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REVIEW ARTICLE

The microbiology of alkaline-fermentation of indigenous seeds used as food condiments in Africa and Asia

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Abstract

Alkaline-fermented food condiments play an important role in the diets of many people in developing and a few developed countries. 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. Studies have been undertaken which have investigated a number of these products like *dawadawa*, *ugba*, *bikalga*, *kinema*, *natto,* and *thua-nao*. In this review, current knowledge about the principal microbiological activities and biochemical modifications which occur during the processing of the alkaline condiments including nutritional, antimicrobial, and probiotic aspects are discussed. The current use of molecular biology methods in microbiological research has allowed unambiguous and more reliable identification of microorganisms involved in these fermentations generating sufficient knowledge for the selection of potential starter cultures for controlled and better production procedures for alkaline-fermented seeds condiments.

Keywords: *Alkaline fermentation; indigenous food;* Bacillus *spp*

1. Introduction

Indigenous technologies can be defined as processing methods and procedures employed by the native inhabitants of a region which constitute an important part of their cultural inheritance. Indigenous or ethno-knowledge is a part of the rural life, and the livelihood and the survival of the rural people depend on this knowledge. Indeed, fermentation is a part of this ethno-knowledge and constitutes one of the oldest forms of food preparation and preservation technologies (Dirar 1993).

Fermentation is generally carried out to bring diversity into the kinds of foods and beverages available; make otherwise inedible foods edible; flavor dishes; enhance the nutritional value; decrease toxicity; preserve food; and decrease cooking times and energy requirements (Steinkraus 1996). A sub-group of these fermented products is the indigenous alkaline-fermented food condiments. Alkaline-fermentation is defined as a fermentation process during which the pH of the substrate increases to alkaline values which may be as high as pH 9 (Amadi et al. 1999; Aniche et al. 1993; Omafuvbe et al. 2000; Sarkar and Tamang 1995). The increase in pH is due to degradation of proteins from the raw material into peptides, amino acids and ammonia (Kiers et al. 2000)

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or due to alkali-treatment during production (Wang and Fung 1996).

In most African and Asian countries, alkaline condiments are common and form an important part of diet of the indigenous people (Dakwa et al. 2005; Odunfa 1983). Because of their sensory characteristics and high nutritional value these products are largely used to flavor soup and stews and constitute significant low cost sources of protein foods in some parts of the world (Azokpota et al. 2006; Tamang and Nikkuni 1998). According to Achi (2005) indigenous fermented condiments consumed by different ethnic groups in Nigeria, have been the pride of culinary traditions for centuries. In most African and Asian countries, the traditional diets of a majority of people rely largely on starchy staples such as cereals, cassava, yam and plantain which are rich in calories but poor in other nutrients (Achi 2005; Dakwa et al. 2005). The indigenous alkaline-fermented food condiments are prepared from many different types of raw materials including seeds from several wild trees (Achi 1992; Ejiofor et al. 1987; Ogunshe et al. 2007; Ouoba et al. 2004) as well as various cultivated plant seeds (Table 1a and Table 1b) (Inatsu et al. 2006; Terlabie et al. 2006). Apart from Japanese *natto* which is widely produced industrially using *Bacillus subtilis* var. *natto* as starter culture (Steinkraus 2004), generally the seeds are spontaneously fermented by microorganisms indigenous to the preparation site (Odunfa 1981).

Despite the nutritional, cultural, and socio-economic importance of the alkaline fermented condiments (Diawara and Jakobsen 2004; Ndir et al. 2000), they constitute a group of poorly characterized food products (Steinkraus 1997; Wang and Fung 1996). Interests and research into indigenous fermented foods have however seen a marked improvement in the last few decades (Dirar 1993; Steinkraus 2004) as this is seen as a source of ethno-knowledge which can be used to enhance food availability and quality, and alleviate malnutrition in many developing countries. Consequently, a truly outstanding review of alkaline-fermented foods was published by Wang and Fung (1996). Since then a number of studies investigating microbial cultures involved in these traditional fermentations, processing equipment, nutritional aspects, and methods for optimizing fermentation conditions have been carried out along with the use of methods based on molecular biology for identification of microbial species (Azokpota et al. 2006; Dakwa et al. 2005; Inatsu et al. 2006; Ouoba et al. 2004; Roy, Moktan, and Sarkar 2007; Terlabie et al. 2006). There is therefore the need to evaluate data currently available on alkaline-fermented foods in light of these developments. The aim of the present review is to assess the importance and current knowledge of alkaline-fermented foods including the main microbiological changes which occur during their production and the technological and functional properties of the bacteria involved in the fermentation.

2. Main alkaline fermented foods of Africa and Asia and predominant microorganisms involved in their fermentation

2.1 Main indigenous alkaline-fermented foods of Africa

2.1.1 West African dawadawa

Dawadawa is an alkaline-fermented condiment which is processed from the seeds of the African locust bean tree and has a final pH above 7 (Azokpota et al. 2006; Omafuvbe et al. 2004). *Dawadawa* is still popular in West Africa and plays an important role in the diet (Dakwa et al. 2005; Odunfa 1988). *Dawadawa* is the name commonly used in Nigeria and Ghana but is also known under different local names such as *iru* in Nigeria (Sanni et al. 2000), *soumbala* in Burkina Faso (Diawara et al. 1992), *netetu* in Senegal (Ndir et al. 1994), *afitin, iru* and *sonru* in Benin (Azokpota et al. 2006), and *Kinda* in Sierra Leone. It contributes significantly to the intake of protein, essential fatty acids, and group-B vitamins besides its flavoring attributes, and is an important source of family income (Akaaimo and Raji 2006; Ndir et al. 1994; Odunfa 1988; Ouoba et al. 2003a; Ouoba et al. 2003b).

Dawadawa production is laborious, energy- and time-consuming and procedures are based on traditional knowledge and experience. There are therefore differences in the procedures employed in different areas and localities (Odunfa 1988; Sawadogo-Lingani et al. 2003). The process is uncontrolled and generally there are no national standards for the product in the different countries. The duration of fermentation, other processing parameters, and to some extent the microbial species involved vary, leading to variations in product quality (Diawara et al. 1992; Sanni 1993). According to Achi (2005) specific process variations which occur are the results of environmental conditions, type of substrates used and local tradition, consequently fermentation takes place under conditions which the producers have found to be favorable for the appropriate growth and activity of the microorganisms.

The traditional *dawadawa* process (Fig. 1) described by Odunfa (1988) involves the cleaning and cooking of African locust bean seeds for 12 to 24 h till they become soft and the seed coat can be easily removed. The cooked seeds are washed, dehulled by hand and the cotyledons cooked again for 2 hours with addition of *kaun* (a local softening agent). The cotyledons are then spread in calabash trays and wrapped in jute sacks or packed in earthenware pots and left to ferment. The fermented seeds are covered with a stringy mucilaginous coating accompanied with a

Table 1a. Indigenous alkaline fermented foods from cultivated plant seeds and the main microorganisms involved.

Raw material	Product name	Place (country/region)	Microorganisms involved	References
Soya bean (*Glycine max*)	Kinema	Himalayan regions, (Nepalis, Lepchas, Bhutias, India)	***B. subtilis****, Enterococcus faecium, Candida parapsilosis, Geotrichum candidum, B. licheniformis, B. cereus, B. circulans, B. thuringiensis, B. sphaericus*	(Dahal et al. 2005; Kiers et al. 2000; Sarkar and Tamang 1995; Sarkar et al. 2002; Sarkar et al. 1994; Sarkar et al. 1997a; Sarkar et al. 1997b)
	Thua-nao	Thailand	***B. subtilis,*** *Bacillus* spp., Lactic acid bacteria	(Chantawannakul et al. 2002; Inatsu et al. 2006; Leejeerajumnean et al. 2001; Visessanguan et al. 2005)
	Natto	Japan	***B. subtilis*** **var natto**	(Kiuchi 2004)
	Dou-shi	China	*B. amyloliquefaciens*	(Peng et al. 2003)
	Soy-dawadawa	Ghana	***B. subtilis****, B. pumilus, B. licheniformis, B. cereus, B. firmus*	(Dakwa et al. 2005)
	Soyadawadawa	Nigeria	***B. subtilis,*** *B. licheniformis, B. circulans, B. pumilus, B. metaterium Staphylococcus saprophyticus, Staphylococcus epidermidis, Micrococcus luteus, Pseudomonas aeruginosa*	(Dike and Odunfa 2003; Jideani and Okeke 1991; Omafuvbe et al. 2000)
Roselle (*Hibiscus sabdariffa*)	Bikalga	Burkina Faso	***B. subtilis****, B. licheniformis, B. cereus, B. pumilus, B. badius, Brevibacillus bortelensis, B. sphaericus, B. fusiformis* and *Staphylococcus* spp.	(Bengaly 2001; Ouoba et al. 2007)
Bambara groundnut (*Vigna subterranea*)	Dawadawa-type product	Nigeria	*B. subtilis, B. licheniformis*	Amadi et al. 1999; Barimalaa et al. 1994)
Melon (*Citrullus vulgaris*) Cotton seed (*Gossypium hirsutum* L.)	Ogiri Owoh	Nigeria Nigeria	***B. subtilis,*** *B. pumilus, B. licheniformis, B. brevis, B. megaterium, B. polymyxa, Staphylococcus* spp., *Pseudomonas aeruginosa B. subtilis, B. Licheniformis, B. Pumilus, Staphylococcus* sp.	(Jideani and Okeke 1991; Omafuvbe et al. 2004; Sanni et al. 2000) (Sanni and Ogbonna 1991)
African yam bean (*Sphenostylis stenocarpa*)	Owoh	Nigeria	*B. licheniformis B. pumilus, B. subtilis, Staphylococcus* sp.	(Ogbonna et al. 2001)
Castor oil bean (*Ricinus communis*)	Ogiri	Nigeria (Anambra State)	***B. subtilis, B. licheniformis, B. metaterium,*** *Staphylococcus* spp., *Pseudomonas aeruginosa*	(jideani and Okeke 1991)

