COMPARATIVE STUDIES ON THE MICFOBIOLOGICAL QUALITY, POST-FERMENTATION CHANGES AND PACKAGING OF SELECTED CONDIMENTS

A DISSERTATION

BY

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DEPARTMENT: MICROBIOLOGY

DECLARATION

I hereby declare that the contents of this thesis is purely a record of my research findings. No part of this work has been submitted or accepted in any previous application for a degree or qualification elsewhere. All quotations and literature cited from other sources are duly acknowledged.

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HELEN ILEIGO INABO

MAY 1991.

C E R T I F I C A T I O N

This thesis titled "Comparative Studies on the Microbiological quality, Post - Fermentation Changes and Packaging of Selected Condiments" By Helen Ileigo Inabo meets the regulations governing the award of the degree of Master of Science of Ahmadu Bello University, Zaria and is approved for its contribution to Scientific knowledge and literary presentation.

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To God be the glory for everything.

DEDICATION

Through the grace of God

To the memories of my late Dad -
J. C. Haruna and my Mum for her love
and moral support.

To my husband - Steve
Our children - Hilda and Sandra,
My sisters and brothers, all for
their love.

ABSTRACT

A total of thirty samples of food condiments comprising of some industrially produced (15) and traditionally fermented indigenous condiments (15) were examined for the incidence of different types of micro-organisms of significance (Total aerobic plate count, Staphylococcus aureus, Bacillus cereus, moulds, bacterial spores Salmonella Shigella, coliforms and faecal coliforms). The commercial flavour enhancers had total aerobic plate count below 1 $x10^4$cfu/g or ml and none of the different groups of micro-organ .sms assayed for while the indigenous condiments had higher counts for these micro-organisms in the range of 2.- x 10^6 - 1.7 . x $10^{7\cdot}$ four samples each of the three indigenous condiments were faecally contaminated with faecal coliform count of 2.- x 10^3. All the indigenous condiments showed various levels of contamination with all the micro-organisms assayed for except Salmonella spp which was found only in one sample of 'ogiri-igbo' at a count of 1.5 x 10^5.

The indigenous condiments (ogiri-igbo, soyabean and locust bean 'dadd-awa) were prepared by traditional methods and in the laboratory. The fermented mash was oven-dried seperately at three temperatures(50°c for $7^{1/2}$ hours, , 70°c for -1/2 hours and 100°2: for $3^{1/2}$ hours). Two batches of oven-dried condiments were produced:

Directly oven-dried and autoclaved, oven-dried condiments. The latter were darker in colour than the former. pH values at the end of fermentation fell to lower values during sun-drying and oven-drying at these temperatures.

At the end of fermentation, the pH values observed for the laboratory and uncontrolled fermented condiments were as follows:
8.3 and 8.- for locust bean 'daddawa', 8.3 and 8.6 for soyabean 'daddawa'; 9.2 and 9.6 for 'ogiri-igbo.

During sun-drying of the fermented mash, the pH values dropped to lower values for both types of fermented condimen:s. These values were 6.1 and 6.- respectively for locust bean 'daddawa', 6.7 and 6.9 respectively for soyabean 'daddawa'. A slight difference in pH (9.0 and 9.5 respectively) was obtained for 'ogiri-igbo' which was not sun-dried but packaged in broad leaves.

The laboratory fermented condiments had higner glutamic acid content than the uncontrolled fermented condiments. These values were 12.00mg/g and 10.50mg/g respectively for locust bean 'daddawa' 13.89mg/g and 12.92 mg/g respectively for soyabean 'daddawa' 9.75mg/g and 8.25 mg/g respectively for 'ogiri-igbo at the end of -8 hours fermentation.

The condiments were packaged in polyethylens non-absorbent film, aluminium foil and a combination of the polyethylene non-absorbent and aluminium foil. Glutamic acid contents of these packaged condiments were not altered after storage for 3 months while those of the unpackaged condiments were altered.

The importance of packaging our indigenous condiments is stressed.

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CHAPTER I

INTRODUCTION

Condiments are flavour or taste enhancers. They are made from proteins rich seeds through fermentation. They may be added directly to dishes or as thickening agents in soups or moulded and used as 'meat' or added to meat in small amounts.

In Africa, many proteinaceous seeds are fermented to make food condiments. The raw materials include locust bean (Parkia biglobosa), melon seeds (Citrullus vulgaris), fluted pumpkin bean (Telfaria occidentale), castor oil seed (Ricinus communis), beniseed (Sesamum indicum), and oil bean (Pentaclethra macrophylla). Cotton seeds may be used in small instances (Odunfa, 1985).

In South East Asia, Soya beans are fermented to give a variety of products which include amongst others -soy sauce (shoyu), miso, natto, tempeh, ontjom and shu-tou-shi (Steinkraus, 1983). Fermentation improves or elevates the digestibility, nutritive value and flavour of the raw seeds. These seeds cannot be consumed in the raw state because they contain toxic substances and antinutritional factors (Coon, 1973, Eka, 1980, Odunfa, 1982).

Protein Energy Malnutrition (PEM) is one of the major nutritional diseases facing many countries in Africa and hence fermented vegetable proteins have great potential as protein supplements in the diet (Steinkraus, 1983).

Recent publications on Nigerian plant protein fermentations include those of Odunfa (1985a) for locust bean 'daddawa' fermentation, Obeta (1983) for 'ugba' fermentation, Popoola and Akueshi (1985) for Soyabean 'daddawa' fermentation and Odunfa (1981) for 'Ogiri' fermentation. Bacillus spp has been established as the fermenting organism in these reports.

With the introduction of industrially-produced flavour enhancers like bouillon cubes in Nigeria, most enlightened urban dwellers neglected the indigenous fermented condiments. However, it was soon realised that the boullion cubes could not substitute for the indigenous taste enhancers in traditional dishes as the latter's typically characteristic aroma forms an inseperable part of the taste. Consequently, the indigenous condiments now feature prominently along with imported ones in nationwide television programmes in which recipes are advertised (Ogbadu, 1985). Comparative analysis have shown that the nutritive value of 'daddawa' is much greater than that of bouillion cubes (Okeoma, 1984).

Considerable quantities of food condiments are commonly used in garnishing/flavouring traditional foods in Nigeria. These condiments are displayed in open containers for sales in the market places. The only likely processing before use is grinding and pounding. Contamination by bacterial spores including agents of food poisoning of such condiments exposed to the atmosphere would be expected as these organisms are known to be in the soil and atmosphere (Frazier, 1967).

Other bacteria like Staphylococcus aureus are likely to be present because of the manner of handling during fermentation, drying of the fermented mash and even during marketing. Moulds in high numbers are also likely to be found as they abound in the soil and atmosphere. Some of these organisms are known to cause food intoxications (B. cereus, S. aureus, mycotoxin-producing fungi) and food infections (Salmonella and Shigella). The involvement of some of these bacteria in food poisoning is well documented (Frazier, 1967), certain dry food items are believed to enhance the production of enterotoxin (Midura et al 1970).

Many of the commercially produced flavour enhancers on the other hand are prepared under hygenic laboratory conditions and packaged and are unlikely to be contaminated with hazardous micro-organisms. The colour change as well as the way the product is presented to consumers unpackaged make the aesthetic quality of the product not particularly appealing. Consequently, the trend in most urban centres is an over-whelming consumer preference for and dependence on other industrially produced flavour enhancers (bouillon cubes) such as Maggi and Knorr cubes.

Apart from the presentation, it has been observed that most consumers prefer the industrially produced flavour enhancers because they are more convenient to use (Oyeleke, 1984). Apparently, processing and packaging of our local condiments in such convenient portions of usage could possibly enhance their aesthetic quality and hence usage.

In view of the observations enumerated above, the present work is aimed at:

a) Comparing the microbiological quality of selected traditional taste enhancers with some bouillon cubes and sauce.

b) Monitoring of post-fermentation changes in the condiments.

c) Devising suitable packaging systems for traditional condiments. Since convenience is one of the reasons for consumer preference and apparent dependence on bouillon cubes in most Nigerian cities (Oyeleke, 1984), it is hoped that the modified processing and packaging techniques would provide this convenience in the use of these condiments and hence break the apparent bondage to imported flavour enhancers among Nigerians.

CHAPTER II

L I T E R A T U R E R E V I E W

2.1 Raw Materials for condiment production

The African locust beans Parkia filicoidea are leguminous seeds of the genus Leguminosae. The seeds are dark in colour and are extremely hard and inedible in the raw state (Ikenebomeh and Kok, 1984). Soyabeans are also leguminous seeds of genus Glycine. The widely cultivated form is known as Glycine max (Morse, 1950). Castor oil seeds (Ricinus communis) are used for the production of 'ogiri-igbo'. Other raw materials usually used for condiment production include Beni-seeds (Sesamum indicum) melon seeds (Citrullus vulgaris) and cotton seeds.

2.2 Nutritive value and food uses of the seeds.

Oyenuga (1968) studied the nutrient contents of unfermented locust beans and compared them with decorticated groundnut seeds. Parkia seeds had higher crude protein and ash but lower crude fibre and ether extract than the decorticated groundnut seeds. The amino acid composition of Parkia seeds were analysed and compared with those of soyabean meal, groundnut meal and hen's egg (Fetuga et al, 1974). The authors found that lysine level was comparable with that of groundnut meal and hen's egg but was deficient in the sulphuramino acids such as methionine and cystine. Annegers (1974) reported that the limiting amino acid in the savannah regions of West Africa is lysine. This is an essential amino acid found to be higher in Parkia seeds than in most other legumes (FAO, 1970; Fetuga et al 1974). The levels in soyabeans and locust beans have been reported as 6.10g per 16gN2 and 6.68g per 16gN2 respectively (Fetuga et al, 1974).

Apparently, the use of 'daddawa' will supplement this deficiency. The lipid content of Parkia seeds has been reported as 60% unsaturated fatty acid with 40% being the essential linoleic acid (Platt, 1980). Soyabean is very rich in protein and oil which is stored in small bodies, interspersed in the cotyledon.

It consists of approximately 42% protein, 35% carbohydrate, 20% oil and 5% ash. It is very high in lysine but deficient in the sulphur amino acids with its limiting amino acid being methionine (Hayword et al, 1936). The oil is highly unsaturated and the carbohydrates are made up of polysaccharides, raffinose, stachyose, glucose and crude fibre (Hayword et al, 1936). Castor oil seeds are poisonous but contain a useful purgative castor oil. Fermentation of the seeds produces 'Ogiri-igbo' (Odunfa, 1982).

The food uses of Parkia spp are numerous. The value of the seeds as a nutritive fodder for domestic animals has been evaluated and found to be a valuable source of protein concentrate for livestock especially during the dry season when other supplements are scarce (Oyenuga, 1968; Fetuga et al, 1974; Oke and Umoh, 1978). The major food use of Parkia seeds in West Africa for human is 'daddawa' which is produced by fermenting the cotyledons of the seeds following the removal of the testa of husk (Platt, 1964). The name also varies according to the localities. For example, it is referred to as 'daddawa' (Hausa) and 'iru' (Yoruba). The Velvety pericarp surrounding the seed is sweet to taste and contains about 20% reducing sugar, 10-20% sucrose and 0.3% vitamin (Platt, 1980). This is a useful source of energy and vitamin C amongst children during the dry season (Fetuga et al, 1974). The sweet pericarp is mixed with honey or made into a fermented alcoholic drink (Dalziel,1937; Platt, 1980). The pericarp may be used for the preparation of a carbohydrate food called 'dorowa' in Northern states of Nigeria. It is usually taken with cereal grains (Fetuga et al, 1974). Besides the use as a fermented product, locust bean seeds can be roasted and used as a coffee substitue known as 'cafe du sudan' (Dalziel, 1937). Indonesians and Malaysians eat wholepods of Parkia speciosa raw or cooked as a vegetable referred to as "petai" and also preserve such whole pods by salting (Douglas, 1972). Soyabean is processed either into flour, milk or paste and then incorporated into local foods. The beans are blanched by boiling for 15-20 minutes, dehulled, and blended into a paste with water or the dehulled beans are dried for 2-3 days and ground into flour. The paste can then be sieved through a muslin cloth after making a slurry of it, to produce soya milk. The milk is heated (boiled) for 15-20 minutes and the scum is continually removed. About 25% of the flour required for any form of pastry or baked foods can be substituted with soya flour without any adverse effect on the taste.

Examples are soybean cakes, biscuits, soya puff-puff and soya pancake. The soya flour can be added to pap. The paste is mixed with cowpea paste for the production of soya 'moimoi' and soya 'akara' which are prepared by steaming and frying respectively (Farynia, 1978).

Dehulled beans can be used to prepare soyabean candies by roasting them or fermented to give soyabean 'daddawa'. Castor oil seeds contain a strong purgative but can be fermented to a useful condiment (Ogiri-Igbo).

2.3 Some Virtues of fermented plant proteins

Fermentation is the application of microbial metabolism to transform simple raw materials into valuable products (Trevan et al, 1987). Fermen-tation can also be viewed as any process for the production of a product by the mass culture of micro-organisms (Trevan et al, 1987). A fermented food is any food that has one or more of its components acted upon by micro-organisms namely bacteria, yeasts and fungi for a period so that the final products have often undergone very considerable changes in chemical composition and other aspects (Van Veen, 1957). The seeds used for production of indigenous condiments usually contain toxic substances but these are removed during fermentation. Oxalic, phytic and hydrocyanic acids have been found in unfermented locust beans, but cooking and fermentation help to reduce the amounts of these toxic substances below lethal levels (Eka, 1980). Soyabeans contain antinutritional factors (eg. phytic acid) which may bind divalent cations and interfere with their metabolism but roasting of the seeds as well as fermentation remove these unwanted substances (Coon, 1973).

