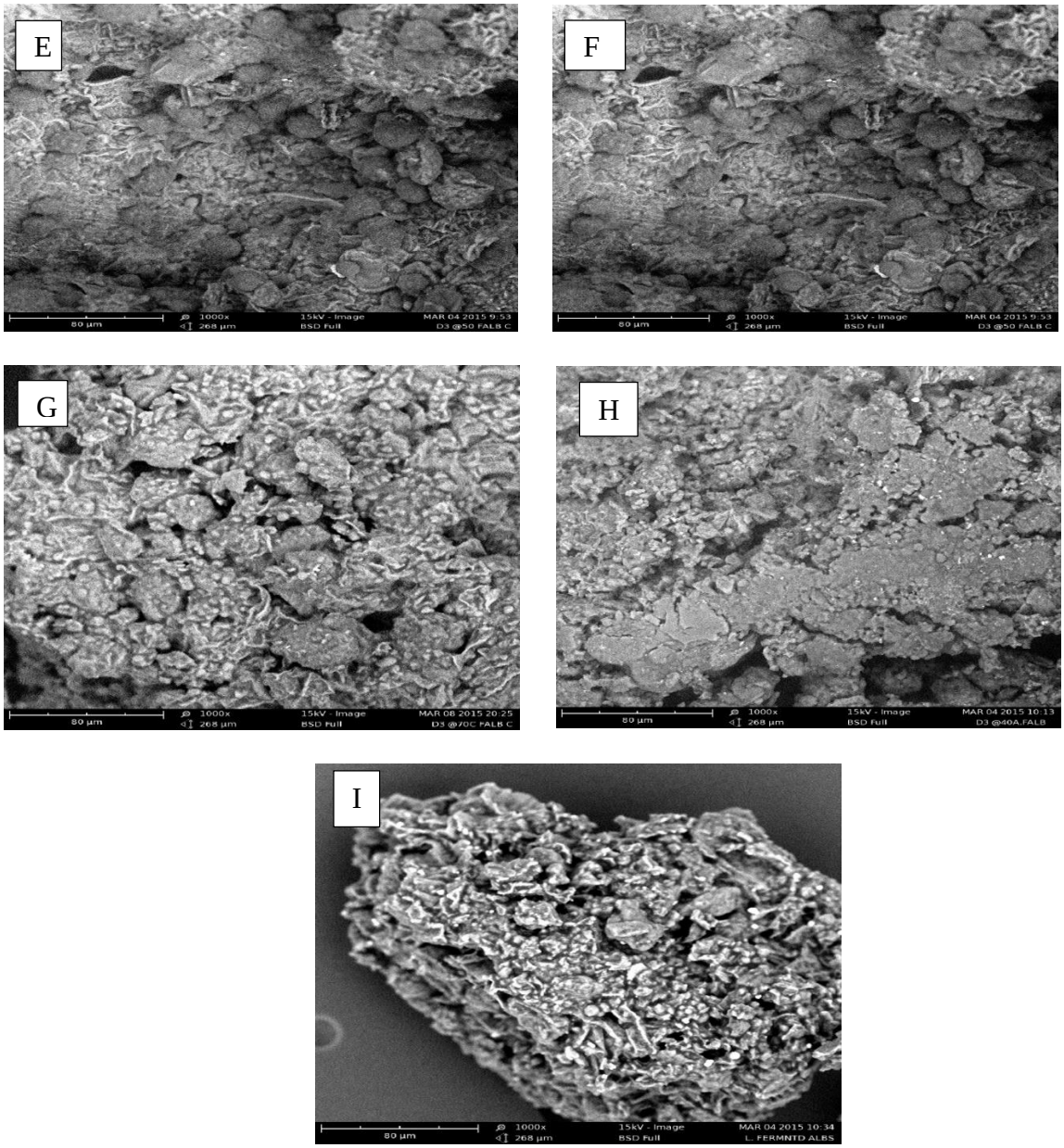


Fig 4.35 - The Morphological Structures of African Locust Bean Seeds

A - The morphological structure of unprocessed African locust bean seeds

B - The morphological structure of processed raw African locust bean seeds

C - The morphological structure of fermented sample on the third day at 40 °C

D - The morphological structure of fermented sample on the fifth day at 40 °C

E - The morphological structure of fermented sample on the third day at 50 °C

F - The morphological structure of fermented sample on the fifth day at 50 °C

G - The morphological structure of fermented sample on the third day at 70 °C

H - The morphological structure of fermented sample on the third day at 40 °C (Anaerobic)

I - The morphological structure of traditionally fermented African locust bean seeds

4.7.1 The Morphological Structure of African locust Bean Seeds

Figure 4.35 A shows the morphological structures of raw unprocessed African locust bean seeds. The sample was neither washed nor cooked. The cotyledon was removed using mortar and pestle and the seeds grinded into powder, this gave the real structure of the seeds. This was carried out before undergoing any reformation or transformation either by the processing stages or fermentation processes. The morphology of the unprocessed seed revealed an agglomerated cohering (stick together) image with a coarse and wrinkled corrugated surface. Fissure were not seen but sporule (small pores) were noticed at some points. At 5000 magnification, presence of pores and micro cracks were very minimal and granulated appearance was seen.

Figure 4.35 B shows the structure of raw African locust bean seeds after processing i.e. dehulling, washing, boiling and grinding.

A wider pore, compared to the raw unprocessed sample was noticed. This was probably due to the processing techniques mentioned above, but an evidence of structural changes as a result of deformation of structure during processing. Structure were crowded into a dense cluster (agglomerate) but cohering. Presences of big pores and cracks were also noticed with a big fissure at the center indicating separation of compounds especially of a broken starch granule. Surface still rough, wrinkled and crumpled. It was observed that the processing altered the structure of the seed since the granular structure disappeared into a wider one with a continuous irregular shape. The protein granules were still present on the surface, confirming the presence of protein.

Day 0 – Figure 4.35 B shows the morphological structure of unfermented sample. This is the starting sample (before fermentation began).

Day 3 – Figure 4.35 C revealed the morphological structure of the third day fermented seeds. Wider agglomerated and non-cohering structure with a wider pores, cracks and increased fissures was noticed, this was probably due to the fermentation process whereby compounds

were been broken down into smaller units such as carbohydrates. Granules were still visible, probably protein but adhere more to the surface of the structure. The large volume of pores space is an indication of uneven distribution of the phase. Structure shows dendritic and a little flowery pattern to the right, this show that the minerals or compounds present at that stage can easily be dissolved or disintegrated into smaller units. The granular appearance was disappearing into a smaller cluster with still a continuous network of irregular pattern.

Day 5 – Figure 4.35 D revealed the morphological structure of sample fermented for five days with fermentation temperature 40 °C.

Wide agglomerated cohering surface pattern with disappearing flowery shape showing that some compounds were been decomposed from their initial forms e.g. protein. This might probably be the reason why products after the third day was unacceptable. More scattered pores were noticed more than the third day, which were more compact and dense, compounds are broken down into smaller units. On the other hand the granular shape or structure disappeared with a continuous network with irregular pattern was formed, with only few adhering protein particles. This is probably the reason for the low protein content after the third day of fermentation. Surface were still wrinkled and crumpled with fissures scattered.

Day 3 – Figure 4.35 E shows the structure of sample fermeted for three days at fermentation temperature 50 °C.

A dendritic flowery with minimal pores and micro cracks. Structure were agglomerated with cohering pattern. Granules appeared bigger with a slightly uniform regular pattern. This shows that compounds were close together with little evidence of disintegration, unlike the third day of fermentation condition 40 °C with large volume of pores. Process rate was probably getting slow since the structure became coarse and compact with still a corrugated surface.

The protein structure were more compact in granular form, which later observed to disintegrate into smaller unit with respect to fermentation duration.

Day 5 – Figure 4.35 F shows the morphological sturctre of sample fermented for five days at fermentation temperature 50 °C.

This shows an agglomerated but non cohering structure. SEM revealed a wrinkled, corrugated with non-uniform pattern. Very wide volume of pores were seen with fissure. Granules of protein were scattered all over the surface of the sample. There was a clear distinction between pores. Mineral or compound disintegration was evident more than in other samples examined, this probably might be the reason for an unacceptable end product. Convoluted surface with disappearing granules, showing degradation of protein.

Day 3 – Figure 4.35 G revealed the morphological structure of the third day fermented sample at fermentation temperature 70 °C. Third day fermentation condition at the highest temperature were also considered. A more flowery pattern was revealed at the surface of the sample examined. Agglomerated with a cohering structure was seen. Since the structure were compact and dense disintegration is probably to be low or absent, hence little or no reaction. This is probably the reason why the product was not acceptable. Narrower pores with more fissures were seen all over the surface.