NB: In bold the main microorganism involved.

strong ammonia-like odor. The duration of fermentation depends on the local conditions and the kind of product desired; 48 h or more for Nigerian *dawadawa* (Odunfa 1988), 18 h for Beninois *afitin* (Azokpota et al. 2006), 72 h for Senegalese *netutu* (Ndir et al. 1994). In Ghana Dakwa et al. (2005) reported that *dawadawa* is sometimes produced from a mixture of locust bean seeds and soybeans or locust bean seeds and groundnuts changing the aroma profile of the final product.

Various studies have been carried out to upgrade the traditional process and make it less labor intensive (Achi 2005; Alabi et al. 2005; Odunfa 1988) and an example of such a procedure is shown in Fig. 2 (Achi 2005). According to Alabi et al. (2005) a modified procedure which reduces processing time and energy requirements has been successfully used to produce *dawadawa* cubes sold in some Nigerian markets. In Burkina Faso, development and introduction of a dehulling machine in the process of production of *soumbala* considerably decreased the first boiling step from 24 h to 6 h and this represents a considerable save of energy and time (Sawadogo-Lingani et al. 2003). Recently, Gernah et al., (2007) showed that the type of material used for fermentation affects both the sensory and nutritional quality of *dawadawa*. *Gmelina arborea* leaves, were reported to give the best overall results at 35°C fermentation for 72 h, followed by *Musa sapienta* leaves, polythene, and jute bags respectively. In some case, salt is added to the fermenting seeds to attenuate the strong smell of *dawadawa*.

Campbell-Platt (1980) identified the dominant microorganisms in numerous *dawadawa* samples from different West African countries and reported that *Bacillus subtilis* accounted for about 31% of all isolates and other *Bacillus* species, 13%. *Bacillus subtilis* represented as high as 61 to 69% of all isolates in some samples and all *Bacillus* species 83 to 93% in some samples. Other organisms found were lactobacilli and pediococci. In Nigerian *dawadawa*, Odunfa (1981) and Ogbadu and Okagbue (1988) have reported

Table 1b. Indigenous alkaline fermented foods from wild trees plant seeds.

Raw material	Product name	Place (country/ region)	Microorganisms involved	References
Mesquite (*Prosopis africana*)	Okpehe (Kpaye, Okpiye)	Nigeria	***B. subtilis, B. licheniformis, B. pumilus,*** *B. megaterium, B. cereus, Staphylococcus epidermidis, Micrococcus luteus, Escherichia coli, Enterobacter cloacae, Klebsiella pneumoniae, Lactobacillus* spp., *Proteus* spp., *Pseudomonas* spp., *Enterococcus* spp., *Staphylococcus* spp., *Micrococcus* spp.,	(Achi 1992; Ogunshe et al. 2007; Oguntoyinbo et al. 2007; Omafuvbe et al. 1999; Oguntoyinbo and Oni 2004)
African oil bean (*Pentaclethra macrophyla*)	Ugba	Nigeria	***B. subtilis,*** *B. pumilus, B. licheniformis, B. brevis, B. megaterium, B. polymyxa, B. coagulans, B. macerans, B. cereus, Lactobacillus* spp., *Micrococcus* spp., *Pseudomonas chlororaphis, Micrococcus roseus, Staphylococci saprophyticus, Staphylococcus* spp.,	(Isu and Njoku 1997; Isu and Ofuya 2000; Mbajunwa et al. 1998; Sanni et al. 2000; Sanni et al. 2002)
African locust bean (*Parkia biglobosa*)	Dawadawa (Iru)	Nigeria	***B. subtilis***, *B. pumilus, B. licheniformis B. brevis, B. megaterium, B. polymyxa, Leuconostoc* spp., *Staphylococcus* spp., *Pseudomonas aeruginosa*	(Jideani and Okeke 1991; Odunfa 1981; Odunfa and Oyewole 1986; Ogbadu and Okagbue 1988; Omafuvbe et al. 2004; Sanni et al. 2000)
	Soumbala	Burkina Faso	***B. subtilis, B. pumilus***, *B. cereus, B. sphaericus, Brevibacillus borstelensis, B. thuringiensis, B. licheniformis, B. badius, Paenibacillus* al*vei, B. firmus, P. larvae, Brevibacillus laterosporus, B. megaterium, B. mycoides,*	(Ouoba et al. 2004; Sarkar et al. 2002)
	Afitin, Iru and Sonru	Benin	***B. subtilis, B. licheniformis,*** *B. cereus*, *Staphylococcus* spp.	(Azokpota et al. 2007)
	Netetu	Senegal	***B. licheniformis, B. coagulans, B. subtilis,*** *B. pumilus, Staphylococcus* spp. *Micrococcus* spp.	(Ndir et al. 1994; Ndir et al. 1997)
Saman tree (*Albizia saman*)	Aisa	Nigeria	***B. cereus* var. *mycoides, B. coagulans, B. licheniformis, B. megaterium, B. pumilus, B. subtilis,*** *Staphylococcus aureus, S. saprophyticus, Escherichia coli, Klebsiella pneumonia, Enterobacter aerogenes, Proteus mirabilis*	(Ogunshe et al. 2006)
Baobab (*Adansonia digitata*)		Nigeria		(Addy et al. 1995; Igboeli et al. 1997; Nnam and Obiakor 2003; Osman 2004)

NB: In bold the main microorganism involved.

B. subtilis, B. licheniformis, B. pumilus, Leuconostoc mesenteroides, L. dextranicus, Staphylococcus spp., and *Micrococcus* spp. as the main microorganisms involved in the fermentation (Table 1b). The predominance of subspecies of *B. subtilis* in the fermentation was confirmed by Odunfa and Oyewole (1986) using biochemical tests.

In Senegalese *netetu*, Ndir et al. (1994) and Ndir et al. (1997) isolated *Bacillus* species as the dominant microorganisms and also isolated *Staphylococcus* spp., *Micrococcus* spp., *Clostridia* spp., and *Streptococci* spp. which they reported as potential spoilage organisms.

Using RAPD-PCR Sarkar et al. (2002) identified *B. subtilis, B. thuringiensis, B. licheniformis, B. cereus, B. badius, B. firmus, B. megaterium, B. mycoides, B. sphaericus, Paenibacillus alvei, Paenibacillus larvae,* and *Brevibacillus laterosporus* in *soumbala* samples from Burkina Faso with *B. subtilis* as the dominant microorganism. Ouoba et al. (2004) confirmed the presence of *B. subtilis* and *B. pumilus* in *soumbala* using ITS-PCR and ITS-PCR RLFP and showed the presence of *Brevibacillus borstelensis* and *B. cereus*. Ouoba et al. (2007b) consider the long cooking period during soumbala production as the process step which leads to the selection of *Bacillus* species as the dominant microorganisms due to their heat-resistant spores.

Azokpota et al. (2006) reported the presence of *Bacillus* spp, and *Staphylococcus* spp. in *afitin*, *iru* and *sonru* from Benin, but showed that during fermentation the presence of *Staphylococcus* spp. decreased significantly after 18h of fermentation. For *iru* and *sonru*, *iku-iru,* and *yanyanku* are added as additives before fermentation and this seems to enhance the initial *Bacillus* spp, and *Staphyloccoccus* spp. count.