Castor oil seeds also contain toxic substances such as ricin, ricinine and ricinoleic acids. These are removed during fermentation (Odunfa, 1982). Apart from the removal of toxic substances from the raw seeds, fermentation also improves the digestibility of the seeds which in their raw states are inedible. Meal made from undehusked Parkia seeds gave low protein utilisation and low biological value when fed to rats (Fetuga et al, 1973) but fermented beans had higher values of digestibility and biological value (Oke and Umoh, 1978). Equally, the nutritive value of the fermented product is higher than the raw seeds. Simic (1971) reported an increase in vitamin content during fermentation of locust beans especially riboflavin.

This vitamin is deficient in the diet of Africans and has been found to be in the highest quantity in Parkia seed (0.51%) and 'daddawa' (0.8%) than in any other food legume or legume products (Platt, 1962 and 1980; Watson, 1980). The fermented condiments available in Nigeria include locust bean 'daddawa', soyabean 'daddawa' 'ogiri-ijebu' 'ogiri-nwan', 'ogiri-saro', 'ogiri-igbo', 'okpehe', and 'ugba'. In advanced countries, better control of food fermentations by use of starter cultures (eg. Manufacture of soya sauce) and application of optimum process conditions has led to improvement in the quality of the foods. In contrast most African fermented foods have not been developed to any appreciable extent as they are at uncontrolled traditional level operations (eg. production of indigenous condiments).

2.4 Microbiological, biochemical and nutritional changes in the production of indigenous condiments.

A lot of microbiological, biochemical and nutritional changes occur in the production of African condiments by fermentation. 'Ogiri' refers to the oily paste made from oil seeds in West Africa. They have strong odour and are used as soup condiments. There are different types of 'ogiri' named after each ethnic group viz: 'ogiri-igbo' (Ibo), 'ogiri-nwan' (Nwan), 'Ogiri-ijebu' (Ijebu), 'Ogiri-igbo' (fermented castor oil seed) is produced by fermenting castor oil seeds (Ricinus communis). These seeds contain toxic substances (ricin, ricinine and ricinoleic acid) which are removed during the fermentation process (Odunfa, 1982). Microbiological examination of the fermented castor oil seeds showed Bacillus spp as the predominant micro-organism. Biochemical changes involve the production of amino acids which can be detected following the analysis of the fermented castor oil seeds by thin layer chromatography. These amino acids include cysteine, glutamine, glutamic acid, phenylalanine, tryptophan and tyrosine. The level of glutamic acid was found to have increased in the fermented castor oil seeds (Anosike and Egwuatu, 1980).

'Ogiri-ijebu' is produced by the fermentation of melon seeds (Citrullus vulgaris). It is consumed by the Ijebu and Ondo tribes in Ondo State. The melon seeds are boiled until soft and wrapped tightly in leaves and left to ferment for about 5 days.

The fermenting mash is smoked for about 2 hours and ground into an oily paste which is now known as 'Ogiri-ijebu' (Odunfa, I98I). Salt, wood and potash may be added. The organisms associated with the fermentation are Bacillus spp, Lactobacillus plantarium, Leuconostoc mesenteroides, Streptococcus faecalis, Pediococcus cerevisae and Staphylococcus aureus (Akindele, I978). However, Odunfa (I985) found Bacillus spp, Escherichia spp, Proteus spp and Pediococcus spp as the fermenting organisms. Fungi have also been reported to be involved in fermentation of melon seeds and these include Absidia corynebifera and Geotrichum candidum but the low oxygen tension and alkaline pH of the fermenting mash probably discouraged fungal growth. The biochemical change during fermentation is an increased activity of the proteinase anzyme which degrade the vegetable proteins to amino acids (Odunfa, I982).

'Ogiri-nwan' is produced by the 'Nwan' tribe in the Eastern part of Nigeria by fermenting fluted pumpkin beans. Microbiological examination of the fermented mash showed Bacillus spp as the fermenting organism. Information on biochemical and nutritional changes in fermenting fluted pumpkin beans is lacking in the literature. 'Ogiri-saro' is produced by fermenting the seeds of Sesame plant (Sesamum indicum). Bacillus spp has been shown to be the predominant fermenting organism. Information on biochemical and nutritional changes in fermenting fluted pumpkin is lacking in the Literature.

Soyabeans (Glycine max) has recently been tried for 'daddawa' production. Traditionally, the seeds are roasted in the frying pan for 3 minutes and then soaked in water for one hour. They are dehulled by pounding lightly in a wooden mortar after which they are washed. The dehulled beans are then boiled for 45 minutes and placed in bamboo basket lined on the inside with washed banana leaves. The baskets are covered with the same leaves and left to ferment at about $32^{o}c \pm 2^{o}c$ for 2 days. Fermentation is carried out by Bacillus spp and Staphylococcus spp (Popoola and Akueshi, $I98^{5}$). Soyabean ranks high among the vegetable protein sources in its protein content. The microorganisms isolated from soyabean 'daddawa' fermentations are similar to those obtained from the locust bean fermentations (Ogbadu and Okagbue, I988).

The oil bean seeds (Pentaclethra macrophylla) are used for 'Ugba' pro-

duction. Obeta (1983) noted that Bacillus spp had been consistently isolated and thus implicated as the fermenting organism. Oyeyiola and Odunfa (1983) also isolated B.subtilis and Staphylococcus spp.

Kolawole and Okonkwo (1984), however, isolated the following organisms: Alcaligenes viscolactis, Pseudomonas aeruginosa, Bacillus cereus, Micrococcus varians, Enterobacter cloacae and a Corynebacterium spp. Njoku et al (1989) isolated Bacillus spp, Corynebacterium spp, Staphylococcus spp. Biochemical changes involved in fermenting oil beans seeds include an increase in the activities of &-amylase, proteolytic and lipolytic enzymes, increased levels of reducing sugar, titratable acidity, pH and amino nitrogen (Njoku and Okemadu, 1989). These authors noted a seven-fold increase in the amino nitrogen level. 'Ugba' is a delicacy in Igbo land and is commonly referred to as oil bean "salad" when served with stockfish and garden egg.

Locust bean 'daddawa' is obtained by fermentation of locust bean seeds (Parkia filicoidea Welw). Microbiological examination of the fermenting mash showed Bacillus spp (B.subtilis and B. pumilus) as the predominant organism (Odunfa, 1985b and Ogbadu, 1988). Antai and Ibrahim (1986) however, isolated B.subtilis and Leuconostoc spp (L.mesenteroides and L.dextranicus). Biochemical changes occuring during fermentation involved the replacement of flatus-inducing oligosaccarides with reducing sugars. The oligosaccharides include raffinose, sucrose and stachyose while the reducing sugars include galactose, glucose and fructose(Odunfa and Adesomoju, 1985). These authors also studied the free fatty acid composition in the fermented mash and found palmitic, stearic, oleic and linoleic acids. Changes in the vitamin content of locust beans during fermentation have been reported and these include an increase in riboflavin content (Simic,1971) and a decrease of thiamine and niacin by 86% and 30% respectively. Analysis of several samples of 'daddawa' from Nigeria showed that riboflavin increased by 60% during fermentation.

Okeoma (1984) noted that the nutritive value of locust bean 'daddawa' was higher than that in bouillon cubes. Proximate analysis of locust bean 'daddawa' were 34.15g/16g N_2 crude protein, 34.80/16g N_2 lipid, 21.07g /16g N_2 - carbohydrate while those of Maggi were 11.56g/16g N_2, crude Protein, 5.52g/16g N_2 lipid, 16.55g/16gN_2 carbohydrate and those of Knorr cube were 19.02g/16gN_2 crude protein, 8.77g/N_2 lipid, 11.22g/N_2 carbohydrate. 'Daddawa' is a flavoursome protein and fat rich condiment which is used in traditional soups that are usually eaten with cereal based dumpling and porridges. The flavour intensifying properties of 'daddawa' has been identified as glutamic acid and small amounts of incidental alcohols produced by the fermenting micro-organisms (Hesseltime, 1965).

FLAVOURING COMPONENTS OF BOUILLON CUBES AND SOYA SAUCE:

The major flavouring compound in bouillon cubes is glutamic acid which is present in the form of a salt-monosodium glutamate. (Food Tech, 1980). Sixty three volatile compounds have been isolated by various investigators from soya sauce. (Steinkraus, 1983). Glycine, arginine, histidine, putrescine and ammonia when conjugated as salts with glutamic acid have a delicious flavour (Steinkraus, 1983).

2.5. THE MICROBIOLOGICAL QUALITY OF DRIED FOODS AND FOOD INGREDIENTS

Obuekwe and Ogbimi (1989) studied the prevalence of B.cereus and S.aureus in some non-fermented condiments (Shrimps, prawns, black pepper and paprika) and noted that B.cereus counts ranged from 0-1.1 x 10^4 for paprika (Capsicum) 1.4 x 10^4 - 2.5 x 10^4 for Caridonia (shrimp) and 1.0 x 10^4 - 2.0 x 10^4 for Macrobranchium spp (prawns). 80% of all the food condiments contained more than 10^6 aerobic bacteria per gram. They observed contamination of Capsicum samples by large numbers of Bacillus cereus and suggested that they may be responsible or contribute to common upsets and diarrhoea. Bacillus has also been isolated from dry milk (Orakwue and Uraih, 1981), pulses and cereals (Blakey and Priest, 1980) and spices (Powers et al, 1976, Seenapa and Kempton, 1981). Staphylococcal food poisoning is a food intoxication in which S.aureus grows in the food and produces a toxin in it.

Coagulase-positive staphylococci have been found in dried pasta products (Gibbs, 1986), Meat and meat products (Kwaga and Adesiyun, 1983). Molds and yeasts have been found on dried foods such as dates (FAO, 1979). Mesophilic aerobic bacterial spores have been found as contaminants of spices, enzyme preparations, gelatin and sugar (Farkas, 1984). Salmonella has been found in dried foods such as non-fat dried milk, dried egg albumin and yolk, macaroni and chocolate (Gibbs, 1986).

2.6. Post-fermentation changes in fermented local condiments

Okeoma (1984) noted a change in colour during the drying of the fermented locust bean samples at 50 - 55°C in an air-oven. Seeds that were dehusked mechanically before cooking and fermentation changed to light dirty yellow while those that were cooked before dehusking and fermentation became darker.

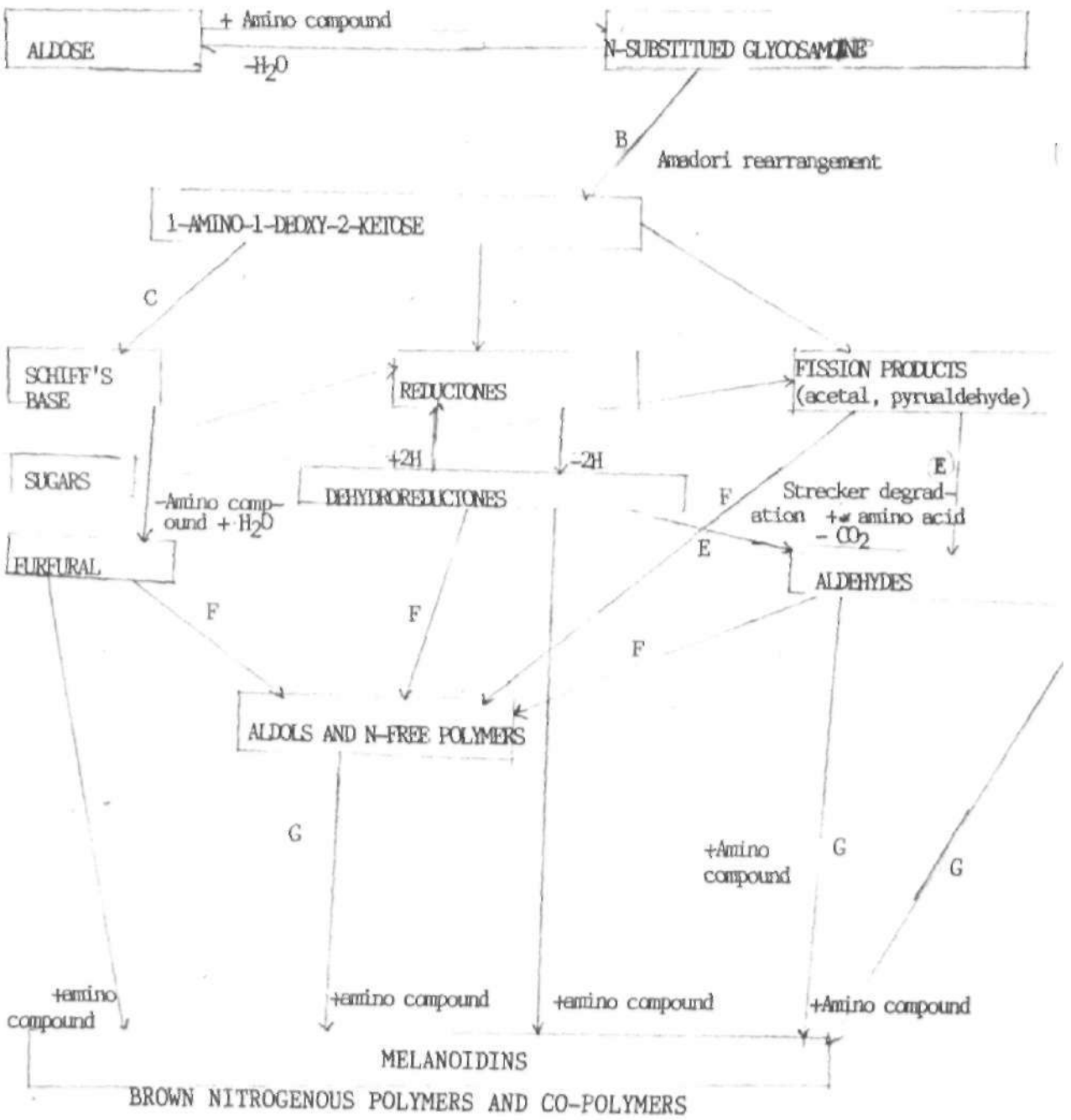
Platt (1980) reported similar colour changes and attributed this to polyphenol oxidation of naturally occuring phenolic substrates. These are probably formed by the breakdown of anthocyanins and flavonoids (Braverman, 1963). In another report, Hulme (1974) stated that all polyphenolases are easily inactivated at temperature between 85°C - 95°C. Non-enzymic browning or Maillard reaction may be responsible for the observed colour change during the drying of the fermented locust beans since fermented samples are known to contain about 43% protein along with increased level of reducing sugar (Odunfa, 1982) and free amino acid (Fetuga et al, 1973).

