UPGRADE OF TRADITIONAL PROCESSING METHODS OF “DADDAWA” AND “OGIRI”

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A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF DOCTOR OF PHILOSOPHY IN MICROBIOLOGY

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MAY, 2017

DECLARATION

I declare that the work in this thesis entitled **Upgrade of Traditional Processing Methods of “Daddawa” and “Ogiri”** has been carried out by me in the Department of Microbiology. The information derived from literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at this or any other institution.

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Name of Student	Signature	Date

CERTIFICATION

This thesis entitled UPGRADE OF TRADITIONAL PROCESSING METHODS OF “DADDAWA” AND “OGIRI” by ORABUIKE Joseph Chisolu meets the regulation governing the award of degree of Doctor of Philosophy in Microbiology of the Ahmadu Bello University and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

To my family and men of goodwill.

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ABSTRACT

Traditional condiments such as “daddawa” and “ogiri” are fermented products of legumes and oil seeds by B*acillus subtilis* and other organisms that contribute distinct taste or specific flavour imparting on socio-sensory attribute of foods and are widely used in various African dishes. Spontaneous fermentation methods were explored resulting in specific taste and varying quality of the product from various sources. “Daddawa” and “ogiri” were purchased from Zaria Central Market, Zaria, Kaduna State and Abagana Central Market, Anambra State. Mannitol Yolk Polymyxin (MYP) agar was mixed with egg, prepared and used to isolate *Bacillus substilis* from these condiments for the purpose of this study. The *Bacillus subtilis* isolates were characterized and mutated in order to improve its fermentative ability and a loopfull was taken and grown for sequencing. African Locust beans and castor oil seeds were cleaned, boiled for 18 hours and made into paste and inoculated with isolated *Bacillus_substilis,* standard strain of *Bacillus substilis* (ABC35615) *and* Mutated *Bacillus substilis* obtained after exposure to UV – light for 30 and 40 minutes and nitrous acid. Seventy five flat bottom flasks were cleaned and twenty five (25g) of paste (African locust bean and Castor oil seed) were separately weighed into each of the flasks. Each of these flasks was allowed to ferment for four days. Samples were taken from various fermented paste in each flask and analysed for proximate composition which had an average of 17.83% moisture, 0.2% ash, 33.90% protein, 23.76% fat and 24.51% carbohydrate. The results obtained were statistically analysed and it was observed that most of the condiments at the varying temperature, pH and the types of organisms used had between 14.26 - 17.29% moisture for “daddawa” and 14.53 - 18.63% moisture for “Ogiri”, 31.73-37.43% protein for “daddawa” and 21.79 – 32.10% protein for “Ogiri” and 22.76 – 23.69% fat for “daddawa”

and the 21.10% - 23.32% fat for "ogiri". The optimal pH for fermentation of these condiment was pH6 and that of temperature was 45^{o}C. When the products were compared, it was observed that the best product was that produced with *Bacillus substilis* mutated with UV – light for 40 minutes. Sensory evaluation result indicated that there was no significant difference observed in the aroma, taste and general acceptability of these condiments. "Daddawa" however, has higher acceptability than "Ogiri". The main fermenting organism for Nigerian indigenous condiment ("Daddawa" and "Ogiri") was *Bacillus subtilis* as found in the study.

Table of Contents

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF APPENDICES

CHAPTER ONE

INTRODUCTION

1.1 Background to the Study

Condiments are spices, herbs, flavouring agents, chemicals, sauces and other materials or blends of flavouring food spices and seasonings, some of which may have been delivered by fermentation, enzymatic action, roasting or heating(Achi, 2005). They contribute distinct taste or character to foods, change or enhance specific flavour(s) of foods, and modify the appearance of foods by adding colour. Most food condiments are strong smelling, sharp tasting substances usually used to improve or adjust the flavour of foods (Achi, 2005; Odebumi *et al*., 2009; Emijiagha, 2009; Oyewole and Isah, 2012; Ojimelukwe, et al, 2011) .Most traditional condiments in use in Nigeria are fermented legumes and oil seeds. These include melon seed (*Citrullus vulgaris*), fluted pumpkin, castor oil seed, cotton seed, African yam bean, *Prosopis africana* seeds (Achi, 2005). Significant contributions have been made in microbiology and biochemistry in fermentation processes of legumes and oils seeds leading to the production of fermented condiments such as "iru", from African locust bean (Odunfa 1986), "ogiri" from melon seed (Barber & Achinewhu 1992), "soumbala" from African locust bean seed, (Ouoba *et al.* 2003) and "daddawa" from soybean (Omafuvbe *et al.* 2000). Fermented locust bean is a well-known condiment with characteristic ammoniacal odour and flavour which enhances the taste of traditional soups and sauces especially those used as a compliments to starchy foods. It is also generally added to soups as low cost meat substitute by low income families in parts of Nigeria (Odebunmi *et al.*, 2009).

Lack of appropriate technologies and comprehensive understanding of the fermentation procedure involved in the production of the condiments have made the up-grading of the village-art method impossible, urbanisation, and population growth have also made these condiments to be in short supply. The resultant effect is introduction of foreign technology which has changed the Nigeria food culture into a mixed grill of both foreign and local dishes (Ojo, 1991) Many researchers have reported on the microbial load, isolation of microorganisms associated with fermentation, sensory evaluation, chemical composition, nutritional evaluation and starter culture development of traditional fermented foods (Aderibigbe and Odunfa, 1990; Odibo *et al.,* 1992; Ouoba *et al.,* 1993; Iwuoha and Eke, 1996; Suberu and Akinyaju, 1996; Wokoma and Aziagbe, 2001; Holzapfel, 2002; Omefuvbe *et al.*, 2002;Naiba, 2003; Achi, 2005; Yahaya, 2006; Adenike *et al.*, 2007; David and Aderibigbe, 2010).

In Nigeria, the raw materials for production of fermented condiments vary from one region to another. In Southeast and some parts of Southwest Nigeria *"Ogiri"* is either made from castor oil seeds, crabs or varieties of melon seeds (Achi *et al.,* 2007; Enujiugha, 2009; David and Aderibigbe, 2010). *"Ogiri"* is also produced in Sierra-Leone from sesame seeds. The production processes are the same as for Nigeria. In Southwest Nigeria, it is known as "*Iru"* and it is produced from locust bean (Odunfa, 1981a). In central Nigeria, it is known as "*Okpehi"* and is produced from *Prosopis africana* (Achi *et al.,* 1992; Odibo *et al.,* 1989; Sanni, 1991). Similarly in northern Nigeria, it is called "*daddawa"*" African locust beans product. (Odunfa, 1981a; Barimalaa *et al.*, 1989).

In the traditional method, fermentation is achieved by indigenous micro-flora and the addition of fermented materials from the previous production. However, a pure starter culture is essential for controlled experimental fermentation (Suberu and Akinyanju, 1996; Omafuvbe *et al.*, 2002). But the use of a single culture would seem too restrictive and the use of mixtures of microorganisms with complementary physiological and metabolic properties seem to be best approach for obtaining a product with the desired nutritional and sensory properties (Achi, 2005).

The selection of starter cultures for large-scale processes may require genetic modification to introduce a number of properties. This may offer nutritional benefit in the form of increased protein production or compatibility to mixed culture fermentation. Fermented condiments have a characteristic organoleptic quality, which probably are the most important factors for consumers (Achi, 2005). Taking into account the increasing demand particularly by urban populations in Nigeria, there are certainly prospects for industrialisation of traditionally fermented condiments.

1.2 Statement of Research Problems

Condiments such as ‘‘Ogiri’’ and “daddawa” provide a distinct volatile characteristic flavour or aroma peculiar to traditional African soups or meals (Enyiugha, 2009). These characteristic flavours cannot be matched by the modern imported condiments that dominate our market (Achi, 2005). The traditional condiments are usually produced from proteinous plants, therefore enhance protein intake. It also impact fermented protein aroma which many Nigerians are used to and seek for in all meals. (Iwuoha and Eke 1996, Naiba, 2003; Ouoba *et al.,* 2003; Achi, 2005). The dominance of modern and western world condiments is mainly due to lack of appropriate technologies for production of local condiments and full understanding of the intricacies in their productions. Therefore, there is need for improvement in the technology, modification of microbial flora associated with fermentation and scale-up processes, if the immigrant and urbanized Nigerian populace will not lose their feeding habits. (Ogbadu, 1988; Achi, 2005).

1.3 Justification of the Study

Migration, urbanisation and modernisation of living standards had made many of our traditional menu, condiments or dishes unattractive, cumbersome to handle and unavailable. The methodologies for production of these traditional condiments are largely village-art (Achi, 2005) there is need for improvement, so that the condiments can meet the market demands and sophistication of modern day living.

A lot of researchers have worked on these condiments but still substrates-microorganism interaction are not well understood, fermentation ability not well established and optimisation of activities of various micro-organisms associated with fermentation need further study (Nout and Sarkar, 1999; Achi, 2005; Dakawa *et al.*, 2005). Among traditional condiments, ‘‘Ogiri’’ and “daddawa” stand out as the most sort by Nigerians.

The condiments are very rich in essential amino acids – glutamic acid, valine, aspartic acid, alanine and lysine which are needed as compliments in food that are deficient in them such as cereals. Also, they are known to be rich in fatty acid especially riboflavin (Anosike and Egwatu, 1980; Annergers, 1974; Odunfa, 1984). Thus there is a need to upgrade their traditional processing methods.

1.4 Aim of the Study

The aim of this work is to upgrade the traditional processing methods of “daddawa” and “ogiri” production.

1.5 Specific objectives of the Study.

The specific objectives of this study are to

1. Isolates, identify and characterize *Bacillus species* from traditional “ogiri” and “daddawa” condiments.
2. Mutate isolated *Bacillus species* using UV- light (260nm) and nitrous acid.

3. Characterize the mutated *Bacillus species* strains using nucleotide sequencing

4. Produce “daddawa” and “ogiri” using standard, isolated and mutated *Bacillus subtilis* strains in laboratory environment.

5. Optimise the fermentation yield of the *Bacillus subtilis* at varying pH and temperatures.

6. Conduct sensory evaluation test on traditional and laboratory prepared condiments.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Food Condiments

The ultimate aim of eating food is to derive adequate nutrients, which the body needs for its normal functioning. Nutrients are components of food needed for growth and to live a normal healthy life (Syazzie et al., 1994). In Nigeria, indigenous foodstuffs provide nutrients such as protein, carbohydrates, fats, water, minerals and vitamins in different proportions (Fagbemi and Oshodi 1991; Adamu *et al.,* 2013). Food condiments or spices have strong smelling, sharp taste usually used to improve or adjust the flavour of foods. They are usually of vegetable origin. Common examples include mustard, nutmeg, ginger, garlic, coriander, locust bean, castor oil beans (Odebunmi *et al.,* 2009). In view of the controversy surrounding the use of monosodium-based seasoning salt (Odebunmi *et al.,* 2009), many homes in Nigeria are now using condiments produced from legumes as a flavourant in traditional soup preparations. (Ojinnaka, 2013). Condiments are also known to contribute to the calorie and protein intake and are generously added to soups as low-cost meat substitute by low-income families in parts of Nigeria (Odunfa 1981). Furthermore, condiments produced from fermented pigeon pea are a good source of vitamin B. There are several reports on the production of fermented condiments from African locust bean "Iru", fermented melon seed ""ogiri" (Odunfa 1981, 1986; Barber and Achinewhu 1992) and fermented soybean "daddawa" (Omafuvbe *et al*., 2000; Oboh, 2006). The production of condiments is largely done on a traditional small-scale household basis under highly variable conditions (Odunfa 1985). The fermentation is usually carried out in a moist solid state, involving contact with appropriate inocula of assorted microorganisms aided by the temperatures of the tropics. The desired state of the fermentation of the condiments is indicated by the formation of mucilage and overtones of ammonia produced as a result of the breakdown of amino acids during the fermentation process (Omafuvbe *et al.*, 2000). The characteristic ammoniacal odour and flavour of condiments enhance the taste of food in which they are used especially the various soups used as a compliment to the starchy root and tuber diets. (Achi, 2005). The ultimate aim of eating food is to derive adequate nutrients, which the body needs for its normal functioning. In Nigeria, the prevalence of malnutrition demands that particular attention be paid to the nutritive value of food.

2.2 Castor Oil Bean (*Ricinus communis*)

The castor oil seed, *Ricinus communis* is a major oil seed and has been known since ancient time. It grows into a tree in the subtropical zones attaining heights of between 11-13 meters **(**Ojinnaka and Ojimelukwe, 2013**)**. *Ricinus communis* is a plant commonly found in both tropical and temperate climates of the world (Lakshmamma and Prayaga, 2006; Raoof and Yasmeen, 2006) and belong to the family *Eurphorbiacae* (Ogunniyi and Nkikang, 2000; **(**Ojinnaka and Ojimelukwe, 2013). The castor plant grows wild in and the plant is known to tolerate varying weather conditions. Specifically, the castor oil seed requires a temperature of between 20 and 26 ^{o}C with low humidity throughout the growing season in order to obtain maximum yields, its cultivation is limited to tropical areas of the developing world (Ogunniyi and Njikang, 2000).

The traditional fermentation of castor oil seed to produce “ogiri” an Igbo condiment, via alkaline fermentation has been discussed by several workers (Odunfa, 1985). Many common foods in West Africa are fermented to increase their nutritive value and keeping quality **(**Ojinnaka and Ojimelukwe, 2013**)**. Such fermentations are brought about by indigenous microorganisms which are present in the substrates. Microbial fermentation of “ogiri” have been found to involve only bacteria; fungi having been regarded as contaminants and therefore do not play any relevant role in its fermentation. Odunfa (1985) first reported that the predominant fermenting organisms in "ogiri" were of *Bacillus* species, *Bacillus subtilis* being the predominant species. Several other workers have also reported *Bacillus subtilis* in the alkaline fermentation of protein rich plant materials (Uaboi-Egbenni *et al.*, 2009; Ouoba *et al.*, 2003). It is reported that proteolysis is the most principal and complex biochemical event occurring during the preparation of some legume based fermented condiments. The degradation products, amino acids, not only have a considerable influence on the nutritional values, but also contribute directly to the taste characteristics, in some cases serving indirectly as precursors of aromatic products (Han *et al.*, 2004). Alkaline, proteolytic fermented foods from legumes are found in various parts of the world and are mainly used as condiments. They play an important role in the diets of many people in developing and a few developed countries. Alkaline fermented foods generally regarded as safe constitute a group of food products popularly consumed in several Asian and African countries (Steinkraus, 2002). The rise in pH during production of these foods is due to the ability of the dominant microorganisms, *Bacillus* spp. to hydrolyze proteins into amino acids and ammonia (Parkouda *et al.*, 2009). The preservative and flavour characteristics of alkaline fermenters are derived in part from the liberation of ammonia and increased pH (Beaumont, 2002).

2.3 Locust Beans (*Parkia biglobosa*)

Parkia biglobosa, named after the famous Scottish botanist and surgeon, Mungo Park by Robert Brown has long been widely recognized as an important indigenous multipurpose fruit tree in many countries of the sub-saharan Africa (Sadiku, 2010). The tree is the source of a natural nutritious condiment which features frequently in the traditional diets of both rural and urban dwellers in at least seventeen West African countries including Nigeria (Sadiku, 2010). *Parkia biglobosa* is a popular food condiment in Nigeria and other West African countries, it is popularly called "Locust bean" in English language and "Iru" in Yoruba language and daddawa in Hausa language. This plant is a perennial leguminous tree which belongs to the sub-family Mimosoideae and family *Leguminosae*. It grows in the savannah region of West Africa up to the southern edge of the Sahel zone (Campbell-platt, 1980). The tree is not normally cultivated but can be seen in population of two or more in the savannah region of West Africa (Hopkins, 1993).

The processing of locust bean involves several stages which include cooking, dehulling, washing, fermentation, salting and refrigerating. Oil seeds such as African locust beans, melon seeds, castor oil seeds, mesquite beans and soybeans are also fermented to give condiments (Omafuvbe *et al.*, 2004). At some stages in the preparation of the seed, fermentation is required to bring out the desired nutritional value and other organoleptic properties such as taste, flavour and texture (Ademola *et al.*, 2011). Previous studies have shown that fermentation improves the digestibility, nutritive value and flavour of the raw seeds (Omafuvbe et al., 2004; Odunfa, 1985; Reddy and Pierson, 1999; Barimala and Antai, 1989). The bacteria responsible for the fermentation in *Parkia biglobosa* has being identified to be *Bacillus subtilis* and *Staphylococcus species* (Odunfa, 1985). There was a general increase in the microbial population throughout the fermentation period (Ogunshe *et al.*, 2006; Ademola *et al.*, 2011). Alabi *et al.* (2005), reported that locust bean is rich in lipid, protein, carbohydrate, soluble sugars and ascorbic acid. The cotyledon is very nutritious, has less fibre and ash contents. The oil content is suitable for consumption since it contains very low acid and iodine content. The oil has very high saponification value and hence would be useful in the soap industry. It has essential acids and vitamins and serves as a protein supplement in the diet of poor families (Diawara *et al.,* 2000). Daddawa is used in soups, sauces and stews to enhance or impart meatiness (Klanjcar *et al.*, 2002).

In Nigeria, the production of fermented locust bean has remained a traditional family art practiced in homes especially in the rural areas with rudimentary utensils (Audu *et al.*, 2004). The methods used vary from one locality to another depending on the culture of the people, their beliefs, taste and the practice of the fore parents who were involved in the same vocation. These variations in the processing techniques in turn bring about variations in the quality of "Iru" (Sadiku *et al.*, 2010).

2.4 Food Fermentation

The ultimate aim of eating food is to derive adequate nutrients, which the body needs for its normal functioning. Nutrients are components of life and essential ingredients for growth (Syazzie *et al.,* 1994). In Nigeria indigenous diets provide nutrients such as protein, carbohydrates, fats, water, minerals and vitamins in different proportion (Fagbemi and Oshodi; Adamu *et al.,* 2013). Fermentation in food processing is the conversion of carbohydrates to alcohol and carbondioxide or organic acids using yeast or bacteria, under anaerobic conditions (William and Dennis, 2011; Wikipedia, 2012). And the fermentation is one of the classic methods of preserving foods in which lactic acid bacteria (LAB) and yeasts are responsible for most of these fermentations (Adenike *et al.*, 2007; Adeleke *et al.,* 2010).

Fermentation plays at least five roles in food processing (Adamu *et al.,* 2013): i) Enrichment of the human diet through development of a wide diversity of flavors, aromas and textures in food. ii) Preservation of substantial amounts of food via lactic acid, alcoholic, acetic acid, alkaline and high salt fermentations. iii) Enrichment of food substrates biologically with vitamins, protein, essential amino acids and essential fatty acids. iv) Detoxification v) Decrease in cooking time and fuel requirements. The weight of the microorganisms in the food is usually small, but the influence on the nature of the food, especially in terms of flavour, and other organoleptic properties, is profound (Okafor, 2009; Oyewole and Isah, 2012).

Nigeria has a variety of people and culture sure that it is difficult to pick one national dish. Each area has its own regional favourite food that depends on customs, tradition and religion (Abdel *et al.,* 2009; Adebayo *et al.,* 2010). The fermentation processes for these foods constitute a vital body of indigenous knowledge used for food preservation, acquired by observations and experience, and passed on from generation to generation (Aworh, 2008; Chelule *et al.,* 2010). Lactic acid bacteria (LAB) isolated from these foods displayed probiotic properties such as hypolipidemic, hepatoproctective and antibacterial and have been found to be effective in treating gastroenteritis in man and animals (Aderiye *et al.,* 2007).

2.5 Fermented Foods in Nigeria

The fermented foods in Nigeria can be classified into groups according to the substrates or raw materials employed (Odunfa, 1985). These include the following:

1. Tubers (cassava products): *gari, lafun, fufu*
2. Cereals (maize, sorghum, millet): *"ogi", pito, burukutu*
3. Legumes (locust beans, soya beans): *iru, daddawa*
4. Fruits (melon): *"ogiri*
5. Beverages: palm wine
6. Animal proteins (milk): cheese

The microflora involved in the fermentation of these foods have been isolated and characterized. Some fermentation processes also have been modernized. (Table 2.1) shows a comprehensive list.

Table 2.1: Fermented foods of Nigerian origin

Substrate	Micro-organism	Product	Shelf life
Cassava	*Streptococcus lacticus* *Geotrichum candidum* *Corynebacterium manihot* *Lactobacillus* spp *Leuconostoc* spp	*Gari* granules *Fufu* paste *Lafun* powder	3 month 1 week 3 month
Cereals: maize, sorghum, millet	*Saccharomyces cerevisiae* *Lactobacillus* spp *Fusarium* spp *Candida mycoderma* *Penicillium* spp	*"ogi"* *Agidi*	2 week
	Rhizopus oryzae *Aspergillus flavus* *Penicillium funiculosum* *Geotrichum candidum* *Candida* spp	*Pito* *Burukutu*	Days
Legumes: locust, beans, soya beans	Coryneform bacteria *Bacillus subtilis* *B. licheniformis* *B. cereus* *Staphylococcus saprophyticus* *Pseudomonas aeruginosa*	*Iru (daddawa)*	1 month
Melon *(Citrullus vulgaris)*	*Bacillus* spp *Escherichia* spp *Proteus* spp *Pediococcus* spp	*"ogiri*	1 month
Oil palm sap	*Saccharomyces cerevisiae* *Candida tropicalis* *C. utilis* *Lactobacillus brevis*	Palm wine	Days

	Bacillus spp *Streptococcus* spp		
Milk	Lactic acid bacteria	Local cheese	Days

Source: (Latunde-Dada, 2014)

The fermentation techniques are often a small scale in household basis, characterized by the use of simple non-sterile equipment, chance or natural inoculums, unregulated conditions, sensory fluctuations, poor durability and unattractive packing of the processed products resulting in food of unpredictable quality (Olanrewaju *et al.,* 2009). With increasing industrialization and urbanization, efforts are presently geared towards the development of large-scale factory production facilities for these foods where the quality of the finished product will be assured (Agarry *et al.,* 2010; Oyewole and Isah, 2012).

