STUDIES ON FERMENTATION OF SOME LEGUMINOUS SEEDS USING MIXED SPECIES OF *BACILLUS* AS STARTER CULTURES

BY

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DECLARATION

I declare that the work in this Dissertation entitled "Studies on fermentation of some leguminous seeds using mixed species of *Bacillus* as starter cultures," has been performed by me in the Department of Microbiology under the supervision of Professor J. B. Ameh, Dr. S.A. Ado and Professor (Mrs) V.J. Umoh.

The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at any university.

_____	_____	_____
Gberikon, Grace Mwuese	Signature	Date

CERTIFICATION

This dissertation entitled: "STUDIES ON FERMENTATION OF SOME LEGUMINOUS SEEDS USING MIXED SPECIES OF *BACILLUS* AS STARTER CULTURES" by Gberikon, Grace Mwuese, meets the regulation governing the Award of the degree of Doctor of philosophy of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

I dedicate this research work to the sweet memories of my late parents Mr. and Mrs S.A Shishi who laid a good educational foundation for me, and also to my late brother Lutor Shishi.

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ABSTRACT

Seeds of three African legumes, namely *P. africana* purchased from Otukpo in Benue state of Nigeria, *P. biglobosa* and *G. max* from Sabon gari Zaria, Kaduna state. Batches of the processed seeds (300g) were subsequently fermented using 5% mixed standard strains of *Bacillus subtilis* and *Bacillus pumilis* (consortium A) and 5% mixed test strains of *Bacillus subtilis* and *Bacillus pumilis* (consortium B) as starters or consortia. *P. biglobosa* and *G.max,* inoculated with consortia fermented within 48h and *P.africana* seeds inoculated with consortia fermented within 84h. From this analysis, it was observed that seeds with consortia fermented faster (48h and 84h) for *P.biglobosa*, *G.max* and *P.africana* respectively, than natural fermentation (72h and 96h). Analysis of variance showed that moisture content, ash, crude lipid, protein and soluble carbohydrates were significantly higher ($p<0.001$) in starter assisted fermentation than in natural fermentation. Mineral elements such as Fe, K, Ca, Na were significantly higher ($p<0.001$) in starter assisted fermentation than natural fermentation. These were compared with condiments obtained from the market and appreciable values for nutrients and mineral element compositions were also obtained.

Different drying conditions namely solar drying, vacuum drying, hot air oven drying, drying under direct sunlight and drying under direct sunlight with net protection were applied to dry products. Dried seeds were blended into powdered form using a sterile blender. Analyses of the powdered condiments showed that nutrient contents were highly appreciable in *G.max* condiments fermented with consortium B, with values ranging from 18% for crude lipid, 29% for crude protein respectively. Crude fibre was highest in *P.biglobosa* condiments fermented with both consortia with values ranging from 16-17%. Soluble carbohydrate had highest value of 32% with condiments of

G.max fermented with consortium B. Mineral elements were also appreciable in powdered condiments, highest mean values of K, Ca were recorded in *P.africana* condiments fermented with consortium A, with values of 23% and 29% respectively. Highest value of Na was recorded in *P.biglobosa* condiments fermented with consortium B with a value of 23%. Traces of iron (Fe) were recorded in all the condiments with values ranging from 1-2%. Highly significant ($p<0.001$) values of these nutrients and minerals were also observed in analysis of market condiments. Essential amino acids were present in fresh and powdered condiments at highly significant values ($p<0.001$) compared to natural fermentation. Leucine had the highest mean value of 9.84g in freshly fermented *G.max* condiments with consortium A. Glutamate, a non-essential amino acid and a flavour enhancer, had the highest mean value of 18.97g in *P.africana* condiment fermented with consortium B. Seeds dried with hot air oven had optimum results in physicochemical parameters during storage. Effect of packaging powdered condiments showed that those packaged in sachets and stored under room and refrigeration temperature had a longer shelf life (six months) than those packaged in small plastic containers with lid (four months). Powdered *G.max* condiments from markets deteriorated faster (two months) than their counterparts produced in the laboratory (six months). Sensory evaluation of soups carried out on these three condiments using nine point hedonic scale showed that *P.africana* was mostly preferred in terms of mouth feel, flavour, taste and overall acceptability and was rated (1) - like very much. A suitable mixed starters consisting of *B.pumilus* and *B.subtilis* can be used to ferment seeds of *P.africana*, *P.biglobosa* and *G.max* for production of organoleptically acceptable condiments with desirable contents of nutrients, minerals and amino acids compositions. Such condiments can be preserved up to six months with sachets packaging, stored under room and refrigeration temperature.

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CHAPTER ONE

INTRODUCTION

In Nigeria, seeds of some legumes such as soya bean seeds (*Glycine max)* African mesquite (*Prosopis africana)*) and locust bean seeds (*Parkia biglobosa*) may account for up to 80% of dietary proteins and this may be the only source of protein for some groups of people. They are commonly used in fermented forms as condiments to enhance flavours and taste of foods (Oniofiok, 1996). Fermented condiments remain key constituents of diets throughout many parts of Asia and Africa. Whereas the process of fermentation probably evolved for the development of taste and aroma, it often results in enhanced nutritive value and elimination of anti nutrient factors (Ogunshe *et al;* 2006). With high contents of protein, legume condiments can serve as a tasty complement to sauces and soups and can be substitutes for fish or meat (Achi, 2005). Traditionally fermented condiments ("*daddawa"* and *"okpehe*") are based on vegetable proteins, and are consumed by different ethnic groups in Nigeria. It has been the pride of culinary traditions for centuries (Campbell-Platt, 1980) and it is evident that these products have played major roles in the food habits of communities in the rural regions. Traditional diets in West Africa often lack varieties and consist of large quantities of staple foods (cassava, yam and maize) which provide the calories, but are poor in other nutrients. Soups are the main source of protein and minerals and one of the ways to improve the diet is to improve the nutrients in the soup through the use of condiments such as fermented legume seeds (Achi, 2005).

Fermentation markedly improves shelf life, digestibility, nutritive value and flavours of the raw seeds. When fermented, the seeds become tasty and protein rich seasoning. They are added to dishes directly or used as thickening agents in soups and stew (Ogbadu, 1988).

Relatively recent microbiological information on Nigerian plant protein fermentations include those reported by Omafuvbe *et al., (*2002); Ouoba *et al;* (2003); Dakwa *et al*., (2005) and Gberikon *et al.*, (2009) In all the reports the involvement of the genus *Bacillus* was established in the fermentation of locust bean seeds and other legumes like soyabean seeds and castor oil seeds. Odunfa (1981) also reported that the organisms involved in fermentation of locust bean seeds have been identified as *Bacillus* and *Staphylococcus* species with species of *Bacillus* being the predominant microorganisms. In view of the fact that the major constituents of vegetable seeds are proteins, the organisms responsible for fermenting them must be capable of utilizing these constituents (Antai and Ibrahim, 1986; Ouoba *et al*. 2003). *Bacillus* species isolated from fermented foods have been reported to be proteolytic and are able to break down oils (Achi,2005).

It is evident that production of fermented condiments is initiated by a diverse microbial flora, which eventually becomes predominantly Gram-positive flora in many African fermented foods (Odunfa, 1985). The contribution of these accompanying flora of bacteria to the properties of the legume product is only partly understood (Iwoha and Eke, 1996). Most probably, they play a role in flavour development, and influence the chemical composition through the substrate modification and synthesis of vitamins (Nout and Rombouts, 1995). However it is necessary to ascertain the contributions of these organisms in vegetable fermentations.

Undoubtedly, traditionally fermented condiments have not been incriminated in food poisoning. The fermentation process for condiment production is still being carried out by the traditional village art. There is need to apply modern biotechnological techniques such as the

use of starter cultures in improving traditional food processing technologies (Achi, 2005). It has been suggested that even though most fermentations in developing countries do not use inocula or extrinsic cultures, these processes could be improved by using starter cultures (Holzapfel, 2002). Starter cultures have been found to reduce fermentation time as well as guarantee product quality,like fermented locust bean and soyabean seeds. In the traditional method of manufacture, fermentation of legume seeds is achieved by indigenous microbial flora or the addition of fermented materials from previous production. Thus it may be assumed that undefined starter cultures have traditionally been employed in the production of these products (Omafuvbe *et al*., 2002; Dakwa *et al*.2005).

A defined starter culture is essential for controlled fermentations. Most authors now agree that there is a predominant development of *Bacillus* species during the various legume fermentation processes **(**Aderigbe and Odunfa, 1990; Odibo *et al*., 1992; Ouoba *et al*., 2003). For soyabean fermentation, single or combined cultures of *Bacillus subtilis, B.licheniformis* have been successfully used. (Sarkar *et al*., 1993). From experience, to avoid spoilage problems by unwanted microorganisms, there is the need to call for further and large-scale intervention studies to reduce the high moisture content in order to increase the shelf life of this product which is enhanced by the use of better packaging materials.

1.1 Statement of the problem.

Although fermented food condiments have contributed a significant proportion of taste to the diet of many people, Nigerians have exhibited an ambivalent attitude in terms of consumer tastes and preferences for such foods (Achi, 2005). The introduction of high technology products, especially processed ones, due to globalization and liberalization of the economy, has radically changed the Nigerian food culture into a mixed grill of both foreign and local dishes (Ojo, 1991). The production of fermented vegetable proteins for use as food condiments is art-based. Remarkably, in many areas of Nigeria today, they are still made in traditional ways, with success depending on observance of good manufacturing practices and control of environmental conditions during the manufacturing process. Starter cultures are not normally used and therefore variations in the quality and stability of the product are often observed.

The traditionally fermented condiments have not attained good commercial status due to their very short shelf life, objectionable packaging materials, stickiness and their characteristic putrid odour (Arogba *et al*., 1995). The condiments often have a stigma attached to them, and are often considered food for the poor.

1.2 **Justification of this study**.

An understanding of the microbial ecology of vegetable fermentations requires knowledge of the fermentation substrates, that is, the seeds of the various plants, as well as the products obtained. It has been suggested that the use of these condiments can be extended as food ingredient included into most fabricated foods in order to further increase their versatility and utility (Giami and Bekebain, 1992; Achi, 1999). For example, fermented fluted pumpkin flour has been incorporated into weaning food formulations (Achinewhu, 1987). As an indigenous condiment, it features prominently with imported ones in nationwide television programmes in which recipes are advertised. Apart from the typical aroma of these

condiments, comparative studies have shown that the nutritive value of fermented legume seeds is greater than that of sodium monoglutamate (Ogbadu, 1988). The protein content increases after fermentation of locust bean seeds; fats and vitamins, particularly riboflavins also increase (Odunfa, 1981). From experience, single starters can be used to initiate fermentation. The use of a mixture of microorganisms with complementary physiological properties as starter cultures seems to be the best approach for obtaining a product with the nutritional and sensory properties desired (Omafuvbe *et al*., 2002).

1.3 Aim of this study

The aim of this study is to ferment locust bean (*Parkia biglobosa*), soya bean (*Glycine max*) and African mesquite (*Prosopis africana*) seeds using mixed *Bacillus* species (*B.subtilis* and *B.pumilus*) as starter cultures.

1.4 Objectives of this study

i. A comparism of properties of strains in two mixed culture, obtained from different sources; standard strain (SX1BS and SX2BP) and test strain (TS001 and TS002).

ii To carry out fermentations of leguminous seeds using mixed *Bacillus* isolates as starter cultures.

iii. To assess the effect of starter cultures and natural fermentation on proximate composition and essential amino acid content of the fermented food.

iv. To determine the effect of processing, packaging and storage of shelf life of the condiments

v. To carry out sensory evaluation of soups prepared with different condiments.

CHAPTER TWO

LITERATURE REVIEW

2.1 Fermented fruits and vegetables

There are several definitions of fruits, the everyday usage of the word "fruits" defines fruits as "The edible product of a plant or tree, consisting of the seeds and its envelope, especially the latter when juicy and pulpy" (FAO, 2009). The scientific definition of a fruit is "The structure that develops from the ovary of an angiosperm as the seeds mature with (false fruit) or without (true fruits) associated structures" (Walker, 1988). A vegetable is a plant cultivated for food especially an edible herb or root used for human consumption.Vegetables it tend to be less sweet than fruits and often require some form of processing to increase their edibility.

Fermenting fruits and vegetables is an ancient tradition which has been prevalent for centuries. It was probably the oldest form of food preservation long before refrigeration became an option. Wine, bread and cheese are probably the oldest examples of fermented foods which have been consumed all over the world for thousands of years (FAO, 2009). It is difficult to pin-point the exact beginning and development of this technology. Anthropologists suggested that probably fruits were the first fermented food consumed (Dirar, 1993). Fermented fruits and vegetables contribute significantly to the diets of rural populations in a number of developing countries (Walker, 1988). Agricultural crops are processed for many different reasons, ranging from removal of antinutritional components and increasing the storage life of the final products to adding values and to increase both employment and income generating opportunities (Ogunshe *et al*., 2006). Fermented vegetables are made with lactic acid bacteria, which is a valuable technique humans have been using for thousands of years. This preservation method has numerous health advantages. Fermented vegetables are rich in nutrients, fiber and digestion –enhancing enzymes (O'Brien,

2003). It was discovered that the fermented vegetables kept longer and also developed other characteristics that enhanced their culinary characteristics. In Korea and other Asian countries, a pungent condiment called *'kimchi'*which is eaten in small amount with many meals, is composed largely of fermented cabbage with some other vegetables as well (FAO, 2009). The Chinese have been fermenting vegetables for thousands of years. It was also used in ancient Rome and medieval Europe (O'Brien, 2003). A wide variety of fermented vegetables are produced in many tropical countries. The raw materials use for their manufacture such as cucumber, olives, onions, pepper, pawpaw, pumpkin are abundantly grown, lactic acid bacteria are naturally indigenous in these vegetables (Ihekoronye and Ngoddy, 1985). In Thailand, fermented cabbage similar to sauerkraut is called *tang-chai* or *kong-chai*. The Philippine *achura* is prepared from grated green pawpaw that has been mixed with salt, red pepper, onions and ginger, and allowed to ferment in wooden barrel. Several fruits such as pineapple, banana, cashew, and mangoes are now being used in many tropical countries in the manufacture of wine and vinegar (Ihekoronye and Ngoddy, 1985).

2.1.1 Examples of some fermented fruits and vegetables

Many varieties of fermented foods are popular around the world, depending upon the fruits and vegetables available in that particular region. Fermented drinks like wine and beer were produced more than 5000 years ago in regions like Babylon, Egypt, Mexico and India (O'Brien, 2003).

- China is considered to be the birthplace of fermented vegetables, using the *Rhizopus* species of moulds to ferment soya beans for '*tempeh*' production.
- Indonesia makes '*tempeh-bongrek'* made by fermenting the pressed cake of coconut and peanuts which remain after oil extraction.
- Nepal makes '*gundruk*', which is a fermented vegetable dish.
- Pineapple peels are fermented into vinegar in South America.

- In America, there is sauerkraut, olives and cucumber.
- India relishes it's *'idlis'* made by fermenting pulses and cereals (FAO, 2009).

2.2 Legume seeds

Grain legumes of the family Leguminosae have been utilized as important food crops and food sources for several thousand years. Grain legumes are the most important legume plants which are cultivated for their seeds (Shi, 2008). Recently more and more nutritionists have paid attention to the biodiversity of legume seeds and their products. Legume seeds have a positive effect on human health because they can be used to treat some disease like cardiovascular disease, diabetes, overweight and obesity (Shi, 2008). Dietary fats play a very important role in human lipid metabolism and health. Diets should be of low fat for health purposes, legume seeds contain low total oil content in which there are low levels of saturated fatty and high content of unsaturated fatty acids (Hymowitze, 1990). Isoflavones are found in most legume seeds in high concentrations, and many studies have confirmed that isoflavones are involved in cancer prevention and body weight control (Duranti, 2006). High protein content is another positive factor of legume seeds. In India legume seeds are also known as "poor man's meat" (Duranti, 2006). In addition, some researchers found that some specific sub units of proteins from legume seeds, such as 7S globulin of soy protein are beneficial in reducing serum cholesterol (Shi, 2008). High fibre content is the fourth positive factor of legume seeds associated with the prevention of bowel cancer and body weight control (Duranti, 2006). Legume seeds as main human food sources provide humans with high quality protein. In addition legume seeds contain other nutrients such as minerals and carbohydrates, which are also beneficial to our health (Smart, 1993). Beans such as soya beans, locust beans and peas are all legume seeds. Every year, total values of legume seeds can be up to two hundred billion dollars in the whole world (Hymowitze, 1990). Legume seeds are divided

into two groups; pulses and non pulses (Table 1). Yielding from 1-12 grains or seeds of variable size, shape and colour within a pod. According to the definination of Food and Agricultural Organizations (FAO) of the United Nations (Duranti, 2006).

2.2.1: Legumes (African locust bean) boost immunity

African locust bean popularly used in seasoning soup has shown promise in boosting cellular immunity in immune – compromised persons as well as in management of diarrhoea, diabetes, and heart attack. Studies showed the aqueous extract of *Parkia biglobosa* leaves induced an increase in both the count of total lymphocytes and TCD4+ in blood. Thus doses of 75 and 100mg/kg Body/Weight (BW) mobilizing more of total lymphocytes and TCD4+ in peripheral TCD4+ blood, and over a period of six days. It would appear that, the leaves of this plant contains immunostimulatory activity molecule. (Muanya, 2011). The bark is used as a mouth wash, vapour inhalant for toothache, or for ear complaints. It is macerated in baths for leprosy and uses for bronchitis, pneumonia, skin infections, sores, washes for fever, diarrhoea and sterility. Roots are used in a lotion for eye sore (Muanya, 2011).

Table 1: Lists of pulses (Duranti, 2006).

Main edible legume seeds (grains/legumes)	**Latin name**
1. Dry beans	
Kidney bean, haricot bean,pinto bean,navy bean.	*Phaseolus vulgaris*
Lima bean, butter bean	*Vigna lunatus*
Adzuki bean	*Vigna angularis*
Mung bean,golden gram,green gram	*Vigna radiate*
Black gram,urd	*Vigna mungo*
Scarlet runner bean	*Phaseolus coccineus*
Rice bean	*Vigna umbellate*
Moth bean	*Vigna acontifolia*
Tepary beans	*Phaseolus acutifolius*
2. Dry broad beans (*Vicia faba*)	
Horse bean	*Vicia faba*
Broad bean	*Vicia faba*
Field bean	*Vicia faba*
3. Dry peas (Pisum spp)	
Garden pea	*Pisum sativum var.sativum*
Protein pea	*Pisum sativum var.arvense*
4. Chickpea	*Cicer arietinum*
5. Dry cowpea, black eye,pea blackeye bean	*Vigna unguiculata spp*
6. Pigeon pea,cajan pea,congo pea	*Cajanus cajan*
7. Lentil	*Lens culinaris*
8. Bambara groundnut,earth pea	*Vigna subterranean*
9. Vetch,common vetch	*Vicia sativa*

10. Lupins	*Lupinus spp*
11. Minor pulses	
Lablab,hyacinth bean	*Lablab purpureus*
Jack bean,sword bean	*Canvalia ensiformis*
Winged bean	*Psophocarpus teragonolobus*
Velvet bean cowitch	*Mucuna pruiens var.utilis*
Yam bean	*Pachyrrizus erosus*
Non-pulse (oil-crops)	
Soyabean	*Glycin max*
Peanut	*Arachis hypogaea*

2.2.2: Positive factors of legume seeds on human health

Low lipid intake is important for good health. Total oil content of legume seeds was lowest compared with that of seeds and grains (Ryne *et al*., 2007). The total oil content of other parts of legumes was less than 1.6g/100g (Table 2). The levels of saturated fatty acids were also very low in all types of legumes moreover and there were high levels of unsaturated fatty acid (>70% of total oil). The content of unsaturated acid in chickpeas was up to 4.3g/100g. Low saturated fatty acids have been identified as an important standard for evaluation of the quality of food. Legume seeds contain low levels of total oil and saturated fatty acids as well as high content of unsaturated fatty acids. Therefore increase in intake of legumes can be beneficial to human health (Ryan *et al.*, 2007).

Table 2 Total oil content (g/100g) and saturated fatty acid composition (%of total) of various seed grains and legumes (Ryan *et al.*, 2007).

Samples	Total oil (g/100g)	saturated fatty acid (S.F.A) (% of total)
Linseed	29.3	12.5
Mustard	15.2	4.9
Poppy	39.5	13.7
Pumpkin	42.3	22.7
Sesame	40.5	15.9
Barley	1.3	22.5
Buckwheat	2.7	21.9
Maize	1.6	15.4
Millet	4.0	10.8
Quinoa	6.3	11.2
Rye	1.3	16.4
Spelt	2.0	17.2
Butter bean	0.9	28.7
Chick pea	5.0	13.7
Kidney bean	1.2	16.7
Lentils	1.4	16.7
Peas	1.5	14.7

2.2.3: Legume seeds: a good source of protein

Protein is the main component of human tissue, most of which is derived from dietary meat (Polhil and Raven, 1981). The Food and Drug Administration (FDA) of United of states suggested intake >6.5g/d of soybeans protein, this could reduce the risk of cardiovascular disease and intake of 25g/d was beneficial to human health (Shi, 2008). Soybean protein is beneficial for control of body weight which is capable of affecting appetite resulting in reduction of food intake (Duranti, 2006).

2.2.4: Dietary fiber

Legume seeds contain high levels of dietary fibre which consist of the indigestible materials of plant cell walls. Fibre which plays an important role in the human digestive system (Tharanathan and Mahadevamma, 2003). Fibre is a combination of various substrates, such as cellulose, hemi-cellulose, pectins, gums, mucilage, resistant starch and other polysaccharides and lignin. There are two groups of dietary fibers; insoluble dietary fibre (IDF) and soluble dietary fiber (SDF) (Tharanathan and Mahadevamma, 2003). Plant cell wall is the main source of IDF and this consists of the complicated mixture of cellulose and non celluloses. It is confirmed that IDF can prevent bowel cancer because it increases faecal bulk therefore diluting faecal content by decreasing interaction between the intestinal mucosa and any other carcinogens (Shi, 2008). Pectins, gums, mucillages and some hemicelluloses fragments are soluble in water and are identifiable as soluble dietary fiber (SDF). This (SDF) is able to delay gastric emptying and is beneficial in reducing the risk of insulin resistance (Witcomb and Erskine, 1984).

2.3 Soya beans

Soya beans vary in growth and habit and the height of the plant varies from below 20 cm (7.9 in) to 2 meters (6.6 ft). The pods, stems, and leaves are covered with fine brown or gray hairs. The leaves are trifoliate, having 3 to 4 leaflets per leaf, and the leaflets are 6–15 cm (2.4–5.9 in) long and 2–7 cm (0.79–2.8 in) broad. The leaves fall before the seeds are mature. The inconspicuous, self-fertile flowers are borne in the axial of the leaf and are white, pink or purple (Riaz, 2007).The fruit is a hairy pod that grows in clusters of 3–5. Each pod is 3–8 cm long (1–3 inches) and usually contains 2–4 (rarely more) seeds 5–11 mm in diameter (Riaz,2007).

Soyabeans occur in various sizes, and in many hull or seed coat colors, including black, brown, blue, yellow, green and mottled. The hull of the mature bean is hard, water resistant, and protects the cotyledon and hypocotyls (or "germ") from damage. If the seed coat is cracked, the seed will not germinate. The scar, visible on the seed coat, is called the helium (colors include black, brown, buff, gray and yellow) and at one end of the helium is the micropyle, or small opening in the seed coat which can allow the absorption of water for sprouting (Henkel,2000).

Remarkably, seeds such as soybeans containing very high levels of protein can undergo desiccation and yet survive and revive after water absorption (Sinclair, 2009).

2.3.1 Chemical composition of soya bean seed

Soybean, mature seeds, raw nutritional value per 100 g (3.5 oz)

Energy	1,866 kJ (446 kcal)
Carbohydrates	30.16 g
Sugars	7.33 g
Dietary fiber	9.3 g
Fat	19.94 g
Saturated	2.884 g
Monounsaturated	4.404 g
Polyunsaturated	11.255 g
Protein	36.49 g
Tryptophan	0.591 g
Threonine	1.766 g
Isoleucine	1.971 g
Leucine	3.309 g
Lysine	2.706 g
Methionine	0.547 g
Phenylalanine	2.122 g
Tyrosine	1.539 g
Valine	2.029 g
Arginine	3.153 g
Histidine	1.097 g
Alanine	1.915 g
Aspartic acid	5.112 g
Glutamic acid	7.874 g

Glycine	1.880 g
Proline	2.379 g
Serine	2.357 g
Water	8.54 g
Vitamin A equiv.	1 μg (0%)
Vitamin B6	0.377 mg (29%)
Vitamin B12	0 μg (0%)
Vitamin C	6.0 mg (10%)
Vitamin K	47 μg (45%)
Calcium	277 mg (28%)
Iron	15.70 mg (126%)
Magnesium	280 mg (76%)
Phosphorus	704 mg (101%)
Potassium	1797 mg (38%)
Sodium	2 mg (0%)
Zinc	4.89 mg (49%)

Percentages are relative to US recommendations for adults.

Source: UN Food & Agricultural Organization (FAO, 2009)

Together, oil and protein account for about 60% of dry soybeans by weight; protein 40% and oil 20%. The remainder consists of 35% carbohydrate and about 5% ash. Soybean cultivars have approximately 8% seed coat or hull, 90% cotyledons and 2% hypocotyl axis or germ (Schwarch, 2004).

The principal soluble carbohydrates of mature soybeans are the disaccharide sucrose (range 2.5–8.2%), the trisaccharide raffinose (0.1–1.0%) composed of one sucrose molecule

connected to one molecule of galactose, and the tetrasaccharide stachyose (1.4 to 4.1%) composed of one sucrose connected to two molecules of galactose. (Hunter, 2009).

Since soluble soya carbohydrates are found in the whey and are broken down during fermentation, soy concentrate, soy protein isolates, tofu, soy sauce, and sprouted soy beans are without flatus activity. (Symolon *et al*., 2004).

The insoluble carbohydrates in soybeans consist of the complex polysaccharides cellulose, hemicelluloses, and pectin. The majority of soybean carbohydrates can be classified as belonging to dietary fiber.

2.3.2 Nutrition

Soybeans are considered by many agencies to be a source of complete protein. A complete protein is one that contains significant amounts of all the essential amino acids that must be provided to the human body because of the body's inability to synthesize them. For this reason, soy is a good source of protein, amongst many others, for vegetarians and vegans or for people who want to reduce the amount of meat they eat (Sneller, 2003). According to the US Food and Drug Administration, soya protein products can be good substitutes for animal products because, unlike some other beans, soy offers a 'complete' protein profile. Soya protein products can replace animal-based foods—which also have complete proteins but tend to contain more fat, especially saturated fat—without requiring major adjustments elsewhere in the diet (Lang, 2006).

The gold standard for measuring protein quality, since 1990, is the Protein Digestibility Corrected Amino Acid Score (PDCAAS) and by this criterion soy protein is the nutritional equivalent of meat, eggs, and casein for human growth and health. Soyabean protein isolate has a biological value of 74, whole soybeans 96, soybean milk 91, and eggs 97(Thompson *et al.,* 2006).

Soya protein is essentially identical to that of other legume seeds; moreover, soybeans can produce at least twice as much protein per acre than any other major vegetable or grain crop, five to ten times more protein per acre than land set aside for grazing animals to make milk, and up to 15 times more protein per acre than land set aside for meat production (Hunter, 2009).

2.3.3 Cultivation

Soyabean output in 2005 :Top Soybean Producers in 2005(million metric tons)

United States	87.7
Brazil	52.4
Argentina	40.4
China	15.5
India	8.3
Paraguay	3.8
Canada	3.5
Bolivia	1.4
European Union	1.2
World Total	221.5

Source: UN Food & Agriculture Organization (FAO, 2009).

Soyabean is an important global crop, providing oil and protein. In the United States, the bulk of the crop is solvent-extracted with hexane, and the "toasted" defatted soy meal (50% protein) then makes possible the raising of farm animals (e.g. chicken, hog, turkey) on an industrial scale never before seen in human history. A very small proportion of the crop is consumed directly by humans. However soyabean products do appear in a large variety of processed foods (Messina, 2010).

During World War II, soybeans became important in both North America and Europe chiefly as substitutes for other protein foods and as a source of edible oil. It was during World War II that the soybean was discovered as fertilizer by the United States Department of Agriculture. In the 1960s, the General Agreement on Tariffs and Trade (GATT), the United States secured tariff-free access for its soyabeans to the European market. In the 1960s the United States exported over 90% of the world's soyabeans. In 2005, top soybean exporters are Brazil (39% of exports), United States (37%) and Argentina (16%), while top importers are China (41% of world imports), European Union (22%), Japan (6%) and Mexico (6%) (Thompson *et al.,* 2006). Cultivation is successful in climates with hot summers, with optimum growing conditions (in mean temperatures) of 20 to 30 °C (68 to 86 °F); temperatures below 20 °C and over 40 °C (68 °F, 104 °F) retard growth significantly (Sing *et al*,2006). They can grow in a wide range of soils, with optimum growth in moist alluvial soils with a good organic content. Soya beans, like most legumes, perform nitrogen fixation by establishing a symbiotic relationship with the bacterium *Bradyrhizobium japonicum* (syn. *Rhizobium japonicum)* (Sing *et al*,2006).However, for best results an inoculum of the correct strain of bacteria should be mixed with the soya bean (or any legume) seed before planting. Modern crop cultivars generally reach a height of around 1 m (3.3 ft), and take 80–120 days from sowing to harvesting (Crawford, 2006). Soybeans are native to East Asia but only 45 percent of soybean production is located there. The other 55 percent of production is in America. The U.S. produced 75 million tons of soybeans in 2000, of which more than one-third was exported. Other leading producers are Brazil, Argentina, Paraguay, China, and India (Minello *et al.,* 2006).

2.3.4 Classification

The word *Glycine* is derived from the Greek - glykys (sweet) and likely refers to the sweetness of the pear-shaped (apios in Greek) edible tubers produced by the native North

American twining or climbing herbaceous legume, *Glycine apios*, now known as *Apios americana*. The cultivated soybean first appeared in Species Plantarum, by Linnaeus, under the name *Phaseolus max L*. The combination *Glycine max* (L.) Merr., as proposed by Merrill in 1917, has become the valid name for this useful plant (Hartman,2009). The genus *Glycine* wild is divided into two subgenera, *Glycine* and *Soja.* The subgenus *Soja (Moench*) includes the cultivated soybean, *Glycine max* (L.) Merr., and the wild soybean, *Glycine soja Sieb*. & *Zucc*. Both species are annual. *Glycine soja* is the wild ancestor of *Glycine max* and grows wild in China, Japan, Korea, Taiwan and Russia. The subgenus *Glycine* consists of at least 16 wild perennial species: for example, *Glycine canescens F.J. Herm*. and *G. tomentella Hayata*, both found in Australia and Papua New Guinea (Hartman,2009). Like some other crops of long domestication, the relationship of the modern soybean to wild-growing species can no longer be traced with any degree of certainty. It is a cultural variety with a very large number of cultivars (Henkel, 2000).The soybeans (U.S.) or soya bean (UK) (*Glycine max)* is a species of legume native to East Asia. The plant is classified as an oilseed rather than a pulse. It is an annual plant that has been used in China for 5,000 years to primarily add nitrogen into the soil as part of crop rotation (Riaz, 2007).

2.3.5 Uses

The use of soya bean at the household level has been limited to many countries in Southeast Asia, where about 77g of soya beans are consumed per capita per day. In addition, both traditional and new soya bean products are marketed in Bangledash, Malaysia, India and other countries of the Far East (Ihekoronye and Ngoddy, 1985). Soya beans are first cooked and fermented or processed by various forms of moist heating. Cooking improves texture and palatability and it is required to improve nutritional quality.

Soya beans can be broadly classified as "vegetable" (garden) or field (oil) types. The vegetable variety types cook more easily, have a mild nutty flavor, better texture, and are

larger in size, higher in protein, and lower in oil than field types (Messina,2010). *Tofu* and soy milk producers prefer the higher protein cultivars bred from vegetable soybeans originally brought to the United States in the late 1930s. The "garden" cultivars are generally not suitable for mechanical combine harvesting because there is a tendency for the pods to shatter upon reaching maturity. Among legumes, the soyabean is also classified as an oilseed, and it is pre-eminent for its high (38–45%) protein content as well as its high (20%) oil content. Soybeans are the second most valuable agricultural export in the United States behind corn (Strom *et al*., 2001). The bulk of the soybean crop is grown for oil production, with the high-protein defatted and "toasted" soy meal used as livestock feed. A smaller percentage of soybeans are used directly for human consumption (Henkel, 2008). Immature soybeans may be boiled whole in their green pod and served with salt, under the Japanese name *edamame.* In China, Japan, and Korea, the bean and products made from it are popular part of the diet. The Chinese invented *tofu*, and also made use of several varieties of soybean paste as seasonings. Japanese foods made from soya include *miso* , *natto*, *kinako* and *edamame* (Henkel, 2008). In Korean cuisine, soybean traditional nonfermented food uses of soybeans include soymilk, and from the latter *tofu* and *tofu* skin or *yuba.* Fermented foods include *shoyu* or soy sauce, *miso, natto,* and *tempeh*, among others. The oil is used in many industrial applications. The main producers of soya are the United States (32%), Brazil (28%), Argentina (21%), China (7%) and India (4%) (Lang, 2006). The beans contain significant amounts of phytic acid, alpha-linolenic acid, and the isoflavones genistein and daidzein (Sing *et al.,* 2006). Soybeans can produce at least twice as much protein per acre than any other major vegetable or grain crop, 5 to 10 times more protein per acre as land set aside for grazing animals to make milk, and up to 15 times more protein per acre than land set aside for meat production(Sinclair,2009). Soya bean sprouts, called *kongnamul*, are also

used in a variety of dishes, and are also the base ingredient in *doenjang*, *cheonggukjang* and *ganjang*. In Vietnam, soya bean is used to make soybean paste (Valsta *et al.,* 2003).

Soya beans are also the primary ingredient involved in the production of soy sauce (or *shoyu)*. Other uses include:

- **Oil**

Among the legumes groundnuts and soya beans have exceptionally high oil content, 45% and 20% respectively of the edible portion of the seeds (Messina, 2010). To extract soybean oil (from the seeds) are cracked, adjusted for moisture content, rolled into flakes and solvent-extracted with commercial hexane. The oil is then refined, blended for different applications, and sometimes hydrogenated. Soybean oils, both liquid and partially hydrogenated, are exported and sold as "vegetable oil," or used in a wide variety of processed foods. The remaining soybean meal is used mainly as animal feed (Heald *et al*., 2007).

- **Meal**

Soybean meal is the material remaining after solvent extraction of oil from soybean flakes, with 50% soy protein content. The meal is 'toasted' (a misnomer because the heat treatment is due with moist steam) and ground in a hammer mill. Soybean meal is an essential element of the American production method of growing farm animals such as poultry and swine on an industrial scale that began in the 1930s; and more recently the aquaculture of catfish. Ninety-eight percent of the U.S. soybean crop is used for livestock feed. Soybean meal is also used in lower end dog foods (Gottstein *et al*., 2003).

- **Flour**

Soy flour refers to defatted soybeans ground finely enough to pass through a 100-mesh or smaller screen with special care taken during solvent removal in order to minimize denaturation of the protein to retain a high Protein Dispersibility Index (PDI), for uses such as

extruder cooking of textured vegetable protein. It is the starting material for production of soy concentrate and soy protein isolates (Gottstein *et al*., 2003).

Infant formula

In Nigeria, a soya bean extract which has come as a result of Research and Development efforts at the Federal Institute of Industrial Research Oshodi is the Soy- Ogi. It is a protein formulation designed for use as weaning food for infants and malnourished children. The formulation involves the wet milling of steeped corn and soya beans into slurry which is allowed to ferment. The fermented corn- soy mashes are fortified with minerals and vitamins, pasteurized and then spray dried (Ihekoronye and Ngoddy, 1985).

Soy-based infant formula (SBIF) is also used for infants who are allergic to pasteurized cow milk proteins (Heald *et al*., 2007). It is sold in powdered, ready-to-eat, and concentrated liquid forms. Some reviews have expressed the opinion that more research is needed to determine what effect the phytoestrogens in soybeans may have on infants. Diverse studies have concluded that there are no adverse effects on human growth, development, or reproduction as a result of the consumption of soy-based infant formula (Messina, 2010). SBIFs provide complete nutrition that adequately supports normal infant growth and development. FDA has accepted SBIFs as safe for use as the sole source of nutrition (Heald *et al*., 2007)

2.3.6 Meat and dairy substitutes and extenders

Soybeans can be processed to produce a texture and appearance similar to many other foods. For example, soybeans are the primary ingredient in many dairy product substitutes (e.g., soy milk, margarine, soy ice cream, soy yogurt, soy cheese, and soy cream cheese) and meat substitutes (e.g. veggie burgers) (Fernandez-Cornejo and Caswell,2006). These substitutes are readily available in most supermarkets. Soy milk does not naturally contain significant amounts of digestible calcium. Many manufacturers of soy milk sell calcium-enriched

products as well. Soya is also used in *tempeh*: the beans (sometimes mixed with grain) are fermented into a solid cake.

Soya products also are used as a low cost substitute in meat and poultry products. Food service, retail and institutional (primarily school lunch and correctional) facilities regularly use such "extended" products. Extension may result in diminished flavor. Vitamin and mineral fortification can be used to make soya products nutritionally equivalent to animal protein. The soy-based meat substitute textured vegetable protein has been used for more than 50 years as a way of inexpensively extending ground beef without reducing its nutritional value (Sasamura *et al*.,2004).

2.3.7 Other products

Soybeans are the beans used in Chinese fermented black beans, *douchi*, not the sometimes confused black turtle beans. Soya beans are also used in industrial products including oils, soap, cosmetics, resins, plastics, inks, crayons, solvents, and clothing. Soybean oil is the primary source of biodiesel in the United States, accounting for 80% of domestic biodiesel production (Sneller, 2003). Soybeans have also been used since 2001 as fermenting stock in the manufacture of a brand of vodka (Schwarz, 2004).

2.3.8 Cattle feed

Cattle are often fed with soy rich meals. Spring grasses are rich in Omega-3 fatty acids whereas soy is predominantly Omega-6 (Riaz, 2007).

2.3.9 Health benefits

Omega-3 fatty acids, for example, alpha-linolenic acid C18-3, all cis, 9,12,15 octadecatrienoic acid (where the omega-3 refers to carbon number 3 counting from the hydrocarbon tail whereas C-15 refers to carbon number 15 counting from the carboxyl acid head) are special fat components that benefit many body functions (Sinclair,2009). However, the effects which are beneficial to health are associated mainly with the longer-chain, more

unsaturated fatty acids eicosapentaenoic acid (20:5n-3, EPA) and docosahexaenoic acid (22:6n-3, DHA) found in fish oil and oily fish. For instance, EPA and DHA, inhibit blood clotting, while there is no evidence that alpha-linolenic acid (18:3n−3, aLNA) can do this (Giampietro *et al.,* 2004). Soybean oil does contain significantly greater amount of omega-6 fatty acids in the oil: 100 g of soybean oil contains 7 g of omega-3 fatty acids to 51 g of omega-6: a ratio of 1:7. Flaxseed, in comparison, has an omega-3: omega-6 ratio of 3:1 (Crawford and Lee, 2003).

- **Isoflavones**

Soybeans also contain the isoflavones, genistein and daidzein, types of phytoestrogen, that are considered by some dietitians and physicians to be useful in the prevention of cancer and by others to be carcinogenic and endocrine disruptive (Crawford, 2006). Soy's content of isoflavones are as much as 3 mg/g dry weigh (Messina *et al* 2010). Isoflavones are polyphenol compounds, produced primarily by beans and other legumes, including peanuts and chickpeas. Isoflavones are closely related to the antioxidant flavonoids found in other plants, vegetables and flowers. Isoflavones such as genistein and daidzein are found in only some plant families, because most plants do not have an enzyme, chalcone isomerase which converts a flavone precursor into an isoflavone (Messina *et al* 2010).

- **Cholesterol reduction**

The dramatic increase in soya food sales is largely credited to the Food and Drug Administration's (FDA) approval of soy as an official cholesterol-lowering food, along with other heart and health benefits (Brachfield and Choate,2007). A study of Indonesian elderly found that *tofu* intake was associated with worse memory, but *tempeh* (a fermented soy product) intake was associated with better memory. This study replicated other studies. From 1992 to 2003, sales have experienced a 15% compound annual growth rate, increasing from $300 million to $3.9 billion over 11 years, as new soy food categories have been introduced.

Soya foods have been repositioned in the market place, thanks to a better emphasis on marketing nutrition (Crawford and Lee,2003).

- **Phytic acid**

Soybeans contain a high level of phytic acid, which has many effects including acting as an antioxidant and a chelating agent (Fargione *et al*., 2008). The beneficial claims for phytic acid include reducing cancer, minimizing diabetes, and reducing inflammation (Hunter, 2000).

2.4: Fermentation

Fermentation is a biochemical change which is brought about by the anaerobic oxidation of carbohydrates by either microorganisms or by enzymes. This is distinct from putrefaction which is the degradation of protein materials (FAO, 2009). The changes caused by fermentation can be both advantageous and disadvantageous. Fermentation initiated by the action of microorganisms occurs naturally and is often part of the process of decay especially in fruits and vegetables**.** However fermentation can be controlled to give beneficial results (FAO, 2009). Fermentation is relatively low energy preservation and a process which leads to increase in shelf life of fermented products which decreases the need for refrigeration or other forms of food technology. It is therefore a highly appropriate technique for use in developing countries and remote areas where access to sophisticated equipment is limited.

Food fermentation is known to serve five main purposes:

- Enrichment of the diet through development of a diversity of flavours, aromas and textures in foods.
- Preservation of substantial amounts of food through lactic acid alcohol, acetic acid and alkaline fermentation.

- Biological enrichment of food substrates with protein, essential amino acids, essential fatty acids and vitamins.
- Elimination of antinutrients.
- A decrease in cooking times and fuel requirements (Schutyser, 2003).

Fermenting vegetables and fruits has brought many benefits to people in developing countries. Fermented foods play an important role in providing food security, enhancing livelihoods and improving the nutrition and social well being of millions of people around the world particularly the marginalized and vulnerable.

2.4.1 Food fermentation: an ancient tradition

Fermentation is one of the oldest forms of food preservation technologies in the world. Traditional fermented foods such as bread, wine and cheese have been prepared and consumed for thousands of years and are strongly linked to culture and tradition, especially in rural households and village communities (Steinkraus, 1992). The development of fermentation technologies was lost in the midst of history. Anthropologists have suggested that it was the production of alcohol that motivated primitive people to settle down and became agriculturalist. Some even think that the consumption of fermented food is prehuman (Stanton, 1985). The first fermented food consumed was probably fermented fruits. Hunter gatherers would have consumed fresh fruits but at times of scarcity they would consume rotten and fermented fruits. Fermented drinks were being consumed over 7000 years ago in Babylon (now Iraq), 5000 years ago in Egypt, 4000years ago in Mexico and 3,500 years ago in Sudan (Dirar, 1993). Bread making probably originated in Egypt over 3,500 years ago (Sugihara, 1985). Several traditional loaves of bread have been found in ancient tombs. Fermentation of milk started in many places with evidence of fermented products in Babylon over 500years ago (Dirar, 1993). There is also evidence of fermented meat products being produced for king

Nebuchadnezer of Babylon. China is thought to be the place of fermented vegetables and the use of *Aspergillus* and *Rhizopus* moulds to make bread (Yokotsuka, 1985). Knowledge about traditional fermentation technologies has been handed down from parent to child for centuries. These fermented products have been adapted over generations. Some products and practices no doubt fell by the wayside. Those that remain today have not only survived the test of time but also more importantly are appropriate to the technical, social and economic conditions of the regions (FAO, 2009).

2.4.2 Improving food security

Eight hundred million people do not have enough food to eat; if we include those not free from hunger the figure will rise to 1.2 billion people. This is one fifth of the world's population. A further two billion are deficient in micro- nutrients (Anon, 1996). In the seventies, food security was viewed mainly in terms of food supply at the national and global levels. Since there is a major shift in understanding food security with more emphasis on access to food rather than purely on production (FAO, 2009). The Food and Agriculture Organization of the United Nations (FAO) among other influential organizations has recognized that the problems of food security cannot be isolated and that it is an integral component of other development issues. FAO highlights the fact that the world food insecurity problem is a result of undemocratic and inequitable distribution and access to resources rather than a problem of global food production (Anon, 1995).

2.4.3 Food preservation

The preservation of foods by fermentation processes are dependent upon the production, by certain microorganisms, of physical and chemical changes which alter the appearance, body and flavour of the original material. The changes may improve the nutrition of the product and they are generally inhibitory to the growth of undesirable microorganisms (Ihekoronye and Ngoddy, 1985). The microorganisms that ferment foods to produce

desirable changes can be distinguished from those that are responsible for food spoilage and food borne diseases. Of the food fermenting organisms the lactic acid producing bacteria, acetic acid producing bacteria, and some alcohol producing yeasts are most important. Certain mould species also play important role in some food fermentation (Ihekoronye and Ngoddy, 1885).

Fermentation is a cheap and energy efficient means of preserving perishable raw materials (Adams, 1997). There are several options for preserving fresh fruits and vegetables, which includes drying, freezing, canning and pickling. However, many of these methods of preservation used are inappropriate for use on small scale especially in developing countries (Ranken *et al.*, 1997). For instance the canning of vegetables at small level has serious food safety implications and contamination with botulism is a possibility (Fleet, 1998). Freezing of fruits and vegetables is not economically viable at a small-scale level. Fermentation requires very little sophisticated equipments both for the process itself and for subsequent storage of the fermented products (FAO, 2009). Fermentation is a technique that has been employed for generations to preserve food for consumption at a later date and also to improve food security (Achi, 2005).