Day 3 – Figure 4.35 H revealed the structure at 40 °C under Anaerobic fermentation condition. Minimal presence of fissures, pores and micro cracks were observed. Agglomerated and cohering structure with wrinkled corrugated surface. No definite pattern and disintegration was observed. The protein granules were present but lesser than in other samples.

Figure 4.35 I - shows the morphological structure of traditionally fermented sample (purchased).

Structure were compact, dense and convoluted. Pores and micro cracks were observed all over the surface, but very small showing even distribution and uniformity. Agglomerated and cohering structure with wrinkled corrugated surface. No definite pattern was observed with no

evidence of disintegration. The impression inferred from this was the presence of a dense protein matrix with partly denatured protein bodies.

Since fermentation is the breaking down of carbohydrate into simple sugar, all the pores or cavity observed with the fissures seen were probably as a result of structural changes during fermentation and the degradation of carbohydrates into simple sugar. Protein granules were present in all the surfaces except for the fifth day with diminishing granules.

Abdoulaye *et al*. (2012) reported that the changes observed in the structure of fermented African locust bean were due to the following: (1) Amylose content (2) interaction between starch chains (3) arrangement of amylose chains within the amorphous domains and (4) lipid-amylose complexes. This is probably why the morphology of fermented and unfermented African locust bean seeds depends on the botanical sources of starch.

4.8 Identification of Organic Functional Groups Present in African Locust Bean (*Parkia biglobosa*) Seeds.

The Organic functional groups were identified and characterized using Fourier Transform Infrared (FTIR) spectroscopy (Bruker VideoMVP™ Single Reflection ATR Microsampler Spectrometer model). This is an extremely accurate and effective way of determining the absence or presence of different functional groups in a molecule.

An infrared spectrum gives valuable information about what functional groups are present in a molecule. Appendix O shows the interpreted table, while R shows the IR spectrum for African locust bean seeds.

The raw unfermented sample – The spectrum has a characteristics strong stretching broad bond of O-H, hydrogen-bonded Alcohol, Phenols and carboxylic acids compound, with absorption frequency range of 3271.68 cm^{-1}. A stretching vibration of C-H, Alkynes with strong and sharp intensity is observed at the same absorption frequency. Similarly, a stretching broad N-H medium but secondary band amine was also noticed. Bands such as stretching frequency of

alkane C-H and alkene =C-H with strong and medium intensity was noticed at absorption frequency of 2924.52 cm^{-1} respectively. All sample analysed shows the same properties as above but at different characteristics absorption of frequency range. This explains the similarity in aroma (odour) during fermentation. The intensity of each compounds found at different stages of fermentation differentiates them from one another, this accounts for the increase in aroma (odour) during and after fermentation.

C-Cl and C-Br (Alkyl halides and aromatics compounds) are the compound which was observed only on the 5^{th} day of fermentation at temperature 50 °C. This probably might be responsible for the strong undesirable odour perceived after the fourth day of fermentation.

TABLE 4.2 Summary of the Identified Compounds in Fermented *P. biglobosa*

FUNCTIONAL GRP	RAW	STARTING SAMPLE	5^{TH} DAY @ 40°C	5^{TH} DAY @ 50°C	5^{TH} DAY @ 60°C	COMMERCIAL SAMPLE
O-H	√	√	√	√	√	√
C-H	√	√	√	√	√	√
N-H	√	√	√	√	√	√
C=O	√	√	√	√	√	√
N-O	√	√	√	√	√	√
-C-H	√	√	√	√	√	√
C-F	√	√	√	√	√	√
C-O	√	√	√	√	√	√
C-Cl	-	-	-	-	√	-
C-Br	-	-	-	-	√	-
C-N	√	√	√	√	√	√
C=C	√	√	√	√	√	√

KEY

(vi) Absent

√ Present

Literature revealed that volatile oils were responsible for the variation in both colour and aroma of fermented African locust bean seeds to 'Iru' Achinewu, (1986) and Nwokeleme *et al*. (2015). Nwokeleme *et al*. (2015) discovered 36 volatile compound in the mixed culture fermentation process of African locust bean seed to 'Iru' which includes 12 hydrocarbons, 10 esters, 5 alcohols, 2 phenols, 2 ketones and 1 furan, amine, acids, thiophene and lactone. While 30 compound were discovered when only *B. subtilis* was used, they comprise 10 hydrocarbons, 8 esters, 3 alcohols, 2 amines, 2 Sulphur compounds, and one of each of acid, aldehyde, phenol, ketone, and furan. They included pyrazines, aldehydes, ketones, esters, alcohols, acids, alkanes, alkenes, amines, pyridines, benzenes, phenols, Sulphur compounds, furans and other compounds.

A total of 12 flavour compounds were identified in *'Iru'* which included alcohols, esters and carbonyls. Esters of fatty acids is one of the largest group of volatile compounds released during the fermentation of African locust bean seeds to 'Iru' which are responsible for the flavour of all fermented foods. This was noticed largely between the 36 – 48 hour of fermentation and persisted in various forms till the end. The sudden appearance of certain aroma in the course of fermentation shows that there were some compounds in the starting substrate which were been enhanced by fermentation and temperature, some disappeared and some appeared.

All the compound discovered in this work are mostly volatile which explains the variation in the odour of condiment with respect to fermentation time.

4.9 Empirical Equation for the Fermentation of African Locust Bean (*Parkia biglobosa)* seeds

The kinetics of fermentation was studied and an empirical formula was developed as a model for the fermentation of African locust bean seeds using only TWO variables (time and temperature) with an n^{th} order equation.

The development of the model was based on the rate of formation of percentage protein since the major reason for the consumption of the fermented seed is its high protein content.

The data generated in the experimental analysis were used to predict the rate of formation of protein with respect to fermentation duration [hour] and temperature of fermentation in [degree Celsius]. The resolved results were very close to the analyzed values.

4.9.1 Fermentation Duration with Respect to Time

Assuming an nth order empirical equation:

$$\%P_T = at^{\propto} + \%P_i \quad \ldots\ldots\ldots\ldots\ldots \quad (4.1)$$

Where:

$\% P_T$	=	Percentage total protein present in the substrate at time't' [%]
a	=	Intercept on the graph of total percentage protein versus time at given temperature
t	=	Fermentation duration [hour] at different temperature
$\propto$	=	The slope on the graph of total percentage protein versus time given temperature
$\% P_i$	=	The initial percentage protein of the substrate

Linearizing equation (4.1)

$$\ln [P_T - P_i] = \ln a + \alpha \ln t \quad \ldots\ldots\ldots\ldots\ldots \quad (4.2)$$

Since all the parameters are known the plot of ln $[P_T - P_i]$ versus ln t gives the slope as 'α' and intercept as 'a'.

TEMPERATURE 40 °C

Equation (4.1) becomes,

$\% P_T = at^{\propto} + \% P_i$

Where:

$\% P_T \equiv$ P

a = intercept = 1

α = slope = 0.769

t = Fermentation time

$\% P_i$ = 32%

IST DAY ≡ 24 hours fermentation time

P = $[1][24]^{0.769} + 32\%$

1 * 11.5181 +32 = 43.52%

2ND DAY ≡ 48 hours fermentation time

P = $[1][48]^{0.769} + 32\%$

1 * 19.6279 +32 = 51.63%

3RD DAY ≡ 72 hours fermentation time

P = $[1][72]^{0.769} + 32\%$

1 * 26.8095 +32 = 58.81%

TEMPERATURE 50 °C

$\% P_T \equiv$ P

a = intercept = 1

α = slope = 0.6522

t = Fermentation time

$\% P_i$ = 32 %

1ST DAY ≡ 24 hours fermentation time

$P_T = at^{\propto} + \%P_i$

P = $[1][24]^{0.6522} + 32\%$

1 * 7.9465 +32% = 39.95%

2ND DAY ≡ 48 hours fermentation time

P = $[1][48]^{0.6522} + 32\%$

1 * 12.4884 +32% = 44.49%

3RD DAY ≡ 72 hours fermentation time

P = $[1][72]^{0.6522} + 32\%$

1 * 16.2687 +32% = 48.27%

FERMENTATION AT AMBIENT TEMPERATURE

Following the above steps, the ambient fermentation temperature also gave a very close range of values with the predicted.