Maillard Reaction

Maillard Reaction is also known as non-enzymic browning involving free amino acids and sugars. These compounds are abundant at the end of fermentation (Fetuga et al, 1973 and Odunfa, 1982). Maillard reaction is best divided into three stages. The initial one comprises glycosylamine formation (A) and subsequent rearrangement (B) to the Amadori compound precursor (1-amino -1-deoxy-2-ketose). The intermediate stage comprises dehydration (C), either by loss of three molecules of water to furfurals or by loss of two to reductions; fission (D), mainly by dealdolisation and Strecker degradation (E).

The final stage consists of the conversion of carbonyl compounds to high molecular weight products (Melanoidins) which could be either nitrogen-free (F) or nitrogenous (G).
This reaction is represented below (Nursten, 1986).

FIG. I: Maillard Reaction



(Adapted from Nursten, 1986)

Nursten (1986) suggested that browning varies with moisture content usually showing a maximum at intermediate values of 0.5 - 0.8. The Maillard reaction is initiated by formation of some Amadori compounds.

2.7. Packaging of fermented local condiments:

Okeoma (1984) observed that there were no observable physical deterioration after 6 months storage of the fermented locust beans at ambient temperature. Polyethylene films, aluminium foil and a combined aluminium foil and Polyethelene film were used to package oven-dried locust bean 'daddawa'. Packaging materials must have the following properties:

They must be compatible with the product, provide protection against mechanical hazards of transport and climate hazards, micro-organisms and flavour gain or loss (Leitch, 1962).

CHAPTER III

MATERIALS AND METHODS

3.1.0. Sampling:

Six (6) different condiments were used for these analysis. Three of these were commercially processed (Knorr cubes, Maggi cubes and Soya sauce) while the other three were traditionally processed (Soya bean 'daddawa', Locust bean 'daddawa' and 'Ogiri-igbo'). The condiments were obtained from the Central Market in Kaduna except the soyabean 'daddawa' and soya sauce. Soyabean 'daddawa' was produced by an expert in traditional fermentation since this condiment could not be obtained in the market, while bottled soya sauce was obtained from the Leventis superstore in Kaduna.

A total of thirty (30) field samples were obtained in approximately one hundred gram portions. Each sample was examined five times according to the specification in FAO manual of Food quality control; Microbiological Analysis (1979). The traditionally fermented condiments were transported to the laboratory in polyethylene non-absorbent films. Samples were obtained weekly over a five-week period.

SECTION A: MICROBIAL COUNTS OF THE CONDIMENTS

3.1.1. Preparation of Food homogenate:

Twenty-five grams or millilitres of each field sample was weighed or pipetted and transfered aseptically into a conical flask containing 225mls of sterile peptone water. The contents of the flask were shaken to release micro-organisms trapped in the condiment and allowed to stand for 10 minutes. The food homogenate (10^{-1}) was thoroughly shaken and ten-fold dilutions prepared. For the traditionally fermented condiments, dilutions up to 10^{-6} and for the commercially processed condiments up to 10^{-3} were made.

The plates were incubated at 35°c for 18-24 hours and two of the suspected colonies of E. coli were transfered on to plate count agar (PCA) slants. These were incubated at 35°C for 24 hours. Gram - stains of each culture were made. The tubes were stored at 4°C until needed. Biochemical identification of the suspected colonies of the coliforms and E. coli were carried out using the following tests: Indole production, methyl-red test, Voges-Proskauer reaction, citrate utilisation test, motility test, sugar fermentation reactions in Kligler iron agar and triple sugar iron agar slants.

3.I.4. Enumeration and identification of Staphylococcus aureus.

Mannitol salt agar (15ml portions in tubes) was poured into sterile petri dishes and allowed to solidify. The surfaces of the agar plates were allowed to dry in an incubator. Prepared plates of Manhitol salt agar were tested for sterility by incubating them at 37°C for 24 hours after which 0.25ml of the appropriate dilutions, 10^{-1} - 10^{-3} for for the commercially processed condiments and 10^{-4} - 10^{-6} dilutions for the traditionally fermented condiments was inoculated in duplicates. The plates were incubated at 37°C for 24 hours after spreading with an alcohol-flamed bent glass rod. Yellow colonies appearing after 24 hours with evidence of mannitol fermentation were counted. Representative colonies were picked from the plates into nutrient broth and the tubes were incubated at 37°C for 24 hours. These were preserved on nutrient agar slants for further identification by biochemical tests which include catalase test, carbohydrate fermentation tests, coagulase test, DNAse production, gelatinase production, growth in increasing concentrations of sodium chloride (3%-15%) haemolysis on blood agar, lecithinase activity, nitrate reduction test and Voges - Proskaeur reaction.

3.1.5 Enumeration and identification of moulds.

Potato dextrose agar (Oxoid) was prepared in 15ml portions, sterilised and kept in a 45°C water bath. One millilitre of the dilutions was transfered into seperate labelled petri dishes.

Fifteen millitres of potato dextrose agar was poured into each plate, mixed thoroughly, allowed to solidify, and incubated at room temperature for 3-5 days. Colonies were counted on selected dishes with 30-300 colonies and recorded as mould count per gram or ml. Moulds were identified by their cultural characteristics and microscopic examination of the stained mycelial preparations.

3.I.6 Enumeration and identification of Salmonella:

Bismuth sulphite agar (15ml portions) and Brilliant green agar (15ml portions) were prepared and poured into plates. The plates were incubated at 37°C overnight to test for sterility. The food homogenate and the dilutions were incubated at 37°C for 24 hours. Transfers were made into tetrathionate broth base (10mls each of the food homogenate and dilutions into separate 90mls of tetrathionate broth base). The tubes were incubated at 42°C for 48 hours. After incubation, 0.25ml each of the enriched broth was aseptically transfered to well dried plates of Bismuth suphite agar and Brilliant green agar plates. These plates were spread with an alcohol-flamed bent glass rod and incubated at 37°C for 24 hours. Colonies with black centres on Bismuth sulphite agar and red colonies on Brilliant green agar were counted as Salmonella spp. Representative colonies were picked onto nutrient agar slants and stored at 4°C for biochemical tests such as lysine decarboxylase test, urease activity, indole production, Voges-Proskaeur reaction, and reactions in triple sugar iron agar.

3.I.7 Enumeration of mesophilic aerobic bacterial spores

Five millilitres of each of the food homogenate was heat-shocked at 80°C for 20 minutes to eliminate vegetative cells. Appropriate tenfold dilutions were carried out. One millilitre of the appropriate dilutions (10^{-1} ; 10^{-2} ; 10^{-3}) for the commercially processed condiments and 10^{-4} - 10^{-6} dilutions for the traditionally processed condiments was inoculated into sterile labelled plates. Melted tubes of nutrient agar were poured into the plates and mixed thoroughly to ensure uniform dispersion of the inoculum.

The plates were allowed to solidify and incubated at 30°C for 24 hours. Plates with 30 - 300 colonies were selected for counting. Smears of selected colonies were stained and observed for spores.

3.I.8 Enumeration and identification of Shigella

Xylose lysine decarboxylase (Oxoid) agar was prepared for Shigella count. About 0.25ml of appropriate dilutions was transfered to the surface of xylose lysine decarboxylase medium, spread and incubated at 37°C for 24 hours. Red colonies were counted as Shigella spp. Representative colonies were picked onto nutrient agar slants and stored at 4°C for biochemical tests such as urease activity, citrate utilisation, indole production, reactions in triple sugar iron agar, Motility test, Voges-Proskauer reaction, sugar fermentations and growth in potassium cyanide broth.

3.I.9 Enumeration and identification of Bacillus cereus

Egg yolk medium with polymyxin B. sulphate was prepared and used to culture B. cereus. Appropriate dilutions were selected and 0.25ml each was transfered with sterile pipette onto the surface of dried egg yolk medium. The inoculum was spread by a sterile bent glass rod. Plates were incubated at 30°C for 24 hours. Plates with 30 - 300 colonies with a dense halo of precipitate (lecinthinase activity) were counted. The counts were worked out bearing in mind the dilution factor. Confirmation of B. cereus isolates was by use of biochemical tests such as gelatin liquefaction, motility, nitrate reduction tests, fermentation and oxidation reaction in Oxidation-fermentation (OF) glucose broth, starch hydrolysis, indole production, urea hydrolysis, carbohydrate fermentation tests and growth in 7.5% sodium chloride.

SECTION B. POST-FERMENTATION CHANGES IN THE INDIGENOUS CONDIMENTS:

3.2.1 Production of the indigenous condiments by traditional methods

3.2.1.1. Locust bean 'daddawa':

The locust bean seeds were boiled overnight for 24 hrs in an earthen-

ware pot to soften the husk. The cooked seeds were dehusked by rubbing with hand after gentle pounding in a wooden mortar. The seeds were rinsed with water to remove the adhering testa and mucilagenous material. The washed seeds were boiled with a few pellets potash for 60 minutes. The seeds were cooled and packed in a bamboo basket lined with fresh, toasted and washed banana leaves. The basket was covered with more fresh, toasted and washed banana leaves and kept in a warm place for 2-days. After fermentation, the strong-odoured brown to black bean mass was pounded into a paste and squeezed into balls and sun-dried for one to two days. This served as the control. Three sets of the fermented beans were collected immediately after fermentation, autoclaved at 121°C for 15 minutes. These were oven-dried seperately at 50°C for 7½ hours; 70°C for 4½ hours and 100°C for 3½ hours until a constant weight was obtained. Three new sets of the fermented beans were directly oven-dried separately at the temperatures specified above. Fifty gram of the fermented mash was oven dried in all the sets.

3.2.1.2 Soyabean 'daddawa':

The seeds were roasted lightly in cooking pan over an open fire for 3 minutes, soaked for one hour, dehulled and boiled for 6 hours. The bamboo basket was lined with fresh washed banana leaves that were lightly toasted over hot-charcoal. The cooked beans were poured into the lined basket while hot, covered with more leaves, and incubated at room temperature. After 48 hours, a dark-brown mass was obtained. A set to be known as the control was prepared by pounding the mash lightly in a mortar and squeezing into small balls, and sun-dried for one to two days. Three sets of the fermented mash were collected immediately after fermentation, auto-claved at 121°C for 15 minutes. These were oven-dried seperately at 50°C for 7½ hours; 70°C for 4½ hours and 100°C for 3½ hours until a constant weight was obtained. Three new sets of the fermented mash were directly oven-dried seperately at the temperatures specified above. Fifty gram of the fermented mash was oven dried in all the sets.

3.2.1.3 'Ogiri-Igbo':

The castor oil seeds were dehusked by breaking the seed coat. The cotyledons were washed and boiled for 12 hours. They were wrapped in washed banana leaves and left to ferment for 48 hours. After fermentation, three sets of fermented mash were collected immediately, autoclaved at 121°C for 15 minutes. These were oven-dried seperately at 50°C for 7½ hours, 70°C for 4½ hours and 100°C for 3½ hours until a constant weight was obtained. Three new sets of the fermented mash was directly oven-dried seperately at the temperature specified above. Fifty gram of the fermented mash was oven-dried in all the sets. Ten grams of the fermented mash was packaged in broad leaves as the control.

3.2.2 PRODUCTION OF THE INDIGENOUS CONDIMENTS UNDER LABORATORY CONDITIONS

3.2.2.I Stock culture preparation of Bacillus subtilis:

Bacillus subtilis was isolated from the fermenting mash of locust beans and the total aerobic plate count was determined by using standard plate count agar (Oxoid). The 24 hour culture was harvested into 10mls of sterile distilled water and stored at 4°C until needed.

3.2.2.2 Locust bean 'daddawa':

The locust bean seeds were autoclaved in excess water in 500ml Erhlenmeyer flasks for one and a half hours at 121°C. The seeds were dehusked by rubbing between the palms and washed free of the testa and adhering mucilagenous materials. Three hundred gram each of the seeds were autoclaved at 121°C for 15minutes in seperate 500ml Erhlenmeyer flasks and inoculated with 2mls of the B.subtilis stock culture (2.8×10^9 cfu/g). The flasks were incubated at 37°C for 48 hours. After fermentation, three sets of the fermented mash were autoclaved immediately at 121°C for 15minutes and oven-dried seperately at 50°C for 7½ hours, 70°C for 4½ hours and 100°C for 3½ hours. Three new sets of the fermented mash were oven-dried directly at these temperatures. Fifty gram each of the fermented mash was oven-dried in all the sets. The control was obtained by sundrying some of the fermented mash for one to two days.

3.2.2.3 Soya bean 'daddawa':

The beans were roasted in a beaker on a hot plate, soaked in tap water for 30 minutes, dehulled and boiled for 6 hours. Three hundred gram each of the beans was autoclaved at 121°C for 15 minutes in 500ml Erhlenmeyer flasks, cooled and inoculated with 2mls of the stock culture of the B.subtilis.

The flasks were incubated at 37°C for 48 hours. Three sets of the fermented mash were autoclaved immediately at 121°C for 15 minutes and oven-dried seperately at 50°C for 7½ hours, 70°C for 4½ hours and 100°C for 3½ hours. Three new sets of the fermented mash were directly oven-dried seperately at these temperatures. Some of the fermented mash was sun-dried. This served as the control. Fifty gram of the fermented mash was oven-dried for all the sets.