2.4.4: Removal of antinutritional factors

Many fruits and vegetables contain a lot of naturally occurring toxins and anti-nutritional compounds. These can be removed or detoxified by the action of microorganisms during fermentation of these products (Ogunshe *et al,* 2006). For instance, the fermentation process that produces the Sudanese product *kawal* removes the toxins from the leaves of *Cassia obtusifolia* (Motarjemn *et al,*1997). Again fermentation is the process that ensures that cassava is safe for consumption. Cassava contains a naturally occurring chemical: cyanogenic glycoside linamarin. When eaten raw or

improperly processed, this substance releases cyanide into the body which can be fatal, correct processing through fermentation removes this chemical (Stienkraus, 1992).

2.4.5:Improving nutrition

The optimum health and nutrition of an individual is dependent upon a regular supply of food and a balanced diet. When diets are sub optimal, the individual's capacity to work and some achievements are greatly reduced (FAO, 2009). Fermentation processes can result in increased levels of vitamins in the final products (Achi, 2005). *Saccharomyces cerevisae* is able to concentrate large quantities of thiamine, nicotinic acid and biotin, and thus enriches products (Dirar, 1993).

2.4.6: Flavour enhancement

When microorganisms metabolize and grow, they release by-products. In food fermentations the byproducts play beneficial roles in preserving and changing texture of food and flavour (Dike and Odunfa, 2003). Improvement of flavour as one of the main reason for fermenting food was suggested by Gberikon *et al.,* (2007). They suggested that desirability of flavour is a matter of opinion and what one is particularly used to. Flavour changes during fermentation, but differences may exist as to whether the product is improved. It is generally viewed that food fermentation improves flavour and texture and also makes the food more enjoyable (Ogunshe *et al*, 2006). During fermentation, many flavour compounds are produced by lactic acid bacteria particularly *Streptococcus lactis* during fermentation of dairy products (Dike and Odunfa, 2003). Esters and aldehydes produced during gari fermentation are believed to impart flavour to the finished products. Glutamic acid produced in locust bean and other legume seed fermentations contributes to the flavour enhancing attributes of the products (Dike and Odunfa, 2003).

Over the years Sudanese women have developed products to replace meat in their diet. These include *'kawal'* (fermented wild legume leaves), *'sigda'* (fermented sesame

presscake), and '*furundu*'(fermented red sorrel seeds). The strong flavour of fermented food products can enhance quality of a dull diet. Fermented vegetable such as pickles, *gundruk* and sauerkraut are used as condiments to enhance the overall flavour of a meal. A small amount of pickle can make a starchy diet (like *dalh* and rice in Asia) much more appealing (Schutyser, 2003).

2.4.7: Digestibility

Microorganisms contain certain enzymes like cellulases, which are incapable of being synthesized by humans (Diawaral *et al*., 2003). Microbial cellulases hydrolyse cellulose into sugars which are then rapidly digestible by humans (Kovac, 1997). Similarly pectinases soften the texture of foods and liberate sugars for digestion. Fermented foods are often more digestible than unfermented foods (Schutyser, 2003). Lactic acid fermented weaning foods are traditionally produced in developing countries both to improve the safety of the food and to improve its digestibility (Kovac, 1997). Starchy porridges are commonly fed to weaning infants in developing countries. The consistencies of this gruel combined with the small capacity of the infants stomach, means that it is physically impossible for the child to consume adequate energy to meet its high demands. By acidifying the porridge through lactic acid fermentation, starch is hydrolyzed into shorter chains of glucose and dextrose, which reduce the viscosity of the porridge and increase its energy density. Thus the child is able to meet its energy requirement (FAO, 2009).

2.4.8: Medicinal benefits

There are many traditional beliefs about the medicinal properties of fermented food products. The Fur ethnic group in Sudan believes strongly that the consumption of fermented foods protects them from certain diseases (Dirar, 1992). '*Koumiiss'* (a fermented fruit sap) is considered to have medicinal properties in Mexico (Adams and Nicolaides,1997). Fermentation is a traditional method of reducing the microbial contamination of porridges in

Kenya (Watson *et al.,* 1996). A study has shown that children fed with fermented gruels had a 33% lower incidence of diarrhoea than those fed with unfermented gruels, owing to the inhibition of pathogenic bacteria by lactic acid forming bacteria (Svanberge, 1992).

2.4.9: Increasing income and employment

The production of fermented fruit and vegetable products provides employment to millions of people around the world (FAO, 2009). Food processing is probably the most important source of employment in Africa, Asia and Latin America. The Food and Agricultural Organization of the United Nations has that value added through marketing and processing raw products can be much greater than the value of primary production (Anon, 1995). For instance in sub-Saharan Africa more than 60% of the work force is employed in small food processing sector, and between one third and two thirds of value added manufacturing is based on agriculture materials (Conroy *et al*, 1995).

Fermented foods are popular throughout the world and production of fermented food products is important in many countries in providing income and employment. In Africa , fermented cassava products (like *gari* and *fufu*) are major component of the diets of more than 800 million people and in some parts of Africa they constitute over 50% of the diet (Oyewole,1992). In Asia the preparation of fermented foods is a wide spread tradition. *Kimchi* (a fermented cabbage product) is the major food product of Korea. Soy sauce (a fermented legume product) is economically important from Indonesia to Japan. Over a billion liters are produced each year in Japan alone. Over 200 million are produced each year in Korea and over 150 million liter in Taiwan. *Miso* (a fermented legume product) is also very important in Asia with over 560,000 tons is produced a year in Japan alone (Anon, 1995). In Latin America, fermented cereal products like alcoholic drinks and fermented milk products are three of the most important sectors of the economy (Oyewole, 1992).

2.5 Diversity of fermented foods

Many fermented foods are consumed around the world. Each nation has its own type of fermented foods representing the staple diet and raw ingredients available in that particular region (Achi, 2005). It is likely that the methods of producing many of the world's fermented foods are unknown and developed by chance (Holzapfel, 2002). Some fermented fruits and vegetables products are the alcoholic beverages such as beers and wines.

Table 3: Examples of fermented fruits and vegetable products from around the world.

Names and region	Type of product
Indian sub- continent	pickled fruit and vegetable
Acar,Achar,Tandal achar,	
Garam,nimbo,achar	
Gundruk	fermented dried vegetable
Lemon pickle,lime pickle,	
Mangoe pickle.	
South East Asia	
Asinan, Burong mangga,Dalok	
Jeruk,kiam-chai,kiam-cheyi	pickled fruit and vegetable
Kong-chai,Naw-mai-dong	
Siam-dong,paw-tsay,phak-dong,	
Phonlami-dong,sajurasin,sambal	
Tempo-jak,santol,Si-sek-chai,Sunki,	
Tang-chai, Tempoyak,vanilla	
Bai-ming, lappet-so,Miang	fermented tea leaves
Nata de coco, Nata de pina	fermented fruit juice.
East Asia	
Bossam-Kimchi,Chonggak-Kimchi	
Dan moogi,Dogchimi,Kachdoo kigactuki	fermented brine.
Kakduggi,kimchi,mootsanji,Muchung-kimchi,	
Oigee,oiji,oiso baegi.	

Africa

Fruit vinegar	vinegar
Hot pepper sauce	
Lamoun makbouse,mauoloh	prickled fruits and vegetable
seeds	
Msir,msalalla olive	
Oils seeds,ogili,ogiri,hibiscus seeds	
Wines	fermented fruits

Americans

Cucumber pickles, Dill pickles	pickled fruits and vegetables
Olives Sauerkraut	
Lupin seeds,oil seeds	pickled oil seeds
Vanilla wine	fermented fruits and vegetables.

Middle East

Kushuk	fermented fruits and vegetables
Lamoun makbouss,mekhalel,olives	pickled fruits and vegetables
Torshi,Tursu .	
Wines	fermented fruits

Europe	
Mushrooms,yeast olives, serruben	moulds,pickled fruits and vegetables.
grape vinegar,wine vinegar	vinegar
wines,citron	fermented fruits.

Source :(FAO, 2009)

2.5.1 Some specific fermented food products of the Tropics

1. Gari, a product obtained when cassava is fermented and fried; made into a pudding with hot water and eaten in Nigeria with vegetable soup, meat or fish. It can also be soaked with cold water and sugar, and be taken as snacks

2. Ogi, a fermented maize starch, made into gruel by stirring with hot water. It is taken with fried beans balls, plantain, meat or fish and constitutes a major breakfast food in Nigeria. It is also a weaning food for babies.

3. Soy-Ogi, a fermented weaning food developed at Federal Institute of Industrial Research Oshodi, Nigeria, from a mixture of maize and soya beans.

4. Wara, a cheese-like product obtained by fermenting milk from either goat or cow. It is usually eaten in Northern part of Nigeria.

5. Fufu, cooked fermented cassava dough eaten with vegetable soup

6. Teff, an Ethiopian fermented maize gruel similar to ogi.

7. Doda, a pancake-like fermented batter of rice and black grain or millet flour consumed in India.

8. Kisra, fermented sorghum bread from Sudan

9. Chokpolo, fermented sorghum drink.

10. Burukutu, a traditional Nigerian beer made from malted millet, guinea corn and corn.

11. Otika, a traditional Nigerian alcoholic beverage from sorghum.

12. Palmwine, a milky alcoholic beverage produced from the inflorescence of raffia or palm tree.

13. Fish sauces, these differ with countries, raw materials used and processes involved differ. The Malaysians extract sauce from anchovies called *audu.* The fermented mysis sauce is called *chenchalok.* In Thailand, it is called *nam pla*. In the Philippines, fermented fish sauce called *patis* is prepared from sardines, anchovies, or shrimps. Apart from fish

sauces, different kinds of fermented fish pastes are produced in these countries. Example is *prahoc* from Cambodia, *bagoong* from Philippines, *trassi-ikan* and *trassi-udang* from Indonesia.

14. Tempeh, a popular food in Indonesia and Malaysia made by fermenting cooked and dehulled soya bean.

15. Soy sauce, represents one of the largest uses of soya beans in the Orient, used as a condiment. It is a dark brown liquid, with a pleasant aroma produced by fermentation of soya

16. Miso, a fermented food product prepared in Taiwan, Philippines, Indonesia, and countries of the Orient. It is essentially a fermented blend of rice, soya beans and sometimes, barley and malt.

17. Sufu, soya bean cheese prepared by mould fermentation of cakes of finely ground precipitated soya beans. *Tahuri,tahuli* and *tokwa* are Philippine names for similar products.

18. Angkak, a coloured fermented rice product used extensively in the Philippines for colouring fermented fish. (Source: Ihekoronye and Ngoddy, 1985).

2.5.2 Fermented vegetable protein foods-Oriental and Africa

Fermentation remains an effective, inexpensive method for extending the shelf life of foods and increasing their nutritional content through probiotic functions. Therefore, fermentation remains a viable practice for developing countries and rural communities with limited facilities (Tamang, 2009). Most fermented vegetable proteins of the world have their origin in African and Asia. Those commonly used in Africa include, among others, condiments and seasoning agents of soups (Ogbadu, 1988). In Asia, the seeds of the popular soya beans (*Glycine max*) are fermented with or without cereals to produce a variety of products with their history dating back hundreds of years (Yokotsuka, 1985). Fermented foods form an important component of the diet in South East Asia, Middle East and Africa.

There are many fermented foods known, some serve as main course meals, others are beverages while others are flavouring food condiments (Platt, 1980). Those which serve as food condiments are made from the fermentation of protein rich seeds. In Indonesia '*tempeh'* is fermented from cooked de-hulled soya beans. The fermented *'tempeh'* is then eaten fresh or deep fried like meat (Wood, 1995). Another popular fermented soya bean product is soy sauce with history dating back thousands of years in the Orient. It is known as '*shoyu'* in Japan and it is fermented from soya beans with or without barley and rice.'*Soumbola'* a product of alkaline fermentation of African locust bean seeds is used as food condiment in Burkina Faso and several other countries of west Africa. It is an important source of protein, and plays an economical, social and cultural role in Burkina Faso (Diawaral *et al*., 2003). '*Ontjom'* is another popular Indonesian fermented food product. It is fermented from a mixture of soya beans. Fermentation of '*ontjom'* is reportedly carried out by species of *Neurospora* and *Rhizopus* (Wood, 1995). Other fermented soyabean product of the Orient are; *Miso,natto,* and *shi-tou-shi. Miso,* a flavouring agent is the fermented soyabean paste of the Orient (Ogbadu, 1988). The fermenting microorganisms are *Aspergillus oryzae, Aspergillus sojae* and *Lactobacillus*, which are introduced along with rice, barley or beans. Like '*tempeh'*and soy sauce, '*miso'* is now produced on industrial scale (Ogbadu, 1988). *'Natto'* is fermented by *Bacillus subtilis*; it is a side dish which is served with either cooked rice or soy-sauce. During '*natto'* fermentation, the *Bacillus* produces a viscous polymer consisting of polyglutamic acid, polypeptide and fructose (Ogbadu, 1988).). '*Shi-tou-shi'* is similar to '*natto'* and it is reportedly fermented by species of *Bacillus* (Yokotsuka, 1985). Soya bean is fermented in the middle belt region to give '*daddawan*' soya, a condiment that enhances the flavour of soups and other dishes. It is fermented by species of *Bacillus* (Achi, 2005). Other fermented legume products used as seasoning and flavour enhancers in West Africa, include *'daddawa'* which is known as '*iru'* in the south west part of Nigeria.

Fermented seeds of African locust bean seeds'*daddawa*' though a Hausa name has being widely adopted by most tribes in northern Nigeria and been erroneously used as '*daddawa*' by non Hausa speaking people (Odunfa, 1985). *'ogiri-igbo'* and '*ogiri-nwan'* are condiments used mainly by the Igbo speaking people of Eastern Nigeria. '*Ogiri –igbo'* is fermented from castor oil (*Ricinus communis*) seeds (Odunfa,1985). While '*ogiri-nwan* is obtained from fermented pumpkin beans (*Telfaira occidentale*) seeds. Another *'ogiri'* common among the Ijebus and Ondo tribes in the forest zone of sourth-western Nigeria is fermented from melon seeds (*Citrullus vulgaris*) and the microorganisms fermenting this '*ogiri'* is *Bacillus* species, which was consistently found in the fermenting mash (Odunfa, 1981). *'Ogiri-saro* on the other hand is usually fermented from the seeds of sesame plant (*Sesamun indicum*) (Odunfa,1985).

- ***Netetou*** : (A fermented *P.biglobosa* condiment in Senegal)

A typical African condiment from *Nere* (Senegal) (*Parkia biglobosa)* is a Leguminous tree that is found throughout Africa from Gambia to Cameroun (Duranti, 2006). While the fruit is consumed for its floury or sweet pulp, the seeds after a lengthy preparation are made into fermented condiment, which is of economic importance and forms a major ingredient in African cooking (FAO, 2009). The method of production varies from one region to another and the condiment is commonly found in African markets where it is known by different names: *netetou* in Senegal, *sumbala* in Mali and Guinea (FAO,2009). *Netetou* has a strong aroma and it is used to strengthen the flavour of sauces that accompany rice and sorghum dishes. A survey in Dakar showed its use in almost all the main recipes of Senegalese cuisine (Duranti, 2006). It is frequently associated with other flavouring agents such as bouillon cubes which are produced and have rapidly increasing sales in Africa. These commercial cubes fit perfectly in local recipes and are sold in small packets (FAO, 2009).

Despite the serious competition from bouillon cubes produced by large companies like Maggi, which use radio and other forms of advertising to promote their products *netetou* remains the veritable 'local cubes' anchored in traditional food habits and popular with all classes (Mesina,2010). *Netetou* has an advantage in that it can be divided into smaller pieces and sold at affordable prices.

- **Sumbala:** (A fermented *P.biglobosa* condiment in Mali)

This is another fermented condiment used widely across West Africa. It is traditionally made from (*Parkia biglobosa)* seeds, and the use of soya bean for this purpose is increasing mainly due to inadequate supply of Parkia *biglobosa* seeds (FAO,2009). The fabrication process involves, boiling, cleaning and then packing away to ferment, the fermentation process giving it a pungent smell. Salt can be added to this product to facilitate storage life (Mesina, 2010). This traditional condiment is sold in balls or patties and they can be kept for several months at a time (in case of quality). It is a traditional ingredient used across West Africa, especially in cooking. The traditional production now faces strong competition from low quality stock cubes due to heavy publicity. *Sumbala* is rich in protein and a variety of dietary minerals, which are completely absent from these bouillon cubes. In recent years however, good quality commercial production has allowed the product to make a come back into every day cuisine (FAO, 2009).

2.5.3 Traditionally fermented vegetable protein condiments in Nigeria

Traditionally, fermented condiments ('*daddawa,iru and ogiri'*) are based on vegetable protein and are consumed by different ethnic groups in Nigeria; they have been the pride of culinary traditions for centuries (Achi,2005). It is evident that these products have played a major role in the food habits of communities in the rural regions serving not only as a nutritious non meat protein substitute, but also as condiments and flavouring or seasoning agents in soups (Achi,1999).

2.5.4 Names and substrates used for fermenting condiments in Nigeria

Throughout Nigeria many names are applied to the multitude of fermented food condiments as shown in the Table 4.

Table 4: Traditional substrates used for food condiments.

Raw material	Local name	References
Soya bean (*Glycine max*)	*daddawa*	Popoola and Akueshi, 1984 Ogbadu and Okagbue, 1988
Mellon seeds (*Citrullus vulgaris*)	*ogiri*	Odunfa, 1981
Castor oil seeds (*Ricinus communis*)	*ogiri-igbo*	Odunfa, 1985
Fluted pumpkin seeds (*Telferia occidentalis*)	*ogiri-ugu*	Barber *et al.,* 1992
African locust bean (*Parkia biglobosa*)	*daddawa (iru*)	Odunfa, 1981
African oil beans (*Pentaclethra macrophylla*)	*ugba/ukpaka*	Obeta, 1983
African yam bean (*Stenophylis stenocarpa*)	*owoh*	Ogbonna *et al*., 2001
Cotton seeds (*Gossypium hirsitium*)	*owoh*	Sanni and Ogbonna, 1991)
African mesquite (*Prosopis africana)*	*okpehe*	Odibo *et al*., 1992)
Bambara groundnut (*Vigna subterranean*)	*daddawa*	Barimalaa *et al*., 1989)

Source :Achi (2005)

2.6 Organisms responsible for food fermentations : An overview

The most common groups of microorganisms involved in food fermentation are:

- Bacteria
- Yeast
- Moulds

2.6.1 Bacteria

Several bacterial families can be found in foods, and majority may be invoved in food spoilage. As a result, the important role of bacteria in (food fermentation) is often overlooked (Omafuvebe *et al*., 2002). The most common bacteria in desirable fermentations are the Lactic acid bacteria which have the ability to produce lactic acid from carbohydrates (Fellow, 1997). Another important bacteria species especially in the fermentation of legume seeds is the genus *Bacillus* (Dike and Odunfa, 2003). Some groups of bacteria are those which bring about alkaline fermentations, that is the genus *Bacillus*. The species that initiates fermentation of legume seeds mostly are *Bacillus subtilis, Bacillus licheniformis* and *Bacillus pumilus* (Omafuvbe, *et al.,* 2002). *Bacillus subtilis* is the dominant species causing the hydrolysis of protein to amino acids and peptides releasing ammonia which increases the alkalinity and makes the substrate unsuitable for the growth of spoilage organisms (Ogunshe *et al*., 2006). Alkaline fermentations are more common with protein rich foods such as soya beans and other legumes (Tamang, 2009). Range of products from alkaline fermentation does not match with those brought about by acid fermentations, they are important in that they provide protein rich, low cost condiments from leaves, seeds and beans which contributes to the diet of millions of people in Africa and Asia (Steinkraus,1996).

2.6.2 Yeasts

Yeasts are unicellular organisms that reproduce asexually by budding. In general, yeasts are larger in size than most bacteria (O'Brien, 2003). Yeast plays an important role in

the food industry, as they produce enzymes that favour desirable chemical reactions such as the leavening of bread. Yeasts and yeast like- fungi are widely distributed in nature. They are present in orchards and vineyards, in the air, the soil and in the intestinal tract of animals (Wood and Hodge, 1995). Like bacteria and moulds, yeast can have beneficial and non beneficial effects in foods. The most beneficial yeast in terms of desirable food fermentation is from the Saccharomycetes family, especially *Saccharomycetes cerevisea* (Nelsson, 1998).

Moulds

Moulds are also important organisms in food industries, both as spoilers and preservers of food (Nout and Rombouts, 1995). Certain moulds produce undesirable toxins and contribute to the spoilage of foods. The *Aspergillus* species are often responsible for undesirable changes in food (Nout and Rombouts, 1995). These moulds are frequently found in foods and can tolerate high concentrations of salt and sugars. However, others impact characteristics flavours to the food, and others produce enzymes such as amylase used for bread making (Kovac, 1997). Moulds are aerobic and therefore require oxygen for growth. They also have the greatest array of enzymes and their colonies can grow on most type of food. Moulds do not play significant role in the desirable fermentations of fruit and vegetable products (Kovac, 1997).

2.7 Conditions required for bacterial fermentations

Microorganisms vary in their optimal pH requirements for growth. For most bacteria, favourable conditions are within the neutral pH of 7. The varied pH requirement, of various groups of microorganisms may be useful in food fermentations because successions of microorganisms may occur as the pH of the environment changes (Dakwa *et al.*, 2005).

2.7.1 Temperature

Different bacteria can tolerate different temperature ranges, which provide enormous scope for a range of fermentations (Odunfa and Adewuyi, 1985). Most bacteria have a

temperature optimum of between 20-30 ^{0}C. There are some (the thermophiles) which prefer higher temperature (50-55 ^{0}C) and those with colder temperature optima (15-20 ^{0}C). Most lactic acid bacteria work best at temperatures of 18-22 ^{0}C. Temperatures above 22 ^{0}C, favour the *Lactobacillus* species (Omafuvbe *et al.,* 2005).

2.7.2 Salt concentration

Lactic acid bacteria tolerate high salt concentrations. The salt tolerance gives them an advantage over other less tolerant species and allows the lactic acid fermenters to begin metabolism, which produces acid, which further inhibit the growth of non-desirable organisms (Omafuvbe, 2005).

2.7.3 Water activity

In general, bacteria require a fairly high water activity (0.9 or higher) to survive (FAO, 2009). There are few species which can tolerate water activity lower than this, but usually the yeasts and moulds will predominate on foods with a lower water activity (Popoola and Akueshi, 1984).

2.7.4 Hydrogen ion concentration (pH)

The optimum pH for most bacteria is near the neutral (pH 7.0). Certain organisms are acid tolerant and will survived at reduced pH levels (Achi, 2005).

2.7.5 Oxygen availability

Some of the fermentative bacteria are anaerobes, while others require oxygen for their metabolic activities (Sarkar *et al*., 1993). Some lactobacilli in particular are microaerophilic, that is, they grow in the presence of reduced amount of atmospheric oxygen. In aerobic fermentation, the amount of oxygen present is one of the limiting factors. It determines the type and amount of biological products obtained, the amount of substrate consumed and the energy released from the reaction (FAO, 2009).

2.7.6 Nutrients

All bacteria require a source of nutrients for their metabolism. The fermentative bacteria require carbohydrates, either simple sugars such as glucose and fructose or complex carbohydrates such as starch or cellulose (Aderibiye and Odunfa, 1990). The energy requirements of microorganisms are very high. Limiting the amount of substrate can check their growth.

2.8: Use of starter cultures in food fermentations

In order to produce fermented legume products of consistent quality, starter cultures (similar to those used in the dairy industry) have being recommended (Omafuvbe *et al.,* 2002). Not only do starter cultures ensure consistency between batches, they speed up fermentation processes. Therefore a pure culture is essential for a controlled fermentation (Dakwar *et al*., 2005).

2.9: Microbiology of fermented foods: An overview

Microbiological studies aimed at isolating and identifying organisms relevant to food fermentations are potentially useful for development of appropriate fermented foods and for improving the scale of production from domestic to factory level (Popoola and Akueshi, 1984).

2.10: The genus *Bacillus*

Bacillus is a genus of gram-positive rod-shaped bacteria and a member of the division Firmicutes. *Bacillus* species can be obligate aerobes or facultative anaerobes and they test positive for the enzyme catalase. Ubiquitous in nature, *Bacillus* includes both free-living and pathogenic species (Grauman, 2007). Under stressful conditions the cells produce oval endospores that can remain dormant for extended periods (Ryan and Ray, 2004).

2.10.1 Scientific classification of *Bacillus*

Domain	Bacteria
Division	Firmicutes
Class	Bacilli
Order	Bacillales
Family	Bacillaceae
Genus	Bacillus
Species	

There are numerous species, these include:

B. aquaemaris

B. brevis

B.caldolyticus

B. centrosporus

B. cereus B. alcalophiles

B. alvei

B. amyloliquefaciens

B. anthracis

B.ciculans

B. coagulans

B.firmus

B. flavothermus

B.globiggi

B.infernus

B. lavae

B.licheniformis

B.megaterium

B.lentus

B.mesentericus

B.mucilaginosus

B.mycoides

B.natto

B.panthothenticus

B. polymyxa

B. pseudoanthracis

B. pumilus

B.schlegelii

B.sphaericus

B.sporothermodurans

B.stearothermophhilus

B. subtilis

B.thermoglucosidasius

B.thuringienses

B.vulgaris

Source Riaz(2007).

2.10.2 Industrial significance of the genus *Bacillus*

Many *Bacillus* species are able to secrete large quantities of enzymes (Omafuvbe *et al.,* 2002). Some *Bacillus* species produce a viscous polymer consisting of polyglutamic acid polypeptide and fructose during fermentation of legume seeds (Heald *et al.,* 2007;Yokutsuka, 1985). *Bacillus amyloliquefaciens* is the source of the natural antibiotic protein barnase (a ribonuclease), alpha amylase use in starch hydrolysis,the protease

subtilisin used with detergent and the Bam HI restriction enzymes used in DNA research (Graumann,2007). *Bacillus* organisms were found to be an inhabitant of the wild African moose's jejunial tract. (Aremu *et al.*,2006).

2.10.3: *Bacillus subtilis* and related species in biology and industry

Bacillus subtilis known as the hay *Bacillus* or grass *bacilli* is a Gram-positive catalase positive bacterium commonly found in the soil (Madigan and Martinko, 2005).*Bacillus subtilis* is a rod – shaped organisms and has the ability to form tough protective endospores, allowing the organisms to tolerate extreme environmental conditions (Madigan and Martinko, 2005).

One of the best understood prokaryotes in terms of molecular biology is *Bacillus subtilis* (Euzeby, 2008). Its superb genetic amenability and relatively large size has provided a powerful tool for investigation. (Euzeby, 2008). This organism has proven highly amenable to genetic manipulations and has therefore become widely adopted as a model organism for laboratory studies especially on sporulation. It is also flagellated, which gives *Bacillus subtilis* the ability to move quickly (Madigan and Martinko,2005).

Other uses of *B.subtilis* and related species

- *Bacillus subtilis* is used as soil inoculants in horticulture and agriculture.
- *Bacillus globigii*, a closely related but pathogenetically distinct species was used as a biowarfare stimulant (Todder,2009).
- Enzymes produced by *Bacillus subtilis*, *Bacillus pumilus*, and *Bacillus licheniformis*, are widely used in legume seed fermentations.
- *Bacillus subtilis* is used as model organisms for laboratory studies.
- A strain of *Bacillus subtilis* formerly known as *Bacillus natto* is used in the commercial production of the Japanese food *natto* as well as the popular Korean food *cheonggukjang*.

- *Bacillus subtilis* strain QST 713 (marketed as QST 713 or serenade) has a natural fungicidal activity, and it is employed as a biological control agent.
- Recombinants *Bacillus subtilis* strain PBE2CIAB was used in the production of polyhydroxyalkanoates (PHA) and they could use malt waste as carbon source to reduce cost of PHA production.
- *Bacillus subtilis* is used to produce amylase enzymes (Todder,2009).

2.10.4 Pathogenicity of *Bacillus* species

Bacillus subtilis and *Bacillus pumilus* are not considered human pathogens; they may contaminate food but they rarely cause food poisoning (Noirot, 2007). *Bacillus subtilis* produces the enzyme subtilisin, and their spore can survive the extreme heating that is used to cook food (Todder, 2009).

2.10.5 Distribution of *Bacillus* species in nature and in foods

Bacillus species are mainly found in the soil, and they are widely distributed in nature. Due to their saprophytic nature, they are found contaminating a wide variety of materials. Some species are also pathogenic. Wakisaka and Koizumi (1982) grouped *Bacillus* species which they isolated from the soil into major and minor soil populations with *B.cereus,B.megaterium*, *B.sphaericus*, and *B.subtilis* considered as the major soil *Bacillus* populations. *Bacillus* populations have also been found in lakes. In a bacteriological study carried out in a lake in USSR, *Bacillus* species ranked highest in number among the total of all the bacteria isolated, with a count of ten *Bacillus* species out of a total number of thirty two bacterial species belonging to nine genera (Koleshko *et al*, 1982). *Bacillus* species are also found in the atmosphere as revealed by microbiological studies of air in grain storage and processing plants, tobacco plants and poultry houses (Todder, 2009). In that study, it was observed that bacteria including *Bacillus* where more numerous than fungi. *Bacillus* species have also been isolated from meat and meat products. In a

bacteriological study of meat extract, *Bacillus subtilis, Bacillus mycoides, Bacillus sphaericus, Bacillus megaterium, Bacillus circulans, Bacillus pumilus* and *Bacillus coagulans* occurred predominantly among *Clostridium* species (Todder, 2009). Thermophilus *Bacillus* species have been reported to cause spoilage of improperly cooked meat (Ogbadu, 1988).

Bacillus species have being isolated from drinks and spices. *Bacillus* species have been isolated from Mexican indigenous alcoholic and non alcoholic beverages (Ulloa, 1981). Some *Bacillus* species have being reported among some water borne organisms frequently contaminating thawing frozen products in water bath (Dietze and Burnie, 1982). Fruits are also frequently contaminated by *Bacillus* species. Soft stem and spoilage in olives have been attributed to *Bacillus polymyxa* and *Bacillus macerans*. These organisms are often found on olives (Vaughan, 1985). *Bacillus* species are generally saprophytic and a few species are pathogenic to human and animals. *Bacillus anthracis* is pathogenic to sheep, cattle and sometimes human beings causing anthrax, a severe pneumonia aggravated by toxins liberated by these bacilli (Todder,2009). The pathogenicity of *Bacillus thuringiensis* and *Bacillus sphaericus* is now so well understood. They are cultivated for their protein products which are used as insecticides (Noirot, 2007).

2.10.6 The genus *Bacillus* in food fermentations

The involvement of the genus *Bacillus* in food fermentation can be attributed to the array of enzymes produced by species of the genus (Diawaral *et al.,* 2003). A number of *Bacillus* species because of the array of enzymes produced, are used to produce enzymes at industrial scale. These of enzymes include; amylase, B.galactosidase, B. gluconase, glucose isomerases, lipase, and proteases (Euzegby, 2008). *Bacillus* species are therefore associated with the fermentation of protein, carbohydrates, lipid-rich vegetable based foods (Omafuvbe *et al.,* 2005). The involvement of *Bacillus* species in the fermentations invoved

in curing of certain fruits used in beverage production, is also known. *Bacillus* species are reported to play a very significant role at the initial stage of fermentation of cherries (FAO, 2009). *Bacillus* species are also involved in other fermentations such as those of legumes and vegetables. *Bacillus circulans. Bacillus subtilis, Bacillus pumilus* and *Bacillus licheniformis* are purportedly involved in fermentation of legume seeds (Achi, 2005). They are also reported to be involved in the fermentation of carbohydrate - based substrates. *Bacillus* are also involved in the fermentation of rice (FAO, 2009). '*Fufu'* a popular fermented cassava product (*Manihot esculenta*) in Eastern Nigeria, is reportedly fermented by *Bacillus* along with other bacteria (Uzuegbu and Eke, 2001). *Bacillus* species are also involved in the fermentation of protein based substrates. Although *Bacillus* species had earlier been reported as spoilage organisms in sausages, they failed to cause spoilage when inoculated into raw sausage mixture (Noirot, 2007). Fermentation of African locust bean (*Parkia biglobosa*) seeds for *daddawa* production is reported to be carried out by *Bacillus subtilis* (Omafuvbe *et al.,* 2005). In the fermentation of soya beans seeds into soya *daddawa, Bacillus* species were also implicated (Dike and Odunfa , 2003). Other vegetable protein fermentations include that of African oil bean plant (*Penthenclethra macrophylla*) seeds for '*ugba'* production in which *Bacillus* species have being implicated as the fermenting organisms (FAO, 2009). In the fermentation of various type of '*ogiri' Bacillus* species were found consistently (Dike and Odunfa , 2003).

Bacillus fermented food products are classified according to the substrate used. *Daddawa* is made from African locust bean seeds whereas *kinema, natto* and *thua nao* are made from soya bean seeds. The *Bacillus* fermented foods are used in different ways. Generally they are used as meat substitute or as flavouring agents in soups as *daddawa, kinema* or *thua nao* or they may be eaten directly with rice in the case of *natto* (Leejeerajumnean, 2000). The

finished product of *Bacillus* fermented soyabeans is greyish and covered with sticky polymer produced by the bacterial cell (Leejeerajumnean, 2000).

2.11: Diversity of *Bacillus* species Isolated from *Okpehe*, a traditional fermented soup condiment from Nigeria

Okpehe is a traditional fermented soup condiment produced from *Prosopis africana* seeds and serves as a tasty and low-cost protein source among the people of eastern and mid western Nigeria (Aderigbibe and Odunfa,1990). In West Africa, other vegetable condiments such as *iru* or *dawadawa* from locust beans (*Parkia biglobosa*), *ogiri* from melon seeds (*Citrulus vulgaris*), *dawadawa* from soybean (*Glycine max*), *soumbola* from soybean, *ugba* from African oil bean *(Pentaclethra macrophylla*), and *owoh* from cotton seeds (*Gossypium hirsutum*) are also products of vegetable seeds fermentation (Achi,2005) .Production of these condiments is by spontaneous fermentation carried out in people's homes using rudimentary utensils under varying hygienic conditions. Therefore, a variable spectrum of microorganisms may be present in such fermentations. Nevertheless, there is general agreement that aerobic spore-forming bacteria, especially *Bacillus* species, are the dominant microorganisms in the fermentation and that proteolytic activity, which leads to release of amino acids, ammonia, and other volatile compounds, is the major biochemical activity during the fermentation (Ouoba *et al.*, 2008). The strains that can bring about the desirable biochemical changes have not been accurately identified, and starter cultures for such fermentations are not yet commercially available (Oguntoyibo, 2010). Because of the spontaneous nature of the fermentation and the often unhygienic production conditions, pathogenic and spoilage strains of *Bacillus* cannot be totally excluded from some fermentation batches. In addition, variation in the quality of the condiments among production or geographical regions is expected. Therefore, use of starter cultures would increase product safety and enhance product quality (Darkwa *et al*., 2005).

The *Bacillus* species isolated from the fermented soup condiment *okpehe* in Nigeria were diverse and included *B. subtilis, B. amyloliquefaciens, B. cereus,* and *B. licheniformis* (Oguntoyinbo, 2010). A significant proportion of the protein intake in developing countries is from plant sources, notably the proteinaceous oil seeds of which many are consumed in the form of fermented vegetable proteins (Oguntoyinbo, 2010). The use of fermented vegetable proteins as seasonings is also widespread in Africa and Asia.

Although *Bacillus subtilis* was reported as the most important species involved in *okpehe* fermentation (Aderibiye and Odunfa, 1990). The dominance of proteolytic *Bacillus* strains in alkaline food fermentations has been well documented in several studies on African fermented *dawadawa, soumbala,* and *bikalga* (Oguntoyinbo, 2010).

2.12: Thua nao: Alkali Fermented soya bean from *Bacillus subtilis*

Thua nao is a Thai alkali fermented product from soybean by *Bacillus* and it has a special flavour and aroma. The microorganisms involved in traditional *thua nao* were mixed natural flora of *Bacillus, B. subtilis, B. megaterium* and *B. cereus* (Leejeerajumnean, 2000). The product had a very strong smell of ammonia; ammonia content in dried product was lower than in the wet product. The increase of ammonia was controlled by fermentation under the atmosphere of CO_2 or adding phosphate buffer (0.1 mol kg-1 wet wt KH_2PO_4, pH 6.5), which had no effect on the growth of *Bacillus*, proteolytic activity or amino acid formation. The major volatile compounds in *thua nao* were different from those found in *natto*, Japanese alkali fermented soybean (Leejeerajumnean, 2000). Alkaline, proteolytic fermented foods from legumes are found in various parts of the world and

they include Japanese natto, Nigerian dawadawa or iru, Nepalese kinema and Thai thua nao. Soybean seeds are cooked and fermented by pure cultures of *Bacillus subtilis,* as for natto in northern Japan (Leejeerajumnean, 2000), or fermented using natural *Bacillus* species, as for *dawadawa/iru* in west and central Africa (Campbell-Platt, 1980; Odunfa, 1981), and *kinema* in Nepal (Tamang, 2009; Sarkar *et al*., 1993). The fermentation of legume seeds with *Bacillus* species changes the texture and organoleptic properties of the original legume seeds. The unpalatable flavour of the unfermented legume is eliminated (Sarkar *et al*., 1993). The fermented product has a different flavour and aroma and requires less cooking time.

Most of the above products are prepared by solid-state fermentation in which the substrate is allowed to ferment spontaneously or by adding *Bacillus* inoculum. The fermented products have a distinct odour. Some products, such as *natto* or *dawadawa* have become commercialized. *Dawadawa* is sold in cubed packages, as are Nestle maggi soup cubes in African markets (Steinkraus, 1992). In Asia, *thua nao* and *natto* are very similar in terms of proteolytic *Bacillus* fermentation but these products have different aroma. *Natto* is a Japanese fermented whole soybean product which is eaten uncooked as a relish with rice, whereas Thai *thua nao* is generally sold as dried disks of ground material and is used as a flavouring agent in soups and curries. Thai fermented soy bean, *thua nao*, is

produced in the north of Thailand. It is consumed as food and used as condiment for enhancing flavour in soups, curries, or as a substitute for shrimp paste (Leejeerajumnean, 2000*).* *Kinema* or *thua nao*, is eaten directly like in the case of *natto* (Steinkraus, 1992). The fermented legumes are consumed as a fresh product without further processing, as with Japanese *natto*, while some products are fried, as with Nepalese *kinema* (Tamang 2009). *Thua nao* is sold as paste or as dried disks, dark brown in colour with a quite different aroma from *natto*, often with a strong proteolytic and ammonia smell. The slime on the beans is a mixture of a-polyglutamic acid and levan, and most of viscous materials are polyglutamic acids containing D- and L- glutamates (Oguntoyibo, 2010). The fermented soybeans have a distinct odour, always accompanied with an ammoniacal odour (Campbell- Platt, 1980; Steinkraus, 1992; Sarkar *et al.,* 1993). The fermentation process was similar to that of Japanese *natto,* however, *natto* is now usually fermented with a pure culture of *Bacillus subtilis, thua nao* is still made by a traditional method with a mixed natural microflora. Fermentation of soya bean was caused by mixed culture of *B.subtilis* and *B. megaterium* , but the predominant organism was *B. subtilis. Natto* has only pure culture of *B. subtilis*. Isolation and identification showed clearly that *B. subtilis* was the predominant species from the beginning to the final products (Leejeerajumnean, 2000).

CHAPTER THREE

MATERIALS AND METHODS

3.1: Sample collection

Samples of locust bean seeds (*Parkia biglobosa*) and (soya bean seeds *(Glycine max*) were purchased from Kasuwan Mata, Sabon gari market Zaria, Kaduna state, and seeds of African mesquite *(Prosopis africana*) was purchased from Otukpo market in Benue State of Nigeria. All the seeds were transported to the laboratory in polythene bags.

3.2: Revalidation and characterization of *Bacillus* isolates

Preliminary characterization of isolates: Test strains of *Bacillus* species ; *B. subtilis* (TS001) *and B. pumilus* (TS002) obtained from the Department of Microbiology Ahmadu Bello University Zaria were compared by re-culturing in nutrient agar broth. The strains were incubated at 37^0C for 24hours. Compared cells were sub-cultured on aerobic plates of nutrient and plate count agars and were incubated at 37^0C for 24hours. This was carried out along side with standard strains of *B.subtilis* (SX1BS) and *B.pumilus* (SX1BP) obtained from Federal Institute of Industrial Research, Oshodi (FIIRO) Lagos, which was used as control.

Representative colonies of microorganisms which developed on the aerobic plates of both nutrient and plate count agar were subjected to initial staining and microscopic examinations.

The isolates were subjected to the following biochemical tests using standard methods as described by Gordon *et al*, (1973).

i. Catalase test: Two drops of 3% hydrogen peroxide were added to 18-hour-old culture of isolates on slides and was observed for gas production.

ii. Coagulase test: A smear of the isolates on slides were made with normal saline, a drop of blood plasma was added. The slides were rocked and observations for coagulation were made.

iii. Motility test: A loopful of the isolates was stabbed mid-way in the tubes containing motility medium, and was incubated at 37^0C for 24 hours. Motility was observed by the organisms moving down the bottom of the tubes and spreading from the stab lines.

iv. Indole test: Nine milliliters of sterile peptone water was dispensed in sterile tubes. The isolates were inoculated in peptone water and were incubated for 24 hour at 37^0C. The isolates in peptone water were brought out of the incubator after 24 hours and 2 drops of Kovac's reagent was added. Observation for colour change was made to either acid or alkaline.

v. Hydrogen Sulphide production: Triple Sugar Iron (TSI) agars slants was prepared and sterilized in test tubes. The TSI was allowed to gel, and a loopful of the isolate was stabbed in the TSI agar. It was incubated at 37^0C for 24 hours. Smell of rotten egg and a black colour was observed for H_2S production.

vi. Starch hydrolysis: The isolates were streaked on the plates of Starch agar, containing 20% soluble starch and incubated at 37^0C for 24 hours. After incubation, the plates were flooded with Gram's iodine. The appearance of blue-black colour indicated the presence of starch, a negative result while clear zones indicated the complete hydrolysis of starch a positive result.

vii. Growths at different Sodium Chloride (NaCl) concentrations: Nine millilitres of peptone water containing different concentrations of NaCl (5%, 10% 15%,20% and 25%) were used. The isolates were inoculated and incubated at 37^0C for 24 hours. Observations for turbidity were made.

viii. Sugar utilization: One gram each of different sugars, lactose, fructose, sucrose, mannitol and rhamnose were dissolved in 100mls peptone water in separate tubes containing Durham tubes and a loop-full of the organisms inoculated into the medium. 1.5ml of 0.2% Bromothymol blue indicator was added into 100ml of basal medium containing sugars, the tubes were incubated at 37^0C for 24hrs. Observation for gas and acid production were made..

3.3 : Preparation of legume seeds for fermentation

i) Locust bean seeds.

The seeds obtained from the market were pre-cleaned by sorting out stones and debris. This was followed by washing and boiling in water for12 hours, renewing the water intermittently until the seeds became soft. The soft seeds were de-hulled by pounding lightly in a wooden

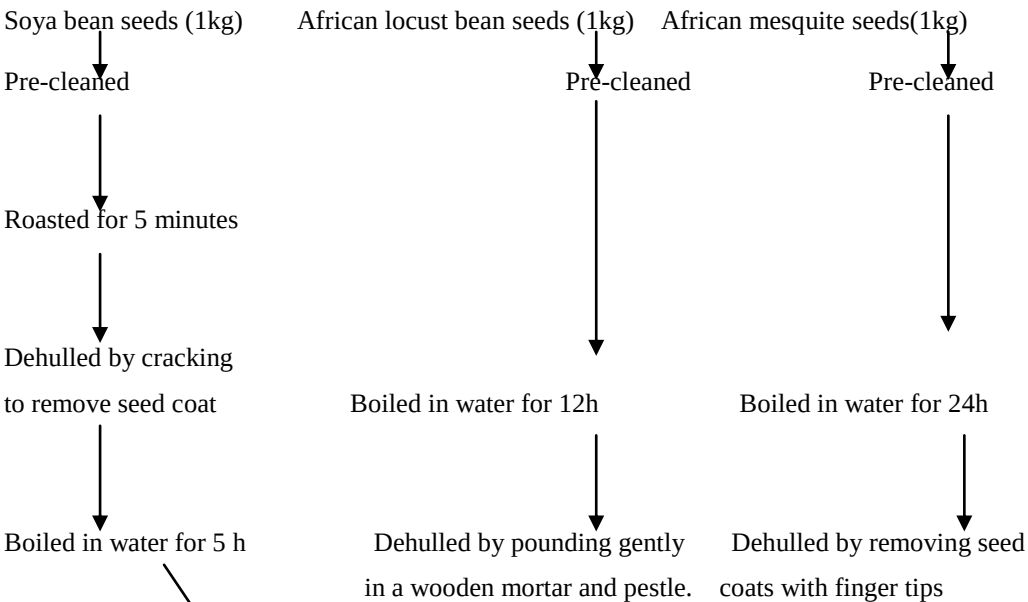
mortar using a wooden pestle. The seed coats were removed, and the cotyledons boiled again for two hours. The re-boiled cotyledons were allowed to cool to 35^0C in an earthen pot lined with sterile foil .

ii) Soya bean seeds.

Soya bean seeds obtained from the market were pre-cleaned by sorting out stones and debris. They were roasted on a hot pan for five minutes. This was followed by dehulling to remove seed coats followed by boiling in water for five hours; the water was renewed intermittently until the seeds became soft. The cotyledons were allowed to cool to 35^0C in an earthen pot lined with sterile foil .

iii) African mesquite.