% P ≡ P

a = intercept = 1

α = slope = 0.556

t = Fermentation time

$\%P_i$ = 32 %

IST DAY ≡ 24 hours fermentation time

P_T = $at^{\propto} + \%P_i$

P = $[1][24]^{0.556} + 32\ \%$

= 5.8533 + 32 % = 37.85 %

2ND DAY ≡ 48 hours fermentation time

P = $[1][48]^{0.556} + 32\ \%$

1 * 8.6054 +32 % = 40.61 %

3RD DAY ≡ 72 hours fermentation time

P = $[1][72]^{0.556} + 32\ \%$

Table 4.3 Comparison of the result for the formation of protein in fermented African locust bean seeds at fermentation temperature 40 °C

Fermentation time [hour]	Model Values [%]	Experimental Values [%]
0 hour	32	32
24 hours	43.52	42.50
48 hours	51.63	52.00
72 hours	58.81	56

Table 4.4 Comparison of the result for the formation of protein in fermented African locust bean seeds at fermentation temperature 50 °C

Fermentation time [hour]	Predicted Values [%]	Experimental Values [%]
0 hour	32	32
24 hours	39.95	38.50
48 hours	44.49	46.10
72 hours	48.27	50

Table 4.5 Comparison of the result for the formation of protein in fermented African locust bean seeds at ambient fermentation temperature

Fermentation time [hour]	Predicted Values [%]	Experimental Values [%]
O hour	32	32
24 hours	37.85	34.60
48 hours	40.61	41.00
72 hours	42.78	43.20

4.10 Statistical Optimization of Fermentation Conditions for Fermented African Locust Bean Seeds (*Iru*)

4.10.1 Results for the Response Surface Methodology

The main interest in this work is the protein composition of the fermented seeds. Therefore the optimization was based only on its experimental results.

Assumption is that the experimentally measured % protein in 'Iru' is a complex function of the day, temperature and concentration of inoculum used during fermentation i.e.:

$$P = f(Time, Ino.conc., Temp.) \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (4.3)$$

The effects of the above variables (operating parameters) on the % Protein composition were studied using Box-Behnken factional factorial design of experiments to optimize the yield. The results were analyzed using MINITAB 17 software.

4.10.2 Experimental Design for the Fermentation Conditions

Response surface regression analysis was used using MINITAB 17 software. Responses were generated as functions of three variables namely: X_1 as Time (fermentation duration), X_2 as Inoculum concentration (g broth/g seed) and X_3 as Temperature (°C).

The response variable (% Protein) was fitted by a second-order polynomials in order to correlate the design variables (X_1, X_2, X_3) which is presented with the model below:

$$Y = \alpha_0 + \alpha_1X_1 + \alpha_2X_2 + \alpha_3X_3 + \alpha_{1,1}X_1X_1 + \alpha_{1,2}X_1X_2 + \alpha_{1,3}X_1X_3 + \alpha_{2,2}X_2X_2 + \alpha_{2,3}X_2X_3 + \alpha_{3,3}X_3X_3 \quad \text{[4.4]}$$

The % Protein composition responses is represented by Y, which is associated with each factor level combinations. α_0, α_1, α_2, α_3, $\alpha_{1,2}$ ….. $\alpha_{3,3}$ are the regression coefficients: X_1, X_2 and X_3 are the factors. X_1X_1, X_1X_2, X_1X_3, X_2X_2, X_2X_3 and X_3X_3 are the interactions of the variables.

Below is the best fitted models obtained from the regression analysis.

Regression Equation (coded variables):

$$\%\ \text{Protein} = 85.7 + 10.42\ X_1 - 3521\ X_2 - 1.29\ X_3 - 1.246\ X_1X_1 - 107600\ X_2X_2 + 0.0007\ X_3X_3 - 465\ X_1X_2 - 0.0005\ X_1X_3 + 117.0\ X_2X_3 \quad \text{[4.5]}$$

R-Sq. = 90.23 %

SOLUTION:

Day: 3.251

Inoculum concentration: 0.005 (g broth/g seed)

Temperature: 40°C

% Protein fit: 51.54

Desirability: 0.977122

Appendix R shows the Box-Behnken Design of the Variables with % Protein as the Response

Appendix S shows the Box-Behnken Design application for the Process Simulation for % Protein Yield

Factors	Notation
Fermentation duration (days)	X_1
Ino. Concentration (g broth/g seed)	X_2
Temperature (°C)	X_3

Appendix T shows the Analysis of Variance (ANOVA) for the Response Surface Regression The Response surface model predicting the % Protein is shown in Appendix T.

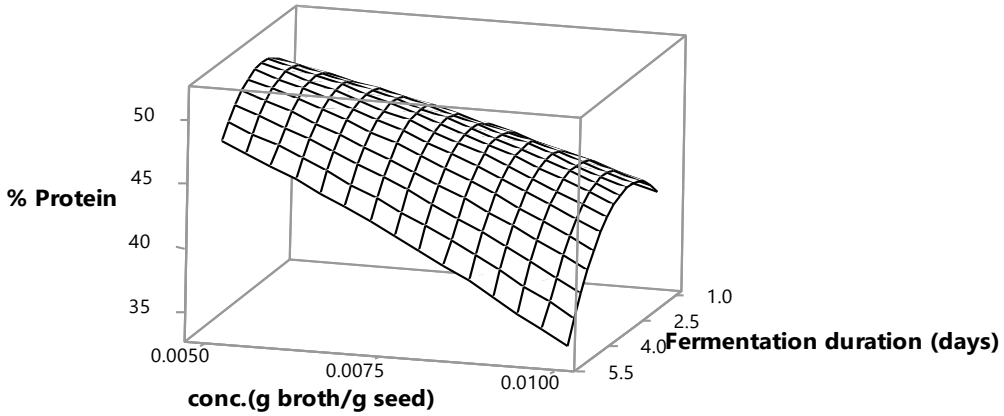
ANOVA was used to test statistical significance of the model. Table 4.7 shows statistically the sum of square (SS), degree of freedom (DF), mean square (MS). F-value, p-value and ANOVA coefficents. The Fisher distribution (F-value and P-value) were used to determine the significance of the model. A large F-value and a small P-value implies that the models are adequate to predict the responses. R^2 indicate the reliability of the model. The closer the R^2 value to 1, the stronger and better the model prediction of the responses.

4.10.3 Optimization of the % Protein yield

A 3-D (3 dimensional) response surface plots of Protein vs. (Day, Conc.); Protein vs. (Day, temp) and Protein vs (conc., temp) were obtained as shown below (Figures A-F). These plots show the predicted effect of process variables (X_1, X_2, X_3) on % Protein as the response. The 3-D plot represent graphically the regression coefficient in equation form in order to obtain the optimum conditions of the variables within the design region.

The result of the optimization shows that more than 3 fermentation days (3.251 days), with Inoculum concentration of 0.005 g broth/g seed and fermentation temperature of 40 ^{0}C will be required to yield an optimum % Protein content of 51.54 %.

A



B

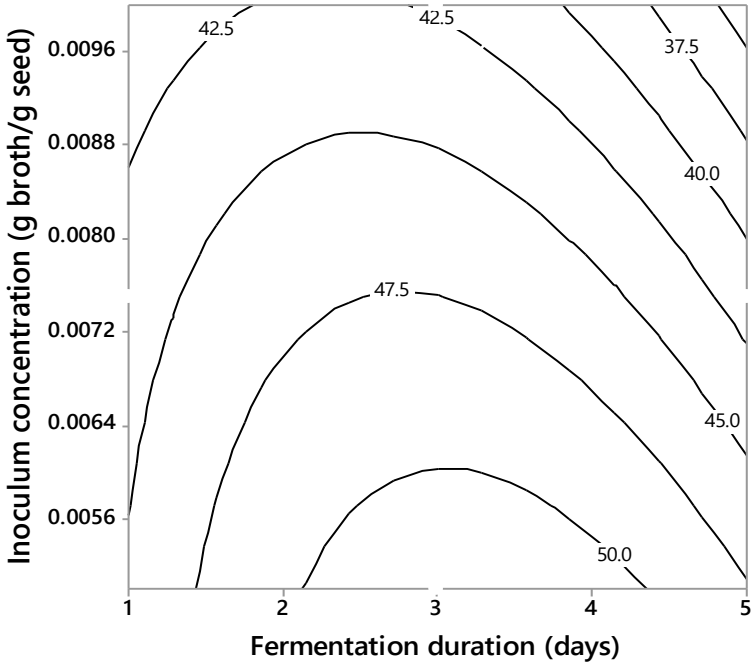
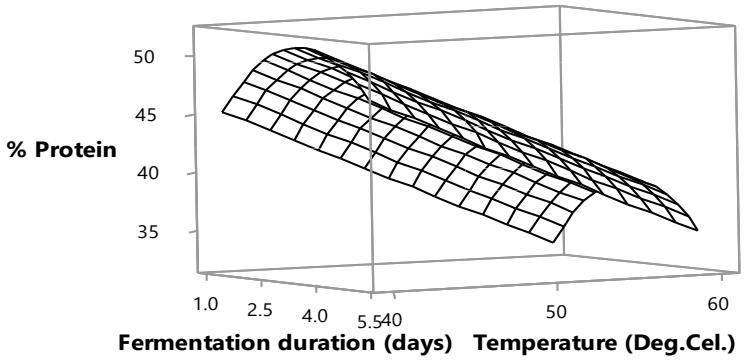


Fig. 4.36 – (A) Surface and (B) Contour plots of % Protein content yield vs fermentation duration and Inoculum concentration at fixed temperature.