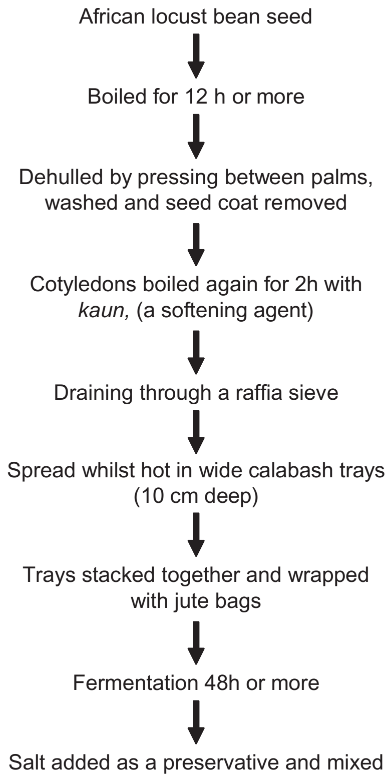

Figure 1. Flow chart for traditional production of dawadawa (Odunfa 1988).

2.1.2. Ugba

Ugba with a pH of about 8 is produced by fermentation of African oil bean seeds, *Pentaclethra macrophylla* Bentham (Ejiofor et al. 1987). It is mainly produced in the south-eastern parts of Nigeria and used to flavor dishes or as a low-cost protein source (Sanni et al. 2002). *Ugba* production basically involves the same unit operations used in *dawadawa* production and is also laborious, time, energy and water consuming. Production takes 5–6 days if *ugba* is to be used as a snack or side-dish and 7– 10 days if it is to be used as soup condiment (Isu and Ofuya 2000). The dominant microbial species have been reported to be *B. subtilis,* but *B. coagulans, B. pumilus, B. megaterium, B. macerans* have been reported to be involved as well (Isu and Njoku 1997; Isu and Ofuya 2000).

2.1.3. Aisa

The Nigerian food seasoning condiment *aisa*, is produced by alkaline fermentation of *Albizia saman* (Jacq.) F. Mull seeds commonly called the rain tree or saman tree (Ogunshe et al. 2006). The process is similar to that of *dawadawa* with fermentation lasting for 5–7 days and final product pH of around 8 (Ogunshe et al. 2006). The main microorganisms responsible for the fermentation are various *Bacillus* species, but *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter aerogenes, Proteus*

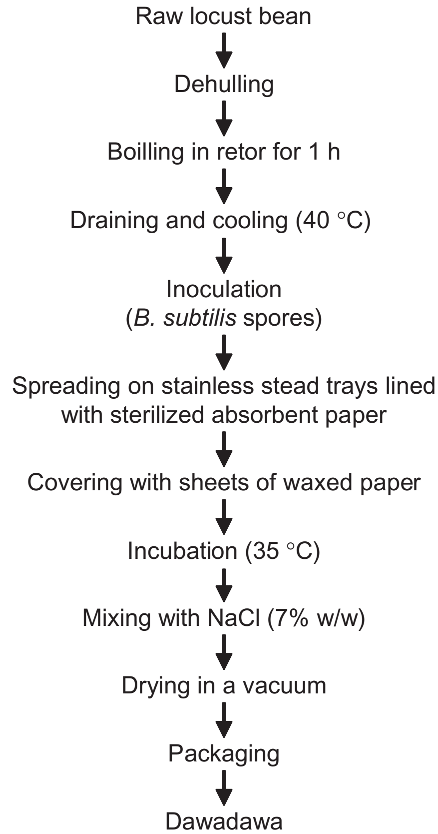

Figure 2. Flow chart of modern production of dawadawa (Achi 2005).

mirabilis, and *Staphylococcus* spp. have also been isolated from *aisa* (Ogunshe et al. 2006).

2.1.4. Okpehe

Okpehe, *kpaye,* or *okpiye* are local Nigerian names of the fermented seeds of *Prosopis africana* with a final pH of between 7.8 and 8.3 (Achi 1992; Oguntoyinbo et al. 2007; Omafuvbe et al. 1999). Oguntoyinbo et al. (2007) described the traditional production as follows: seeds are boiled for 15–24 h until soft, dehulled by pressing between the palms after which the cotyledons are washed and boiled again for 3–5 h. They are then spread in wide calabash trays or earthenware pots lined with pawpaw leaves and the trays tightly wrapped with jute bags and fermentation allowed to proceed for 3–5 days. *Bacillus* species including *B. subtilis, B. licheniformis, B. megaterium,* and *B. puhilus,* are mainly responsible for the fermentation, but *Staphylococcus epidermis, Micrococcus* spp., *Enterobacter cloacae, Klebsiella pneumonia, Lactobacillus* spp., *Escherichia coli, Proteus* spp., and *Pseudomonas* spp. have all been isolated and identified on the basis of phenotypic traits (Achi 1992; Ogunshe et al. 2007; Omafuvbe et al. 1999).

2.1.5. Ogiri

Ogiri is a food condiment produced by the alkaline-fermentation of melon seeds (*Citrullus vulgaris*) in Nigeria. It is used for flavoring as well as a protein supplement (Omafuvbe et al. 2004). For *ogiri* production, shelled melon seeds are cleaned by sorting and boiled for 6h till they become soft. The cooked seeds are covered with *Thaumaloccus demoelli* leaves and left to ferment for 3 days. Omafuvbe et al. (2004) found the pH of the final product to be around 7.9. On the contrary Abaelu et al., (1990) reported product pH values of 5.4, 5.6–6.4, and 6.4 after fermenting ground seeds with pure cultures of *B. pumilus, B. licheniformis,* and *B. subtilus*, respectively. The difference in final pH might be explained by the use of spontaneous fermentation *versus* the use of pure starter cultures where the organisms present in the spontaneously fermented product have adapted to the ecological niche whereas the pure cultures are introduced into a new environment needing time to adapt.

2.1.6. Soydawadawa

Soydawadawa is a fermented food condiment with a pH of between 8.2 and 8.9 made from soybeans (*Glycine max* L Merr) (Dike and Odunfa 2003; Omafuvbe et al. 2000). Omafuvbe et al. (2000) reported that *soydawadawa* is produced using traditional methods on a household level in the major soybean-producing areas of Nigeria, and described the traditional processing: soybeans are sorted, washed, soaked in water for 12 h and dehulled by hand. They are then cooked for 2 h and incubated in calabashes lined with plantain leaves to ferment for 72 h. In Ghana, soybeans are roasted or boiled rather than soaked in water overnight to enable dehulling (Dakwa et al. 2005). According to Dakwa et al. (2005) there is preference for *soydawadawa* prepared by roasting rather than boiling the grains before dehulling as based on a consumer survey conducted.

In Nigeria Omafuvbe et al. (2000) reported the predominance of *B. subtilis, B. licheniformis* and *B. pumilus* and the presence of relatively low numbers of *Micrococcus luteus* and *Staphylococcus epidermidis* in *soydawadawa*. In Ghana the most frequently isolated microorganisms from *soydawadawa* are *B. subtilis, B. pumilus, B. licheniformis, B. cereus,* and *B. firmis* (Amoa-Awua et al. 2006; Dakwa et al. 2005; Terlabie et al. 2006).

2.1.7. Bikalga

Bikalga also known as *dawadawa-botso* in Niger, *datou* in Mali, *furundu* in Sudan and *mbuja* in Cameroon, is one of the most popular condiments in Burkina Faso and is a product of the alkaline fermentation of *Hibiscus sabdariffa* L. commonly known as Roselle or sorrel (Parkouda et al. 2008). *Bikalga* is used to flavor many dishes and constitutes a valuable source of protein (22–30%), lipids, carbohydrates, essential amino and fatty acids in the diet (AbuTarboush et al. 1997; Yagoub, et al. 2004). *Bikalga* production involves cleaning of the seeds, cooking overnight (12 to 24 h) with addition of liquid ash as a softening and alkalizing agent, 3–4 days fermentation, crushing, moulding, steaming overnight, and sundrying. During food preparation, *bikalga* is steeped in lukewarm water for a few minutes and the steep water used to prepare stews, soups, sauces, and other foods (Parkouda et al. 2008). Using ITS-PCR, rep-PCR and DNA sequencing, Ouoba et al., (2008) identified the main microorganisms involved in *bikalga* fermentation as *B. subtilis, B. licheniformis, B. cereus, B. pumilus, B. badius, B. sphaericus, B. fusiformis,* and *Brevibacillus bortelensis,* with predominance of *B. subtilis*. Associated microorganisms have been characterized to be staphylococci (Bengaly 2001).

2.1.8. Owoh

Owoh is a food condiment with a pH of about 9.0 popular in mid-western Nigeria and is processed by the alkaline fermentation of cotton seeds (*Gossypium hirsutum* L.). The seed is not eaten in its natural state because of its content of anti-nutritional substances, in particular gossypol, which inhibit digestion but the fermented seeds are edible (Sanni and Ogbonna 1991). *Owoh* is prepared by boiling cotton seeds for about 2 h until they become soft. The boiled seeds are then soaked in water overnight and subsequently dehulled by hand. The cotyledons are wrapped in banana leaves and boiled again for 1–2 h. After draining the packets are placed in earthenware pots or calabash trays, covered with jute sacks and allowed to ferment for 2–3 days into *owoh*. The mash may be ground and moulded into balls and to extend the shelf life it is smoked over charcoal (Sanni et al. 1991). Sanni et al. (1991) identified *Bacillus subtilis, B. licheniformis,* and *B. pumilus* as the dominant microorganisms in *owoh*. Staphylococci were isolated as well.