The seeds obtained from the market were pre-cleaned by sorting out stones and debris. This was followed by washing and boiling in water for 24 hours, renewing the water intermittently until the seeds became soft. The soft seeds were dehulled by removing seed coats with finger tips. The cotyledons were reboiled for four hours and were also allowed to cool to 35^0C in an earthen pot lined with sterile foil.



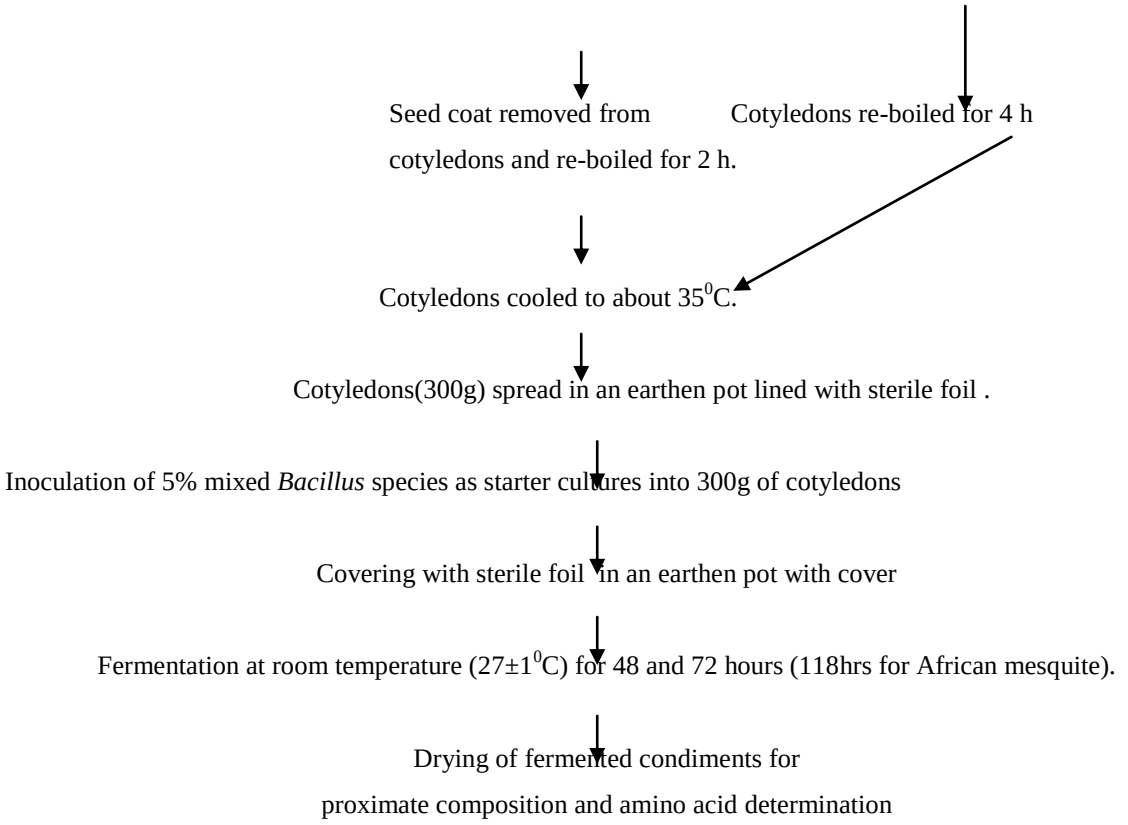


Figure 1: Flow chart for seed preparations and fermentations for production of *"daddawa"* (*Parkia biglobosa* and *Glycine max*) and *"okpehe"* (*Prosopis africana*) using mixed *Bacillus subtilis* and *Bacillus pumilus* as starters.

3.4 : Preparation of *Bacillus* inoculum

The inoculum used for each fermentation contained 2.7×10^7 cells/ml; the cell population was calibrated using McFarland standards (No 7) which was prepared by adding 0.7ml of 1% anhydrous barium chloride ($BaCl_2$) to 9.3ml of 1% sulphuric acid (H_2SO_4) (Todder, 2009).

The inoculum used formed 5.0% of fermenting materials and consisted (15ml of 24hr old cultures of organism into 300g of unfermented seeds) consortium A (standard mixture of *B.subtilis* and *B.pumilus* combined) and consortium B (Test mixture of *B.subtilis* and *B.pumilus* combined).

3.5: Controlled fermentations of *P.africana, P.biglobosa* and *G.max* seeds using starters

The fermentation process was set up using both consortium A and B seperately. The organisms were inoculated into 300g of the unfermented seeds and were wrapped with sterile aluminum foil and placed in an earthen pot with cover. Fermentation was allowed to progress at room temperature (28 ± 2^0C) for 48hrs for *P. biglobosa* and *G. max*, 118hrs for *P.africana* in the laboratory at the Department of Microbiology, Ahmadu Bello University, Zaria. After fermentation, proximate analyses for crude protein, carbohydrates, crude lipids, fiber, ash and moisture content were carried out. Also mineral element contents of the fermented seeds such as sodium, potassium, calcium, iron and lead were carried out according to AOAC (1980) methods.

3.6 : Microbiological monitoring of fermentation.

Microbiological analysis was carried out at intervals of 12hrs to monitor growth of the starter cultures from the start to the end of the fermentation process.

During the 48 and 118 hours of fermentation, samples of ten grams each were taken aseptically at intervals of 12 hours and put into 90ml sterile peptone water. The suspension was shaken vigorously to dislodge microorganisms, thus forming the stock concentration. A tenfold serial dilution was prepared to obtain dilutions up to tenfolds. Aliquots of 0.1ml of 10^{-5} and 10^{-6} dilutions were plated in duplicates on nutrient agar plates (Oxiod), plate count agar (Oxiod); for isolation and determination of count of bacteria. Potato dextrose agar containing chloramphenicol (0.5mg/ml) to suppress growth of bacteria was used for isolation of fungi. The plating was done using a hockey glass stick spreader. The nutrient and plate count agar plates were incubated at 37^0C for 24 hours. Potato dextrose agar plate were incubated at room temperature (27 ± 2^0C) for one week.

3.7:Physicochemical and proximate analyses of *P.africana, P.biglobosa* and *G.max* seeds during fermentation with starters in the laboratory.

i Temperature

Nine thermometers (Seward immersion model) were cleaned with ethanol and were inserted into nine fermenting mashes in earth pots covered with aluminum foil and containing *P.biglobosa G.max* and *P.africana* seeds for monitoring temperature changes during the fermentations. The temperature monitoring for all seeds was carried out at intervals of 12 hours from 0 to 118 hours.

ii. pH

pH meter (Pye Unicam model 291 equipped with a glass electrode) was first calibrated using standard buffers of pH 4.0 and 9.2. Readings were also taken at intervals of 12 hours. This was done by mixing one gram of each type of fermenting seeds of locust bean, soyabean and African mesquite taken from the fermenting mash in 10ml of sterile distilled water. The pH of the suspension was then determined.

iii. Moisture content

The method described by AOAC, (1980) was adopted. A clean crucible was dried to a constant weight in an air oven at 110^0C, cooled in a dessicator and weighted (W_1). Two grams of finely ground sample using a sterile blender was accurately weighed into the previously labeled crucible and reweighed (W_2). The crucible-containing sample was dried in an oven set at 55^0C to a constant weight (W_3). The percentage moisture content was calculated thus:

$$\% \text{ Moisture Content} = \frac{W_2 - W_3}{W_2 - W_1} \times 100$$

Where W_1 – weight of crucible

W_2 – weight of crucible + sample

W_3 – weight of crucible + dried sample

iv. Ash content

The AOAC (1980) method was used. A porcelain crucible was dried in an oven at 100^0C for 10 minutes, cooled in a dessicator and weighed (W_1). Two grams of the finely ground sample was placed into the previously weighed porcelain crucible and reweighed (W_2). It was first charred and then transferred into a furnace, which was then set at 550^0C and the sample was left in the furnace for eight hours to ensure proper ashing. The crucible containing the ash was then removed cooled in the dessicator and was weighed (W_3). The percentage ash content was calculated as;

$$\% \text{ Ash content} = \frac{W_3 - W_1}{W_2 - W_1} \times 100$$

Where W_1 – weight of crucible

W_2 – Weight of crucible + sample

W_3 – weight of ash + crucible

V. Determination of nitrogen and crude protein

The method adopted by AOAC (2007) was used. Exactly 1.5g of the ground defatted sample in an ashless filter paper was dropped into 300ml Kjeldahl flask. Twenty five millitres conc. H_2SO_4 and 3g of digesting mixed catalyst ($CuS0_4$ and Na_2S0_4, 1:10) weighed separately into an ashless filter was also dropped into the Kjeldahl flask which was then transferred to the kjeldahl digestion apparatus. The sample was digested until a clear green colour was obtained. The digest was cooled and diluted with 100ml distilled water.

Distillation of digest

Twenty milliliters of the digest was measured and then transferred into 500ml Kjeldahl flask containing anti bumping chips, and 40ml of 40% sodium hydroxide (NaOH) was slowly added. A 250ml conical flask countaining a mixture of 50ml of 2% boric acid with mixed indicator was used to trap the ammonia to be liberated. The Erlenmeyer (500ml) flask and the Kjeldahl flask were then placed on the Kjeldahl distillation apparatus with the tubes inserted

into the conical flask and the kjeldahl flask. The distillation was carried out until 125ml of the distillate was trapped in the boric acid solution. From the point when boric acid turns green, 10 minutes was allowed for complete distillation of the ammonia present in the digest. The distillate was then titrated with 0.1M HCl. A blank was also carried out; the protein content was calculated with respect to percentage crude nitrogen as follows:

$$\% N_2 = \frac{14 \times M_x V_t \times T_v \times 100}{\text{Weight of test sample (mg)} \times V_a}$$

% crude protein = % nitrogen (N_2) x 6.25

Where M = actual molarity of acid

T_v =titre volume of HCL used

V_t = total volume of diluted digest

V_a = aliquot volume distilled

vi. Determination of crude fiber content

The method described by AOAC (1980) was used. Two gramms of the finely ground sample was weighed out into a round bottom flask. 100ml of 0.25% sulphuric acid solution was added and the mixture was boiled under reflux for 30mins. The hot solution was quickly filtered under suction. The insoluble matter was washed several times with hot water until it was acid free. It was quantitatively transferred into the flask and 100ml of hot 0.31 Na0H solution was added and the mixture boiled again under reflux for 30mins and was quickly filtered under suction. The insoluble residue was washed with boiling water until it was base free. It was dried to a constant weight in the oven at 100^0C, cooled in a dessicator and weighed (C_1). The weighed sample (C_1) was then incinerated in a muffled furnace at 550^0C for 2h. It was cooled in a dessicator and reweighed (C_2).

Calculation: the loss in weight on incineration = C_1- C_2. The calculation was carried out thus:

$$\% \text{ Crude fiber} = \frac{C_1 - C_2 \times 100}{\text{Weight of original sample}}$$

vii. Determination of crude lipid content

The crude lipid was determined by adopting the method AOAC (1980). A clean, dried 500ml round bottom flask containing few anti-bumping granules was weighed (W_1) and 300ml pet ether for the extraction was poured into the flask fitted with Soxhlet extraction unit. The extraction thimble containing 20g was fixed into the Soxhlet extraction unit. The round bottom flask and a condenser were connected into the soxhlet extractor and cold water circulation was put on. The heating mantle was switched on and the heating rate adjusted until the solvent was refluxing at a steady rate. Extraction was carried out for 6h. The round bottom flask containing oil was cooled and then weighed (W_2).

$$\% \text{ crude lipid} = \frac{W_2 - W_1}{\text{Weight of sample}}$$

Viii. Determination of carbohydrate (by difference)

The total carbohydrate content was determined by difference. The sum of the percentages moisture, ash, crude fiber, crude lipid, crude protein was subtracted from 100 (AOAC, 2007).

Calculation:

% Total carbohydrate = 100 - % moisture + % ash + % fat + % protein + % fiber.

(Nitrogen-free extract- NFE).

ix Determination of peroxide value

This is defined as the mg per/kg of oil, and it is the measure of the formation of hydrogen peroxide groups that are the initial products of lipid oxidation.

Procedure:

One gram of the sample was weighed into a 250cm^3 conical flask and one gram of powdered KI and solvent mixture (2:1 glacial acetic acid and trichloromethane) was added. The solution was then placed on a water bath for few minutes to dissolve properly.20cm^3 of 5% KI was

added and the sample was then titrated with 0.002N $Na_2S_2O_3$ using starch as indicator. The peroxide value of the content was calculated using the formula as described by AOAC (2007).

$$\text{Peroxide value} = \frac{(\text{sample titer} - \text{blank titer}) \times 1000 \times \text{Normality}}{\text{Weight of sample}}$$

X. Titratable acidity (TTA)

Ten gram of the sample was weighed into a 250cm^3 conical flask. 25ml diethyl ether with 25ml ethanol mixture was added. The mixture was then boiled on a hot plate until all the oil dissolved completely. Three drops of phenolphthalein indicator was added, the solution was titrated with 0.1N NaOH, with constantly shaking until a pink colour persisted for 30mins. The percentage of titratable acid was calculated using the formula AOAC (2007).

$$\%\ \text{Total TTA} = \frac{\text{titer value} \times \text{Normality of NaOH} \times 28.2\text{mg}}{\text{Weight of sample}}$$

Xi Determination of mineral content using Atomic Absorbtion Spectrophotometer(AAS)

The method AOAC 1980 was adopted; fresh samples of fermented products were dried at room temperature for seven days and were crushed to a fine powder. The powder was ignited in a muffle funance at 550^oC for one hour and was transferred into 250ml beaker. 5ml of nitric acid (conc.) was added, 15ml of conc. HCl was added and the whole was kept on a hot plate at 100^oC to dryness. 10ml of distilled water was added and was filtered warm into 100ml volumetric flask.

The filtered samples were aspirated into the flame through the air steam as a fine mist and were passed into the burner through a mixing chamber. The air was allowed to meet the fuel

gas (acetylene) supplied to the burner at a given pressure and the mixture was burnt. The radiation from the resulting flame was allowed to pass through a lens, and finally through an optical filter which permits only the radiation characteristics of the element under investigation to pass through the photocell. The atoms held are irradiated with the light produced by the cathode lamp. These atoms held absorbed some of the incident radiation, and the amount absorbed is proportional to the concentration of the sample in mg/l. The output from the photocell was measured on a suitable digital readout system and was finally printed out via a printer.

Xi. Determination of essential amino acids using gas chromatography

Amino acid extraction (AOAC, 2007)

Fresh samples were dried in an oven using low heat of 20^0C for five days. The dried and pulverized samples were made to be free of water by ensuring constant weight for a period of time in the laboratory. 10g of the samples was weighed into 250ml conical flask and was defatted with 30ml petroleum spirit with Soxhlet extractor that was equipped with a thimble, after hydrolysing by using 30ml of deionised water.

The amino acid content of the samples was recovered by extraction with 30ml methylene chloride before concentrating the samples to 1ml for gas chromatography analyses.

Xi. Determination of shelf life of fermented seed condiments

The fermented condiments were subjected to microbial analyses every two months for a period of six months to monitor deterioration during storage. Analysis was done by weighing 10g of the sample into 90ml peptone water. The suspension was shaken vigorously to dislodge microorganisms, thus forming the stock suspension. Tenfold serial dilutions was prepared up to 10^{-10}. Aliquots of 0.1ml of selected dilutions 10^{-5} and 10^{-6} in duplicates were plated onto nutrient agar, (Oxiod), and plate count agar (Oxiod); for isolation and

enumeration of bacteria. Potato dextrose agar was also used for isolation of fungi. The plating was done using a hockey glass stick spreader. The nutrient and plate count agar plates were incubated at 37^0C for 24 hours. Chloramphenicol (0.05mg/ml) was incorporated into potato dextrose agar plates to suppress growth of bacteria and the plates were incubated at room temperature (27 ± 2^0C) for one week. Physicochemical properties such as moisture level, ash, crude protein, crude lipid, peroxide value and titratable acid will be determined. Mineral elements namely Ca, Fe, K, Na and Pb were determined using methods adopted by AOAC (1980) and AOAC (2007). These were also analysed in the condiments every two months for a period of six months.

3.8: Dehydration of freshly fermented seeds of *P.biglobosa, G.max* and *P.africana* using different methods

- **Hot air oven - drying**

Fifty grams of freshly fermented seeds of *P.biglobosa*, *G.max* and *P.africana* seeds containing both consortia, and a control that fermented naturally, were weighed into nine Petri dishes cleaned with ethanol. The Petri dishes containing the fermented samples were placed in a hot air oven at a temperature of 45^0C for a period of one week. The samples were re-weighed again and again until a constant weight was obtained.

- **Direct sun - drying**

Fifty grams of freshly fermented seeds of *P.biglobosa*, *G.max* and *P.africana* seeds containing both consortia, and a control that fermented naturally, were weighed into nine Petri dishes cleaned with ethanol. The dishes containing the samples were exposed to

direct sunlight on a pouch in the Department of Microbiology, Ahmadu Bello University, Zaria for a period of two weeks to ensure total drying. Atmospheric temperature of the environment was also taken by placing a thermometer in a beaker containing distilled water. The products were reweighed until a constant weight was obtained.

- **Drying using a vacuum pump**

Fifty grams of freshly fermented seeds of *P.biglobosa*, *G.max* and *P.africana* seeds containing both consortia, and a control that fermented naturally, were weighed into nine Petri dishes cleaned with ethanol. The samples contained in the Petri dishes were placed in a desicator with a vacuum pump machine connected to it, and were dried for a period of one week. The samples were reweighed until a constant weight was obtained.

- **Drying using a solar dryer**

  Fifty grams of freshly fermented seeds of *P.biglobosa*, *G.max* and *P.africana* seeds containing both consortia, and a control that fermented naturally, were weighed into nine Petri dishes cleaned with ethanol. The samples were placed in a solar dryer box, and a thermometer was also placed in the box to monitor temperature changes. Another thermometer was placed in a 100ml beaker containing distilled water outside the box to monitor atmospheric temperature. The fermented fresh samples dried within a period of five days, and they were repeatedly weighed until a constant weight was obtained.

- **`Sundrying of seeds protected with net**

Fifty grams of freshly fermented seeds of *P.biglobosa*, *G.max* and *P.africana* seeds containing both consortia, and a control that fermented naturally, were weighed into nine Petri dishes cleaned with ethanol. The fermented samples were exposed to direct sunlight but were protected with a soft net with little meshes. Atmospheric temperature was also taken by placing a thermometer in a 100ml beaker containing distilled water around the drying environment. Drying was done in a period of two weeks and repeated weighing was carried out, until a constant weight was obtained

3.9: Powdering blending and packaging of dried fermented seeds of *P.africana, P.biglobosa* and *G.max*.

The dried fermented seeds of *P.africana, P.biglobosa* and *G.max* were blended into powders using a sterile blender. Ten gram of each type of powder was packaged into small plastic containers with seals sterilized with 70% ethanol. Air tight sealing into aluminum foil sachets was also done using a sealing machine. The packaged condiments were stored at refrigeration temperature (9 ± 2^0C) and on the shelf at ambient temperature. (27 ± 2^0C). As a control, fermented samples purchased from Sabongari were also powdered packaged and stored under the same conditions.

3.10: Sensory evaluation using nine point hedonic scale

Powdered condiments from seeds fermented naturally, and those from starter assisted fermentation will be used to prepare *egusi* soup (composition of soup include; one cup of blended *egusi*, four table spoonful of palmoil, ten gram of condiment, half teaspoonful of salt, one bulb of onion, one teaspoonful of powdered pepper, pumpkin leaves, four gram of powdered crayfish, one kilogram of dried antelope bush meat and three cups of water). A positive control (composition of soup include; one cup of blended *egusi*, five table spoonful of palmoil, two cubes of maggi, half teaspoonful of salt, one bulb of onion, one teaspoonful of powdered pepper and crayfish, two cups of water) and a negative control

(composition of soup include; one cup of blended *egusi*, five table spoonful of palmoil, a teaspoonful of salt, one bulb of onion, one teaspoonful of powdered pepper and crayfish, two cups of water) was also set up. Sensory evaluation of the soups was conducted using nine point hedonic scale (Like extremely- (1); Like very much- (2); Like moderately- (3); Like slightly- (4) ;Neither like nor dislike -(5) ;Dislike slightly- (6); Dislike moderately- (7) ;Dislike very much- (8); Dislike extremely -(9);) scale in other to deermine the effect of the condiments on taste, colour, texture and overall acceptability of the soups.

Twenty panelists were selected to participate in the sensory evaluation test. They comprised both male and female students, and also some members of staff of Ahmadu Bello University, Zaria who are regular consumers of these condiments.

3.11 Statistical analysis: Analysis of variance (ANOVA) was used to compare these seed condiments by quality, nutritional values, sensory and shelf-life stability.

CHAPTER FOUR

RESULTS

4.1: Comparison of the test and the standard strains *Bacillus*

Gram's reactions of all the isolates (TS001, SX1BS, TS002 and SX2BP). All the strains had creamy colonies and were Gram positive rods. Endospores location of all the isolates was central. Catalase and coagulase reactions were positive for all the isolates. Motility test for all the isolates gave a positive result when stabbed midway on a motility medium, but reacted negatively when indole test was carried out on them. Incubation of isolates on triple sugar iron (TSI) agar after 24 hours did not produce a rotten egg smell or black colouration; this gave the isolates a negative result for H_2S production. Some of the isolates were not able to hydrolyse starch, this is a clear indication that the isolates are of different species. Inoculation

and incubation of the isolates in different concentration of NaCl (5%, 10%, 15% and 20%) and after 24 hours showed turbidity in some concentrations (5% and 10%) and there was no turbidity in concentrations of 15% and 25%. Sugar utilization and acid production in a basal medium (oxidative and fermentative) for some of the isolates were positive while some were negative. Those isolates (TS001 and XS1BS) that hydrolyzed starch had a identity of *Bacillus subtilis,* while isolates (TS002 and XS2BP) that were not able to hydrolyze starch had identity of the organism *Bacillus pumilus*. Similarly, those organisms that fermented all the sugars gave identity of the organism *B. subtilis* while those that did not ferment all the sugars gave identity of the organism *B. pumilus* (Table 4.1).

Table 4.1: Comparison of the test and the standard strains *Bacillus* used in this study

Tests	**Isolates**							
	TS001		TS002		SX1BS		SX2BP	
Gram's reaction / morphology	+ rods		+ rods		+ rods		+ rods	
Endospores	Present		Present		Present		Present	
Spore position	Central		Central		Central		Central	
Pigmentation	Cream		cream		Cream		Cream	
Catalase reaction	+		+		+		+	
Coagulase test	+		+		+		+	
Motility test	+		+		+		+	
Indole test	+		+		+		+	
H_2S production	-		-		-		-	
Starch hydrolysis	+		-		+		-	
Growth in 5% Nacl	+		+		+		+	
10% Nacl	+		+		+		+	
15% Nacl	-		-		-		-	
20% Nacl	-		-		-		-	
O/F medium	O	F	O	F	O	F	O	F

Sucrose	+	+	+	+	+	+	+	+
Rhamnose	+	+	+	-	+	+	+	-
Lactose	+	+	+	-	+	+	+	+
Glucose	+	+	+	+	+	+	+	+
Mannitol	+	+	+	-	+	+	+	-
Fructose	+	+	-	-	+	+	-	-
Identity	***B.subtilis***		***B. pumilus***		***B.subtilis***		***B. pumilus***	

Key: + positive; - negative; TS001- test strain of *B. subtilis*, TS002- test strain *B. pumilus;* SX1BS- standard strain of *B. subtilis*,SX2BP - standard strains of *B. pumilus*; O F – oxidative and fermentative medium

4.2: Changes in temperature during fermentation with inoculated and uninoculated *P.africana,P.biglobosa and G.max* seeds.

Figure 2 shows that fermentation of *P.africana* seeds inoculated separately with consortia A and B, and that inoculated naturally started when the temperature was 35^{o}C. The fermentation got to the peak at 84h when the temperature was 48^{0}C, for seeds inoculated with the consortia. In contrast, the seeds undergoing natural fermentation attained the highest temperature of 47^{0}C in 98h. By 120h, temperature in all the fermentations had dropped to 24^{0}C .

Figure 3 shows that fermentation of *P.biglobosa* seeds inoculated separately with consortia A and B, and that inoculated naturally started when the temperature was 35^{o}C. The fermentation got to the peak at 48h when the temperature was 48^{0}C, for seeds inoculated with the consortia. In contrast, the seeds undergoing natural fermentation attained the highest

temperature of 48^0C in 72h. By 96h, temperature in all the fermentations had dropped to 22^0C .

Figure 4 shows that fermentation of *G.max* seeds inoculated separately with consortia A and B, and that inoculated naturally started when the temperature was 35^oC. The fermentation got to the peak at 48h when the temperature was 48^0C, for seeds inoculated with the consortia. In contrast, the seeds undergoing natural fermentation attained the highest temperature of 48^0C in 72h. By 96h, temperature in all the fermentations had dropped to 20^0C .

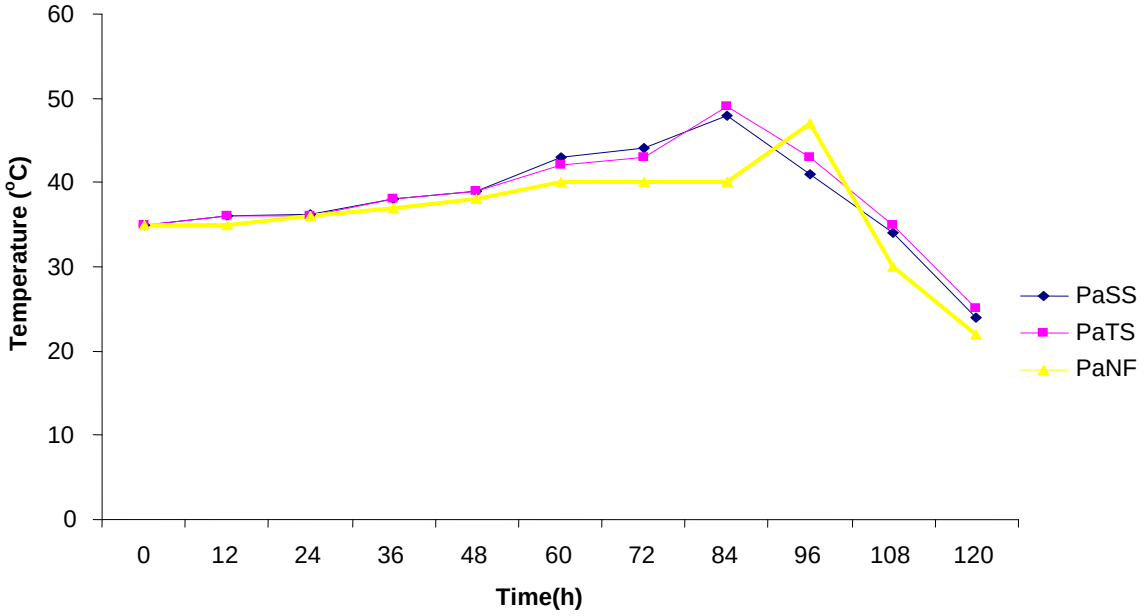


Figure 2: Temperature changes(during natural and inoculated fermentations of *P. africana* seeds

Key

PaSS- *P.africana* seeds fermented with mixed culture of a mixture of standard strain of *B.subtilis* and *B.pumilus* (consortium A); PaTS- *P.africana* seeds fermented with mixture of test strain of *B.subtilis* and *B.pumilus* (consortium B); PaNF- *P.africana* seeds undergoing natural fermentation.

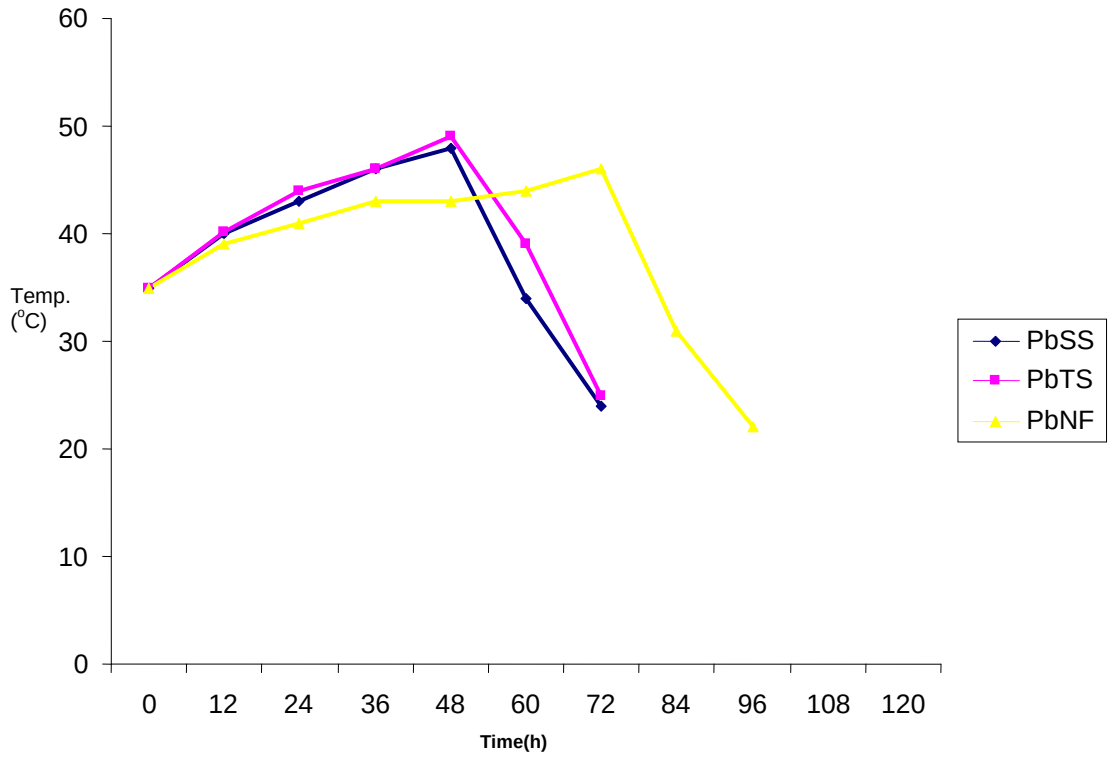


Figure 3: Temperature changes (during natural and inoculated fermentations of P.*biglobosa* seeds)

Key

PbSS- *P.biglobosa* seeds fermented with mixed culture of a mixture of standard strains of *B.subtilis* and *B.pumilus* (consortium A); PbTS- *P.biglobosa* seeds fermented with mixture of test strain of *B.subtilis* and *B.pumilus* (consortium B); PbNF- *P.biglobosa* seeds undergoing natural fermentation.

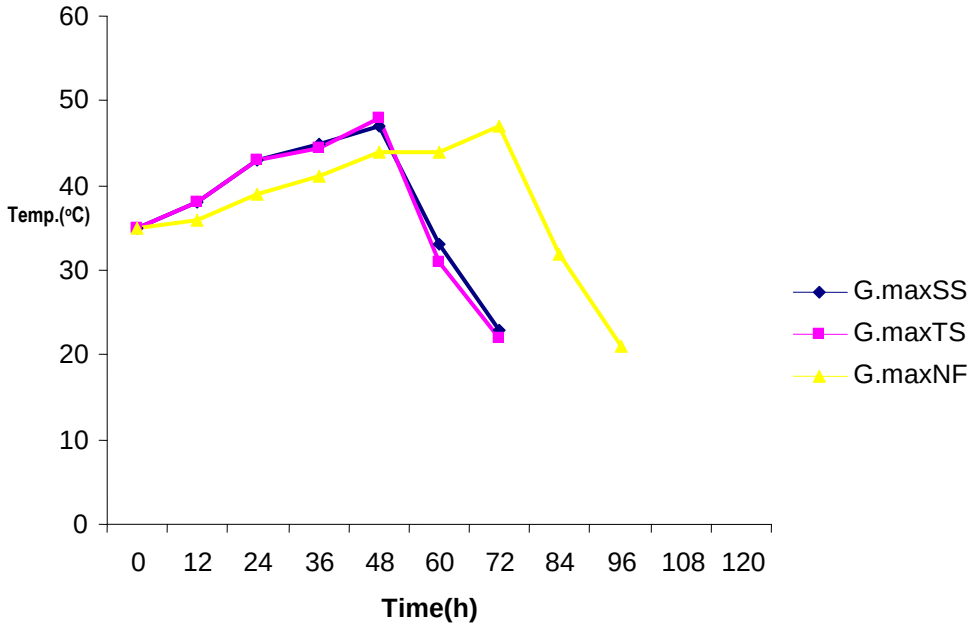


Figure 4 Temperature changes (during natural and inoculated fermentations of *G.max* seeds)

Key

GmSS- *G.max* seeds fermented with mixed culture of a mixture of standard strain of *B.subtilis* and *B.pumilus* (consortium A); GmTS- *G.max* seeds fermented with mixture of test strain of *B.subtilis* and *B.pumilus* (consortium B); GmNF- *G.max* seeds undergoing natural fermentation.

4.3: Changes in pH during fermentation with inoculated and uninoculated *P.africana,P.biglobosa and G.max* seeds.

In figure 5, fermentation of *P.africana* seeds shows that as fermentation time progressed there was a steady increase in pH from 6.3 in both consortia and the seeds without inocula. As fermentation got to its peak, pH rose to 7.9 with consortium B and 8.0 with consortium A. seeds without inocula at 48h, pH rose to 8.0 and didn't drop up to 120h for all fermentations.

Figure 6, shows that in fermentation of *G.max* seeds, as fermentation time progressed there was a steady increase in pH from 6.3 in both consortia and the seeds without inocula. As fermentation got to its peak, pH rose to 8.1 with consortium A and B at 24-72h. Seeds without inocula at 72h, pH rose to 7.8 and didn't drop up to 120h for all fermentations.

Figure 7, shows that in fermentation of *P.biglobosa* seeds seeds, as fermentation time progressed there was a steady increase in pH from 6.3 in both consortia and the seeds without inocula. As fermentation got to its peak, pH rose to 8.2 with consortium A and B at 24-72h. Seeds without inocula at 72h, pH rose to 8.0 and didn't drop up to 120h for all fermentations.

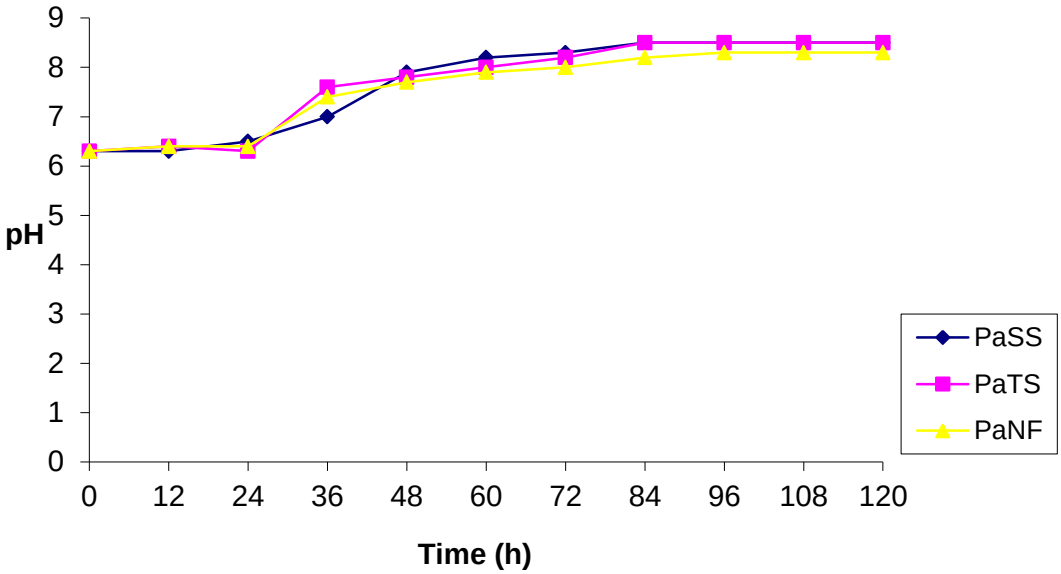


Figure 5 pH changes (during natural and inoculated fermentations of *P.africana* seeds)

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Key

PaSS- *P.africana* seeds fermented with a mixed culture of a mixture standard strain of *B.subtilis* and *B.pumilus* (consortium A); PaTS- *P.africana* seeds fermented with mixture of test strain of *B.subtilis* and *B.pumilus* (consortium B); PaNF- *P.africana* seeds undergoing natural fermentation.

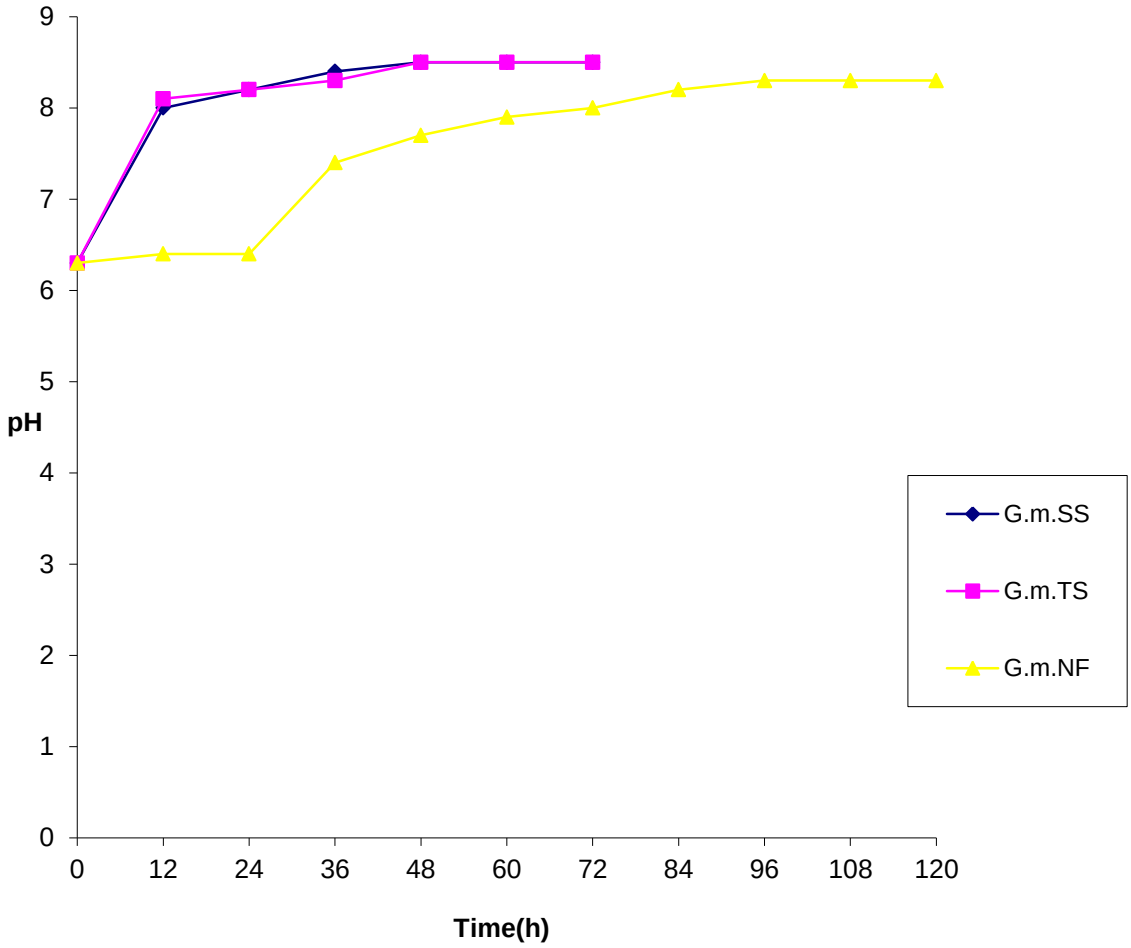

Figure 6 pH changes (during natural and inoculated fermertation of *G.max* seeds)

Key:

GmSS- *G.max* seeds fermented with a mixed culture of a mixture of standard strain of *B.subtilis* and *B.pumilus* (consortium A); GmTS- *G.max* seeds fermented with mixture of test strain of *B.subtilis* and *B.pumilus* (consortium B); GmNF- *G.max* seeds undergoing natural fermentation.

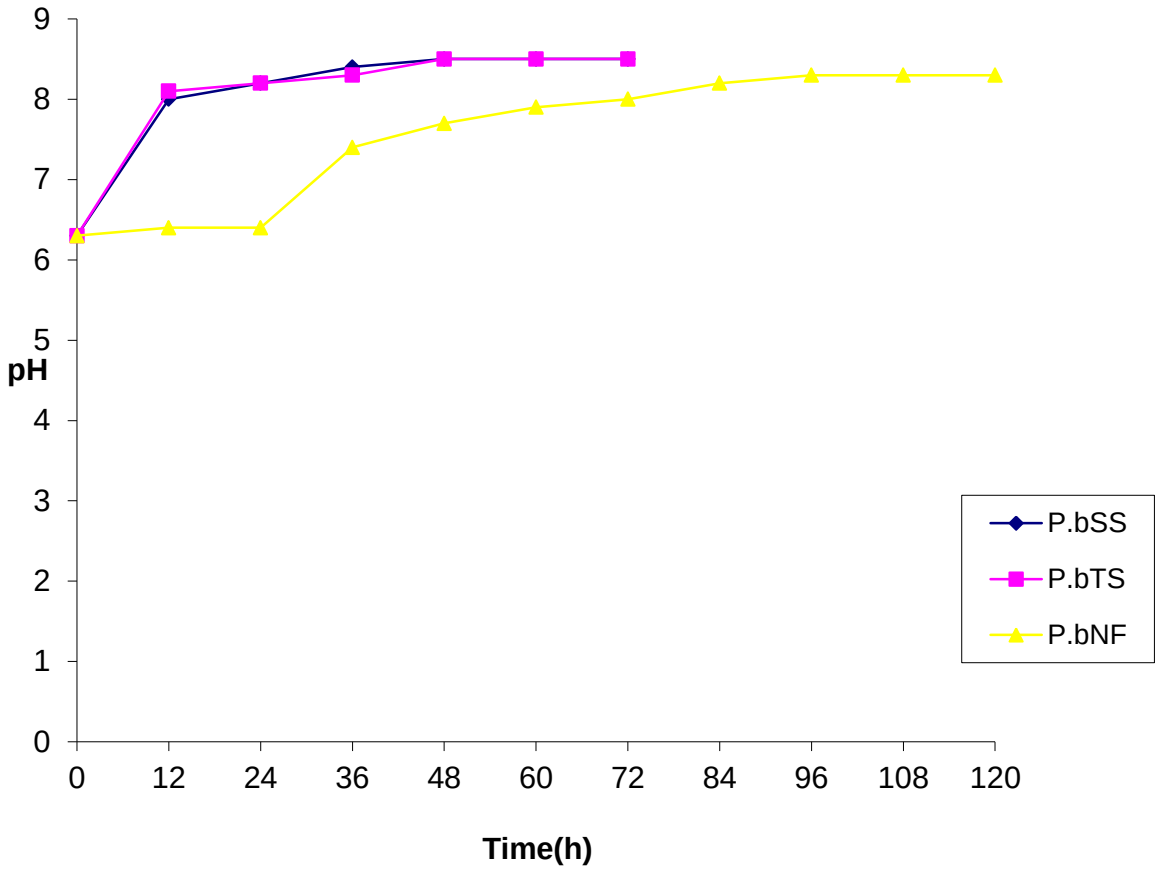

Figure 7 pH changes (during natural and inoculated fermentation of *P.biglobosa* seeds)

Key

PbSS- *P.biglobosa* seeds fermented with a mixed culture of a mixture of standard strains of *B.subtilis* and *B.pumilus* (consortium A); PbTS- *G.P.biglobosa* seeds fermented with a mixture of test strain of *B.subtilis* and *B.pumilus* (consortium B); PbNF- *P.biglobosa* seeds undergoing natural fermentation.

4.4: Growth of bacteria during fermentation of *P. africana, P.biglobosa* and *G.max* seeds with or without starter cultures.

Tables 4.2, 4.3 and 4.4 show that at time 0h of fermentation of For *P. africana* fermenting mash, counts at 0h, was low 2.1×10^{7} for all seeds with inocula and 1.6×10^{7} for seeds undergoing natural fermentation. As fermentation time progressed, seeds with inocula got to the peak at 84h with counts of 3.5×10^{8} and 3.7×10^{8} respectively for consortium A and B. For *P. africana* seeds undergoing natural fermentation, fermentation got to the peak at 96h with counts of 3.5×10^{8}. At 96h-132h, bacterial counts dropped drastically. As the time progresses fermenting mashes started producing a foul odour for both consortia and seeds without inocular.

P. biglobosa and *G. max* with inocular, count got to the peak at 48h and seeds undergoing natural fermentation, counts got to the peak at 72h. As fermentation time keeps progressing, fermenting mashes started producing foul odour.

Table 4.2: Growth of bacteria during fermentation of *P. africana*, with or without starter cultures.