2.5.1 Traditional method for production of "ogiri" from castor oil seed and "daddawa" from African locust bean

Traditionally, castor oil seeds are prepared by shelling the seeds and then the cotyledons are boiled for 24 – 48h. The boiled cotyledons were then milled and placed in an earthen ware pot covered with banana leaves then allowed to ferment for 48-98h at room temperature (25 ± 2^{o}C) before been wrapped in various leaves and stored for 48-96h before marketing The flow chart for traditional production of "ogiri" is shown below (Fig. 2.1)

Castor Oil Seeds

↓

Shelling

↓

Boiling for 48-96h

↓

Milling

↓

Fermenting for 48-96h

↓

Wrapping

↓

Keeping over a cooking fire place

↓

Aging for 48-96h

↓

Storing over a cooking fire place

Figure 2.1: The Flow chart for Production of “ogiri”

Source: Achi, (2005)

‘Daddawa’ condiments are traditionally prepared by boiling of the locus bean seeds in water for 28h. After the initial boiling, the boiled seeds are dehulled by pressing between the palms and later washed to remove seed coats. The washed cotyledons are then boiled for 2h and draining of water is done by using raffia sieve. The cotyledons are spread hot into a wide calabash and covered with jute sacks. Fermentation was allowed to proceed under room temperature (30 ± 2^oC for 72h) (Fig. 2.2).

African locust bean seeds

↓

Addition of water

↓

Boiling for 12h or more

↓

De-hulling by pressing between palms of hands

↓

Washing for seed coat removal

↓

Addition of water

↓

Boiling of the cotyledons for 2h or more

↓

Draining with raffia sieve

↓

Spread hot in calabash tray (10cm deep)

↓

Trays stacked together and wrapped with jute bags

↓

Fermenting for 48-72h

↓

Daddawa

Figure 2.2: Flow chart for preparation of “daddawa”

Source: Achi, (2005)

2.6 Classification of Food Fermentation

Steinkraus (1997) classified fermentations according to the following categories;

1. Fermentation producing textured vegetable protein meat substitutes in legume/cereal mixtures

2. High salt/ savory meat-flavored/ amino acid/ peptide sauce and paste fermentations

3. Lactic acid fermentations

4. Alcoholic fermentations

5. Acetic acid/ vinegar fermentations

6. Alkaline fermentations

7. Leavened breads

8. Flat unleavened breads

2.7 Alkaline Fermentations

Alkaline fermentations are generally safe, especially those involving *bacilli* fermenting protein rich beans and seeds. Africa has a number of very important foods and condiments that are not only used to flavour soups and stews but also serves as low-cost sources of protein in the diet (Odunfa, 1981a).

Among these are Nigerian "daddawa", Ivory Coast "soumbara" and West African "iru" made by fermentation of soaked, cooked locust bean *Parkia biglobosa* seeds with bacteria belonging to genus *Bacillus*, typically *Bacillus subtilis*. Others includes Nigerian "“ogi”ri" made by fermentation of melon seed (*Citrullus vulgaris*); Nigerian "ugba" made by fermentation of the oil bean (*Pentacletha macrophylla*); Sierra Leone "ogiri-saro" made by fermentation of sesame seed (*Sesamum indicum*); Nigerian "ogiri-igbo" made by fermentation of castor bean (*Ricinus communis*) seeds and Nigerian "ogiri-nwan" made by fermentation of the fluted pumpkin bean (*Telfaria occidentale*) seeds. Soybeans can also be substituted for locust beans and examples of the product produces includes Japanese "natto", "Thai Thua-Nao" and Indian "kenima". The essential microorganisms involved are *Bacillus subtilis* and related *bacilli*. The organisms are very proteolytic and the proteins are hydrolyzed to peptides and amino acids. Ammonia is releases and the pH rapidly reaches as high as 8.0. The combination of high pH and free ammonia along with very rapid growth of the essential microorganisms at relatively high temperatures above 40°C make it very difficult for other microorganisms that might spoil the product to grow. Thus, the products are quite stable and well-preserved especially when dried. They are safe foods even though they may be manufactured in unhygienic environment.

2.8 Microorganisms Involved in Fermented Foods

Microorganisms are living creatures that are microscopic in size and are heterogeneous organisms that can be in form of plant or animal such as algae, fungi (mould and yeasts), and bacteria (Wikipedia, 2011; Oyewole and Isah, 2012). The multiplication of microorganisms in food is greatly influenced by the inherent (intrinsic factors) and environmental conditions. (Onyenekwe et al., 2012). In general, microorganisms multiply most rapidly in moist, nutritionally-rich, neutral pH and warm, oxygen-rich environment (Nester *et al.,* 2007; Oyewole and Isah, 2012).

The commonest organisms responsible for fermentation of foods are acid-forming bacteria such as the genera lactic acid bacteria (*Lactobacillus, Lactococcus, Leuconostoc, Enterococcus, Streptococcus, Aerococcus* and *Pediococcus)* (Chelule *et al.,* 2010; Agarry *et* *al.,* 2010; Wikipedia, 2011) known as obligate fermenters, flavorful organisms (aromatic compound microorganisms) and *Propionibacterium* species (Bukola and Abiodun, 2008). The yeasts are mainly of the species *Saccharomyces, Candida, Kluyeromyces* and *Debaryomyces* (Omemu *et al*., 2007; Chelule *et al.,* 2010).

Moulds have been used mainly in milk and cheese fermentation (William and Dennis, 2011) and these include *Penicillium, Mucor, Geotrichium, and Rhizopus* species (Chelule *et al.,* 2010) of more importance are the lactic acid bacteria. The lactic acid bacteria (LAB) are a group of Gram positive bacteria, non-spore forming, cocci or rods. The genera *Lactobacillus, Leuconostoc, Pediococcus* and *Streptococcus* are the main species that plays important role in fermented foods of tropical origin (Nwachukwu *et al*., 2010).

Indigenous natural fermentation takes place in a mixed colony of microorganisms such as moulds, bacteria and yeasts (William and Dennis, 2011). These bacteria are not harmful to the consumers and have enzymes such as proteases, amylases and lipases that hydrolyze food complexes into simple nontoxic products with desirable textures and aroma that makes them palatable for consumption (Nwachukwu *et al.,* 2010).

2.9 Importance of Locally Fermented Foods

Locally fermented Foods have a role to play in reducing poverty, ignorance and disease, so that a nation can live in dignity (Anukam and Reid, 2009; Olanrewaju *et al*., 2009).

The locally fermented foods are a significant item in both local and international trade. Some of these significance of fermented foods include;

2.9.1 Poverty alleviation

It provides a source of income and means of poverty alleviation contributing to food security of the nations. (FAO, 2010). The preparation of this locally fermented foods like "kunu" has become a very well-known technology in many homes in the rural communities and more recently in the urban areas where commercial production due to support from the government through the poverty alleviation scheme. (Essien *et al*., 2011).

2.9.2 Food supplements

The locally fermented foods serve as food supplement, like the use of ""ogi"" as a weaning food in Southern Nigeria to supplement breastfeeding (Falana *et al.,* 2011). The fermented foods make nutrient more readily available in the diet. (Egbere, 2008). Traditional fermented protein-rich foods offer excellent opportunities for improving the diets of people in tropical countries providing rich sources of starch, vitamins, proteins and minerals (Oladejo and Adetunji, 2012).

2.9.3 Reduction in mortality rate

Eating an enzyme-rich diet like fermented foods, prevent the enlargement of pancreas by decreasing the load on pancreas and preserving the body's own natural enzyme, thereby reducing the risk of chronic diseases (Kristen, 2011).

2.9.4 Provision of employment opportunities

Small scale food industries involved in food fermentations, reduce rural-urban migration and the associated social problems by generating employment opportunities in the rural areas (Aworh, 2008).

2.9.5 Industrialization

Fermented foods play a unique role in promoting industrial development in Nigeria through employment generation, value added processing and training of skilled manpower. Their impact is felt greatly in the urban areas (Aworh, 2008). Small-scale food industries that involve low capital investment and that rely on traditional food processing technologies are crucial to rural development in Nigeria (Aworh, 2008).

2.9.6 Market improvement

The availability of different locally fermented foods displayed on supermarket shelf is the proof of diversity of commodity items, convenience food and prepared food available in the market. (Frazier *et al.*, 2008).

2.9.7 Food availability

They are vital to reducing post-harvest food losses and increasing food availability (Aworh, 2008). Fermented foods are of great significant because they provide and preserve vast quantities of nutritious foods in a wide diversity of flavors, aromas and textures which enrich the human diet (Yabaya, 2008).

2.9.8 Food security

Fermented foods promote and improve food processing and preservation at all levels of operation, which are essential components of national strategic plans for food security which aims at achieving national food security (Adeyemi, 2008). Fermentation technology also has the potential of meeting a nation's food supply demand if adequately developed into the industrial scale. Such as condiments which have being increasingly marketed throughout the country and beyond in informal ways. Fermented foods also constitute a major portion of peoples' diets all over the world and provide 20 – 40% of the total food supply (Abdel *et al.*, 2009).

2.10 Advantages and Disadvantages of fermented foods

2.10.1 Advantages of Food Fermentation

Some advantages of food fermentation include: general improvement in the shelf life, texture, taste and aroma, nutritional value and digestibility and lowering of the anti-nutritional components of cereal products (Oyewole and Isah, 2012).

2.10.1.1 Provision of nutritional quality

A number of foods especially cereals are poor in nutritional value, which constitute the main staple diet of the low income populations (Chelule *et al.*, 2010). However, lactic acid bacteria fermentation has been shown to improve the nutritional value and digestibility of these foods (Nout, 2009). The enzymes, which include amylases, proteases, phytases and lipases, modify the primary food products through hydrolysis of polysaccharides, proteins, phytates and lipids respectively (Adeyemi, 2008). The quantity and quality of the food proteins as expressed by biological value, and often the content of water soluble vitamins is generally increased, while the anti-nutrient factors (ANFs) such as phytic acid and tannins in food decline during fermentation leading to increased bioavailability of minerals such as calcium, phosphorus, zinc, iron, amino acids and simple sugars (Santos *et al.*, 2008; Soetan and Oyewole, 2009; Murwan and Ali, 2011).

2.10.1.2 Detoxification during food fermentation

Food and feeds are often contaminated with a number of toxins like fumonisins, ochratoxin A, zearalenone and aflatoxins, either naturally or through infestation by microorganisms such as moulds, yeast, bacteria and viruses (Ari *et al.*, 2012). Using lactic acid bacteria in fermentation detoxified toxins and preserves the nutritive value and flavour of foods (Chelule *et al.*, 2010). In addition to this, fermentation irreversibly degrades mycotoxins without adversely affecting the nutritional value of the food (Ari *et al.*, 2012) and without leaving any toxic residues.

2.10.1.3 Organoleptic properties enhancement

Fermentation makes the food palatable by enhancing its organoleptic properties such as aroma, textures and flavor (Chelule *et al.*, 2010). These organoleptic properties make fermented food more popular than the unfermented one in terms of consumer acceptance (Osungbaro, 2009).

2.10.1.4 Preservative properties

The preservative activity of local fermentation has been observed in some fermented products such as cereals and fruits (Adeyemi, 2012). The lowering of the pH to below 4 through acid production inhibits the growth of pathogenic organisms which causes food spoilage, food poisoning and diseases, this prolongs the shelf life of fermented food. (Abdel, 2009; Olukoya et al., 2011).

2.10.1.5 Production of antibiotics

Some of the inhibitory compounds produced by fermented foods against other bacteria include hydrogen peroxide and bacteriocins (Olanrewaju *et al.*, 2009). A myriad of beneficial activities such as immuno-modulatory, antiallergenic, antimicrobial, antihypertensive and anti-tumorigenic effects have also been reported (Osuntoki, 2010). One of the supporting use of LAB fermentation to prevent diarrheal diseases because they modify the composition of intestinal microorganisms and thus, act as deterrents for pathogenic enteric bacteria (Olukoya *et al.*, 2011). In these regards, lactic acid bacteria are applied as barriers against non-acid tolerant bacteria, which are ecologically eliminated from the medium due to their sensitivity to acidic environment (Agarry *et al.*, 2010).

In addition, fermentation has been reported to be more effective in the removal of Gram negative than the Gram-positive bacteria, which are more resistant to fermentation processing

.As such, fermented food can control diarrheal diseases in children. Moreover, lactic acid bacteria are also known to produce proteineous antimicrobial agents such as bacteriocins, peptides that elicit antimicrobial activity against food spoilage organisms and foodborne pathogens, but do not affect the producing organisms (Egbere, 2008; Wikipedia, 2012).

2.10.1.6 Health benefits of fermented foods

Many of the fermented products consumed by different ethnic groups have therapeutic values, some of the most widely known are fermented milks (yoghurt, curds) which contain high concentrations of probiotic bacteria that can lower the cholesterol level (Jyoti, 2010), improvement of nutrients absorption and digestion, restores the balance of bacteria in the gut to hinder constipation, abdominal cramps, asthma, allergies, lactose and gluten intolerance (Abdel *et al.*, 2009). The slurries of carbohydrate based fermented Nigerian foods such as "ogi", "fufu" and "wara" have been known to exhibit health promoting properties such as control of gastroenteritis in animals and human (Aderiye *et al.*, 2007; Olukoya *et al.*, 2011; 'Oyewole and Isah, 2012). Raw fermented foods are also rich in enzymes and our body needs enzymes to properly digest, absorb and make full use of food.

2.10.2 Disadvantages of Food Fermentation

2.10.2.1 Crude handling of traditionally fermented foods

According to Achi (2005), the following factors work against traditional fermented foods: Inadequate raw material grading and cleaning contributing to the presence of foreign matters (such as insects, stones) in the final products. Crude handling and processing techniques employed, lack of durability (shelf life), lack of homogeneity and unattractive presentation. Thus, the production environment usaully fails to observe good manufacturing practice

(GMP) or code of hygiene; lack of knowledge of the culture of the processes and their characteristics; absence of any control in the processing variables like pH, temperature, humidity, and quality of water; lack of awareness of the nutritional and toxicol”ogi”cal implications of fermentation and its products (Olanrewaju, 2009; Oyewole and Isah, 2012).

2.10.2.2 Health defect

Chronic cyanide toxicity is also associated with several health conditions including “konzo”, an irreversible paralysis of the legs (Aworh, 2008). The problem of food-borne diseases like cases of botulism (Wikipedia, 2012) and death due to diarrhea (Oluwafemi *et al.*, 2011).

2.10.2.3 Food spoilage

The negative effects include spoilage of food products and contamination by pathogenic microorganisms (Adeyemi, 2012). This is achieved by increasing their numbers, utilizing nutrients, causing enzymatic changes, and contributing off-flavours through breakdown of a product or synthesis of new compounds. Oxidation of food constituents is also a key event in food spoilage which may reduce the nutritional value and safety of the food by producing undesirable flavours and toxic substances while addition of synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) to foods have been reported to also have side effects (Osuntoki & Korie 2010).

2.10.2.4 Laborious and time consuming

Labor requirements for traditional processing locally fermented foods like cassava into gari, fufu, lafun and other products are huge and usually cumbersome (Aworh, 2008; Oyewole and Isah, 2012). Though most fermented foods have health-promoting benefits; their global consumption is declining as traditional food systems give way to the influence of a western diet and fast foods (Jyoti, 2010).

2.10.2.5 Socio-economics of the consumer base

The consumer base of traditionally fermented foods in most developing countries is largely poor. Price, rather than food safety or nutritional security and quality that make a substantial contribution to their food, is therefore a major preoccupation of this group when purchasing food (FAO, 2010).

2.11 Factors Affecting the Development of Fermented Foods

2.11.1 Temperature

Different bacteria can tolerate different temperatures, which provide enormous scope for a range of fermentations (Lee *et al.*, 2011). While most bacteria have a temperature optimum of between 20 to 30ºC, there are some (thermophiles) which prefer higher temperatures (50 to 55ºC) and also those with colder temperature optima (15 to 20ºC). Most lactic acid bacteria work best at temperatures of 18 to 22ºC. The *Leuconostoc species* which initiate fermentation have an optimum of 18 to 22ºC and also temperatures above 22ºC, favours the *Lactobacillus species* (Food and Drug Administration, 2011).

2.11.2 Hydrogen ion concentration (pH)

The optimum pH for most bacteria is near the neutral point (pH 7.0). Certain bacteria are acid tolerant and will survive at reduced pH levels. Notable acid-tolerant bacteria include the *Lactobacillus* and *Streptococcus species*, which plays a significant role in the fermentation of dairy and vegetable products (FDA, 2011).

2.11.3 Salt concentration

Lactic acid bacteria tolerate high salt concentrations. The salt tolerance gives them an advantage over other less tolerant species and allow the lactic acid fermenters to initiate fermentation, which produces acid and further inhibits the growth of non-desirable organisms. *Leuconostoc* is noted for its high salt tolerance and for this reason, initiates the majority of lactic acid fermentations (FDA, 2011).

2.11.4 Water activity

In general, bacteria require a fairly high water activity (0.9 or higher) to survive. There are a few species which can tolerate water activities lower than this, but usually the yeasts and fungi will predominate on foods with a lower water activity (FDA, 2011).

2.11.5 Oxygen availability

Some of the fermentative bacteria are anaerobes; while others are aerobes that requires oxygen for their metabolic activities (Wikipedia, 2012). *Lactobacilli* in particular, are microaerophilic. That is they grow in the presence of reduced amounts of atmospheric oxygen. In aerobic fermentations, 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 (Lee *et al.*, 2011; FDA, 2011).

2.11.6 Nutrients

Bacteria require sources of nutrients for metabolism (Egbere, 2008). The fermentative bacteria require carbohydrates – either simple sugars such as glucose and fructose or complex carbohydrates such as starch or cellulose (Oyewole and Isah, 2012). The energy requirements of micro-organisms are very high. Limiting the amount of substrate available can check their growth (FDA, 2011). Fermentation extends the shelf life, adds a variety of flavours, and enhances the nutritional value of processed foods. Despite extensive research on isolating and characterizing the wide range of micro-organisms involved in the fermentation of these foods, the techniques employed in the cottage industries of Nigeria remain traditional and crude. Simple and non-sterile materials are used, and there is great reliance on the use of natural inocula under uncontrolled fermentative conditions. Other features of this system include contamination, varied sensory characteristics, unattractive packaging and presentation, and short shelf life of the products. It is imperative that modern biotechnological techniques be integrated into the traditional processing procedures to upgrade their efficiency and productivity. The production and use of starter cultures and the maintenance of optimum conditions for microbial activity will enhance the qualities of fermented foods produced in the cottage industries of Nigeria (Latunde-Dada, 2014).

In traditional fermentation processes, natural micro-organisms are employed in the preparation and preservation of different types of food. These processes add to the nutritive value of foods as well as enhance the flavour and other desirable qualities associated with digestibility and edibility. The fermentation techniques are often characterized by the use of simple, non-sterile equipment, chance or natural inoculum, unregulated conditions, sensory fluctuations, poor durability, and unattractive packaging of the processed products (Nout, 1985). Nigeria is endowed with a wide range of fermentable indigenous staple foods that serve as raw materials for agro-allied cottage industries. These industries utilize small-scale equipment and provide alternative equipment for rural communities while adding value to such local produce (Latunde-Dada, 2014).

2.12 Development of fermentation processes

Traditional fermentation processes at the cottage level use simple operations and equipment. Fermentation is usually carried out on a solid substrate or under submerged conditions. The temperature, pH, and aeration are not optimized for efficient output. The fermentation processes for *"ogiri"*, and palm wine have been upgraded and modernized (Okafor and Ejiofor, 1990). *Iru is* one of the traditional fermented condiments used to flavour soups and stews in Nigeria. The traditional alkaline fermentation of locust beans using *Bacillus subtilis* (Kuboye, 1985; Oyewole and Odunfa, 1991; Steinkraus, 1991) was modernized in the production of daddawa cubes. The greatest advance, however, is the production on an industrial scale of *iru (daddawa)* as cubes by Cadbury Nigeria. The advertisement of daddawa cubes utilizes attractive packaging to appeal to the consumer's preference and taste. This also enhances saleability, particularly among sophisticated housewives. For other products, however, the main problem is the scaling-up of the fermentation processes, which involves heavy capital investment in equipment design, regulates conditions of moisture, pH, temperature, oxygen transfer, aeration, and agitation, and calculating microbial growth characteristics (Latunde-Dada, 2014)

The fermentation of local cheese is perhaps the most rudimentary of the fermentative processes. The availability of milk products in Nigeria is limited, and the warm climate is unsuitable for the preparation of cheese. Fermented cheeses are usually the soft variety. Cheeses take a long period to mature. The milk is heated in pots, and the juice of *Calotropis procera* or pawpaw leaves is introduced to curdle the milk. The curdled milk is then heated for at least 20 minutes and allowed to drain before it is mounded into different shapes (Latunde-Dada, 2014)

2.13 Nutritional Enhancement of Fermented Foods

Traditional fermented protein-rich foods offer excellent opportunities for improving the diets of people in tropical countries. Various attempts have been made to increase the protein level of cassava-based products, particularly *gari*. Growth of the fungus *Aspergillus niger* for 24 to 30 hours on cassava flour with an initial content of 2 - 3% protein and 80 - 90% carbohydrate resulted in a product containing 18 - 20% protein and 30 - 35% carbohydrate (Latunde-Dada, 1991).