C



D

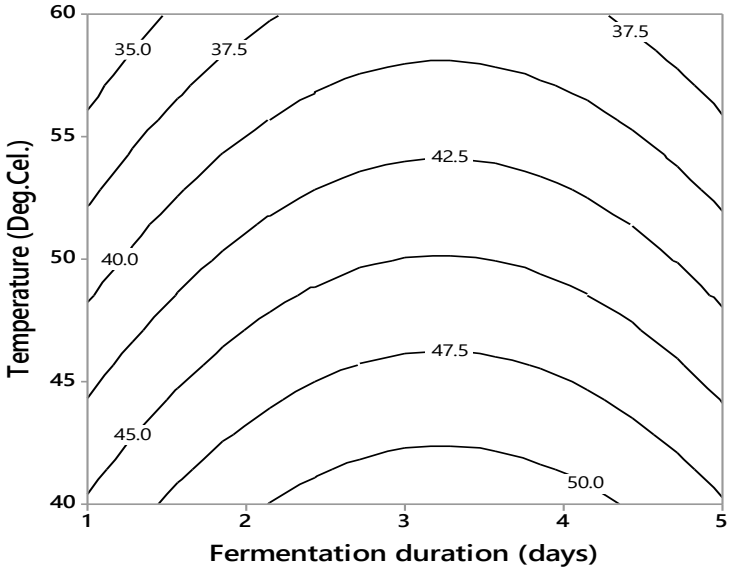
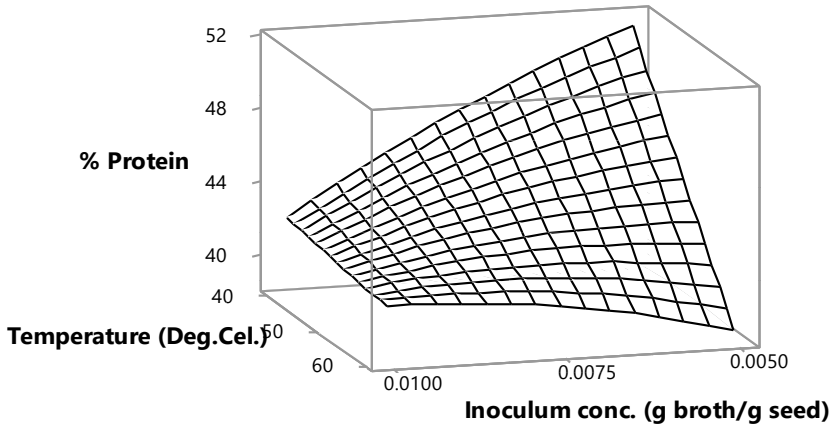


Fig. 4.37 – (C) Surface and (D) Contour plots of % Protein content yield vs fermentation duration and temperature at fixed volume

E



F

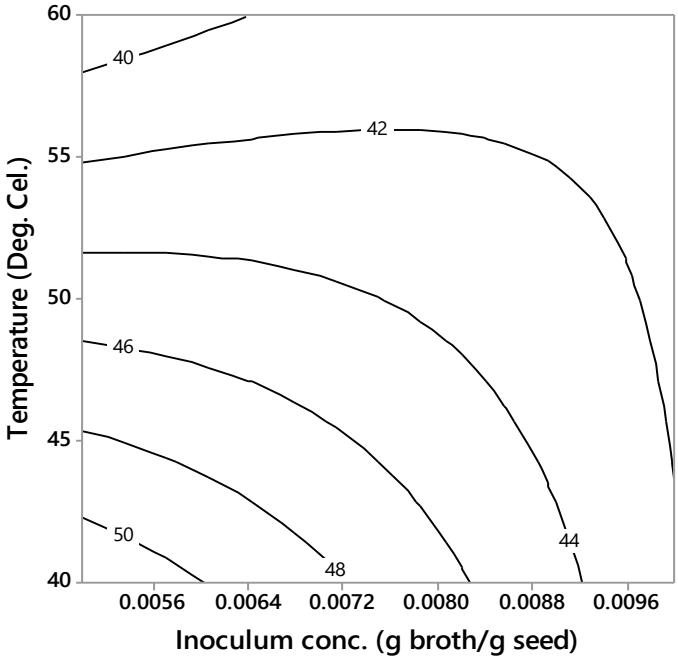


Fig. 4.38 – (E) Surface and (F) Contour plots of % Protein content yield vs Inoculum concentration and temperature at a fixed volume.

Table 4.8 Properties of Bacteria Isolated from ‘Iru’

Samples	Colony Morphology	Cell Characteristics	Size (mm)	Gram Reaction	Probable Organism
3^{RD} Day @ 40 °C	Creamy, pin point and submerge in Agar.Long opaque rod	Rods in singles	0.1	+ve rods	*Bacillus subtilis*
3^{RD} Day @ 50 °C	Creamy Convex, pin point and submerge in Agar. Opaque rough rods	Long rods	0.5	+ve rods	*Bacillus subtilis* a*nd Bacillus pumilus*
Commercial	Whitish (creamy) convex and smooth	Cocci in clusters	0.5	+ve cocci	*Staphylococcus saprophyticus*

Table 4.8 revealed *Bacillus subtilis* as the dominant microorganism responsible for the fermentation of African locust bean seeds to ‘Iru’, a protein based condiment.

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The results obtained of this research study confirmed and upgraded other previous research works that have been carried out on the fermentation of African locust bean.

Fermentation of African locust bean with *Bacillus subtilis* improves both the nutritional values and organoleptic property of fermented seeds, especially the protein content which increased form 32 % to about 52 % in the aerobic fermentation process. An increase in the consumption of African locust beans will reduce the risk of nutrient deficiency in consumers.

The optimum operating conditions for the fermentation of African locust bean seeds were developed. Experimentally, the best conditions were found to be: Fermentation duration of 3 days, with inoculum concentration of 0.005 g broth/g seed and operating fermentation temperature as 40 °C. These results correlated with the optimized conditions of 3.251 days for fermentation duration, 0.005 g broth/g seed of inoculum concentration and 40 °C operating temperature using the MINITAB 17 software.

The results obtained in this study confirmed that the microorganism responsible for the fermentation of *Parkia biglobosa* were: *Bacillus species (subtilis, pumilus and licheniformis)* and *Saccharomyces Cerevisiae.* The physiological and sensory analysis carried out showed that the sample fermented with *Bacillus* spp. was the best.

A total of 10 Flavour compounds were identified in 'Iru' which included alcohols, esters and carbonyls.

Total dryness of the fermented condiment will preserve the seed and increase their utilization especially in protein deficient food formulation. This will reduce scarcity (due to very short shelf life), and improve its availability all year round.

Aerobic fermentation produced alkaline Iru with a higher fibre, fat and carbohydrate content than the Iru produced during anaerobic fermentation and other legumes.

5.2 Recommendations

It is recommended that *Bacillus subtilis* cultures should be made more available commercially so that industrial/large scale production of 'Iru' having consistent quality would be feasible.

'Iru' has a high amount of protein and thus is recommended as a supplement to meat for the populace to reduce protein- calorie malnutrition.

Investigation should be extended to include a variety of organisms as starter culture such as *Lactobacillus,* Azetobacter, fungi etc. at different temperature for the fermentation of 'Iru'.

Due to the economic relevance of *Parkia biglobosa* and its fermented products, a pilot project should be set up to produce the condiment using the available data from this study as baseline information.

Equipment for the processing such as de-hulling should be designed.

Boiling time should be reduced since useful minerals tend to leach into the boiling water during processing and also investigation should be carried out on further uses of the chaft (hull) produced as waste during processing.

Research should be carried out to see the effects of salting, drying and other preservation methods on the aerobically fermented condiment using the optimized condition obtained in this work.

Iru should be packaged in appealing ways so as to make it more presentable and as marketable as imported food flavourings.

Finally, it is recommended that *'Iru'* should be made into cubes and packaged well to attract prospective buyers in order to upgrade the powdered one produced in this work.