Time (h)	Bacterial counts (cfu/g) with inocula Consortium A *P. africana* (cfu/g)	Consortium B *P. africana* (cfu/g)	Bacterial counts (cfu/g) with natural fermentation *P. africana* (cfu/g)	PDA (cfu/g)
0	2.1×10^{7}	2.1×10^{7}	1.6×10^{7}	NG
12	2.1×10^{6}	2.1×10^{6}	1.9×10^{6}	NG
24	2.3×10^{6}	2.3×10^{6}	1.9×10^{6}	NG
36	2.7×10^{7}	2.8×10^{7}	2.0×10^{6}	NG
48	3.0×10^{7}	3.1×10^{7}	2.6×10^{7}	NG
60	3.3×10^{7}	3.4×10^{7}	3.0×10^{7}	NG
72	3.3×10^{7}	3.4×10^{7}	3.3×10^{7}	NG
84	3.5×10^{8}	3.7×10^{8}	3.3×10^{7}	NG
96	3.7×10^{7}	3.7×10^{7}	3.5×10^{8}	NG
108	3.7×10^{7}	4.0×10^{7}	3.7×10^{7}	NG
120	3.9×10^{6}	4.0×10^{6}	3.7×10^{6}	NG
132	FO	FO	3.7×10^{6}	NG

Values are means of triplicate determinations.

Key- FO – foul odour; NG – no growth

Table 4.3: Growth of bacteria during fermentation of *P.biglobosa,* with or without starter cultures.

	Bacterial counts (cfu/g) with inocula		Bacterial counts (cfu/g) with natural fermentation	
	Consortium A	Consortium B		
Time (h)	*P. biglobosa*	*P. biglobosa*	*P. biglobosa*	
	(cfu/g)	(cfu/g)	(cfu/g)	PDA (cfu/g)
0	2.1×10^{7}	2.1×10^{7}	1.6×10^{7}	NG
12	2.8×10^{6}	2.9×10^{6}	1.9×10^{6}	NG
24	3.8×10^{7}	3.1×10^{7}	2.0×10^{6}	NG
36	3.3×10^{7}	3.5×10^{7}	2.9×10^{7}	NG
48	3.3×10^{8}	4.0×10^{8}	3.0×10^{7}	NG
60	3.4×10^{6}	4.1×10^{7}	3.5×10^{7}	NG
72	3.4×10^{6}	4.1×10^{7}	3.7×10^{8}	NG
84	FO	FO	3.8×10^{6}	NG
96	FO	FO	3.6×10^{5}	NG
108	FO	FO	FO	NG
120	FO	FO	FO	NG
132	FO	FO	FO	NG

Values are means of triplicate determinations.

Key- FO – foul odour; NG – no growth

Table 4.4: Growth of bacteria during fermentation of *G. max*, with or without starter cultures.

Time (h)	Bacterial counts (cfu/g) with inocula		Bacterial counts (cfu/g) with natural fermentation	
	Consortium A	Consortium B		
	G. max	*G. max*	*G. max*	
	(cfu/g)	(cfu/g)	(cfu/g)	PDA (cfu/g)
0	2.1×10^{7}	2.1×10^{7}	1.8×10^{7}	NG
12	2.5×10^{6}	2.3×10^{6}	2.0×10^{6}	NG
24	2.6×10^{7}	2.7×10^{7}	2.4×10^{6}	NG
36	2.7×10^{7}	3.0×10^{7}	2.9×10^{7}	NG
48	3.4×10^{8}	3.3×10^{8}	3.0×10^{7}	NG
60	3.3×10^{7}	3.4×10^{7}	3.5×10^{7}	NG
72	3.5×10^{6}	3.6×10^{5}	3.9×10^{8}	NG
84	FO	FO	3.9×10^{7}	NG
96	FO	FO	3.9×10^{6}	NG
108	FO	FO	FO	NG
120	FO	FO	FO	NG
132	FO	FO	FO	NG

Values are means of triplicate determinations.

Key- FO – foul odour; NG – no growth

4.5: Physical appearance and sensory properties of unfermented naturally fermented and consortia fermented seeds of *P.africana, P.biglobosa* and *G.max*.

Unfermented seeds of *P.biglobosa* and *G.max* were hard in texture and had uniform brown colour. They have grey outer convering and no pungent smell. Unfermented seeds of *P.africana* were also hard in texture, deep brown in colour with no pungent smell and grayish outer covering.

Physical appearance of *P.biglobosa* fermented with consortia A and B for two days was soft in texture, brownish colour with greyish outer covering, and an ammoniacal smell. *P.africana* seeds fermented with consortia A and B were within five days, soft in texture, black in colour with no greyish outer covering and gave out a strong ammoniacal smell. Physical characteristics with *G.max* seeds fermented with consortia A and B was within two days and were also soft in texture, brown in colour like that of *P.biglobosa* with greyish outer covering and a pungent smell. Seeds of *P.biglobosa* and *G.max* subjected to natural fermentation fermented within three days giving same characteristics with those fermented with consortia. *P. africana* seeds fermented naturally within six days giving same characteristics with those fermented with consortia.

4.6: Bacterial plate counts from samples of freshly fermented *P.africana, P.biglobosa* and *G.max* from Sabon gari market, Zaria.

Bacterial counts from condiments of *P.africana, P.biglobosa* and *G.max* obtained from local markets shows that mean bacterial counts were highest in condiments of P.africana which had a mean value of 6.1×10^{8}. Lowest bacterial mean counts was observed in condiments of P.biglobosa with counts of 5.7×10^{8} . There was no mould growth on PDA. (Table 4.5).

Table 4.5: Bacterial plate counts from samples of freshly fermented *P.africana, P.biglobosa* and *G.max* from Sabon gari market, Zaria.

Samples	Plate counts(cfu/g)	Plate counts(cfu/g)
P.africana	6.1×10^{8}	NG
P.biglobosa	$5.7.1\times10^{8}$	NG
G.max	6.0×10^{8}	NG

Key

NG- no growth.

4.7 Proximate composition of unfermented, naturally fermented and starter fermented seeds of *P. africana, P. biglobosa* and *G. max*

Figure 8 shows that moisture content was high in all the unfermented seeds with values ranging from 60 – 70%.Crude lipid values were appreciable in all the seeds. However highest mean values exist in *P. biglobosa* and *G.max* seeds fermented with both consortia (FSTS2, FSTS3) with values of 27 and 23% respectively. Crude protein was also high in all the seeds, highest mean value of 43% was observed in FSSS1 and FSSS2. Crude fibre was within the range of 0.1 - 0.5% in all the seeds.Soluble carbohydrates was high in NFS5 and FSSS2 with values of 10 and 12% respectively.

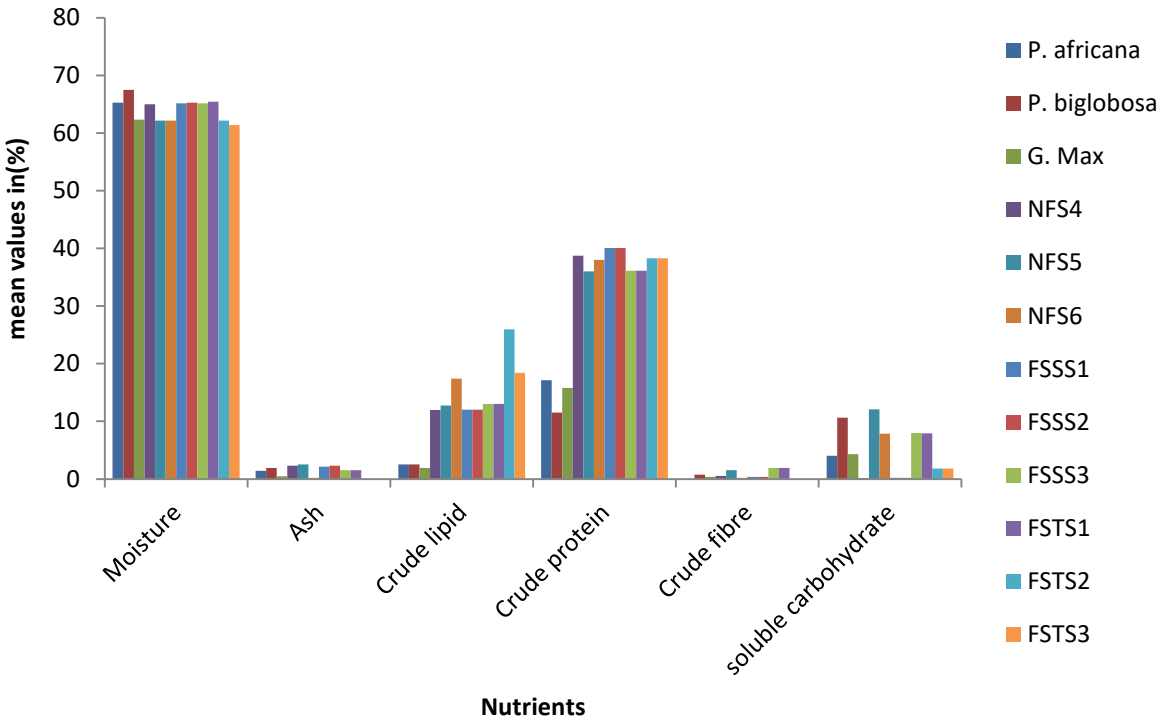

Figure 8: Proximate composition of unfermented, naturally fermented and starter fermented seeds of *P. africana*, *P. biglobosa* and *G. max*

Key

NFS4-Condiment from natural fermentation of *P.africana*; NFS5- Condiment from natural fermentation of *P.biglobosa*

NFS6-Condiments from natural fermentation of *G.max*; FSSS1- Condiment from fermentation of *P.africana* with consortium A (standard strains *B.subtilis* and *B.pumilus*); FSSS2- Condiment from fermentationof *P.biglobosa* with consortium A(standard strains *B.subtilis* and *B.pumilus*);; FSSS3- Condiment from fermentation of *G.max* with consortium A(standard strains *B.subtilis* and *B.pumilus*); FSTS1- Condiment from fermentationof *P.africana* with consortium B(test strains *B.subtilis* and *B.pumilus*) FSTS2- Condiment from fermentation of *P.biglobosa* with consortium B(test strains *B.subtilis* and *B.pumilus*); FSTS3- Condiment from fermentation of *G.max* with consortium B test strains *B.subtilis* and *B.pumilus*).

4.8: Mineral composition of unfermented, naturally fermented, and starter fermented seeds of *P. africana, P. biglobosa* and *G. max*

In figure 9, Iron was detected in the fermented seeds as well in the fermented under different conditions, but values were generally low and did not exceed 0.1%. Potassium was appreciable with highest mean value in starter assisted fermented seeds of *P.biglobosa* (FSSS2) which had a value of 12%. Calcium (Ca) had highest values of 42-43% FSTS3 and FSSS2 respectively. Sodium (Na) was high in seeds of FSTS2 and FSSS1 with values ranging from 13 – 14%. Only traces of Lead (Pb) were found in all the seeds.

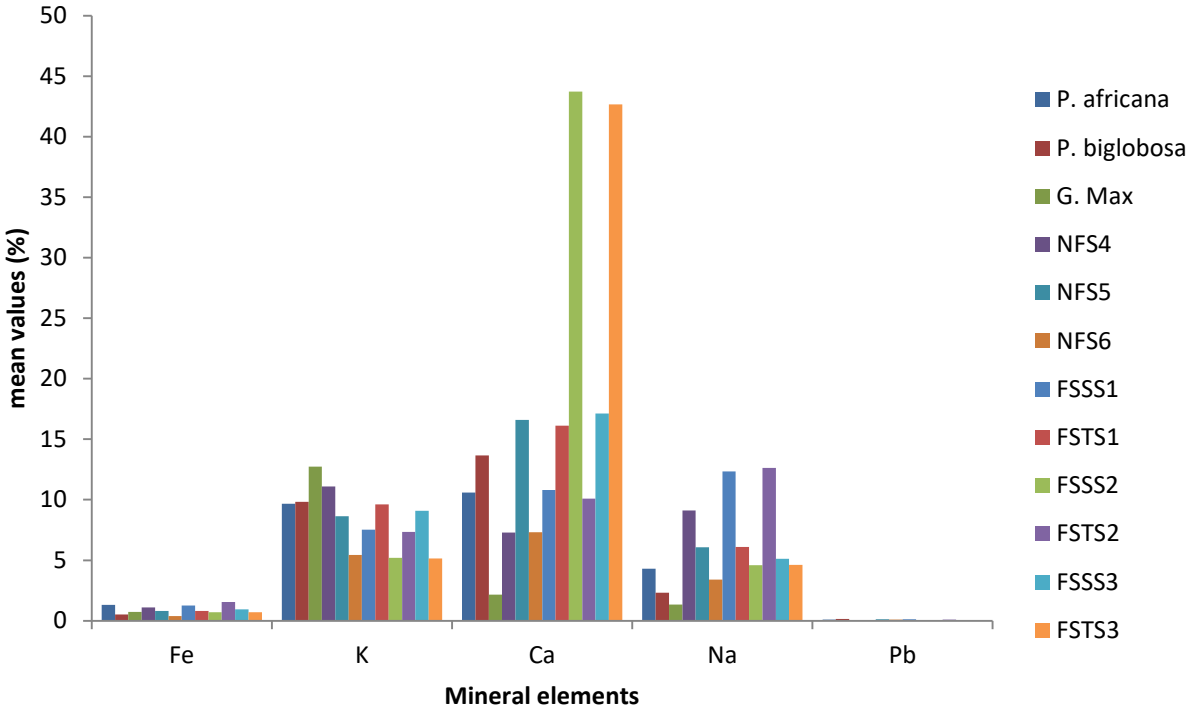

Figure 9: Mineral composition of unfermented, naturally fermented, and starter fermented seeds of *P. africana, P. biglobosa* and *G. max*

Key

NFS4-Condiment from natural fermentation of *P.africana*; NFS5- Condiment from natural fermentation of *P.biglobosa*

NFS6-Condiments from natural fermentation of *G.max*; FSSS1- Condiment from fermentation of *P.africana* with consortium A (standard strains *B.subtilis* and *B.pumilus*); FSSS2- Condiment from fermentationof *P.biglobosa* with consortium A(standard strains *B.subtilis* and *B.pumilus*);; FSSS3- Condiment from fermentation of *G.max* with consortium A(standard strains *B.subtilis* and *B.pumilus*); FSTS1- Condiment from fermentationof *P.africana* with consortium B(test strains *B.subtilis* and *B.pumilus*) FSTS2- Condiment from fermentation of *P.biglobosa* with consortium B(test strains *B.subtilis* and *B.pumilus*); FSTS3- Condiment from fermentation of *G.max* with consortium B test strains *B.subtilis* and *B.pumilus*).

4.9: Proximate composition of freshly fermented *P. africana, P. biglobosa* and *G. max* seeds from Sabon gari market, Zaria.

All the products have comparable levels of moisture with values ranging from 62 – 64%. Crude protein with values ranging from 35 – 37%. Crude lipid was high in *P.biglobosa* seeds with a value 15% as compared to other seeds. Ash content was highest in *G.max* seeds with a value of 62% crude fibre with a value of 9%. Soluble carbohydrates were highest in *P.africana* and *P.biglobosa* seeds with values ranging from 12% and 10% respectively.

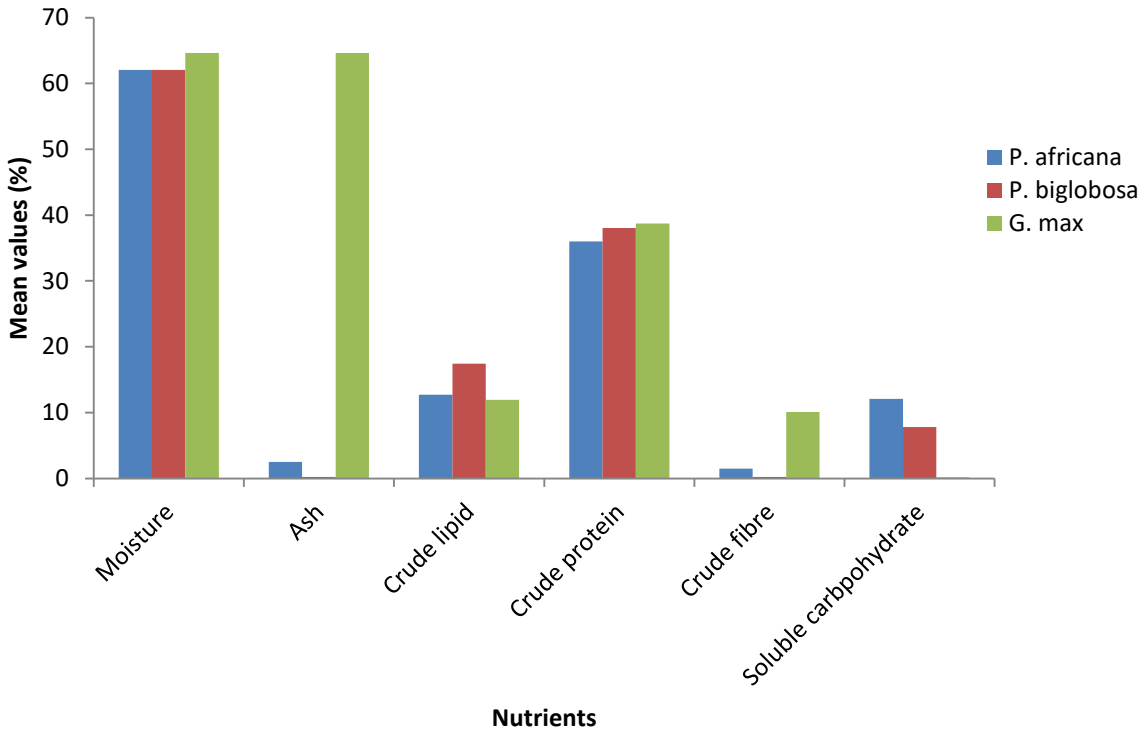

Figure 10: Proximate composition of freshly fermented *P. africana, P. biglobosa* and *G. max* seeds from Sabon gari market, Zaria

4.10: Mineral composition of fermented seeds of *P. africana, P. biglobosa* and *G. max* from Sabon gari market, Zaria.

Figure 11 shows that Iron (Fe) was detected but values were generally low. Potassium (K) had highest mean values in *P.biglobosa* seeds with 7%. Highest mean value of 43% calcium was observed in *G.max* seeds, followed by *P.biglobosa* with a mean value of 14%. Sodium (Na) was high in *P. africana* seeds with a mean value of 13%. Lead (Pb) content was very low.

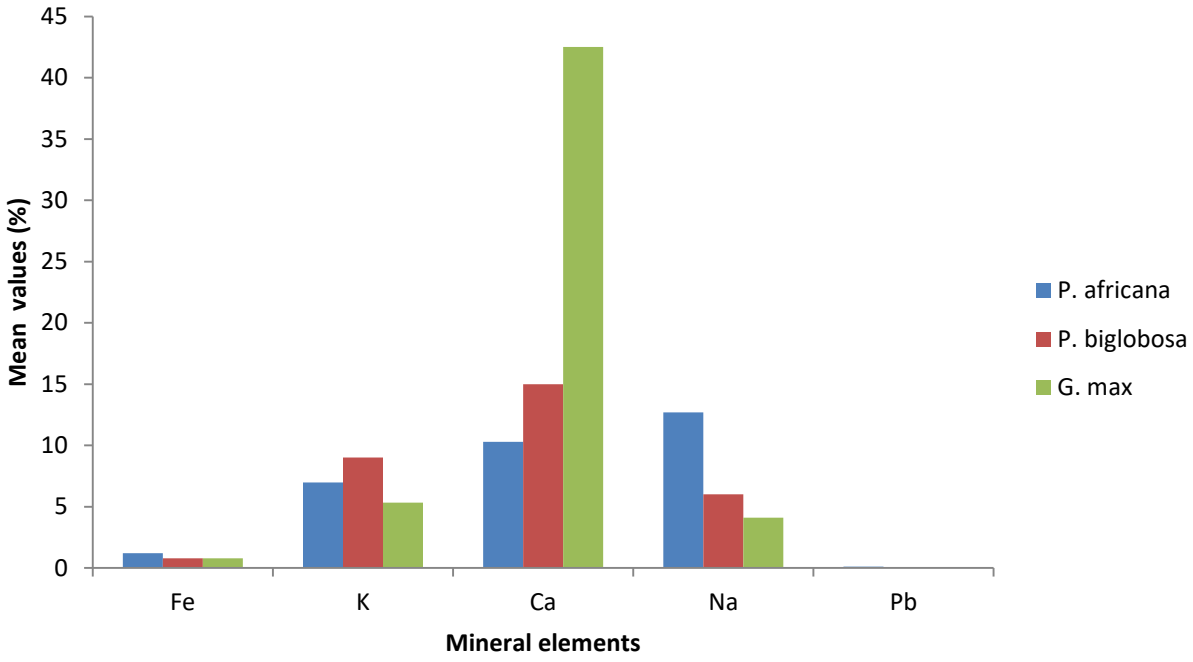

Figure 11: Mineral composition of fermented seeds of *P. africana, P. biglobosa* and *G. max* from Sabon gari market, Zaria.

4.11 : Effect of drying conditions on microbial population and physicochemical properties of fermented *P. africana*, *P. biglobosa* and *G. max* condiments with or without consortia before storage

Table 4.3 shows that effect of drying conditions on physicochemical properties of *P.africana*, *P.biglobosa* and *G.max* condiments fermented with or without consortia before storage showed that highest pH values were obtained with condiments of *P.africana* fermented with both consortia; mean pH value of 6.24 and 6.23 respectively subjected to oven dried condition. Peroxide value and titratable acid were relatively low in condiments of *P.africana*, *P.biglobosa* and *G.max* subjected to oven dried conditions. There was no moulds growth on potatoe dextrose agar. For market condiments pH was low, moisture, peroxide values, titratable acidity and bacteria count were relatively higher than others. There was no mould growth on potato dextrose agar.

Table 4 . 6 : Effect of drying conditions on pre-storage and physicochemical properties of *P. africana*, *P. biglobosa* and *G. max* fermented with or witout consortia.

Drying conditions	condiments	Means of fermentation	pH	Moisture (%)	Peroxide value(meq kg)	Titratable acidity(mg lactic acid g)	PCA (Cfu/g)	PDA (Cfu/g)
Solar	*P. africana*	consortium A	5.23±0.00	0.19±0.02	4.29±0.01	1.10±0.00	1.2×10^5	NG
	P. biglobosa		5.26±0.05	0.27±0.00	4.30±0.02	1.11±0.02	1.0×10^5	NG
	G. max		5.31±0.05	0.28±0.01	4.31±0.00	1.17±0.00	1.1×10^5	NG
	P. africana	consortium B	5.23±0.00	0.20±0.00	4.27±0.04	1.12±0.00	1.2×10^5	NG
	P. biglobosa		5.25±0.02	0.26±0.02	4.30±0.02	1.11±0.01	1.1×10^6	NG
	G. max		5.30±0.01	0.28±0.01	4.32±0.00	1.13±0.00	1.5×10^6	NG
	P. africana	NF	5.24±0.00	0.20±0.02	4.27±0.01	1.15±0.05	1.2×10^5	NG
	P. biglobosa		5.20±0.02	0.26±0.02	4.30±0.02	1.11±0.01	1.3×10^6	NG
	G. max		5.35±0.00	0.27±0.00	4.32±0.00	1.16±0.02	1.1×10^5	NG
Oven	*P. africana*	consortium A	6.24±0.00	0.21±0.02	4.18±0.01	1.10±0.05	1.4×10^5	NG
	P.biglobosa		5.19±0.02	0.20±0.02	4.20±0.02	1.10±0.00	1.0×10^6	NG
	G.max		5.35±0.05	0.21±0.00	4.20±0.00	1.10±0.00	1.1×10^5	NG
	P.africana	consortium B	6.23±0.00	0.19±0.01	4.10±0.04	1.10±0.02	1.0×10^5	NG
	P.biglobosa		5.55±0.02	0.20±0.05	4.19±0.02	1.10±0.00	1.1×10^6	NG
	G.max		5.38±0.00	0.22±0.01	4.20±0.01	1.10±0.01	1.1×10^5	NG
	P.africana	NF	5.29±0.00	0.20±0.00	4.20±0.00	1.10±0.04	1.0×10^5	NG
	P..biglobosa		5.55±0.02	0.21±0.03	4.20±0.04	1.10±0.00	1.0×10^6	NG
	G.max		5.38±0.00	0.20±0.01	4.21±0.00	1.10±0.01	1.1×10^6	NG
Vacuum	*P.africana*	consortiumA	5.20±0.00	0.20±0.02	4.30±0.00	1.10±0.00	1.1×10^5	NG
	P.biglobosa		5.25±0.02	0.24±0.01	4.33±0.00	1.11±0.01	1.2×10^6	NG
	G.max		5.36±0.00	0.24±0.00	4.30±0.01	1.11±0.02	1.3×10^6	NG
	P.africana	consortiumB	5.20±0.00	0.21±0.02	4.31±0.01	1.11±0.00	1.2×10^5	NG
	P.biglobosa		5.23±0.01	0.24±0.00	4.32±0.00	1.10±0.01	1.1×10^6	NG
	G.max		5.36±0.00	0.23±0.01	4.31±0.02	1.11±0.02	1.3×10^5	NG
	P.africana	NF	5.21±0.00	0.25±0.02	4.29±0.01	1.10±0.01	1.0×10^5	NG
	P.biglobosa		5.27±0.01	0.24±0.00	4.32±0.00	1.11±0.00	1.1×10^5	NG
	G.max		5.36±0.00	0.22±0.01	4.35±0.04	1.11±0.02	1.0×10^6	NG
Sun (N)	*P.africana*	consortiumA	5.23±0.05	0.27±0.05	4.33±0.02	1.11±0.03	1.4×10^5	NG
	P.biglobosa		5.21±0.05	0.27±0.05	4.29±0.02	1.12±0.03	1.2×10^6	NG
	G.max		5.25±0.02	0.28±0.02	4.31±0.01	1.15±0.00	1.5×10^5	NG
	P.africana	consortiumB	5.20±0.05	0.20±0.05	4.30±0.02	1.12±0.03	1.2×10^6	NG
	P.biglobosa		5.22±0.03	0.27±0.05	4.31±0.01	1.11±0.03	1.0×10^6	NG
	G.max		5.26±0.00	0.21±0.02	4.33±0.02	1.14±0.02	1.5×10^6	NG
	P.africana	NF	5.25±0.00	0.24±0.02	4.27±0.01	1.12±0.01	1.1×10^5	NG
	P.biglobosa		5.22±0.03	0.27±0.05	4.31±0.01	1.11±0.03	1.0×10^6	NG
	G.max		5.26±0.00	0.22±0.00	4.30±0.05	1.17±0.01	1.7×10^6	NG
Sun (O)	*P.africana*	consortiumA	5.23±0.05	0.20±0.05	4.32±0.02	1.12±0.03	1.3×10^6	NG
	P.biglobosa		5.21±0.00	0.25±0.01	4.33±0.02	1.13±0.03	1.1×10^6	NG
	G.max		5.24±0.01	0.28±0.02	4.31±0.00	1.15±0.02	1.5×10^6	NG
	P.africana	consortiumB	5.20±0.05	0.24±0.05	4.30±0.02	1.12±0.03	1.3×10^6	NG
	P.biglobosa		5.25±0.03	0.26±0.05	4.35±0.05	1.11±0.03	1.0×10^6	NG
	G.max		5.36±0.02	0.21±0.05	4.32±0.02	1.17±0.01	1.4×10^6	NG
	P.africana	NF	5.25±0.01	0.24±0.01	4.26±0.04	1.10±0.01	1.1×10^6	NG
	P.biglobosa		5.22±0.03	0.25±0.05	4.31±0.02	1.10±0.05	1.0×10^5	NG
	G.max		5.30±0.05	0.21±0.06	4.32±0.05	1.12±0.01	1.5×10^5	NG
Market samples	*P.africana*		4.25±0.01	1.26±0.00	5.20±0.04	1.20±0.04	1.8×10^6	NG
	P.biglobosa		4.01±0.03	1.29±0.02	5.31±0.02	1.22±0.05	1.7×10^6	NG
	G.max		5.10±0.05	1.27±0.00	5.40±0.05	1.39±0.01	1.9×10^6	NG

values are means of triplicate determinations

Key

Consortium A- seeds fermented with standard strains of *B.subtilis* and *B.pumilus*; consortium B-seeds fermented with test strains of *B.subtilis* and *B.pumilus;* NF-naturally fermented; PCA- plate count agar; PDA- potato dextrose agar; NG- no growth. Sun drying (O)- sundrying without net; Sun drying (N)- sundrying with net.

4.12: Effect of drying conditions on microbial populations and physicochemical properties of pre storage condiments fermented with or without consortia .

Physicochemical properties of condiments dried under different drying conditions showed that pH, peroxide value and bacteria counts on PCA were highly significant ($p<0.001$). No moulds were observed.

Duncan multiple range tests (DMRT) indicated that condiments subjected to oven drying condition was ranked the best among other drying conditions and was significantly different ($p <0.001$) from other drying conditions (Appendix 31) .

For condiments obtained from the market, pH, moisture, peroxide value, titratable acidity and bacteria counts were appreciable different. No mould growth on PDA plates.

4. 13: Effects of different modes of fermentation, drying and packaging on shelf life properties of powdered *P.africana* condiments at two months storage.

Tables 4.7 – 4.9 shows that after two months of storage of *P.africana* condiments produced with consortia and those produced produced by natural fermentation dried, and packaged under different conditions pH was highest in condiments produced and subjected to oven drying condition (OvRe) packaged in sachets, and stored at refrigeration temperature; with a mean pH value of 5.52 with consortium A. The lowest mean pH value of 4.23 occurred in condiments of *P.africana* from the market, with the lowest mean value being 4.23. Moisture content, peroxide value, titratable acidity, bacteria count on PCA was generally lowest in condiments produced in laboratory under oven drying conditions. Physicochemical properties of market condiments had highest mean values as compared to laboratory produced ones. Peroxide value, titratable acidity and bacteria counts on PCA were highly significant ($p<0.001$). No moulds were detected on potato dextrose agar for condiments produced in the laboratory under all the various conditions and for condiments obtained from the market. For condiments fermented with consortium B, peroxide value was highest in VaccRM packaged in both plastic and satchets with a value of 4.43. Titratable acidity was highest in SUNDRE and SUNRM packaged in both plastics and satchets. No mould growth was observed. In condiments with natural fermentation peroxide values were highest in VaccRM packaged in both plastics and sachet. Titratable acidity was also highest in SUNDRE and SUNRM packaged in both plastics and sachets. (Tables 4.7 - 4.9).

Table 4.7: Effects of drying and packaging conditions (at two months storage) on shelf life properties of *P.africana* condiments produced with consortium A.

Seeds	Means of Fermentation	Duration of storage(months)	Drying Conditions	packaging	Temperature (^{0}C) of storage	pH	Moisture (%)	Peroxide value(meq kg)	Titratable acidity(mg lactic acid g)	PCA (Cfu/g)	PDA(Cfu/g)
P. africana	Consortium A	2	SLRE	Sachet	8.64±0.30	5.11±0.00	0.23±0.02	4.30±0.01	1.10±0.01	1.2×10^5	NG
				plastic	8.65±0.33	5.03±0.03	0.26±0.00	4.32±0.03	1.11±0.00	1.3×10^5	NG
			SLRM	Sachet	27.66±0.01	5.13±0.01	0.26±0.03	4.33±0.34	1.10±0.00	1.0×10^5	NG
				Plastic	27.67±0.31	5.11±0.00	0.25±0.03	4.61±0.01	1.10±0.00	1.2×10^5	NG
			VaccRE	Sachet	8.67±0.33	5.23±0.03	0.30±0.06	4.42±0.00	1.11±0.01	1.2×10^5	NG
				plastic	8.01±0.00	5.26±0.03	0.31±0.06	4.43±0.02	1.13±0.01	1.4×10^5	NG
			VaccRM	Sachet	27.67±0.33	5.52±0.03	0.30±0.06	4.42±0.01	1.12±0.01	1.0×10^4	NG
				Plastic	27.67±0.33	5.37±0.03	0.30±0.06	4.43±0.04	1.12±0.01	1.6×10^6	NG
			OVRe	Sachet	8.00±0.00	5.45±0.06	0.10±0.00	4.27±0.01	1.10±0.03	1.0×10^4	NG
				plastic	9.00±0.00	5.40±0.04	0.11±0.01	4.28±0.00	1.11±0.02	1.2×10^5	NG
			OVRM	Sachet	27.33±0.33	5.43±0.04	0.10±0.00	4.29±0.01	1.10±0.04	1.0×10^4	NG
				Plastic	27.32±0.33	5.43±0.02	0.10±0.02	4.29±0.00	1.11±0.03	1.1×10^6	NG
			SUCRE	Sachet	8.67±0.33	5.23±0.01	0.30±0.06	4.32±0.01	1.11±0.00	1.4×10^5	NG
				plastic	8.77±0.32	5.10±0.04	0.31±0.04	4.32±0.00	1.13±0.01	1.5×10^6	NG
			SUCRM	Sachet	28.00±0.00	5.07±0.03	0.31±0.02	4.32±0.02	1.10±0.00	1.2×10^5	NG
				Plastic	29.00±0.02	5.11±0.00	0.31±0.06	4.32±0.01	1.11±0.02	1.5×10^6	NG
			SUDRE	Sachet	8.33±0.33	5.30±0.06	0.27±0.03	4.32±0.01	1.10±0.01	1.7×10^6	NG
				plastic	8.32±0.31	5.10±0.06	0.28±0.03	4.33±0.01	1.13±0.04	1.5×10^6	NG
			SUDRM	Sachet	27.67±0.33	5.22±0.07	0.27±0.03	4.31±0.01	1.12±0.01	1.3×10^6	NG
				Plastic	27.67±0.33	5.20±0.00	0.29±0.03	4.31±0.01	1.12±0.00	1.6×10^6	NG
			MarRE	Sachet	9.00±0.00	5.00±0.06	1.17±0.09	6.11±0.00	1.13±0.01	1.9×10^5	NG
				plastic	9.00±0.00	4.60±0.10	1.13±0.07	6.11±0.01	1.14±0.01	2.1×10^6	NG
			MarRM	Sachet	27.33±0.33	5.01±0.32	1.12±0.06	6.11±0.00	1.14±0.00	1.7×10^6	NG
				Plastic	27.33±0.33	4.23±0.33	1.13±0.04	6.12±0.01	1.18±0.03	2.2×10^7	NG

Values are means of triplicate determinations.

Key

SLRE-solar dried refrigerator; SLRM-solar dried room temperature; VaccRM- vacuum dried room temperature; VaccRE –vacuum dried refrigerator;OVRE- oven dried refrigerator; OvRM- oven dried room temperature; SUCRE- sun dried with cover refrigeration; SUCRM – sun dried with cover room temperature; SUDRE- sun dried direct refrigeration; SUDRM-sun dried direct room temperature;MarRE- market sample refrigeration; MaRM- market sample room temperature; PCA- plate count agar; PDA-potato dextrose agar; NG- no growth.

Table 4.8: Effects of drying and packaging conditions (at two months storage) on shelf life properties of *P.africana* condiments produced with consortium B.

Seeds	M eans of Fermentation	Duration of storage(months)	Drying Condition	Packaging	Temperature (^{0}C) of storage	pH	Moisture (%)	Peroxide value(meq kg)	Titratable acidity(mg lactic acid g)	PCA (Cfu/g)	PDA(Cfu/g)
P.africana	Consortium B	2	SLRE	Sachet	8.64±0.31	5.51±0.02	0.24±0.03	4.30±0.00	1.10±0.00	1.1×10^5 1.1×10^5	NG
				Plastic	8.65±0.33	5.03±0.03	0.26±0.02	4.32±0.00	1.12±0.00		NG
			SLRM	Sachet	27.66±0.00	5.13±0.01	0.26±0.03	4.63±0.34	1.10±0.00	1.0×10^5	NG
				Plastic	27.67±0.31	5.10±0.00	0.26±0.03	4.31±0.01	1.10±0.00	1.2×10^5	NG
			VaccRE	Sachet	8.67±0.33	5.23±0.03	0.30±0.06	4.42±0.02	1.11±0.01	1.3×10^5	NG
				Plastic	8.01±0.00	5.26±0.03	0.30±0.06	4.43±0.02	1.12±0.01	1.0×10^5	NG
			VaccRM	Sachet	27.67±0.33	5.23±0.03	0.30±0.06	4.43±0.01	1.12±0.01	1.0×10^5	NG
				Plastic	27.67±0.33	5.27±0.03	0.30±0.06	4.43±0.01	1.12±0.01	1.6×10^5	NG
			OVRe	Sachet	8.00±0.00	5.50±0.06	0.10±0.00	4.29±0.00	1.09±0.03	1.1×10^4	NG
				Plastic	9.00±0.00	5.53±0.03	0.10±0.00	4.29±0.00	1.10±0.03	1.0×10^4	NG
			OVRM	Sachet	27.33±0.33	5.63±0.03	0.10±0.00	4.29±0.01	1.10±0.03	1.0×10^4	NG
				Plastic	27.32±0.33	5.53±0.02	0.10±0.00	4.29±0.00	1.10±0.03	1.1×10^4	NG
			SUCRE	Sachet	8.67±0.33	5.03±0.01	0.30±0.06	4.32±0.01	1.11±0.00	1.4×10^6	NG
				Plastic	8.67±0.33	5.10±0.06	0.31±0.06	4.32±0.00	1.11±0.01	1.4×10^5	NG
			SUCRM	Sachet	28.00±0.00	5.07±0.03	0.31±0.06	4.32±0.02	1.11±0.00	1.4×10^6	NG
				Plastic	28.00±0.01	5.51±0.00	0.31±0.06	4.32±0.01	1.11±0.02	1.5×10^6	NG
			SUDRE	Sachet	8.33±0.33	5.30±0.06	0.27±0.03	4.32±0.01	1.12±0.01	1.7×10^6	NG
				Plastic	8.32±0.31	5.10±0.06	0.26±0.03	4.33±0.01	1.12±0.01	1.5×10^6	NG
			SUDRM	Sachet	27.67±0.33	5.22±0.07	0.27±0.03	4.31±0.01	1.12±0.01	1.3×10^6	NG
				Plastic	27.67±0.33	5.20±0.00	0.27±0.03	4.31±0.01	1.12±0.01	1.4×10^6	NG

Values are means of triplicate determinations

Key
SLRE-solar dried refrigerator; SLRM-solar dried room temperature; VaccRM- vacuum dried room temperature; VaccRE –vacuum dried refrigerator;OVRE- oven dried refrigerator; OvRM- oven dried room temperature; SUCRE- sun dried with cover refrigeration; SUCRM – sun dried with cover room temperature; SUDRE- sun dried direct refrigeration; SUDRM-sun dried direct room temperature;; PCA- plate count agar; PDA-potato dextrose agar NG – no growth.

Table 4.9: Effects of drying and packaging conditions (at two months storage) on shelf life properties of *P.africana* condiments produced without consortia.

Seeds	Means of Fermentation	Duration of storage(months)	Drying Condition	Packaging	Temperature (^{0}C) of storage	pH	Moisture (%)	Peroxide value(meq kg)	Titratable acidity(mg lactic acid g)	PCA (Cfu/g)	PDA (Cfu/g)
P.africana	NF	2	SLRE	Sachet	8.67±0.33	5.53±0.03	0.29±0.03	4.30±0.00	1.11±0.00	1.0×10^5	NG
				plastic	8.67±0.33	5.03±0.03	0.29±0.03	4.30±0.00	1.12±0.00	1.0×10^5	NG
			SLRM	Sachet	27.67±0.00	5.43±0.03	0.27±0.03	4.64±0.34	1.10±0.00	1.0×10^6	NG
				Plastic	27.67±0.33	5.10±0.00	0.27±0.03	4.31±0.01	1.11±0.00	1.1×10^6	NG
			VaccRE	Sachet	8.67±0.33	5.33±0.03	0.30±0.06	4.43±0.02	1.12±0.01	1.0×10^5	NG
				plastic	8.00±0.00	5.37±0.03	0.30±0.06	4.43±0.02	1.12±0.01	1.1×10^6	NG
			VaccRM	Sachet	27.67±0.33	5.53±0.03	0.30±0.06	4.43±0.01	1.12±0.01	1.0×10^5	NG
				Plastic	27.67±0.33	5.27±0.03	0.30±0.06	4.43±0.01	1.12±0.01	1.2×10^5	NG
			OvRe	Sachet	8.00±0.00	5.50±0.06	0.10±0.00	4.28±0.00	1.10±0.03	1.0×10^4	NG
				plastic	8.00±0.00	5.43±0.03	0.10±0.00	4.29±0.00	1.10±0.03	1.0×10^4	NG
			OVRM	Sachet	27.33±0.33	5.40±0.03	0.10±0.00	4.29±0.01	1.11±0.03	1.0×10^4	NG
				Plastic	27.33±0.33	5.55±0.03	0.10±0.00	4.29±0.00	1.10±0.03	1.1×10^4	NG
			SUCRE	Sachet	8.67±0.33	5.03±0.03	0.30±0.06	4.32±0.01	1.11±0.00	1.2×10^5	NG
				plastic	8.67±0.33	5.10±0.06	0.30±0.06	4.33±0.01	1.11±0.00	1.1×10^5	NG
			SUCRM	Sachet	28.00±0.00	5.07±0.03	0.30±0.06	4.32±0.01	1.11±0.00	1.0×10^5	NG
				Plastic	28.00±0.00	5.50±0.00	0.30±0.06	4.32±0.01	1.11±0.00	1.1×10^5	NG
			SUDRE	Sachet	8.33±0.33	5.30±0.06	0.27±0.03	4.32±0.01	1.12±0.01	1.2×10^6	NG
				plastic	8.33±0.33	5.20±0.06	0.27±0.03	4.32±0.01	1.12±0.01	1.1×10^6	NG
			SUDRM	Sachet	27.67±0.33	5.23±0.07	0.27±0.03	4.31±0.01	1.12±0.01	1.1×10^6	NG
				Plastic	27.67±0.33	5.20±0.06	0.27±0.03	4.31±0.01	1.12±0.01	1.2×10^6	NG

Values are means of triplicate determinations

Key

SLRE-solar dried refrigerator; SLRM-solar dried room temperature; VaccRM- vacuum dried room temperature; VaccRE –vacuum dried refrigerator;OVRE- oven dried refrigerator; OvRM- oven dried room temperature; SUCRE-sun dried with cover refrigeration; S UCRM – sun dried with cover room temperature; SUDRE- sun dried direct refrigeration; SUDRM-sun dried direct room temperature; PCA- plate count agar; PDA-potato dextrose agar; NG – no growth.

4.14: Effects of different modes of fermentation, drying and packaging on shelf life properties of powdered *P.africana* condiments at four months storage

P.africana condiments produced in the laboratory and stored for four months showed increase in moisture content, peroxide value and titratable acidity compared with values obtained after two months storage. Plastic packaging yielded consistently higher values regardless of the method of drying. Also bacteria counts on PCA were higher under plastic packaging than sachets. Highest mean values for moisture content and peroxide value for laboratory produced *P.africana* condiments after four months were 6.37 and 10.57 respectively for sundried covered with net at room temperature (SUCRM) for consortium A. For *P.africana* condiments produced with consortium B and by natural fermentation, peroxide value, titratable acidity, bacterial counts on PCA were also higher. Condiments of *P.africana* purchased from the market and stored for four months at room temperature showed significant increases in physicochemical properties as compared to laboratory produced condiments. There was no mould growth on potato dextrose agar for both laboratory and market purchased condiments of *P.africana*. (Tables 4.10- 4.12).

Table 4.10 : Effects of drying and packaging conditions (at four months storage) on shelf life properties of *P.africana* condiments produced with consortium A.

Seeds	Means of Fermentation	Duration of storage(months)	Drying Condition	Packaging	Temperature (^{0}C) of storage	pH	Moisture (%)	Peroxide value(meq kg)	Titratable acidity(mg lactic acid g)	PCA (Cfu/g)	PDA (Cfu/g)
P.africana	Consortium A	4	SLRE	Sachet	9.67±0.33	5.67±0.03	0.30±0.06	4.50±0.00	1.11±0.00	1.2×10^5	NG
				Plastic	9.67±0.33	5.17±0.03	3.07±0.07	7.17±0.03	1.21±0.00	1.2×10^6	NG
			SLRM	Sachet	29.67±0.33	5.60±0.00	0.37±0.03	4.71±0.03	1.11±0.00	1.1×10^5	NG
				Plastic	29.67±0.33	5.53±0.03	4.33±0.12	7.37±0.13	1.30±0.00	1.4×10^6	NG
			VaccRE	Sachet	8.67±0.33	5.83±0.03	0.27±0.03	4.51±0.00	1.11±0.00	1.2×10^5	NG
				plastic	8.67±0.33	5.07±0.03	4.07±0.03	7.27±0.03	1.21±0.00	1.4×10^6	NG
			VaccRM	Sachet	27.33±0.33	5.67±0.07	0.37±0.03	4.60±0.01	1.12±0.00	1.0×10^5	NG
				Plastic	27.33±0.33	5.10±0.00	4.87±0.03	7.97±0.07	1.27±0.00	1.4×10^6	NG
			OvRE	Sachet	8.33±0.33	6.10±0.03	0.23±0.03	4.34±0.01	1.10±0.00	1.0×10^4	NG
				Plastic	8.33±0.33	5.13±0.03	3.77±0.03	7.50±0.00	1.23±0.00	1.1×10^4	NG
			OVRM	Sachet	27.33±0.33	5.43±0.03	0.23±0.03	4.35±0.00	1.11±0.00	1.0×10^5	NG
				Plastic	27.33±0.33	5.41±0.03	4.10±0.06	7.80±0.10	1.20±0.00	1.2×10^5	NG
			SUCRE	Sachet	8.33±0.33	5.40±0.06	0.43±0.03	4.38±0.01	1.11±0.00	1.2×10^6	NG
				Plastic	8.33±0.33	5.53±0.03	4.53±0.03	9.50±0.00	1.31±0.00	1.5×10^6	NG
			SUCRM	Sachet	28.00±0.00	5.37±0.03	0.47±0.07	4.36±0.01	1.11±0.00	1.3×10^5	NG
				plastic	28.00±0.00	5.73±0.03	6.17±0.03	10.57±0.03	1.36±0.00	1.6×10^6	NG
			SUDRE	Sachet	8.33±0.33	5.53±0.03	0.43±0.03	4.37±0.03	1.11±0.00	1.2×10^5	NG
				plastic	8.33±0.33	5.67±0.03	6.37±0.03	10.43±0.03	1.30±0.00	1.7×10^6	NG
			SUDRM	Sachet	27.67±0.33	5.20±0.06	0.37±0.03	4.31±0.00	1.11±0.00	1.2×10^5	NG
				Plastic	27.67±0.33	5.53±0.03	6.27±0.03	4.00±0.00	1.37±0.00	1.6×10^6	NG
			MarRE	Sachet	8.67±0.33	4.10±0.06	1.27±0.03	9.20±0.06	1.14±0.01	1.9×10^5	NG
				plastic	8.67±0.33	4.03±0.32	8.00±0.00	14.57±0.03	1.30±0.00	2.9×10^6	NG
			MarRM	Sachet	28.67±0.33	4.10±0.37	1.57±0.07	9.53±0.03	1.15±0.00	1.8×10^6	NG
				plastic	28.67±0.33	4.10±0.40	8.10±0.06	18.10±0.00	1.38±0.00	3.0×10^6	NG

Values are means of triplicate determinations

Key

SLRE-solar dried refrigerator; SLRM-solar dried room temperature; VaccRM- vacuum dried room temperature; VaccRE –vacuum dried refrigerator;OVRE- oven dried refrigerator; OvRM- oven dried room temperature; SUCRE- sun dried with cover refrigeration; SUCRM – sun dried with cover room temperature; SUDRE- sun dried direct refrigeration; SUDRM-sun dried direct room temperature;MarRE- market sample refrigeration; MaRM-market sample room temperature; PCA- plate count agar; PDA-potato dextrose agar; NG- no growth.