Another approach is the supplementation of cassava with protein-rich foods, for example, supplementation of *gari* with soya protein. *Soya-"ogi"*, a combination of maize and soya beans, was developed by the Federal Institute of Industrial Research, Oshodi, to increase the protein level of *"ogi"*. The germination and fermentation of cereals enhance the availability of elemental iron (Latunde-Dada, 1991), the deficiency of which is responsible for the high incidence of anaemia in tropical countries.

2.14 Mutation

In genetics, a mutation is a change of the nucleotide sequence of the genome of an organism, virus, or extrachromosomal genetic element. Mutations result from unrepaired damage to DNA or to RNA genomes (typically caused by radiation or chemical mutagens), errors in the process of replication, or from the insertion or deletion of segments of DNA by mobile genetic elements (Bertram, 2000; Burrus and Waldor, 2004; Aminetzach *et al*., 2005). Mutations may or may not produce discernible changes in the observable characteristics (phenotype) of an organism. Mutations play a part in both normal and abnormal biological processes including: evolution, cancer, and the development of the immune system. Mutation can result in several different types of change in sequences. Mutations in genes can either have no effect, alter the product of a gene, or prevent the gene from functioning properly or completely. Mutations can also occur in congenic regions. One study on genetic variations between different species of *Drosophila* suggests that, if a mutation changes a protein produced by a gene, the result is likely to be harmful, with an estimated 70 percent of amino acid polymorphisms that have damaging effects, and the remainder being either neutral or weakly beneficial (Sawyer *et al.*, 2007. Due to the damaging effects that mutations can have on genes, organisms have mechanisms such as DNA repair to prevent or correct (revert the mutated sequence back to its original state) mutations (Bertram, 2000).

2.14.1 Description of Mutation

Mutations can involve the duplication of large sections of DNA, usually through genetic recombination Hastings *et al*., 2009).These duplications are a major source of raw material for evolving new genes, with tens to hundreds of genes duplicated in animal genomes every million years (Carroll *et al*., 2005). Most genes belong to larger families of genes of shared ancestry (Harrison and Gerstein, 2002). Novel genes are produced by several methods, commonly through the duplication and mutation of an ancestral gene, or by recombining parts of different genes to form new combinations with new functions (Long *et al*., 2003; Orengo and Thornton, 2005). Domains act as modules, each with a particular and independent function, that can be mixed together to produce genes encoding new proteins with novel properties (Wang and Caetano-Anolles, 2009). The human eye uses four genes to make structures that sense light: three for color vision and one for night vision; all four arose from a single ancestral gene (Bowmaker, 1998). Another advantage of duplicating a gene (or even an entire genome) is that this increases redundancy; allows one gene in the pair to acquire a new function while the other copy performs the original function (Gregory and Hebert, 1999; Hurles, 2004). Other types of mutation occasionally create new genes from previously noncoding DNA (Liu *et al*., 2008; Siepel, 2009). Changes in chromosome number may involve even larger mutations, where segments of the DNA within chromosomes break and then rearrange. In the Homininae, two chromosomes fuse to produce human chromosome, this fusion did not occur in the lineage of the other apes, and they retain these separate chromosomes (Zhang. 2004). In evolution, the most important role of such chromosomal rearrangements may be to accelerate the divergence of a population into new species by making populations less likely to interbreed, thereby preserving genetic differences between these populations (Ayala and Coluzzi, 2005).

Sequences of DNA that can move about the genome, such as transposons, make up a major fraction of the genetic material of plants and animals, and may have been important in the evolution of genomes (Hurst and Werren, 2001). More than a million copies of the Alu sequence are present in the human genome, and these sequences have now been recruited to perform functions such as regulating gene expression (Hasler and Strub, 2006). Another effect of these mobile DNA sequences is that when they move within a genome, they can mutate or delete existing genes and thereby produce genetic diversity (Aminetzach *et al*., 2005). Nonlethal mutations accumulate within the gene pool and increase the amount of genetic variation (Eyre-Walker and Keightley, 2007). The abundance of some genetic changes within the gene pool can be reduced by natural selection, while other "more favorable" mutations may accumulate and result in adaptive changes. For example, a butterfly may produce offspring with new mutations. The majority of these mutations will have no effect; but one might change the color of one of the butterfly's offspring, making it harder (or easier) for predators to see. If this color change is advantageous, the chance of this butterfly's surviving and producing its own offspring are a little better, and over time the number of butterflies with this mutation may form a larger percentage of the population.

Neutral mutations are defined as mutations whose effects do not influence the fitness of an individual. These can accumulate over time due to genetic drift. It is believed that the overwhelming majority of mutations have no significant effect on an organism's fitness. Also, DNA repair mechanisms are able to mend most changes before they become permanent mutations, and many organisms have mechanisms for eliminating otherwise-permanently mutated somatic cells.

CHAPTER THREE

MATERIALS AND METHODS

3.0 MATERIALS

The materials used for this experiment are beakers of various sizes, petri dishes, oven, grinders, test tubes, autoclave, refrigerator, microbial media, reagents, pipettes and tips, hand gloves and centrifuges.

3.1 Collection of Samples

Ten samples of ‘Ogiri’ were collected from five different locations in Oyeagu market Abagana in Anambra State and 10 wraps of ‘daddawa’ from five different spots in Zaria main market, Kaduna State.

3.2 METHODS

3.2.1 Isolation, Identification and Characterization of *Bacillus species* in the Condiments

Each sample weighing 25g was aseptically transferred into 225ml of 0.1% sterile buffered peptone water and shaken thoroughly to make 10^{-1} dilution. One millilitre (1ml) of the 10^{-1}

dilution was transferred to 9ml of 0.1 sterile peptone water. The solution was decimally diluted till 10^{-5} ml. (Harrigan and McCance, 1976). Duplicate plates for isolation of *Bacillus species* were prepared on MYP (Mannitol Yolk Polymyxin) agar with polymycin B supplement and incubated at 37^{o}C for 24h. Distinct colonies were picked from the plates using sterile wire-loop and streaked on nutrient agar plates to obtain pure cultures. Pure cultures were isolated by repeated sub-culturing on the same medium and the isolate were stored on nutrient agar slants at 5^{o}C. Sub-culturing was done monthly in order to preserve them for use in subsequent experiments (Ogbadu, 1988; Enujiugha, 2009).

The following tests were carried out to characterize the isolates:

3.2.1.1 Cultural Characteristics

The growth of the organism on nutrient agar and MacConkey agar was observed for morphological characteristics including pigment formation and odour of colonies.

3.2.1.2 Aerobic Growth

The growth of the organisms on the surface of the nutrient agar at 37^{o}C was observed and recorded as aerobic growth (Idise, 2010).

3.2.1.3 Anaerobic growth

The inoculated plates were incubated in an aerobic jar containing anaerobic gaspak at 37^{o}C for 24h and observed for growth.

3.2.1.4 Biochemical tests

The following tests were carried out in accordance with procedures reported by Idise, (2010) Gram staining, endospore test, catalase test, oxidase test, oxidative-fermentative test, glucose, mannitol, lactose fermentation test and citrate utilization test.

3.2.1.4.1 Gram staining

A smear of the organism was prepared on a grease free slide and fixed. This was then flooded with crystal violet and allowed to stand for 30 seconds. The stain was then poured off. The slide was slanted, flooded with Gram’s iodine and allowed to stand for 30 seconds. This was washed off with water and flooded with acetone-alcohol (50:50) and tilted form side to side until the colour ceased to appear. The fixed slide was then was then counter stained with safranin and washed off with water. The slide was allowed to air dry and observed with oil immersion lens under the microscope. Gram-positive organisms appeared blue while Gram negative organisms appeared red.

3.2.1.4.2 Endospore test

A smear of the organism was prepared on a grease free slide and fixed. The slide was placed over boiling water in a test-tube while resting on the rack with the bacterial film uppermost. After about 40 seconds, large droplets of water condensed on the underneath of the slide. The slide was then flooded with 5% aqueous solution of Malachite green and allowed to stand for 2 minutes w the water continued to boil. The slide was then removed from the boiling water and washed with running water. The slide was allowed to air dry and using oil immersion lens, it was observed under the microscope. Endospores stained green while vegetative cells stained red.

3.2.1.4.3 Catalase test

Exactly 2ml of hydrogen peroxide solution was poured into a test tube. Then using a sterile glass rod, several colonies of the test organisms were removed and immersed into hydrogen peroxide solution and observed for bubbles. Catalase positive organisms produced bubbles while catalase negative organisms did not (Idise, 2010).

3.2.1.4.4 Motility test

Nutrient agar slants were prepared for each organism by using an inoculating needle in which a luxuriant growth of the organisms were picked up and stabbed onto the agar at right angle to the bottom of the tube. This was then incubated at 37^{o}C for 28h and observed for pattern of growth of the organism. Motile organisms grew away from the line of stabbing while non-motile organisms grew along the line of stabbing.

3.2.1.4.5 Oxidase test

A piece of test paper was held with forceps and touched onto an area of heavy growth of the test organism. Change of colour to purple within 10 seconds was taken as positive result while change of colour within 10 to 6 seconds was taken as delayed positive and a change in colour in more than 60 seconds will be taken as negative change.

3.2.1.4.6 Starch hydrolysis

Freshly grown test *Bacilillus* cultures from isolated *Bacillus* slants were streak inoculated on *Lactobacillus* agar incorporated with 1% starch and incubated at 37ºC for 30h. After incubation, the plates were flooded with few drops of Gram's iodine and observed for clear zones around the colony (Idise, 2010). Presence of clear zones around the colony was scored as positive for starch hydrolysis.

3.2.1.4.7 Oxidative – Fermentative (OF) test

Two tubes of oxidative fermentative medium were inoculated. The inoculation was carried out as in motility test. One of the tubes was overlaid with sterile paraffin oil to exclude all oxygen. Both tubes were incubated at 37^{o}C for 48 h. The result was interpreted as follows:

Open Tube	Closed Tube	Results
Yellow	Green	Oxidative
Yellow	Yellow	Fermentative
Green	Green	No action on glucose

(Idise, 2010**)**

3.2.1.4.8 Glucose, mannitol, lactose fermentation test

Two test tubes containing the test substrates (glucose, mannitol, lactose) were inoculated with the organisms. The inoculation was stabbed within one centimeter of the bottom of the test tube. One of the tubes was overlaid with sterile paraffin to exclude all oxygen. Both tubes were incubated at $37^{o}C$ for 48 h. The result was interpreted as follows:

Open Tube	Closed Tube	Results
Yellow	Green	Oxidative (O)
Yellow	Yellow	Fermentative (F)
Green	Green	No action on glucose

(Idise, 2010)

3.2.1.4.9 Citrate utilization test

Simmon's citrate agar plate (green colour) was streaked with the organism and incubated at 37^{o}C for 48 h. Growth in the medium was accompanied with a rise in pH resulting in change in colour of the medium from its initial green colour to deep blue. It is noteworthy that the medium was lightly inoculated to avoid a carryover of nutrient which may lead to a false positive result (Idise, 2010).

3.2.2 Modern Kit Characterization

In the modern kit characterization process, the microgen Bacillus – ID identification system consists of 2 microwell test strips were used. The strips 2 microwell test strips (labelled BAC 1 and BAC 2) each containing 12 dehydrated substances for the performance of either carbohydrates fermentation tests or other biochemical tests. The last well in the second microwell test strip was a carbohydrate fermentation control well for use as a reference well in the interpretation of these tests. The selection of the substrate included in the microwell test strip has been determined using computer based analysis of all available substrates for the identification or differentiation of this group of organism.

Identification of isolates was achieved by recording the results visualized by colour change after 24h and 48h incubation at 30^{O}C and the addition of appropriate reagents (Kovac's Nitrate and VP reagents) after 48h.

3.2.3 Mutagenic Treatment of Isolated *Bacillus species*

3.2.3.1 Mutation with UV radiation at 260nm

This was carried out using a modification of the procedure reported by Ado (2004). Briefly, the organism was grown in nutrient broth for 28h and the microbial counts recorded. Ten millilitres of 5.9 x 10^{14} cfu/ml and 4 x 10^{10}cfu/ml of *Bacillus subtilis* were aseptically transferred into separate sterile petri dishes and placed at a distance of 6cm from the source of

UV light in a dark room and withdrawn at 10, 20, 30, 40, 50 and 60 minutes respectively. The UV irradiated organisms were then transferred into a sterile twenty millilitre test tube in a dark room and treated with 0.2% (w/v) caffeine and allowed to stand at room temperature (30 ± 2^{o}C) in the dark for 5 h. Irradiated cells were centrifuged at 1500rpm for 3 to 5 minutes, re-suspended in normal saline and re-centrifuged. The supernatant discarded. The treated organisms were then incubated at 18^{o}C for 16h in a nutrient media and then counted to calculate the survival rate.

3.2.3.2 Mutation with nitrous acid

The organism was grown in nutrient broth for 18 – 24h and the microbial counts recorded. Aliquot of 0.15ml of filtered sterilized freshly prepared aqueous 2.0M sodium nitrate ($NaNO_3$) was added to the growing organism in the broth with acetate buffer (0.2ml, pH 4.4). After 20 minutes of exposure at 30^{o}C, the reaction was terminated by serial dilution in Tris hydrochloric (HCl) buffer (0.2M, pH 8.0). Aliquots of 0.1ml of the diluted suspension were plated on nutrient agar containing 0.2% soluble starch. The inoculated plates were incubated at 30^{o}C for 72h. The samples were stored in slants for further use at 5^{o}C (Idise, 2010).

3.2.4 Screening For Amino Acid Requirement

The test organisms were grown on nutrient media with specific amino acid addition.

3.2.5 Optimization of Growth Parameter on the Growth of Mutants and Isolated Organisms

After identification, the *Bacillus subtilis* strains were grown under different parameters such as temperature, pH, carbon source and nitrogen sources. Also the fermentation of the substrates (locust bean paste and castor oil bean paste) for the purpose of production of condiments, the organisms were subjected to the same conditions in order to ascertain the best condition for the production of the condiments (Achi, 2005).

3.2.6 DNA Isolation and Molecular Characterization of Mutant Strains

The stored organism in the slants were taken for DNA isolation and characterization; in the following sequence;

3.2.6.1 Deoxyribonucleic Acid (DNA) isolation

Deoxyribonucleic Acid (DNA) extraction is the removal of deoxyribonucleic acid (DNA) from the cells or viruses in which it normally resides. The bacterial genomic DNA was isolated according to the modified method of Idise et al, (2010). Briefly, 1.5ml of an overnight *Bacillus subtilis* culture grown in tris buffered saline (TBS) was collected and washed with TE buffer (10mM tris HCl, 1mM EDTA, pH 8.0). Bacterial DNA was isolated by lysozyme (Sigma Australia) and proteinase K (Promega Australia) treatment, followed by cetyltrimethylammonium bromide (CTAB) chloroform extraction and isopropanol in TE buffer. The quality of the DNA prepared was verified by measuring the absorbance ratio at 260/280nm.

The DNA extraction started by setting the heating block at 55^{o}C. Three hundred micro litre of buffy coat was added in a 1.5µl tube. This was placed on the heating block for 3h. The lysate was transferred as much as possible to a new tube. Seventy five micro litres of 5M NaCl was added and mixed by flicking the tube. This was centrifuged at 20,000rpm speed for 10 minutes. The lysate was carefully transferred into another tube. Care was taken not to disturb the pellets as the pellet contains proteins and other cellular debris. Two volumes of ethanol was added, (to a total of 300µl lysate, 600µl of -2^{o}C cold ethanol) was added and mixed by inverting the tube several times. The tube was spun at speed of 20,000rpm for 10 – 30 minutes in a refrigerated centrifuge in the same condition. The ethanol was removed and 400µl of 70% ethanol added. This was spun at a speed of 20,000rpm for 5minutes at 4^oC. As much ethanol as possible was removed and the tube was spun for 30s at 20,000rpm. All the traces of ethanol were removed. The DNA was dried out by leaving the tube open for 3-5 minutes. The pellet was re-suspended in 20-50µl of sterile water. To estimate the quantity and integrity of the DNA extracted, 1µl, 5µl and 10µl was run on agarose gel using 100ng-800ng for PCR.

3.2.6.2 Polymerase Chain Reaction

Two 16s rRNA universal primers 341f and 962r were used (Xi-Yang Wu *et al*., 2005).

B-K1/F, 5^I – TCACCAAGGCRACGATGCG – 3^I

19 – mer forward primer

B-K1/R1, 5^I – CGTATTCACCGCGGCATG – 3^I

18 – mer reverse primer

(Xi-Yang Wu *et al*., 2005; Mafra *et al*., 2008).

There are three major steps in a polymerase chain reaction which were repeated for a large number of cycles (60 circles). This was done in an automated cycler, which can heat and cool the tubes with the reaction mixture in a very short time. The steps were;

3.2.6.2.1 Denaturation

During this process, the double helical DNA melted and unwounded to single stranded DNA called template.

3.2.6.2.2 Annealing

The primers during this cooling process, annealed to the single stranded DNA forming ionic bonds, thereby copying the complementary DNA templates.

3.2.6.2.3 Extension

This is the ideal working temperature to activate the taq polymerase, which extended the primers and replicated the DNA. The polymerase added dNTPs from 5' to 3', reading the template from 3' to 5' side, bases were added complementary to the template.

3.2.7 PCR Premix

The PCR premix is a mixture that was prepared in a lyophilized format. The mixture contained Taq DNA polymerase, reaction buffer, dNTPs (dATP, dGTP, dCTP, dTTP), loading buffer $MgCl_2$ and tracking dye for efficient PCR amplification.

For the reaction set up, the templates, specific primers and water were added to the premix. In order, to make a 20µl reaction for example the following were needed; dH_2O – 17µl, primer 1 - 0.5µl, primer 2 – 0.5µl, template - 2µl.

PCR conditions: Pre-denaturation was done for 5 minutes at 94^{o}C. Denaturation was done at 0.5 to 1minutes at 50 - 68^{o}C (approximately 5^{o}C below primer temperature). Extension was done for 1 minute at 77^{o}C and final extension was done for 5 minutes at 72^{o}C.

3.2.7.1 Reconstitution of Primers

When primers are ordered, they are shipped dry, which means they have to be re-suspended. In order to get primers at the proper stock concentration, the company gave information about the primer and how deionized water can be used to in dissolving it.

The dilution was made in the hood. To make the stock dilution, the amount of water written on the tube (in µl) of sterile water was added to the primer tube in order to get a 100µM solution of the primer. After the addition of the sterile water, the tube was vortexed to re-suspend the primer well and spun briefly. The tubes were labelled with the name of the primer and the concentration in µl using a permanent marker. From the stock dilution, a working solution was made with 5µM and made to 100µl with sterile water and stored at -20^{o}C.

3.2.8 Sequencing

DNA sequencing includes several methods and technologies that are used for determining the order of the nucleotide bases, adenine, guanine, cytosine and thymine in a molecule of DNA. The dye termination method was used for the sequencing.

The dye termination sequencing utilizes labelling of the chain terminator dNTPs, which permits sequencing in a single reaction, rather than four reactions as in the labelled primer method.

In this method, each of the four deoxynucleotide chain terminator was labelled with a fluorescent dye, each of which emits light at a different wavelengths.

3.2.8.1 Dye terminator cycle sequencing with quick start kit.

Sequencing reaction was prepared in a 2.0ml tube. All reagents were kept on ice while preparing the sequencing reactions and were added in the following orders; dH_2O 0 - 9µl, DNA templates 0.5 – 10.0µl, primers 2.0µl, DTCS quick start master mix 8.0µl.

The sequencing reaction was set up in the PCR machine in the order; 96^{o}C for 20 seconds; 50^{o}C for 20 seconds x 30 cycle; 60^{o}C for 4 minutes.

3.2.8.2 Ethanol precipitation

A labelled sterile 0.5ml tube was prepared for each sample. Fresh stop solution/glycogen mixture was prepared as follows per sequencing reaction: 2µl of 100mM Na_2-EDTA and 1µl of 20mg/ml of glycogen. To each of the labelled tubes, 5µl of the stop solution/glycogen mixture was added. The sequencing reaction was transferred to each of the tubes and mixed thoroughly. 60µl of cold 95% (v/v) ethanol stored at -20^{oC} was added and mixed thoroughly. This was immediately centrifuged at 14,000rpm at 4^{o}C for 15 minutes. The supernatant was carefully removed with the aid of a micropipette. The pellet was rinsed with 200µl of 70% (v/v) ethanol stored at -20^{o}C, it was centrifuged at 14,000rpm at 4^{o}C for 2 minutes. The supernatant was carefully removed with a micropipette. It was vacuum dried until dried. The sample was re-suspended in 40µl of the sample loading solution.