2.1.9. Other African indigenous alkaline-fermented foods

There are several other indigenous alkaline-fermented seeds which constitute a group of less known food condiments and which have received little scientific investigation (Tables 1a and 1b). Among these products *owoh*-type-product from African yam bean (*Sphenostylis stenocarpa*) seeds, *dawadawa*-like product from bambara groundnut (*Vigna subterranean* L), *ogiri* from Castor oil bean (*Ricinus communis*), *dadawa* from *Acacia nilotica* seeds, *ogiri* from sesame seeds, and *maari* from baobab seeds should be mentioned (Barimalaa et al.,1994; Jideani and Okeke 1991; Ogbonna et al. 2001; Yabaya 2006).

2.2. Main indigenous alkaline-fermented foods of Asia

2.2.1. Kinema

Kinema is produced by the traditional alkaline fermentation of soybeans (*Glycine max* L) by the people of the eastern Himalayan regions of the Darjeeling hills and Sikkim in India, Nepal and Bhutan who use it in local dishes as a seasoning agent and as a low-cost source of protein (Dahal, et al. 2005; Sarkar et al. 1997b; Tamang and Nikkuni 1996). *Kinema* has a pH between 7.9 and 8.5 and is produced by cleaning of soybean grains, washing, soaking overnight (12–20 h), boiling till soft (90 min) and crushing lightly into grits. The grits are wrapped in fern or banana leaves and sackcloth, and left to ferment for 1–3 days until the beans are covered with a stringy, mucilaginous coating and a typical *kinema* flavor dominated by ammonia appears (Sarkar et al. 1995). Addition of a small amount of firewood ash to the crushed beans is optional (Sarkar et al. 2002). Fresh kinema can be kept for 2 to 3 days during summer and up to 1 week during winter. For food preparation, fresh kinema is fried in oil with other ingredients and eaten with rice and vegetables (Dahal et al. 2005).

In *kinema* from the eastern Himalayan region, Sarkar et al., (1994) isolated 502 bacterial strains representing *Bacillus subtilis* and *Enterococcus faecium,* and 198 yeast strains representing *Candida parapsilosis* and *Geotrichum candidum*. In another study Sarkar et al. (1995) reported the same organisms to be involved in *kinema* fermentation. More recently Sarkar et al. (2002) have identified *B. subtilis, B. lichenisformis, B. cereus, B. circulans, B. thuringensis,* and *B. sphaericus* in *kinema* with predominance of *B. subtilis* using RAPD-PCR. Tamang et al. (1996; 1998) have produced kinema of acceptable quality with a high level of soluble proteins using *Bacillus subtilis* as starter culture for controlled fermentation at 40°C for 20 h followed by maturation at 5°C for 1 day.

2.2.2. Natto

Natto is a typical and popular soybean fermented food in Japan. It is eaten with steamed rice for breakfast or used to flavor dishes (Wang and Fung 1996). Its equivalent is *chungkukjang* in Korea and parts of China (Jung et al. 2006). Traditionally, *natto* was also used to treat heart and vascular diseases, to relieve fatigue and as an anti-beriberi agent. *Natto* has a long history extending back for more than 2000 years and its special taste and flavor produced by *Bacillus* spp. are well liked in Japan (Sumi et al. 1987). *Natto* production is now carried out industrially using starter cultures of *Bacillus subtilis* var *natto* and is commercialized all over the world (Steinkraus 1996; Wang and Fung 1996).

Natto is produced by first cleaning and soaking whole soybean grains in water overnight at ambient temperature. Subsequently the seeds are cooked under steam pressure of 0.98–1.47 Bar for 20–30 min. The cooked seeds are cooled to 45°C and inoculated with *B. subtilis* var. *natto* and fermented for 18–20 h at 40–45 °C (Wang and Fung 1996). A few studies have been carried out to investigate the health benefits of *natto.* As an example a subtilisin-like serine protease, nattokinase, has been reported to have pro-fribrinolitic effect in *in vivo* and *in vitro* studies (Fujita et al. 1993; Pais et al. 2006; Sumi et al. 1987). Mamiya and Nishimura (2007) reported enhancement of locomotor activity in mice fed with *natto*. *Natto* intake has also been reported to support bone formation in menopausal women and to prevent postmenopausal bone loss possibly due to the presence of menaquinone or bioavailable isoflavones in *natto* (Ikeda et al. 2006; Katsuyama et al. 2004).

2.2.3. Thua-nao

The alkaline-fermented food condiment made from soybeans in Thailand is called *thua-nao* (Leejeerajumnean et al. 2001). It is widely used to flavor vegetable soups and hot dishes in the northern regions of Thailand as a substitute for fermented fish (Wang and Fung 1996). Soybeans are washed, soaked overnight, boiled for about 3–4 h, mashed and wrapped in banana leaves to ferment. Fermentation lasts for 2–3 days and the product may further be allowed to ferment outdoors exposed to sunlight, resulting in a dried form of *thua nao* which can be kept for several months. Fresh *thua-nao* can be consumed by steaming or roasting and the dried product is an important ingredient in various local dishes. Chantawannakul et al., (2002) and Inatsu et al. (2006) have reported that the main microorganism responsible for fermentation of *thua-nao* is *Bacillus subtilis.*

3. Raw materials of alkaline fermented foods

Most of the indigenous alkaline-fermented foods discussed above are derived from the seeds of various wild trees, but cultivated plants are also an important source of these seeds and grains (Table 1a and Table 1b). These plants and trees with great agronomic and commercial potential as food crops abound in Africa and Asia (Dirar 1993; Steinkraus 2004). Studies carried out to control and improve their propagation, conservation and fruit production are discussed briefly below.

3.1. Wild trees

3.1.1. African locust bean

The African locust bean, *Parkia biglobosa (*Jacq.) Benth (Fig. 3) is an indigenous tree legume which belongs to the sub-family *Mimosoideae* in the *Leguminosae* family (Timmer et al. 1996). The tree can reach up to 20 m in

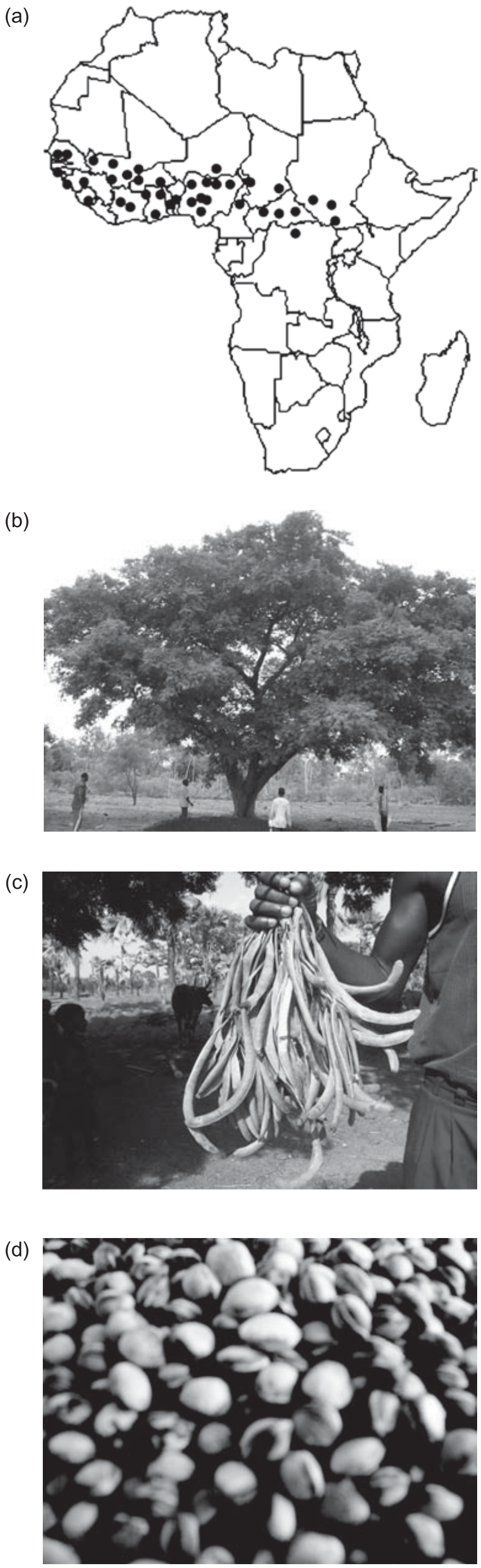

Figure 3. (a) Distribution of *Parkia biglobosa* (adapted from Hall et al. 1997), (b) *Parkia biglobosa* tree, (c) *Parkia biglobosa* pods, (d) *Parkia biglobosa* seeds.

height (Iwuoha and Eke 1996) and is widely distributed through the Sudan and Guinean savanna. This extends from the western coast of Africa in Senegal across to Sudan (Fig. 3a). The locust bean trees play an important role in the cycling of nutrients from deep soils by holding soil particles together with their roots to prevent soil erosion (Teklehaimanot 2004). African locust bean trees are usually carefully conserved by the local inhabitants due to their importance as valuable source of reliable food, especially the seeds, which are used to produce African *dawadawa* (Campbell-Platt 1980). African locust bean seeds contain 35–40% of proteins, 30–32% of lipids, 16–24% of carbohydrates and 4–6% of minerals (Diawara and Jakobsen 2004). The amount of fruit produced per year ranges from 15 to 130 kg per tree (Teklehaimanot et al. 2004). Investigations have shown that it is possible to domesticate locust trees through selection and breeding (Pettersson and Knudsen 2001; Teklehaimanot et al. 1996; Teklehaimanot et al. 2000). These studies have also helped to develop vegetative propagation methods that allow multiplication of superior trees and on-farm domestication. Timmer et al. (1996) showed that pruning of locust trees increase fruit production.