Table 4.11: Effects of drying and packaging conditions (at four months storage) on shelf life properties of *P.africana* condiments produced with consortium B.

Seeds	Means of Fermentaion	Duration of storage(months)	Drying Conditions	Packaging	Temperature (°C) of storage	pH	Moisture (%)	Peroxide value(meq kg)	Titratable acidity(mg lactic acid g)	PCA (Cfu/g)	PDA(Cfu/g)
P.africana	consortiumB	4	SLRE	Sachet	9.67±0.33	5.57±0.03	0.31±0.06	4.52±0.01	1.12±0.00	1.4×10^6	NG
				Plastic	9.67±0.33	5.17±0.03	3.07±0.07	7.17±0.03	1.21±0.00	1.5×10^6	NG
			SLRM	Sachet	29.67±0.33	5.61±0.00	0.37±0.03	4.71±0.03	1.11±0.00	1.2×10^5	NG
				Plastic	29.67±0.33	5.22±0.03	5.32±0.12	7.37±0.13	1.30±0.00	1.6×10^6	NG
			VaccRE	Sachet	8.67±0.33	5.63±0.03	0.28±0.03	4.50±0.01	1.11±0.00	1.2×10^5	NG
				plastic	8.67±0.33	5.07±0.03	4.07±0.03	7.27±0.03	1.21±0.00	1.3×10^6	NG
			VaccRM	Sachet	27.33±0.33	5.67±0.07	0.37±0.03	4.60±0.01	1.12±0.00	1.4×10^6	NG
				Plastic	27.33±0.33	5.10±0.00	5.86±0.03	7.97±0.07	1.26±0.00	1.7×10^5	NG
			OvRE	Sachet	8.33±0.33	6.53±0.03	0.24±0.03	4.34±0.01	1.11±0.00	1.1×10^5	NG
				Plastic	8.33±0.33	5.11±0.03	3.77±0.03	7.50±0.00	1.21±0.00	1.2×10^6	NG
			OVRM	Sachet	27.33±0.33	5.42±0.03	0.23±0.03	4.34±0.00	1.11±0.01	1.1×10^5	NG
				Plastic	27.33±0.33	5.13±0.03	4.10±0.06	7.80±0.10	1.20±0.01	1.0×10^5	NG
			SUCRE	Sachet	8.33±0.33	5.40±0.06	0.44±0.03	4.38±0.01	1.11±0.00	1.2×10^6	NG
				Plastic	8.33±0.33	5.23±0.03	4.52±0.03	9.51±0.00	1.31±0.00	1.7×10^5	NG
			SUCRM	Sachet	28.00±0.00	5.37±0.03	0.47±0.07	4.35±0.01	1.10±0.00	1.2×10^5	NG
				plastic	28.00±0.00	5.33±0.03	5.17±0.03	10.57±0.02	1.34±0.00	1.6×10^6	NG
			SUDRE	Sachet	8.33±0.33	5.53±0.03	0.43±0.03	4.37±0.02	1.11±0.00	1.2×10^5	NG
				plastic	8.33±0.33	5.37±0.03	4.37±0.03	12.40±0.03	1.30±0.00	1.8×10^6	NG
			SUDRM	Sachet	27.67±0.33	5.20±0.06	0.37±0.03	4.33±0.00	1.11±0.00	1.1×10^6	NG
				Plastic	27.67±0.33	5.53±0.03	6.67±0.03	4.00±0.00	1.36±0.00	1.6×10^6	NG

Values are means of triplicate determinations

Key

SLRE-solar dried refrigerator; SLRM-solar dried room temperature; VaccRM- vacuum dried room temperature; VaccRE –vacuum dried refrigerator;OVRE- oven dried refrigerator; OvRM- oven dried room temperature; SUCRE- sun dried with cover refrigeration; SUCRM – sun dried with cover room temperature; SUDRE- sun dried direct refrigeration; SUDRM-sun dried direct room temperature; PCA- plate count agar; PDA-potato dextrose agar; NG – no growrh

Table 4.12: Effects of drying and packaging conditions (at four months storage) on shelf life properties of *P.africana* condiments produced without consortia.

Seeds	Means of Fermentation	Duration of storage(months)	Drying Condition	Packaging	Temperature (^{0}C) of storage	pH	Moisture (%)	Peroxide value(meq kg)	Titratable acidity(mg lactic acid g)	PCA (Cfu/g)	PDA (Cfu/g)
P.africana	NF	4	SLRE	Sachet	9.67±0.33	5.60±0.03	0.35±0.06	4.60±0.00	1.11±0.00	1.2×10^5	NG
				Plastic	9.67±0.33	5.17±0.03	3.07±0.07	7.17±0.03	1.21±0.00	1.3×10^6	NG
			SLRM	Sachet	29.67±0.33	5.65±0.00	0.37±0.03	4.71±0.03	1.11±0.00	1.2×10^5	NG
				Plastic	29.63±0.33	5.82±0.03	4.30±0.12	7.33±0.13	1.31±0.00	1.4×10^5	NG
			VaccRE	Sachet	8.63±0.33	5.63±0.03	0.25±0.02	4.50±0.00	1.11±0.00	1.1×10^5	NG
				plastic	8.67±0.33	5.07±0.03	4.07±0.03	7.29±0.02	1.20±0.00	1.1×10^6	NG
			VaccRM	Sachet	27.33±0.33	5.67±0.07	0.37±0.03	4.60±0.01	1.13±0.00	1.2×10^6	NG
				Plastic	27.33±0.33	6.10±0.00	5.87±0.03	7.97±0.07	1.24±0.00	1.5×10^5	NG
			OvRE	Sachet	8.33±0.33	5.53±0.03	0.23±0.03	4.34±0.01	1.10±0.00	1.0×10^6	NG
				Plastic	8.33±0.33	5.13±0.03	1.77±0.03	5.53±0.00	1.21±0.00	1.0×10^5	NG
			OVRM	Sachet	27.33±0.33	5.43±0.03	0.23±0.03	4.30±0.00	1.11±0.00	1.1×10^5	NG
				Plastic	27.33±0.33	5.13±0.03	1.10±0.06	5.80±0.10	1.20±0.00	1.2×10^5	NG
			SUCRE	Sachet	8.33±0.33	5.40±0.06	0.43±0.03	4.38±0.01	1.11±0.00	1.1×10^6	NG
				Plastic	8.33±0.33	5.53±0.03	4.53±0.03	9.50±0.00	1.30±0.00	1.5×10^6	NG
			SUCRM	Sachet	28.00±0.00	5.37±0.03	0.47±0.07	4.36±0.01	1.10±0.00	1.2×10^5	NG
				plastic	28.00±0.00	6.73±0.03	5.17±0.03	10.57±0.03	1.35±0.00	1.3×10^6	NG
			SUDRE	Sachet	8.33±0.33	5.53±0.03	0.43±0.03	4.37±0.03	1.11±0.00	1.0×10^5	NG
				plastic	8.33±0.33	5.69±0.03	4.37±0.03	12.43±0.03	1.30±0.00	1.4×10^5	NG
			SUDRM	Sachet	27.67±0.33	6.25±0.06	0.37±0.03	4.31±0.00	1.11±0.00	1.1×10^6	NG
				Plastic	27.67±0.33	5.53±0.03	5.77±0.03	4.00±0.01	1.34±0.00	1.4×10^6	NG

Values are means of triplicate determinations

Key:

SLRE-solar dried refrigerator; SLRM-solar dried room temperature; VaccRM- vacuum dried room temperature; VaccRE –vacuum dried refrigerator;OVRE- oven dried refrigerator; OvRM- oven dried room temperature; SUCRE- sun dried with cover refrigeration; SUCRM – sun dried with cover room temperature; SUDRE- sun dried direct refrigeration; SUDRM-sun dried direct room temperature; PCA- plate count agar; PDA-potato dextrose agar; NG – no growth.

4.15: Effects of different modes of fermentation, drying and packaging on shelf life properties of powdered *P.africana* condiments at six months storage

Storage for six months of *P.africana* condiments produced in the laboratory with consortia or by natural fermention showed highly significant difference ($p<0.001$) in pH and bacteria count on PCA. Increases in moisture content, peroxide value, titratable acidity were particularly evident in plastic packaged condiments of *P.africana* stored at room temperature under all the drying conditions tested, as compared to storage of *P.africana* condiments at two and four months. Market condiments of *P.africana* also showed highly significant difference ($p<0.001$) of peroxide value, titratable acidity and bacteria count on PCA. Increases in moisture content, peroxide value and titratable acidity were appreciably high in plastic packaging stored at room temperature. Bacteria count on PCA was highest in market condiments of *P.africana* with a count of 3.5×10^6 and was highly significant ($p<0.001$) in plastic packaging stored at room temperature as shown in the ANOVA table. Mould counts were 0 for laboratory and market condiments of *P.africana* after six months storage (Tables 4.13 - 4.15).

Table 4.13: Effects of drying and packaging conditions (at six months storage) on shelf life properties of *P.africana* condiments produced with consortium A.

Seeds	Means of Fermentation	Duration of storage(months)	Drying Conditions	Packaging	Temperature (^{0}C) of storage	pH	Moisture (%)	Peroxide value(meq kg)	Titratable acidity(mg lactic acid g)	PCA (Cfu/g)	PDA(Cfu/g)
P.africana	Consortium A	6	SLRE	Sachet	8.33±0.33	5.87±0.06	0.47±0.07	4.37±0.02	1.11±0.00	1.2×10^6	NG
				plastic	8.33±0.33	5.80±0.03	6.40±.010	13.57±0.03	1.30±0.00	1.5×10^6	NG
			SLRM	Sachet	27.33±0.33	5.83±0.03	0.50±0.06	4.42±0.01	1.13±0.00	1.1×10^5	NG
				Plastic	27.33±0.33	5.07±0.03	7.07±0.03	18.03±0.03	1.39±0.00	1.7×10^5	NG
			VaccRE	Sachet	8.00±0.00	5.87±0.03	0.43±0.03	4.40±0.00	1.13±0.00	1.1×10^5	NG
				plastic	8.00±0.00	5.00±0.00	6.77±0.03	18.23±0.03	1.41±0.00	1.6×10^6	NG
			VaccRM	Sachet	28.67±0.33	5.90±0.00	0.43±0.03	4.33±0.02	1.11±0.00	1.1×10^5	NG
				Plastic	28.67±0.33	5.57±0.06	7.53±0.03	18.87±0.03	1.44±0.01	1.8×10^5	NG
			OvRe	Sachet	8.67±0.33	6.73±0.03	0.33±0.03	4.23±0.02	1.10±0.00	1.1×10^4	NG
				plastic	8.67±0.33	5.53±0.03	3.33±0.00	6.27±0.07	1.12±0.00	1.1×10^5	NG
			OVRM	Sachet	27.33±0.33	5.57±0.07	0.50±0.03	4.40±0.00	1.10±0.27	1.1×10^5	NG
				Plastic	27.33±0.33	5.70±0.10	3.13±0.03	7.13±0.03	1.11±0.00	1.4×10^6	NG
			SUCRE	Sachet	9.00±0.00	5.77±0.03	0.43±0.03	4.37±0.01	1.10±0.01	1.2×10^5	NG
				plastic	9.00±0.00	5.63±0.07	5.13±0.03	15.07±0.03	1.10±0.00	1.7×10^5	NG
			SUCRM	Sachet	28.33±0.33	5.60±0.06	0.40±0.00	4.39±0.00	1.11±0.00	1.3×10^5	NG
				Plastic	28.33±0.33	5.47±0.03	6.33±0.33	18.43±0.07	1.46±0.01	1.5×10^6	NG
			SUDRE	Sachet	8.33±0.33	5.83±0.03	0.37±0.09	4.40±0.00	1.11±0.00	1.0×10^5	NG
				plastic	8.33±0.33	6.13±0.03	5.80±0.00	17.70±0.10	1.43±0.02	1.8×10^5	NG
			SUDRM	Sachet	28.00±0.00	5.60±0.06	0.40±0.00	4.37±0.01	1.11±0.00	1.1×10^6	NG
				Plastic	28.00±0.00	5.73±0.06	6.13±0.03	18.27±0.09	1.49±0.00	1.9×10^6	NG
			MarRE	Sachet	8.67±0.33	5.80±0.06	1.37±0.03	7.15±0.00	1.14±0.01	1.5×10^5	NG
				plastic	8.67±0.33	4.87±0.03	9.00±0.00	17.43±0.07	1.52±0.00	3.0×10^5	NG
			MarRM	Sachet	28.67±0.33	4.73±0.03	1.23±0.03	7.14±0.01	1.14±0.01	2.7×10^6	NG
				Plastic	28.67±0.33	4.90±0.00	9.17±0.17	19.33±0.17	1.55±0.00	3.1×10^5	NG

Values are means of triplicate determinations

Key

SLRE-solar dried refrigerator; SLRM-solar dried room temperature; VaccRM- vacuum dried room temperature; VaccRE –vacuum dried refrigerator;OVRE- oven dried refrigerator; OvRM- oven dried room temperature; SUCRE- sun dried with cover refrigeration; SUCRM – sun dried with cover room temperature; SUDRE- sun dried direct refrigeration; SUDRM-sun dried direct room temperature;MarRE- market sample refrigeration; MaRM- market sample room temperature; PCA- plate count agar; PDA-potato dextrose agar;NG - no growth.

Table 4.14: Effects of drying and packaging conditions (at six months storage) on shelf life properties of *P.africana* condiments produced with consortium B

Seeds	Means of Fermentation	Duration of storage(months)	Drying Condition	**Packaging**	Temperature (^{0}C) of storage	pH	Moisture (%)	Peroxide value(meq kg)	Titratable acidity(mg lactic acid g)	PCA (Cfu/g)	PDA(Cfu/g)
P.africana	Consortium B	6	SLRE	Sachet	8.32±0.30	5.88±0.05	0.39±0.07	4.30±0.01	1.10±0.02	1.2×10^5	NG
				plastic	8.33±0.31	5.87±0.03	6.40±.010	13.57±0.03	1.39±0.02	1.7×10^6	NG
			SLRM	Sachet	27.30±0.30	5.80±0.01	0.52±0.03	4.42±0.01	1.13±0.01	1.3×10^5	NG
				Plastic	27.33±0.33	5.07±0.00	6.07±0.06	18.03±0.03	1.39±0.00	1.7×10^5	NG
			VaccRE	Sachet	8.00±0.00	5.87±0.03	0.40±0.03	4.40±0.00	1.13±0.00	1.2×10^5	NG
				plastic	8.00±0.00	5.00±0.00	6.77±0.00	18.23±0.03	1.41±0.00	1.5×10^5	NG
			VaccRM	Sachet	28.67±0.33	5.40±0.01	0.42±0.00	4.33±0.02	1.11±0.00	1.2×10^5	NG
				Plastic	28.67±0.30	5.57±0.02	6.51±0.03	18.87±0.03	1.44±0.01	1.7×10^5	NG
			OvRe	Sachet	8.67±0.31	5.73±0.03	0.30±0.02	4.23±0.02	1.10±0.01	1.0×10^6	NG
				plastic	8.67±0.33	5.53±0.03	5.50±0.00	6.20±0.02	1.32±0.00	1.6×10^5	NG
			OVRM	Sachet	27.33±0.32	5.57±0.07	0.30±0.03	4.39±0.00	1.23±0.25	1.2×10^5	NG
				Plastic	27.33±0.31	5.70±0.10	6.00±0.01	16.13±0.03	1.32±0.00	1.5×10^6	NG
			SUCRE	Sachet	9.00±0.00	5.57±0.03	0.43±0.03	4.38±0.01	1.10±0.01	1.3×10^6	NG
				plastic	9.00±0.00	5.63±0.07	6.13±0.03	15.07±0.02	1.40±0.00	1.8×10^5	NG
			SUCRM	Sachet	28.33±0.33	5.60±0.06	0.40±0.00	4.39±0.00	1.11±0.00	1.2×10^6	NG
				Plastic	28.33±0.33	5.87±0.03	6.33±0.31	18.43±0.07	1.40±0.01	1.6×10^6	NG
			SUDRE	Sachet	8.33±0.33	5.83±0.01	0.37±0.09	4.30±0.00	1.12±0.00	1.1×10^5	NG
				plastic	8.33±0.33	4.13±0.01	7.70±0.00	17.60±0.10	1.43±0.02	1.9×10^5	NG
			SUDRM	Sachet	28.00±0.00	5.40±0.06	0.40±0.00	4.32±0.01	1.11±0.00	1.0×10^6	NG
				Plastic	28.00±0.00	5.13±0.06	6.10±0.03	18.20±0.09	1.49±0.00	1.8×10^6	NG

Values are means of triplicate determinations.

KeySLRE-solar dried refrigerator; SLRM-solar dried room temperature; VaccRM- vacuum dried room temperature; VaccRE –vacuum dried refrigerator;OVRE- oven dried refrigerator; OvRM- oven dried room temperature; SUCRE- sun dried with cover refrigeration; SUCRM – sun dried with cover room temperature; SUDRE- sun dried direct refrigeration; SUDRM-sun dried direct room temperature; PCA- plate count agar; PDA-potato dextrose agar; NG- no growth.

Table 4.15: Effects of drying and packaging conditions (at six months storage) on shelf life properties of *P.africana* condiments produced without consortia.

Seeds	Means of Fermention	Duration of storage(months)	Drying Condition	Packaging	Temperature (^{0}C) of storage	pH	Moisture (%)	Peroxide value (meq kg)	Titratable acidity(mg lactic acid g)	PCA (Cfu/g)	PDA(Cfu/g)
P.africana	NF	6	SLRE	Sachet	8.33±0.30	5.87±0.03	0.45±0.06	4.37±0.00	1.11±0.01	1.3×10^4	NG
				plastic	8.31±0.33	5.83±0.03	6.42±.010	13.57±0.02	1.40±0.00	1.4×10^6	NG
			SLRM	Sachet	27.29±0.33	5.80±0.01	0.50±0.06	4.42±0.01	1.10±0.00	1.2×10^5	NG
				Plastic	27.33±0.30	5.07±0.00	7.07±0.03	18.03±0.03	1.33±0.03	1.5×10^6	NG
			VaccRE	Sachet	8.00±0.00	5.87±0.03	0.33±0.03	4.70±0.02	1.11±0.01	1.0×10^5	NG
				plastic	8.00±0.01	5.10±0.00	6.77±0.03	18.19±0.02	1.41±0.00	1.4×10^6	NG
			VaccRM	Sachet	28.67±0.33	5.90±0.01	0.33±0.03	4.30±0.00	1.11±0.00	1.2×10^4	NG
				Plastic	28.67±0.33	5.57±0.02	6.23±0.00	18.77±0.03	1.44±0.01	1.6×10^5	NG
			OvRe	Sachet	8.67±0.33	5.73±0.03	0.33±0.03	4.20±0.02	1.11±0.02	1.1×10^4	NG
				plastic	8.67±0.33	5.53±0.03	3.30±0.00	6.27±0.04	1.22±0.00	1.1×10^5	NG
			OVRM	Sachet	27.33±0.33	5.57±0.07	0.33±0.01	4.40±0.00	1.23±0.27	1.2×10^5	NG
				Plastic	27.33±0.33	5.70±0.10	3.33±0.00	7.13±0.01	1.39±0.00	1.1×10^6	NG
			SUCRE	Sachet	9.00±0.00	5.57±0.03	0.43±0.03	4.37±0.01	1.11±0.01	1.1×10^5	NG
				plastic	9.00±0.00	5.63±0.07	6.17±0.01	15.07±0.03	1.40±0.00	1.4×10^6	NG
			SUCRM	Sachet	28.33±0.33	5.60±0.06	0.30±0.00	4.39±0.00	1.11±0.00	1.2×10^5	NG
				Plastic	28.33±0.33	5.87±0.03	6.33±0.33	18.43±0.07	1.46±0.01	1.5×10^6	NG
			SUDRE	Sachet	8.33±0.33	5.83±0.01	0.37±0.03	4.40±0.04	1.11±0.02	1.1×10^5	NG
				plastic	8.33±0.33	5.13±0.01	5.80±0.00	17.70±0.10	1.43±0.02	1.4×10^5	NG
			SUDRM	Sachet	28.00±0.00	5.40±0.06	0.40±0.00	4.37±0.01	1.11±0.01	1.1×10^5	NG
				Plastic	28.00±0.00	5.53±0.06	6.13±0.01	18.27±0.07	1.32±0.04	1.6×10^6	NG

Values are means of triplicate determinations

Key

SLRE-solar dried refrigerator; SLRM-solar dried room temperature; VaccRM- vacuum dried room temperature; VaccRE –vacuum dried refrigerator;OVRE- oven dried refrigerator;OvRM - oven dried room temperature; SUCRE- sun dried with cover refrigeration; SUCRM – sun dried with cover room temperature; SUDRE- sun dried direct refrigeration; SUDRM-sun dried direct room temperature; PCA- plate count agar; PDA-potato dextrose agar;NG – no growth.

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4.16 : Effects of different modes of fermentation, drying and packaging on shelf life properties of powdered *P.biglobosa* condiments at two months storage

Storage for two months of *P.biglobosa* condiments fermented naturally and with consortia , to highly significant ($p<0.001$) values of pH, and bacteria count on PCA. Highest mean value of pH was observed in *P.biglobosa* condiments produced with consortium A; with a mean value of 5.90 was given by oven dried condiments packaged in sachets and stored at refrigeration temperature. Moisture content, peroxide value and titratable acidity were lowest with *P.biglobosa* condiments produced with both consortia and by natural fermentation and subjected to oven drying conditions (Tables 4.16-4.18).

Market condiments of *P.biglobosa* had lowest mean pH value (Table 4.13) under plactic packaging stored at room temperature. Peroxide value, titratable acidity, bacterial plate counts on PCA, were also highly significant ($p<0.001$) in market condiments of *P.biglobosa*. Highest mean values were observed in market condiments as compared to laboratory products condiments (Tables 4.16- 4.18).

Table 4.16: Effects of drying and packaging conditions (at two months storage) on shelf life properties of *P.biglobosa* condiments produced with consortium A

Seeds	Means of Fermentation	Duration of storage(months)	Drying Conditions	Packaging	Temperature (^{0}C) of storage	pH	Moisture (%)	Peroxide value(meq kg)	Titratable acidity(mg lactic acid)	PCA (Cfu/g)	PDACfu/g)
P.biglobosa	Consortium A	2	SLRE	Sachet	8.67±0.33	5.53±0.03	0.23±0.03	4.20±0.00	1.11±0.00	1.1×10^5	NG
				plastic	8.67±0.33	5.03±0.03	0.57±0.28	4.31±0.00	1.11±0.00	1.3×10^6	NG
			SLRM	Sachet	28.00±0.58	5.43±0.03	0.77±0.03	4.23±0.00	1.11±0.00	1.0×10^5	NG
				Plastic	28.00±0.58	5.10±0.00	0.80±0.06	4.31±0.01	1.11±0.00	1.3×10^6	NG
			VaccRE	Sachet	8.00±0.00	5.53±0.03	0.37±0.03	4.31±0.00	1.12±0.00	1.3×10^6	NG
				plastic	8.00±0.00	5.47±0.03	0.37±0.03	4.31±0.00	1.12±0.00	1.2×10^7	NG
			VaccRM	Sachet	27.00±0.00	5.53±0.03	0.43±0.03	4.21±0.00	1.12±0.00	1.0×10^5	NG
				Plastic	27.00±0.00	5.17±0.03	0.43±0.03	4.31±0.00	1.12±0.00	1.4×10^6	NG
			OvRe	Sachet	8.00±0.00	5.90±0.06	0.10±0.07	4.19±0.00	1.11±0.03	1.0×10^4	NG
				plastic	8.00±0.00	5.20±0.03	0.12±0.07	4.19±0.00	1.16±0.03	1.2×10^6	NG
			OVRM	Sachet	27.33±0.33	5.23±0.03	0.13±0.07	4.17±0.00	1.11±0.03	1.1×10^4	NG
				Plastic	27.33±0.33	5.33±0.03	0.13±0.07	4.29±0.01	1.16±0.03	1.0×10^6	NG
			SUCRE	Sachet	8.00±0.00	5.23±0.03	0.57±0.03	4.32±0.01	1.12±0.01	1.3×10^5	NG
				plastic	8.00±0.00	5.10±0.06	0.43±0.03	4.32±0.01	1.12±0.01	1.4×10^6	NG
			SUCRM	Sachet	27.33±0.33	5.07±0.03	0.43±0.03	4.32±0.01	1.12±0.01	1.4×10^5	NG
				Plastic	27.33±0.33	5.00±0.00	0.43±0.03	4.32±0.01	1.12±0.01	1.4×10^6	NG
			SUDRE	Sachet	9.33±0.33	5.30±0.06	0.37±0.03	4.31±0.00	1.11±0.01	1.5×10^5	NG
				plastic	9.33±0.33	5.20±0.06	0.37±0.03	4.31±0.00	1.11±0.01	1.5×10^6	NG
			SUDRM	Sachet	27.67±0.33	5.23±0.07	0.37±0.03	4.31±0.00	1.11±0.01	1.3×10^5	NG
				Plastic	27.67±0.33	5.20±0.06	0.37±0.03	4.31±0.00	1.11±0.01	1.6×10^6	NG
			MarRE	Sachet	9.00±0.00	4.20±0.06	1.17±0.09	7.11±0.00	1.15±0.00	1.9×10^5	NG
				plastic	9.00±0.00	4.17±0.03	1.20±0.10	7.11±0.00	1.15±0.00	1.3×10^7	NG
			MarRM	Sachet	27.33±0.33	5.13±0.03	1.20±0.10	7.11±0.00	1.15±0.00	1.7×10^6	NG
				Plastic	27.33±0.33	4.13±0.03	1.20±0.10	7.20±0.01	1.15±0.00	1.9×10^6	NG

Values are means of triplicate determinations

Key

SLRE-solar dried refrigerator; SLRM-solar dried room temperature; VaccRM- vacuum dried room temperature; VaccRE –vacuum dried refrigerator;OVRE- oven dried refrigerator; OvRM- oven dried room temperature; SUCRE- sun dried with cover refrigeration; SUCRM – sun dried with cover room temperature; SUDRE- sun dried direct refrigeration; SUDRM-sun dried direct room temperature;MarRE- market sample refrigeration; MaRM- market sample room temperature; PCA- plate count agar; PDA-potato dextrose agar; NG – no growth.

Table 4.17: Effects of drying and packaging conditions (at two months storage) on shelf life properties of *P.biglobosa* condiments produced with consortium B

Seeds	Means of Fermentation	Duration of storage(months)	Drying conditions	Packaging	Temperature (^{0}C) of storage	pH	Moisture (%)	Peroxide value(meq kg)	Titratable acidity(mg lactic acid g)	PCA (Cfu/g)	PDA(Cfu/g)
P.biglobosa	Consortium B	2	SLRE	Sachet	8.57±0.30	5.50±0.01	0.13±0.00	4.20±0.00	1.10±0.01	1.0×10^5	NG
				Plastic	8.68±0.31	5.03±0.02	0.52±0.27	4.27±0.01	1.12±0.01	1.4×10^6	NG
			SLRM	Sachet	28.00±0.58	5.43±0.03	0.77±0.03	4.53±0.00	1.11±0.00	1.0×10^6	NG
				Plastic	28.00±0.58	5.10±0.00	0.80±0.06	4.31±0.04	1.11±0.00	1.3×10^7	NG
			VaccRE	Sachet	8.00±0.01	5.51±0.02	0.39±0.03	4.41±0.03	1.14±0.02	1.4×10^6	NG
				Plastic	8.00±0.00	5.89±0.01	0.39±0.02	4.31±0.01	1.12±0.01	1.2×10^7	NG
			VaccRM	Sachet	27.00±0.00	5.43±0.01	0.47±0.00	4.23±0.01	1.12±0.00	1.2×10^6	NG
				Plastic	27.00±0.01	5.27±0.01	0.43±0.03	4.35±0.04	1.12±0.00	1.7×10^7	NG
			OvRe	Sachet	8.00±0.00	5.50±0.06	0.33±0.07	4.16±0.01	1.10±0.01	1.0×10^4	NG
				Plastic	8.00±0.00	5.43±0.03	0.33±0.07	4.19±0.00	1.11±0.02	1.2×10^6	NG
			OVRM	Sachet	27.33±0.33	5.58±0.03	0.33±0.07	4.19±0.01	1.11±0.03	1.1×10^5	NG
				Plastic	27.33±0.33	5.25±0.03	0.33±0.07	4.30±0.04	1.13±0.05	1.0×10^5	NG
			SUCRE	Sachet	8.00±0.00	5.03±0.03	0.57±0.03	4.30±0.01	1.10±0.03	1.2×10^4	NG
				plastic	8.00±0.00	5.10±0.06	0.43±0.03	4.33±0.01	1.11±0.01	1.4×10^5	NG
			SUCRM	Sachet	27.33±0.31	5.48±0.02	0.43±0.04	4.32±0.02	1.12±0.01	1.4×10^6	NG
				Plastic	27.33±0.32	5.34±0.04	0.41±0.02	4.22±0.01	1.12±0.00	1.3×10^6	NG
			SUDRE	Sachet	9.32±0.30	5.35±0.05	0.37±0.03	4.30±0.05	1.11±0.01	1.5×10^5	NG
				plastic	9.30±0.33	5.20±0.08	0.33±0.03	4.33±0.04	1.11±0.01	1.6×10^6	NG
			SUDRM	Sachet	27.65±0.31	5.29±0.07	0.35±0.03	4.30±0.02	1.10±0.02	1.3×10^6	NG
				Plastic	27.66±0.33	5.20±0.06	0.37±0.03	4.29±0.01	1.11±0.01	1.6×10^7	NG

Values are means of triplicate determinations

Key

SLRE-solar dried refrigerator; SLRM-solar dried room temperature; VaccRM- vacuum dried room temperature; VaccRE –vacuum dried refrigerator;OVRE- oven dried refrigerator; OvRM- oven dried room temperature; SUCRE- sun dried with cover refrigeration; SUCRM – sun dried with cover room temperature; SUDRE- sun dried direct refrigeration; SUDRM-sun dried direct room temperature; PCA- plate count agar; PDA-potato dextrose agar; NG – no growth.

Table 4.18: Effects of drying and packaging conditions (at two months storage) on shelf life properties of *P.biglobosa* condiments produced without consortia

Seeds	Means of Fermentation	Duration of storage(months)	Drying Conditions	Packaging	Temperature (^{0}C) of storage	pH	Moisture (%)	Peroxide value(meg kg)	Titratable acidity(mg lactic acid g)	PCA (Cfu/g)	PDA(Cfu/g)
P.biglobosa	NF	2	SLRE	Sachet	8.67±0.30	5.53±0.00	0.33±0.00	4.20±0.02	1.10±0.03	1.0×10^5	NG
				plastic	8.67±0.33	5.03±0.00	0.53±0.22	4.21±0.01	1.11±0.02	1.1×10^6	NG
			SLRM	Sachet	28.00±0.58	5.43±0.03	0.70±0.03	4.33±0.00	1.12±0.01	1.1×10^5	NG
				Plastic	28.00±0.58	5.10±0.02	0.84±0.06	4.45±0.02	1.11±0.00	1.1×10^6	NG
			VaccRE	Sachet	8.00±0.00	5.53±0.03	0.37±0.03	4.35±0.02	1.12±0.04	1.0×10^5	NG
				plastic	8.00±0.00	5.60±0.05	0.38±0.03	4.32±0.06	1.12±0.00	1.2×10^6	NG
			VaccRM	Sachet	27.00±0.00	5.63±0.03	0.41±0.00	4.20±0.02	1.10±0.02	1.3×10^5	NG
				Plastic	27.00±0.00	5.27±0.03	0.43±0.03	4.38±0.06	1.11±0.00	1.4×10^6	NG
			OvRe	Sachet	8.00±0.00	5.80±0.06	0.33±0.04	4.15±0.02	1.10±0.01	1.0×10^5	NG
				plastic	8.00±0.00	5.30±0.03	0.33±0.05	4.18±0.02	1.11±0.03	1.3×10^6	NG
			OVRM	Sachet	27.33±0.33	5.73±0.00	0.33±0.04	4.16±0.02	1.11±0.04	1.0×10^5	NG
				Plastic	27.33±0.33	5.33±0.03	0.33±0.07	4.25±0.03	1.15±0.00	1.2×10^6	NG
			SUCRE	Sachet	8.00±0.00	5.03±0.01	0.55±0.00	4.30±0.01	1.12±0.01	1.2×10^5	NG
				plastic	8.00±0.00	5.10±0.06	0.43±0.03	4.32±0.01	1.10±0.00	1.1×10^6	NG
			SUCRM	Sachet	27.33±0.33	5.07±0.01	0.40±0.05	4.31±0.01	1.12±0.01	1.0×10^4	NG
				Plastic	27.33±0.33	5.50±0.04	0.43±0.03	4.32±0.01	1.12±0.02	1.4×10^6	NG
			SUDRE	Sachet	9.33±0.33	5.40±0.06	0.39±0.02	4.31±0.02	1.11±0.04	1.1×10^5	NG
				plastic	9.33±0.33	5.27±0.04	0.37±0.03	4.30±0.06	1.10±0.00	1.1×10^6	NG
			SUDRM	Sachet	27.67±0.33	5.22±0.08	0.37±0.02	4.31±0.00	1.11±0.01	1.2×10^5	NG
				Plastic	27.67±0.33	5.26±0.06	0.37±0.04	4.31±0.01	1.12±0.00	1.2×10^6	NG

Values are means of triplicate determinations

Key

SLRE-solar dried refrigerator; SLRM-solar dried room temperature; VaccRM- vacuum dried room temperature; VaccRE –vacuum dried refrigerator;OVRE- oven dried refrigerator; OvRM- oven dried room temperature; SUCRE- sun dried with cover refrigeration; SUCRM – sun dried with cover room temperature; SUDRE- sun dried direct refrigeration; SUDRM-sun dried direct room temperature;; PCA- plate count agar; PDA-potato dextrose agar; NG – no growth.

4.17 : Effects of different modes of fermentation, drying and packaging on shelf life properties of powdered *P.biglobosa* condiments at four months storage

P.biglobosa condiments in the laboratory stored for four months showed increase in moisture content, peroxide value and titratable acid as compared to values for two months storage particularly with plastic packaged stored at room temperature. Temperature and pH were highly significant ($p<0.001$). Highest mean values for moisture content and peroxide value for laboratory produced *P.biglobosa* condiment stored for four months were 9.67 and 8.3 respectively for sundried covered with net at room temperature (SUCRM) for consortium A. *P.biglobosa* condiment produced with consortium B and natural fermentation, the same increase in physicochemical properties was equally observed. *P.biglobosa* condiments purchased from the market stored at four months and at room temperature showed significant increase in physicochemical properties as compared to laboratory produced condiments. No mould growth on potato dextrose agar for both laboratory and market purchased condiments of *P.biglobosa*. (Tables 4.19-4.21).

Table 4.19: Effects of drying and packaging conditions (at four months storage) on shelf life properties of *P.biglobosa* condiments produced with consortium A.

Seeds	Means of Fermentation	Duration of storage(months)	Drying Conditions	Packaging	Temperature (^{0}C) of storage	pH	Moisture (%)	Peroxide value(meg kg)	Titratable acidity(mg lactic acid)	PCA (Cfu/g)	PDA(Cfu/g)
P.biglobosa	Consortium A	4	SLRE	Sachet	8.67±0.33	5.53±0.03	0.43±0.03	4.30±0.00	1.11±0.00	1.0×10^5	NG
				Plastic	8.67±0.33	5.03±0.03	6.03±0.03	7.21±0.01	1.31±0.01	1.2×10^6	NG
			SLRM	Sachet	28.00±0.58	5.43±0.03	0.37±0.03	4.24±0.01	1.11±0.00	1.2×10^5	NG
				Plastic	28.00±0.58	5.10±0.03	7.23±0.03	7.81±0.01	1.41±0.01	1.5×10^6	NG
			VaccRE	Sachet	8.67±0.33	5.53±0.03	0.37±0.03	4.20±0.00	1.12±0.00	1.2×10^5	NG
				plastic	8.67±0.33	5.37±0.03	6.43±0.03	7.91±0.00	1.32±0.00	1.4×10^6	NG
			VaccRM	Sachet	27.33±0.33	5.53±0.03	0.43±0.03	4.21±0.00	1.12±0.00	1.0×10^5	NG
				Plastic	27.33±0.33	5.27±0.03	7.30±0.06	7.31±0.00	1.42±0.00	1.3×10^6	NG
			OvRE	Sachet	8.33±0.33	5.70±0.06	0.27±0.03	4.11±0.00	1.14±0.26	1.0×10^4	NG
				Plastic	8.33±0.33	5.53±0.03	5.07±0.03	6.29±0.00	3.37±0.00	1.2×10^5	NG
			OVRM	Sachet	27.33±0.33	5.73±0.03	0.27±0.03	4.10±0.10	1.17±0.27	1.1×10^4	NG
				Plastic	27.33±0.33	5.53±0.03	5.03±0.03	6.31±0.00	1.39±0.00	1.3×10^5	NG
			SUCRE	Sachet	8.67±0.33	5.03±0.03	0.40±0.06	4.32±0.01	1.11±0.00	1.3×10^6	NG
				Plastic	8.67±0.33	5.10±0.03	7.47±0.07	8.32±0.01	1.41±0.00	1.6×10^7	NG
			SUCRM	Sachet	28.00±0.00	5.07±0.03	0.33±0.03	4.22±0.01	1.11±0.01	1.3×10^5	NG
				plastic	28.00±0.00	5.50±0.00	7.67±0.03	8.31±0.01	1.46±00.0	1.5×10^6	NG
			SUDRE	Sachet	8.33±0.33	5.30±0.06	0.40±0.00	4.12±0.00	1.13±0.01	1.2×10^5	NG
				plastic	8.33±0.33	5.20±0.06	7.26±0.03	8.31±0.00	1.41±0.00	1.7×10^6	NG
			SUDRM	Sachet	27.67±0.33	5.23±0.07	0.40±0.00	4.21±0.00	1.12±0.01	1.2×10^5	NG
				Plastic	27.67±0.33	5.20±0.06	7.67±0.03	8.35±0.00	1.51±0.00	1.7×10^6	NG
			MarRE	Sachet	9.00±0.00	5.20±0.06	1.17±0.09	7.10±0.00	1.24±0.13	1.8×10^5	NG
				plastic	9.00±0.00	6.23±0.32	8.20±0.06	10.37±0.07	3.63±0.01	3.1×10^6	NG
			MarRM	Sachet	27.33±0.33	5.80±0.35	1.30±0.00	7.11±0.00	1.13±0.00	1.7×10^5	NG
				plastic	27.33±0.33	5.13±0.03	8.67±0.17	10.50±0.00	1.68±0.00	2.1×10^5	NG

Values are means of triplicate determinations

Key

SLRE-solar dried refrigerator; SLRM-solar dried room temperature; VaccRM- vacuum dried room temperature; VaccRE –vacuum dried refrigerator;OVRE- oven dried refrigerator; OvRM- oven dried room temperature; SUCRE-sun dried with cover refrigeration; SUCRM – sun dried with cover room temperature; SUDRE- sun dried direct refrigeration; SUDRM-sun dried direct room temperature;MarRE- market sample refrigeration; MaRM- market sample room temperature; PCA- plate count agar; PDA-potato dextrose agar; NG- no growth.

Table 4.20: Effects of drying and packaging conditions (at four months storage) on shelf life properties of *P.biglobosa* condiments produced with consortium B.

Seeds	Means of Fermentation	Duration of storage(months)	Drying Conditions	Packaging	Temperature (^{0}C) of storage	pH	Moisture (%)	Peroxide value(meq kg)	Titratable acidity(mg lactic acid g)	PCA (Cfu/g)	PDA(Cfu/g)
P.biglobosa	Consortium B	4	SLRE	Sachet	*8.64±0.30	5.35±0.01	0.43±0.03	4.33±0.02	1.11±0.00	1.2×10^5	NG
				Plastic	8.67±0.31	5.37±0.03	6.03±0.01	7.21±0.01	1.31±0.04	1.4×10^6	NG
			SLRM	Sachet	28.00±0.58	5.43±0.00	0.39±0.03	4.34±0.05	1.11±0.00	1.2×10^5	NG
				Plastic	28.00±0.58	5.10±0.10	7.23±0.00	7.81±0.01	1.41±0.03	1.5×10^6	NG
			VaccRE	Sachet	8.67±0.33	5.33±0.03	0.35±0.03	4.22±0.00	1.12±0.06	1.2×10^5	NG
				plastic	8.67±0.33	5.23±0.02	6.43±0.02	7.91±0.02	1.32±0.00	1.3×10^6	NG
			VaccRM	Sachet	27.33±0.33	5.43±0.00	0.43±0.03	4.21±0.00	1.12±0.06	1.1×10^5	NG
				Plastic	27.33±0.30	5.27±0.01	7.38±0.05	7.33±0.02	1.42±0.01	1.3×10^6	NG
			OvRE	Sachet	8.33±0.33	5.82±0.05	0.15±0.00	4.10±0.00	1.11±0.20	1.1×10^4	NG
				Plastic	8.33±0.31	5.21±0.02	2.07±0.02	5.29±0.01	1.17±0.01	1.4×10^5	NG
			OVRM	Sachet	27.33±0.33	5.67±0.04	0.17±0.03	4.13±0.10	1.17±0.22	1.1×10^4	NG
				Plastic	27.33±0.33	5.00±0.03	2.03±0.04	5.37±0.03	1.39±0.02	1.3×10^5	NG
			SUCRE	Sachet	8.67±0.30	5.02±0.02	0.40±0.02	4.22±0.01	1.12±0.01	1.4×10^5	NG
				Plastic	8.67±0.33	5.10±0.03	7.40±0.02	8.31±0.01	1.41±0.00	1.7×10^6	NG
			SUCRM	Sachet	28.00±0.00	5.07±0.01	0.23±0.03	4.22±0.09	1.12±0.01	1.3×10^5	NG
				plastic	28.00±0.00	5.50±0.00	7.67±0.03	8.30±0.01	1.46±0.01	1.4×10^6	NG
			SUDRE	Sachet	8.33±0.33	5.30±0.06	0.40±0.06	4.12±0.00	1.13±0.00	1.2×10^5	NG
				plastic	8.33±0.33	5.22±0.06	8.26±0.02	8.30±0.05	1.40±0.01	1.8×10^6	NG
			SUDRM	Sachet	27.67±0.33	5.25±0.00	0.40±0.00	4.11±0.00	1.12±0.02	1.2×10^5	NG
				Plastic	27.67±0.33	5.23±0.01	8.67±0.00	8.21±0.03	1.41±0.05	1.9×10^6	NG

Values are means of triplicate determinations

Key

SLRE-solar dried refrigerator; SLRM-solar dried room temperature; VaccRM- vacuum dried room temperature; VaccRE –vacuum dried refrigerator;OVRE- oven dried refrigerator; OvRM- oven dried room temperature; SUCRE- sun dried with cover refrigeration; SUCRM – sun dried with cover room temperature; SUDRE- sun dried direct refrigeration; SUDRM-sun dried direct room temperature; PCA- plate count agar; PDA-potato dextrose agar; NG – no growth..

Table 4.21: Effects of drying and packaging conditions (at four months storage) on shelf life properties of *P.biglobosa* condiments produced without consortia.