3.2.8.3 Sample Preparation.

The re-suspended samples were transferred into appropriate wells of the sample plate (PN 609801). Each of the re-suspended samples was overlaid with one drop of mineral oil from the kit. The sample plate was then loaded into the instrument and then started.

3.2.9 Electrophoresis

The solution (4g of agarose gel) was heated in a boiling water bath until agarose was completely dissolved. This was allowed to cool in a water bath set at 55^{o}C. The gel casting was prepared by sealing ends of gel chamber with tape. Appropriate number of combs were placed in gel tray. Five micro- liters of ethidium bromide was added to the cooled gel and poured into gel tray. This was allowed to cool for 30 minutes at 30^{o}C. The combs were placed in the electrophoresis chamber and covered with buffer (TBE). Isolated DNA and standard (Ladder) were loaded into gel. This was electrophoresed for 1 hour. Deoxyribonucleic Acid (DNA) bands were visualized using gel imaging system.

3.2.10 Production of 'daddawa' from locust bean seeds

African locust bean seeds (*Parkia biglobosa*) was purchased in Zaria central market, Kaduna State and processed into "daddawa" by the modified Achi, (2005) method. Processing of the seeds was carried out by boiling for 19h during which water was removed twice. The testa was then removed by pounding lightly in a wooden mortar using a wooden pestle. The seeds were then washed to remove the testa from the cotyledon. The cotyledons were then re-boiled for 2 h and the water discarded. Fermentation was carried out by weighing out 2500gm of the boiled locust bean was crushed into paste and later boiled again for sterilization. Three hundred grams each were transferred aseptically into seven sterile 250ml flat bottom flask. The flask was covered with aluminum foil to keep away contaminants. The inoculation of fermenting *Bacillus* strains were done inside sterile flat bottom flasks containing the locust bean seed paste. Then the fermentation was done under varying temperature (25, 35 & 45), varying pH (5, 6, 7, & 8) and normal pH. (Fig 3.3)

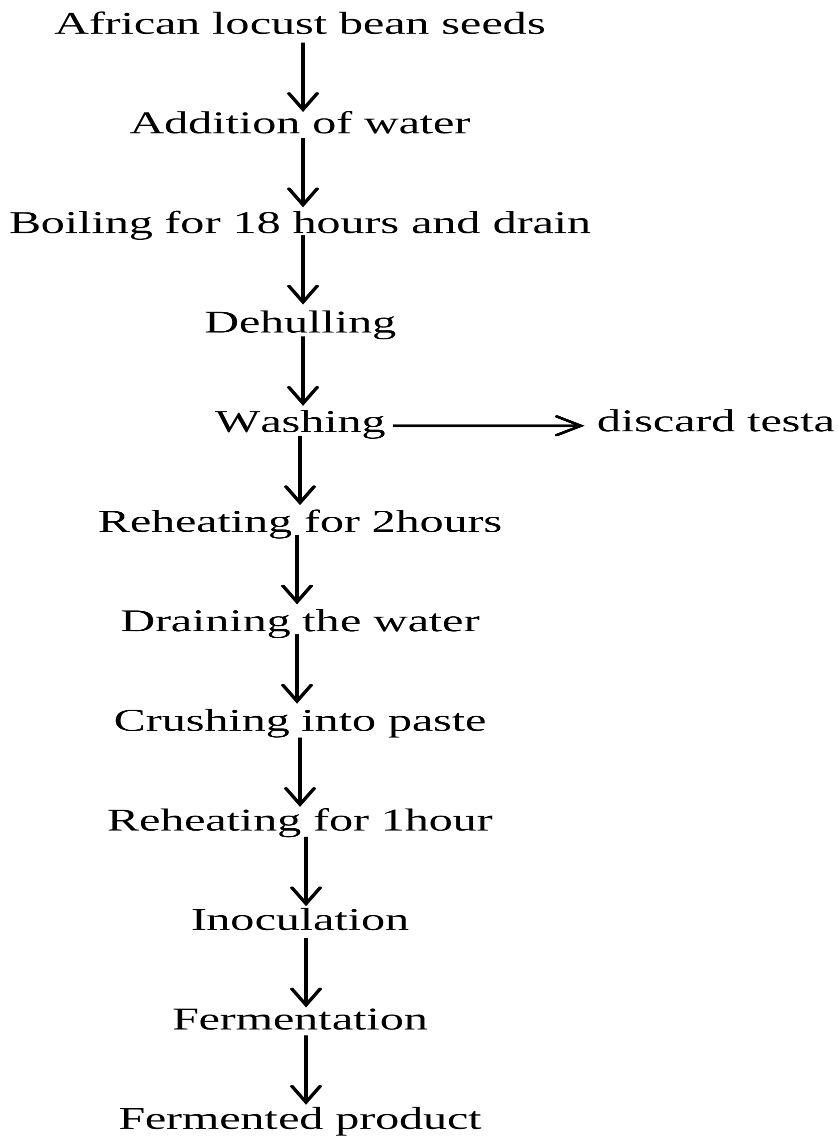

Figure 3.3: Production of Daddawa from Locust Beans (Achi 2005)

3.2.11 Production of 'Ogiri' under controlled condition

Castor oil seeds were purchased in Oye-Agu market, Abagana, Anambra State. Pre-processing of the seeds was done by shelling and separation from the shells. The processing of the seeds started with boiling of the seeds with water for 48 h. The boiled seeds were milled using wooden mortar and wooden pestle. The fermentation was carried out by weighing out 2500gm of the boiled locust bean and then crushed into paste and later boiled again for sterilization. Three hundred grams each was transferred aseptically into seven sterile flat bottom flasks that have been cleaned and sterilized. The flask was covered with aluminum foil paper to keep away contaminants. The inoculation of fermenting *Bacillus* strains was done inside sterile flat bottom flasks containing the castor oil seeds paste. Then the fermentation was done under varying temperature (25, 35 & 45), varying pH (5, 6, 7, & 8) and normal pH.

Castor oil seeds

↓

Shelling

↓

Boiling with H_2O for 48 hours

↓

Milling

↓

Sterilize by boiling for 1hour

↓

Fermenting for 48 – 96hours

↓

Packing for aging

↓

Product

Figure 3.4: Production of ogiri from Castor Oil Seeds

3.2.12 Physiochemical Composition of “daddawa” and “ogiri”

3.2.12.1 Temperature

Thermometer (Seward immersion model) was cleaned with ethanol and then inserted into the 30 flat bottom flask containing the locust bean seeds, castor oil seeds (*Ricinus communis*) ‘daddawa’ and ‘Ogiri’ to monitor the temperature. The temperature was monitored at intervals of 12h till the end of the fermentation period.

3.2.12.2 pH

The pH meter was first calibrated using standard buffers of pH 4.0 and 9.0.Reading was taken at intervals of 12h till the end of the fermentation. This was done by mixing 1gm of the fermenting mash in 10ml of sterile distilled water. The pH of the suspension was determined with pyeunicam pH meter (USA).

3.2.12.3 pH Adjustment

pH of the samples was measured using pH meter. A standard solution of HCl was prepared and allowed to stand, and then a standard solution of $NaCl_2$ was also prepared.

The pH of the sample was measured, and then a little solution of either HCl or $NaCl_2$ was added to adjust the pH to a required standard.

3.2.13 Proximate Composition of “daddawa” and “ogiri”

3.2.13.1 Moisture content

Principle: Dehydration of the test sample in an electrically heated drying oven at 105 ± 2^oC under atmospheric pressure for 8 h.

Method: The AOAC (2010) air oven method was used for the analysis. Two grams of the grounded samples were weighed into a pre-heated cooled and weighed porcelain crucibles and dried in an oven for 8h at a regulated temperature of 105±2^oC. They were removed, cooled in a desiccator and weighed. The process of drying, cooling and weighing continued until a constant weight was obtained.

The percentage moisture content of the sample was calculated as follows:

% moisture content (dry basis) = $\frac{W_1 - W_3}{W_2 - W_1}$ x 100

Where W_1 = weight of crucible

W_2 = weight of crucible plus sample before drying

W_3 = weight of crucible plus sample after drying

3.2.13.2 Ash content

Principle: Incineration of a test sample at a temperature of 550-600^oC for 5 h, until complete disappearance of the organic matter in the residue. The residue is the ash content which consists of inorganic component in the form of oxides, sulphates, phosphates, chlorides or silicates.

The AOAC (2010) method was used for the analysis. Briefly, two grams of the sample were weighed into a pre-heated cooled and weighed porcelain crucible. The crucible and its contents were charred on a Bunsen burner flame in a fume cupboard to drive of the smoke. After ignition, the crucibles and the contents were transferred to into a muffle furnace at 550^oC for 3 h until a white ash result. The crucibles were removed and placed in a desiccator to cool and then reweighed.

% Ash content (dry basis) = $\frac{M_{ash}}{M_{dry}}$x 100

Where M_{ash} refers to the mass of the ashed sample and M_{dry} refers to the original mass of the dried sample.

3.2.13.3 Determination of crude protein content

Principle: The protein-containing sample is digested using concentrated H_2SO_4 in the presence of $CuSO_4$ and Na_2SO_4 as catalyst to raise the boiling point. Standard alkali is added to the digest and the product is steam-distilled into a standard solution of boric acid. The titration of the acid gives an indication of the amount of ammonia (NH_3) distilled and hence the nitrogen content from which crude protein is calculated.

$$\text{Nitrogen in sample} \xrightarrow[\text{Mixed catalyst}]{\text{Conc. } H_2SO_4} NH_3$$

The ammonia is produced in the form of ammonium sulphate ($(NH_4)_2SO_4$)

$$2NH_3 + H_2SO_4 \longrightarrow (NH_4)_2SO_4$$

When the ammonium sulphate is reacted with an alkali, such as 40% NaOH solution, ammonia (NH_3) is evolved and then trapped into boric acid.

$$(NH_4)_2SO_4 + 2NaOH \longrightarrow NaSO_4 + 2H_2O + 2NH_3$$

Then $H_3BO_3 + NH_3 \longrightarrow NH_4^+\ H_2BO_3^-$

Boric acid ammonia boric acid complex

When the complex is steam-distilled, the NH_3 comes out as NH_4OH which is titrated with HCl.

Thus, $NH_4OH + HCl \longrightarrow NH_4Cl + H_2O$

Method: The method used for the analysis was as reported by Onyeike and Osuji (2003). Exactly 1.50g of samples were weighed in an ashless filter paper and transferred into a 300ml digestion flask, then 25ml of concentrated H_2SO_4 and 3.0g of mixed catalyst (Na_2SO_4 and $CuSO_4$ (10:1)) were added. The flask and the contents were placed on the Kjeldahl heater and heated until a clear green solution was obtained. It was allowed to cool and the digest were measured into a 250ml conical flask and four drops of a mixture of equal volumes of screened 0.2% methyl red, 0.1% methylene blue indicator solutions added. Twenty millilitres of the diluted digest were measured into 500ml distillation flask and 40ml of 40% NaOH slowly added with a syringe. The flask was heated to distil out the NH_3 evolved which was collected as distillate in the boric acid solution placed at the tip of the receiver adaptor. A change in colour from pink to green indicates the end of the distillation. The percentage nitrogen (%) in the samples was calculated as illustrated below;

Calculation

$$\% \text{ Nitrogen} = \frac{\text{Titre value} \times 1.40 \times \text{total volume of diluted digest}}{\text{Sample weight (mg)} \times \text{aliquote volume distilled}} \times 100$$

% crude protein = % Nitrogen x 6.25

Where 6.25 is the conversion factor for leguminous foods

3.2.13.4 Determination of crude lipid

Principle: The food sample is dried, grounded and its fatty substances extracted with petroleum (40-60^oC) using soxhlet apparatus. The solvent which now forms part of the extract (fat) is removed by evaporation and the residue (fat) is weighed.

Methodology: The AOAC (2010) method was used for this analysis. Five grams of the samples were weighed into a thimble and placed in soxhlet apparatus. The lipid contained in the dry sample was exhaustively extracted using petroleum ether (40-60^oC) for 5-6 h. The extractant (petroleum ether) was distilled off and the flask re-weighed. The percentage lipid was calculated.

Thus:

% lipid (fat) = $\frac{\text{Weight of lipid}}{\text{Weight of sample}}$ x 100

3.2.13.7 Total Carbohydrate

The total Carbohydrates were determined by difference.

% Total Carbohydrates = 100 – (% moisture + % ash content + % protein + % lipid)

3.2.14 Sensory Evaluation

Sensory evaluation of “Egusi” soup which was prepared with the laboratory produced “daddawa” and “ogiri” as well as the market ones was conducted using the 9-points hedonic scale, for the evaluation where 1 represents dislike extremely, 2 dislike very much, 3 dislike moderately, 4 dislike, 5 neither like nor dislike, 6 like, 7 moderately like, 8 like very much and 9 like extremely and the results obtained were statistically analysed.

3.2.15 Statistical analysis of Data

Data obtained were statistically analyzed using SPSS (version 11). Multiple comparisons of mean±SEM were carried out by two way ANOVA. A probability level of less than 5% was considered significant. Duncan multiple range test of post Hoc test ($p<0.05$). The results were also presented in tables, plates and figures.

CHAPTER FOUR

RESULTS

4.1 Isolation, Characterization and Identification of the Bacteria Isolates from the Condiments

The isolates were tentatively identified as *Bacillus cereus*, *Bacillus licheniformis, Bacillus laterosporus, Bacillus firmus* and *Bacillus subtilis*. All the *Bacillus* isolates were positive to Gram reaction, sucrose, motility, catalase and nitrate test, they were negative to indole, melezitose and methyl D mannose test. These tests results are presented in Table 4.1. Table 4.2 is the microgen identification of the isolates indicating that the isolates *B. subtilis, B. licheniformis and B. cereus*

4.2 Mutation, PCR Amplification and Nucleotide Sequencing of Wild and Mutant *Bacillus subtilis*

After the exposure of the organism to UV light and nitrous acid, it was observed that the plate containing organism that was exposed to UV light and nitrous acid for 20 minutes had little heavily clouded growth. The plate containing organism that was exposed to UV light and nitrous acid for 30 minutes had moderate number of cell growth. The plate containing organisms that was exposed to UV light and nitrous acid for 40 minutes had a scanty growth and was judged to have a good number of cells killed by the exposure. The plate containing organisms that was exposed to UV light and nitrous acid for 50 minutes had very little number of growth. The plate exposed to UV light for 60 minutes had no colony and that of the nitrous acid had four colonies.

Table 4.1: Preliminary biochemical characteristics of bacteria isolated from the condiments

Test	**Test Code**									
	D_1	D_2	D_3	D_4	D_5	O_1	O_2	O_3	O_4	O_5
Gram reaction	+	+	+	+	+	-	+	+	+	+
Shape	Rod	Rod	Cocci	Rod	Cocci	Rod	Rod	Rod	Rod	Rod
Sucrose	+	+	-	+	-	+	+	+	-	+
Motility	+	+	-	+	-	+	+	+	+	+
Catalase	+	+	+	+	+	+	+	+	+	+
Voges Proskauer	-	-	-	-	-	+	-	-	-	-
Starch hydrolysis	+	+	-	+	-	-	+	+	+	+
Nitrate	+	+	+	+	+	+	+	+	+	+
Protease	+	-	+	+	+	+	-	-	+	-
Glucose	-	-	+	+	+	+	+	-	-	-
Citrate Utilization	+	+	-	+	_	+	+	+	+	+
Probable identity	*B.subtilis*	*B.subtilis*	*S. aureus*	*B. subtilis*	*S. aureus*	*B. cereus*	*B. subtilis*	*B. subtilis*	*B. subtilis*	*B. subtilis*

KEY: D = daddawa, D_{1-5} = daddawa from location 1 – 5
O = "ogiri", O_{1-5} = "ogiri" from location 1 – 5
+ = Positive
- = Negetive

Table 4.2: Characterization of *Bacillus* Isolates using Microgen Identification Kit

Sample Code	Ara	Cel	Ino	Man	Mns	Raf	Rha	Sal	Sor	Suc	Tre	Xyl	Ado	Gal	Mdm	MDG	Inu	Mlz	Ind	ONPG	Arg	Cit	VP	Nit	Tentative identification
D_1	-	+	+/-	+	+	+/-	-	+	+	+	+	+/-	-	-	-	+	+	-	-	+	-	-		+	*B. subtilis*
D_2	-	+	+/-	+	+	+/-	-	+	+	+	+	+/-	-	-	-	+	+	-	-	+	-	-		+	*B. subtilis*
D_3	+	+	-	-	+	+	-	+	-	+	+	-	-	+	-	-	-	-	-	+	+	+	+	+	*B. licheniformis*
D_4	-	+	-	-	-	-	-	+	-	+	-	+	-	+	-	-	-	-	-	+	+	-	+	+	*B. cereus*
D_5	-	+	+/-	+	+	+/-	-	+	+	+	+	+/-	-	-	-	+	+	-	-	+	-	-		+	*B. subtilis*
O_1	-	+	+/-	+	+	+/-	-	+	+	+	+	+/-	-	-	-	+	+	-	-	+	-	-		+	*B. subtilis*
O_2	-	+	-	-	+	-	+	+	-	-	+	-	-	-	-	-	-	-	-	+	+	-	+	+	*B. laterosporus*
O_3	+	-	-	-	-	+	-	-	-	+	+	-	-	-	-	-	+	-	-	+	+	-	+	+	*B. firmus*
O_4	+	+	-	-	+	+	-	+	-	-	+	-	-	+	-	-	-	-	-	+	+	+	+	+	*B. licheniformis*
O_5	-	+	+/-	+	+	+/-	-	+	+	+	+	+/-	-	-	-	+	+	-	-	+	-	-		+	*B. subtilis*

Key: Ara = arabinose, Cel = cellobiose, Ino = inositol, Man = mannose, Raf = raffinose, Rha = rhamnose, Sal = salicin, Sor = sorbitol, Suc = sucrose, Tre = trehalose, Xyl = xylose, Ado = adonitol, Gal = galactose, Mdm = methyl D manoside, MDG = Methyl-D-Glucoside, Inu = inulin, Mlz = melezitose, Ind = indole, ONPG = O-nitrophenyl-β-D-galactopyranoside;, Arg = Arginine dihydrolase, Cit = citrate utilization, VP = Voges Proskauer, Mns = mannose, Nit = nitrate, D_{1-5} = Daddawa, O_{1-5} = "ogiri"

Table 4.3 Percentage survival rate of *Bacillus Subtilis* after mutation

Time/min	UV – light (%)	Nitrous acid (%)
10	70	85
20	43	57
30	25	36
40	14	20
50	6	10
60	0	4

4.2.1 Mutagenic treatment of the wild strain isolates.

The effects of the mutagenic treatment are presented in Table 4.3. The effects of Ultraviolet (UV) radiation (260nm) on survival rate after exposure by the parent strains at different times 10, 20, 30 40, 50, and 60, presented showed % survival that ranges from 70 to 0%. The effect of nitrous acid (2.0M, NHO_2) treatment on survival rate after exposure by the parent strains at different time interval (10,20,30,40,50 and 60min) presented in table 4.3a showed percentage survival ranging from 85% to 4%. The result in table 4.3 showed that nitrous acid treatment of strains had a higher survival percentage.

4.3 Screening for amino acid requirement by parent and modified strains

The requirement of essential amino acids – leucine, lysine, tryptophan, methionnine, histidine, valine and phenylalaine for growth by the parent and mutated strains is shown in Table 4.4. It was observed that most of the organisms required all the tested amino acids for growth except BsN30, BsN40, BsN50, BsN60, BsUV30, BsUV40, BsUV50 and BsUV60 which do not require histidine, lysine, methionine and tryptophan respectively. This showed the mutagenized strains having amino acid requirement different from their parents, hence, their selection for further analysis.

The electrophoresis results shows that the organisms are all the same as distance travelled in the electrophoresis frame are the same, as shown in plate 1 above.

Standard *Bacillus* Strain (ABC 35615) Obtained on 25th April, 2013 from a sample obtained in South Africa was sequenced and the genome strand amounting to 271 nucleotides Chain.

The Wild Strain of *Bacillus subtilis* had genome strand of 632 nucleotides chains and at 562nd Position, an unknown genome N occurs which also appears at 572nd position. This genome also occurs at 621st position. This nucleotide strand were not known in standard strand of *Bacillus Subtilis*. When this Strain were mutated using UV-light (260m) for 30 minutes this genome now occurs at 62nd position (CNG) and 439th position (CNC) and 463rd position (GNA). Now the genome chain is 601 instead of 632 nucleotides chains which is for wild *Bacillus subtilis*. This shows a reduction in genome chains and change in position of the unknown gene N, for mutation using UV-light (260nm) for 40 minutes the genome reduce d to 564 and there were introduction of new unknown gene F at 354th position and 401st position. Another gene C1occured at 399th position and N gene found in the wild strains still remained at 487th position. For Nitrous acid mutation, there was a general increase in number of genome from 631 to 653. A new genome G1 (GG1T) appeared at 36th position in the nucleotide chain. Also I in the (CCIA) genome appeared at 306th position and another new genome E (AET) appeared at 625th position.

Generally this indicate that standard strain of ***Bacillus Subtilis*** are quite different from all others including wild strain which has 631 nucleotides chain. There was location changes for these genomes and creation of new ones in the three mutation methodologies which shows that actually there was mutation in the organism. All these shows that there are mutations in the wild *Bacillus subtilis* strains, also the mutated organisms.