5.3 Contribtions to Knowledge

This study made contributions to the society and to human knowledge in the following ways:

1. An improved processing method was developed through the optimized conditions for hygienic industrial processing techniques.
2. Good preservation method which will lead to a longer shelf life of condiment was developed.
3. Suitable starter culture for the fermentation of African locust bean seeds was discovered.
4. Industrial database parameters were developed.
5. A simple model for the prediction of the production of protein was developed. This will reduce the rigorous experiment and minimize cost.

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APPENDIX

Appendix A: Determination of Fermentation Rate

Table A-1: Effect of fermentation on Substrate weight loss using *Bacillus subtilis at* different temperatures

Aerobic

Samples	WT Loss (g) at Tempt. 40 ^{0}C	WT LOSS (g) at Tempt. 50 ^{0}C	WT Loss (g) at TemptT. 60 ^{0}C	WT Loss (g) at Tempt.70 ^{0}C
Day 0	0.000	0.000	0.000	0.000
Day 1	0.536	0.319	0.179	0.215
Day 2	0.802	0.508	0.214	0.284
Day 3	0.971	0.842	0.373	0.384
Day 4	1.389	1.012	0.639	0.591
Day 5	1.402	1.562	0.747	0.798

Anaerobic

Samples	WT Loss (g) at Tempt. 40 ^{0}C	WT Loss (g) at Tempt. 50 ^{0}C	WT Loss (g) at Tempt. 60 ^{0}C	WT Loss (g) at Tempt. 70 ^{0}C
Day 0	0.000	0.000	0.000	0.000
Day 1	0.288	0.244	0.361	0.593
Day 2	0.288	0.318	0.543	0.699
Day 3	0.349	0.546	0.602	0.711
Day 4	0.508	0.711	0.893	0.874
Day 5	0.638	0.778	1.096	1.114

Table A-2: Effect of fermentation temperature on Substrate weight loss using *Saccharomyces cerevisiae as* starter culture

Samples	WT Loss (g) at Tempt. 40 ^{0}C	WT Loss (g) at Tempt. 50 ^{0}C	WT Loss (g) at Tempt. 60 ^{0}C
Day 0	0.000	0.000	0.000
Day 1	0.370	0.241	0.063
Day 2	0.481	0.283	0.080
Day 3	0.503	0.198	0.033
Day 4	0.381	0.100	0.022
Day 5	0.289	0.003	0.001

Table A-3: Effect of fermentation temperature on Substrate weight loss using the mixture of *Bacillus subtilis* and *Saccharomyces cerevisiae as* starter culture

Samples	WT Loss (g) at Tempt. 40 ^{0}C	WT Loss (g) at Tempt. 50 ^{0}C
Day 0	0.000	0.000
Day 1	0.781	0.971
Day 2	1.068	0.793
Day 3	1.438	0.769
Day 4	1.991	0.697
Day 5	2.059	0.535

Table A-4: Effect of Ambient fermentation temperature on Substrate weight loss

Samples	WT Loss (g) at Ambient Tempt.
Day 0	0
Day 1	0.064
Day 2	0.080
Day 3	0.133
Day 4	0.218
Day 5	0.282

Table A-5: Effect of Inoculum concentration (g broth/g) seed on Substrate weight loss (*B.subtilis*)

At **40 ^{0}C**

Samples	INO_ 0.0025	INO_0.005	INO_0.0075	INO_0.01
Day 0	0.000	0.000	0.000	0.000
Day 1	0.353	0.536	0.260	0.119
Day 2	0.757	0.802	0.528	0.140
Day 3	0.796	0.971	0.554	0.152
Day 4	1.143	1.389	0.672	0.215
Day 5	1.202	1.402	0.780	0.330

At 50 ^{0}C

Samples	INO_0.0025	IN0_0.005	INO_0.0075	IN0_0.01
Day 0	0.000	0.000	0.000	0.000
Day 1	0.166	0.244	0.302	0.530
Day 2	0.262	0.318	0.378	0.551
Day 3	0.386	0.546	0.728	0.572
Day 4	0.697	0.711	0.794	0.823
Day 5	0.720	0.778	0.812	0.870

Table A-6: Effect of fermentation temperature on the pH value of the substrate in **Aerobic** fermentation condition (*Bacillus subtilis*)

Samples	pH at 40 ^{0}C	pH at 50 ^{0}C	pH at 60 ^{0}C	pH at 70 ^{0}C
Day 0	5.36	5.36	5.35	5.36
Day 1	5.82	5.75	5.84	5.55
Day 2	5.92	6.02	6.48	5.88
Day 3	6.13	7.13	7.25	6.2
Day 4	7.7	7.79	7.51	6.35
Day 5	8.3	8	7.88	6.77

Table A-7: Effect of fermentation temperature on the pH value of the substrate using *Saccaromyces cerevisiae* as starter culture

Samples	pH at 40 ^{0}C	pH at 50 ^{0}C	pH at 60 ^{0}C
Day 0	6.03	6.03	6.03
Day 1	6.35	5.96	5.65
Day 2	6.73	5.72	5.55
Day 3	6.89	6.02	5.23
Day 4	7.55	6.35	5.28
Day 5	7.01	6.27	5

Table A-8: Effect of ambient fermentation temperature on the pH value of the substrate

Samples	pH at Ambient
Day 0	5.16
Day 1	6.8
Day 2	7.44
Day 3	7.49
Day 4	7.74
Day 5	7.99

Table A-9: Effect of Inoculum concentration on the pH value of the substrate

At 40 ^{0}C

Samples	pH at 0.0025	pH at 0.005	pH at 0.0075	pH at 0.01
Day 0	5.36	5.36	5.36	5.36
Day 1	5.5	5.82	5.5	5.4
Day 2	6.7	5.41	5.71	5.4
Day 3	7.8	6.13	6.5	5.5
Day 4	8.01	7.7	6.9	5.54
Day 5	8.2	8.3	7.5	6.01

At 50 ^{0}C

Samples	pH at 0.0025	pH at 0.005	pH at 0.0075	pH at 0.01
Day 0	5.36	5.36	5.36	5.36
Day 1	6.01	5.7	5.5	5.39
Day 2	7.1	6.7	5.6	5.52
Day 3	7.34	7.5	5.73	5.78
Day 4	7.59	8.54	5.82	6.22
Day 5	7.88	8.7	6.03	6.43

Appendix G. The Physiological Analysis of Aerobic fermentation using *B.subtilis*

Sample fermented at **40 °C**

	Odour/Aroma	Colour	Appearance
Starting sample	beany smell	creamy brown	not edible
Day 1	lightly plesant	brownish	acceptable
Day 2	lightly plesant	browner	acceptable
Day 3	plesant	browner	acceptable
Day 4	plesant choking	dark brown	managable
Day 5	plesant choking	darker	slightly acceptable

Sample fermented **at 50 °C**

	Odour/Aroma	Colour	Appearance
Starting sample	beany smell	creamy brown	not edible
Day 1	plesant	brownish	acceptable
Day 2	plesant	brown	acceptable
Day 3	plesant choking	browner	acceptable
Day 4	plesant choking	darker	slightly acceptable
Day 5	not plesant choking	darker	not acceptable

Sample fermented **at 60 °C**

	Odour/Aroma	Colour	Appearance
Starting sample	beany smell	creamy brown	not edible
Day 1	plesant	browner	slightly acceptable
Day 2	plesant	browner	slightly acceptable
Day 3	plesant choking	dark brown	not acceptable
Day 4	plesant choking	dark brown	not acceptable
Day 5	roasting odour	dark brown	not acceptable

Sample fermented at **70 °C**

	Odour/Aroma	Colour	Appearance
Starting sample	beany smell	creamy brown	not edible
Day 1	mildy plesant	brown	not acceptable
Day 2	roasting odour	brown	not acceptable

Day 3	roasting odour	brown	not acceptable
Day 4	bad roasting odour	brown	not acceptable
Day 5	bad roasting odour	brown	not acceptable

Appendix H: The Physiological Analysis of Anaerobic fermentation using *B.subtilis*

Samples fermented **at 40 °C**

	Odour/Aroma	Colour	Appearance
Starting sample	beany smell	creamy brown	not edible
Day 1	pungent	brownish	acceptable
Day 2	pungent	brownish	acceptable
Day 3	pungent	brownish	not acceptable
Day 4	pungent	brownish	not acceptable
Day 5	choking pungent	brownish	not acceptable