Seeds	Means of Fermentation	Duration of storage(months)	Drying Conditions	Packaging	Temperature (0C) of storage	pH	Moisture (%)	Peroxide value(meq kg)	Titratable acidity(mg lactic acid g)	PCA (Cfu/g)	PDA(Cfu/g)
P.biglobosa	NF	4	SLRE	Sachet	*8.67±0.33	5.50±0.00	0.43±0.04	4.35±0.02	1.13±0.05	1.1×10^{6}	NG
				Plastic	8.47±0.30	5.01±0.06	6.03±0.02	7.22±0.01	1.30±0.01	1.4×10^{6}	NG
			SLRM	Sachet	28.00±0.58	5.43±0.03	0.37±0.03	4.24±0.05	1.10±0.06	1.1×10^{5}	NG
				Plastic	28.00±0.55	5.10±0.03	7.20±0.00	7.81±0.01	1.41±0.01	1.5×10^{5}	NG
			VaccRE	Sachet	8.67±0.33	5.13±0.03	0.30±0.03	4.20±0.00	1.12±0.00	1.1×10^{5}	NG
				plastic	8.67±0.33	5.37±0.03	6.43±0.00	7.81±0.04	1.41±0.07	1.2×10^{6}	NG
			VaccRM	Sachet	27.33±0.33	5.53±0.03	0.40±0.00	4.21±0.00	1.12±0.00	1.2×10^{6}	NG
				Plastic	27.33±0.33	5.27±0.02	7.20±0.01	7.30±0.00	1.32±0.01	1.7×10^{5}	NG
			OvRE	Sachet	8.33±0.33	5.80±0.06	0.13±0.00	4.15±0.02	1.14±0.26	1.0×10^{6}	NG
				Plastic	8.33±0.33	5.63±0.03	5.01±0.03	5.19±0.01	1.17±0.01	1.1×10^{6}	NG
			OVRM	Sachet	27.33±0.30	5.73±0.03	0.27±0.00	4.10±0.12	1.37±0.27	1.2×10^{5}	NG
				Plastic	27.33±0.33	5.43±0.03	3.06±0.02	5.31±0.00	1.39±0.00	1.3×10^{6}	NG
			SUCRE	Sachet	8.67±0.31	5.03±0.03	0.30±0.04	4.22±0.01	1.14±0.20	1.1×10^{6}	NG
				Plastic	8.62±0.33	5.10±0.03	7.40±0.08	8.32±0.01	1.41±0.00	1.5×10^{6}	NG
			SUCRM	Sachet	28.00±0.00	5.07±0.03	0.33±0.01	4.22±0.05	1.11±0.01	1.0×10^{6}	NG
				plastic	28.00±0.00	5.50±0.00	7.67±0.03	8.30±0.01	1.36±0.06	1.3×10^{6}	NG
			SUDRE	Sachet	8.33±0.33	5.30±0.06	0.40±0.00	4.16±0.00	1.13±0.01	1.0×10^{5}	NG
				plastic	8.33±0.33	5.20±0.06	8.26±0.03	8.31±0.05	1.41±0.02	1.4×10^{5}	NG
			SUDRM	Sachet	27.67±0.34	5.23±0.07	0.40±0.00	4.21±0.00	1.12±0.01	1.1×10^{6}	NG
				Plastic	27.67±0.33	5.20±0.06	8.67±0.03	8.33±0.10	1.51±0.00	1.6×10^{6}	NG

Values are means of triplicate determinations

Key

SLRE-solar dried refrigerator; SLRM-solar dried room temperature; VaccRM- vacuum dried room temperature; VaccRE –vacuum dried refrigerator;OVRE- oven dried refrigerator; OvRM- oven dried room temperature; SUCRE- sun dried with cover refrigeration; SUCRM – sun dried with cover room temperature; SUDRE- sun dried direct refrigeration; SUDRM-sun dried direct room temperature; PCA- plate count agar; PDA-potato dextrose agar; NG- no growth.

4.18: Effects of different modes of fermentation, drying and packaging on shelf life properties of powdered *P.biglobosa* condiments at six months storage

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Storage of *P.biglobosa* condiments produced in the laboratory for six months after fermentation with both consortia and under natural conditions showed highly significant difference ($p<0.001$) in temperature and pH. Moisture content, peroxide value and titratable acid were particularly high in plastic packaged condiments of *P.biglobosa* stored at room temperature with all the drying conditions used as compared to storage of *P.biglobosa* condiments at two and four months. Market condiments of *P.biglobosa* also showed highly significant increase in moisture content, peroxide value and titratable acid in plastic packaging stored at room temperature. Bacteria count on PCA was highest and highly significant ($p<0.001$) in market condiments of *P.biglobosa;* a count of 2.0×10^6 was recorded in plastic packaging stored at room temperature. Mould counts were not observed for laboratory and market purchased condiments of *P.biglobosa* at six months (Tables 4.22-4.24).

Table 4.22 : Effects of drying and packaging conditions (at six months storage) on shelf life properties of *P.biglobosa* condiments produced with consortium A.

Seeds	Means of Fermentation	Duration of storage(months)	Drying Conditions	Packaging	Temperature (^{0}C) of storage	pH	Moisture (%)	Peroxide value(meq kg)	Titratable acidity(mg lactic g)	PCA (Cfu/g)	PDA(Cfu/g)
P.biglobosa	Consortium A	6	SLRE	Sachet	*8.67±0.33	5.80±0.06	0.43±0.03	4.25±0.00	1.11±0.00	1.2×10^5	NG
				plastic	8.57±0.33	5.87±0.03	9.40±0.10	13.57±0.03	1.61±0.00	1.4×10^6	NG
			SLRM	Sachet	27.33±0.33	5.83±0.03	0.47±0.07	4.33±0.02	1.11±0.00	1.1×10^5	NG
				Plastic	27.33±0.33	6.07±0.03	9.07±0.03	18.03±0.03	1.68±0.00	1.9×10^6	NG
			VaccRE	Sachet	8.33±0.33	5.27±0.03	0.47±0.03	4.30±0.00	1.11±0.00	1.2×10^5	NG
				plastic	8.33±0.33	5.13±0.07	9.43±0.37	18.23±0.03	1.61±0.00	1.4×10^6	NG
			VaccRM	Sachet	28.33±0.33	5.20±0.00	0.47±0.07	4.30±0.00	1.11±0.00	1.2×10^5	NG
				Plastic	28.33±0.33	5.17±0.07	9.53±0.03	18.87±0.03	1.70±0.00	1.8×10^5	NG
			OvRe	Sachet	9.00±0.00	6.73±0.03	0.37±0.03	4.19±0.00	1.11±0.27	1.0×10^4	NG
				plastic	9.00±0.00	5.53±0.03	3.50±0.00	5.27±0.0	1.32±0.00	1.4×10^5	NG
			OVRM	Sachet	27.33±0.33	5.87±0.03	0.37±0.03	4.19±0.00	1.44±0.27	1.1×10^4	NG
				Plastic	27.33±0.33	5.70±0.10	3.47±1.68	5.43±0.07	1.49±0.00	1.3×10^6	NG
			SUCRE	Sachet	8.33±0.33	5.57±0.03	0.47±0.03	4.25±0.03	1.10±0.00	1.2×10^5	NG
				plastic	8.33±0.33	5.13±0.07	9.13±0.03	15.07±0.03	1.36±0.00	1.6×10^6	NG
			SUCRM	Sachet	28.33±0.67	5.60±0.06	0.43±0.03	4.35±0.00	1.11±0.00	1.3×10^5	NG
				Plastic	28.33±0.67	5.27±0.03	9.67±0.33	18.43±0.07	1.41±0.00	1.5×10^6	NG
			SUDRE	Sachet	8.33±0.33	5.17±0.03	0.47±0.03	4.35±0.00	1.12±0.00	1.2×10^5	NG
				plastic	8.33±0.33	6.13±0.03	9.00±0.40	17.70±0.10	1.40±0.00	1.8×10^6	NG
			SUDRM	Sachet	27.67±0.33	5.60±0.06	0.40±0.00	4.32±0.02	1.17±0.00	1.2×10^5	NG
				Plastic	27.67±0.33	5.73±0.03	9.13±0.03	18.27±0.09	1.42±0.00	1.7×10^6	NG
			MarRE	Sachet	8.67±0.33	4.80±0.06	1.70±0.00	7.33±0.02	1.14±0.00	1.5×10^5	NG
				plastic	8.67±0.33	4.83±0.03	10.00±0.00	19.97±0.24	1.78±0.00	1.9×10^6	NG
			MarRM	Sachet	28.33±0.33	4.73±0.03	1.70±0.00	7.33±0.02	1.12±0.00	1.9×10^6	NG
				Plastic	28.33±0.33	4.90±0.00	10.00±0.00	22.33±0.17	1.79±0.10	2.0×10^6	NG

Values are means of triplicate determinations

Key:SLRE-solar dried refrigerator; SLRM-solar dried room temperature; VaccRM- vacuum dried room temperature; VaccRE –vacuum dried refrigerator;OVRE- oven dried refrigerator; OvRM- oven dried room temperature; SUCRE- sun dried with cover refrigeration; SUCRM – sun dried with cover room temperature; SUDRE- sun dried direct refrigeration; SUDRM-sun dried direct room temperature;MarRE- market sample refrigeration; MaRM-market sample room temperature; PCA- plate count agar; PDA-potato dextrose agar; NG – no growth.

Table 4.23: Effects of drying and packaging conditions (at six months storage) on shelf life properties of *P.biglobosa* condiments produced with consortium B.

Seeds	Means of Fermentation	Duration of storage(months)	Drying Conditions	Packaging	Temperature (^{0}C) of storage	pH	Moisture (%)	Peroxide value(meq kg)	Titratable acidity(mg lactic acid g)	PCA (Cfu/g)	PDA(Cfu/g)
P.biglobosa	Consortium B	6	SLRE	Sachet	*8.60±0.30	5.60±0.04	0.33±0.00	4.28±0.00	1.14±0.01	1.1×10^5	NG
				plastic	8.57±0.35	5.87±0.01	9.41±0.12	13.54±0.00	1.61±0.00	1.3×10^6	NG
			SLRM	Sachet	27.33±0.33	5.83±0.03	0.47±0.07	4.33±0.02	1.11±0.00	1.2×10^5	NG
				Plastic	27.33±0.33	5.07±0.03	9.07±0.03	18.03±0.03	1.68±0.03	1.8×10^6	NG
			VaccRE	Sachet	8.33±0.33	5.57±0.03	0.47±0.03	4.30±0.04	1.11±0.01	1.2×10^6	NG
				plastic	8.33±0.33	5.73±0.04	9.43±0.33	17.23±0.03	1.41±0.00	1.3×10^6	NG
			VaccRM	Sachet	28.33±0.33	5.90±0.00	0.47±0.08	4.30±0.02	1.11±0.02	1.2×10^5	NG
				Plastic	28.33±0.33	5.57±0.03	9.53±0.00	18.77±0.03	1.70±0.00	1.4×10^6	NG
			OvRe	Sachet	9.00±0.00	5.83±0.03	0.37±0.03	4.19±0.00	1.10±0.27	1.0×10^4	NG
				plastic	9.00±0.00	5.53±0.02	4.50±0.03	5.25±0.04	1.40±0.00	1.2×10^5	NG
			OVRM	Sachet	27.33±0.33	5.57±0.03	0.37±0.03	4.19±0.00	1.11±0.27	1.1×10^4	NG
				Plastic	27.33±0.33	5.70±0.10	4.47±1.68	5.43±0.07	1.37±0.02	1.4×10^6	NG
			SUCRE	Sachet	8.33±0.30	5.57±0.00	0.47±0.03	4.25±0.03	1.10±0.00	1.2×10^5	NG
				plastic	8.33±0.33	5.36±0.07	9.13±0.03	15.07±0.03	1.33±0.00	1.8×10^6	NG
			SUCRM	Sachet	28.33±0.65	5.30±0.07	0.43±0.03	4.35±0.00	1.11±0.04	1.3×10^5	NG
				Plastic	28.33±0.67	5.27±0.03	9.67±0.32	18.42±0.07	1.71±0.00	1.6×10^6	NG
			SUDRE	Sachet	8.33±0.33	5.49±0.02	0.47±0.03	4.35±0.05	1.11±0.05	1.2×10^5	NG
				plastic	8.33±0.32	5.13±0.03	9.00±0.45	17.70±0.10	1.60±0.00	1.6×10^5	NG
			SUDRM	Sachet	27.67±0.33	5.30±0.06	0.40±0.00	4.32±0.00	1.10±0.03	1.2×10^6	NG
				Plastic	27.67±0.30	5.17±0.00	9.13±0.03	18.27±0.09	1.62±0.00	1.6×10^6	NG

Values are means of triplicate determinations

Key

SLRE-solar dried refrigerator; SLRM-solar dried room temperature; VaccRM- vacuum dried room temperature; VaccRE –vacuum dried refrigerator;OVRE- oven dried refrigerator; OvRM- oven dried room temperature; SUCRE- sun dried with cover refrigeration; SUCRM – sun dried with cover room temperature; SUDRE- sun dried direct refrigeration; SUDRM-sun dried direct room temperature; PCA- plate count agar; PDA-potato dextrose agar;NG- no growth.

Table 4.24: Effects of drying and packaging conditions (at six months storage) on shelf life properties of *P.biglobosa* condiments produced without consortia.

Seeds	Means of Fermentation	Duration of storage(months)	Drying Condition	Packaging	Temperature (^{0}C)of storage	pH	Moisture (%)	Peroxide value(meq kg)	Titratable acidity(mg lactic acid g)	PCA (Cfu/g)	PDA(Cfu/g)
P.biglobosa	NF	6	SLRE	Sachet	*8.69±0.33	5.81±0.02	0.42±0.02	4.21±0.03	1.10±0.00	1.2×10^5	NG
				plastic	8.57±0.34	5.77±0.03	9.45±0.14	13.57±0.03	1.63±0.01	1.3×10^6	NG
			SLRM	Sachet	27.30±0.33	5.83±0.03	0.47±0.07	4.44±0.02	1.11±0.00	1.0×10^5	NG
				Plastic	27.33±0.30	6.06±0.00	9.07±0.01	18.03±0.02	1.68±0.00	1.4×10^6	NG
			VaccRE	Sachet	8.33±0.33	5.87±0.03	0.47±0.03	4.31±0.00	1.11±0.30	1.1×10^5	NG
				plastic	8.33±0.33	5.73±0.07	9.63±0.32	18.20±0.02	1.61±0.00	1.2×10^7	NG
			VaccRM	Sachet	28.33±0.33	5.90±0.00	0.47±0.05	4.32±0.00	1.11±0.05	1.1×10^6	NG
				Plastic	28.33±0.33	5.37±0.07	9.54±0.02	18.77±0.03	1.70±0.00	1.6×10^5	NG
			OvRe	Sachet	9.00±0.00	5.73±0.03	0.33±0.03	4.19±0.00	1.10±0.27	1.1×10^4	NG
				plastic	9.00±0.00	5.53±0.00	3.50±0.00	5.27±0.03	1.32±0.02	1.2×10^5	NG
			OVRM	Sachet	27.33±0.33	5.97±0.03	0.37±0.01	4.19±0.00	1.11±0.27	1.2×10^4	NG
				Plastic	27.33±0.30	5.72±0.10	4.47±1.64	5.43±0.05	1.47±0.00	1.3×10^5	NG
			SUCRE	Sachet	8.33±0.33	5.57±0.01	0.43±0.02	4.25±0.04	1.15±0.05	1.1×10^5	NG
				plastic	8.33±0.33	5.68±0.07	9.11±0.03	15.07±0.03	1.36±0.00	1.4×10^6	NG
			SUCRM	Sachet	28.33±0.67	5.60±0.03	0.43±0.03	4.35±0.00	1.14±0.03	1.2×10^5	NG
				Plastic	28.33±0.67	5.87±0.03	8.64±0.00	18.43±0.08	1.71±0.00	1.7×10^6	NG
			SUDRE	Sachet	8.33±0.35	5.80±0.00	0.48±0.01	4.35±0.05	1.14±0.02	1.2×10^5	NG
				plastic	8.33±0.33	5.13±0.03	9.01±0.40	17.80±0.10	1.60±0.00	1.4×10^5	NG
			SUDRM	Sachet	27.67±0.30	5.60±0.06	0.40±0.02	4.32±0.00	1.12±0.00	1.1×10^5	NG
				Plastic	27.67±0.34	5.73±0.03	9.11±0.03	18.29±0.09	1.72±0.02	1.7×10^6	NG

Values are means of triplicate determinations

Key:

SLRE-solar dried refrigerator; SLRM-solar dried room temperature; VaccRM- vacuum dried room temperature; VaccRE –vacuum dried refrigerator;OVRE- oven dried refrigerator; OvRM- oven dried room temperature; SUCRE-sun dried with cover refrigeration; SUCRM – sun dried with cover room temperature; SUDRE- sun dried direct refrigeration; SUDRM-sun dried direct room temperature; PCA- plate count agar; PDA-potato dextrose agar; NG- no growth

4.19: Effects of different modes of fermentation, drying and packaging on shelf life properties of powdered *G .max* condiments at two months storage

Table 4.25 shows that *G.max* condiments produced in the laboratory with both consortia and natural fermentation stored for two months showed highly significant difference ($p<0.001$) in temperature and pH. Lowest mean value for pH was observed in *G.max* condiments with vacuum dried condition packaged in plastic containers and stored at room temperature with consortium A, which had a mean value of 4.07. Highest mean value for moisture content was observed in market condiment which had a mean value of 1.23 in plastic packaging stored at room temperature. Peroxide value and titratable acidity were also highest and highly significant ($p<0.001$) in market purchased *G.max* condiment. Mould growth 1.0×10^4 was also observed in market condiments in plastic packaging stored at room temperature. There was no mould growth on potato dextrose agar for laboratory produced condiments of *G.max* (Tables 4.25-4.27)

Table 4.25: Effects of drying and packaging conditions (at two months storage) on shelf life properties of *G.max* condiments produced with consortium A.

Seeds	Means of Fermentation	Duration of storage(months)	Drying Condition	Packaging	Temperature(^{0}C) of storage	pH	Moisture (%)	Peroxide value(meq kg)	Titratable acidity(mg lactic acid g)	PCA (Cfu/g)	PDA(Cfu/g)
G.max	Consortium A	2	SLRE	Sachet	8.33±0.33	5.23±0.03	0.23±0.03	5.16±0.01	1.12±0.00	1.2×10^5	NG
				plastic	8.33±0.33	5.13±0.03	0.23±0.03	5.46±0.01	1.21±0.00	1.4×10^6	NG
			SLRM	Sachet	27.00±0.00	5.13±0.03	0.27±0.07	5.27±0.03	1.15±0.00	1.1×10^5	NG
				Plastic	27.00±0.00	5.10±0.00	0.60±0.31	5.47±0.03	1.41±0.00	1.1×10^6	NG
			VaccRE	Sachet	8.67±0.33	5.13±0.03	0.30±0.06	5.31±0.00	1.12±0.00	1.2×10^5	NG
				plastic	8.67±0.33	4.17±0.03	0.50±0.06	5.37±0.01	1.32±0.00	1.4×10^6	NG
			VaccRM	Sachet	27.33±0.33	5.13±0.03	0.33±0.09	5.11±0.00	1.12±0.00	1.3×10^5	NG
				Plastic	27.33±0.33	4.07±0.03	0.30±0.06	5.31±0.00	1.12±0.00	1.3×10^6	NG
			OvRe	Sachet	8.00±0.00	5.70±0.06	0.13±0.03	5.13±0.00	1.10±0.03	1.0×10^5	NG
				plastic	8.00±0.00	5.03±0.03	0.13±0.03	5.16±0.00	1.11±0.03	1.4×10^6	NG
			OVRM	Sachet	27.33±0.33	5.23±0.03	0.13±0.03	5.14±0.01	1.10±0.03	1.1×10^5	NG
				Plastic	27.33±0.33	5.03±0.03	0.43±0.03	5.20±0.00	1.50±0.03	1.5×10^6	NG
			SUCRE	Sachet	8.67±0.33	5.03±0.03	0.23±0.03	5.31±0.01	1.12±0.00	1.2×10^6	NG
				plastic	8.67±0.33	5.10±0.06	0.63±0.03	5.42±0.01	1.22±0.00	1.4×10^7	NG
			SUCRM	Sachet	27.33±0.33	5.07±0.03	0.23±0.03	5.29±0.00	1.14±0.00	1.3×10^6	NG
				Plastic	27.33±0.33	5.10±0.00	0.63±0.03	5.31±0.01	1.32±0.00	1.3×10^6	NG
			SUDRE	Sachet	8.67±0.33	5.10±0.06	0.13±0.03	5.11±0.00	1.12±0.00	1.2×10^5	NG
				plastic	8.67±0.33	5.00±0.06	0.66±0.03	5.38±0.00	1.12±0.00	1.4×10^6	NG
			SUDRM	Sachet	27.67±0.33	5.13±0.07	0.33±0.03	5.21±0.00	1.15±0.00	1.3×10^6	NG
				Plastic	27.67±0.33	5.10±0.06	0.73±0.03	5.41±0.00	1.42±0.00	1.2×10^6	NG
			MarRE	Sachet	9.00±0.00	4.20±0.06	1.17±0.09	7.10±0.00	1.14±0.00	1.5×10^5	NG
				plastic	9.00±0.00	4.90±0.35	1.17±0.09	7.41±0.00	1.36±0.00	1.7×10^7	NG
			MarRM	Sachet	27.00±0.00	4.80±0.35	1.13±0.03	7.11±0.00	1.16±0.00	2.1×10^6	NG
				Plastic	27.00±0.00	3.10±0.35	1.23±0.03	7.31±0.00	1.46±0.00	2.1×10^7	1.0×10^4

Values are means of triplicate determinations

Key

SLRE-solar dried refrigerator; SLRM-solar dried room temperature; VaccRM- vacuum dried room temperature; VaccRE –vacuum dried refrigerator;OVRE- oven dried refrigerator; OVRM- oven dried room temperature; SUCRE- sun dried with cover refrigeration; SUCRM – sun dried with cover room temperature; SUDRE- sun dried direct refrigeration; SUDRM-sun dried direct room temperature;MarRE- market sample refrigeration; MaRM- market sample room temperature; PCA- plate count agar; PDA-potato dextrose agar; NG – no growth.

Table 4.26: Effects of drying and packaging conditions (at two months storage) on shelf life properties of *G.max* condiments produced with consortium B.

Seeds	Means of Fermentation	Duration of storage (months)	Drying Condition	Packaging	Temperature(^{0}C) of storage	pH	Moisture(%)	Peroxide value(meq kg)	Titratable acidity(mg lactic acid g)	PCA (Cfu/g)	PDA(Cfu/g)
G.max	Consortium B	2	SLRE	Sachet	*8.33±0.31	5.13±0.02	0.20±0.01	5.16±0.00	1.10±0.00	1.2×10^5	NG
				plastic	8.33±0.30	5.12±0.01	0.33±0.03	5.44±0.01	1.32±0.04	1.3×10^6	NG
			SLRM	Sachet	27.00±0.00	5.13±0.03	0.25±0.04	5.27±0.03	1.11±0.00	1.2×10^5	NG
				Plastic	27.00±0.00	5.11±0.01	0.60±0.31	5.65±0.01	1.30±0.00	1.3×10^6	NG
			VaccRE	Sachet	8.67±0.33	5.13±0.03	0.10±0.06	5.31±0.00	1.12±0.01	1.2×10^5	NG
				plastic	8.67±0.31	5.03±0.01	0.31±0.01	5.64±0.00	1.41±0.00	1.4×10^6	NG
			VaccRM	Sachet	27.33±0.33	5.12±0.01	0.12±0.09	5.21±0.01	1.13±0.03	1.3×10^5	NG
				Plastic	27.33±0.31	5.01±0.00	0.31±0.01	5.60±0.04	1.30±0.00	1.4×10^6	NG
			OvRe	Sachet	8.00±0.00	5.42±0.04	0.11±0.03	5.12±0.03	1.10±0.04	1.1×10^4	NG
				plastic	8.00±0.01	5.53±0.01	0.33±0.04	5.31±0.02	1.23±0.03	1.4×10^5	NG
			OVRM	Sachet	27.33±0.33	5.22±0.01	0.10±0.03	5.14±0.04	1.16±0.05	1.1×10^5	NG
				Plastic	27.33±0.33	5.03±0.03	0.43±0.03	5.21±0.00	1.44±0.03	1.2×10^6	NG
			SUCRE	Sachet	8.67±0.33	5.03±0.02	0.23±0.02	5.31±0.01	1.12±0.00	1.2×10^5	NG
				plastic	8.67±0.33	5.11±0.06	0.61±0.03	5.40±0.04	1.42±0.04	1.4×10^6	NG
			SUCRM	Sachet	27.33±0.33	5.07±0.02	0.13±0.02	5.29±0.00	1.15±0.00	1.4×10^4	NG
				Plastic	27.33±0.33	5.00±0.00	0.43±0.03	5.32±0.01	1.52±0.01	1.2×10^5	NG
			SUDRE	Sachet	8.67±0.33	5.11±0.06	0.20±0.03	5.31±0.02	1.12±0.00	1.1×10^6	NG
				plastic	8.67±0.33	5.10±0.05	0.63±0.04	5.50±0.00	1.43±0.02	1.3×10^5	NG
			SUDRM	Sachet	27.67±0.33	5.23±0.07	0.23±0.03	5.21±0.00	1.12±0.00	1.1×10^6	NG
				Plastic	27.67±0.33	5.00±0.06	0.43±0.01	5.30±0.04	1.31±0.04	1.4×10^7	NG

Values are means of triplicate determinations

Key

SLRE-solar dried refrigerator; SLRM-solar dried room temperature; VaccRM- vacuum dried room temperature; VaccRE –vacuum dried refrigerator;OVRE- oven dried refrigerator; OvRM- oven dried room temperature; SUCRE- sun dried with cover refrigeration; SUCRM – sun dried with cover room temperature; SUDRE- sun dried direct refrigeration; SUDRM-sun dried direct room temperature; PCA- plate count agar; PDA-potato dextrose agar; NG – no growth..

Table 4.27: Effects of drying and packaging conditions (at two months storage) on shelf life properties of *G.max* condiments produced without consortia.

Seeds	Means of Fermentation	Duration of storage(months)	Drying Conditions	Packaging	Temperature(0C) of storage	pH	Moisture (%)	Peroxide value(meq kg)	Titratable acidity(mg lactic acid g)	PCA (Cfu/g)	PDA(Cfu/g)
G.max	NF	2	SLRE	Sachet	*8.33±0.33	5.11±0.02	0.23±0.01	5.16±0.01	1.11±0.00	1.1×10^5	NG
				plastic	8.33±0.33	5.13±0.03	0.42±0.03	5.44±0.00	1.31±0.01	1.0×10^5	NG
			SLRM	Sachet	27.00±0.00	5.01±0.03	0.14±0.00	5.17±0.03	1.10±0.00	1.2×10^5	NG
				Plastic	27.00±0.00	5.10±0.01	0.60±0.31	5.25±0.01	1.31±0.02	1.1×10^6	NG
			VaccRE	Sachet	8.67±0.33	5.03±0.03	0.12±0.06	5.31±0.02	1.12±0.00	1.0×10^5	NG
				plastic	8.67±0.33	5.06±0.02	0.30±0.04	5.35±0.01	1.41±0.00	1.1×10^6	NG
			VaccRM	Sachet	27.33±0.33	5.13±0.03	0.13±0.09	5.11±0.02	1.12±0.01	1.0×10^5	NG
				Plastic	27.33±0.33	5.17±0.03	0.31±0.03	5.31±0.00	1.32±0.00	1.3×10^6	NG
			OvRe	Sachet	8.00±0.00	5.51±0.02	0.11±0.03	5.12±0.01	1.10±0.03	1.0×10^4	NG
				plastic	8.00±0.00	5.43±0.03	0.13±0.00	5.13±0.00	1.16±0.02	1.0×10^5	NG
			OVRM	Sachet	27.33±0.33	5.43±0.03	0.10±0.03	5.12±0.02	1.17±0.03	1.0×10^5	NG
				Plastic	27.33±0.33	5.32±0.00	0.33±0.03	5.20±0.00	1.36±0.03	1.1×10^6	NG
			SUCRE	Sachet	8.67±0.33	5.03±0.03	0.13±0.01	5.31±0.02	1.12±0.01	1.2×10^5	NG
				plastic	8.67±0.33	5.10±0.02	0.41±0.03	5.41±0.01	1.42±0.00	1.1×10^6	NG
			SUCRM	Sachet	27.33±0.33	5.06±0.03	0.13±0.02	5.19±0.02	1.11±0.00	1.0×10^6	NG
				Plastic	27.33±0.33	5.10±0.00	0.40±0.02	5.33±0.01	1.32±0.01	1.3×10^7	NG
			SUDRE	Sachet	8.67±0.33	5.10±0.06	0.13±0.00	5.20±0.03	1.10±0.00	1.2×10^5	NG
				plastic	8.67±0.33	5.12±0.05	0.31±0.01	5.42±0.02	1.32±0.00	1.1×10^7	NG
			SUDRM	Sachet	27.67±0.33	5.13±0.06	0.10±0.00	5.21±0.00	1.12±0.01	1.0×10^5	NG
				Plastic	27.67±0.33	5.00±0.06	0.53±0.03	5.41±0.01	1.30±0.01	1.2×10^6	NG

Values are means of triplicate determinations

Key

SLRE-solar dried refrigerator; SLRM-solar dried room temperature; VaccRM- vacuum dried room temperature; VaccRE –vacuum dried refrigerator;OVRE- oven dried refrigerator; OvRM- oven dried room temperature; SUCRE- sun dried with cover refrigeration; SUCRM – sun dried with cover room temperature; SURE- sun dried direct refrigeration; SUDRM-sun dried direct room temperature ;PCA-plate count agar;PDA-potato dextrose agar; NG – no growth..

4.20 : Effects of different modes of fermentation, drying and packaging on shelf life properties of powdered *G .max* condiments at four months storage

G.max condiments produced in the laboratory with both consortia and by natural fermentation and stored for four months showed highly significant difference ($p<0.001$) in pH. Lowest mean value of pH was observed in *G.max* condiments with vacuum dried condition packaged in plastic and stored at room temperature with consortium A, which had a mean value of 4.93. Highest mean values for peroxide and titratable acidity for laboratory produced condiments with consortium B were 7.31 and 1.60 respectively. For natural fermentation highest mean peroxide and titratable acidity were 7.31 and 1.90 respectively. Highest mean value for moisture content was observed in market condiment which had a mean value of 10.50. Peroxide value and titratable acid was also highest in market *G.max* condiment and were highly significant ($p<0.001$). Moulds growth 1.0×10^4 was also observed in market condiments stored both at room and refrigeration temperature at four months of storage as compared to storage at two months. There was no mould growth on potato dextrose agar for laboratory produced condiments of *G.max* (Tables 4.28-4.30).

Table 4.28: Effects of drying and packaging conditions (at four months storage) on shelf life properties of *G.max* condiments produced with consortium A.

Seeds	Means of Fermentation	Duration of storage	Drying Conditions	Packaging	Temperature (^{0}C) of storage	pH	Moisture (%)	Peroxide value(meq kg)	Titratable acidity(mg lactic acid g)	PCA (Cfu/g)	PDA(Cfu/g)
G.max	Consortium A	4	SLRE	Sachet	*8.67±0.33	5.13±0.03	0.33±0.07	5.30±0.00	1.12±0.00	1.3×10^5	NG
				Plastic	8.67±0.33	5.03±0.03	9.13±0.03	6.21±0.01	1.41±0.01	1.3×10^7	NG
			SLRM	Sachet	28.00±0.58	5.13±0.03	0.43±0.03	5.34±0.01	1.11±0.00	1.2×10^5	NG
				Plastic	28.00±0.58	5.10±0.00	9.17±0.07	6.61±0.01	1.49±0.00	1.7×10^6	NG
			VaccRE	Sachet	8.67±0.33	5.13±0.03	0.47±0.03	5.30±0.01	1.12±0.01	1.2×10^5	NG
				plastic	8.67±0.33	5.17±0.03	9.43±0.03	6.30±0.01	1.42±0.00	1.9×10^6	NG
			VaccRM	Sachet	27.33±0.33	4.93±0.03	0.47±0.03	5.33±0.02	1.13±0.00	1.1×10^5	NG
				Plastic	27.33±0.33	5.17±0.03	9.30±0.06	7.31±0.00	1.52±0.00	1.5×10^6	NG
			OvRE	Sachet	8.33±0.33	5.50±0.06	0.23±0.03	5.20±0.00	1.13±0.27	1.0×10^4	NG
				Plastic	8.33±0.33	5.13±0.03	8.07±0.03	6.29±0.00	1.42±0.01	1.3×10^5	NG
			OVRM	Sachet	27.33±0.33	5.53±0.03	0.20±0.00	5.20±0.00	1.10±0.00	1.1×10^5	NG
				Plastic	27.33±0.33	5.03±0.03	9.47±0.07	6.29±0.00	1.52±0.01	1.3×10^6	NG
			SUCRE	Sachet	8.67±0.33	5.03±0.03	0.50±0.06	5.22±0.01	1.11±0.00	1.4×10^6	NG
				Plastic	8.67±0.33	5.10±0.06	10.47±0.07	6.32±0.01	1.41±0.00	1.6×10^7	NG
			SUCRM	Sachet	27.67±0.33	5.07±0.03	0.53±0.03	5.22±0.01	1.37±0.27	1.3×10^5	NG
				plastic	27.67±0.33	5.00±0.00	9.67±0.0	6.38±0.00	1.61±0.00	1.8×10^6	NG
			SUDRE	Sachet	8.67±0.33	5.10±0.06	0.47±0.03	5.21±0.00	1.11±0.00	1.2×10^5	NG
				plastic	8.67±0.33	5.10±0.06	9.17±0.32	6.41±0.01	1.41±0.00	1.9×10^6	NG
			SUDRM	Sachet	27.67±0.33	5.23±0.07	0.43±0.03	5.30±0.00	1.10±0.00	1.3×10^5	NG
				Plastic	27.67±0.33	5.20±0.06	9.67±0.03	6.31±0.00	1.61±0.00	1.8×10^6	NG
			MarRE	Sachet	9.00±0.00	5.20±0.06	1.23±0.03	7.10±0.00	1.18±0.00	1.9×10^5	NG
				plastic	9.00±0.00	3.57±0.03	10.50±0.00	10.37±0.13	1.63±0.01	3.9×10^6	1.0×10^4
			MarRM	Sachet	27.33±0.33	3.13±0.03	1.43±0.07	7.11±0.00	1.17±0.00	1.9×10^6	NG
				plastic	27.33±0.33	3.13±0.03	10.23±0.15	11.17±0.03	1.73±0.01	5.7×10^5	1.0×10^4

Values are means of triplicate determinations

Key:

SLRE-solar dried refrigerator; SLRM-solar dried room temperature; VaccRM- vacuum dried room temperature; VaccRE –vacuum dried refrigerator;OVRE- oven dried refrigerator; OvRM- oven dried room temperature; SUCRE- sun dried with cover refrigeration; SUCRM – sun dried with cover room temperature; SUDRE- sun dried direct refrigeration; SUDRM-sun dried direct room temperature;MarRE- market sample refrigeration; MaRM- market sample room temperature; PCA- plate count agar; PDA-potato dextrose agar; NG – no growth

Table 4.29 : Effects of drying and packaging conditions (at four months storage) on shelf life properties of *G.max* condiments produced with consortium B.

Seeds	Means of Fermentation	Duration of storage(months)	Drying Conditions	Packaging	Temperature (^{0}C) of storage	pH	Moisture (%)	Peroxide value(meq kg)	Titratable acidity(mg lactic acid g)	PCA (Cfu/g)	PDA(Cfu/g)
G.max	Consortium B	4	SLRE	Sachet	8.67±0.33	5.11±0.02	0.34±0.05	5.32±0.01	1.11±0.02	1.2×10^5	NG
				Plastic	8.67±0.33	5.03±0.03	9.13±0.02	6.21±0.04	1.41±0.01	1.4×10^6	NG
			SLRM	Sachet	28.00±0.58	5.11±0.03	0.43±0.03	5.34±0.01	1.11±0.01	1.2×10^5	NG
				Plastic	28.00±0.58	5.10±0.01	9.16±0.06	6.60±0.00	1.47±0.00	1.8×10^6	NG
			VaccRE	Sachet	8.67±0.33	5.13±0.03	0.47±0.03	5.30±0.01	1.12±0.01	1.3×10^5	NG
				plastic	8.67±0.33	5.16±0.04	9.45±0.02	6.30±0.01	1.43±0.02	1.7×10^6	NG
			VaccRM	Sachet	27.33±0.33	5.13±0.03	0.47±0.03	5.31±0.01	1.13±0.00	1.3×10^5	NG
				Plastic	27.33±0.33	5.17±0.01	9.31±0.06	7.31±0.02	1.54±0.03	1.7×10^6	NG
			OvRE	Sachet	8.33±0.33	5.22±0.06	0.23±0.01	5.23±0.00	1.12±0.27	1.1×10^5	NG
				Plastic	8.33±0.33	5.23±0.03	8.05±0.03	6.27±0.01	1.44±0.01	1.2×10^6	NG
			OVRM	Sachet	27.33±0.33	5.23±0.01	0.20±0.00	5.20±0.00	1.11±0.02	1.0×10^5	NG
				Plastic	27.33±0.33	5.01±0.03	8.48±0.01	6.27±0.03	1.52±0.01	1.7×10^6	NG
			SUCRE	Sachet	8.67±0.33	5.03±0.00	0.51±0.02	5.22±0.01	1.10±0.02	1.4×10^6	NG
				Plastic	8.67±0.33	5.11±0.06	9.47±0.07	6.44±0.01	1.41±0.00	1.6×10^7	NG
			SUCRM	Sachet	27.67±0.33	5.07±0.01	0.51±0.02	5.22±0.01	1.37±0.27	1.4×10^5	NG
				plastic	27.67±0.33	5.12±0.00	9.67±0.0	6.35±0.04	1.60±0.01	1.9×10^6	NG
			SUDRE	Sachet	8.67±0.33	5.12±0.06	0.45±0.01	5.20±0.03	1.12±0.00	1.2×10^5	NG
				plastic	8.67±0.33	5.20±0.04	10.14±0.32	6.41±0.01	1.40±0.01	1.9×10^6	NG
			SUDRM	Sachet	27.67±0.33	5.13±0.07	0.43±0.02	5.32±0.01	1.10±0.00	1.4×10^5	NG
				Plastic	27.67±0.33	5.12±0.06	10.66±0.03	6.31±0.00	1.60±0.02	1.9×10^6	NG

Values are means of triplicate determinations

Key

SLRE-solar dried refrigerator; SLRM-solar dried room temperature; VaccRM- vacuum dried room temperature; VaccRE –vacuum dried refrigerator;OVRE- oven dried refrigerator; OvRM- oven dried room temperature; SUCRE- sun dried with cover refrigeration; SUCRM – sun dried with cover room temperature; SUDRE- sun dried direct refrigeration; SUDRM-sun dried direct room temperature; PCA- plate count agar; PDA-potato dextrose agar; NG – no growth.

Table 4.30: **Effects of drying and packaging conditions (at four months storage) on shelf life properties of *G.max* condiments produced without consortia.**

Seeds	Means of Fermentation	Duration of storage(months)	Drying Conditions	Packaging	Temperature (^{0}C) of storage	pH	Moisture (%)	Peroxide value(meq kg)	Titratable acidity(mg lactic acid g)	PCA (Cfu/g)	PDA(Cfu/g)
G.max	NF	4	SLRE	Sachet	*8.67±0.33	5.11±0.00	0.31±0.06	5.33±0.01	1.11±0.02	1.2×10^5	NG
				Plastic	8.67±0.33	5.03±0.02	9.13±0.02	6.21±0.01	1.41±0.01	1.5×10^6	NG
			SLRM	Sachet	28.00±0.58	5.13±0.03	0.44±0.03	5.34±0.01	1.11±0.04	1.1×10^5	NG
				Plastic	28.00±0.58	5.11±0.00	9.17±0.07	6.60±0.03	1.45±0.00	1.9×10^6	NG
			VaccRE	Sachet	8.67±0.33	5.13±0.03	0.47±0.02	5.30±0.01	1.12±0.01	1.3×10^5	NG
				plastic	8.67±0.33	5.17±0.03	9.42±0.03	6.31±0.01	1.42±0.03	1.5×10^6	NG
			VaccRM	Sachet	27.33±0.33	5.12±0.01	0.47±0.04	5.33±0.02	1.12±0.00	1.4×10^5	NG
				Plastic	27.33±0.33	5.17±0.03	9.30±0.02	7.31±0.04	1.52±0.01	1.8×10^6	NG
			OvRE	Sachet	8.33±0.33	5.31±0.06	0.22±0.03	5.20±0.00	1.63±0.27	1.1×10^4	NG
				Plastic	8.33±0.33	5.23±0.02	8.07±0.01	6.27±0.01	1.41±0.01	1.3×10^5	NG
			OVRM	Sachet	27.33±0.33	5.23±0.03	0.21±0.00	5.20±0.00	1.90±0.03	1.0×10^5	NG
				Plastic	27.33±0.33	5.02±0.01	9.47±0.07	6.27±0.02	1.52±0.01	1.5×10^6	NG
			SUCRE	Sachet	8.67±0.33	5.03±0.03	0.50±0.03	5.22±0.01	1.11±0.00	1.4×10^6	NG
				Plastic	8.67±0.33	5.10±0.06	10.46±0.07	6.32±0.01	1.41±0.05	1.6×10^7	NG
			SUCRM	Sachet	27.67±0.33	5.06±0.02	0.53±0.01	5.21±0.02	1.37±0.27	1.3×10^5	NG
				plastic	27.67±0.33	5.10±0.00	9.67±0.00	6.38±0.00	1.60±0.00	1.8×10^6	NG
			SUDRE	Sachet	8.67±0.33	5.10±0.06	0.46±0.01	5.21±0.01	1.10±0.00	1.2×10^5	NG
				plastic	8.67±0.33	5.11±0.04	9.17±0.32	6.42±0.01	1.41±0.01	1.4×10^6	NG
			SUDRM	Sachet	27.67±0.33	5.10±0.04	0.40±0.00	5.31±0.01	1.12±0.02	1.3×10^6	NG
				Plastic	27.67±0.33	5.10±0.06	9.67±0.03	6.31±0.00	1.61±0.00	1.5×10^6	NG

Values are means of triplicate determinations

Key:

SLRE-solar dried refrigerator; SLRM-solar dried room temperature; VaccRM- vacuum dried room temperature; VaccRE –vacuum dried refrigerator;OVRE- oven dried refrigerator; OvRM- oven dried room temperature; SUCREh-sundriedwithcoverrefrigeration;SUCRM –sun dried wih ver room temperature; SUDRE- sun dried direct refrigeration; SUDRM-sun dried direct room temperature PCA- plate count agar; PDA-potato dextrose agar; NG- no growth

4.21: Effects of different modes of fermentation, drying and packaging on shelf life properties of powdered *G .max* condiments at six months storage

At six months storage of *G.max* condiment produced in the laboratory with both consortia and condiments from natural fermentation, there was also significant difference ($p<0.001$) in pH and bacteria count on PCA. Highest mean values of moisture content, peroxide value, and titratable acid were observed in both laboratory and market produced condiments of *G.max* at six months. Condiments of *G.max* fermented with consortium A shows highest mean values of peroxide (25.87) and titratable acidity (1.97). Also highest mean value for peroxide value and titratable acidity were 26.40 and 1.99 respectively. For condiments with natural fermentation, highest mean values for peroxide and titratable acidity were 25.86 and 1.94. Highest peroxide value and titratable acidity for market condiments were 29.47 and 1.99 respectively.Mould growth of 1.0 x 10^4 on potato dextrose agar was evident in both laboratory and market condiments particularly those packaged in plastic containers and stored at room temperature (Tables 4.31-4.33).

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Table 4.31 Effects of drying and packaging conditions (at six months storage) on shelf life properties of *G.max* condiments produced with consortium A.

Seeds	Means of Fermentation	Duration of storage(months)	Drying Condition	Packaging	Temperature (0C) of storage	pH	Moisture (%)	Peroxide value(meq kg)	Titratable acidity(mg lactic acid g)	PCA (Cfu/g)	PDA(Cfu/g)
G.max	Consortium A	6	SLRE	Sachet	*8.67±0.33	5.10±0.06	0.50±0.06	5.35±0.03	1.71±0.00	1.2×10^5	NG
				plastic	8.67±0.33	5.17±0.03	9.40±0.10	20.23±0.32	1.71±0.00	1.4×10^5	NG
			SLRM	Sachet	27.33±0.33	5.13±0.03	0.677±0.03	5.37±0.01	1.71±0.00	1.2×10^5	NG
				Plastic	27.33±0.33	5.07±0.03	9.07±0.03	23.03±0.03	1.78±0.00	1.4×10^6	1.0×10^4
			VaccRE	Sachet	8.67±0.33	5.17±0.03	0.47±0.03	5.40±0.00	1.12±0.00	1.2×10^5	NG
				plastic	8.67±0.33	5.13±0.07	9.77±0.03	24.23±0.03	1.78±0.00	1.3×10^6	NG
			VaccRM	Sachet	28.67±0.33	5.10±0.00	0.47±0.03	5.30±0.00	1.24±0.00	1.1×10^5	NG
				Plastic	28.67±0.33	5.07±0.07	9.53±0.03	25.87±0.03	1.80±0.00	1.0×10^6	NG
			OvRe	Sachet	8.67±0.33	5.73±0.03	0.37±0.03	5.24±0.01	1.23±0.27	1.0×10^5	NG
				plastic	8.67±0.33	5.53±0.03	7.50±0.00	19.27±0.07	1.32±0.00	1.1×10^6	NG
			OVRM	Sachet	27.33±0.33	5.57±0.07	0.33±0.03	5.31±0.01	1.90±0.00	1.1×10^5	NG
				Plastic	27.33±0.33	5.60±0.10	9.43±0.03	22.50±0.00	1.59±0.00	1.2×10^6	NG
			SUCRE	Sachet	9.00±0.00	5.57±0.03	0.37±0.03	5.32±0.02	1.60±0.00	1.4×10^5	NG
				plastic	9.00±0.00	5.13±0.07	10.13±0.03	24.90±0.20	1.80±0.00	1.5×10^5	NG
			SUCRM	Sachet	27.67±0.67	5.20±0.06	0.40±0.06	5.27±0.03	1.12±0.01	1.3×10^5	NG
				Plastic	28.33±0.67	5.17±0.03	9.67±0.33	22.43±0.07	1.91±0.00	1.4×10^6	1.0×10^4
			SUDRE	Sachet	8.00±0.00	5.23±0.03	0.50±0.00	5.27±0.00	1.16±0.00	1.2×10^5	NG
				plastic	8.00±0.00	5.13±0.03	9.67±0.07	22.70±0.10	1.70±0.00	1.9×10^6	NG
			SUDRM	Sachet	27.67±0.33	5.10±0.06	0.50±0.00	5.35±0.03	1.16±0.00	1.3×10^5	NG
				Plastic	27.67±0.33	5.13±0.03	9.13±0.03	23.20±0.15	1.97±0.00	1.4×10^6	NG
			MarRE	Sachet	8.67±0.33	5.80±0.06	1.53±0.03	7.50±0.00	1.40±0.00	1.8×10^5	NG
				plastic	8.67±0.33	5.17±0.03	9.00±0.00	28.60±0.06	1.78±0.00	2.0×10^5	1.0×10^4
			MarRM	Sachet	28.67±0.33	5.00±0.03	1.83±0.03	7.42±0.01	1.45±0.00	1.9×10^5	1.0×10^4
				Plastic	28.67±0.33	4.90±0.00	10.70±0.10	29.47±0.15	1.99±0.00	2.1×10^6	1.0×10^4

Values are means of triplicate determinations

Key: SLRE-solar dried refrigerator; SLRM-solar dried room temperature; VaccRM- vacuum dried room temperature; VaccRE –vacuum dried refrigerator;OVRE- oven dried refrigerator; OvRM- oven dried room temperature; SUCRE- sun dried with cover refrigeration; SUCRM – sun dried with cover room temperature; SUDRE- sun dried direct refrigeration; SUDRM-sun dried direct room temperature;MarRE- market sample refrigeration; MaRM- market sample room temperature; PCA- plate count agar; PDA-potato dextrose agar; NG – no growth.