Table 4.4: Screening for amino acid requirement of Parent, Nitrous acid and UV light treated strains of *Bacillus Subtilis*

	Nitrous Acid							UV light					
Amino acid	P	BsN10	BsN20	BsN30	BsN40	BsN50	BsN60	BsUV10	BsUV20	BsUV30	BsUV40	BsUV50	BsUV60
Histidine	+	+	+	+	+	+	–	+	+	+	+	+	–
Lysine	+	+	+	–	+	+	–	+	+	–	+	+	–
Methionine	+	+	+	+	+	–	+	+	+	–	+	–	+
Phenylalanine	+	+	+	+	–	+	+	+	+	+	–	+	+
Valine	+	+	+	–	+	–	+	+	+	+	–	–	+
Leucine	+	+	+	+	+	+	+	+	+	+	+	+	–
Tryptophan	+	+	+	+	–	+	+	+	+	–	–	+	+

Key: P- isolated parent organism, Bs- *Bacillus subtilis*, N- nitrous acid treated, UV= UV-light treated, Time of treatment =10, 20, 30, 40, 50, and 60. + = positive, - = Negative

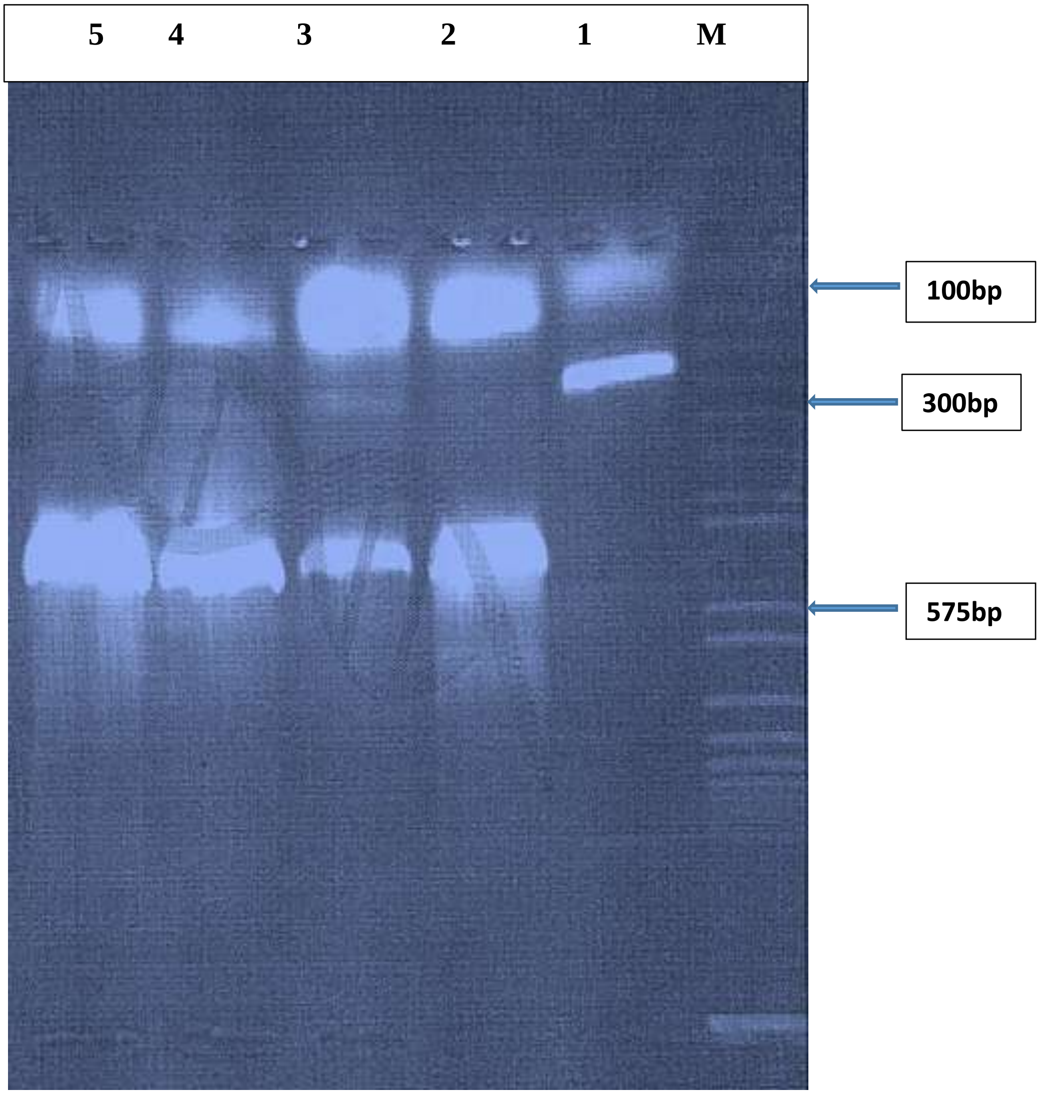

Figure 4.1: PCR Amplification of *Bacillus subtilis* and its Mutant strains

Key: M=Biomarker (100bp ladder), 1=Standard strain (ABC35615), 2=wild strain, 3= mutated at UV light 30mins, 4=mutated at UV light 40mins, 5=Nitrous acid mutated strain, bp=base pair.

• CTTCCGCAGTCGAGTTGCACACTGCGATCCGAACTGACACAGATATCGG
ATTGGCTAAACCTTGCGGTCTTGCAGCCCTTTGTTCTGTCCATTGTAGCA
CGTGTGAGCCCAGGTCATAAGGGGCATGATGATTTGACGTCATCCCCAC
CTTCCTCCGGTTTGTCACCCGCCGCCACCTTAGAGTGCCCAACAGAATG
CTGGCAACTAAGATCAAGGGTTGCGCTCGTTGCGGGACTTACCCCAAC
ATCTCACGACACGAGCTGACGACAAC

Figure 4.1: Nucleotide Sequence of *Bacillus subtilis* (standard strain)

Key: A=Adenine, T=Thymine, G=Guanine, C=cytosine

27

• ACCAATTCAGGTATGCCTGAATGGACACTAATAAGGTCCCGAGAGGTGG
GACC
CAGAGATCTCTCTGAGAATGTACCCTTGTCAAACCTTATGATATTCCCGCTT
GGTGCTGAGTATTCTCGATGTGGTACACCTGCCTGTAAGACTGGGATAAC
TCCGGAAACCGGAGCTAATACCGGATAGTTCCTTGAACCGCATGGTTAAG
GATGAAAGCGGTTTCGGCTGTCACTTACAGATGGACCCGCGGCGCATTA
GCTAGTGGTGGGGTAATGGCTCACCAAGGCGACGATGCGTAGCCGACCT
GAGAGGGTGACGGCCACACTGGGACTGAGACACGGCCCAGACTCCTAC
GGGAGGCAGCAGTAAATCTTCCGCAATGGACGAAAGTCTGACGGAGCA
ACGCCGCGTGAGTGATGAGGTTTTCGGATCGTAAAGCTCTGTTGTTAGG
GAAGAACAAGTGCGAGAGTAACTGCTCGCACCTTGACGGTACCTAACCA
GAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGG
CAAGCGTTGTCCGGAATTATNGGGCNTAAAGGGCTCCCAGGCGGTTCTT
AAGTTTGATGTGAAAGCCCCGCTCANCCGGGAGGTCT

Figure 4.2: Nucleotide Sequence of *Bacillus subtilis* (wild strain)

Key: A=Adenine, T=Thymine, G=Guanine, C=cytosine

29

- AAAAACGGACGGACCCGAAACGGACCCAGGAACGACCGCACTGCATC
CTGTATGATTGACCNGCACACAGGAAGTGACGCGGCGGACTGGTGAGT
GACACGTGGCTGATTCCTGCCCATGAAGACTGGGATGAACTCGGGAAA
CGGGGGCTAATACCGGATAACATTTGAACGCATGTTCGAATGAAGGCGC
TTCGCTGTACTATGATGACCGCGTGCATAGCTGTGGTGAGTACGGCTCCC
AAGGCAACCAATCCTTACCCAACCTGAGAGGGTGATCGGCCACACTGG
GGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAAT
CTCCGCAATGGACGAAAGTCTGACGGAGCACGCCGTGAGTGATGAAGG
CTTCCGGGTCGTAAAACTCTGTTGTTAGGGAAGAACAGTGGTTGAATTA
GTTGNACCTGAACGTACCTACCAGAAGCNCGGCTACTACGTGACTAGTA
GTGCAGCTATCGATATGGCTAAGGCCGTGTCTATTAGGAGGTACTGTGC
GAATAGGAAGATGATCGTACTATCTAATGGACATGCAGCATCGTGATACT
AGCATTGACACGTATCTAA

Figure 4.3 Nucleotide Sequence of *Bacillus subtilis* (mutated with uv-light 30mins)
Key: A=Adenine, T=Thymine, G=Guanine, C=cytosine

31

- CGAGTTGCAGCTACAATCCGAACTGAGAACGACTTTATCGGATTAGC
TCCCTCTCGCGAGTTGGCAACCGTTTGTATCGTCCATTGTAGCACGT
GTGTAGCCCAGGTCATAAGGGGCAGGATGATTTGACGGCATCCCCA
CCTTTCTCCGGTTTGTCACCGG
CAGTCACCTTAGAGTGCCCAACTAAATGATGCAACTAAGATCAAGG
GTTGCGCTCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCF
GACGACAACCATGACCACCTGTCACCGTTGCCCCCGAAGGGGAAA
CCATATCTCTACAGTGGTCAGGATGTCAAGACCTGGTAAGGTTCTTC
GCGTGCTFCAAATAAACCACATGCCCACGCTTGTGCGGGCCCCCGTC
AATTCC1TFGAGTTCAGTCTTGCGACCGTACTCCCCACGCGGAGTGC
TTAATGCGTTAGCTGCACACTAAGGGGCGGAAACCCCCTAACACTTA
NCACTCATCGTTTACGGCGTGGACTACCAGTATCTAATCTGTTTGCTC
CCCACGTTTCGCGCCTCAGCGTCTGTTACA

Figure 4.4 Nucleotide Sequence of *Bacillus subtilis* (mutated with UV-light 40mins)
Key: A=Adenine, T=Thymine, G=Guanine, C=cytosine

33

- AGGCGAGTTGCAGCCTACAATCCGAACTGAGAACGG1TTTATGAGATTA
GCTCCACCTCGCGGTCTTGCAGCTCTTTGTACCGTCCATTCTAGCACGTG
TCCCAGCCAGGTCATAAGGGGCATGATGATTTGACGTCATCCCCACCTTC
CTCCGGTTTGTCACCGGCAGTCACCTTAGAGTGCCCAACTGAATGATGG
CAACTAAGATCAAGGG
TTGCGCTCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACG
ACAACCATGCACCACCTGTCACTCTGCTCCCGAAGGAGAAGCCCIATCTC
TAGGGTTGTCAGAGGATGTCAAGACCTGGTAAGGTTCTTCGCGTTGCTT
CGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCCTT
TGAGTTCAGCCTTGCGCCGTACTCCCCAGGCGGAGTGCTTAATGCGTTA
ACTTCAGCACTAAAGGGCGGAAACCCTCTAACACTTAGCACTCATCGTT
TACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTC
GCGCCTCACTGTCAGTTACAGACCAGAAAGTCGCTTCGCCACTGGTGAT
CCTCCATTCTCTACGCAETCACCGCTACACAGGAATTCACTTTCCT

Figure 4.5: Nucleotide Sequence of *Bacillus subtilis* (mutated with nitrous acid)
Key: A=Adenine, T=Thymine, G=Guanine, C=cytosine

35

4.4 Upgrading of Traditional Processing Methods.

4.4.1 Proximate Composition of unfermented "daddawa" and "ogiri"

Table 4.5 shows the percentage compositions of unfermented "daddawa" and "ogiri". The proximate compositions unfermented "daddawa" include moisture (14.26%), ash (0.80%), lipid (22.13%), protein (29.40%) and carbohydrate (33.41%). Similarly, the percentage compositions of "ogiri" was moisture (14.55%), ash (1.04%), lipid (23.06%), protein (27.76%) and carbohydrates (33.59%). However, "daddawa" had higher protein content than "ogiri". Moisture, ash, lipid and carbohydrate contents were higher in "ogiri" than in "daddawa".

There was a significant difference ($P<0.05$) in the percentage composition of the proximate compositions of the test condiments.

4.4.1.2 Proximate Compositions of *Bacillus subtilis* fermented "daddawa" and "ogiri"

Table 4.6 shows the proximate composition of *Bacillus subtilis* (wild, mutated and standard strain) fermented "daddawa" and "ogiri". "Daddawa", moisture content was highest (16.68%) and lowest (16.13%) in *Bacillus subtilis* (uv-30mins) fermented "daddawa" and *Bacillus subtilis* (nitrous acid) respectively. Highest (1.14%) and lowest (0.70%) ash contents were observed in *Bacillus subtilis* (mutated with nitrous acid) and *Bacillus subtilis* (wild strain) respectively. The highest (23.85%) and lowest (23.18%) lipid contents were observed in *Bacillus subtilis* (uv-40mins) and *Bacillus subtilis* (wild strain) respectively.

Table 4.5: Proximate Composition in percentage (%) of Unfermented ("daddawa" and "ogiri")

Proximate Compositions	"daddawa"	"ogiri"	P-value
Moisture	14.26±0.10	14.55±0.21	0.033
Ash	0.80±0.13	1.04±0.09	0.026
Lipid	22.13±0.34	23.06±0.32	0.054
Protein	29.40±0.34	27.76±0.29	0.038
Carbohydrate	33.41±0.19	33.59±0.24	0.046

P<0.05= significance, Values are expressed as Means ± SD (Standard deviation) of triplicate determinations.

Table 4.6: Proximate Composition (%) of *Bacillus subtilis* Fermented ("daddawa" and "ogiri")

	Moisture		Ash		Lipid		Protein		Carbohydrate	
Test organism	"daddawa"	"ogiri"	"daddawa"	"ogiri"	"daddawa"	"ogiri"	"daddawa"	"ogiri"	"daddawa"	"ogiri"
*B. subtilis(*wild*)*	16.60±0.00[a]	17.22±0.10[a]	0.70±0.11[c]	1.56±0.09[c]	23.18±0.20[a]	22.69±0.21[a]	31.73±0.07[a]	30.24±0.01[b]	28.5±0.02[a]	28.29±0.05[a]
B. subtilis (UV 30mins)	16.68±0.11[a]	17.55±0.01[a]	0.81±1.01[c]	1.79±0.12[a]	23.41±0.08[a]	23.40±0.10[b]	31.20±0.01[a]	30.29±0.04[b]	28.96±0.03[a]	26.97±0.04[c]
B. subtilis (UV 40mins)	16.65±0.01[a]	17.24±0.50[a]	0.96±1.20[b]	1.81±0.02[a]	23.85±0.01[a]	22.74±0.10[a]	30.91±0.00[a]	31.33±0.02[a]	27.97±0.05[b]	27.67±0.02[b]
B. subtilis (Nitrous acid)	16.13±0.23[a]	17.34±0.21[a]	1.14±0.03[a]	1.51±0.30[b]	23.68±1.00[a]	22.88±0.05[a]	30.79±0.04[a]	21.79±0.01[c]	27.01±0.01[b]	27.01±0.01[b]
B. subtilis (Standard strain)	16.62±0.02[a]	16.89±0.03[b]	0.94±1.05[b]	0.94±0.15[c]	23.64±0.00[a]	22.52±0.08[a]	30.84±0.01[a]	30.31±0.03[b]	26.81±0.04[b]	26.81±0.03[b]
P-value	0.047	0.054	0.049	0.051	0.038	0.056	0.047	0.040	0.044	0.062

Means having different superscripted letters along the columns are significantly different at P<0.05. Values are expressed as Means ± SD (Standard deviation) of triplicate determinations.

The highest (31.73%) and the lowest (30.84%) protein contents were observed in *Bacillus subtilis* (uv- 40mins) and *Bacillus* (standard strain). Highest (28.96%) and lowest (27.01%) carbohydrate contents were observed in *Bacillus subtilis* (uv-30mins) and *Bacillus subtilis* (mutated with nitrous acid) fermented “daddawa”.

In “ogiri” sample, moisture content was highest (17.55%) and lowest (17.22%) in *Bacillus subtilis* (uv-30mins) and *Bacillus subtilis* (wild) respectively. Highest (1.81%) and lowest (0.94%) ash contents were observed in *Bacillus subtilis* (uv-40mins) and *Bacillus subtilis* (standard strain) respectively. The highest (22.88%) and lowest (22.69%) lipid contents were observed in *Bacillus subtilis* (nitrous acid) and *Bacillus subtilis* (wild strain) respectively and the highest (31.33%) and lowest (21.79%) protein contents were observed in *Bacillus subtilis* (uv-40mins) and *Bacillus subtilis* (nitrous acid) respectively. In addition the highest (28.29%) and lowest (26.81%) carbohydrate contents were observed in *Bacillus subtilis* (wild) and *Bacillus subtilis* (standard). However, the various strains of the *Bacillus subtilis* revealed significant difference ($P<0.05$) in the percentage compositions of the proximate parameters.

4.4.3 Proximate Analysis of Fermented “daddawa” Inoculated with Wild, Mutated and Standard *Bacillus subtilis* at varying pH

The results of percentage proximate compositions of “daddawa” inoculated with various strains of *Bacillus subtilis* at varying pH are presented in Tables 4.7a to 4.7e).

Table 4.7a: Proximate Composition (%) of "daddawa" inoculated with Wild, Mutated and standard *Bacillus subtilis* at Normal pH (5.4)

Organisms	Proximate parameters (Mean±SD)				
	Moisture	Ash	Lipid	Protein	Carbohydrate
B. subtilis (Wild)	16.86±1.16[a]	0.54±0.00[c]	21.47±1.73[c]	32.24±1.21[c]	28.89±1.11[b]
B. subtilis (UV 30mins)	16.99±0.44[a]	0.66±0.30[b]	23.67±2.35[b]	28.85±0.30[d]	29.83±3.23[a]
B. subtilis (UV 40mins)	15.60±0.52[b]	0.75±0.22[a]	24.30±1.64[a]	37.43±10.14[a]	21.92±1.63[d]
B. subtilis (Nitrous acid)	16.41±0.70[a]	0.50±0.10[c]	22.93±1.19[c]	31.49±3.07[c]	28.67±2.15[b]
B. subtilis (Standard strain) (ABC35615)	16.80±1.20[a]	0.77±0.57[a]	23.76±2.11[b]	34.69±0.26[b]	23.98±2.24[c]
P-Value	**0.041**	**0.043**	**0.051**	**0.034**	**0.044**

Means having different superscripted letters along the columns are significantly different at P<0.05. Values are expressed as Means ± SD (Standard deviations) of triplicate determination

Table 4.7b: Proximate Composition (%) of "daddawa" Inoculated with Wild, Mutated and Standard *Bacillus subtilis* at pH 5

Organisms	Proximate parameters (Means±SD)				
	Moisture	Ash	Lipid	Protein	Carbohydrate
B. subtilis (Wild)	16.26 ± 1.20^{b}	0.80 ± 0.17^{b}	24.30 ± 2.39^{a}	33.36 ± 1.81^{a}	47.28 ± 1.14^{a}
B. subtilis (UV 30mins)	16.70 ± 0.37^{b}	0.80 ± 0.48^{b}	24.20 ± 0.45^{a}	29.55 ± 1.50^{c}	28.75 ± 2.02^{c}
B. subtilis (UV 40mins)	16.05 ± 1.32^{b}	0.65 ± 0.28^{d}	22.40 ± 2.05^{b}	29.66 ± 1.28^{c}	31.24 ± 2.22^{b}
B. subtilis (Nitrous acid)	15.67 ± 0.32^{c}	0.71 ± 0.54^{c}	23.70 ± 1.05^{b}	30.96 ± 3.29^{b}	28.96 ± 3.19^{c}
B. subtilis (Standard strain) (ABC35615)	17.23 ± 0.69^{a}	0.84 ± 0.26^{a}	24.60 ± 1.14^{a}	30.12 ± 1.56^{b}	27.21 ± 1.31^{d}
P-Value	**0.038**	**0.024**	**0.052**	**0.019**	**0.018**

Means having different superscripted letters along the columns are significantly different at $P<0.05$. Values are expressed as Means ± SD (Standard deviation) of triplicate determinations.

Table 4.7c: Proximate Composition (%) of "daddawa" Inoculated with Wild, Mutated and Standard *Bacillus subtilis* at pH 6

Organisms	Proximate parameters (mean±SD)				
	Moisture	Ash	Lipid	Protein	Carbohydrate
B. subtilis (wild)	16.43±1.02^{b}	0.85±0.22^{d}	23.57±1.90^{b}	32.46±0.65^{a}	26.69±1.25^{e}
B. subtilis (UV 30mins)	16.30±0.95^{b}	0.67±0.24^{e}	22.30±2.94^{c}	30.50±2.01^{c}	30.23±2.31^{b}
B. subtilis (UV 40mins)	17.02±0.96^{a}	1.43±0.81^{a}	23.81±1.71^{b}	31.26±3.00^{b}	36.48±3.10^{a}
B. subtilis (Nitrous acid)	16.55±1.00^{b}	1.12±0.94^{b}	24.81±0.10^{a}	30.44±2.00^{c}	27.08±1.19^{d}
B. subtilis (Standard strain) (ABC35615)	16.20±1.20^{b}	0.88±0.39^{c}	21.23±0.63^{c}	32.06±1.93^{a}	29.63±1.11^{c}
P-Value	**0.02**	**0.010**	**0.025**	**0.020**	**0.032**

Means having different superscripted letters along the columns are significantly different at $P<0.05$. Values are expressed as Means ± SD (Standard deviation) of triplicate determinations.