3.2.2.4 'Ogiri-igbo'

The dehusked castor oil seeds were boiled for 8-hours in a beaker over a hot plate. Three hundred gram each of the seeds were autoclaved at 121°C for 15 minutes in 500ml Erhlenmeyer flasks, cooled and inoculated with 2mls of the B.subtilis stock culture. The flasks were incubated at 37°C for 48 hours. Three sets of the fermented mash were autoclaved immediately at 121°C for 15 minutes and oven-dried separately at 50°C for 7½ hours, 70°C for 4½ hours and 100°C for 3½ hours. Three new sets of the fermented mash were directly oven-dried seperately at these temperatures. Ten grams of the fermented mash was packaged in broad leaves. This served as the control. Fifty gram each was oven-dried for all the sets.

3.2.3 Determination of pH:

Ten grams each of the sun-dried or oven-dried condiments prepared under laboratory conditions and by traditional methods was blended with 40mls of distilled water after which pH readings were determined using the pH meter (PYE UNICAM MODEL 292 MK 2). The pH of the fresh condiments and the ogiri-igbo packaged in leaves was determined.

3.2.4. Determination and Estimation of glutamic acid:

Glutamic acid contents of the fresh and dried condiments were estimated qualitatively and quantitatively. The qualitative assay involved chromatographic comparison of spots of the fresh and dried condiment samples with the pure glutamic acid standard. Whatman No. 1 chromatography papers were used (AOAC, 1980). These were prepared ruled and labelled using pencil. They were spotted with two microlitres (2.0ul) of glutamic acid standard prepared by acidifying 10mls of 0.1M solution of the standard with 10 drops of 19% hydrochloric acid and heating to dissolve. The test samples were milled and extracted with potassium dehydrogen phosphate (pH 6.4). Five grams each of the milled test sample was mixed with 20mls of the buffer and shaken on Stuart orbital shaker for 1 hour. The homogenates were filtered and the volume of the extracts noted. One millilitre of the extracts was evaporated to 0.5ml on a Gallenhemp water bath at 100°C for 2 minutes. The concentrates were spotted in 2.0ul amounts on the labelled chromatogram after which they were allowed to air-dry (AOAC, 1980).

The chromatograms were developed in n-Butanol; acetic acid: water (60:15:25 by vol) for 4 hours in an ascending chamber. The developed chromatograms were again air-dried and sprayed uniformly with 0.2% ninhydrin (w/v in ethanol) in a chamber. After drying, the chromatograms were placed in an oven at 105°C for for about 3 minutes to develop purple spots. The width and intensity of the purple spots (which have the same Rf values with the standard) were scored + or ++ on a comparative basis (AOAC, 1980).

The quantitative assay was carried out by eluting the glutamic acid spot of the test sample from the paper chromatograms in one millilitre (1ml) each of 98% ethanol. The absorbance of the eluants was read off on an Sp-6 uv spectrophotometer at a wavelength of 420nm. For the calibration curve, the absorbance of varying concentrations (0.04mg /ml, 0.08mg/ml; 0.12mg/ml; 0.16mg/ml and 0.20mg/ml) of glutamic acid standard were read at the same wavelength used in plotting the graph.

The calibration curve was obtained after applying the equation y' = bx + c:

$$\text{where } b = \frac{NE\ Xy - E\ x\ Ey}{NE\ x\ 2 - (EX)^2}$$

$$c = \frac{Ey - b \cdot EX}{N} \quad \text{(Meddis, 1975)}$$

Where X is value of concentration of glutamic acid and y is the observed value of absorbance readings of the glutamic acid standard and N is the number of glutamic acid standard solutions of known concentrations.

Concentrations of the glutamic acid extrapolated from the calibration curve at given absorbance readings were used to find the real concentration in the test samples and these were worked out by the following equation.

Concentration of glutamic acid (mg/ml) = a/bxcxd = e
Concentration of glutamic acid (mg/g) =e x f/g =h

a= Concentration of glutamic acid extrapolated from graph at a given absorbance value.

b= Volume of concentrate spotted (2.0 ul).

c= Conversion factor (I.Oml =.IO^3 ul)

d= Final volume of concentrate before spotting (0.5ml).

e= Concentration of glutamic acid (ng/ml)

f= Volume of extract of test samples with buffer

g= Weight of test sample shaken with buffer (5g)

h= Concentration of glutamic acid (ng/g).

3.3.3 PACKAGING OF THE LABORATORY FERMENTED CONDIMENTS

Five grams of the various fermentation-terminated condiments using heat (50°C) were packaged in aluminium foil, polythylene non-absorbent films, and a combination of aluminium foil and polyethylene non-absorbent film in powdered form or cubes. The cubes were made by using a sterile metal box with the dimensions (1.4cm by 1.4cm) of a standard bouilon cube (Maggi cubes) and these were wrapped in aluminium foil. The aesthetic appearance of the packaged condiments was recorded by photography. The glutamic acid content of the packaged condiments was determined after 3 months.

CHAPTER IV

R E S U L T S

Higher microbial counts were obtained from the traditionally fermented condiments than the commercially processed ones. Table 4.I shows the range of microbial counts in the condiments. The commercially processed flavour enhancers had mean total aerobic plate count below I x 10^4 cfu/g or ml. (Maggi and Knorr cube samples had mean total aerobic plate count of I.4 x 10^I cfu/g while soya sauce had mean total aerobic plate count of I.0 x 10^I cfu/ml). The following organisms were not isolated from the samples collected: Moulds, bacterial spores, Salmonella, Shigella, S. aureus, Coliforms, faecal coliforms, E.coli and B.cereus. For the indigenous condiments, soyabean 'daddawa' had total aerobic plate count that ranged from I.7 x 10^6 to 2.8 x 10^7 cfu/g with a mean count of 2.4 x 10^6 cfu/g; for locust bean 'daddawa, the total aerobic plate count ranged from I.2 x 10^5 to 2.7 x 10^7 cfu/g with a mean count of 3.5 x 10^6 cfu/g; for 'ogiri-igbo', the total aerobic plate count ranged from I.8 x 10^6 to I.4 x 10^7 cfu/g with a mean count of I.7 x 10^7 cfu/g. These counts showed that 'Ogiri-igbo' had a slightly higher total aerobic plate count than soyabean and locust bean 'daddawa'.

Coliform counts for the traditionally fermented condiments were as follows: Soyabean 'daddawa' counts ranged from 7 to 2,400 ccoliforms/g with a mean count of I.47I coliforms/g; Locust bean 'daddawa' counts ranged from 0 to 2,400 coliforms/g with a mean count of I,442 coliforms/g; 'ogiri-igbo' counts ranged from 64 to 2,400 coliforms/g with a mean count of I,673 coliforms/g.

These counts showed that 'ogiri-igbo' had slightly higher coliform count than soyabean and locust bean 'daddawa'.

Faecal coliform counts for the traditionally fermented condiments were as follows: Soyabean 'daddawa' counts ranged from 0 to 2,400 faecal coliforms/g with a mean count of 563 faecal coliforms/g ; locust bean 'daddawa' counts ranged from 0 to 2,400 faecal coliforms/g with a mean count of 720 faecal coliforms/g; 'ogiri-igbo' counts ranged from 0 to 2,400 faecal coliforms/g with a mean count of 604 faecal coliforms/g. . These counts showed that locust bean 'daddawa' had slightly higher faecal coliform count than soya bean 'daddawa' and 'ogiri-igbo'.

S. aureus counts for soyabean 'daddawa' ranged from 1.8 x 10^4 to 2.3 x 10^6 with a mean count of 3.3 x 10^5 cfu/g; locust bean 'daddawa' counts ranged from 2.8 x 10^7 cfu/g 'ogiri-igbo' had counts ranging from 4.2 x 10^6 to 3.0 x 10^7 cfu/g with a mean count of 3.5 x 10^6 cfu/g. These counts showed that 'ogiri-igbo' had slightly higher S. aureus count than soyabean and locust bean 'daddawa'.

Mould counts for soyabean 'daddawa' ranged from 1.0 x 10^2 to 2.0 x 10^6 cfu/g with a mean count of 1.2 x 10^3 cfu/g while locust bean 'daddawa' had counts ranging from 1.9 x 10^3 to 2.4 x 10^6 cfu/g with a mean count of 2.1 x 10^3 cfu/g. 'ogiri-igbo' had counts ranging from 2.2 x 10^3 to 4.2 x 10^5 cfu/g with a mean count of 1.9 x 10^3 cfu/g. These counts showed that locust bean 'daddawa' had slightly higher mould count than soyabean and ogiri-igbo'.

Mesophilic aerobic bacterial spore counts for soyabean 'daddawa' ranged from 1.5 x 10^5 to 1.6 x 10^7 cfu/g with a mean count of 1.9x10^5. Locust bean 'daddawa' had counts that ranged from 1.6 x 10^4 to 1.7 x 10^7 cfu/g with a mean count of 2.2 x 10^6 cfu/g while 'ogiri-igbo' had counts ranging from 1.4 x 10^4 to 2.8x10^7 cfu/g with a mean count of 2.0 x 10^6 cfu/g. These counts showed that locust bean 'daddawa' had slightly higher mesophilic aerobic bacterial spore count.

B. cereus counts for soyabean 'daddawa' ranged from 7.6 x 10^4 to 3.9 10^5 cfu/g with a mean count of 3.6 x 10^5 cfu/g; locust bean 'daddawa' had counts ranging from 3.2 x 10^4 to 3.2 x 10^5 cfu/g with a mean count of 3.2 x 10^4 cfu/g while 'ogiri-igbo' had counts ranging from 1.6 x 10^4 to 5.2 x 10^5 cfu/g with a mean count of 4.2 x 10^5 cfu/g.

No Shigella spp was isolated from the traditionally fermented condiments. No Salmonella spp was found in locust bean and soyabean 'daddawa' but from one of the 'ogiri-igbo' samples with a count of I.5 x 10^5 cfu/g. The moisture content of the indigenous condiments were as follows Locust bean 'daddawa' (16%); soyabean 'daddawa' (10%) and 'Ogiri-igbo' (24%) while those of maggi and knorr cubes were 2.5% and 3.0% respectively.

Table 4.2 shows the post-fermentation changes in fresh and dried locust bean 'daddawa'. At the end of fermentation of the seeds, the pH was 8.3 and 8.4 respectively for the laboratory fermented and uncontrolled fermented locust bean 'daddawa'. The glutamic acid contents for both types of the condiment were 12.00mg/g (laboratory fermented) and 10.50mg/g (uncontrolled fermented). The pH of the 'daddawa' dropped from 8.3 and 8.4 to 6.1 and 6.4 respectively for the laboratory and uncontrolled fermented condiment during sun-drying. Direct oven-drying of the fermented mash at different temperatures showed similar drops in pH when compared with the sun-dried 'daddawa' but the differences between the pH values of the condiments dried at these temperatures were not significant using test at 5% level of significance (α=0.5). Laboratory fermented locust bean 'daddawa' had pH values of 6.4, 6.3 and 6.1 respectively when dried seperately at 50°C for 7½ 70°C for 4½ hours and 100°C for 3½hours while the uncontrolled fermented locust bean 'daddawa' had pH values of 6.2; 6.1 and 6.0 respectively when dried seperately at the temperatures listed above. The glutamic acid content of the directly oven-dried 'daddawa' were as follows: Laboratory fermented locust bean 'daddawa' had glutamic acid content of 15.90mg/g, 16.28mg/g and 13.69mg/g when dried seperately at 50°C for 7½ hours, 70°C for 4½ hours and 100°C for 3½ hours respectively while the uncontrolled fermented 'daddawa' had glutamic acid content of 14.40mg/g, 15.00mg/g and 11.48mg/g when dried seperately at 50°C for 7½ hours, 70°C for 4½ hours and 100°C for 3½ hours.

Autoclaved and oven-dried, laboratory fermented locust bean 'daddawa' had glutamic acid content of 12.95mg/g, 11.78mg/g and 10.28mg/g when dried seperately at 50°C for 7½ hours, 70°C for 4½ hours and 100°C for 3½ hours, 70°C for 4½ hours and 100°C for 3½ hours while the uncontrolled fermented 'daddawa' had glutamic acid content of 11.88mg/g,

I0.98mg/g and 9.60mg/g when dried seperately at the three temperatures listed above.

Table 4.3 shows the post fermentation changes in fresh and dried soyabean 'daddawa'. At the end of fermentation of the seeds, the pH was 8.3 and 8.6 respectively for the laboratory fermented and uncontrolled fermented soyabean 'daddawa'.
The glutamic acid contents for both types of the condiments were I3.50-mg/g and I2.75mg/g respectively. The pH of the 'daddawa' dropped from 8.3 and 8.6 to 6.7 and 6.9 respectively for both types of 'daddawa during sun-drying. Direct oven-drying of the fermented mash at different temperatures showed similar drops in pH when compared with the sun-dried 'daddawa' but the differences between the pH values of condiment dried at these temperatures were not significant. Laboratory fermented soyabean 'daddawa' had pH values of 7.9; 7.7 and 7.6 respectively when dried seperately at 50°C for 7½ hours; 70°C for 4½ hours and I00°C for 3½ hours, while the uncontrolled fermented soyabean 'daddawa' had pH values of 7.8; 7.5 and 7.4 respectively when dried seperately at the temperatures listed above.

The glutamic acid content of the directly oven-dried 'daddawa' were as follows: Laboratory fermented soyabean 'daddawa' had glutamic acid content of I7.40mg/g, I7.80mg/g and I6.30 when dried seperately at 50°C for for 7½ hours, 70°C for 4½ hours and I00°C for 3½ hours respectively while the uncontrolled fermented 'daddawa' had glutamic acid content of I6.80mg/g, I7.20mg/g and II.90mg/g, respectively when dried seperately at the temperatures listed above.

Autoclaved and oven-dried laboratory fermented soyabean 'daddawa' had glutamic acid content of I3.30mg/g, I2.40mg/g and II.02mg/g respectively when dried seperately at 50°C for 7½ hours, 70°C for 4½ hours and I00°C for 3½ hours while the uncontrolled fermented 'daddawa' had glutamic acid content of I2.95mg/g; I0.24mg/g respectively when dried separatley at the temperatures listed above.