Table 4.32 : Effects of drying and packaging conditions (at six months storage) on shelf life properties of *G.max* condiments produced with consortium B.

Seeds	Means of Fermentation	Duration of storage(months)	Drying Conditions	Packaging	Temperature (^{0}C) of storage	pH	Moisture (%)	Peroxide value(meq kg)	Titratable acidity(mg lactic acid g)	PCA (Cfu/g)	PDA(Cfu/g)
G.max	Consortium B	6	SLRE	Sachet	*8.67±0.33	5.11±0.04	0.52±0.04	5.33±0.01	1.12±0.02	1.2×10^5	NG
				plastic	8.67±0.33	5.17±0.03	9.40±0.10	20.23±0.32	1.71±0.01	1.5×10^5	NG
			SLRM	Sachet	27.33±0.33	5.11±0.01	0.47±0.00	5.37±0.00	1.12±0.00	1.2×10^5	NG
				Plastic	27.33±0.33	5.07±0.03	9.04±0.03	23.02±0.03	1.75±0.02	1.9×10^5	NG
			VaccRE	Sachet	8.67±0.33	5.55±0.00	0.47±0.03	5.40±0.00	1.12±0.00	1.2×10^5	NG
				plastic	8.67±0.33	5.13±0.07	10.75±0.00	21.23±0.02	1.77±0.00	1.8×10^5	NG
			VaccRM	Sachet	28.67±0.33	5.10±0.00	0.47±0.03	5.31±0.00	1.12±0.01	1.1×10^5	NG
				Plastic	28.67±0.33	5.53±0.06	9.23±0.03	25.87±0.01	1.81±0.00	2.0×10^5	1.0×10^4
			OVRe	Sachet	8.67±0.33	5.13±0.03	0.35±0.01	5.24±0.01	1.63±0.23	1.0×10^4	NG
				plastic	8.67±0.33	5.56±0.03	7.50±0.00	19.25±0.04	1.31±0.00	1.2×10^4	NG
			OVRM	Sachet	27.33±0.33	5.47±0.04	0.33±0.01	5.31±0.01	1.70±0.00	1.1×10^5	NG
				Plastic	27.33±0.33	5.13±0.10	8.11±0.03	22.50±0.02	1.99±0.02	1.5×10^5	NG
			SUCRE	Sachet	9.00±0.00	5.17±0.00	0.37±0.03	5.33±0.02	1.16±0.00	1.1×10^5	NG
				plastic	9.00±0.00	5.10±0.07	9.11±0.03	24.90±0.21	1.80±0.00	1.5×10^6	NG
			SUCRM	Sachet	27.67±0.67	5.10±0.06	0.40±0.04	5.24±0.03	1.16±0.02	1.3×10^5	NG
				Plastic	28.33±0.67	4.88±0.01	9.55±0.30	26.40±0.06	1.93±0.03	1.8×10^5	1.0×10^4
			SUDRE	Sachet	8.00±0.00	5.13±0.00	0.50±0.01	5.37±0.00	1.13±0.00	1.2×10^5	NG
				plastic	8.00±0.00	5.13±0.03	9.67±0.07	22.70±0.12	1.72±0.03	1.9×10^5	NG
			SUDRM	Sachet	27.67±0.33	5.60±0.06	0.53±0.02	5.33±0.01	1.13±0.00	1.2×10^5	NG
				Plastic	27.67±0.33	4.72±0.02	10.13±0.03	23.20±0.12	1.95±0.01	1.9×10^6	1.0×10^4

Values are means of triplicate determinations

Key

SLRE-solar dried refrigerator; SLRM-solar dried room temperature; VaccRM- vacuum dried room temperature; VaccRE –vacuum dried refrigerator;OVRE- oven dried refrigerator; OvRM- oven dried room temperature; SUCRE- sun dried with cover refrigeration; SUCRM – sun dried with cover room temperature; SUDRE- sun dried direct refrigeration; SUDRM-sun dried direct room temperature; PCA- plate count agar; PDA-potato dextrose agar; NG – no growth.

Table 4.33: Effects of drying and packaging conditions (at six months storage) on shelf life properties of *G.max* condiments produced without consortia

Seeds	Means of Fermentation	Duration of storage(months)	Drying Condition	Packaging	Temperature (^{0}C) of storage	pH	Moisture (%)	Peroxide value(meq kg)	Titratable acidity(mg lactic acid)	PCA (Cfu/g)	PDA(Cfu/g)
G.max	NF	6	SLRE	Sachet	8.67±0.33	5.11±0.04	0.41±0.05	5.34±0.01	1.16±0.01	1.2×10^5	NG
				plastic	8.67±0.33	5.16±0.03	9.40±0.11	20.23±0.02	1.73±0.00	1.6×10^6	NG
			SLRM	Sachet	27.33±0.33	5.13±0.01	0.46±0.01	5.36±0.00	1.16±0.05	1.2×10^5	NG
				Plastic	27.33±0.33	5.07±0.03	9.47±0.03	23.03±0.03	1.78±0.00	1.7×10^4	NG
			VaccRE	Sachet	8.67±0.33	5.14±0.00	0.47±0.00	5.40±0.01	1.11±0.02	1.2×10^5	NG
				plastic	8.67±0.33	5.13±0.07	9.70±0.03	21.22±0.03	1.78±0.00	1.9×10^6	NG
			VaccRM	Sachet	28.67±0.33	5.10±0.00	0.47±0.03	5.30±0.01	1.12±0.01	1.1×10^6	NG
				Plastic	28.67±0.33	4.56±0.02	9.83±0.00	25.86±0.03	1.81±0.01	1.9×10^5	1.0×10^4
			OvRe	Sachet	8.67±0.33	5.13±0.01	0.25±0.03	5.24±0.01	1.13±0.27	1.0×10^5	NG
				plastic	8.67±0.33	5.53±0.03	7.50±0.00	19.27±0.05	1.33±0.03	1.2×10^5	NG
			OVRM	Sachet	27.33±0.33	5.48±0.02	0.32±0.00	5.30±0.01	1.13±0.00	1.1×10^5	NG
				Plastic	27.33±0.33	5.10±0.10	8.12±0.02	22.40±0.04	1.54±0.01	1.4×10^6	NG
			SUCRE	Sachet	9.00±0.00	5.17±0.03	0.37±0.02	5.30±0.00	1.17±0.00	1.3×10^5	NG
				plastic	9.00±0.00	5.12±0.05	9.10±0.00	24.91±0.21	1.80±0.01	1.6×10^6	NG
			SUCRM	Sachet	27.67±0.67	5.10±0.01	0.40±0.04	5.20±0.02	1.18±0.04	1.3×10^6	NG
				Plastic	28.33±0.67	5.17±0.02	9.67±0.33	22.92±0.07	1.90±0.05	1.8×10^6	1.0×10^4
			SUDRE	Sachet	8.00±0.00	5.12±0.03	0.50±0.00	5.37±0.01	1.13±0.00	1.2×10^5	NG
				plastic	8.00±0.00	4.13±0.02	9.65±0.04	22.75±0.10	1.74±0.02	1.8×10^6	1.0×10^4
			SUDRM	Sachet	27.67±0.33	5.00±0.06	0.50±0.00	5.34±0.04	1.13±0.00	1.1×10^6	NG
				Plastic	27.67±0.33	5.11±0.02	9.73±0.01	23.20±0.15	1.94±0.01	1.4×10^7	1.0×10^4

Values are means of triplicate determinations.

Key

SLRE-solar dried refrigerator; SLRM-solar dried room temperature; VaccRM- vacuum dried room temperature; VaccRE –vacuum dried refrigerator;OVRE- oven dried refrigerator; OvRM- oven dried room temperature; SUCRE- sun dried with cover refrigeration; SUCRM – sun d ried with cover room temperature; SUDRE- sun dried direct refrigeration; SUDRM-sun dried direct room temperature; PCA- plate count agar; PDA-potato dextrose agar; NG – no growth

4:22: Proximate composition of powdered condiments of *P.africana, P.biglobosa,* and *G.max* produced in the laboratory with or without starters.

The condiments with the highest crude lipid were *G.max* fermented with and without consortia, that is, NFS6, FSSS3 and FSTS3, and values ranges from 16 – 18%. Condiment with the lowest crude lipid content was in *P.africana* fermented with and without consortia with a value of 10%. The condiments generally have appreciable values of crude protein, highest mean values of crude protein were observed in *G.max* condiments fermented with and without consortia, with a value of 26%. In crude fiber, highest mean values was in condiment of *P.biglobosa* fermented with and without cosortia (NFS4,FSSS2, FSTS2) values ranging from 14 – 15%. Soluble carbohydrates were appreciably high in *G. max* condiments fermented with consortium B (FSTS3) and without consortia (NFS6) which had values ranging from 27 – 30% respectively (Figure 12).

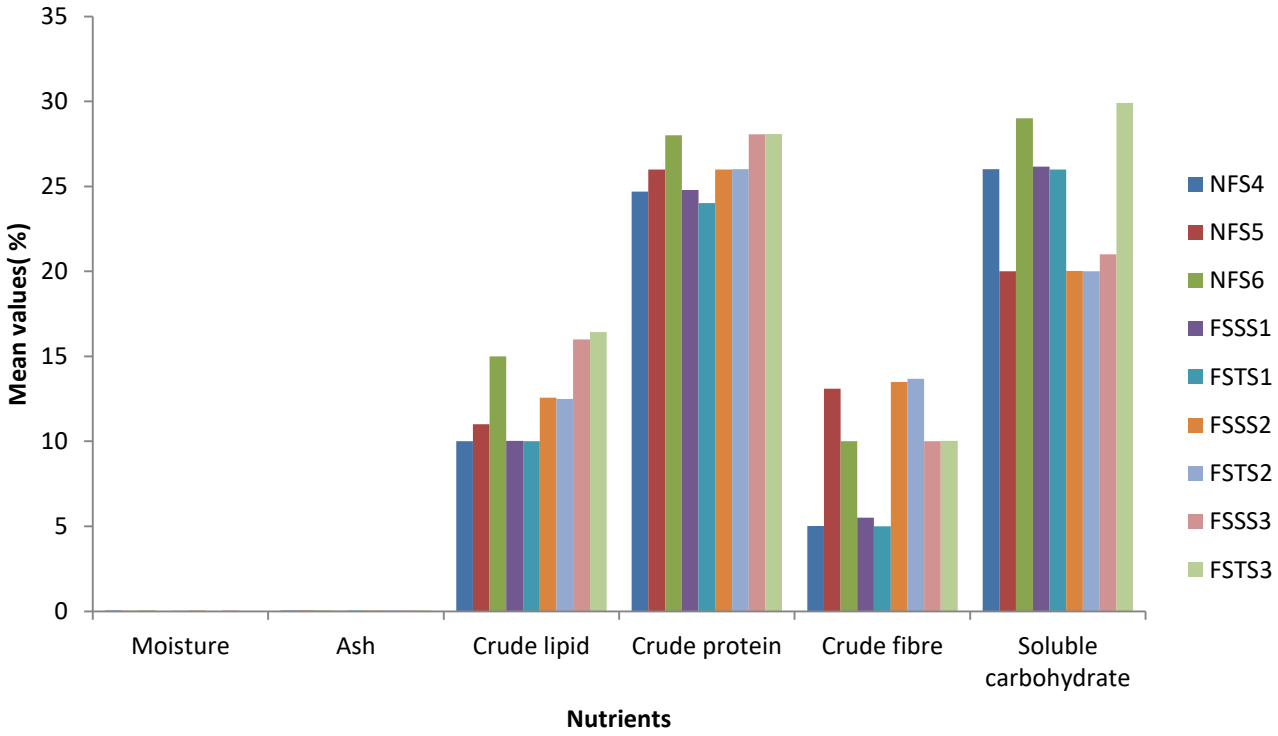

Figure 12: Proximate composition of powdered condiments of *P. africana, P. biglobosa,* and *G.max* fermented naturally and with consortia.

Key

NFS4-Condiment from natural fermentation of *P.africana*; NFS5- Condiment from natural fermentation of *P.biglobosa*

NFS6-Condiments from natural fermentation of *G.max*; FSSS1- Condiment from fermentation of *P.africana* with consortium A (standard strains *B.subtilis* and *B.pumilus*); FSSS2- Condiment from fermentationof *P.biglobosa* with consortium A(standard strains *B.subtilis* and *B.pumilus*);; FSSS3- Condiment from fermentation of *G.max* with consortium A(standard strains *B.subtilis* and *B.pumilus*); FSTS1- Condiment from fermentationof *P.africana* with consortium B(test strains *B.subtilis* and *B.pumilus*) FSTS2- Condiment from fermentation of *P.biglobosa* with consortium B(test strains *B.subtilis* and *B.pumilus*); FSTS3- Condiment from fermentation of *G.max* with consortium B test strains *B.subtilis* and *B.pumilus*).

4.23: Proximate composition of powdered condiments of *P.africana, P. biglobosa* and *G.max* purchased from Sabon gari market, Zaria

Proximate compositions of market condiments show that moisture was generally low and ranged from 1-2% in the three condiments. *G.max* condiments yielded the highest level of crude lipid, crude protein and soluble carbohydrates in this study (figure 13), mean values being 13, 28 and 27% respectively.

P. biglobosa and *P.africana* were comparable in their content of crude protein (22 and 20 mean percent respectively). The former had a higher crude lipid content of 13% while the later had a higher soluble carbohydrate content of 27%. Crude fibre was highest in *P.biglobosa* with a mean value of 13% (Figure 13).

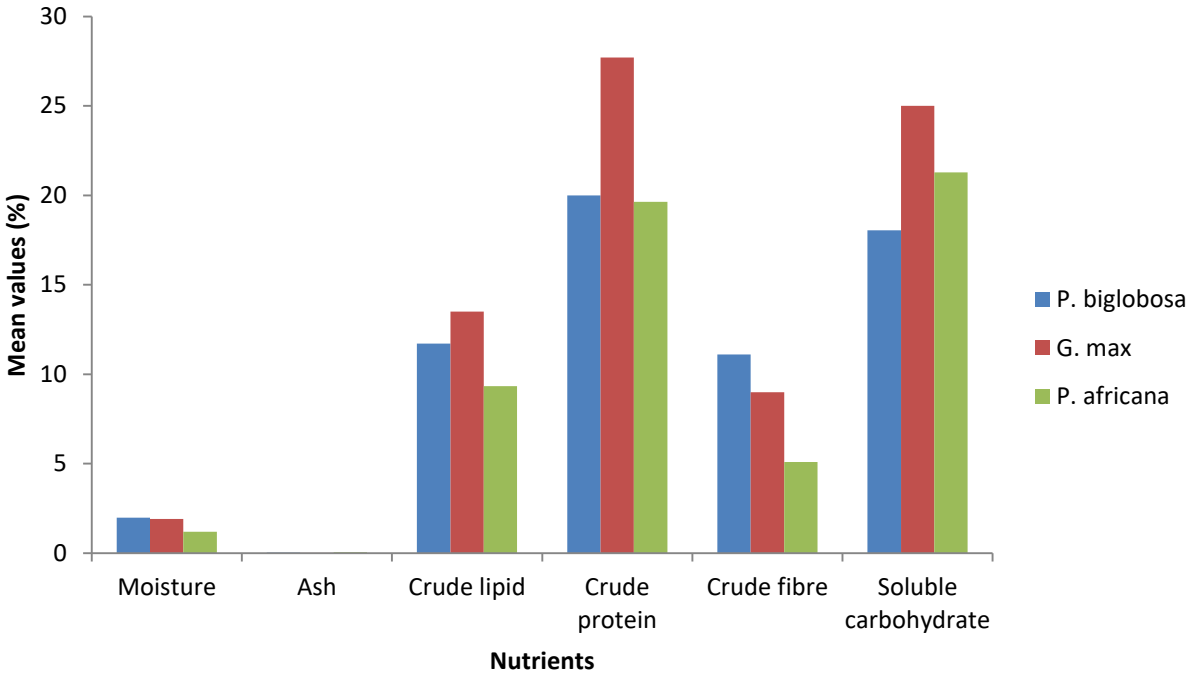

Figure 13: Proximate composition of dried powdered condiments of *P.africana, P. biglobosa* and *G.max* from Sabon gari market, Zaria.

4.24: Mineral element composition of powdered condiments from *P.africana, P.biglobosa, G.max* fermented in the laboratory with or without consortia.

Figure 14 shows that mineral element compositions of powdered condiments produced in the laboratory with and without consortia shows that condiments were generally rich in potassium (K), calcium (Ca) and sodium (Na). Only traces of lead (Pb) were recorded. Levels of iron (Fe) also minimal and were generally below 2.0 ppm.

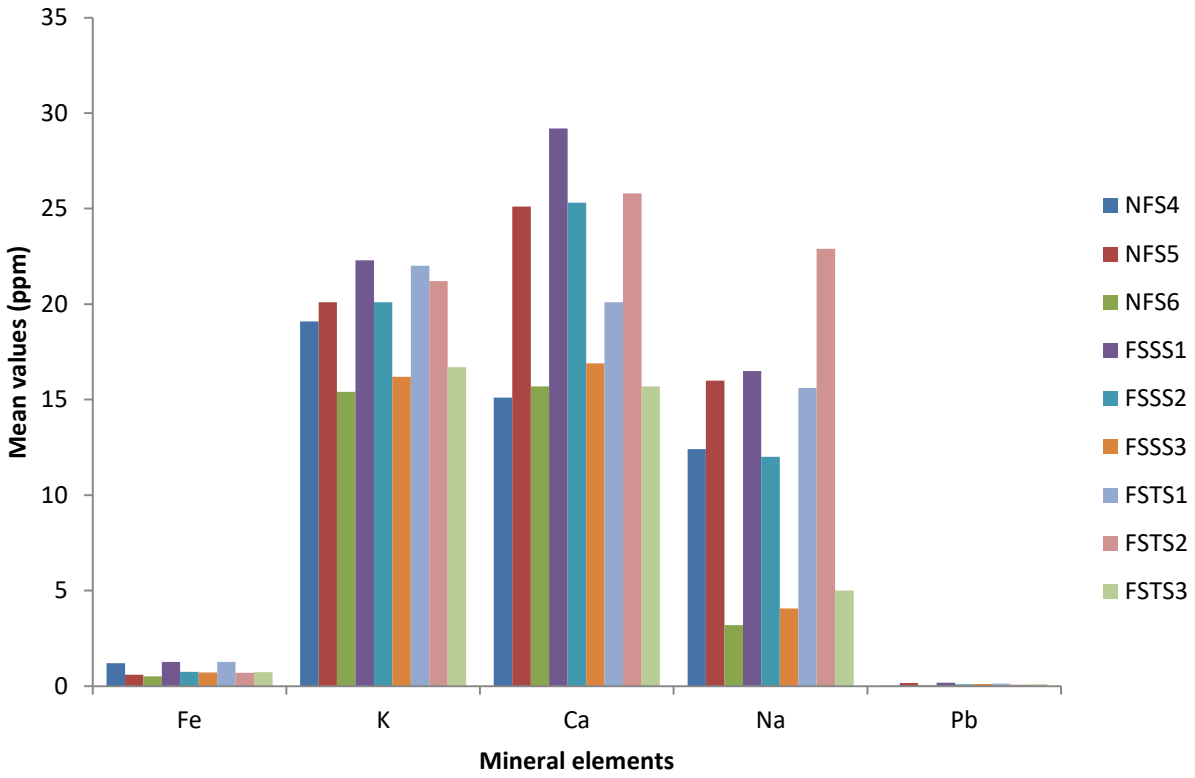

Figure 14: Mineral element composition of powdered condiments from *P.africana, P.biglobosa, G.max* fermented with or without consortia.

Key

NFS4-Condiment from natural fermentation of *P.africana*; NFS5- Condiment from natural fermentation of *P.biglobosa*

NFS6-Condiments from natural fermentation of *G.max*; FSSS1- Condiment from fermentation of *P.africana* with consortium A (standard strains *B.subtilis* and *B.pumilus*); FSSS2- Condiment from fermentationof *P.biglobosa* with consortium A(standard strains *B.subtilis* and *B.pumilus*);; FSSS3- Condiment from fermentation of *G.max* with consortium A(standard strains *B.subtilis* and *B.pumilus*); FSTS1- Condiment from fermentationof *P.africana* with consortium B(test strains *B.subtilis* and *B.pumilus*) FSTS2- Condiment from fermentation of *P.biglobosa* with consortium B(test strains *B.subtilis* and *B.pumilus*); FSTS3- Condiment from fermentation of *G.max* with consortium B test strains *B.subtilis* and *B.pumilus*).

4.25 : Mineral element compositions of powdered condiments of *P.africana, P.biglobosa* and *G.max* from Sabon gari market, Zaria.

Figure 15 shows that mineral element compositions of condiments obtained from the market shows that iron (Fe), calcium (Ca) and sodium (Na) were generally high in *P.africana* condiments with mean values ranging from 2, 27 and 17ppm respectively. Condiment of *P.biglobosa* is highest in potassium (K) with a mean value of 23ppm. Only traces of lead (Pb) were recorded in all the condiments.

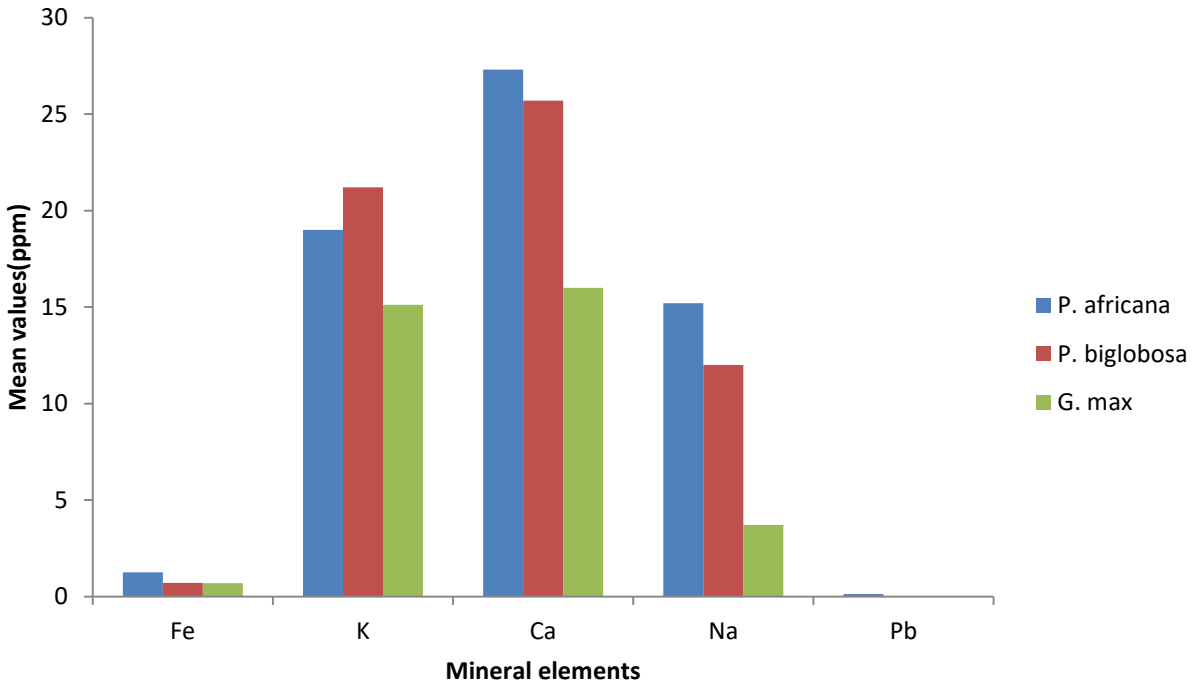

Figure 15: Mineral element compositions of dried condiments of *P.africana, P.biglobosa* and *G.max* from Sabon gari market, Zaria.

4. 26 : Essential amino acid composition of fresh and powdered condiments of *P.africana, P.biglobosa* and *G.max* produced with or without consortia.

Essential amino acids were compared in both fresh and dry condiments of *P.africana*, *P.biglobosa* and *G.max* condiments produced in the laboratory (Table 4.34). Higher mean values of essential amino acid were generally recorded in freshly fermented condiments than in their dried counterparts. Among the essential amino acids, leucine was found to have the highest mean value on 9.84 in non dried (that is, fresh) *G.max* seeds fermented with consortium A. Glutamate a non essential amino acid was generally high in all fermentation; the highest value of 18.97 was determined in *P.africana* fermented with consortium B. Also the lowest mean value of glutamate being 16.10 was (determined for *P.biglobosa* fermented with consortium B) compared with the highest value of 9.84 recorded for leucine earlier.

Table 4.34:Essential amino acid composition of freshly fermented seeds and fermented powdered condiments of *P.africana, P.biglobosa* and *G.max* with or without consortia

Plant	Means of fermentation	Condiments	Valine	Threonine	Isoleucine	Leucine	Lysine	Methionine	Phenylalanine	Histidine	Tyrosine	Cystein	Glutamate
P.africana	consortiumA	PCCA	4.19 ±0.00	2.72 ±0.01	3.74±0.00	6.82±0.03	3.42±0.00	1.84±0.03	4.18±0.00	*2.08 ±0.00	2.98±0.00	2.87±0.03	16.89±0.00
		FFS	6.64 ±0.03	4.98 ±0.00	6.46±0.01	8.77±0.04	5.93±0.00	3.32±0.09	7.92±0.03	4.64 ±0.04	4.29±0.03	6.93±0.00	18.87±0.00
P.africana	consortiumB	PCCB	4.20± 0.01	2.72± 0.01	3.78±0.00	6.85±0.02	3.40±0.00	1.84±0.03	4.20±0.00	2.11±0.02	2.90±0.00	2.88±0.01	16.90±0.01
		FFS	6.61± 0.00	4.99±0.00	6.42±0.01	8.70±0.03	5.95±0.00	3.31±0.09	7.90±0.02	4.67±0.02	4.30±0.01	6.95±0.00	18.97±0.00
P.africana	NF	FDS	3.13 ±0.00	1.43 ±0.00	2.74±0.30	5.81±0.00	2.43±0.00	1.28±0.03	3.71±0.27	1.94 ±0.03	1.88±0.03	4.12±0.36	16.69±0.00
		FFS	5.90 ±0.03	3.96 ± 0.07	5.84±0.03	6.85±0.04	2.43±0.06	2.70±0.00	3.94±0.01	2.92 ±0.01	2.72±0.03	4.92±0.00	18.92±0.02
P.biglobosa	consortiumA	PCCA	3.98 ±0.00	2.66±0.00	3.48±0.01	6.89±0.00	6.85±0.04	1.74±0.00	4.16±0.00	1.83 ±0.00	2.62±0.02	4.50±0.03	16.11±0.00
		FFS	5.93 ±0.00	4.60±0.29	5.91±0.00	8.96±0.03	8.31±0.00	3.92±0.01	6.78±0.00	3.79 ±0.00	4.98±0.00	6.72±0.00	17.68±0.00
P.biglobosa	consortiumB	PCCB	3.96±0.00	2.66±0.00	3.44±0.00	6.90±0.02	6.85±0.00	1.78±0.01	4.18±0.00	1.84±0.00	2.65±0.01	4.50±0.00	16.10±0.01
		FFS	5.99±0.00	4.62±0.00	5.90±0.00	8.91±0.01	8.29±0.00	3.90±0.00	6.70±0.00	3.80±0.01	4.90±0.00	6.75±0.03	17.70±0.00
P.biglobosa	NF	FDS	2.99 ±0.00	1.65±0.01	2.50±0.01	5.99±0.01	2.93±0.00	0.98±0.00	3.16±0.00	0.91 ±0.04	1.93±0.00	3.88±0.01	16.15±0.00
		FFS	4.78 ±0.00	3.48±0.01	3.01±0.00	6.23±0.00	2.98±0.00	1.27±0.04	4.23±0.00	1.22 ±0.01	3.21±0.00	4.13±0.00	17.01±0.00
G.max	consortiumA	PCCA	4.33 ±0.00	2.78±0.01	3.89±0.00	7.14±0.00	4.24±0.01	2.44±0.00	4.44±0.00	2.34 ±0.01	3.02±0.00	5.27±0.00	16.78±0.00
		FFS	6.33 ±0.00	4.56±0.00	6.88±0.01	9.84±0.01	5.59±0.06	4.22±0.00	7.71±0.00	4.96 ±0.00	6.34±0.00	9.36±0.01	17.68±0.01
G.max	consortiumB	PCCB	4.30±0.01	2.79±0.00	3.90±0.01	7.10±0.01	4.20±0.00	2.45±0.00	4.41±0.00	2.30±0.02	3.00±0.01	5.30±0.00	16.79±0.02
		FFS	6.30±0.00	4.60±001	6.71±0.00	9.80±0.00	5.61±0.01	4.25±0.02	7.69±0.01	4.86±0.00	6.50±0.00	9.29±0.00	17.60±0.00
	NF	FDS	3.94 ±0.04	1.79±0.07	2.97±0.01	6.88±0.01	2.51±0.00	1.82±0.03	3.36±0.00	1.97 ±0.00	2.71±0.00	4.15±0.01	16.82±0.01
		FFS	5.01 ±0.00	2.16±0.01	3.18±0.00	7.82±0.01	4.20±0.00	4.79±0.00	6.23±0.00	2.33 ±0.01	4.88±0.01	5.29±0.03	17.01±0.00

Values are means of triplicate determinations

Key

ConsortiumA- standard strains of *B.subtilis* and *B.pumilus;* consortiumB- test strains of *B.subtilis* and *B.pumilus;* PCCA- powdered condiments femented with consortium A; PCCB- powdered condiments femented with consortium B;FFS- fermented fresh seeds; FDS – fermented dried seeds; NF- natural fermentation

All the essential amino acids found in fresh and dry condiments were highly significant ($p<0.001$) (Appendix 53).

Glutamate a non essential amino acid was also highly significant ($p<0.001$) (Appendix 53).

4.27: Sensory evaluation of *egusi* soup prepared with powdered condiments produced by means of starters, and that prepared with naturally fermented condiments.

After assessment of the colour of various fermented seeds and appropriate powders (composition of soup include; one cup of blended *egusi*, four table spoonful of palmoil, ten gram of condiment, half teaspoonful of salt, one bulb of onion, one teaspoonful of powdered pepper, pumpkin leaves, four gram of powdered crayfish, one kilogram of dried antelope bush meat and three cups of water). Colour for *P.africana* condiments fermented with consortium A (CCA1), *P.africana* condiments fermented with consortium B (CCB1) and *P.africana* condiments fermented naturally (CNF4) had the highest mean values of 4.67, 4.33 and 4.20 respectively. Mouth feel and taste for *P.africana* condiments fermented with consortium A (CCA1) and *P.africana* condiments fermented with consortium B (CCB1) scored 1.00. Flavour and overall acceptability for CCA1 and CCB1 scored the lowest values. Negative control without condiments and mono sodium glutamate (C-) had the highest mean value of 5.56. Positive control with mono sodium glutamate (C+) only, had a mean value of 5.38 (Table 4.35).

Table 4.35: Sensory evaluation of *egusi* soup prepared with powdered condiments produced with starter assisted fermentation and condiments produced by natural fermentation.

Products	Colour	Texture	Odour	Mouth feel	Taste	Flavour	Overall Acceptability
CCA1	4.67	1.00	2.30	1.00	1.00	1.27	1.11
CCA 2	1.33	2.00	2.67	3.00	2.33	3.33	2.44
CCA 3	1.00	2.67	1.00	2.33	1.33	2.00	2.06
CCB1	4.33	2.33	2.67	1.00	1.00	1.27	1.10
CCB2	2.00	2.33	2.67	2.00	1.67	2.33	2.17
CCB 3	2.67	3.00	4.00	3.00	3.00	4.00	3.28
CNF 4	4.40	2.00	2.67	1.20	1.30	1.40	1.15
CNF 5	2.00	2.67	2.33	1.67	1.20	2.00	1.95
CNF 6	1.33	1.00	1.67	1.20	1.33	2.00	1.22
C+	-	-	-	4.00	4.33	4.33	5.38
C –	-	-	-	5.33	5.00	5.67	5.56
SEM±	0.18	0.17	0.25	0.25	0.27	0.27	0.19

Values are means of triplicate determination.

SEM= standard error of mean.

Key

Like extremely- (1); Like very much- (2); Like moderately- (3); Like slightly- (4) ;Neither like nor dislike -(5) ;Dislike slightly- (6); Dislike moderately- (7) ;Dislike very much- (8); Dislike extremely -(9); CCA1-*P.africana* condiments fermented with consortium A(standard strains of *B.pumilus* and *B.subtilis*); CCB1 *P.africana* condiments fermented with consortium B; CCA2- *P.biglobosa* condiments fermented with consortium A; (standard strains of *B.pumilus* and *B.subtilis)* CCB2 - *P.biglobosa* condiments fermented with consortium B; (test strains of *B.pumilus* and *B.subtilis)* CCA3 *G.max* condiments fermented with consortium A(standard strains of *B.pumilus* and *B.subtilis)*; CCB3- *G.max* condiments fermented with consortium B; (test strains of *B.pumilus* and *B.subtilis)* CNF4- *P.africana* condiments from natural fermentation; CNF5- *P.biglobosa* condiments from natural fermentation CNF6- *G,max* condiments from natural fermentation; C+ positive control with mono sodium glutamate; C- negative control without condiments and mono sodium glutamate,

CHAPTER FIVE

DISCUSSION

5.1 Identification of bacteria (test and standard strains)

Test and standard strains of bacteria obtained were characterized and identified using standard methods as described by Gordon *et al*., (1973).The major significant difference in the characteristics of these organisms was observed in starch hydrolysis. One of the organisms (*B.subtilis*) hydrolysed starch rapidly, while the other one was not able to hydrolyse the substrate. In the case of sugar fermentation and utilization one of the organisms (*B.subtilis*) was able to ferment all the sugars while the other organisms could not ferment all. On the basis of colony morphologies and cell characteristics, these organisms were characterized and identified as *B.subtilis* and *B. pumilus* respectively.

5.1.2 Indigenous organisms involved in the fermentation of leguminous seeds (*P.africana,P.biglobosa* and *G.max*)

Bacteria of the genus *Bacillus* was isolated from fresh samples of fermented *P.africana, P.biglobosa* and *G.max*. Species that were commonly isolated were *B.subtilis, B.pumilus, B. licheniformis* and *B.circulans* with *B.subtilis* predominating over other species of *Bacillus* (Omafvbe *et al.,* 2002). In this study only *B. subtilis,* combined with *B. pumilus* were used as consortia (consortium A: standard strains, and consortium B: test strains). Results from earlier studies showed that these two organisms fermented and produced products with very high sensory qualities. Both consortia were used in this study to develop starters for fermentation; they all increased in counts and were recovered at the end of the fermentation. *Bacillus* species have been found to be associated with fermentation of plant seeds for production of condiments. Oguntoyibo *et al.,* 2007 isolated a number of *Bacillus* species from fermenting African oil bean tree (*Pentaclethra macrophylla)* for '*ugba'* production. Achi (1999) isolated *Bacillus* species in fermentation of *P.africana* seeds for production of '*okpehe'.* Achi (2005)

isolated *Bacillus* species in the fermentation of "*okpehe* and *daddawa*". Oguntoyibo (2010) also isolated *Bacillus subtilis* in fermenting mash of *P.africana* for *"okpehe"* production. In the isolation, only bacterial growth was obtained, there was no fungal growth on potatoes dextrose Agar) which confirm earlier studies. The fact that fungi were not found in the fermentation of locust bean seeds suggests that it safe from the risk of mycotoxins. Although most of the very many soya bean fermented products of Asia are fermented by moulds (*Aspergillus* and *Mucor* species) a few are also carried out by bacteria (Yokotsuka, 1985). However, it has been noted that while fermented foods of South – East Asia are generally fermented by moulds, those of Africa are fermented by bacteria; this fact was noted as an important difference between African fermented foods and those of South –East Asia (Odunfa, 1985). Starter cultures are used to initiate soya fermentations in Asia and some African countries. Fermentations that do not require conscious introduction of starters would depend on chance inoculation by the microbial flora of the fermenting environments.

B. subtilis, and *B. pumilus* used as consortia in this study could be developed as starter cultures to enhance fermentation activities by reducing fermentation time and enhancing product quality.

5.1.3 Temperature, pH, plate count on PCA and PDA in relation to fermentation of *P.africana,P.biglobosa* and *G.max* with consortia A,B and under natural conditions.

In the controlled fermentations carried out in this study by inoculating consortium A and B inoculated into 300g unfermented seeds of *P.africana, P. biglobosa, G.max* and in fermentation of seeds under natural conditions, all the were sealed properly in earth pots lined with aluminum foil. In this fermentation, it was observed that adequate covering of the system to exclude as much air as possible, was essential for obtaining a good fermentation free of bacterial contaminants (Ogbadu, 1988). As fermentation time progressed, there was a rapid rise in pH from 6.3 to 8.4, temperature also increased from 35^0C to 49^0C and inoculum size increased from 2.1×10^7 to 4.5×10^7 cfu/g in all the fermenting mashes containing both consortia and the mashes that fermented naturally.

The rapid rise in pH from 6.3 to the alkaline region of 8.4 in all the fermenting mashes containing consortia A, B and the mashes that fermented naturally as observed in this study is peculiar to vegetable protein fermentation as was observed by Hesseltine, (1965). Odunfa (1981) also reported that during fermentation heat evolved and pH increases, which agrees with earlier studies. The pH of the fermentation medium increased from slightly acidic pH (6.3) at zero time to alkaline pH (8.4) after 48 hours of fermentation. The rise in pH could be due to rapid proteolytic activities, thus leading to release of ammonia and other volatile compounds making the environment unfavourable to moulds and growth of other unwanted organisms, thus agreeing with the report of Alozie *et al.*, (1980), that the inhibitory factor to mould growth is attributed to the alkaline pH. Odunfa, (1985) also reported that the major biochemical change in fermentation of locust bean for '*daddawa*' production was protein and amino acid metabolism, which is also characterized by strong ammonia smell and increase in pH, again in agreement with earlier study.

There was a rise in temperature from 35 to 49^0C as fermentation progressed for all the seeds with consortia and the ones that fermented naturally. The increase in temperature is due to increase in metabolic activities during which heat was evolved. (Odunfa,1981). Temperature dropped drastically as soon as fermentation processes was completed as a result of reduced metabolic activities shown in figures 2-7.

The persistence of consortia A and B at the end of the fermentation of *P.africana, P.biglobosa* and *G.max* seeds showed that they were responsible for the fermentations. Seeds of *P.africana, P.biglobosa* and *G.max* that fermented naturally also increased in counts of *Bacillus* till the end of fermentation. This is because even though starters were not inoculated into the seeds, fermentation took place, this showed that these organisms are indigenous to legume seeds and that they aided in their fermentations. They usually survived fermentation processing and carryout fermentation of these seeds (Ogbadu,

1988). A single species of *Bacillus*, namely *Bacillus subtilis* can initiate and end fermentation of locust bean seeds as reported by Odunfa, (1981). Experience has shown that mixed species of *Bacillus* enhances fermentation activities more than single species. *B. subtilis* has also been associated with fermenting soybean for *natto* production as reported by Hesseltine, (1965), wheat-milk mixture of *Rishk* preparation and fermenting rice in Ecuador stated by Ogbadu (1988). In a similar work, Obeta, (1983), reported that *Bacillus* species were found to be associated with fermentation of plant seeds, such as locust bean seeds and other legume seeds.

5.1.4: Benefits of utilizing starter cultures (*B.pumilus* and *B.subtilis*)

The development and introduction of combined *Bacillus* species as consortia is to speed up fermentation activities (Holzapfel, 2002). It was observed in this study that fermenting mashes inoculated with consortia A and B fermented within 48h. Mashes that fermented naturally completed the process within 72h as shown in figures 2-7. This is because starter cultures optimize production processes and they speed up fermentation by their abilities to break down protein to amino acids faster than seeds that fermented naturally.

5.1.5: Nutritional benefits of consortia assisted fermentation of *P.africana, P.biglobosa* and *G.max* seeds

Results of proximate analyses carried out in this study with freshly fermented seeds of *P.africana*, *P.biglobosa*, *G.max* and those of natural fermentation showed that crude protein (10-40%), crude lipids (2-29%), carbohydrates (1-12%), fibre (1%) and ash (2%). It can be concluded that fermentation of the legume seeds results in enhanced nutritional quality of the seeds as compared to unfermented seeds. Similarly, seeds fermented with consortia gave significantly higher nutritional values than seeds that fermented naturally because of the activities of the inoculated microorganisms. Platt,(1964) referred to this contribution by organisms in fermentations as “biological ennoblement,” showing increased

nutrients in fermented foods over the unfermented counterparts. Similarly, (Tamang, 2009) stated that increased nutritional content during fermentation is as a result of probiotic functions. Nutrient enhancement in fermented foods was also reported in an Indian cereal protein (Ogbadu,1988). Odunfa (1984) and Omafuvbe *et al.*,2002 also reported that protein, fats, vitamins especially riboflavins increased significantly during fermentation of legume seeds.

Dieticians recommended that certain mineral elements are required in the body for proper functioning of the body and for optimizing health conditions. Legume seeds are rich in some of these chemical elements. As shown in this study, potassium (5-12%), calcium (2-40%), and sodium (1-11%) were significantly high in naturally fermented and starter assisted fermented seeds of *P.africana* than in seeds of *P.biglobosa* and *G.max* . Lead (Pb) content was very low in all the seeds that is naturally fermented and seeds fermented with consortia. Low lead (Pb) content showed that legume seeds are relatively safe for consumption and that they are grossly deficient in the element. Similarly, fermented seeds of *P.africana, P.biglobosa* and *G.max* obtained from the market were not significantly different in nutrients from the seeds fermented in the laboratory. This is because same seeds used by the market people were the same used in the laboratory and their fermentation is mediated by the same organisms.

5.1.6: Effect of drying conditions and packaging on shelf life of powdered condiments of *P.africana, P.biglobosa,G.max* fermented with consortia and under natural conditions.

Different drying conditions (solar drying, sun drying with net protection, direct sun drying without net, vacuum drying and oven drying) were applied to fermented condiments. Seeds dried in hot air oven at a temperature of 45^0C for seven days gave products with best results. This is because the hot air released by the oven dried the environment and inhibited growth of unwanted organisms. Therefore during processing, if the fermented seeds are not allowed to dry properly or if they are not handled hygienically, moulds and other spoilage organisms would set in as shown in this study. This would result in shorter shelf life, and when consumed, the products constitute a health hazard. Duncan

multiple range tests (DMRT) indicated that condiments fermented with consortium A and subjected to oven drying condition was ranked the best among other drying conditions and was significantly different ($p < 0.001$) from other drying conditions (Appendix 31).