Table 4.7d: Proximate Composition (%) of "daddawa" Inoculated with Wild, Mutated and Standard *Bacillus subtilis* at pH 7

Organisms	Proximate parameters (Mean±SD)				
	Moisture	Ash	Lipid	Protein	Carbohydrate
B. subtilis (wild strain)	17.02 ± 1.14^{a}	0.97 ± 0.00^{e}	23.11 ± 2.21^{a}	29.61 ± 1.03^{d}	29.29 ± 2.14^{a}
B. subtilis (UV 30mins)	16.47 ± 1.29^{b}	1.10 ± 0.35^{d}	22.63 ± 2.01^{b}	30.70 ± 3.36^{c}	29.10 ± 1.31^{a}
B. subtilis (UV 40mins)	16.82 ± 0.93^{b}	1.61 ± 0.46^{a}	23.61 ± 1.78^{a}	31.4 ± 0.53^{b}	26.56 ± 0.79^{c}
B. subtilis (Nitrous acid)	15.42 ± 0.26^{c}	1.43 ± 0.54^{b}	23.32 ± 1.56^{a}	32.01 ± 1.98^{a}	27.82 ± 1.52^{b}
B. subtilis (Standard strain) (ABC35615)	16.79 ± 1.12^{b}	1.26 ± 0.39^{c}	23.79 ± 1.83^{a}	31.79 ± 2.59^{b}	26.37 ± 0.68^{c}
P-Value	**0.010**	**0.040**	**0.017**	**0.050**	**0.013**

Means having different superscripted letters along the columns are significantly different at $P<0.05$. Values are expressed as Means ± SD (Standard deviation) of triplicate determinations.

Table 4.7e: Proximate Composition (%) of "daddawa" Inoculated with Wild, Mutated and Standard *Bacillus subtilis* at pH 8

Organisms	Proximate Parameters (Mean±SD)				
	Moisture	Ash	Lipid	Protein	Carbohydrate
B. subtilis (wild strain)	16.25 ± 1.13^{b}	0.63 ± 0.54^{e}	23.45 ± 2.36^{b}	30.81 ± 1.88^{c}	28.86 ± 1.56^{b}
B. subtilis (UV 30mins)	16.94 ± 1.36^{b}	0.88 ± 0.17^{c}	24.30 ± 1.48^{a}	31.26 ± 2.14^{b}	26.62 ± 2.17^{d}
B. subtilis (UV 40mins)	17.05 ± 1.20^{a}	0.98 ± 0.98^{b}	22.39 ± 2.37^{c}	32.62 ± 3.11^{a}	26.96 ± 2.09^{d}
B. subtilis (Nitrous acid)	16.61 ± 0.10^{b}	1.17 ± 0.14^{a}	23.65 ± 0.95^{b}	31.16 ± 3.35^{b}	27.41 ± 3.13^{c}
B. subtilis (Standard strain) (ABC35615)	16.10 ± 0.57^{b}	0.78 ± 0.33^{d}	22.54 ± 0.93^{c}	30.21 ± 2.02^{c}	30.37 ± 2.41^{a}
P-Value	**0.049**	**0.025**	**0.036**	**0.030**	**0.033**

Means having different superscripted letters along the columns are significantly different at $P<0.05$. Values are expressed as Means ± SD (Standard deviation) of triplicate determinations.

Table 4.7a shows the results of the percentage proximate compositions of "daddawa" at normal pH (natural condition of the "daddawa" without pH adjustment). The Table reveals that the "daddawa" inoculated with *B. subtilis* (wild strain) had the highest moisture content of 16.86% while *B. subtilis* (mutated at UV light for 40minutes) had the lowest moisture content of 15.60%. The highest (0.77%) and lowest (0.50%) ash content were observed in "daddawa" inoculated with *B. subtilis* (standard strain) and *B. subtilis* (mutated with nitric acid) respectively. "daddawa" sample inoculated with *B. subtilis* (mutated at UV light for 40mins) had the highest lipid content of 24.30% while the "daddawa" inoculated with *B. subtilis* (Wild) had the lowest lipid content of 21.47%. The highest (37.43%) and lowest (28.85%) protein content were observed in *B. subtilis* (mutated at UV light for 40minutes) and *B. subtilis* (mutated at UV light for 30minutes) inoculated "daddawa" respectively. The highest (29.83%) and lowest (21.92%) carbohydrate contents were recorded by *B. subtilis* (wild strain) and *B. subtilis* (UV 40mins) inoculated "daddawa" respectively. However, the various strains of the *Bacillus subtilis* revealed significant difference ($P<0.05$) in the percentage compositions of the proximate parameters at varying pH.

The results in Table 4.7b shows that the percentage proximate composition of fermented "daddawa" inoculated with various strains *Bacillus subtilis* at pH 5. The highest (17.23%) and lowest (17.23%) moisture contents were recorded by *B. subtilis* (Standard strain) and *B. subtilis* (Wild) inoculated "daddawa" respectively. The highest (0.84%) and lowest (0.65%) ash contents were recorded by *B. subtilis* (Standard strain) and *B. subtilis* (UV 40mins) inoculated "daddawa" respectively. While the highest (24.60%) and lowest (22.40%) lipid contents were given by *B. subtilis* (Standard strain) and *B. subtilis* (UV 40mins) inoculated "daddawa" respectively, the highest (33.36%) and lowest (29.55%) were given by *B. subtilis*

(Wild strain) and *B. subtilis* (mutated at UV 30mins). The highest (47.28%) and lowest (27.21%) carbohydrate contents were recorded by *B. subtilis* (wild strain) and *B. subtilis* (Standard strain) inoculated “daddawa” respectively.

Percentage proximate analysis of “daddawa” at pH 6 is shown in Table 4.7c. The Table reveals that the highest (17.02%) and lowest (16.20%) moisture contents was recorded by *B. subtilis* (mutated at UV 40mins) and *B. subtilis* (standard strain) respectively; the highest (1.43%) and lowest (0.67%) ash contents were recorded *B. subtilis* (mutated at UV 40mins) and *B. subtilis* (mutated at UV 30mins) respectively; highest (24.81%) and lowest (21.23%) of lipid contents were given by *B. subtilis* (mutated with Nitric acid) and *B. subtilis* (standard strain) respectively; the highest (32.46%) protein content was recorded by *B. subtilis* (wild strain) while the lowest (21.26%) was recorded by *B. subtilis* (mutated at UV 40mins). The highest (36.48%) and lowest (26.69%) carbohydrate contents were recorded by *B. subtilis* (UV 40mins) and *B. subtilis* (wild) inoculated “daddawa” respectively. However, the various strains of the *Bacillus subtilis* revealed significant difference ($P<0.05$) in the percentage compositions of the proximate parameters.

Table 4.7d shows the results of the percentage proximate compositions of “daddawa” at pH 7. *B. subtilis* (wild strain) inoculated “daddawa” had the highest moisture content of 17.02% while *B. subtilis* (mutated with nitrous acid) inoculated “daddawa” gave the lowest moisture content of 15.42%. The highest (1.61%) and lowest (0.97%) ash content were observed in “daddawa” inoculated with *B. subtilis* (mutated at UV 40mins) and *B. subtilis* (mutated with nitric acid) respectively. *B. subtilis* (Standard strain) gave the highest lipid content of 23.79% while the “daddawa” inoculated with *B. subtilis* (mutated UV 30mins) gave the lowest lipid content of 22.63%. The highest (32.01%) and lowest (29.61%) protein content were observed in *B. subtilis* (mutated with nitrous acid) and *B. subtilis* (wild strain) respectively. Highest (29.29%) and lowest

(26.37%) carbohydrate content were observed in "daddawa" inoculated with *B. subtilis* (wild) and *B. subtilis* (standard) respectively.

The percentage proximate compositions of "daddawa" at pH 8 is presented in Table 4.7e. The highest (17.05%) and lowest (16.10%) moisture contents were recorded by *B. subtilis* (mutated at UV 40mins) and *B. subtilis* (standard strain) respectively; the highest (1.17%) and lowest (0.63%) ash contents were recorded *B. subtilis* (mutated with nitrous acid) and *B. subtilis* (wild strain) respectively; highest (24.30%) and lowest (22.39%) of lipid contents were given by *B. subtilis* (mutated with nitrous acid) and *B. subtilis* (Standard strain) respectively; the highest (32.46%) protein content was recorded by *B. subtilis* (Wild strain) while the lowest (21.26%) was recorded by *B. subtilis* (mutated at UV 40mins). Highest (30.37%) and lowest (26.62%) carbohydrate content were observed in "daddawa" inoculated with *B. subtilis* (standard) and *B. subtilis* (mutated at UV 30mins) respectively. However, the various strains of the *Bacillus subtilis* revealed significant difference ($P<0.05$) in the percentage compositions of the proximate parameters.

4.4.4 Proximate Analysis of "ogiri" Inoculated with Wild, Mutated and Standard *Bacillus subtilis* at varying pH

The results of percentage proximate compositions of "ogiri" inoculated with various strains of *Bacillus subtilis* at varying pH are presented in Tables 4.8a to 4.8e).

Table 4.8a: Proximate Composition (%) of "ogiri" Inoculated with Wild, Mutated and Standard *Bacillus subtilis* at Normal pH (6.1)

Organisms	Proximate Parameters (Mean±SD)				
	Moisture	Ash	Lipid	Protein	Carbohydrate
B. subtilis (wild strain)	16.78±0.52	1.62±0.60^{a}	24.13±0.55^{a}	30.86±3.55	26.61±0.53^{c}
B. subtilis (UV 30mins)	16.79±1.07	1.42±0.39^{c}	22.72±2.03	30.33±0.65	28.74±1.31^{b}
B. subtilis (UV 40mins)	14.31±3.75	1.67±0.58^{a}	21.93±1.97	30.43±2.13	31.66±2.13^{a}
B. subtilis (Nitrous acid)	18.61±0.38	1.50±0.50^{b}	22.97±1.38	33.80±4.53	23.12±0.48^{e}
B. subtilis (Standard strain)	17.75±0.70	1.33±.0.57^{d}	22.38±1.42	33.25±0.66	25.29±0.19^{d}
P-Value	**0.034**	**0.023**	**0.030**	**0.025**	**0.029**

Means having different superscripted letters along the columns are significantly different at P<0.05. Values are expressed as Means ± SD (Standard deviation) of triplicate determinations.

Table 4.8b: Proximate Composition (%) of "ogiri" inoculated with Wild, Mutated and Standard *Bacillus subtilis* at pH 5

Organisms	Proximate Parameters (Mean±SD)				
	Moisture	Ash	Lipid	Protein	Carbohydrate
B. subtilis (wild strain)	17.85±2.43^{b}	2.41±0.68^{a}	22.31±2.65^{c}	30.78±3.05^{c}	26.63±2.01^{b}
B. subtilis (UV 30mins)	17.61±1.73^{b}	1.97±1.00^{c}	24.41±1.58^{a}	29.36±0.99^{d}	26.65±1.44^{b}
B. subtilis (UV 40mins)	18.63±1.10^{a}	2.12±0.91^{b}	22.48±1.42^{c}	31.90±2.66^{b}	24.87±0.35^{c}
B. subtilis (Nitrous acid)	15.51±0.49^{c}	1.03±0.24^{d}	23.45±2.51^{b}	30.19±0.61^{c}	29.82±0.96^{a}
B. subtilis (Standard strain)	17.67±1.39^{b}	1.08±0.81^{c}	22.49±0.92^{c}	32.52±1.83^{a}	26.24±1.78^{b}
P-Value	**0.026**	**0.031**	**0.010**	**0.017**	**0.011**

Means having different superscripted letters along the columns are significantly different at $P<0.05$. Values are expressed as Means ± SD (Standard deviation) of triplicate determinations

Table 4.8c: Proximate Composition (%) of "ogiri" inoculated with Wild, Mutated and Standard *Bacillus subtilis* at pH 6

Organisms	Proximate Parameters (Mean±SD)				
	Moisture	Ash	Lipid	Protein	Carbohydrate
B. subtilis	15.93 ± 0.26^{c}	0.91 ± 0.40^{e}	23.59 ± 1.96^{b}	29.39 ± 2.45^{b}	30.18 ± 2.63^{a}
B. subtilis (UV 30mins)	16.62 ± 0.52^{b}	1.99 ± 1.03^{b}	23.77 ± 1.77^{b}	29.86 ± 3.00^{b}	27.76 ± 1.39^{d}
B. subtilis (UV 40mins)	17.68 ± 2.50^{a}	1.81 ± 0.94^{d}	24.38 ± 3.00^{a}	30.05 ± 3.75^{a}	26.08 ± 0.74^{e}
B. subtilis (Nitrous acid)	17.47 ± 0.98^{a}	2.48 ± 0.60^{a}	21.49 ± 1.32^{c}	29.14 ± 2.00^{b}	29.42 ± 0.41^{b}
B. subtilis (Standard strain)	16.62 ± 1.73^{b}	1.76 ± 1.07^{c}	21.86 ± 1.00^{c}	30.81 ± 2.20^{a}	28.95 ± 0.67^{c}
P-Value	**0.03**	**0.37**	**0.41**	**0.15**	**0.34**

Means having different superscripted letters along the columns are significantly different at $P<0.05$. Values are expressed as Means ± SD (Standard deviation) of triplicate determinations.

Table 4.8d: Proximate Composition (%) of "ogiri" inoculated with Wild, Mutated and Standard *Bacillus subtilis* at pH 7

Organisms	Proximate Parameters (Mean±SD)				
	Moisture	Ash	Lipid	Protein	Carbohydrate
B. subtilis	18.51±1.25[a]	1.59±0.55[a]	21.71±2.10[d]	30.21±1.85[b]	27.98±3.10[b]
B. subtilis (UV 30mins)	17.35±2.00[b]	1.45±4.12[b]	22.29±2.80[c]	30.68±1.53[b]	28.23±0.84[a]
B. subtilis (UV 40mins)	17.31±1.88[b]	1.45±0.36[b]	23.92±1.79[b]	30.64±1.63[b]	26.68±0.60[c]
B. subtilis (Nitrous acid)	15.78±0.46[d]	0.75±0.50[d]	24.77±0.48[a]	31.35±2.94[a]	27.35±1.67[b]
B. subtilis (Standard strain)	16.80±0.53[c]	0.95±0.47[c]	23.06±1.85[b]	30.77±2.34[b]	28.42±0.92[a]
P-Value	**0.031**	**0.017**	**0.022**	**0.010**	**0.029**

Means having different superscripted letters along the columns are significantly different at $P<0.05$. Values are expressed as Means ± SD (Standard deviation) of triplicate determinations.

Table 4.8e: Percentage (%) Proximate Composition of "ogiri" inoculated with Wild, Mutated and Standard *Bacillus subtilis* at pH 8

Organisms	**Proximate Parameters (Mean±SD)**				
	Moisture	**Ash**	**Lipid**	**Protein**	**Carbohydrate**
B. subtilis	16.89±1.40[c]	1.30±0.26[d]	21.70±1.40[c]	29.99±2.41[d]	30.12±0.88[a]
B. subtilis (UV 30mins)	17.73±2.33[b]	2.11±1.46[a]	23.83±3.02[a]	30.89±2.09[c]	25.44±0.13[d]
B. subtilis (UV 40mins)	16.49±0.75[c]	2.02±0.24[b]	20.97±0.89[d]	31.53±3.08[b]	28.99±0.47[c]
B. subtilis (Nitrous acid)	18.29±1.96[a]	1.78±0.39[c]	21.75±1.52[c]	32.83±2.45[a]	25.35±0.61[d]
B. subtilis (Standard strain)	16.13±1.28[d]	2.05±0.64[b]	22.82±2.14[b]	29.31±0.36[d]	29.61±0.23[b]
P-Value	**0.32**	**0.25**	**0.31**	**0.26**	**0.29**

Means having different superscripted letters along the columns are significantly different at $P<0.05$. Values are expressed as Means ± SD (Standard deviation) of triplicate determinations.

Table 4.8a shows the result of the percentage proximate compositions of “ogiri” at normal pH.(6.1) *B. subtilis* (wild strain) inoculated “ogiri” had the highest moisture content of 17.02% while *B. subtilis* (mutated with nitrous acid) inoculated “ogiri” gave the lowest moisture content of 15.42%. The highest (1.61%) and lowest (0.97%) ash content were observed in *B. subtilis* (mutated at UV 40mins) and *B. subtilis* (mutated with nitrous acid) respectively. *B. subtilis* (Standard strain) had the highest lipid content of 23.79% while the “ogiri” inoculated with *B. subtilis* (mutated at UV 30mins) had the lowest lipid content of 22.63%. The highest (32.01%) and lowest (29.61%) protein content were observed in *B. subtilis* (mutated with nitric acid) and *B. subtilis* (wild strain) respectively. Highest (29.29%) and lowest (26.37%) carbohydrate content were observed in “ogiri” inoculated with *B. subtilis* (wild) and *B. subtilis* (standard) respectively.

Table 4.8b shows the result of the percentage proximate compositions of “ogiri” at pH 5. *B. subtilis* (UV 40mins) inoculated “ogiri” had the highest moisture content of 18.63% while *B. subtilis* (mutated with nitrous acid) inoculated “ogiri” gave the lowest moisture content of 15.51%. The highest (2.41%) and lowest (1.03%) ash content were observed in “ogiri” inoculated with *B. subtilis* (wild strain) and *B. subtilis* (mutated with nitric acid) respectively. “ogiri” sample inoculated with *B. subtilis* (UV 40mins) gave the highest lipid content of 24.41% while the “ogiri” inoculated with *B. subtilis* (wild strain) gave the lowest lipid content of 22.31%. The highest (32.52%) and lowest (29.36%) protein content were observed in *B. subtilis* (standard strain) and *B. subtilis* (UV 40mins) inoculated “ogiri” respectively. Highest (29.82%) and lowest (24.87%) carbohydrate content were observed in “ogiri” inoculated with *B. subtilis* (mutated with nitrous acid) and *B. subtilis* (UV 40mins) respectively. However, the various strains of the

Bacillus subtilis revealed significant difference ($P<0.05$) in the percentage compositions of the proximate parameters at varying pH

The percentage proximate compositions of "ogiri" at pH 6 is presented in Table 4.8c. The table shows that the highest (17.68%) and lowest (15.93%) moisture contents were recorded by *B. subtilis* (mutated at UV 40mins) and *B. subtilis* (standard strain) respectively; the highest (2.48%) and lowest (1.81%) ash contents were recorded *B. subtilis* (mutated with nitrous acid) and *B. subtilis* (UV 40mins) respectively; highest (24.38%) and lowest (21.49%) of lipid contents were given by *B. subtilis* (UV 40mins) and *B. subtilis* (mutated with nitrous acid) respectively; the highest (30.81%) protein content was recorded by *B. subtilis* (standard strain) while the lowest (29.14%) was recorded by *B. subtilis* (mutated with nitrous acid). Highest (30.18%) and lowest (26.08%) carbohydrate content were observed *B. subtilis* (wild) and *B. subtilis* (mutated at UV 40mins) respectively.

Table 4.8d presents the percentage proximate compositions of "ogiri" at pH 7. The highest (18.51%) and lowest (15.78%) moisture contents were recorded by *B. subtilis* (wild) and *B. subtilis* (nitrous acid) respectively; the highest (1.59%) and lowest (0.75%) ash contents were recorded *B. subtilis* (wild) and *B. subtilis* (nitrous acid) respectively; highest (24.77%) and lowest (21.71%) of lipid contents were given by *B. subtilis* (mutated with nitrous acid) and *B. subtilis* (wild strain) respectively; the highest (31.35%) protein content was recorded by *B. subtilis* (nitrous acid) while the lowest (30.21%) was recorded by *B. subtilis* (wild strain). Highest (28.42%) and lowest (26.68%) carbohydrate content were observed in "ogiri" inoculated with *B. subtilis* (standard strain) and *B. subtilis* (mutated at UV 40mins) respectively. However, the various strains of the *Bacillus subtilis* revealed significant difference (P<0.05) in the percentage compositions of the proximate parameters.

Table 4.8e presents the percentage proximate compositions of "ogiri" at pH 8. The table reveals that the highest (18.29%) and lowest (16.89%) moisture contents were recorded by *B. subtilis* (nitrous acid) and *B. subtilis* (wild) respectively; the highest (2.11%) and lowest (1.30%) ash contents were recorded *B. subtilis* (UV 30mins) and *B. subtilis* (wild) respectively; highest (23.83%) and lowest (20.97%) of lipid contents were given by *B. subtilis* (UV 30mins) and *B. subtilis* (UV 40mins) respectively; the highest (32.83%) protein content was recorded by *B. subtilis* (nitrous acid) while the lowest (29.31%) was recorded by *B. subtilis* (standard strain). Highest (30.12%) and lowest (25.35%) carbohydrate content were observed in *B. subtilis* (wild strain) and *B. subtilis* (nitrous acid) respectively. However, the various strains of the *Bacillus subtilis* revealed significant difference (P<0.05) in the percentage compositions of the proximate parameters

4.4.5 Proximate Analysis of "daddawa" Inoculated with Wild, Mutated and Standard *Bacillus subtilis* at varying Temperature

The results of percentage proximate compositions of "ogiri" inoculated with various strains of *Bacillus subtilis* at varying pH are presented in Tables 4.9a to 4.9e).