Samples fermented **at 50 °C**

	Odour/Aroma	Colour	Appearance
Starting sample	beany smell	creamy brown	not edible
Day 1	mild beany smell	butter light	not acceptable
Day 2	choking pungent	butter light	not acceptable
Day 3	choking pungent	butter light	not acceptable
Day 4	choking pungent	butter light	not acceptable
Day 5	choking pungent	butter light	not acceptable

Samples fermented **at 60 °C**

	Odour/Aroma	Colour	Appearance
Starting sample	beany smell	creamy brown	not edible
Day 1	mild beany smeel	butter light brown	not acceptable
Day 2	mild beany smell	butter light brown	not acceptable
Day 3	mild beany smell	butter light brown	not acceptable
Day 4	bad smell	butter light brown	not acceptable
Day 5	bad choking roasting smell	butter light brown	not acceptable

Samples fermented **at 70 °C**

	Odour/Aroma	Colour	Appearance
Starting sample	beany smell	creamy brown	not edible

Day 1	offensive	butter light brown	not acceptable
Day 2	offensive	butter light brown	not acceptable
Day 3	offensive	butter light brown	not acceptable
Day 4	offensive	butter light brown	not acceptable
Day 5	offensive (2 layers observed, brown and dark)	butter light brown	not acceptable

Appendix I: The Physiological Analysis of Fermentation using *Saccharomyces cerevisiae*

Samples fermented **at 40 °C**

	Odour/Aroma	Colour	Appearance
Starting sample	beany smell	creamy brown	not edible
Day 1	very mild	brown	acceptable
Day 2	lightly plesant	brown	acceptable
Day 3	plesant	brown	acceptable
Day 4	plesant choking	brown	managable
Day 5	lightly choking	brown	slightly acceptable

Samples fermented **at 50 °C**

	Odour/Aroma	Colour	Appearance
Starting sample	beany smell	brownish	not edible
Day 1	not plesant	lighter	acceptable
Day 2	lightly choking	lighter	not acceptable
Day 3	lightly choking	lighter	not acceptable
Day 4	choking	lighter	not acceptable
Day 5	strongly choking	lighter	not acceptable

Samples fermented **at 60 °C**

	Odour/Aroma	Colour	Appearance
Starting sample	beany smell	brownish	not edible
Day 1	not plesant	darker	not acceptable
Day 2	not plesant	darker	not acceptable
Day 3	not plesant	darker	not acceptable
Day 4	not plesant	darker	not acceptable
Day 5	roasting odour	darker	not acceptable

Appendix j: The Physiological Analysis of Fermentation using mixture of S*accharomyces cerevisiae and Bacillus subtilis*

Samples fermented **at 40 °C**

	Odour/Aroma	Colour	Appearance
Starting sample	beany smell	creamy brown	not edible
Day 1	mildly pungent	brown	fair
Day 2	pungent	brown	fairly acceptable
Day 3	choking pungent	brown	fairly acceptable
Day 4	choking pungent	brown	fairly acceptable
Day 5	badly choking	brown	not acceptable

Samples fermented **at 50 °C**

	Odour/Aroma	Colour	Appearance
Starting sample	beany smell like	brownish	not edible
Day 1	not plesant	dark brown	not acceptable
Day 2	not plesant	dark brown	not acceptable
Day 3	not plesant	dark brown	not acceptable
Day 4	not plesant	dark brown	not acceptable
Day 5	not plesant	dark brown	not acceptable

Appendix K: The Physiological Analysis of natural fermentation (without inoculum)

	Odour/Aroma	Colour	Appearance
Starting sample	beany smell	brownish	not edible
Day 1	slightly beany	brown	acceptable
Day 2	not too plesant	brown	acceptable
Day 3	fairly plesant	brown	not too bad
Day 4	fairly plesant	brown	not too bad
Day 5	not plesant	lighter	not acceptable

Appendix L. The Physiological Analysis of dried samples

2 Hours drying time (17 %)

Storage duration (days)	Flavour	Colour	Texture	Appearance	Comments
0	pleasant	brown	coarse	acceptable	good
5	pleasant	darker	coarse	acceptable	good
10	pleasant	darker	coarse	acceptable	bad
15	not pleasant	darker	coarse	not acceptable	deteriorati-on noticed
20	not pleasant	darker	coarse	not acceptable	mold growth
25	not pleasant	darker	coarse	not acceptable	maggot infestation
30	not pleasant	darker	coarse	not acceptable	maggot infestat - ion

4 Hours drying time (8.2 %)

Storage duration (days)	Flavour	Colour	Texture	Appearance	Comments
5	pleasant	darker	coarse	acceptable	good
10	pleasant	darker	coarse	acceptable	good
15	pleasant	darker	coarse	acceptable	good
20	fairly pleasant	darker	coarse	fairly acceptable	rancid smell
25	off flavour	darker	coarse	fairly acceptable	no maggot
30	not pleasant	darker	coarse	not acceptable	no maggot

6 Hours drying time (7.2 %)

Storage duration (days)	Flavour	Colour	Texture	Appearance	Comments
0	pleasant	dark brown	soft	acceptable	good
5	pleasant	darker	soft	acceptable	good
10	pleasant	darker	soft	acceptable	good
15	pleasant	darker	soft	acceptable	good
20	pleasant	darker	soft	acceptabe	good
25	off flavour	darker	soft	acceptabe	rancid smell

30	not pleasant	darker	soft	not acceptabe	no maggot/ rancid smell

8 Hours drying time (4.9 %)

Storage duration (days)	Flavour	Colour	Texture	Appearance	Comments
0	pleasant	dark brown	soft	acceptable	good
5	pleasant	darker	soft	acceptable	good
10	pleasant	darker	soft	acceptable	good
15	pleasant	darker	soft	acceptable	good
20	pleasant	darker	soft	acceptable	good
25	pleasant	darker	soft	acceptable	good
30	pleasant	darker	soft	acceptable	good

10 Hours drying time (0.7 %)

Storage duration (days)	Flavour	Colour	Texture	Appearance	Comments
0	pleasant	dark brown	soft	acceptable	good
5	pleasant	darker	soft	acceptable	good
10	pleasant	darker	soft	acceptable	good
15	pleasant	darker	soft	acceptable	good
20	pleasant	darker	soft	acceptable	good
25	pleasant	darker	soft	acceptable	good
30	pleasant	darker	soft	acceptable	good

12 Hours drying time (0 %)

Storage duration (days)	Flavour	Colour	Texture	Appearance	Comments
0	pleasant	dark brown	soft	acceptable	good
5	pleasant	darker	soft	acceptable	good
10	pleasant	darker	soft	acceptable	good
15	pleasant	darker	soft	acceptable	good
20	pleasant	darker	soft	acceptable	good
25	pleasant	darker	soft	acceptable	good
30	pleasant	darker	soft	acceptable	good

Appendix M. The Physiological Analysis of the Purchased Sample

1 Hours drying time (17 %)

Storage duration (days)	Flavour	Colour	Texture	Appearance	Comments
0	pleasant	brownish	coarse	acceptable	good
5	off flavour	dark brown	coarse	not acceptable	deteriora-tion noticed
10	choking	darker	coarse	not acceptable	mold growth
15	not pleasant	darker	coarse	not acceptable	mold growth
20	not pleasant	darker	coarse	not acceptable	maggot infestati-on
25	not pleasant	darker	coarse	not acceptable	maggot infestati-on
30	not pleasant	darker	coarse	not acceptable	maggot infestati-on

4 HOURS DRYING TIME (8.2 %)

Storage duration (days)	Flavour	Colour	Texture	Appearance	Comments
0	pleasant	brown	coarse	acceptable	good
5	not pleasant	dark brown	coarse	fair	fair
10	off flavour	darker	coarse	not acceptable	bad
15	off flavour	darker	coarse	acceptable	deterior-ation noticed
20	off flavour	darker	coarse	fairly acceptable	mold growth
25	off flavour	darker	coarse	fairly acceptable	no maggot
30	not pleasant	darker	coarse	not acceptable	no maggot

6 HOURS DRYING TIME (7.2 %)

Storage duration (days)	Flavour	Colour	Texture	Appearance	Comments
0	pleasant	brown	soft	acceptable	good
5	pleasant	dark brown	soft	acceptable	good

10	pleasant	darker	soft	fair	fair
15	off flavour	darker	soft	fair	fair
20	not pleasant	darker	soft	fair	good
25	off flavour	darker	soft	fair	no maggot
30	off flavour	darker	soft	fair	no maggot