3.1.2. Saman tree

Samanea saman (Jacq.) Merr. (syn. *Albizia saman* (Jacq.) F.v. Muell.) commonly called the Saman tree is a leguminous tree species with potential in agroforestry (Durr 2001). It belongs to the *Leguminosae* family and the *Mimosoideae* sub-family (Cascante et al. 2002). Also called the rain tree, it is a large tree native to tropical America, and which has now become widespread throughout the humid and sub-humid tropics (Durr 2001). The height of the tree can reach up to 20–25 m if grown individually or up to 40 m in natural forests and plantations (Durr 2001; Janzen 1982). The pods are typically 10– 20 cm long with a sticky sweet pulp filling the mesocarp and a total of 12 to 20 red-brown seeds (Durr 2001; Ogunshe et al. 2006). These seeds are usually used to produce *aisa*.

3.1.3. Baobab

Adansonia digitata L. called the baobab tree in both English and French is very characteristic of the Sahelian region (Fig. 4) and belongs to the family of *Bombacaceae* (Diop et al. 2006). The baobab is a very massive tree with a very large trunk (up to 10 m diameter) which can grow up to 25 m in height and may live for hundreds of years. The fruit is ovoid and contains about a hundred seeds (Diop et al. 2006). Nkafamiya et al., (2007) reported that baobab seeds contain 21.75, 5.01, and 6.71 g/100 g of protein, ash and fiber, respectively, and concluded that the seeds could be an alternative sources of human food despite the presence of some anti-nutrients which

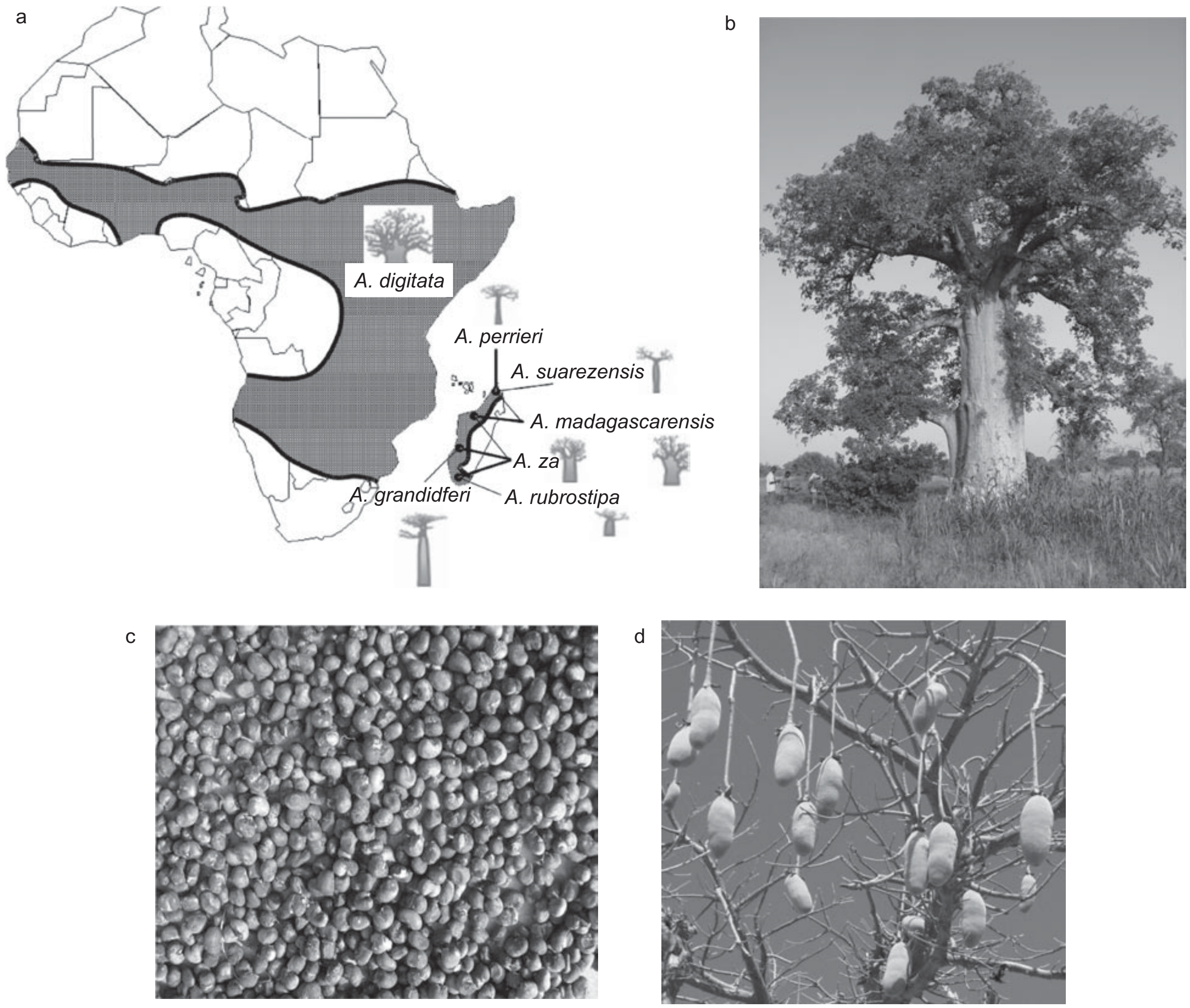

Figure 4. (a) Distribution of baobab in Africa (Diop et al. 2006), (b) Baobab tree, (c) Baobab seeds, (d) Baobab fruits.

are below the established toxic levels (oxalate, phytate, saponin and tannin representing 10.31, 2.00, 7.16, and 2.84% of seeds respectively) (Nkafamiya et al. 2007). The baobab tree can be propagated by seeding or vegetative multiplication (Diop et al. 2006; Ishii and Kambou 2007). Seeds of *Adansonia digitata* are used to produce *maari*.

3.1.4. African oil bean

The African oil bean, *Pentaclethra macrophylla* Benth, is a large leguminous timber tree belonging to the *Fabaceae* family and *Mimosoidae* sub-family. The tree is found in the humid and some parts of the sub-humid zones of West and Central Africa and can reach up to about 21 m in height and 60 cm in girth. The pods are about 40–50 cm long and 5–10 cm wide and contain between 6 and 10 flat glossy brown seeds which vary in size. The seeds contain essential fatty acids and twenty different amino acids and are a potential source of protein and calories (Enujiugha and Agbede 2000; Enujiugha 2003). African oil bean trees are available as both wild and cultivated plants (Enujiugha 2003). Nigerian *ugba* is obtained from the alkaline fermentation of seeds of African oil bean.

3.1.5. Mesquite

Prosopis africana, commonly called Mesquite, belongs to the *Fabaceae-Minosoidae* family. The natural distribution of *P. africana* extends from the sahelian and savannah woodlands of Senegal in West Africa to Ethiopia in East Africa. From Ethiopia it extends north to Egypt and Sudan and south to Lake Victoria in Kenya (Tchoundjeu et al. 1997). The mesquite tree can reach up to 20 m in height. Each tree produces up to an average of 300 pods and each pod (15 × 3 cm) contains 10–15 seeds (Tchoundjeu et al. 1997), which can be fermented into *okpehe*. According to Barminas et al., (1998) the proximate composition of mesquite seeds in terms of protein (20.54%), ash (5.67%), lipid (12.93%), and fiber (6.51%) is comparable to Africa locust beans seeds. Due to the importance of the mesquite tree, the International Centre for Research in Agroforestry (ICRAF, Mali) has made a germplasm collection from 34 sites in order to conserve the genetic diversity within this species before the valuable genetic resources are lost (Tchoundjeu et al. 1997).