Table 4.4 shows the post-fermentation changes in fresh and dried 'ogiri igbo'. At the end of fermentation of the seeds, the pH was 9.2 and 9.6 respectively for the laboratory fermented and uncontrolled fermented 'ogiri-igbo'. The glutamic acid contents for both types of the condiment were 9.75mg/g and 8.25mg/g respectively. The pH of the controls were 9.0 and 9.5 respectively for both types of the condiments. The differences between these values and those obtained after fermentation were not significant. Direct oven-drying of the fermented mash at different temperatures showed drops in pH when compared with the control but the differences between the pH values of the condiment dried at these temperatures were not significant. Laboratory fermented 'ogiri-igbo' had pH values of 8.6; 7.6 and 7.3 respectively when dried seperately at 50°C for 7½ hours, 70°C for 4½ hours and 100°C for 3½ hours while the uncontrolled fermented 'ogiri-igbo' had pH values of 8.3, 7.3 and 7.2 respectively when dried seperately at temperatures listed above.

The glutamic acid contents of the directly oven-dried 'ogiri-igbo' were as follows: Laboratory fermented 'ogiri-igbo' had glutamic acid content of 11.00mg/g , 11.80mg/g and 10.20mg/g when dried seperately at 50°C for 7½ hours, 70°C for 4½ hours and 100°C for 3½ hours respectively, while uncontrolled fermented 'ogiri-igbo' had glutamic acid content of 10.60mg/g, 11.00mg/g and 9.6mg/g when dried seperately at these temperatures. Autoclaved and oven-dried, laboratory fermented ogiri-igbo had glutamic acid content of 7.50mg/g, 6.60mg/g and 5.80mg/g when dried seperately at 50°C for 7½ hours, 70°C for 4½ hours and 100°C for 3½ hours while the uncontrolled fermented 'ogiri-igbo' had glutamic acid content of 7.00mg/g, 6.25mg/g and 5.20mg/g when dried seperately at the temperatures listed above.

The condiments used in this study were oven-dried to a constant weight at these temperatures. 50g each of soyabean and locust bean 'daddawa' was reduced to 17g (mean weight) while 50g of 'ogiri-igbo' was reduced to 30g (mean weight).

Plate I shows the physical appearance of both the directly oven-dried condiments and autoclaved oven-dried condiments. The latter were darker in colour than the former.

Plate 2 shows the laboratory fermented and packaged 'ogiri-igbo'. Plate 3 shows the laboratory fermented and packaged locust bean 'daddawa' while plate 4 shows the laboratory fermented and packaged soyabean 'daddawa'. Plate 5 shows the naked cubes before packaging.

Table 4.5 shows the glutamic acid concentration during storage of laboratory fermented, oven-dried condiments. There was no change in the glutamic acid concentration of the condiments packaged in various materials after storage for 3-months while the unpackaged condiments (control) showed slight changes.
The glutamic acid concentration in the packaged and stored condiments were as follows: locust bean 'daddawa' (I5.90mg/g); soyabean 'daddawa' (I7.40mg/g) and 'ogiri-igbo' II.00mg/g while the values of their controls after 3 months storage were I5.00mg/g, I6.80mg/g and I0mg/g respectively.

Table 4.6 shows the glutamic acid concentration during storage of laboratory fermented autoclaved, oven-dried condiments. Similarly, there was no change in the glutamic acid concentration of the condiments packaged in the various materials after storage for 3 months, but slight changes were observed in the unpackaged condiments. The glutamic acid concentration in the packaged and stored condiments were as follows: locust bean 'daddawa' (I2.95mg/g); soyabean 'daddawa' (I3.30mg/g) and 'ogiri-igbo' (7.50mg/g); while the values for their controls were II.20mg/g; I2.95mg/g and 7.00mg/g respectively.

The differences between the concentrations of the glutamic acid before packaging and after storage for 3 monthswere statistically non-significant.

Appendix A shows the detailed microbial counts in the condiments examined. Appendix B shows the calibration curve for glutamic acid standard.

Appendix C shows the biochemical tests employed for the identification of Staphylococcus aureus isolates obtained from the indigenous condiments. Coagulase-positive S.aureus were isolated from locust bean 'daddawa' and 'ogiri-igbo' samples but none from soyabean 'daddawa'. Five locust bean 'daddawa' isolates and two 'ogiri-igbo' isolates exhibited β.haemolysis on blood agar while none of the soyabean 'daddawa' isolates showed no haemolysis at all. The soyabean and locust bean 'daddawa' samples and four 'ogiri-igbo' isolates showed Dnase activity. Three locust bean 'daddawa' isolates, one soyabean 'daddawa' isolates and four 'ogiri-igbo' isolates exhibited lecithinase activity. All isolates from the three indigenous condiments fermented mannitol in mannitol salt agar and also liquefied gelatin.

Appendix D shows the biochemical tests used for the identification of B.cereus isolated from the indigenous condiments. All isolates were Gram-positive spore-forming rods exhibited urease activity, lecithinase activity and liquefied gelatin. Four locust bean 'daddawa' isolates, and all isolates from soyabean 'daddawa' hydrolysed starch. Four soyabean 'daddawa' isolates, two locust bean 'daddawa' isolates and all 'ogiri-igbo' isolates hydrolysed caesin (Litmus milk reaction).

Appendix E shows the biochemical tests employed for the identification of coliforms isolated from the indigenous condiments. Escherichia coli was detected in four samples each of the condiments while Enterobacter aerogenes was detected in one sample each of the condiments.

Appendix F shows the biochemical tests employed for the identification of Salmonella spp isolated from 'ogiri-igbo' sample. The isolate was negative for the following tests: Indole production, urease activity, Voges-Proskauer reaction and positive for lysine decarboxylase test with typical blackening of the butt in triple sugar iron agar.

Appendix G shows the identification of moulds isolated from the indigenous condiments. Rhizopus spp was identified from soyabean 'daddawa' (5 stains), locust bean 'daddawa' (4 strains) and 'ogiri-igbo' (3 strains). Aspergillus spp was identified from soyabean 'daddawa' (I strain), locust bean 'daddawa' (2 strains) and 'ogiri-igbo' (4 strains). Penicillium spp was identified from soyabean 'daddawa'.

TABLE 4.1 RANGE OF MICROBIAL COUNTS & MOISTURE CONTENT IN THE DIFFERENT CONDIMENTS. (cfu/g or ml).

NAME OF SAMPLE	TAPC	COLIFORM COUNT (1)	FAECAL COLIFORM COUNT (2)	S.aureus COUNT	MOULD COUNT	SPORE COUNT	B.cereus COUNT	Salmonella COUNT	Shigella COUNT	MOISTURE CONTENT %
KNORR CUBES	$1.0x10^{1}$ - $3.0x10^{2}$ ($1.4x10^{1}$)	0	0	0	0	0	0	0	0	2.5
MAGGI CUBES	$1.0x10^{1}$ - $3.0x10^{2}$ ($1.4x10^{1}$)	0	0	0	0	0	0	0	0	3.5
SOYA SAUCE	$1.0x10^{1}$	0	0	0	0	0	0	0	0	- *
SOYA BEAN 'daddawa'	$1.7x10^{6}$ - $2.8x10^{7}$ ($2.4x10^{6}$)	7-2,400 (1.471)	0-2,400 (563)	$1.8x10^{4}$ - $2.3x10^{5}$ ($3.3x10^{5}$)	$1.0x10^{2}$ - $2.0x10^{6}$ $1.2x10^{5}$)	$1.5x10^{5}$ - $1.6x10^{7}$ ($1.9x10^{5}$)	$7.6x10^{4}$ - $3.9x10^{5}$ ($3.6x10^{5}$)	0	0	10.0
LOCUST BEAN 'daddawa'	$1.2x10^{5}$ - $2.7x10^{7}$ ($3.5x10^{6}$)	0-2,400 (1,442)	0-2,400 (720)	$2.8x10^{5}$ - $2.9x10^{7}$ ($3.0x10^{6}$)	$1.9x10^{3}$ - $2.4x10^{6}$ $2.1x10^{3}$)	$1.6x10^{4}$ - $1.7x10^{7}$ ($2.2x10^{6}$)	$3.2x10^{4}$ - $3.2x10^{5}$ ($3.2x10^{4}$)	0	0	15.0
'OGIRI' IGBO'	$1.8x10^{6}$ - $1.4x10^{7}$ ($1.7x10^{7}$)	65-2400 (1,673)	0-2,400 (604)	($4.0x10^{5}$ - $3.0x10^{6}$ ($3.5x10^{6}$)	$2.2x10^{3}$ - $4.2x10^{7}$ ($1.9x10^{6}$)	$1.4x10^{4}$ - $2.8x10^{5}$ $2.0x10^{5}$	$1.6x10^{5}$ - $5.2x10^{5}$ ($4.2x10^{5}$)	0-$1.5x10^{5}$ ($3.0x10^{1}$)	0	24.0

* MEAN COUNTS ARE INDICATED IN PARANTHESES.
(i)- Counts in coliforms/g or ml
(ii)-Counts in faecal coliforms/g or ml
* MOISTURE CONTENT NOT DONE FOR LIQUID CONDIMENT.

TABLE 4.2. POST FERMENTATION CHANGES IN FRESH AND DRIED LOCUST BEAN 'BADDAWA'

CONDITION OF THE CONDIMENT	*pH		GLUTAMIC ACID CONCENTRAION (mg/g)	
	Laboratory fermented	uncontrolled fermented	Laboratory fermented	uncontrolled fermented
1. After 48 hours fermentation.	8.3	8.4	12.00	10.50
2. Control (Sun-dried)	6.1	6.4	12.75	11.20
3. Oven-dried at:				
$50^{o}c$	6.4	6.2	15.90	14.40
$70^{o}c$	6.3	6.1	16.28	15.00
$100^{o}c$	6.1	6.0	13.69	11.48
4. Autoclaved and Oven dried at:				
$50^{o}c$	7.4	7.6	12.95	11.88
$70^{o}c$	7.2	7.2	11.78	10.98
$100^{o}c$	7.0	6.6	10.28	9.60

*Values are average of duplicate readings.

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TABLE 4.3. POST-FERMENTATION CHANGES IN FRESH AND DRIED SOYA-BEAN 'DADDAWA'

CONDITION OF THE CONDIMENT	*pH		GLUTAMIC ACID CONCENTRATION (mg/g)	
	Laboratory fermented	uncontrolled fermented	Laboratory fermented	uncontrolled fermented
1. After 48 hours fermentation	8.3	8.6	13.50	12.75
2. Control (Sun-Dried)	6.7	6.7	13.87	12.92
3. Oven dried at:				
$50^{o}c$	7.9	7.8	17.40	16.80
$70^{o}c$	7.7	7.5	17.80	17.20
$100^{o}c$	7.6	7.4	16.50	11.90
4. Autoclaved and Over-dried at:				
$50^{o}c$	8.2	8.5	13.30	12.95
$70^{o}c$	8.2	8.5	12.40	12.00
$100^{o}c$	8.1	8.4	11.02	10.24

*Values are averages of duplicate readings'

TABLE 4.4. POST FERMENTATION CHANGES IN FRESH AND DRIED 'OGIRI IGBO'

CONDITION OF THE CONDIMENT	*pH		*GLUTAMIC ACID CONCENTRATION (mg/g)	
	Laboratory fermented	uncontrolled fermented	Laboratory fermented	Uncontrolled fermented
1. After 48 hours fermentation.	9.2	9.6	9.75	8.25
2. Control (packaged) in broad leaves)	9.0	9.5	9.75	8.25
3. Oven dried at:				
$50^{o}c$	8.6	8.3	11.00	10.60
$70^{o}c$	7.6	7.3	11.80	11.00
$100^{o}c$	7.3	7.2	10.20	9.6
4. Autoclaved and Oven-dried at:				
$50^{o}c$	7.1	7.6	7.50	7.00
$70^{o}c$	6.9	7.5	6.60	6.25
$100^{o}c$	6.8	7.4	5.80	5.20

*Values are averages of duplicate readings.

TABLE 4.5. GLUTAMIC ACID CONCENTRATION DURING STORAGE OF LABORATORY FERMENTED, OVEN-DRIED CONDIMENTS

Name of food Condiment	Post-fermentation heat treatment	Type of packaging material	Glutamic and Concentration (mg/g) before packaging	After Storage for 3 months
1. Locust bean 'daddawa'	1. Control (Sun-dried)	-	15.90	15.00
	2. Oven-dried at 50°c	*1. Aluminium foil	15.90	15.90
		*2. Polyethylene non-absorbent film	15.90	15.90
		*3. Cubes in aluminium foil	15.90	15.90
		*4. A combination of (1) and (2)	15.90	15.90
2. Soyabean daddawa'	1. Control (Sun-dried)	-	17.40	16.80
	2. Oven-dried at 50°c	*1. Aluminium	17.40	17.40
		*2. polyethylene non-absorbent film	17.40	17.40
		*3. Cubes in aluminium foil.	17.40	17.40
		*4 A combination of (1) and (2)	17.40	17.40
B. Ogiri-Igbo	1. Control (no heat treatment).	Packaged in broad leaves	11.00	10.20
	2. Oven-dried at 50°c	*1. Aluminium foil	11.00	11.00
		*2. Polyethylene non-absorbent film	11.00	11.00
		*3. Cubes in aluminium foil	11.00	11.00
		*4. A combination of (1(and (2)	11.00	11.00

*Condiments are in powdered form in these packaging materials

ABSTRACT

A total of thirty samples of food condiments comprising of some industrially produced (15) and traditionally fermented indigenous condiments (15) were examined for the incidence of different types of micro-organisms of significance (Total aerobic plate count, Staphylococcus aureus, Bacillus cereus, moulds, bacterial spores, Salmonella Shigella, coliforms and faecal coliforms). The commercial flavour enhancers had total aerobic plate count below 1 x10^{4}cfu/g or ml and none of the different groups of micro-organisms assayed for while the indigenous condiments had higher counts for these micro-organisms in the range of 2.→ x 10^6 - 1.7 x 10^7. Four samples each of the three indigenous condiments were faecally contaminated with faecal coliform count of 2.→ x 10^3. All the indigenous condiments showed various levels of contamination with all the micro-organisms assayed for except Salmonella spp which was found only in one sample of 'ogiri-igbo' at a count of 1.5 x 10^5.