8 HOURS DRYING TIME (4.9 %)

Storage duration (days)	Flavour	Colour	Texture	Appearance	Comments
0	pleasant	dark brown	coarse	acceptable	good
5	pleasant	darker	coarse	acceptable	good
10	pleasant	darker	coarse	acceptable	good
15	pleasant	darker	coarse	acceptable	good
20	pleasant	darker	coarse	acceptable	good
25	pleasant	darker	coarse	fair	whitish mucilage noticed
30	off flavour	darker	coarse	fair	whitish mucilage noticed

10 Hours drying time (0.7 %)

Storage duration (days)	Flavour	Colour	Texture	Appearance	Comments
0	pleasant	dark brown	coarse	acceptable	good
5	pleasant	darker	coarse	acceptable	good
10	pleasant	darker	coarse	acceptable	good
15	pleasant	darker	coarse	acceptable	good
20	pleasant	darker	coarse	acceptable	good
25	pleasant	darker	coarse	acceptable	good

30	pleasant	darker	coarse	acceptable	good

12 Hours drying time (0 %)

Storage duration (days)	Flavour	Colour	Texture	Appearance	Comments
0	pleasant	very dark	powdery	acceptable	good
5	pleasant	darker	powdery	acceptable	good
10	pleasant	darker	powdery	acceptable	good
15	pleasant	darker	powdery	acceptable	good
20	pleasant	darker	powdery	acceptable	good
25	pleasant	darker	powdery	acceptable	good
30	pleasant	darker	powdery	acceptable	good

Appendix N. The Physiological Analysis of the undried Sample

Storage duration (days)	Flavour	Colour	Texture	Appearance	Comments
0	Pleasant	brownish	soft	acceptable	good
5	Choking	light brown	soft	not acceptable	maggot infestation
10	very choking	light brown	soft	not acceptable	maggot infestation
15	not pleasant	light brown	soft	not acceptable	maggot infestation
20	not pleasant	light brown	soft	not acceptable	maggot infestation
25	not pleasant	light brown	soft	not acceptable	maggot infestation
30	not pleasant	light brown	soft	not acceptable	maggot infestation

Appendix O. Identification of Organic Functional Groups present in African locust bean (*Parkia biglobosa*) seeds.

Unprocessed African locust bean seed [Raw]

S

FUNCTIONAL GROUP	TYPE OF VIBRATION	XTERISTICS ABSORPTIONS (cm^{-1})	INTENSITY
O-H suggest the presence of Alcohol, phenols and carboxylic acids	Stretch, H-bonded	3271.68	Strong and broad
C-H Alkynes	Stretch	3271.68	Strong and sharp
N-H Amine	stretch	3271.68	Medium and secondary
C-H and =C-H Alkanes and alkenes	stretch	2924.52 and 2854.19	Strong and medium
C=O Carbonyl, Ketones, Aldehydes, Esters.	stretch	1744.03 and 1631.37	Strong [most intense absorption in the spectrum]
N-H Primary amines C=C Alkenes N-O NITRO	Bend Stretch Asymmetric Stretch	1631.37 1542.67	Multiple sharp medium peaks Variable Strong 2 bonds
-C-H Alkanes. C-F Alkyl halide.	Bend Stretch	1391.58	Variable Strong
C-O Alcohols, carboxylic acids, esters and ethers. C-H wag ($-CH_2X$) Alkyl halides. C-N Aliphatic amines.	Stretch Stretch	1238.39	Strong [falls within the finger print region] a strong absorption medium-weak
C-O Ether. C-N Amines.	Stretch Stretch	1051.25	Strong [Falls within the finger print region] a strong absorption

STARTING SAMPLE (PROCESSED) OF AFRICAN LOCUST BEAN SEED

FUNCTIONAL GROUP	TYPE OF VIBRATION	XTERISTICS ABSORPTIONS (cm^{-1})	INTENSITY
O-H suggest the presence of Alcohol, phenols and carboxylic acids	Stretch, H-bonded	3274.51	Strong and broad
C-H Alkynes	Stretch	3274.51	Strong and sharp
N-H Amine	stretch	3274.51	Medium and secondary
C-H and =C-H Alkanes and alkenes	stretch	2924.18 and 2853.58	Strong and medium
C=C Alkenes	Stretch	1625.07	Variable
N-O NITRO	Asymmetric Stretch	1535.70	Strong 2 bonds
-C-H Alkanes C=C and C-C (in ring) Aromatic	Bend Stretch	1450.76	Medium –weak, multiple sharp bond
C-F Alkyl halide. -C-H Alkane.	Stretch bending	1391.30	Strong [FP region] variable
C-O Alcohol, carboxylic acids, esters, ethers. C-H wag (-CH2X) Alkyl halides. C-N Aliphatic amines.	Stretch Stretch	1238.52	Strong FP
C-O Alcohol, carboxylic acids, esters, ethers. C-H wag (-CH_2X) Alkyl halides. C-N Aliphatic amines.	Stretch Stretch	1158.86	Strong FP
C-N Aliphatic amines. C-O Alcohol. C-F Alkyl halide. C-O Ester	Stretch Stretch	1057.80	Strong FP Strong Two bands or more

LOCALLY FERMENTED [PURCHASED SAMPLE] AFRICAN LOCUST BEAN SEEDS

FUNCTIONAL GROUP	TYPE OF VIBRATION	XTERISTICS ABSORPTIONS (cm^{-1})	INTENSITY
O-H suggest the presence of Alcohol, phenols and carboxylic acids	Stretch, H-bonded	3271.40	Strong and broad
C-H Alkynes	Stretch	3271.40	Strong and sharp
N-H Amine	Stretch	3271.40	Medium and secondary
C-H and =C-H Alkanes and alkenes	Stretch	2922.83 and 2853.05	Strong and medium
C=C A lkenes	Stretch	1621.09	Variable
-C-H Alkanes C=C and C-C (in ring) Aromatic	Bend Stretch	1410.41	Medium –weak, multiple sharp bands FP
C-O Alcohol, carboxylic acids, esters, ethers. C-H wag ($-CH_2X$) Alkyl halides. C-N Aliphatic amines.	Stretch Stretch	1239.54	Strong FP
C-O Alcohol, carboxylic acids, esters, ethers. C-H wag ($-CH_2X$) Alkyl halides. C-N Aliphatic amines.	Stretch Stretch	1151.49	Strong FP
C-N Aliphatic amines. C-O Alcohol, carboxylic acids, esters, ethers. C-F Alkyl halide. C-O Ester	Stretch Stretch	1098.42	Strong FP Strong Two bands or more

C-F Alkyl halide. C-O Esters, Alcohol, carboxylic acids, ethers. C-N Aliphatic amines.	Stretch Stretch Stretch	1033.18	Strong Two bands or more FP

THIRD DAY at 50 °C

FUNCTIONAL GROUP	**TYPE OF VIBRATION**	**XTERISTICS ABSORPTIONS (cm^{-1})**	**INTENSITY**
O-H suggest the presence of Alcohol, phenols and carboxylic acids	Stretch, H-bonded	3271.05	Strong and broad
C-H Alkynes	Stretch	3271.05	Strong and sharp
N-H Amine	Stretch	3271.05	Medium and secondary
C-H and =C-H Alkanes and alkenes	Stretch	2922.99 and 2852.99	Strong and medium
C=C Alkenes	Stretch	1624.68	Variable
N-O Nitro compounds. C=C Aromatic	Asymmetric stretch	1536.10	Strong, two bands. Medium-weak, multiple sharp bands.
-C-H Alkanes C=C and C-C (in ring) Aromatic	Bend Stretch	1443.91 and 1401.74	Medium –weak, multiple sharp bands. Variables. FP
C-O Alcohol, carboxylic acids, esters, ethers. C-H wag ($-CH_2X$) Alkyl halides. C-N Aliphatic amines.	Stretch Stretch	1239.70	Strong FP
C-O Alcohol, carboxylic acids, esters, ethers. C-H wag ($-CH_2X$) Alkyl halides. C-N Aliphatic amines.	Stretch Stretch	1159.77	Strong FP

C-N Aliphatic amines. C-O Alcohol, carboxylic acids, esters, ethers. C-F Alkyl halide. C-O Ester	Stretch Stretch	1057.82	medium Strong FP Strong Two bands or more

THIRD DAY at 60 °C

FUNCTIONAL GROUP	TYPE OF VIBRATION	XTERISTICS ABSORPTIONS (cm^{-1})	INTENSITY
C-N Aliphatic amines. C-O Alcohol, carboxylic acids, esters, ethers. C-F Alkyl halides. C-O Ester.	Stretch Stretch	1059.34	medium Strong FP Strong Two bands or more
C-CI Alkyl halides. C-Br Alkyl halides.	Stretch Stretch	589.25	strong