3.2. Cultivated plants

3.2.1. Soybean

Soybean, *Glycine max* (L.) Merr, belongs to the *Fabaceae* family and *Faboideae* subfamily. Soybeans are an important ingredient in the diet of people in many parts of the World (Karr-Lilienthal et al. 2005). While soybean grains are commonly used to produce many fermented foods like *natto, tempeh, miso,* and *kinema* in some Asian countries (Steinkraus 1996), they are being promoted as an alternative to locust beans as raw material for *dawadawa* production in West Africa (Dakwa et al. 2005). Due to their high content of protein (44.6%), fat (21%), ash (3%), and crude fiber (5.9%), many studies have been carried out to use them to produce fermented foods (Dakwa et al. 2005; Kiers et al. 2000; Omafuvbe et al. 2000; Sarkar et al. 1994; Sarkar et al. 1995; Sirisomboon et al. 2007). These studies reported that fermented products from soybeans can be used to address the problem of protein malnutrition beside their important role as flavor enhancer.

3.2.2. Bambara groundnut

Vigna subterranea commonly known as bambara groundnuts or bambara beans is an indigenous African crop. It is drought tolerant and easy to cultivate. The average yearly yield is 650–850 kg/ha (Baryeh 2001). Bambara groundnuts serve as an important source of protein in the diets of a large percentage of the population in Africa, particularly poorer people who cannot afford expensive animal protein (Amadi et al. 1999; Amarteifio et al. 1997; Barimalaa et al. 1994). Amarteifio et al. (1997) reported that bambara groundnut contains 17.4% protein, 53.1% carbohydrate, 6.1% fat, 6.1% fiber, 3.4% ash, 0.098% calcium, 0.007% iron, 1.2% potassium, and 0.003% sodium. *Dawadawa-type* is a product obtained from the fermentation of Bambara groundnut.

3.2.3. Roselle

Roselle or sorrel botanically known as *Hibiscus sabdariffa* is an erect annual herb plant belonging to the Malvaceae family which grows widely in Central and West Africa, South East Asia, and elsewhere (Alwandawi et al. 1984). Alwandawi et al. (1984) reported that the seed yields are 1250 to 2500 kg/hectare for Indian cultivars. The calyces of the flower are consumed worldwide as a cold beverage or hot drink (Ali et al. 2005; Omobuwajo et al. 2000). Their use as treatment for several complaints has been reported by Ali et al. (2005). Whole seeds with 93% dry matter have an approximate composition of 28.69% proteins, 21.93% crude fat, 6.32% ash, and 26.39% total carbohydrates (Parkouda et al. 2008). Used to produce *bikalga*, Roselle seeds have been reported to be a good source of proteins and lipids as well as K, Na, Mg, and Ca (Alwandawi et al. 1984; Eladawy and Khalil 1994).

3.2.4. Other cultivated plants

Seeds from other cultivated plants are also used as raw material for fermented food condiments (Table 1a and Table 1b) including cotton seeds, *Gossypium hirsutum* L. (Sanni et al. 1991), melon seeds, *Citrullus vulgaris* (Abaelu et al. 1990; Omafuvbe et al. 2004), African yam bean, *Sphenostylis stenocarpa* (Ogbonna et al. 2001), castor oil bean, *Ricinus communis* (Aniche et al. 1993), and sesame seeds (Elfaki et al. 1991).

4. Main modifications during transformation

4.1. Biochemical changes during transformation

4.1.1. Proteins

Bacillus species are responsible for degradation of proteins during alkaline fermentation (Odunfa 1985; Ouoba et al. 2003b). Several studies have recorded increases in protease activity during alkaline fermentation (Odunfa 1985; Oguntoyinbo et al. 2007). Odunfa (2005) and Wang and Fung (1996) consider proteolysis to be the most important metabolic activity during alkaline fermentation. Omafuvbe et al. (2000) and Terlabie et al. (2006) recorded increasing protease activity throughout 72 h of *soydawadawa* fermentation. During locust bean fermentation, both Odunfa (1985) and Ouoba et al. (2003b) reported decreases in proteolytic activity after 36–48 h following initial increase in the enzyme activity. Comparing proteolytic activity of different species, Ouoba et al. (2003b) reported that proteolysis during fermentation of African locust bean seeds is species and strain dependant. *Bacillus subtilis* strains showed higher proteolytic activity than *B. pumilus* leading to a release of a higher quantity of amino acids.

Several studies have reported increasing levels of free amino acids, non-protein and soluble nitrogen content during alkaline fermentation (Abaelu et al. 1990; Azokpota et al. 2006; Gernah et al. 2007; Ogbonna et al. 2001; Ogunshe et al. 2007; Omafuvbe et al. 2000; Sarkar and Tamang 1995; Terlabie et al. 2006). Ouoba et al. (2003b) recorded increases in the concentration of cysteine, methionine, leucine, isoleucine, tyrosine, phenylalaline and even lysine which is limiting in plant foods during soumbala fermentation. Achi (2005) reported loss of lysine and other essential amino acids on prolonged fermentation which is of concern since lysine is reported by Diawara et al. (1992) to be limiting in some African dishes. It is generally accepted that proteolysis due to *Bacillus* spp. results in the production of amino acids and ammonia from proteins which is responsible for the increase in pH during alkaline fermentations (Aderibigbe and Odunfa 1990; Ouoba et al. 2003b).

Consumption of the indigenous alkaline condiments is often limited by their atypical odor which some people find objectionable. The odor is partly due to proteolysis of proteins and utilization of released amino acids by *Bacillus* spp. leading to formation of ammonia and rise in pH. Above pH levels of about 8–8.3, sufficient ammonia is present to give along with other volatile compounds a strong ammonia-like odor to the product, which readily reaches objectionable levels (Campbell-Platt 1980; Odunfa 1986; Sarkar, Cook and Owens 1993; Ouoba et al. 2005).

According to Allagheny et al., (1996) by limiting the growth and metabolism of the bacteria while not inhibiting the action of flavor generating proteolytic enzymes, production of ammonia can be restricted. This is achieved in *natto* production by holding the fermented beans at sufficiently low temperature during maturation. By adding glycerol as humectant to partially fermented locust bean or soybean cotyledons or by restricting oxygen supply, Allagheny et al. (1996) reported that bacterial growth and ammonia formation were arrested while enzymatic activity continued during *dawadawa* fermentation.

4.1.2. Carbohydrates

Most seeds used to produce alkaline-fermented condiments are rich in carbohydrates of which a large proportion are non-digestible oligosaccharides (Sarkar et al. 1997a). The main poly- and oligosaccharides contained in some of the seeds are stachyose, raffinose, sucrose and arabinogalactan (Aderibigbe et al. 1990; Karr-Lilienthal et al. 2005; Kiers et al. 2000; Odunfa 1983; Terlabie et al. 2006). In addition soybean seeds contain verbascose (Karr-Lilienthal et al. 2005). Some of these carbohydrates are non-digestible and have been shown to be associated with abdominal distension and flatulence in humans (Naczk et al. 1997; Sarkar et al. 1997a). During fermentation microorganisms hydrolyze non-digestible carbohydrates into sugars which are readily digestible by humans and in addition positively influence the texture of the product by softening the tissue (Odunfa 1983; Ouoba et al. 2007a; Sarkar et al. 1997a). *Bacillus* spp. are producers of amylase, galactanase, galactosidase, glucosidase, and fructofuranosidase, enzymes involved in degradation of carbohydrates during alkaline fermentation (Aderibigbe et al. 1990; Kiers et al. 2000; Omafuvbe et al. 2000; Sarkar et al. 1997a).

Omafuvbe et al. (2000) and Terlabie et al. (2006) recorded decreases in the total sugar concentration in soybeans during fermentation. Carbohydrate loss during processing has been attributed to leaching of some soluble carbohydrates during prolonged cooking and hydrolyses of starch to fermentable sugars which are utilized as carbon sources by the fermentative microorganisms (Odunfa 1985; Odunfa and Adewuyi 1985; Yagoub et al. 2004). Omafuvbe et al. (2000), Dakwa et al. (2005) and Terlabie et al. (2006) all recorded rapid increases in α-amylase activity in soydawadawa during the first 48 hours of fermentation. Omafuvbe et al. (2000) and Ouoba et al. (2007a) demonstrated the degradation of oligosaccharides (galactamannan, arabinogalactan, stachyose, and raffinose) by *Bacillus* spp. to simple sugars (melibiose, fructose, galactose).

4.1.3. Lipids

Several workers have reported that the crude fat content and fatty acid profiles change during alkaline fermentation (Ogunshe et al. 2007; Omafuvbe et al. 2000; Terlabie et al. 2006). The dominant microbial species involved in the alkaline fermentations have been shown to possess lipolytic activity (Abaelu et al. 1990; Gernah et al. 2007; Ndir et al. 2000; Omafuvbe et al. 2004; Sarkar et al. 1995; Sarkar et al. 1994). Antai and Ibrahim (1986) attributed oil degradation in African locust beans during fermentation to enzymes produced by *Staphylococcus* or *Leuconostoc* species. More recently Ouoba et al. (2003a) reported high lipolytic activity during degradation of locust bean oil by *B. subtilis* and *B. pumilus.* This activity was also species and strain dependant, *B. pumilus* showing significant higher lipolytic activity than isolates of *B. subtilis*. In both *kinema* and *dawadawa* fermentation increase in crude fat concentration has been reported (Azokpota et al. 2006; Gernah et al. 2007; Sarkar et al. 1994), While decreasing concentrations have been reported in *ogiri* and African yam bean *owoh* (Ogbonna et al. 2001; Omafuvbe et al. 2004).