The indigenous condiments (ogiri-igbo, soyabean and locust bean 'daddawa') were prepared by traditional methods and in the laboratory. The fermented mash was oven-dried seperately at three temperatures (50°c for 7½ hours, 70°c for →½ hours and 100°c for 3½ hours). Two batches of oven-dried condiments were produced:

Directly oven-dried and autoclaved, oven-dried condiments. The latter were darker in colour than the former. pH values at the end of fermentation fell to lower values during sun-drying and oven-drying at these temperatures.

At the end of fermentation, the pH values observed for the laboratory and uncontrolled fermented condiments were as follows:
8.3 and 8.→ for locust bean 'daddawa', 8.3 and 8.6 for soyabean 'daddawa'; 9.2 and 9.6 for 'ogiri-igbo.

During sun-drying of the fermented mash, the pH values dropped to lower values for both types of fermented condiments. These values were 6.1 and 6.→ respectively for locust bean 'daddawa', 6.7 and 6.9 respectively for soyabean 'daddawa'. A slight difference in pH (9.0 and 9.5 respectively) was obtained for 'ogiri-igbo' which was not sun-dried but packaged in broad leaves.

The laboratory fermented condiments had higher glutamic acid content than the uncontrolled fermented condiments. These values were 12.00mg/g and 10.50mg/g respectively for locust bean 'daddawa' 13.89mg/g and 12.92 mg/g respectively for soyabean 'daddawa', 9.75mg/g and 8.25 mg/g respectively for 'ogiri-igbo at the end of →8 hours fermentation.

The condiments were packaged in polyethylene non-absorbent film, aluminium foil and a combination of the polyethylene non-absorbent and aluminium foil. Glutamic acid contents of these packaged condiments were not altered after storage for 3 months while those of the unpackaged condiments were altered.

The importance of packaging our indigenous condiments is stressed.

TABLE 4.6. GLUTAMIC ACID CONCENTRATION DURING STORAGE OF LABORATORY FERMENTED AUTOCLAVED, OVEN-DRIED CONDIMENTS

Name of food condiment	Post-fermentation heat treatment	Type of packaging material	Glutamic acid concentration (mg/mL) Before packaging	After storage for 3 months
1. Locust bean 'daddawa'	1. Control (Sun-dried)	-	12.95	11.20
	2. Autoclaved and Oven-dried at 50°c	*1. Aluminium foil	12.95	12.95
		*2. Polyethylene non-absorbent film	12.95	12.95
		*3. Cubes in aluminium foil	12.95	12.95
		*4, A combination of (1) and (2) .	12.95	12.95
2. Soyabean 'daddawa'	1. Control (Sun-Dried)	-	13.30	12.95
	2. Autoclaved and oven-dried(50°c)	*1. Aluminium foil	13.30	13.30
		*2. Polyethylene Non-absorbent film	13.30	13.30
		*3. Cubes in Aliminium foil	13.30	13.30
		*4. A combination (1) and (2)	13.30	13.00
3. Ofiri-Igbo	1. Control (no heat treatment)	Packaging in broad leaves	7.50	7.00
	2. Oven-dried at 50°c	*1. Aluminium foil	7.50	7.50
		*2. Polyethylene Non-absorbent film	7.50	7.50
		*3 Cubes in Aluminium Foil	7.50	7.50
		*4 A combination of 1&2	7.50	7.50

*Condiments are in powdered form in these packaging materials

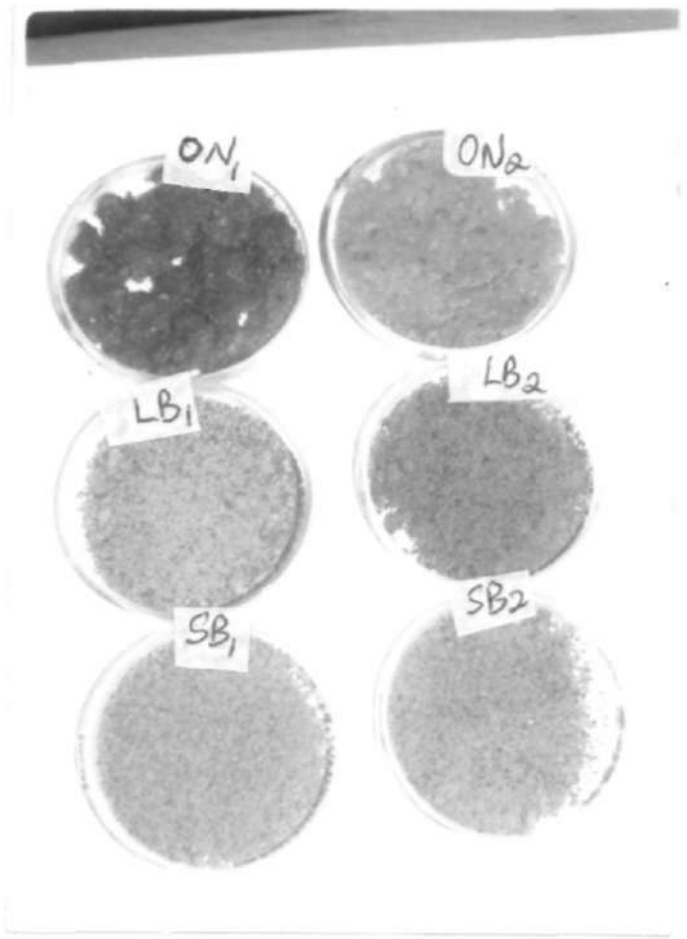


PLATE I: EFFECT OF AUTOCLAVING ON PHYSICAL APPEARANCE OF OVEN-DRIED CONDIMENTS.

- Autoclaved, oven-dried condiments are darker in colour than the directly oven-dried condiments.

KEY

ON_I - Autoclaved, oven-dried laboratory fermented 'ogiri-igbo'

ON_2 - Oven-dried laboratory fermented 'ogiri-igbo'.

LB_I - Autoclaved, oven-dried laboratory fermented locust bean 'daddawa'.

LB_2 - Oven-dried laboratory fermented locust bean 'daddawa'

SB_I - Autoclaved, oven-dried laboratory fermented soyabean 'daddawa'

SB_2 - Oven-dried laboratory fermented soyabean 'daddawa'



PLATE 2: LABORATORY FERMENTED AND PACKAGED 'OGIRI-IGBO'

KEY:-

ON_I - AUTOCLAVED, OVEN DRIED LABORATORY FERMENTED 'OGIRI-IGBO' PACKAGED:

ON_{Ia} - As cubes in aluminium foil

ON_{Ib} - In alumimium foil

ON_{Ic} - In combined polythylene non-absorbent film and aluminium foil

ON_{Id} - In polyethylene non-absorbent film.

ON_2 - DIRECTLY OVEN-DRIED LABORATORY FERMENTED OGIRI-IGBO PACKAGED:

ON_{2a} - As cubes in aluminium foil

ON_{2b} - In aluminium foil

ON_{2c} - In combined polyethylene non-absorbent film and aluminium foil

ON_{2d} - In polyethylene non-absorbent film.

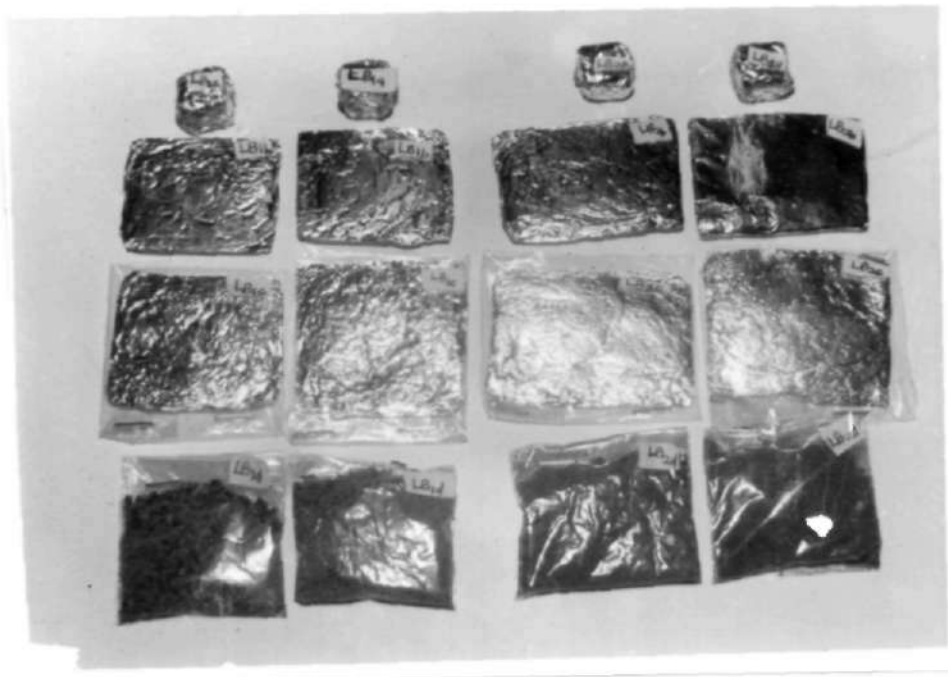


PLATE 3: LABORATORY FERMENTED AND PACKAGED LOCUST BEAN 'DADDAWA'.

KEY:

LB_1 - Autoclaved, oven-dried laboratory fermented locust bean 'daddawa' packaged:

LB_{1a} - as cubes in aluminium foil

LB_{1b} - in powdered form in aluminium foil

LB_{1c} - in powdered form in combined polyethylene film and aluminium foil

LB_{1d} - in powdered form in polyethylene non-absorbent film

LB_2 - Directly oven-dried laboratory fermented locust bean 'daddawa' packaged:

LB_{2a} - as cubes in aluminium foil

LB_{2b} - in powdered form in aluminium foil

LB_{2c} - in powdered form in combined polyethylene non-absorbent film and aluminium foil.

LB_{2d} - in powdered form in polyethylene non-absorbent film.

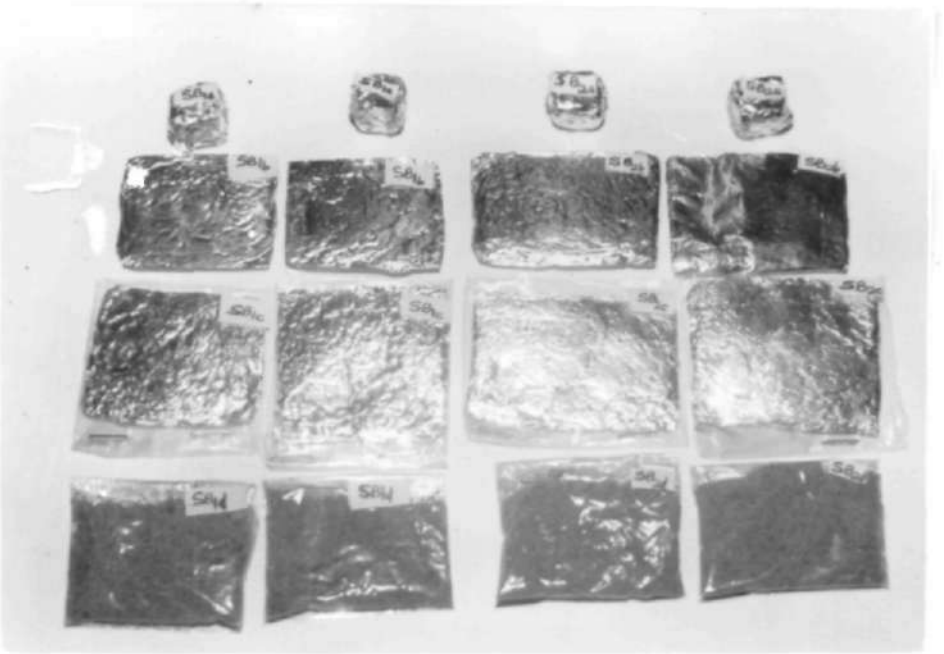


PLATE: 4: LABORATORY FERMENTED AND PACKAGED SOYA BEAN 'DADDAWA'

KEY:

SB_I - AUTOCLAVED, OVEN DRIED LABORATORY FERMENTED SOYABEAN 'DADDAWA' PACKAGED:

SB_{Ia} - As cubes in Aluminium foil

SB_{Ib} - In powdered form in aluminium foil

SB_{Ic} - In powdered form in combined polyethylene non-absorbent film and aluminium foil.

SB_{Id} - In powdered form in polyethylene non-absorbent film.

SB_2 - DIRECTLY OVEN-DRIED LABORATORY FERMENTED SOYABEAN 'DADDAWA' PACKAGED:

SB_{2a} - As cubes in aluminium foil

SB_{2b} - In powdered form in aluminium foil

SB_{2c} - In powdered form in combined polyethylene non-absorbent film and aluminium foil

SB_{2d} - In powdered form in polyethylene non-absorbent film.