Results of condiments taken before storage showed that many samples of *G.max* had significant amount of peroxide value and titratable acidity before storage . This means that some acid producing activities were going on in *G.max* condiments from consortia and condiments during fermentation with or without starters. The packaging conditions can affect shelf life of products. Condiments packaged in sachets had a longer shelf life (six months) than those packaged in sterile plastic containers (two months) (for market condiments). This is due to the fact that the sachet were sealed airtight giving no room for entry of air, moisture and unwanted organisms. Condiments packaged in sterile plastic containers were covered, but the covers did not prevent air from entering, thereby leading to increase in moisture content by the second month particularly for *G.max* condiment purchased from the market. The increase in moisture content permitted growth of mould and unwanted bacteria, leading to a shorter shelf life. Condiments packaged in sachets and stored in the refrigerator at refrigeration temperature ($9^0C \pm 1^0C$) were in the same conditions as those packaged in sachets and stored on the shelf at room temperature ($27^0C \pm 2^0C$). This is due to the fact that since air is completely excluded from entering the sachets, contamination of condiments was minimal. Peroxide and titratable acidity values which are indices for deterioration were not significantly high ($p > 0.001$) for products packaged in sachets and stored at room and refrigeration temperature throughout the six months of storage for all the condiments. In contrast, the indices were significantly high ($p < 0.001$) for products of *G.max* packaged in plastic containers and stored at room and refrigeration temperatures for six months. Peroxidation value is a good indicator for fat deterioration (Kolapo *et al*., 2007), and can be used as an indicator of condiments spoilage. This was particularly evident for condiments of *G.max* at six months of storage in

plastic containers. At that period of storage mean value for peroxide content in *G.max* rose up to 25.87 with VaccRM packaged in plastic container fermented with consortium A and 29.47 packaged in plastic countainer and stored at room temperature for market samples. Pearson (1985) opined that for fatty foods rancidity sets in at peroxide value of 20-40meq kg. The high lipid content in condiments of *G.max* will lead to rancidity more rapidly than would be the case in condiments of *P.africana* and *P.biglobosa* if storage lasts for a longer time. Powdered condiments of *P.africana* had low peroxide and titratable acid values, thereby giving the product a longer shelf life over others. Dried condiments of *G.max* purchased from Sabon- gari market deteriorated faster (two months) than those produced under controlled conditions in the laboratory (six months). This is because laboratory products were fermented using starters under optimum conditions and were handled hygienically. Market samples were fermented using chance inoculation, and hygienic processes were not observed. This led to rapid deterioration of all products and high plate counts of spoilage organisms especially the condiments that were packaged in plastic containers and stored at room temperature on the shelf. Therefore if local processors can subject their products to hygienic methods of preparation, thorough drying and packaging, shelf life of their products can be prolonged.

Fermentation with consortia and those of natural fermentation were ranked equal with respect to their stability storage, which is also in line with the report of Omafuvebe *et al*., (2002).

5.1.7 : Proximate composition of fermented dried powdered condiments of *P.africana, P.biglobosa* and *G.max* produced from the laboratory

Results of proximate composition carried out in this study with fermented dried powdered condiments of *P.africana*, *P.biglobosa*, and *G.max* fermented with consortia and the powders of the condiments fermented naturally showed that crude protein, crude lipids, carbohydrates and fibre were appreciably high. Powdered condiment with the highest values of crude lipid and crude protein were condiments of

G.max fermented with consortia A and B respectively. This shows that dried powdered condiments are rich in nutrients and that even after drying, nutrients are not lost. Similarly, legume seed condiments are rich in some minerals. In this study it was shown that Fe, K, Ca, Na were highly significant ($p<0.001$). Highest mean values of Ca, Fe and Na were observed in dried powdered condiments of *P.africana* fermented with consortia A and B; this is in agreement with the report of Odunfa (1985). Dried powdered condiments of *P.africana*, *P.biglobosa*, and *G.max* purchased from Sabon gari market had significant amounts of nutrients and minerals in them. There was no difference in proximate and mineral composition between laboratory and market condiments in this study. This might probably be due to the fact that the same organisms mediate natural or local fermentation giving the same nutritional information as condiments produced in the laboratory.

5.1.8: Amino acid compositions and sensory quality (using nine point hedonic scale) of condiments of *P.africana, P.biglobosa* and *G.max.*

Legume condiments have substantial amount of essential amino acids in them (Messina 2010). All the essential amino acids were highly significant ($p<0.001$). Glutamate, a non essential amino acid, is a flavour enhancer (Nelson and Cox, 2005). *P.africana* condiment had the highest mean value of glutamate, this probably explains why *P.africana* fermented condiment aroma was sweeter and stronger than that *P.biglobosa* and *G.max*. Legume condiments are used to enhance flavour in dishes and soups because of their sweet strong aroma.

It was observed in sensory evaluation carried out using nine points hedonic scale, that *P.africana* condiments fermented with standard strains of *B.subtilis* and *B.pumilus* (CCA1), *P.africana* condiments fermented with test strains of *B.subtilis* and *B.pumilus* (CCB1), and *P.africana* condiments from natural fermentation (CNF4) had values ranging from 1.00 - 1.27 for mouth feel, taste, flavor and overall acceptability. On the hedonic scale, like very much was rated (1) and condiments of CCA1, CCB1 and CNF4 prepared with *P.africana* with consortia A, B and by natural fermentation were liked

very much and were most preferred by people. C- being a negative control without condiments and sodium mono glutamate was rated 5.56 for overall acceptability. C+ a positive control with sodium monoglutamate only was rated 5.38 for overall acceptability. On the hedonic scale (5) was rated neither like nor dislike. This implies that people prefer fermented legumes as flavour enhancers in dishes. In this study condiments from fermented legume seeds of *P.africana* were rated (1) and control (C+) with mono sodium glutamate only and control (C-) without mono sodium glutamate and without condiments were rated (5).

CHAPTER SIX

SUMMARY

Two distinct starter cultures from different sources were compared with respect to their physiological characteristics. Seeds of *P.africana. P.biglobosa* and *G.max* were analysed, and defined starters were used for production of condiments. Seeds were fermented using starters under different conditions and fermented seeds were analysed for proximate, minerals and amino acids compositions. Different drying conditions (vaccum drying, oven drying, solar drying, sun drying with net protection and direct sun drying) were used to dry condiments. Dried seeds were converted to powders using a sterile blender.Powdered condiments were packaged and stored under different drying conditions for a period of two, four and six months. Condiments were analysed prior to storage, and relevant parameters were reassessed within storage for two, four and six months.

The condiments were used for preparing *egusi* soup and consumer acceptance of the soup was evaluated as indicators of organoleptic qualities of the condiments.

6.1: CONCLUSION

Starter cultures from different sources have retained similar physiological properties over sometime. Among the physiological properties; *Bacillus pumilus* characteristically differs from *Bacillus subtilis* due to starch hydrolysis. These starter cultures at 5% inoculum or consortia when used on these seeds carryout fermentation faster, that is achieving 48h fermentation as oppose to 72h in natural fermentation. Oven drying and packaging in sachets seems to be most appropriate for improving the shelf life. Bacteria numbers, pH, moisture, peroxide and titratable acidity in oven dried condiments were very low as compared to other drying conditions. Oven dried can preserve condiments for six months as compared with sun drying with net protection and sundrying without net protection especially for *G.max* condiments. *Egusi* soup produced with condiments fermented with different means was analysed. A panel of tasters indicated that egusi soup produced by means of consortia A, B and by natural fermentation of *P.africana* was the most acceptable.

6.2: RECOMMENDATION

i. Use of starters for producing these condiments is recommended because of shorter fermentation time, microbiological safety. The shorter the fermentation time, the guarantee to development of cottage industries.

ii. Microbiological safety would enhance the use of these condiments by the elite of Nigerians who have become ambivalent towards the use of these condiments.

iii. It will also help the community to conserve some foreign exchange.

iv. Part of future study is to remove the odour, if organic acid, example citric acid is added to reduce the odour.

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APPENDIX 1

Processed unfermented seeds of *G.max.*

APPENDIX 2

Processed unfermented seeds of *P.africana.*

APPENDIX 3

Processed unfermented seeds of *P.biglobosa*.

APPENDIX 4

Fermented seeds of *P.africana*.

APPENDIX 5

Fermented seeds of *P.biglobosa*.

APPENDIX 6

Fermented seeds of *G.max.*

APPENDIX 7

Drying of fermented *P.africana, P.biglobosa* and *G.max* seeds using a vacuum pump**.**

APPENDIX 8

Drying of fermented *P.africana, P.biglobosa* and *G.max* seeds using direct sun light covered with net.

APPENDIX 9

Drying of fermented *P.africana*, *P.biglobosa* and *G.max* seeds using direct sun light without cover.

APPENDIX 10

Drying of fermented *P.africana, P.biglobosa* and *G.max* seeds using hot air oven

APPENDIX 11

Packaging of *P.africana, P.biglobosa* and *G.max* powdered condiments using sterile plastic containers.

APPENDIX 12

Packaging of *P.africana*, *P.biglobosa* and *G.max* powdered condiments using aluminum foil as sachets.

Appendix 13: Proximate composition of unfermented, naturally fermented and starter assisted fermented seeds of *P. africana*, *P. biglobosa* and *G. max*

	State of seeds												
	unfermented			Naturally fermented seeds			Starter assisted fermented seeds						
Parameters	*P. africana*	*P. biglobosa*	*G. max*	NFS4	NFS5	NFS6	FSSS1	FSTS1	FSSS2	FSTS2	FSSS3	FSTS3	***P***
Moisture	65.30 ± 0.26	^{h}67.50 ± 0.00	62.30 ± 0.30	65.0 ± 0.50	62.20± 0.27	62.20± 0.27	65.10 ± 0.10	65.30± 0.20	65.30± 0.20	65.43± 0.21	62.20± 0.26	^{l}62.13± 0.15	$< 0.001^{S}$
Ash	1.44 ± 0.00	1.93 ± 0.03	0.46 ± 0.01	2.30 ± 0.00	^{h}2.50 ± 0.01	0.22 ± 0.00	2.15± 0.12	2.30 ± 0.00	1.51± 0.01	1.52 ± 0.02	0.04 ± 0.50	^{l}0.01 ± 0.50	$< 0.001^{S}$
Crude lipid	2.55 ± 0.02	2.52 ± 0.02	^{l}1.91 ± 0.01	11.96 ± 0.01	12.74 ± 0.01	17.43 ± 0.01	12.03 ± 0.05	12.01 ± 0.00	13.03 ± 0.02	13.00 ± 0.00	^{h}18.43 ± 0.01	^{h}18.43 ± 0.01	$< 0.001^{S}$
Crude protein	17.13 ± 0.01	^{l}11.53 ± 0.01	15.81 ± 0.00	38.70 ± 0.20	36.00 ± 0.00	38.03 ± 0.05	40.05 ± 0.01	^{h}40.06 ± 0.0	36.14 ± 0.01	36.13 ± 0.01	38.27 ± 0.02	38.29 ± 0.01	$< 0.001^{S}$
Crude fibre	^{l} 0.01 ± 0.00	0.36 ± 0.01	0.38 ± 0.01	0.51 ± 0.00	1.50 ± 0.00	0.21± 0.01	0.38 ± 0.00	0.38 ± 0.01	1.92 ± 0.00	^{h}1.93 ± 0.00	0.03 ± 0.01	0.02 ± 0.01	$<0.011^{S}$
Soluble carbohydrate	4.04 ± 0.02	10.63 ± 0.01	4.31± 0.00	^{l}0.15 ± 0.00	^{h}12.09 ± 0.07	7.83 ± 0.00	0.17 ± 0.01	0.17 ± 0.01	7.99 ± 0.00	7.95 ± 0.02	1.82 ± 0.02	1.81 ± 0.00	$<0.002^{S}$

Values are means of triplicate determinations.

Keys

h = mean value with the highest composition.

l = mean value with the least composition.

NFS4 - Naturally fermented seeds of *P. africana* ; NFS5 - Naturally fermented seeds of *P. biglobosa*; NFS6 - Naturally fermented seeds of *G. max;* FSSS1 - Fermented seeds of *P. africana* with standard strain (consortium A)*;* FSTS1 - Fermented seeds of *P. africana* with test strain (consortium B)*;* FSSS2 - Fermented seeds of *P. biglobosa* with standard strain (consortium A); FSTS2 - Fermented seeds of *P. biglobosa* with test strain (consortium B); FSSS3 - Fermented seeds of *G. max* with standard strain (consortium A); FSTS3 - Fermented seeds of *G. max* with test strain (consortium B).

Appendix 14: ANOVA on Proximate composition of unfermented, naturally Fermented and starter assisted fermented seeds of *P. africana*, *P. biglobosa* and *G. max*

Nutrients	Sum of squares	df	Sum of mean	F	p-value
Moisture	123.406	11	11.219	86.982	<0.001
Ash	29.059	11	2.642	17291.086	<0.001
Crude lipid	1647.081	11	149.735	10.577	<0.001
Crude protein	3722.626	11	338.421	92366.489	<0.001
Crude fiber	17.036	11	1.549	17985.463	<0.001
Soluble carbohydrate	594.750	11	54.068	148584.205	<0.001

Appendix 15: Mineral element composition of unfermented, naturally fermented and starter assisted fermented seeds of *P. africana, P. biglobosa* and *G. max*

	State of seeds												
	Unfermented			Naturally fermented seeds			Starter assisted fermented seeds						*p*
Minerals	*P. africana*	*P. biglobosa*	*G. max*	NFS4	NFS5	NFS6	FSSS1	FSSS2	FSSS3	FSTS1	FSTS2	FSTS3	
Fe	1.17±0.10	0.51±0.00	0.75±001	1.02±0.12	0.80±0.02	^{l}0.38±0.00	1.27±0.00	0.85±0.05	0.71±0.00	^{h}1.52±0.11	0.95±0.01	0.73±0.00	< 0.001^{S}
K	9.68±0.00	9.83±0.01	^{h}12.9±0.05	11.1±0.00	8.67±0.04	5.47±0.05	7.53±0.01	9.60±0.01	5.23±0.03	7.35±0.04	9.11±0.00	^{l}5.11±0.05	< 0.001^{S}
Ca	10.6±0.02	13.7±0.10	^{l}2.16±0.00	7.30±0.01	16.6±0.01	7.32±0.01	10.8±0.02	16.1±0.02	^{h}43.8±0.04	10.1±0.00	17.1±0.03	42.9±0.02	<0.001^{S}
Na	4.31±0.05	2.30±0.03	^{l}1.34±0.02	9.12±0.00	6.06±0.00	3.39±0.10	12.5±0.05	6.11±0.00	4.60±0.01	^{h}12.6±0.05	5.11±0.04	4.61±0.00	< 0.001^{S}
Pb	0.11±0.05	^{h}0.14±0.00	0.04±0.01	^{l}0.00±0.01	0.11±0.02	0.01±0.00	0.12±0.00	0.03±0.02	0.03±0.00	0.11±0.02	0.01±0.01	0.03±0.01	<0.002^{S}

Values are means of triplicate determinations

Key: h = mean with the highest value
l = mean with the least value
NFS4-naturally fermented seeds of *P. africana*; NFS5- naturally fermented seeds of *P. biglobosa*
NFS6- naturally fermented seeds of *G. max*; FSSS1- seeds of *P. africana* fermented with consortium A; FSSS2- fermented seeds of *P. biglobosa* fermented with consortium A; FSSS3- fermented seeds of *G. max* with consortium A; FSTS1- fermented seeds of *P. africana* with consortium B FSTS2- fermented seeds of *P. biglobosa* with consortium B; FSTS3- fermented seeds of *G. max* with consortium B.

Appendix 16: ANOVA on mineral elements composition in unfermented, naturally fermented and starter assisted fermented seeds of *P. africana*, *P. biglobosa* and *G. max*

Mineral elements	Sum of squares	df	Sum of mean	F	p-value
Fe	3.864	11	0.351	63.373	<0.001
K	189.835	11	17.258	5607.193	<0.001
Ca	5753.179	11	523.016	28940.342	<0.001
Na	430.430	11	39.130	4339.746	<0.001
Pb	0.074	11	0.007	13.791	<0.001

Appendix 17: Proximate composition of freshly fermented *P. africana, P. biglobosa* and *G. max* seeds from Sabon gari market,Zaria.

	Market samples		
Nutrients	*P. biglobosa*	*G. max*	*P. africana*
moisture	62.10±0.01	62.07± 0.11	64.67 ± 0.57
Ash	2.50 ± 0.00	0.21 ± 0.00	64.67± 0.57
Crude lipids	12.72 ± 0.02	17.41 ± 0.01	11.92 ± 0.03
Crude protein	36.00 ± 0.00	38.03 ± 0.01	38.71 ± 0.01
Crude fiber	1.51 ± 0.01	0.22 ± 0.01	10.10 ± 1.31
Soluble carbohydrates	12.08 ± 0.01	7.81 ± 0.01	0.16 ± 0.01

Values are means of triplicate determinations.

Appendix 18 : ANOVA on proximate composition of freshly fermented *P. africana, P. biglobosa* and *G. max* seeds from Sabon gari market, Zaria.

Nutrients	Sum of squares	df	Sum of mean	F	p-value
Moisture	13.453	2	6.727	3363.289	<0.001
Ash	8021.224	2	4010.612	36100000	<0.001
Crude lipid	52.776	2	26.388	87960.333	<0.001
Crude protein	11.936	2	5.968	24415.409	<0.001
Crude fibre	173.660	2	86.830	868300.000	<0.001
Soluble carbohydrate	218.842	2	109.421	1094209.000	<0.001

Appendix 19: Mineral composition of fermented seeds of *P. africana, P. biglobosa* and *G. max* from Sabon gari market,Zaria

Mineral elements	Samples *P. africana*	*P. biglobosa*	*G. max*
Fe	1.21±0.01	0.79±0.04	0.79±0.01
K	6.98±0.00	9.01±0.02	5.34±0.00
Ca	10.1±0.05	15.0±0.00	42.8±0.02
Na	12.7±0.00	6.01±0.01	4.10±0.01
Pb	0.11±0.02	0.01±0.02	-0.02±0.00

Values are means of triplicate determinations.

Appendix 20: ANOVA on mineral composition of fermented seeds of *P. africana, P. biglobosa* and *G. max* from Sabon gari market, Zaria (ppm)

Mineral elements	Sum of squares	df	Sum of mean	F	p-value
Fe	0.364	2	0.182	1639.600	<0.001
K	20.495	2	10.248	46113.950	<0.001
Ca	1815.180	2	907.590	17017.313	<0.001
Na	122.364	2	61.182	917731.500	<0.001
Pb	0.016	2	0.008	37.000	<0.001

Appendix 21: Effect of the interaction of drying conditions on physicochemical properties of condiments fermented with or without consortia before storage (Temperature)

Duncan grouping	Mean	N	Factor
A	27.9319	27	Vaccum dried
B	27.7789	27	Oven dried
B	27.7704	27	Solar dried
C	27.6915	27	Sun dried (N)
D	27.5563	27	Sun dried (O)

Key:

(N)- Covered with net and dried; (O) – dried open.

Appendix 22: pH

Duncan grouping	Mean	N	Factor
A	5.5767	27	Oven dried
B	5.2726	27	Vaccum dried
BC	5.2681	27	Solar dried
BC	5.2578	27	Sun dried (N)
C	5.2541	27	Sun dried (O)

Key:

(N)- Covered with net and dried; (O) – dried open.

Appendix 23: Moisture

Duncan grouping	Mean	N	Factor
A	0.2652	27	Solar dried
AB	0.2574	27	Sun dried (N)
AB	0.2548	27	Sun dried (O)
B	0.2433	27	Vaccum dried
C	0.2148	27	Oven dried

Key:

(N)- Covered with net and dried; (O) – dried open.

Appendix 24: Peroxide value

Duncan grouping	Mean	N	Factor
A	4.3185	27	Vaccum dried
A	4.3174	27	Sun dried (O)
A	4.3100	27	Sun dried (N)
AB	4.2126	27	Solar dried
B	4.1522	27	oven dried

Key:

(N)- Covered with net and dried; (O) – dried open.

Appendix 25: Titratable acidity

Duncan grouping	Mean	N	Factor
A	1.1315	27	Sun dried (N)
A	1.1300	27	Sun dried(O)
AB	1.1204	27	Solar dried
B	1.1133	27	Vaccumdried
B	1.1085	27	Oven dried

Key:

(N)- Covered with net and dried; (O) – dried open.

Appendix 26: Bacterial counts

Duncan grouping	Mean	N	Factor
A	891222.22	27	Sun dried (N)
AB	846777.78	27	Sun dried (O)
B	543148.15	27	Solar dried
B	542407.41	27	Vaccumdried
B	526814.81	27	Oven dried

Key

PCA – plate count agar; (N)- covered with net and dried; (O) – dried open; consortium A- standard strains of *B.pumilus* and *B.subtilis*

Appendix 27:ANOVA on effect of the interaction of drying conditions on physicochemical properties of condiments fermented with consortia and naturally fermented before storage

Variable	Sum of squares	df	Mean square	F	p-value
Temperature	6.431	16	0.402	932.099	< 0.001
pH	2.040	16	0.127	249.400	< 0.001
Moisture	0.007	16	0.000	0.852	0.625
Peroxide value	1.939	16	0.121	4.323	< 0.001
Titratable acidity	0.008	16	0.001	1.481	0.123
Bacterial counts	1.459×10^{13}	16	9.118×10^{11}	158.252	< 0.001

Key:
PCA- plate count agar

Appendix 28: ANOVA for physicochemical properties of market condiments of *P.africana, P.biglobosa* and *G.max* before storage

Variable	Sum of squares	df	Mean square	F	p-value
Temperature	5.097	3	2.549	6199.324	< 0.001
pH	1.947	3	0.971	1004.379	< 0.001
Moisture	0.011	3	0.006	127.750	< 0.001
Peroxide value	0.052	3	0.026	24.853	<0.001
Titratable acidity	0.062	3	0.031	29.564	<0.001
Bacterial counts	6.289×10^{12}	3	3.145×10^{12}	277476.088	< 0.001

Key:
PCA- plate count agar

Appendix 29: Duncan's Multiple Range Test for Temperature (Laboratory produced condiments)

Duncan Grouping	Mean	N	Factor

Duncan Grouping	Mean	N	Condition
A	27.7638	162	solar dried stored at room temp.
B A	27.6861	162	direct sun dried stored at room temperature.
B C	27.4988	162	vacuum dried stored at room temperature.
C	27.2083	162	oven dried stored at room temp.
D	26.8559	162	sun dried covered with net at room temperature
E	9.2199	162	direct sun dried stored at refrigeration temp.
G F	8.6938	162	solar dried stored at refrigeration temp.
G F			
G F	8.5978	162	sun dried covered with net at refrige temp.
G			
G	8.4367	162	vacuum dried stored at refrigeration temp.
G	8.4071	162	oven dried stored at refrigeration temp.

Means of_

Duncan Grouping	Mean	N	Fermentation
A	18.13279	648	naturally fermented.
A			
A	18.09256	648	consortium A
A			
A	18.02972	647	consortium B

Duncan Grouping	Mean	N	Months
A	18.38273	648	6
B	17.96241	648	2
B			
B	17.90975	647	4

Duncan Grouping	Mean	N	seeds
A	18.38378	648	*P.africana*
B	18.09068	648	*G.max*
C	17.78023	647	*P.biglobosa*

Duncan Grouping	Mean	N	packaging
A	18.09166	972	sachets
A			
A	18.07844	971	plastics

Key: consortium A- standard strains of *B.pumilus* and *B.subtilis*; consortium B- test strain of *B.pumilus* and *B.subtilis*

Appendix 30: Duncan's Multiple Range Test for Temperature (Market condiments)

Duncan grouping	Mean	N	Factor
			Seeds
A	18.5017	3	*P. africana*
B	18.1843	3	*G. max*
C	18.0736	3	*P. biglobosa*
			Storage condition
A	27.8176	162	Room
B	8.8717	162	Refrigeration
			Month
A	18.5718	108	6
B	18.2251	108	4
C	17.9609	108	2
			Packaging
A	18.3398	162	Sachet
B	18.2312	162	Plastic

Appendix 31: Duncan's Multiple Range Test for pH (Laboratory produced condiments)

Duncan Grouping Mean N Factor

Drying_

Duncan Grouping	Mean	N	Condition
A	5.92346	162	oven dried stored at refrigeration temp.
B	5.83404	161	vacuum dried stored at room temp.
C	5.68093	162	sun dried covered stored at room temp.
D	5.54265	162	solar dried stored at room temp.
D			
D	5.53543	162	direct sun dried stored at refrigeration temp.
D			
D	5.53407	162	direct sun dried stored at room temp.
D			
D	5.49832	161	solar dried stored at refrigeration temp.
E	5.34123	162	sun dried covered stored at refrigeration temp.
E			
E	5.31562	162	oven dried stored at room temp.
E			
E	5.31167	162	vacuum dried stored at refrigeration temp

Means of_

Duncan Grouping	Mean	N	Fermentation
A	5.59590	648	consortium A
A			
A	5.58491	648	naturally fermented
A			
A	5.58432	646	consortium B

Duncan Grouping	Mean	N	Months
A	5.95610	648	6
B	5.45870	647	4
C	5.34977	647	2

Duncan Grouping	Mean	N	seeds
A	5.66628	648	*P. africana*
B	5.55272	647	*P. biglobosa*
B			
B	5.54601	647	*G. max*

Duncan Grouping	Mean	N	packaging
A	5.67473	971	Plastics
B	5.50203	971	Sachets

Key: consortium A- standard strains of *B.pumilus* and *B.subtilis*; consortium B- test strain of *B.pumilus* and *B.subtilis*

Appendix 32: Duncan's Multiple Range Test for Moisture (Laboratory produced condiments)

Duncan Grouping Mean N Factor

Duncan Grouping	Mean	N	Drying_ Condition
B			
B	6.631	161	vacuum dried stored at room temp.
B			
B	6.247	162	direct sundried stored at room temp.
B			
B	6.133	162	solar dried stored at room temp.
B			
B	6.004	161	sundried covered stored at room temp.
B			
B	4.301	162	direct sundried stored at refrigeration temp.
B			
B	4.030	161	vacuum dried stored at refrigeration temp.
B			
B	4.012	162	sundried covered stored at refrigeration temp.
B			
B	3.518	162	oven dried stored at room temp.
B			
B	2.835	162	solar dried stored at refrigeration temp.
B			
B	2.566	162	oven dried stored at refrigeration temp.

Duncan Grouping	Mean	N	Means of_ Fermentation
A	7.871	646	consortium B
A			
A	5.205	648	consortium A
A			
A	5.165	646	naturally fermented

Duncan Grouping	Mean	N	Months
A	10.016	646	6
A			
A	7.728	647	4
B	0.500	647	2

Duncan Grouping	Mean	N	seeds
A	8.098	646	*G.max*
A			
A	5.643	646	*P.biglobosa*
A			
A	4.503	648	*P.africana*

Duncan Grouping	Mean	N	packaging
A	11.614	969	plastics
B	0.557	971	sachets

Key: consortium A- standard strains of *B.pumilus* and *B.subtilis*; consortium B- test strain of *B.pumilus* and *B.subtilis*

Appendix 33: Duncan's Multiple Range Test for Peroxide value (Laboratory produced condiments)

Key: consortium A- standard strains of *B.pumilus* and *B.subtilis*; consortium B- test strain of *B.pumilus* and *B.subtilis*

Duncan Grouping	Mean	N	Factor

Drying_

Duncan Grouping	Mean	N	Condition
A	18.565	162	direct sundried stored at room temp.
B	8.062	162	direct sundried stored at refrig temp.
B			
B	7.907	162	vacuum dried stored at room temp.
B			
B	7.785	162	sundried covered stored at room temp.
B			
B	7.589	162	solar dried stored at room temp.
B			
B	7.511	162	vacuum dried stored at refrigeration temp.
B			
B	7.497	162	sundried covered stored at refrigeration temp
B			
B	7.277	162	oven dried at room temp.
B			
B	7.011	162	oven dried at refrigeration temp.
B			
B	6.925	162	solar dried stored at refrigeration temp.

Means of_

Duncan Grouping	Mean	N	Fermentation
A	10.798	648	consortium A
A			
A	8.055	648	naturally fermented
A			
A	8.016	648	consortium B

Duncan Grouping	Mean	N	Months
A	15.082	648	6
B	6.794	648	4
B			
B	4.994	648	2

Duncan Grouping	Mean	N	seeds
A	10.323	648	*G.max*
A			
A	8.860	648	*P.biglobosa*
A			
A	7.686	648	*P.africana*

Duncan Grouping	Mean	N	packaging
A	12.790	972	plastics
B	5.124	972	sachets

Appendix 34: Duncan's Multiple Range Test for Peroxide (Market condiments)

Duncan grouping	Mean	N	Factor
			Seed
A	11.2632	3	*G. max*
B	10.5638	3	*P. africana*
C	9.4033	3	*P. biglobosa*
			Storage condition
A	10.7223	162	Room
B	10.2239	162	Refrigeration
			Month
A	14.2603	108	6
B	9.9178	108	4
C	6.9972	108	2
			Packaging
A	18.3398	162	plastic
B	13.2312	162	Sachet

Appendix 35:Duncan's Multiple Range Test for Titratable acidity(Laboratory produced condiments)

Duncan Grouping	Mean	N	Factor

Drying_

Duncan Grouping	Mean	N	Condition
A	1.9438	162	vacuum dried stored at room temp.
A			
B A	1.7552	162	oven dried stored at refrigeration temp.
B A			
B A	1.4847	162	oven dried stored at room temp.
B A			
B A	1.4801	162	direct sundried stored at room temp.
B A			
B A	1.3844	162	sundried cover stored at room temp.
B			
B			
B	1.2394	162	solar dried stored at room temp.
B			
B	1.2335	161	direct sundried stored at refrigeration temp.
B			
B	1.2290	162	vacuum dried stored at refrigeration temp.
B			
B	1.2251	162	sundried cover stored at refrigeration temp.
B			
B	1.2217	162	solar dried stored at refrigeration temp.

Means of_

Duncan Grouping	Mean	N	Fermentation
A	1.5214	648	consortium B
A			
A	1.3784	647	naturally fermented
A			
A	1.2980	648	consortium A

Duncan Grouping	Mean	N	Months
A	1.5554	648	4
A			
A	1.4641	648	6
B	1.1780	647	2

Duncan Grouping	Mean	N	seeds
A	1.6154	648	*G.max*
A			
B A	1.3594	647	*P.biglobosa*
B			
B	1.2230	648	*P.africana*

Duncan Grouping	Mean	N	packagig
A	1.4955	972	plastics
A			
A	1.3029	971	sachets

Key: consortium A- standard strains of *B.pumilus* and *B.subtilis*; consortium B- test strain of *B.pumilus* and *B.subtilis*

Appendix 36: Duncan's Multiple Range Test for Titratable acidity (Market condiments)

Duncan grouping	Mean	N	Factor
			Seed
A	1.3571	3	*G. max*
B	1.3111	3	*P. biglobosa*
C	1.2021	3	*P. africana*
			Storage condition
A	1.2998	162	Room
A	1.2888	162	Refrigeration
			Month
A	1.3763	108	6
B	1.3109	108	4
C	1.1803	108	2
			Packaging
A	1.4378	162	plastic
B	1.1508	162	Sachet

Appendix 37: Duncan's Multiple Range Test for PCA(Laboratory produced condiments)

Duncan Grouping Mean N Factor

Duncan Grouping	Mean	N	Drying_ Condition
A	1198827	162	direct sundried stored at room temp.
B	1089451	162	sundried covered stored at refrig temp.
B	1056235	162	sundried covered stored at room temp.
C	822790	162	solar dried stored at refrig.temp.
C	813586	162	vacuum dried stored at refrig.temp.
D	665432	162	vacuum dried stored at room.temp.
D	628580	162	solar dried stored at room.temp.
ED	613951	162	oven dried stored at room temp.
EF	520623	162	oven dried stored at refrig temp.
F	511373	161	direct sundried stored at refrig temp.

Duncan Grouping	Mean	N	Means of_ Fermentation
A	982025	647	Consortium B
B	911909	648	Consortium A
B			
B	879151	648	Naturally fermented

Duncan Grouping	Mean	N	Months
A	1017037	648	6
B	902828	647	4
C	853097	648	2

Duncan Grouping	Mean	N	Seeds
A	1004352	648	*G.max*
B	908611	648	*P. biglobosa*
C	859934	647	*P. africana*

Duncan Grouping	Mean	N	packaging
A	1198273	971	Plastics
B	650673	972	sachets

Key: consortium A- standard strains of *B.pumilus* and *B.subtilis*; consortium B- test strain of *B.pumilus* and *B.subtilis*

Appendix 38 : Duncan's Multiple Range Test for PCA (market condiments)

Duncan grouping	Mean	N	Factor
			Seed
A	1756728.97	3	*G. max*
B	1454824.07	3	*P. africana*
C	1435000.00	3	*P. biglobosa*
			Storage condition
A	1.2998	162	Room
A	1.2888	162	Refrigeration
			Month
A	2031388.89	3	6
B	1355386.79	3	2
C	1253644.86	3	4
			Packaging
A	2053518.52	162	Plastic
B	1056369.20	162	Sachet

Appendix 39: ANOVA on effects of packaging and storage conditions on shelf life of powdered condiments obtained from fermented seeds of *P.africana P.biglobosa and G.max* with consortia A, B and naturally fermented stored at two, four and six months in the laboratory

Variable	Sum of squares	df	Mean square	F	p-value
Temperature	176703.1304	331	533.8463	288.85	< 0.0001
pH	561.3919	331	1.6960	31.35	< 0.0001
Moisture	677286.2700	331	2046.1800	1.31	0.0005
Peroxide value	652412.5540	331	1971.0340	1.18	0.0223
Titratable acidity	2812.5221	331	8.4971	1.24	0.0053
Bacterial counts	$1.273x\ 10^{15}$	331	3.846×10^{12}	20.74	< 0.0001

Key:

ANOVA- analysis of variance; PCA- plate count agar

Appendix 40: ANOVA on effects of packaging and storage conditions on shelf life of powdered condiments obtained from fermented seeds of *P.africana, P.biglobosa* and *G.max* purchased from the market and stored at two, four and six months

Variable	Sum of squares	df	Mean square	F	p-value
Temperature	4.254	4	2.127	26.957	< 0.001
pH	0.370	4	0.185	0.968	0.381
Moisture	1.9166.378	4	9583.189	1.011	0.365
Peroxide value	236.560	4	118.280	52.490	< 0.001
Titratable acidity	1.432	4	0.716	68.513	< 0.001
Bacterial counts	8.663×10^{12}	4	4.331×10^{12}	8.944	< 0.001

Key:

ANOVA- analysis of variance; PCA- plate count agar

Appendix 41: proximate composition of powdered condiments of *P.africana,P.biglobosa,*and *G.max* condiments allowed to ferment with consortia and natural fermentation.

Nutrients	Powdered condiments from natural fermentation			Powdered condiments from Starter assisted fermentation					
	NFS4	NFS5	NFS6	FSSS1	FSTS1	FSSS2	FSTS2	FSSS3	FSTS3
Moisture content	0.04 ± 0.01	0.03± 0.01	0.04± 0.02	0.02 ± 0.00	0.03± 0.02	0.04± 0.00	0.02± 0.03	0.04± 0.01	0.02± 0.05
Ash	0.05 ± 0.00	0.04 ± 0.01	0.05 ± 0.00	0.03± 0.12	0.05 ± 0.00	0.04± 0.01	0.04 ± 0.02	0.04 ± 0.50	0.05 ± 0.50
Crude lipid	10.01 ± 0.00^{D}	11.01 ± 0.01^{C}	15.00 ± 0.01^{C}	10.03 ± 0.04^{D}	10.00 ± 0.00^{D}	12.56 ± 0.01^{D}	12.50 ± 0.00^{D}	16.00 ± 0.01^{B}	16.43 ± 0.03^{A}
Crude protein	24.70 ± 0.02^{D}	26.00 ± 0.04^{B}	28.01 ± 0.00^{A}	24.79 ± 0.00^{C}	24.02 ± 0.01^{C}	26.00 ± 0.01^{H}	26.01 ± 0.02^{I}	28.06 ± 0.05^{A}	28.08 ± 0.01^{A}
Crude fiber	5.01 ± 0.00^{F}	13.10 ± 0.01^{A}	10.00 ± 0.02^{B}	5.50 ± 0.02^{E}	5.00 ± 0.05^{F}	13.50 ± 0.04^{A}	13.67 ± 0.00^{A}	10.00 ± 0.01^{B}	10.02 ± 0.04^{B}
Soluble carbohydrate	26.01 ± 0.02^{C}	20.00 ± 0.05^{G}	29.00 ± 0.01^{A}	26.17 ± 0.00^{B}	26.00 ± 0.00^{C}	20.02 ± 0.01^{F}	20.01 ± 0.02^{E}	21.00 ± 0.02^{G}	29.91 ± 0.02^{A}

Values are means of triplicate determinations

*values with same letter in a row are not statistically different. Values with different letters in a row are statistically different.

Keys

NFS4 - Powdered condiment from natural fermentation of *P. africana* ; NFS5 - Powdered condiment from natural fermentation of *P.biglobosa*; NFS6 - Powdered condiment from natural fermentation of *G.max;* FSSS1 – Powdered condiment from *P.africana* with consortium A*;* FSTS1 – Powdered condiment from *P.africana* with consortium B*;* FSSS2 – Powdered condiment from *P.biglobosa* with consortium A; FSTS2 – Powdered condiments from *P.biglobosa* with consortium B; FSSS3 – Powdered condiment from *G.max* with consortium A; FSTS3 – Powdered condiments from *G.max* with consortium B. consortium A- standard strains of *B.pumilus* and *B.subtilis*

consortium B;- test strains of *B.pumilus* and *B.subtilis*

Appendix 42: ANOVA table on proximate composition of powdered condiments of *P.africana,P.biglobosa,*and *G.max* condiments fermented with consortia and natural fermentation in the laboratory.

Nutrients	Sum of squares	df	Mean square	F	p-value
Moisture	0.002	8	0.000	1.277	0.315
Ash	0.001	8	0.000	2.250	0.072
Crude lipid	887.598	8	110.950	516490.272	< 0.001
Crude Protein	896.743	8	112.093	315261.070	< 0.001
Crude fibre	2661.450	8	332.681	477786.914	< 0.001
Soluble carbohydrate	1408.759	8	176.095	251564.155	< 0.001

Appendix 43: Proximate composition of powdered condiments of *P.africana, P. biglobosa* and *G.max* purchased from Sabon gari market, Zaria.

	Market samples		
Nutrients	*P.biglobosa*	*G.max*	*P.africana*
Moisture	1.98 ± 0.01^{A}	1.91 ± 0.10^{B}	1.20 ± 0.02^{C}
Ash	0.03 ± 0.05^{A}	0.02 ± 0.00^{C}	0.05 ± 0.01^{B}
Crude lipids	11.72 ± 0.02^{B}	13.51 ± 0.05^{A}	9.33 ± 0.03^{C}
Crude protein	20.00 ± 0.00^{B}	27.71 ± 0.01^{A}	19.64 ± 0.00^{C}
Crude fibre	11.10 ± 0.04^{A}	9.00 ± 0.02^{B}	5.10 ± 0.03^{C}
Soluble carbohydrates	18.05 ± 0.01^{C}	25.0 ± 0.00^{A}	21.29 ± 0.01^{B}

Values are means of triplicate determinations.

*Means with the same letter in the same row are not significantly different at $p \leq 0.05$.

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Appendix 44: ANOVA table on proximate compositions for market purchased condiments

Nutrients	Sum of squares	df	Mean square	F	p-value
Moisture	1.162	2	0.581	454.809	< 0.001
Ash	0.002	2	0.001	4.136	0.074
Crude lipid	26.428	2	13.214	11892.790	< 0.001
Crude protein	124.699	2	62.349	1870479.000	< 0.001
Crude fibre	55.674	2	27.837	32964.882	< 0.001
Soluble carbohydrate	72.564	2	36.282	544231.500	< 0.001

Post hoc analysis and DMRT was used to rank the condiments based on the nutrient contents. *G. max* was ranked the condiment with the highest ($p = 0.001$) nutrients content.

Appendix 45: Mineral element composition of powdered condiments from *P.africana, P.biglobosa, G.max* fermented with consortia and powdered condiments from natural fermentation produced in the laboratory .

Minerals	Powdered condiments from natural fermentation			Powdered condiments from Starter assisted fermentation					
	NFS4	NFS5	NFS6	FSSS1	FSSS2	FSSS3	FSTS1	FSTS2	FSTS3
Fe	1.20±0.10^{A}	0.60±0.00^{C}	0.52±0.01^{C}	1.26±0.02^{A}	0.74±0.03^{B}	0.72±0.05^{B}	1.27±0.10^{A}	0.70±0.02^{b}	0.73±0.00^{B}
K	19.1±0.00^{E}	20.1±0.02^{D}	15.4±0.05^{H}	22.3±0.05^{A}	20.1±0.01^{G}	16.2±0.01^{C}	22.0±0.04^{D}	21.2±0.00^{B}	16.7±0.02^{F}
Ca	15.1±0.05^{H}	25.1±0.01^{B}	15.7±0.01^{G}	29.2±0.02^{A}	25.3±0.02^{B}	16.9±0.02^{C}	30.1±0.01^{A}	25.8±0.05^{B}	15.7±0.01^{G}
Na	12.4±0.00^{C}	16.0±0.05^{A}	3.20±0.02^{I}	16.5±0.04^{A}	12.0±0.01^{H}	4.07±0.01^{F}	15.6±0.05^{B}	11.9±0.04^{C}	5.01±0.06^{G}
Pb	0.01±0.01^{G}	0.16±0.02^{A}	0.05±0.00^{F}	0.17±0.00^{A}	0.09±0.02^{C}	0.11±0.00^{F}	0.12±0.02^{E}	0.07±0.01^{B}	0.10±0.01^{D}

Values are means of triplicate determinations.

*Means with the same letter in the same row are not significantly different at $p \leq 0.05$.

.**Key**

NFS4-Condiment from natural fermentation of *P.africana*; NFS5- Condiment from natural fermentation of *P.biglobosa* NFS6-Condiments from natural fermentation of *G.max*; FSSS1- Condiment from fermentation of *P.africana* with consortium A (standard strains *B.subtilis* and *B.pumilus*); FSSS2- Condiment from fermentationof *P.biglobosa* with consortium A(standard strains *B.subtilis* and *B.pumilus*);; FSSS3- Condiment from fermentation of *G.max* with consortium A(standard strains *B.subtilis* and *B.pumilus*); FSTS1- Condiment from fermentationof *P.africana* with consortium B(test strains *B.subtilis* and *B.pumilus*) FSTS2- Condiment from fermentation of *P.biglobosa* with consortium B(test strains *B.subtilis* and *B.pumilus*); FSTS3- Condiment from fermentation of *G.max* with consortium B test strains *B.subtilis* and *B.pumilus*).

Appendix 46: ANOVA table on mineral element compositions of powdered condiments from *P.africana, P.biglobosa, G.max* fermented with consortia and powdered condiments from natural fermentation produced in the laboratory.

Minerals	Sum of squares	df	Mean square	F	p-value
Fe	2.107	8	0.263	99.477	< 0.001
K	158.280	8	19.785	23429.605	< 0.001
Ca	915.900	8	114.480	156119.318	< 0.001
Na	672.981	8	84.123	61056.732	< 0.001
Pb	0.062	8	0.008	46.250	< 0.001

DMRT was used to rank the condiments based on the mineral compositions. *P.africana* fermented with standard strains of *B.subtilis* and *B.pumilus* (FSSS1) was ranked the condiment with the highest ($p<$ 0.001) mineral content in laboratory produced condiments

Appendix 47: Mineral element compositions of condiments of *P.africana, P.biglobosa* and *G.max* purchased from Sabon gari market,Zaria .

Mineral elements	Samples *P.africana*	*P.biglobosa*	*G.max*
Fe	1.26 ± 0.01^{A}	0.70 ± 0.01^{B}	0.69 ± 0.01^{B}
K	19.0 ± 0.05^{B}	21.2 ± 0.06^{A}	15.12 ± 0.01^{C}
Ca	27.3 ± 0.05^{A}	25.7 ± 0.00^{B}	16.00 ± 0.04^{C}
Na	15.2 ± 0.01^{A}	12.0 ± 0.01^{B}	3.70 ± 0.00^{C}
Pb	0.12 ± 0.00^{A}	0.01 ± 0.03^{B}	0.03 ± 0.02^{B}

Values are means of triplicate determinations..

*Means with the same letter in the same row are not significantly different at $p \leq 0.05$.

Appendix 48: ANOVA on mineral composition in *P.africana, P. biglobosa* and *G.max* dried condiments purchased from Sabon gari market.

Minerals	Sum of squares	df	Mean square	F	p-value
Fe	0.639	2	0.319	3193.000	< 0.001
K	56.861	2	28.430	23051.676	< 0.001
Ca	224.340	2	112.480	82075.610	< 0.001
Na	211.380	2	105.690	1585350.000	< 0.001
Pb	0.016	2	0.008	34.714	0.001

Appendix 49: ANOVA of mean of amino acids in laboratory prepared condiments

Amino acid	Sum of squares	df	Mean square	F	p-value
Valine	73.015	17	4.295	21475.025	< 0.001**
Threonine	74.024	17	4.354	828.523	< 0.001**
Isoleucine	123.982	17	7.293	1050.760	< 0.001**
Leucine	82.245	17	4.838	11929.170	< 0.001**
Lysine	194.880	17	11.464	23098.210	< 0.001**
Methionine	69.835	17	4.108	205.017	< 0.001**
Phenylalanine	151.686	17	8.923	2158.715	< 0.001**
Histidine	86.466	17	5.086	15784.883	< 0.001**
Tyrosine	98.878	17	5.816	29912.758	< 0.001**
Cystein	182.599	17	10.741	1447.154	< 0.001**
Glutamate	41.588	17	2.446	36695.191	< 0.001**

** = highly significant difference ($p<0.001$) exists in the means of the amino acids in the condiments.

DMRT(Duncan multiple range tests) showed that freshly fermented seed of *G. max* that was fermented with consortium A yielded the highest quantity of essential amino acids, as compared to dried condiments.

Appendix 50: Sensory evaluation form using nine (9) point hedonic scale for three different African condiments (soya bean, locust bean and African mesquite in powdered form) with *egusi* soup

Questionnaire for hedonic scale

Name……. Date……

Product……..

Taste this sample (s) and check how much you like or dislike each one by going through the nine point hedonic scale. Please indicate your likes and dislikes by using the numbers attached to the scale.

Sensory attributes

Hedonic scale	Colour	Texture	Odour	Mouth feel	Taste	Flavour
Remarks						
Like extremely (1)						
Like very much (2)						
Like moderately (3)						
Like slightly (4)						
Neither like nor dislike (5)						
Dislike slightly (6)						
Dislike moderately (7)						
Dislike very much (8)						
Dislike extremely (9)						