Table 4.9a: Proximate Composition (%) of "daddawa" Inoculated with Wild, Mutated and Standard *Bacillus subtilis* at 25^{o}C

Organisms	Proximate Parameters (Mean±SD)				
	Moisture	Ash	Lipid	Protein	Carbohydrate
B. subtilis	16.05±1.09^{a}	0.49±0.10^{e}	21.90±2.02^{c}	31.09±1.99^{b}	31.37±1.07^{a}
B. subtilis (UV 30mins)	16.66±0.93^{a}	0.98±0.28^{c}	23.66±4.30^{a}	29.07±0.76^{c}	29.63±3.03^{b}
B. subtilis (UV 40mins)	16.69±1.04^{a}	0.87±0.33^{d}	23.95±1.90^{a}	32.93±2.11^{a}	25.56±1.72^{e}
B. subtilis (Nitrous acid)	15.83±0.87^{b}	1.61±0.51^{a}	23.20±1.24^{a}	31.59±3.06^{b}	27.91±0.08^{c}
B. subtilis (Standard strain)	16.88±1.07^{a}	1.02±0.64^{b}	22.85±1.00^{b}	32.39±1.84^{a}	26.86±2.22^{d}
P-Value	**0.52**	**0.02**	**0.40**	**0.02**	**0.05**

Means having different superscripted letters along the columns are significantly different at $P<0.05$. Values are expressed as Means ± SD (Standard deviation) of triplicate determinations.

Table 4.9b: Proximate Composition (%) of "daddawa" Inoculated with Wild, Mutated and Standard *Bacillus subtilis* at 35^{o}C

Organisms	Proximate Parameters (Mean±SD)				
	Moisture	Ash	Lipid	Protein	Carbohydrate
B. subtilis (wild)	17.50±0.40^{a}	0.80±0.26^{b}	23.41±2.47^{b}	32.10±2.28^{a}	26.19±0.16^{c}
B. subtilis (UV 30mins)	16.30±1.07^{b}	0.70±0.26^{c}	23.09±1.53^{b}	31.81±2.66^{b}	28.10±0.07^{b}
B. subtilis (UV 40mins)	16.61±1.09^{b}	0.74±0.32^{d}	22.98±1.90^{c}	30.62±2.78^{c}	29.05±0.24^{a}
B. subtilis (Nitrous acid)	16.15±0.71^{b}	0.66±0.43^{e}	24.17±1.28^{a}	30.07±1.53^{c}	28.95±1.11^{b}
B. subtilis (Standard strain)	16.78±0.87^{b}	0.84±0.30^{a}	22.86±2.82^{c}	30.34±2.58^{c}	29.18±0.81^{a}
P-Value	**0.025**	**0.05**	**0.048**	**0.053**	**0.051**

Means having different superscripted letters along the columns are significantly different at P<0.05. Values are expressed as Means ± SD (Standard deviation) of triplicate determinations.

Table 4.9c: Proximate Composition (%) of "daddawa" Inoculated with Wild, Mutated and Standard *Bacillus subtilis* at 45^oC

Organisms	Proximate Parameters (Mean±SD)				
	Moisture	Ash	Lipid	Protein	Carbohydrate
B. subtilis (wild)	16.25 ± 0.99^b	0.81 ± 0.24^d	24.23 ± 1.00^b	32.60 ± 2.15^a	26.70 ± 0.85^b
B. subtilis (UV 30mins)	17.08 ± 0.52^a	0.65 ± 0.17^e	23.48 ± 2.43^c	29.63 ± 1.02^c	29.16 ± 0.64^a
B. subtilis (UV 40mins)	16.64 ± 1.01^b	1.27 ± 0.66^a	22.98 ± 1.88^d	29.82 ± 0.96^c	29.29 ± 0.43^a
B. subtilis (Nitrous acid)	16.41 ± 0.94^b	1.14 ± 0.66^b	23.67 ± 0.75^c	31.80 ± 2.72^b	24.18 ± 0.71^c
B. subtilis (Standard strain)	16.21 ± 0.95^b	0.97 ± 0.28^c	25.84 ± 5.78^a	32.01 ± 2.23^a	24.38 ± 1.01^c
P-Value	**0.013**	**0.014**	**0.016**	**0.014**	**0.054**

Means having different superscripted letters along the columns are significantly different at $P<0.05$. Values are expressed as Means ± SD (Standard deviation) of triplicate determinations.

Table 4.9a shows the percentage proximate compositions of “daddawa” at 25^{o}C. The Table shows that the highest (16.69%) and lowest (15.83%) moisture contents were recorded by *B. subtilis* (UV 40mins) and *B. subtilis* (nitrous acid) respectively; the highest (1.61%) and lowest (0.49%) ash content was recorded *B. subtilis* (nitrous acid) and *B. subtilis* (wild) respectively; highest (23.95%) and lowest (21.90%) lipid contents were recorded by *B. subtilis* (UV 40mins) and *B. subtilis* (wild strain) respectively; similarly the highest (32.93%) protein content was recorded by *B. subtilis* (UV 40mins) while the lowest (29.07%) was recorded by *B. subtilis* (UV 30mins). In addition the highest (31.37%) and lowest (25.56%) carbohydrate content were observed in “daddawa” inoculated with *B. subtilis* (wild strain) and *B. subtilis* (mutated at UV 40mins) respectively

Table 4.9b also depicts the percentage proximate compositions of “daddawa” at 35^{o}C. The Table shows that the highest (17.50%) and lowest (16.15%) moisture contents were recorded by *B. subtilis* (wild strain) and *B. subtilis* (mutated with nitrous acid) respectively; the highest (0.84%) and lowest (0.66%) ash contents were recorded by *B. subtilis* (standard strain) and *B. subtilis* (nitrous acid) respectively; highest (24.17%) and lowest (22.86%) of lipid contents were given by *B. subtilis* (nitrous acid) and *B. subtilis* (standard strain) respectively; the highest (32.10%) protein content was recorded by *B. subtilis* (wild strain) while the lowest (30.07%) was recorded by *B. subtilis* (nitrous acid). Highest (29.18%) and lowest (26.19%) carbohydrate content were observed in “daddawa” inoculated with *B. subtilis* (standard strain) and *B. subtilis* (wild strain) respectively.

The percentage proximate compositions of “daddawa” at 45^{o}C is also illustrated in Table 4.9c. The table shows that the highest (17.08%) and lowest (16.21%) moisture contents were recorded by *B. subtilis* (UV 30mins) and *B. subtilis* (standard) respectively; the highest (1.27%) and lowest (0.65%) ash contents were recorded *B. subtilis* (UV 40mins) and *B. subtilis* (UV 30mins) respectively; highest (25.84%) and lowest (22.98%) of lipid contents were given by *B. subtilis* (standard) and *B. subtilis* (UV 40mins) respectively; the highest (32.60%) protein content was recorded by *B. subtilis* (standard strain) while the lowest (29.63%) was recorded by *B. subtilis* (UV 30mins). Highest (29.29%) and lowest (24.18%) carbohydrate content were observed in "daddawa" inoculated with *B. subtilis* (UV 40mins) and *B. subtilis* (mutated with nitrous acid) respectively. However, the various strains of the *Bacillus subtilis* revealed significant difference ($P<0.05$) in the percentage compositions of the proximate parameters

4.4.6 Proximate Analysis of "ogiri" Inoculated with Wild, Mutated and Standard *Bacillus subtilis* at varying Temperature

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The results of percentage proximate compositions of "ogiri" inoculated with various strains of *Bacillus subtilis* at varying pH are presented in Tables 4.10a to 4.10c).

Table 4.10a: Proximate Composition (%) of "ogiri" Inoculated with Wild, Mutated and Standard *Bacillus subtilis* at 25°C

Organisms	Proximate Parameters (Mean±SD)				
	Moisture	Ash	Lipid	Protein	Carbohydrate
B. subtilis	16.34±1.59^{b}	1.40±0.69^{e}	22.51±1.26^{b}	30.02±2.41^{a}	29.73±2.11^{a}
B. subtilis (UV 30mins)	17.60±0.94^{a}	1.94±0.63^{a}	21.43±1.13^{c}	29.79±0.95^{b}	29.24±1.53^{a}
B. subtilis (UV 40mins)	15.92±3.62^{c}	1.76±0.59^{b}	23.88±2.36^{a}	30.91±2.27^{a}	27.53±2.91^{c}
B. subtilis (Nitrous acid)	16.67±1.36^{b}	1.44±0.74^{d}	23.32±2.22^{a}	29.40±1.62^{b}	29.17±1.88^{a}
B. subtilis (Standard strain)	16.49±1.34^{b}	1.48±0.75^{c}	22.61±1.43^{b}	30.44±1.54^{a}	28.98±1.95^{b}
P-Value	**0.049**	**0.053**	**0.015**	**0.020**	**0.018**

Means having different superscripted letters along the columns are significantly different at P<0.05. Values are expressed as Means ± SD (Standard deviation) of triplicate determinations

Table 4.10b: Proximate Composition (%) of "ogiri" Inoculated with Wild, Mutated and Standard *Bacillus subtilis* at 35°C

Organisms	Proximate Parameters (Mean±SD)				
	Moisture	Ash	Lipid	Protein	Carbohydrate
B. subtilis	17.89±1.52[a]	1.62±0.30[b]	22.03±2.29[b]	31.20±3.14[a]	27.26±3.01[c]
B. subtilis (UV 30mins)	16.58±1.21[b]	1.35±0.41[c]	23.34±2.06[a]	29.32±1.22[c]	29.41±2.98[a]
B. subtilis (UV 40mins)	17.35±1.62[a]	1.61±0.42[b]	22.20±1.90[b]	30.84±2.42[b]	28.00±3.11[b]
B. subtilis (Nitrous acid)	17.29±1.99[a]	1.68±0.53[a]	22.68±1.96[b]	31.26±2.83[a]	27.09±1.97[c]
B. subtilis (Standard strain)	17.00±0.89[a]	1.19±0.57[d]	23.09±1.59[a]	30.61±1.85[b]	28.11±2.97[b]
P-Value	**0.052**	**0.045**	**0.053**	**0.050**	**0.054**

Means having different superscripted letters along the columns are significantly different at $P<0.05$. Values are expressed as Means ± SD (Standard deviation) of triplicate determinations

Table 4.10c: Proximate Composition (%) of "ogiri" Inoculated with Wild, Mutated and Standard *Bacillus subtilis* at 45^oC

Organisms	Proximate Parameters (Mean±SD)				
	Moisture	Ash	Lipid	Protein	Carbohydrate
B. subtilis	17.43 ± 1.22^b	1.67 ± 0.99^b	23.52 ± 2.04^b	29.51 ± 1.45^d	27.87 ± 2.07^a
B. subtilis (UV 30mins)	18.46 ± 1.85^a	2.07 ± 1.29^a	25.44 ± 0.24^a	31.76 ± 1.63^b	22.27 ± 2.64^c
B. subtilis (UV 40mins)	17.37 ± 1.61^b	2.05 ± 0.83^a	22.14 ± 1.94^c	30.97 ± 3.11^c	27.47 ± 1.99^a
B. subtilis (Nitrous acid)	18.14 ± 1.24^a	1.41 ± 1.00^c	22.65 ± 1.77^c	31.72 ± 2.60^b	26.08 ± 0.02^b
B. subtilis (Standard strain)	17.17 ± 1.69^b	1.63 ± 1.00^b	21.87 ± 1.03^d	32.95 ± 1.93^a	26.38 ± 0.30^b
P-Value	**0.053**	**0.036**	**0.020**	**0.028**	**0.031**

Means having different superscripted letters along the columns are significantly different at $P<0.05$. Values are expressed as Means ± SD (Standard deviation) of triplicate determinations

Table 4.10a depict the percentage proximate compositions of “ogiri” at 25^{o}C. The Table shows that the highest (17.60%) and lowest (16.34%) moisture contents were recorded by *B. subtilis* (UV 40mins) and *B. subtilis* (wild strain) respectively. The highest (1.94%) and lowest (1.40%) ash contents were also recorded by *B. subtilis* (UV 30mins) and *B. subtilis* (wild strain) respectively; similarly the highest (23.88%) and lowest (21.43%) lipid contents were given by *B. subtilis* (UV 40mins) and *B. subtilis* (UV 30mins) respectively; and the highest (30.91%) protein content was recorded by *B. subtilis* (UV 30mins) while the lowest (29.40%) was recorded by *B. subtilis* (nitrous acid). The highest (29.73%) and lowest (27.53%) carbohydrate content were also observed in “ogiri” inoculated with *B. subtilis* (wild strain) and *B. subtilis* (mutated at UV 40mins) respectively.

The percentage proximate compositions of “ogiri” at 35^{o}C is shown in Table 4.10b. The Table depict that the highest (17.89%) and lowest (16.58%) moisture contents were recorded by *B. subtilis* (wild strain) and *B. subtilis* (UV 30mins) respectively; similarly the highest (1.68%) and lowest (1.19%) ash contents were recorded *B. subtilis* (nitrous acid) and *B. subtilis* (standard strain) respectively; highest (23.34%) and lowest (22.03%) lipid contents were given by *B. subtilis* (UV 30mins) and *B. subtilis* (wild strain) respectively; and the highest (31.26%) protein content was recorded by *B. subtilis* (nitrous acid) while the lowest (29.32%) was recorded by *B. subtilis* (UV 30mins). The highest (29.41%) and lowest (27.09%) carbohydrate content were also observed in “ogiri” inoculated with *B. subtilis* (UV 30mins) and *B. subtilis* (mutated nitrous acid) respectively. However, the various strains of the *Bacillus subtilis* revealed significant difference ($P<0.05$) in the percentage compositions of the proximate parameters

Table 4.10c depicts the percentage proximate compositions of “ogiri” at 45°C. The table shows that the highest (18.46%) and lowest (17.37%) moisture contents were recorded by *B. subtilis* (UV 30mins) and *B. subtilis* (UV 40mins) respectively; the highest (2.07%) and lowest (1.41%) ash contents were recorded *B. subtilis* (UV 30mins) and *B. subtilis* (nitrous acid) respectively; highest (25.44%) and lowest (21.87%) of lipid contents were given by *B. subtilis* (UV 30mins) and *B. subtilis* (standard strain) respectively; the highest (32.95%) protein content was recorded by *B. subtilis* (standard strain) while the lowest (29.51%) was recorded by *B. subtilis* (wild strain). Highest (27.87%) and lowest (22.27%) carbohydrate content were observed in “ogiri” inoculated with *B. subtilis* (wild strain) and *B. subtilis* (mutated at UV 30mins) respectively. However, the various strains of the *Bacillus subtilis* revealed significant difference ($P<0.05$) in the percentage compositions of the proximate parameters

4.5 Optimization of Processing Conditions

Two way Analysis of variance (ANOVA) was carried out to know the best and optimized pH and temperature in terms of the nutritive components of the condiments (“daddawa” and “ogiri”). The detailed results are described and presented below.

4.5.1 Optimization of Processing Conditions of “daddawa”

The results of percentage proximate compositions of “daddawa” inoculated with various strains of *Bacillus subtilis* at varying pH are presented in Tables 4.11a to 4.11c).

Table 4.11a: Comparative analysis of pH with Proximate Compositions of Fermented "daddawa"

Proximate Parameters	pH (Mean±SD)					P-Value
	Normal pH(5.4)	pH 5	pH 6	pH 7	pH 8	
Moisture	16.48 ± 0.50^a	16.20 ± 0.08^b	16.28 ± 1.23^b	16.59 ± 1.24^a	16.56 ± 1.19^a	0.036
Ash	0.49 ± 0.10^c	0.98 ± 0.28^a	0.98 ± 0.33^a	0.87 ± 0.51^b	1.02 ± 0.64^a	0.027
Lipid	23.69 ± 2.00^a	23.28 ± 1.21^a	22.55 ± 2.14^b	23.88 ± 1.41^a	22.16 ± 1.74^b	0.040
Protein	32.68 ± 2.86^a	29.92 ± 1.70^c	32.69 ± 1.83^a	31.20 ± 1.20^b	30.59 ± 1.93^b	0.051
Carbohydrate	26.66 ± 2.11^c	29.62 ± 0.08^b	37.50 ± 1.03^a	27.46 ± 0.32^c	29.67 ± 1.11^b	0.058

Means having different superscripted letters across rows are significantly different at $P<0.05$. Values are expressed as Means ± SD (Standard deviation) of triplicate determinations.

Table 4.11b: Comparative Analysis of Temperature (^{o}C) with Proximate Compositions of Fermented "daddawa"

Proximate Parameters	Temperature (Mean±SD)			P-Value
	25	35	45	
Moisture	16.27 ± 0.72^{a}	16.48 ± 1.05^{a}	16.84 ± 1.00^{a}	0.048
Ash	0.49 ± 0.10^{c}	0.67 ± 0.24^{b}	0.75 ± 0.40^{a}	0.036
Lipid	23.69 ± 2.00^{a}	23.23 ± 2.34^{b}	22.76 ± 1.47^{c}	0.042
Protein	32.68 ± 2.86^{b}	31.14 ± 2.55^{c}	35.01 ± 2.24^{a}	0.031
Carbohydrate	26.87 ± 0.91^{b}	28.48 ± 1.88^{a}	24.64 ± 0.23^{c}	0.028

Means having different superscripted letters across rows are significantly different at $P<0.05$. Values are expressed as Means ± SD (Standard deviation) of triplicate determinations.

Tables 4.11a indicates the comparative analysis of pH of fermented “daddawa” with its proximate parameters. The Table revealed that the highest (16.59%) and lowest (16.20%) moisture contents were observed at pH 7 and pH 5 respectively. The highest (1.02%) and lowest (0.49%) ash contents were observed at pH 8 and normal pH. The highest (23.88%) lipid content was observed at pH 7 while the lowest (22.16%) was observed at pH 8. The highest (32.69%) and lowest (29.92%) protein contents were observed at pH 6 and pH 5 respectively. Moreover, the highest (37.5%) and lowest (26.66%) carbohydrate contents were observed at pH 6 and normal pH respectively. pH 6 had the highest values of percentage proximate compositions, hence, the optimized temperature. However, the various proximate compositions were significantly difference ($P<0.05$) at the varying pH.

The result in Table 4.11b depicts the comparative analysis of temperature of fermented “daddawa” with its proximate parameters. In this table, the highest (16.85%) and lowest (16.27%) moisture contents were noted at temperatures of 45^{o}C and 25^{o}C respectively. The highest (0.75%) and lowest (0.49%) ash contents were noted at temperatures of 45^{o}C and 25^{o}C respectively. Highest (35.01%) and lowest (31.14%) protein contents were noted at temperatures of 45^{o}C and 35^{o}C respectively. Highest (28.48%) and lowest (24.64%) carbohydrate contents were noted at temperatures of 35^{o}C and 45^{o}C respectively. The Temperature of 45^{o}C had the highest values of percentage proximate compositions, hence, the optimized temperature. However, the various proximate compositions were significantly difference ($P<0.05$) in the varying temperature

4.5.2 Optimization of Processing Conditions of “ogiri”

The results of percentage proximate compositions of “ogiri” inoculated with various strains of *Bacillus subtilis* at varying pH are presented in Tables 4.12a to 4.12b.

Table 4.12a: Comparative Analysis of pH with Proximate Compositions of Fermented "ogiri"

Proximate Parameters	pH (Mean±SD)					P-Value
	Normal pH	pH 5	pH 6	pH 7	pH 8	
Moisture	16.34±1.73[b]	16.85±1.59[b]	15.67±0.60[c]	17.94±1.15[a]	16.22±1.13[b]	0.048
Ash	1.72±0.56[b]	1.52±0.74[c]	1.71±0.95[b]	1.18±0.49[d]	1.88±0.49[a]	0.036
Lipid	23.32±1.44[a]	23.12±1.22[a]	22.52±2.52[b]	23.68±2.03[a]	21.10±0.76[c]	0.044
Protein	30.11±1.76[b]	31.77±2.18[a]	28.27±0.77[d]	30.65±1.14[b]	29.76±0.81[c]	0.039
Carbohydrate	28.51±2.01[b]	26.74±2.07[c]	31.83±0.89[a]	26.55±1.11[c]	31.04±1.34[a]	0.053

Means having different superscripted letters across rows are significantly different at $P<0.05$. Values are expressed as Means ± SD (Standard deviation) of triplicate determinations.

Table 4.12b: Comparative Analysis of Temperature (^oC) with Proximate Compositions of Fermented "ogiri"

Proximate Parameters	Temperature (Mean±SD)			P-Value
	25	35	45	
Moisture	16.87 ± 1.18^b	17.34 ± 0.93^a	16.34 ± 3.61^c	0.051
Ash	1.17 ± 0.41^c	1.63 ± 0.26^b	1.72 ± 0.56^a	0.029
Lipid	22.60 ± 1.81^b	22.55 ± 1.74^c	23.32 ± 1.44^a	0.054
Protein	30.96 ± 2.94^c	32.13 ± 2.28^b	32.11 ± 3.54^a	0.039
Carbohydrate	38.40 ± 0.54^a	26.35 ± 1.34^c	26.51 ± 2.85^b	0.050

Means having different superscripted letters across rows are significantly different at $P<0.05$. Values are expressed as Means ± SD (Standard deviation) of triplicate determinations.