FIFTH DAY at 50°C

FUNCTIONAL GROUP	TYPE OF VIBRATION	XTERISTICS ABSORPTIONS (cm^{-1})	INTENSITY
C-O Alcohol, carboxylic acids, esters, ethers. C-H wag ($-CH_2X$) Alkyl halides. C-N Aliphatic amines.	Stretch Stretch	1100.07	Strong FP medium
C-N Aliphatic amines. C-O Alcohol, carboxylic acids, esters, ethers. C-F Alkyl halide. C-O Ester	Stretch Stretch	1032.71	medium Strong FP Strong Two bands or more

FIFTH DAY at 40°C

FUNCTIONAL GROUP	TYPE OF VIBRATION	XTERISTICS ABSORPTIONS (cm^{-1})	INTENSITY
O-H suggest the presence of Alcohol, phenols and carboxylic acids	Stretch, H-bonded	3329.66	Strong and broad
C-H Alkynes	Stretch	3329.66	Strong and sharp
N-H Amine	Stretch	3265.54	Medium and secondary
C-H and =C-H Alkanes and alkenes	Stretch	2922.73	Strong and medium
C=C Alkenes	Stretch	1630.51	Variable
C-C Aromatics. C=C Aromatics. -C-H Alkanes.	Stretch (in-ring) Stretch Bend	1413.03	Medium Medium-weak, multiple bands
C-O Alcohol, carboxylic acids, esters, ethers. C-H wag ($-CH_2X$) Alkyl halides. C-N Aliphatic amines. C-F Alkyl halide	Stretch Stretch Stretch	1097.87	Strong FP Medium Strong
C-N Aliphatic amines. C-O Alcohol, carboxylic acids, esters, ethers. C-F Alkyl halide. C-O Ester.	Stretch Stretch	1032.56	medium Strong FP Strong Two bands or more

FIFTH DAY at 60 °C

FUNCTIONAL GROUP	TYPE OF VIBRATION	XTERISTICS ABSORPTIONS (cm^{-1})	INTENSITY
O-H suggest the presence of Alcohol, phenols and carboxylic acids	Stretch, H-bonded	3274.90	Strong and broad
C-H Alkynes	Stretch	3274.90	Strong and sharp
N-H Amine	Stretch	3274.90	Medium and secondary
C-H and =C-H Alkanes and alkenes	Stretch	2921.40	Strong and medium
C=C Alkenes	Stretch	1628.10	Variable
N-O NITRO	Asymmetric Stretch	1543.83	Strong 2 bonds
-C-H Alkanes. C=C and C-C (in ring) Aromatic	Bend Stretch	1456.08	Medium –weak, multiple sharp bond Variable
-C-H Alkanes. C-F Alkyl halide	Bend Stretch	1398.42 and 1360.82	Variable Strong
C-O Alcohol, carboxylic acids, esters, ethers. C-H wag ($-CH_2X$) Alkyl halides. C-N Aromatic amines	Stretch Stretch Stretch	1256.09	Strong FP
C-O Alcohol. C-F Alkyl halide. C-N Aliphatic amines.	Stretch Stretch	1075.28	Strong Strong

=C-H Alkenes. N-H Pri & sec. amines.	Bend wag	874.39	Strong Strong and broad
=C-H Alkenes. N-H Pri & sec. amines C-H Aromatics. C-CI Alkyl halides	Bend Wag Oop stretch	769.96	Medium Strong and broad strong
=C-H Alkenes. N-H Pri & sec. amines. C-H Aromatics. C-CI Alkyl halides. C-H Alkanes	Bend Wag oop stretch rock	690.13	Medium Strong and broad Strong Medium medium
C-Br Alkyl halides	Stretch	590.55	medium

FOURTH DAY at 50 °C

FUNCTIONAL GROUG10:P	TYPE OF VIBRATION	XTERISTICS ABSORPTIONS (cm^{-1})	INTENSITY
O-H suggest the presence of Alcohol, phenols and carboxylic acids	Stretch, H-bonded	3279.99	Strong and broad
C-H Alkynes	Stretch	3279.99	Strong and sharp
N-H Amine	Stretch	3279.99	Medium and secondary
C-H and =C-H Alkanes and alkenes	Stretch	2852.06	Strong and medium
C=C Alkenes	Stretch	1629.81	Variable
N-O Nitro compounds	Asymmetric Stretch	1541.31	Strong
-C-H Alkanes	Bend	1457.42	Medium –weak, multiple sharp bands

C=C and C-C (in ring) Aromatic	Stretch		FP
C-O Alcohol, carboxylic acids, esters, ethers. C-H Alkyl halides. C-N Aliphatic amines. -C-H Alkane	Stretch wag (-CH_2X) Stretch Bending	1398.53	Strong FP variable
C-O Alcohol, carboxylic acids, esters, ethers. C-H Alkyl halides. C-N Aliphatic amines. -C-H Alkane.	Stretch wag (-CH_2X) Stretch Bending	1361.27	Strong FP variable
C-F Alkyl halide. C-N Amine. C-O Alcohol, carboxylic acids, esters, ethers.	Stretch Stretch stretch	1257.00 and 1201.05	Strong Medium-weak Strong FP (2 bands or more)
C-O Alcohol, carboxylic acids, esters, ethers. C-H Alkyl halides. C-N Aliphatic amines	Stretch wag (-CH2X) Stretch	1156.46	Strong FP (2 bands or more) medium medium
C-N Aliphatic amines. C-O Alcohol, carboxylic acids, esters, ethers.	Stretch Stretch	1075.89 and 1028.02	Medium Strong FP (2 bands or more)
=C-H Alkenes. N-H Pri & sec. amines.	Bend wag	874.78	Strong Strong and broad
=C-H Alkenes. N-H	Bend Wag	771.02	Strong Strong and broad

Pri & sec. amines. C-H Aromatics.	oop		strong
=C-H Alkenes. N-H Pri & sec. amines. C-H Aromatics. C-CI Alkyl halides. -C≡C-H:C-H	Bend Wag Oop Stretch Bend	690.64	Strong Strong and broad Strong Medium Broad and strong
C-CI Alkyl halides. C-Br Alkyl halides.	Stretch Stretch	590.77	Medium Medium

Appendix V: Publications and Conference contributions

(i) **Authors:** **Modupe, E. Ojewumi**, Prof. Abiodun, J. Omoleye, Prof. Adesola, A. Ajayi (2016)**.**

Article Paper Title: The Study of the Effect of Moisture Content on the Biochemical Deterioration of Stored Fermented Parkia Biglobosa Seeds**.**

Journal: Open Journal of Engineering Research and Technology
Volume 1, Issue 1. (www.ojert.com)

Status: Published

(ii) **Authors:** **Modupe, E. Ojewumi**, Prof. Abiodun, J. Omoleye, Prof. Adesola, A. Ajayi (2016)**.**

Article Paper Title: The Effect of Different Starter Cultures on the Protein Content in Fermented African Locust bean (Parkia biglobosa) Seeds

Journal: International Journal of Engineering Research and Technology (IJERT) (http://www.ijert.org) IJERTV5IS040435.
Vol. 5 Issue 04, April.

Status: Published

(iii) **Authors:** **Modupe, E. Ojewumi**, Prof. Abiodun, J. Omoleye, Prof. Adesola, A. Ajayi, Ayoola, A.A., Adekeye, B.T. ., Adeyemi, A. O. (2016)**.**

Article Paper Title: Effect of Various Temperatures on the Nutritional Compositions of Fermented *Parkia biglobosa* seed

Journal: International Food Research Journal.

Status: In Press.

Conference attended:

(iv) **Authors:** **Modupe, E. Ojewumi**, Prof. Abiodun, J. Omoleye, Prof. Adesola, A. (2016)**.**
(CU-ICADI) International Conference on African Development Issues
Ota, Nigeria. http://cu-icadi.covenantuniversity.edu.ng

Date: May 9–11 2016.

Conference Paper Title: Optimum Fermentation Temperature for the Protein Yield of *Parkia biglobosa* Seeds (Iyere)

(v) Patented Research work.

IR Key

Unprocessed African Locust bean

African Locust Bean One SS is the Starting Sample (Day 0)

African Locust Bean – 2 is the sample fermented for 3 days at temperature 50 ºC

African Locust Bean – 3 Local is the Purchased saample

African Locust Bean – 4 is the sample fermented for 3 days at temperature 60 ºC

African Locust Bean – 5 is the sample fermented for 5 days at temperature 50 ºC

African Locust Bean – 6 is the sample fermented for 5 days at temperature 40 ºC

African Locust Bean – 7 is the sample fermented for 5 days at temperature 60 ºC

African Locust Bean – 8 is the sample fermented for 3 days at temperature 40 ºC