Generally, increasing free fatty acids levels during alkaline fermentation have been reported (Dirar 1993; Ogunshe et al. 2007; Sarkar et al. 1995). However, for *dawadawa* contradicting results have been published (Odunfa and Adesomoju 1985; Antai et al. 1986). Odunfa et al. (1985) reported a decrease while Antai et al. (1986) reported an increase in the fatty acid concentration. Ouoba et al. (2003a) have attributed the different results to the predominance of different *Bacillus* spp. having different lipolytic activities.

The composition of the free fatty acids in locust bean seeds according to Aiyelaagbe et al., (1996) consists of 89.8% saturated fatty acids, 8.9% polyunsaturated fatty acids and no monounsaturated fatty acids whereas Ndir et al. (2000) have reported 39.2%, 44.7%, and 15.5% of these fatty acids respectively. In the fermented product, *netetu*, Ndir et al. (2000) reported the presence of 30–42.9% saturated fatty acids, 41.9–46.8% polyunsaturated fatty acids and 13.2–14.8% monounsaturated fatty acids. The presence of palmitic, stearic, arachidic, behenic, lignoceric, linolenic, and gadoleic acids and high concentrations of linoleic and oleic acids have been reported in *netetu* and *soumbala* by Ndir et al. (2000) and Ouoba et al. (2003a). In addition Ouoba et al. (2003a) found palmitoleic and myristic acids in *soumbala* and reported that no free fatty acids with less than 14 carbon chains could be detected. Ndir et al. (2000) also found the concentration of tocopherol in *netetu* to be very low, 17.7–30.6 mg × 100 g^{-1}. The presence of linoleic and oleic acids which can be converted into polyunsaturated fatty acids enhance the nutritive value of the products since they are essential for human nutrition (Gurr 1993). Chin et al. (1992) showed that conjugated linoleic acid has an anticarcinogen and anticarcinogenic effect in several animal models.

4.1.4. Formation of aroma compounds

The widespread use of indigenous alkaline condiments in many African and Asian countries is due to their pleasant taste, peculiar odor and important nutritional qualities (Azokpota et al. 2008; Dirar 1993; Ouoba et al. 2005; Owens et al. 1997). Flavor of indigenous condiments has mainly been attributed to various volatile compounds produced through the metabolic activities of *Bacillus* spp. during fermentation (Azokpota et al. 2008; Beaumont 2002; Leejeerajumnean et al. 2001; Ouoba et al. 2005).

Beaumont (2002) regards the amino acid content of *dawadawa*, in particular glutamate which contributes to flavor enhancement, as well as peptides and aroma volatile constituents to be responsible for the flavor of the product. In *afitin*, *iru* and *sonru*, Azokpota et al. (2008) identified 2,5–dimethylpyrazine, tetramethylpyrazine, 3-methylbutanal, 2-decanone, 3,5-dimethylphenylmethanol, ethyl linoleate and chlorobenzene as important aroma compounds and mentioned other pyrazines, aldehydes, ketones, esters, alcohols, acids, alkanes, alkenes, benzenes, phenols, sulphurs, and furans. In addition to these compounds Ouoba et al. (2005) found amines and pyridines in *soumbala*. It has been reported that aroma volatiles of fermented African locust bean seeds vary qualitatively and quantitatively according to the species and strain of *Bacillus* involved in the fermentation (Ouoba et al. 2005). The heat treatment during the production contributes to the flavor by formation of pyrazines originating from reactions between sugars and amino acids (Ouoba et al. 2005; Owen et al. 1997). Owen et al. (1997) reported a high production of acetoin, 2,5-dimethylpyrazine and trimethylpyrazine during *Bacillus subtilis* fermentation of soybean seed, While Leejeerajumnean et al. (2001) reported the major volatile compounds in fermented soybeans to be 3-hydroxybutane, 2-methylbutanoic acid, pyrazines, dimethyldisulphide, and 2-pentylfuran. Dakwa et al. (2005) found pre-treatment of soybeans by either boiling or roasting before fermentation to affect the volatile aroma profile of *soy-dawadawa* and detected the presence of 3-methyl butan-1-ol, 2-methyl-1-propanol, benzaldehyde, 5-methyl-2-phenyl-2-hexenal, 3-methylbutyl pentanoate, hexadecanoic acid, trimethyl pyrazine, and tetracosane amongst others in both types of *soy-dawadawa*. The major volatile compounds in *natto* and *thua-nao* have been reported by Owens et al. (1997) to be ketones, acids, and pyrazines. According to Leejeerajumnean et al. (2001) some volatile aroma compounds are lost during sun-drying of such products. Free fatty acids are also known to contribute positively to the production of characteristic flavors in food but high levels of free fatty acids may cause rancidity easily (Nawar 1985).

4.2. Physical changes

Important features of alkaline-fermentations are changes which occur in the texture of the products during fermentation as the seeds soften. Mbajunwa et al., (1998) and Njoku et al., (1990) demonstrated the ability of *B. subtilis* to soften the tissue of African oil beans leading to the desired texture of *ugba* and suggested that the strain may possess pectic and proteolytic enzymes that readily hydrolyzed the pectin and protein components of the beans. Mbajunwa et al. (1998) further suggested that the color of *ugba* develops during cooking due to non-enzymic browning reactions involving the amino groups of amino acids and the non-reducing sugar constituents of the oil bean and microbial activity. Various alkaline fermentations involving *B. subtilis* as main microorganisms for production of foods as *ugba*, *natto*, and *soumbala* are characterized by the extensive production of whitish mucilaginous polymers. In the case of *ugba* the polymers are assumed to be polysaccharides, which lead to the sticking together of the slices (Mbajunwa et al. 1998). For *natto*, Tanimoto et al. (2001) reported that the mucilage observed is composed of 44% of carbohydrates and 54% of proteins. A major part of the proteins content is constituted of glutamic acid assumed to be poly-γ-glutamic acid.

5. Indigenous alkaline-fermented condiments as functional foods

The raw materials used to produce the alkaline condiments are substrates in which *Bacillus* spp. grow and produce metabolites and enzymes recognized to have beneficial effects on health (Achi 2005; Dahal et al. 2005; Wang and Fung 1996). *Bacillus* spp. including *B.*

subtilis are used as probiotic for human and farm animals (Sanders et al. 2003, Vaseeharan and Ramasamy 2003). Specific probiotic effects have not yet been documented for strains isolated from alkaline African fermented food. However, Guo et al. (2006) showed that *B. subtilis* from fermented foods in Asia produces metabolites which inhibit *E. coli, Salmonella typhimurium,* and *Staphylococcus aureus* and suggested *B. subtilis* as a promising alternative to antibiotics which can also be used as a probiotic. Ko et al. (2004) and Pais et al. (2006) showed that nattokinase, (a pro-fibrinolytic enzyme) extract from *natto* might be used to combat diseases such as cardio- and cerebrovascular diseases. Ikeda et al., (2006) and Katsuyama et al. (2004) reported that *natto* contains several biologically active components which contribute individually or synergistically to health and that *natto* intake may help promote bone formation in menopausal women as well as prevent postmenopausal bone loss.

African and Asian alkaline condiments often have numerous advantages over the raw materials from which they are made (Addy et al. 1995; Ko et al. 2004; Igboeli et al. 1997; Mamiya et al. 2007; Nnam et al. 2003; Pais et al. 2006; Sarkar et al. 1997b; Sarkar et al. 1998). The fermentation can simultaneously create or improve flavor and texture, appearance, aroma and nutritional status, destroy or mask undesirable flavors, reduce or eliminate carbohydrates causing flatulence, decrease the required cooking time and increase shelf life (Wang and Fung 1996; Steinkraus 1996). Akaaimo et al. (2006), Ogbonna et al. (2001), Omafuvbe et al. (2000) and Sarkar et al. (1995) reported that some fermented condiments are a good source of protein, lipid, carbohydrates and mineral elements such as iron, potassium, calcium, magnesium, sodium and zinc. Sarkar et al. (1998) recorded increases in vitamin B_2 (riboflavin) and vitamin B_3 (niacin) during kinema production, While vitamin B_1 (thiamine) decreased during soaking and increased during the subsequent fermentation.

Addy et al. (1995) reported degradation of trypsin inhibitor while Nnam et al. (2003) reported reduction of the antinutrients, phytate and tanins, during fermentation of baobab seeds.