Tables 4.12a shows the comparative analysis of pH of fermented “ogiri” with its proximate parameters. The Table revealed that the highest (17.94%) and lowest (16.22%) moisture contents were observed at pH 7 and pH 8 respectively. Highest (1.88%) and lowest (1.18%) ash contents were observed at pH 8 and normal pH. The highest (23.68%) lipid content was observed at pH 7 while the lowest (21.10%) was observed at pH 8. The highest (31.77%) and lowest (28.27%) protein contents were observed at pH 5 and pH 6 respectively. Furthermore, the highest (31.83%) and lowest (26.55%) carbohydrate contents were observed at pH 6 and pH 7 respectively. pH 6 had the highest values of percentage proximate compositions, hence, the optimized temperature. However, the various proximate compositions were significantly difference ($P<0.05$) at the varying pH.

The result in Table 4.12b depicts the comparative analysis of temperature of fermented “ogiri” with its proximate parameters. The table reveals that the highest (17.34%) and lowest (16.34%) moisture contents were observed at temperatures of 35^oC and 45^oC respectively. The highest (1.72%) and lowest (1.17%) ash contents were noted at temperatures of 45^oC and 25^oC respectively. Highest (23.32%) and lowest (22.55%) lipid contents were noted at temperatures of 45^oC and 35^oC respectively. Highest (32.13%) and lowest (30.13%) protein contents were noted at temperatures of 35^oC and 25^oC respectively. Highest (38.40%) and lowest (26.35%) carbohydrate contents were noted at temperatures of 25^oC and 35^oC respectively. The temperature of 45^oC had the highest values of percentage proximate compositions, hence, the optimized temperature. However, the various proximate compositions were significantly difference ($P<0.05$) in the varying temperature

Table 4.13: Sensory Evaluation of Market and Laboratory Prepared "daddawa" and "ogiri"

Sensory Parameter	A	B	C	D	P-value
Taste	$7.67{\pm}1.1^{a}$	$7.07{\pm}2.64^{c}$	$7.53{\pm}1.98^{b}$	$6.91{\pm}3.04^{d}$	0.46
Mouth feel (consistency)	$7.47{\pm}0.70^{a}$	$7.07{\pm}2.07^{b}$	$7.47{\pm}1.12^{a}$	$7.00{\pm}2.17^{c}$	0.54
Aroma	$7.67{\pm}0.38^{a}$	$7.00{\pm}1.86^{b}$	$6.73{\pm}1.50^{c}$	$6.52{\pm}1.65^{d}$	0.07
Acceptability	$7.60{\pm}0.83^{a}$	$7.33{\pm}0.67^{b}$	$7.60{\pm}0.69^{a}$	$6.88{\pm}0.73^{c}$	0.62

A=Market purchased "daddawa", B=Market purchased "ogiri", C=laboratory prepared "daddawa", D=laboratory prepared "ogiri". Means having different superscripted alphabets across row are significantly different at $P<0.05$. Values are expressed as Means ± SD (Standard deviation) of triplicate determinations

4.6 Sensory Evaluation of “Egusi” soup seasoned with the laboratory prepared and Market Purchased Condiments

Table 4.13 shows the sensory evaluation of the laboratory prepared and market purchased condiments by a 15 man panelist using a 9-point hedonic scale. The Table showed that market purchased “daddawa” (A) had the best taste (7.76) while laboratory prepared “ogiri” (D) had the least taste (6.91). Mouth feel of the condiment was best in laboratory prepared and market purchased “daddawa” (7.47) each and least (7.00) in laboratory prepared “ogiri”. Market purchased “daddawa” and laboratory prepared “ogiri” had the best (7.67) and least (6.52) aroma respectively. Market purchased (A) and laboratory prepared (C) “daddawa” had the best overall acceptability (7.66) each while the laboratory prepared (D) “ogiri” gave the least overall acceptability of (6.88). However, the sensory evaluation of the various condiments (“daddawa” and “ogiri”) revealed significant difference ($P<0.05$) in the sensory parameters.

CHAPTER FIVE

DISCUSSION

5.1 Isolation and Characterizations of Microorganisms from the Condiments

Fermented foods are food substrates that are been acted upon by edible microorganisms whose enzymes, particularly amylases, proteases, lipases hydrolyze the polysaccharides, proteins and lipids to nontoxic products with flavours, aromas and textures pleasant and attractive to the human consumer.(Agarry et al, 2010, Achi et al, 2007, Achi, 2005) Fermented foods are essential components of the diet in many countries and are consumed either as main dishes or as condiments (Steinkraus, 1996; Ojimelukwe *et al.,* 2011). Indigenous fermented foods were developed through traditional technologies which were preserved over the years in order to maintain their uniqueness and identity (Valyasevi and Rolle, 2002). They are prepared from both plant and animal materials, using processes in which microorganisms play active roles in the physical, nutritional and organoleptic modification of the starting materials (Aidoo, 1994). Legume seeds have continued to make significant contributions to human nutrition (Nwosu and Ojimelukwe, 1993). Seeds of legumes may account for 80% of dietary protein and may be the only source of protein for some groups. Their cooked forms are eaten as meals and are commonly used in fermented form as condiments to enhance the flavour of foods (Oniofiok *et al.*, 1996, Ojimelukwe *et al.*, 2011).

The *Bacillus species* isolated from the test condiments ("daddawa" and "“ogiri”") in this work were observed to be Gram positive, rod-shaped, nitrate-positive and endospore forming. This is in agreement with the findings of Rao *et al.* (1998) and Allaf (2011). Many species of this genus exhibit a wide range of physiologic abilities that allow them to live in many natural environment, (Ahn *et al.*, 2001). The *Bacillus species* play an increasingly important role in food and beverage industries (Grass *et al.,* 2004). *Bacillus subtilis* has been identified to be the main fermenting bacterium during indigenous production of “ogiri”; a traditional soup flavouring rich in protein. Evaluation of the use of starter and broth cultures of this bacterium in the production of '“ogiri”' was undertaken by Ogueke *et al.* (2013) with the view to improve the fermentation process and quality of the product. Earlier studies on the fermentation of locust bean and soybeans (Ariahu, *et al*,. 1999; Ouoba, *et al.,* 2003; Omafuvbe, 2006) have also identified *Bacillus spp* as the main microorganisms responsible for the fermentation of “daddawa”.

Similarly, various researchers have identified different microorganisms in fermented “daddawa” and “ogiri”. These include *Bacillus spp*., *E.coli, Proteus spp*., *Pediococcus spp*. and *Alcaligenes spp*. (Barber and Achinewhu, 1992; Sanni *et al.,* 2000). However, Ogueke *et al.* (2013), identified *Bacillus subtilis* and *B. licheniformis* as the main bacteria involved in the fermentation because of their ability to produce the requisite enzymes for the breakdown of proteins and production of the various flavor compounds associated with condiments. Also, Nwosu and Ojimelukwe (2000) reported that *Bacillus* and *Acinetobacter spp*, together with some yeast and fungi (*Rhizopus*) were identified in the fermented product developed from portions of melon (*Citrullus vulgaris*) and groundnut (*Arachis hypogea*).

5.2 Effect of mutation on *Bacillus subtilis* Fermented Condiments

The mutated *Bacillus subtilis* species performed well with the wild and standard strains during fermentation of the condiments and also gave considerable proximate compositions of “daddawa” and “ogiri”. Among the mutated strains, *Bacillus subtilis* (uv-40mins) performed better than *Bacillus subtilis* (uv-30mins) and *Bacillus subtilis* (nitrous acid). Although there was no statistical difference (P<0.05) between the test strains and the fermented condiments.

The PCR amplification and nucleotide sequences of the various strains of *Bacillus subtilis* revealed that the wild and mutated *Bacillus subtilis* are related to the standard *Bacillus subtilis* strain. The only difference is in their band distances and thickness. It was also noted that the nucleotide chains of wild and mutated *Bacillus subtilis* were longer than the standard strain and as well have different nucleotide arrangements. This may be due to the fact that although the wild and mutated *Bacillus subtilis* were of the same organism, the only difference is in their mutation.

Mutation is a change of the nucleotide sequence of the genome of an organism or extrachromosomal genetic element. Mutations result from unrepaired damage to DNA or to RNA genomes (typically caused by radiation or chemical mutagens), errors in the process of replication, or from the insertion or deletion of segments of DNA by mobile genetic elements (Bertram, 2000; Burrus and Waldor, 2004; Aminetzach *et al*., 2005). Many of the mutations may be deleterious making the organism less adapted to its environment and some may even be lethal. Some may be neutral in their effects and may confer no immediate advantage, but may help to generate a wide range of useful recombinant genotypes through the subsequent process of independent segregation and crossing over of genes (Orengo and Thornton, 2005)

However, some of the mutations occur from rearrangement of bases in the DNA. A small or large sequence of bases may be inverted as a result of chromosome breakage, and reunion of the broken ends may involve different DNA molecules in a reciprocal rearrangement or in loss of a fragment. Duplication of a DNA sequence is another common mechanism for change in the structure of a gene leading to gene mutation (Long *et al.,* 2003). Sanjuan (2010) reported that mutation can result in several different types of change in sequences. Mutations in genes can either have no effect, alter the product of a gene, or prevent the gene from functioning properly or completely. It can lead to the duplication of large sections of DNA, usually through genetic recombination (Hastings *et al*., 2009). Changes in chromosome number may involve even larger mutations, where segments of the DNA within chromosomes break and then rearrange. Sequences of DNA that can move about the genome, such as transposons, make up a major fraction of the genetic material of plants and animals, and may have been important in the evolution of genomes (Harrison and Gerstein, 2002).

Due to the damaging effects that mutations can have on genes, organisms have mechanisms such as DNA repair to prevent or correct (revert the mutated sequence back to its original state) mutations (Burrus and Waldor, 2004).

5.3 Proximate Compositions of the Test Condiments

The percentage proximate compositions of the “daddawa” and “ogiri” fermented with the various strains of *Bacillus subtilis* (wild, mutated and standard strains) indicated that the condiments are rich source of carbohydrate and protein rich foods with high moisture and fat content. However, it was observed that there were variations in the values of the proximate compositions of these condiments after fermentation with *Bacillus subtilis* at varying pH and temperatures.

The reduction in the carbohydrate content after fermentation of "Daddawa" and "ogiri" may be due to the activities of the microorganisms which derive their energy from carbohydrate metabolism (Azokpota *et al.,* 2006).

The significant difference and increase observed in the moisture content of African locust beans after fermentation is in line with the report of Antai and Ibrahim (1986), Ogunjobi *et al.* (2005) and Jonathan *et al.* (2011), this result differs from the report of Omafuvbe *et al.*, (1998), who reported a decrease in the moisture content of African locust beans after 72h of fermentation.

The fermentation process also increased the protein content of the fermented "daddawa" and "ogiri". This is in agreement with other researchers who observed similar trend (Enujiugha, 2003; Omafuvbe, *et al.* 2004; Fagbemi, *et al.* 2005; Pelig-Ba, 2009; Ojumelukwe *et al.*, 2011). The increase in protein content will enhance the nutritional quality and increase utilization, of the condiment. Achi (2005) also observed that fermentation for four days had the highest increase in protein and tannin as well as decreases in ash, lipid and also non-protein nitrogen. In addition, Pelczer *et al.* (1993), Ibrahim and Antai (2005), Omafuvbe *et al.* (2006) and Yabaya (2006) also observed a marked decrease in the total sugar content and increase in crude and protein levels. When condiments were produced from plant seeds researchers reported that the microorganisms grew rapidly and produced a high percentage yield of protein. These was attributed to the ability of the fermenting organisms to hydrolyze protein, thereby liberating ammonia smell which accounted for the enhance food taste, flavour and aroma of such products (Wakshama *et al.*, 2010).

Carbohydrate was found to decrease in the fermented "daddawa" and "ogiri". The decrease in the carbohydrate content may be as a result of the ability of the microorganisms to convert it into simple sugars and hence, utilize it as their major carbon sources during fermentation to increase the microbial masses. This is in agreement with the works of Omafuvbe *et al.* (1998), Ogunjobi *et al.* (2005), Yabaya (2006) and Azokpota *et al.* (2006) and that of Jonathan *et al.* (2011) and Amao *et al.* (2013), who reported a decrease in the percentage carbohydrate contents of different beans after fermentation to produce different condiments. They reported that the reduction in carbohydrate content may as well be due to the hydrolytic effect of microbial amylase converting carbohydrate into sugars. Similar findings of Yabaya (2006) reported that consumption of soluble sugars by the microorganisms may be responsible for the reduction in the soluble sugars. This observation was supported by earlier reports (Sanni and Ogbonna, 1991; Omafuvbe, *et al.* 2004). The *Bacillus subtilis* used for the fermentation is a common saprophile that has the ability to produce exoenzyme which can hydrolyse starch and casein leading to the rapid growth of the microorganism and also reduction in the concentration of sugars.

The formation of some volatile aroma compounds belonging to ketones and aldehydes groups which are generally formed via Millard reactions between saccharides and peptides may also play a role (Ledauphin *et al.*, 2003; Ojumelukwe *et al.*, 2011) in the reduction of carbonhydrates. Also, the decrease may likely be due to cooking and loss of carbohydrates in the cooking water and by fermentation where carbohydrates were metabolized by the microorganisms. (Parkouda, *et al.,* 2008; Ojumelukwe *et al.,* 2011). Nwosu and Ojimelukwe (1993) observed a similar trend in fermentation of melon and groundnut into '"ogiri"' as the total carbohydrate content was appreciably reduced by the fermentation process. Achinewhu (1986) also reported the presence of a reasonable amount of maltose, fructose, glucose and other identified sugars in '"ogiri"'. Ogbonnaya, *et al.* (2010) also reported a reduction in the total carbohydrate content of fermented locust bean. Ademola *et al.* (2011) also reported a significant decrease in carbohydrate content in salted and unsalted fermented locust beans. Dakare *et al.* (2011) reported that the decrease in carbohydrate content of the condiments may be due to fermentation by the microorganisms that might have used them as nutrients or convert them to fatty acids and amino acids as indicated by the increase in crude lipid and crude protein content respectively, after fermentation.

5.4 pH and Temperature Optimization

The important characteristics of most organisms is their strong dependence on pH for their cell growth and production of secondary metabolites. A change in pH of the medium plays an important role in fermentation of substrates by microorganism through their effects on cellular growth or activity. In this present study, the results of the effect of pH on the *Bacillus subtilis* inoculated condiment clearly revealed that maximum proximate composition of the condiments occurred mostly at pH 6, followed by pH 7 and 8.

Thisresult collaborated the findings of David and Aderibigbe (2010) who reported that the pH of unfermented substrates of ranged between 6.1-6.4 and 6.8-7.8 during fermentation, Omezuruike *et. al.* (2008) also reported a pH level of 5.5 at the onset of fermentation which increased to 8.0 at the end of fermentation, during the production of “anyi”, Omafuvbe *et al.* (2002), also reported an initial pH range of 6.50-6.55 which increased to 7.50-8.00 after 72 hours of fermentation Antai and Ibrahim (1986) also reported a pH of 7.1 at 0h, and a of pH 7.9 after 72h of fermentation, Jonathan *et al.* (2011) also reported that the pH decreased from 6.7 at 0h to 4.4 at the end of fermentation of bambara nuts (96h) for the production of “daddawa”. Omafuvbe *et al.* (2002) reported an increase in pH of fermented soybeans after 72 hours of fermentation also inoculated *Bacillus spp*. as single inocula showed different ability to increase the final pH value with different proteolytic enzyme activities and free amino acids production.

In addition, the pH of the culture medium also determines the electrical charge of the cells and the oxidation–reduction potential which can affect nutrient absorption and enzymatic reaction (Salehizadeh and Shojaosadati, 2001; Husam and Ahmed, 2013). Different organisms have different pH optima and decrease or increase in pH on either side of the optima results in poor microbial growth (Praveesh *et al.,* 2011).

It was observed that the temperature of the fermenting “daddawa” and “ogiri” increased as the fermentation process progressed. The proximate composition values were highest at 45^{o}C. The increase in temperature being attributed to heat or the liberation of carbon dioxide by the fermenting organisms. This might have been as a result of exerted pressure on the substrate. This is in agreement with Yabaya (2006) who reported that rise in temperature observed was due to the growth and development of the microorganisms.

Temperature increases have also been reported by Odunfa (1981). Similar trends have also been observed in other plant materials (Achi, 1992; Ogueke and Nwagwu, 2007; Ojimelukwe *et al.,* 2011). The rise in temperature indicates that the fermentation reaction is exothermic with the changes being due to metabolic activities of the microorganisms (Achi, 1992). The heat generated in the fermenting mash possibly provided the conditions for the optimal activity of the proteolytic enzymes (Odunfa, 1985). An initial increase in temperature followed by a gradual decrease thereafter has been reported during ‘“ogiri”’ fermentation (Odunfa and Oyeyiola, 1985; Njoku and Okemadu, 1989). During ‘“daddawa”’ production, Odunfa (1981) reported a continuous increase in temperature during fermentation process. According to Amao *et al.* (2013) fermentation of plant seeds for condiment production is a solid substrate fermentation with an exothermic process, where the temperature of the fermenting seed increases gradually from ambient temperature to about 30^{o}C to 45^{o}C (Odunfa and Oyewole, 1986). Desired fermentation state is indicated by the formation of mucilage and production of ammonia as a result of the breakdown of amino acids (Omafuvbe, 1998). Apart from the improvement of sensory properties, condiments add nutritional values to foods, providing dietary fibre, energy, minerals and vitamins (Kolapo *et al.,* 2007). Iru is an important source of riboflavin and it contains the highest riboflavin content when compared to some other common plant foods (Pelig-Ba, 2009).

5.5 Sensory Evaluation

The preparations of food by fermentation processes are dependent upon the production by certain microorganism of chemical and physical changes that altered the appearance, body and flavour of the original material. These changes may improve the nutrition of the product and they are generally inhibitive to the growth of undesirable microorganisms (Babalola and Giwa, 2012).

Cooked “egusi” soup seasoned with “daddawa” and “ogiri” was organoleptically evaluated by a panel of 15 tasters who compared it to “daddawa” and “ogiri” prepared traditionally using a score line of 1(dislike extremely) to 10 (like extremely) according to the modified methods of Njoku *et al.* (1991), Wokoma and Aziagba (2002) and Yabaya (2006). Four attributes were assessed, namely, taste, mouth feel, aroma, and acceptability. The result of this present study (Table 4.14) condiments had the highest overall acceptability as well as aroma, taste and consistency than “ogiri”. This is similar to the research work of Oboh (2006) who reported that among the condiments produced, locust bean condiments had the highest acceptability (aroma and taste) and they are closely followed with condiments produced from soybean and melon seed, while pigeon pea-based condiment had the least acceptability. According to Wakshama *et al.* (2010), the changes in colour, state of solidness and smell are normal characteristics associated with production of “daddawa”. High protein content in fermented foods causes increase in proteinase activity during fermentation with the release of more amino acids and nitrogenous compounds which may have odour or smell depending on the fermentation period (Asagbra *et al.,* 2012). The nitrogenous compound produced may have affected the taste and overall acceptability of the egusi soup prepared with “ogiri”. The higher fat contents in the “ogiri” condiments may have contributed to the better taste, flavour and aroma of the soup prepared. According to Odunfa (1983), Onawola *et al.* (2011) and Asagbra *et al.* (2012) who reported that during fermentation, there was evidence of lipase activity which indicate production of free fatty acids. These may react with some other components of the fermenting mash to form esters which produce the characteristic aroma of the food condiments.

Jonathan *et al.* (2011) noted that there was no significant differences in general acceptability of the “iru” produced from bambara nut to that produced from African locust beans. It is also in line with that of Aderibigbe and Odunfa (1999) who suggested that though high lipase activity can lead to rancidity in fermented foods, it may also lead to the production of some volatile fatty acids, providing flavor and aroma. (Amao *et al.*, 2013).

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The main organisms isolated from Nigerian indigenous condiments are *Bacillus subtilis*, *Bacillus licheniformis* and *Bacillus ceres.*

Mutation has a profound effect on the test organism, UV- light and nitrous acid had effect on the organism nucleotide strains; sequencing of the mutated strains showed changes in the nucleotides where genes had been substituted or deleted.

The Micro-organisms were characterized and found to be a *Bacillus subtilis*.

"Daddawa" and "ogiri" were produced with standard *Bacillus subtilis* (ABC35615), mutated (UV- light and nitrous acid) *Bacillus subtilis* and wild *Bacillus subtilis* isolates under laboratory conditions.

Optimization of fermentation yield using varying temperatures and pH (acidity) shows that the best temperatures for the "Daddawa/Ogiri" production was 45^{O}C and best pH was 6.

The condiments produced and the purchased ones from the market were used in preparing soups and the taste panel of 15 untrained personnel were used to assay the condiments. It was observed that there was no remarkable difference between the market “daddawa” and the laboratory produced “daddawa” but for “ogiri” there was a remarkable difference.

6.2 Recommendations

The following recommendations are made

1. A system based on the optimal pH (6) and temperature (45^{o}C) is suggested for efficient application of the *Bacillus subtilis* in fermentation of food condiments

2. Since indigenous fermented foods form part of the rich nutritional culture of most groups in Nigeria, efforts should be concentrated on improving rural fermentation technologies.

3. A compendium of traditional fermented foods will be a welcome development. Just as germplasm is conserved in gene banks, information on rural food-processing methods should, in the same way, be stored in “food banks”

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