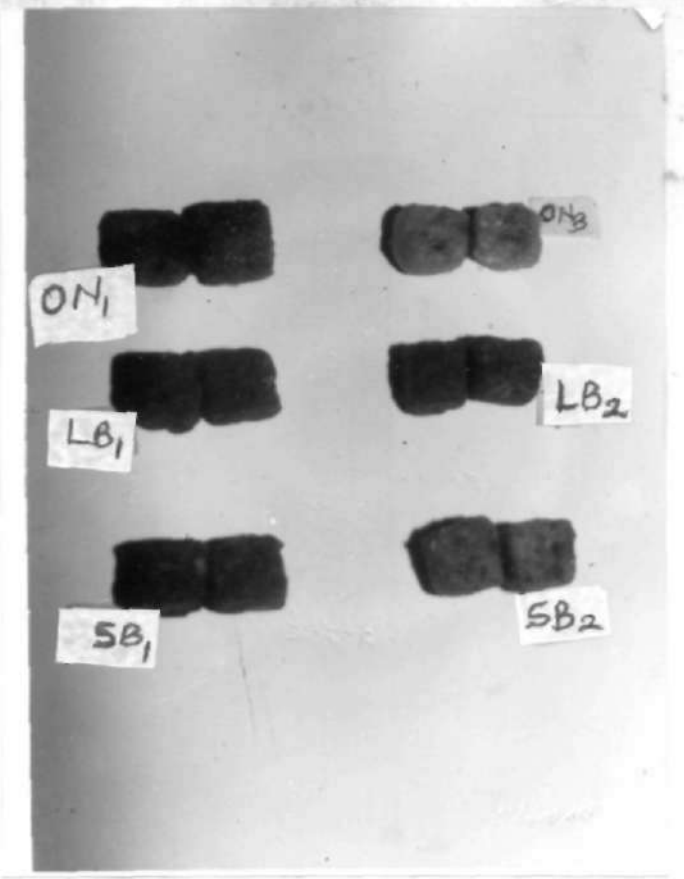


PLATE 5: NAKED CUBES OF THE OVEN-DRIED CONDIMENTS

KEY:

ON_I - Autoclaved, oven-dried laboratory fermented 'ogiri-igbo'

ON_2 - Oven-dried laboratory fermented 'ogiri-igbo'.

LB_I - Autoclaved, oven-dried laboratory fermented locust bean 'daddawa'

LB_2 - Oven-dried laboratory fermented locust bean 'daddawa'

SB_I - Autoclaved, oven-dried laboratory fermented soyabean 'daddawa'

SB_2 - Oven-dried laboratory fermented soyabean 'daddawa'.

CHAPTER V.

D I S C U S S I O N

Higher microbial counts were obtained with the indigenous condiments than the industrially produced flavour enhancers. The 100% recovery for the former may be attributed to contamination as a result of septic or improper handling, surviving fermenting flora, exposure after fermentation (soyabean 'daddawa' and locust bean 'daddawa'). Since the products in question are all fermented, the high total aerobic plate count recorded for them can be due to the surviving spores of the fermenting organism. It has been established that the total aerobic plate count alone cannot be used to assess microbial quality especially where fermentation is involved (ICMSF, 1978).

Results show that four samples each of the indigenous condiments were faecally contaminated. The presence of faecal coliforms (E. coli) indicates direct or indirect post-processing contamination with faecal matter. The presence of faecal coliforms in a food product may indicate that pathogenic enteric bacteria may be present (WHO, 1968, HPB, 1980; Odumosu, 1980).

Staphylococcus aureus was isolated from the indigenous condiment with counts above 10^4 cells/g. Some of the strains were coagulase-positive except those isolated from soyabean 'daddawa' samples. The correlation of coagulase production with enterotoxigenicity of S. aureus has been suggested by a number of workers (Adesiyun, 1984 a and b and Adewumi et al 1989).

Their presence in these condiments up to 10^7 cells/g, a figure above the acceptable ICMSF limit of 10^5 cells/g is indicative of possible health hazard. The strains that are coagulase positive may produce enterotoxins and staphylococcal enterotoxins are known to be heat-stable and thus can withstand some heat during cooking or reheating of foods. Other enzymes produced by S. aureus which correlate with pathonecity are lecithinase and DNase. Presence of Staphylococcus spp in foods is attributable to poor handling during processing.

The organism could gain access to the food condiments through handlers who sneeze or cough during preparation as staphylococci are normal flora of the nasal passage, hands, skin and wounds where they are commensals and harmless (Lajide, 196-, Talabi and Bassey 198-). Staphylococcus aureus has been isolated from various food-stuffs and utensils (Onuorah et al, 1987; Warburton and Weiss, 1988). The utensils used for preparation of the condiments may serve as sources of contamination. The isolation of coagulase-positive staphylococci from these condiments shows that great improve ment is needed in the sanitary practices employed in the preparation of these fermented food condiments.

The high counts of mesophilic aerobic spores obtained may be due to the surviving spores of the fermenting organisms most of which repeatedly are spore formers (Odunfa, 1981a and b; Obeta, 1983; Ogbadu, 1988) or spores of other contaminating Bacillus spp commonly found in the enviroment. Farkas (198-) noted that heat-resistant bacterial spores are the notable contaminants in dry food ingredients and processing aids such as spices, condiments, texturizing agents, sugars and enzymic preparation.

All the indigenous condiments examined had B.cereus counts above the acceptable limit of 10^{-} cells/g. These food condiments may serve as souces of B. cereus food poisoning, particularly for dishes that are lightly heat processed. Strains of B.cereus have been associated with food poisoning (Hauge, 1955; Blakey and Priest, 1980). The prevalence of B.cereus in food condiments has been studied by Obuekwe and Ogbimi (1989). They found large numbers of B.cereus in paprika and suggested that they may be responsible or contribute to common stomach upset and diarrhoea which accompany ingestion of high-paprika containing foods. Improper heat treatment is still hazardous since the spores may be forced to germinate and liberate toxin following heat-shocking during re-heating and cooling of our foods.

The range of mould count obtained for the condiments in this study may serve as the level of contamination during sun-drying or packaging of the condiments in leaves. Some species of Aspergillus and Penicillium both of which were isolated from the condiments in this study may produce mycotoxins under favourable conditions.

A third genus isolated was Rhizopus spp which may easily be inactivated during cooking. No Shigella spp was recovered from the condiments. No Salmonella spp was isolated from soyabean and locust bean 'daddawa' but from one of the 'ogiri-igbo' samples. The presence of Salmonella spp in foods or food condiments is not desirable as they cause severe gastro enteritis, although they can easily be inactivated by heat during cooking. There was however, a high count of faecal coliforms whose presence may indicate the presence of pathogens.

The moisture content of the indigenous condiments used for microbiological analysis could support microbial growth and metabolism. The bouillion cubes had lower moisture content and are also properly packaged hence the low total aerobic plate count as well as the total exclusion of organisms of significance. Removal or limitation of water prevents endogenous degradation of foods and also the activities of contaminating micro-organisms (Gibbs, 1986). Practically, all deteriorative processes which take place in food-stuffs are influenced in one way or the other by the concentration and mobility of the water in the food-stuff. As a rough orientation, spoilage is caused by the growth and development of bacteria and moulds on the food and by enzymatic or non -enzymatic reactions; at low water concentrations quality losses are caused mainly by auto-oxidative reactions and physical deterioration (Frazier, 1967 and Gibbs, 1986).

Okeoma (198-) noted that no deteriorative changes occured in locust bean 'daddawa' when stored at ambient temperature for 6 months. 'Ogiri-igbo' had higher moisture content than locust bean and soyabean 'daddawa' and is more likely to be spoilt as it is less stable at higher water concentrations.

Slightly higher microbial counts were observed for 'ogiri-igbo' samples than locust bean and soyabean 'daddawa'. This may be due to the higher moisture content (2-%) which could support microbial growth and activity.

Browning of the condiments was observed during drying for each type of condiment after fermentation. Okeoma (198-) reported similar colour changes during the oven-drying of locust bean 'daddawa' at 50-55°c . Arogba et al (1990) noted similar colour changes during the oven-drying of ogiri (melon), soyabean and locust bean 'daddawa' at 100°c. These colour changes may be due to Maillard reaction leading to the production of Amadori compounds. These are compounds resulting from the degradation of 1-amino-1-deoxy-ketose either by fission, deaodolisation and removal of water. (Nursten, 1986). Non-enzymic browning involves the reactions between amino acids and sugars. These compounds are abundant at the end of fermentation (Fetuga et al, 1973 and Odunfa, 1982). The browning became intensified when the fermented mash was autoclaved before drying. (plate 1-ON,LB,SB). Pasteurisation of soya sauce was reported to double the colour intensity (Steinkraus, 1983). This report agrees with the observation in this study. It can be safely deduced from this study that moist heat when applied to fermented vegetable proteins tend to increase their colour intensity.

Perhaps the concentration of Amadori compounds resulting from Maillard reaction is increased by autoclaving thus darkening the product. These darkening changes affect the aesthetic appeal to the consumers and thus affect their choice of the product, i.e. the autoclaved oven-dried condiments are darker in colour than the directly oven-dried condiments.

The fall in pH observed during the oven-drying of the fermented mash when compared with the values obtained at the end of fermentation may be due to loss of ammonia from the fermented mash.

Ammonia has been reported to be released during fermentation of vegetable proteins (Hesseltime, 1965). Soyabean 'daddawa' had the highest glutamic acid content when compared with values obtained for locust bean 'daddawa' and 'ogiri-igbo'. A comparative study of the sensory attributes of these condiments by Jideani and Okeke (1989) showed that soyabean 'daddawa' had the highest value in the sensory evaluation preference rating followed by locust bean 'daddawa', with melon condiment (ogiri) being the least preferred. Their study showed a correlation between the glutamic acid content and the preference of flavour in these condiments. Higher glutamic acid contents in the laboratory fermented condiments may be due to autoclaving of the seeds before fermentation. Steinkraus (1983) showed that cooking under pressure increase the digestibility of proteins in soyabean during fermentation.

The directly oven-dried condiments had slightly higher glutamic acid than the autoclaved, oven-dried condiments. This may be due to the fact that the fermenting organism was not inactivated by autoclaving before oven-drying in the latter. The former had better aesthetic appeal than the latter.

Autoclaving of the fermented mash before oven-drying tends to decrease the level of glutamic acid in the condiments. Steinkraus (1983) noted that small amounts of amino acids may be destroyed by deamination and aminocarboxyl reactions during processing of soya sauce. This destruction of amino acids represent a loss in nutritive value.

The glutamic acid contents of the post-fermentation heat-processed and packaged condiments were not altered while the glutamic acid contents for the controls were altered. The significance of this results lies in the importance of packaging our indigenous condiments as the unpackaged ones may have their glutamic acid content altered by micro-organisms metabolising part of this flavouring amino acid. The advantages offered by the packaged condiments include exclusion of contamination, (low microbial load), non-alteration of the glutamic acid content, non-alteration of the flavouring ability and provision of convenient, attractive packages as well

as space saving for the cubed condiment for the consumers. Other advantages of the packaged condiments are that the odour of the condiment is sealed off and the transparency of the plastic film enables the consumer to visualise the colour of the condiment which may aid their choice. It is sugested that oven-drying of the fermented mash be done at 50°C for 7½ hours for both types of oven-dried condiments as this has less browning than those dried at 70°c and 100°c

Comparatively, the differences in pH values and the glutamic acid contents of both the laboratory and traditionally fermented condiments were not significant. This is because the parameters used in this study may have no-significant effect on the quality of the condiment. Both types of condiments may be different in respect of other parameters such as the microbial load of the final product.

The recommended monosodium glutamate (MSG) Dietary Intake has been established by the American Institue of Food Technologists Expert panel on Food safety and nutrition as 7.5g MSG/day (Food Technol, 1980). The level of monosodium glutamate in all the samples were within the acceptable limit.

CONCLUSION

It was established that the traditionally fermented condiments were highly contaminated when compared with the, commercially processed flavour enhancers.

These indigenous condiments may be contaminated by poor handling during preparation, marketing and lack of packaging.

It is recommended that they are fermented under controlled condition and packaged appropriately for large scale production.

The changes occuring after fermentation give an insight to the production parameters for pilot-scale drying of the fermented mash.

It is recommended that indigenous condiments be produced by oven-drying at $50^{o}c$ for $7\frac{1}{2}$ h before packaging.

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APPENDIX A. DETAILED MICROBIAL COUNTS IN THE DIFFERENT CONDIMENTS

COUNTS IN cfu/g or cfu/ml:

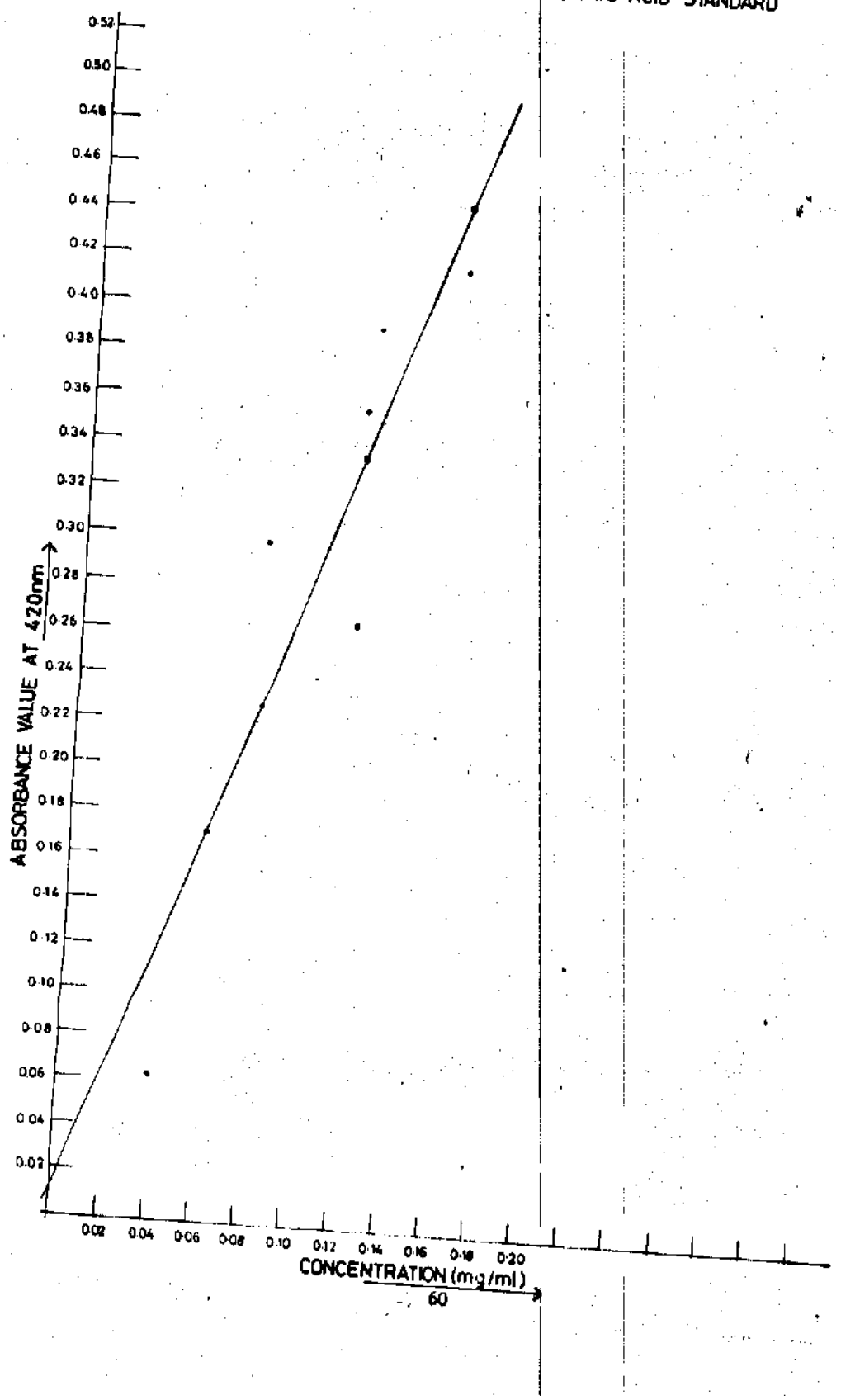
NAME OF SAMPLE	TOTAL AEROBIC PLATE COUNT	COLIFORM COUNT (1)	FAECAL COLIFORM COUNT (2)	S.aureus COUNT	MOULD COUNT	SALMONELLA COUNT	SHIGELA COUNT	MESOPHILIC STORE COUNT	B. Cereus COUNT
Knorr cubes (5).	1.0×10^{1}	0	0	0	0	0	0	0	0
	3.0×10^{2}	0	0	0	0	0	0	0	0
	1.0×10^{1}	0	0	0	0	0	0	0	0
	1.0×10^{1}	0	0	0	0	0	0	0	0
	1.0×10^{1}	0	0	0	0	0	0	0	0
MAGGI CUBES (5).	3.0×10^{2}	0	0	0	0	0	0	0	0
	1.0×10^{1}	0	0	0	0	0	0	0	0
	1.0×10^{1}	0	0	0	0	0	0	0	0
	1.0×10^{1}	0	0	0	0	0	0	0	0
	1.0×10^{1}	0	0	0	0	0	0	0	0
SOYA SAUCE (5).	1.4×10^{1}	0	0	0	0	0	0	0	0
	1.0×10^{1}	0	0	0	0	0	0	0	0
	1.0×10^{1}	0	0	0	0	0	0	0	0
	1.0×10^{1}	0	0	0	0	0	0	0	0
	1.0×10^{1}	0	0	0	0	0	0	0	0
SOYABEAN 'daddawa' (5)	2.4×10^{7}	2.4×10^{3}	1.5×10^{2}	3.6×10^{5}	1.8×10^{3}	0	0	1.8×10^{6}	3.9×10^{5}
	2.7×10^{6}	2.4×10^{3}	2.4×10^{2}	2.3×10^{6}	1.0×10^{3}	0	0	1.6×10^{7}	3.2×10^{5}
	1.7×10^{6}	7	0	1.8×10^{4}	1.1×10^{5}	0	0	1.5×10^{5}	7.6×10^{4}
	2.5×10^{7}	1.5×10^{2}	2.3×10^{1}	4.8×10^{5}	1.0×10^{2}	0	0	2.3×10^{5}	2.0×10^{5}
	2.8×10^{6}	2.4×10^{3}	2.4×10^{3}	4.0×10^{5}	1.2×10^{3}	0	0	2.6×10^{6}	1.3×10^{5}
LOCUST BEAN 'daddawa' (5).	2.7×10^{7}	2.4×10^{3}	2.4×10^{3}	2.9×10^{7}	1.9×10^{3}	0	0	1.5×10^{7}	3.2×10^{5}
	2.8×10^{6}	2.4×10^{3}	1.1×10^{3}	2.8×10^{7}	1.2×10^{3}	0	0	1.7×10^{7}	2.8×10^{5}
	1.2×10^{6}	0	0	2.8×10^{5}	4.8×10^{5}	0	0	1.6×10^{5}	3.0×10^{4}
	1.8×10^{6}	9	4	5.8×10^{5}	1.0×10^{3}	0	0	5.5×10^{7}	3.2×10^{3}
	9.0×10^{6}	2.4×10^{3}	9.3×10^{1}	1.0×10^{7}	1.6×10^{3}	0	0	1.1×10^{7}	3.8×10^{4}
OGIRI-IGBO (5).	1.4×10^{3}	2.4×10^{3}	1.5×10^{2}	3.0×10^{7}	2.2×10^{3}	0	0	1.2×10^{7}	1.6×10^{4}
	1.8×10^{6}	1.1×10^{1}	4.6×10^{2}	5.4×10^{6}	2.8×10^{3}	0	0	2.8×10^{7}	3.2×10^{5}
	2.8×10^{6}	6.4×10^{3}	0	4.4×10^{5}	2.5×10^{6}	0	0	1.4×10^{4}	5.2×10^{5}
	1.2×10^{7}	2.4×10^{3}	2.4×10^{3}	4.0×10^{5}	1.2×10^{2}	0	0	2.6×10^{6}	2.8×10^{5}
	1.5×10^{7}	2.4×10^{3}	9	1.0×10^{7}	1.0×10^{3}	$*1.0\times10^{5}$	0	2.2×10^{6}	8.4×10^{4}

* NUMBER OF SAMPLES EXAMINED FOR THE DIFFERENT ORGANISMS.

(1)- Counts are in coliforms/g or coliforms/ml.

(2)- Counts are in faecal coliforms/g or faecal coliforms/ml.

APPENDIX B: CALIBRATION CURVE FOR GLUTAMIC ACID STANDARD



APPENDIX C. BIOCHEMICAL IDENTIFICATION OF Staph aureus ISOLATED FROM THE INDIGENOUS CONDIMENTS

TESTS EMPLOYED	SOYABEAN 'daddawa' ISOLATES					LOCUST BEAN 'daddawa' ISOLATES					,OGIRI-IGBO, ISOLATES				
	SB_1	SB_2	SB_3	SB_4	SB_5	LB_1	LB_2	LB_3	LB_4	LB_5	ON_1	ON_2	ON_3	ON_4	ON_5
1. B-haemolysis on Blood Agar	-	-	-	-	-	+	+	+	+	+	-	+	-	-	+
2. Acid and gas from:-															
(1) Glucose (aerobic)	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG
(2) Glucose (anaerobic)	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG+	AG
2b Acid and gas from:-															
(1)Mannitol (anaerobic)	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
(2)Mannitol (anaerobic)	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
3. Coagulase (tube)	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+
4. Dnase production	+	+	+	+	+	+	+	+	+	+	+	+			+
5. Growth on Mannitol salt Agar (MSA)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
6. Mannitol fermentation in MSA	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+
7. GROWTH in Nacl															
3%	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
5%	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
10%	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+
15%	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+
8. Lecithinase activity	-	-	-	-	+	-	+	+	-	+	-	+	+	+	+
9. Nutrient - Gelatin Liquefaction ($22^{o}c$)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
10.Nitrate reduction	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
11.Voges-proskaeur reaction	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
12.Oxidation fermentation in OF glucose broth	O	O	O	O	F	F	F	F	F	F	F	F	F	F	F
13.Catalase test.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

O = OXIDATION: F = FERMENTATION.

APPENDIX D _ BIOCHEMICAL IDENTIFICATION OF Bacillus cereus ISOLATED FROM THE INDIGENUOS CONDIMENTS

TEST EMPLOYED	SOYABEAN 'daddawa' ISOLATES					LOCUST BEAN 'daddawa' ISOLATES					OGIRI-IGBO, ISOLATES				
	SB_1	SB_2	SB_3	SB_4	SB_5	LB_1	LB_2	LB_3	LB_4	LB_5	ON_1	ON_2	ON_3	ON_4	ON_5
1. Gram reaction.	GRAM POSITIVE SPORE FORMING RODS.					GRAM POSITIVE SPORE FORMING RODS.					GRAM POSITIVE SPORE FORMING RODS				
2. VP test.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3. Citrate Utilisation	+	+	+	+	+	-	+	+	-	-	+	+	-	-	+
4. Acid from: (1) Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
(2) Mannitol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
(3) Xylose	-	-	-	-	+	-	-	-	-	-	+	+	+	+	-
5. Nitrate reduction.	-	+	-	-	+	-	-	-	-	-	+	+	+	+	-
6. Growth on Blood Agar.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
7. B-haemolysis on Blood Agar under anaerobic Conditions.	-	+	-	-	-	+	+	+	+	+	-	+	+	+	+
8. Urease activity	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
9. Casein hydrolysis.	-	+	+	+	+	+	+	-	-	-	+	+	+	+	+
10. Lecithinase activity.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
11. Indole production.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12. Motility test.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
13. Gelatin - Liquefection	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
14. Starch hydrolysis	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+
15. Catalase Activity	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
16. Growth in 7.5% Nacl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
17. OF glucose broth (oxidation fermentation reaction)	F E R M E N T A T I O N.					F E R M E N T A T I O N.					F E R M E N T A T I O N.				

APPENDIX E. BIOCHEMICAL IDENTIFICATION OF THE COLIFORM ISOLATES FROM THE INDIGENOUS CONDIMENTS

TEST EMPLOYED	SOYABEAN 'daddawa' ISOLATES					LOCUST BEAN 'daddawa' ISOLATES					OGIRI-IGBO ISOLATES				
	SB_1	SB_2	SB_3	SB_4	SB_5	LB_1	LB_2	LB_3	LB_4	LB_5	ON_1	ON_2	ON_3	ON_4	ON_5
1. Gas production at 44.5°c	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+
2. Indole production	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+
3. Methyl Red Test.	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+
4. Voges-Proskaeur reaction.	-	-	+	-	-	-	-	+	-	-	-		+	-	-
5. Citrate Utilisation.	-	-	+	-	-	-	-	+	-	-	-	-	+	-	-
6. Reaction in Kligler Iron Agar	A/A;G	A/A;G	A/A;G	A/A;G	A/A;G	A/A;G	A/A;G	A/A;G	A/A;G	A/A;G	A/A;G	A/A;G	A/A;G	A/A;G	A/A;G
7. Reaction in Triple Sugar Iron Agar.	A/A;G	A/A;G	A/A;G	A/A;G	A/A;G	A/A;G	A/A;G	A/A;G	A/A;G	A/A;G	A/A;G	A/A;G	A/A;G	A/A;G	A/A;
8. Motility Test.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
9. Colonial Appearance On EMB - Agar.	1	1	2	1	1	1	2	1	1	1	1	2	1	1	1
10. Inference	Escherichia coli	Ent. aerogenes		Escherichia coli		E.coli	Ent. aerogenes	Escherichia Coli			Escherichia coli	Ent. aerogenes	Escherichia* coli		

FOOT NOTES:
(a) A/A;G represents Acid Production in both the slope and butt together with gas production.
(1) Small, ... purple colonies with dark centers and a metallic sheen.
(2) Large, pink mucoid colonies with no metallic sheen.

APPENDIX F.

BIOCHEMICAL IDENTIFICATION OF SALMONELLA SPP ISOLATED FROM 'OGIRI-IGBO'

Test Employed		Reaction
1. Indole production		-
2. Urease activity		-
3. Voges-Proskaeur Test		-
4. Lysine Decarboxylase		+
5. Triple sugar Iron Agar reaction		
Butt:	yellow	+
	black	+
	Cracks	+
Slant :	Red	+

Appendix G

IDENTIFICATION OF MOULDS ISOLATED FROM THE INDIGENOUS CONDIMENTS

Kashim Ibrahim Library, Bello University.

SOURCE	ISOLATE	MACROSCOPY	MICROSCOPY	IDENTITY
SOYABEAN 'daddawa'	SB_1	White, fluffy mycelial mats with black sporangia on further incubation.	Non-septate mycelia with sporangia laden with spores.	Rhizopus spp
	SB_2	"	"	"
	SB_3	"	"	"
	SB_4	1. "	"	"
		2. Green mycelial mats.	Spores radiating from hyphal ends.	1. Rhizopus spp 2. Aspergillus spp
	SB_5	1. White, fluffy mycelial mats with black sporangia.	1. Non-septate mycelia with sporangia laden with spores.	1. Rhizopus spp
		2. Blue, mycelial mats with white outline.	2. Broom like conidia on conidiophores	2. Penicillium spp.
LOCUST BEAN 'daddawa'	LB_1	White, fluffy mycelial mats with black sporangia.	Non-septate mycelia with sporangia laden with spores.	Rhizopus spp.
	LB_2	"	"	"
	LB_3	Green fluffy mycelial mats.	Spores radiating from hyphal ends.	Aspergillus spp.
	LB_4	White, fluffy mucelial mats with black sporangia.	Non-septate mycelia with sporaniga laden with spores.	Rhizopus spp
	LB_5	1. Black, fluffy mycelial mats. 2. White, fluffy mycelial mats.	1. Conidia radiating from conidiophores. 2. Non-septate mycelia with sporangia laden with spores.	1. Aspergillus spp 2. Rhizopus spp
'OGIRI-IGBO'	ON_1	1. Green, fluffy mycelial mats. 2. White, fluffy mycelial mats.	1. Spores radiating from conidiophores. 2. Non-septate mycelia with black spores.	Aspergillus spp Rhizopus spp
	ON_2	"	"	"
	ON_3	Green fluffy mycelial mats.	Conidia radiating from conidiophores.	Aspergillus spp
	ON_4	1. Black, fluffy mycelial mats. 2. "	Non-septate mycelia with sporangia laden with spores.	Rhizopus spp "
	ON_5	1. Black, fluffy mycelial mats. 2. Green, fluffy mycelial mats.	1. Spores radiating from conidiophores. 2. "	Aspergillus spp.