6. Food safety of alkaline fermented products

It is generally recognized that alkaline-fermentation produces safe products (Steinkraus 1997). For most alkaline fermented foods, raw materials undergo a long cooking time (up to 40 h in some cases) prior to fermentation and this contributes to an elimination of non-spore–forming pathogenic bacteria. Fermented products are most of the time cooked in various dishes and this allows an elimination of pathogenic contaminants after drying and storage. In addition, the alkaline fermented products are stable and well-preserved due to the antimicrobial effects of the dominant microorganisms towards harmful bacteria and moulds. Ouoba et al. (2007b) found *B. subtilis* and *B. pumilus* isolated from soumbala to be able to inhibit and inactivate both Gram-positive and Gram-negative bacteria including *Micrococcus luteus, Staphylococcus aureus, Bacillus cereus, Enterococus facium, Listeria monocytogenes, Escherichia coli, Salmonella typhimurium, Shigella dysenteriae, Yersinia enterocolitica,* as well as ochratoxigenic moulds such as *Aspergillus ochraceus*. Guo et al., (2006) showed that *B. subtilis* from fermented foods produces metabolites which inhibit *E. coli, Salmonella typhimurium* and *Staphylococcus aureus* and suggested *B. subtilis* as a promising alternative to antibiotics which can also be used as a probiotic. Ndir et al. (1994) reported the ability of *Bacillus* strains isolated from *netetu* to produce antifungal substances including iturin A, surfactin, and another compound with the same retention time as mycosubtilin and bacillomycin L. Nout et al. (1998) showed the capacity of *B. subtilis* to reduce growth of *B. cereus* and prevent its production of enterotoxin during *kinema* fermentation. It has also been reported that predominant *B. subtilis* do not produce enterotoxins and or cereulide (Ouoba et al. 2008). In addition the high pH value and free ammonia present in alkaline fermented foods makes it rather difficult for microorganisms which might otherwise cause spoilage of the product to grow (Steinkraus 1996). During fermentation elimination or decrease of antinutritional factor is also observed making then the food more edible (Odunfa 1988; Ouoba et al. 2007a; Sarkar et al. 1997a; Steinkraus 1996).

Even though alkaline fermented products generally can be considered safe as mentioned above, they can sometimes involve a risk of food borne disease. This is a problem common to all types of traditional fermented foods. One factor, which may contribute to make alkaline fermented foods unsafe, is the fact that the production is made in homes using rudimentary equipment under poor hygienic conditions (Sanni 1993; Steinkraus 1997). This allows a constant recontamination of the fermented seeds. Besides, producers are not trained to apply at least Good Manufacturing Practices (GMP). Another major problem is that the fermentation is totally uncontrolled with variable microorganisms present. A presence of fermenting but potentially pathogenic spore forming bacteria such as *B. cereus* is sometimes observed (Azokptota et al. 2007; Ouoba et al. 2007). *B. cereus* at levels of 10^{6-8} CFU/g has been detected (Oguntonyinbo and Oni 2004). This bacterial species is able to cause vomiting and diarrhoea through the production of emetic toxin (cereulide) and enterotoxins, respectively (Elling-Schulz et al. 2004, Granum 2001; Ouoba et al. 2008). It is important to avoid cereulide production in foods, because cereulide in contrast to enterotoxins is stable to heat, extreme pH (pH 2–11), and is not degraded by the digestive enzymes, pepsin and trypsin (Shinagawa et al. 1995; Shinagawa et al. 1996). Cereulide production in African locust beans by an emetic *B. cereus* strain isolated from *sonru* showed to be dependent on the fermentation temperature being strongly inhibited at 40°C (unpublished results). Consumption of foods contaminated with *B. cereus* will not necessarily result in diarrhoea (Langeveld et al 1996), and interestingly no report on sickness related to *B. cereus* from alkaline fermented foods has been documented. Presence of potential pathogenic microorganisms like *Staphylococcus* spp. *Escherichia coli, Salmonella* spp., *Shigella* spp., and *Clostridium* spp. has also been reported especially during drying and storage (Antai et al. 1986; Ndir et al. 1994, Ndir et al. 1997; Odunfa 1981; Roy et al. 2007). However, these bacteria will be easily eliminated during cooking of dishes to which the fermented product is added.

The safety of alkaline traditional fermented foods can be improved by various actions. First, producers should be trained to ferment foods under hygienic conditions based upon guidelines of GMP and HACCP. For production of *soumbala* HACCP as quality assurance system has been proposed and documented (Diawara et al. 1998). Further, the development of better equipment should be encouraged: i.e., improved fermentors constructed with maximum emphasis on maintenance, sanitation and reliability for the process, and minimum capital investment and operating cost. One major factor, which could positively improve the quality of products, is a development of starter cultures for controlled fermentation. Microorganisms might be well identified and selected to conduct the fermentation and to produce a desirable safe product. The results of these positive actions will lead to fermented products with improved nutritional and hygienic quality as well as stability.

7. Starters for controlled fermentation

Several studies have been carried out to isolate microbial strains which can be used as starter cultures for the production of the various alkaline fermented condiments based on their technological and antimicrobial properties (Amoa-Awua et al. 2006; Gernah et al. 2007; Oguntoyinbo et al. 2007; Tamang and Nikkuni 1998).

Japanese *natto* is already produced industrially using pure cultures of *B. subtilis* var. *natto* (Wang and Fung 1996; Kiuchi 2004). This involves about 500 companies with the ten largest companies producing about 260,000 metric tons yearly accounting for 85% of the total production in Japan (Kiuchi 2004). Kiuchi (2004) has elucidated the differences between the *natto* starter culture and other strains of *B. subtilis*. The specific characteristics of *natto* (flavor, physical aspect) were obtained only by the use of *Bacillus subtilis* var. *natto*.

Sarkar et al. (1994) and Sarkar et al. (1995) have reported that strains of *B. subtilis* are the main organisms responsible for producing *kinema* with satisfactory organoleptic quality. Tamang and Nikkuni (1996) have proposed *B. subtilis* KK-2:B10 and *B. subtilis* GK-2:B10 as starter cultures for *kinema* production based on biochemical and sensory analysis. Similarly, Visessanguan et al., (2005) have proposed *B. subtilis* TISTRO (BIOTEC 7123) as one of the most suitable starter cultures for *thua-nao* production. Sarkar et al. (1997a) found *B. subtilis* DK-W1 capable of degrading compounds responsible for flatus resulting from soybean ingestion.

In various studies, Ouoba et al. (2003a; 2003b; 2007a; 2005; 2007b) showed the ability of *B. subtilis* and *B. pumilus* to degrade African locust bean oil, proteins and carbohydrates, and also their ability to produce a pleasant aroma and antimicrobial substances against pathogens. They have proposed *B. subtilis* B7 and B15 as suitable starter cultures for the controlled production of *soumbala*.

For *ugba* production, Sanni et al. (2002) suggested strains of *B. subtilis*, especially *B. subtilis* MM-4:B12, as potential starter cultures based on their enzymatic activities and ability to produce stickiness. Amoa-Awua et al. (2006) have screened 42 *Bacillus* isolates from *soydawadawa* and proposed *B. subtilis* $24BP_2$ and *B. subtilis* $FpdP_2$ as the best starter cultures for *soydawadawa* production. Further work by Terlabie et al. (2006) rated *B. subtilis* $FpdP_2$ as the best starter culture.

8. Conclusion: Perspectives and Research Needs

To date a number of investigations have been carried out to study the biochemical and microbiological changes which occur during indigenous alkaline fermentations in both laboratory and commercial production. These investigations have provided information on the nutritional importance of these indigenous food condiments, their safety and antimicrobial properties, and suitable starter cultures which can be used to produce a number of these products have been identified. The studies generally suggest improvement of the traditional procedures to enhance the nutritional quality as well as the microbiological safety of the products. However, this should be done without unduly altering the traditional appearance and taste of the products. Training of producers is also recommended as one of the most effective interventions to ensure safety and defined quality of these alkaline foods.

The indigenous alkaline fermented condiments are being promoted in some countries because of their beneficial role in the diet, and there is increasing demand for such products in both rural and urban areas (Diawara and Jakobsen 2004). However, to generate sufficient data for industrial production, optimal processing parameters such as temperature and time for cooking, effect of heat treatment on nutritional value, effect of sun drying on final product quality, selection of raw materials including cultivar, water quality, softening agents and food additives, packaging, shelf life as well GMP and HACCP for each product should be investigated further. Such investigations will help to develop these indigenous products as functional foods for the international market. For some of the products the dominant microbial strains responsible for the fermentation are yet to be confirmed by molecular biology-based methods which are currently basic tools in microbiological research. The use of DGGE for example in a culture-independent approach will give more information on microbial population dynamics during fermentation. Unfortunately such procedures are still largely unavailable in most of the laboratories in the developing countries due to limited financial resources and other factors, and there is still the need for collaboration between laboratories in developing and developed countries to investigate these indigenous fermented foods.

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