N PAKISTAN JOURNAL OF UTRITION

Microbial Studies and Biochemical Characteristics of Controlled Fermented *Afiyo*- a Nigerian Fermented Food Condiment from *Prosopis africana* (Guill and Perr.) Taub

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Abstract: One hundred and fifteen bacterial strains isolated from fermenting *Prosopis africana* during a controlled production of *okpehe*, a Nigerian food-seasoning condiment most popular among the middle belt states of Nigeria were characterized as *Bacillus subtilis*, *Bacillus pumilus*, *Bacillus licheniformis*, *Bacillus megaterium*, non-sporing *Staphylococcus* species and *Escherichia coli* according to their differences in morphological, microscopic and biochemical characteristics using the bacterial taxonomic tools. There was no isolation of fungi throughout the fermentation period. The biochemical changes and enzymatic activities in fermenting *okpehe* mash were investigated. Reducing sugars increased from 2.0 mg g^{-1} to 11.6 mg g^{-1} during the first 2 days of fermentation but subsequently decreased to 7.8 mg g^{-1} at day 6 while total soluble sugars decreased from 13.4 mg g^{-1} at day 1 to 5.8 mg g^{-1} at day 6. The most significant biochemical activity during the fermentation was the rapid and steady increase in the quantity of free amino acids throughout the fermentation period from 43.7 mg g^{-1}- 70.0 mg g^{-1}. Proteinase activities increased from 0.51 − 0.71 U ml^{-1}. α-amylase activities were not consistent but had their peaks at days 1 and 3, while lipase activities were maximal at days 3 and 5 of fermentation. The role of each associated bacteria in the fermenting *okpehe* indicated *B. licheniformis*, *B. megaterium* and *Bacillus subtilis* as the most active bacteria involved in the controlled fermentation without masking the fermented cotyledons after 3-6 months storage, but smoking as post fermentation treatment changed the colour of the condiment from dark brown to black.

Key words: Bacteria, biochemical, enzymatic, controlled fermentation, food condiment, *Prosopis africana*

Introduction

Prosopis africana (mimosaceae) Guill and Perr Syn. P. Oblonga Benth is a savannah tree, 40-60 feet high and up to 7 feet in girth. Though the common name of *Prosopis africana* is Mesquite, (Schuster, 1969; Burkart, 1976), its native Nigerian names are *kiriya* (Hausa), *kohi* (Fulani), *sam chi lati* (Nupe), *kpaye* (Tiv), ayan (Yoruba) and *ubwa* (Ibo) etc. (Keay, 1964; Ogunshe, 1989). *Afiyo* as called by the Hausas or *okpehe* as known by the Idomas of Benue State is a fermented food flavouring condiment most popular in the middle belt of Nigeria. It is produced from *Prosopis africana*, which is a leguminous oil seed, fermented in most parts of Benue, Niger, Kaduna states, and northernmost part of Kwara state. This fermented product of *Prosopis africana* is a strong smelling mash of sticky dark brown seed and fermentation is in moist solid state by chance inoculation, supposedly by various species of microorganisms (Ogunshe, 1989).

According to Odunfa (1985), over thirty different fermented foods have been recorded. Some of them are highly placed condiments while some serve as main meals (Odunfa, 1985). Most of the fermented vegetable proteins reported are from leguminous seeds. Of the thousand known legumes, less than twenty are used extensively today. Those in common use include peanuts, soyabeans, locust beans, oil beans, cowpeas, lentils, *alfalfa* (lucerne), etc. Some of the most important food condiments are *ogiri*, which is produced from melon seeds; *iru* or *dawadawa*, produced from locust bean (*Parkia biglobosa*); *ugba* produced from oil-bean seeds (*Pentaclethra macrophylla*); *ogiri-igbo* produced from castor oil seed (*Ricinus communis*) etc. (Odunfa, 1985). Several indigenous research studies have been carried out on the production of fermented condiments-*iru*-from various local legume and non-legume seeds (Eka, 1980; Odunfa, 1981a,b; Barber and Achinewhu, 1992; Omafuvbe *et al.*, 2000; 2002), however, to date, no fundamental study on the specific roles of the fermenting bacterial flora on the fermenting / fermented condiment(s), with the ultimate aim of developing indigenous starter culture(s) for the various popular and mostly-consumed condiments has been documented. The main objective of the present study is therefore to carry out a controlled fermentation using *afiyo* as a pilot study.

Materials and Methods

Samples collection: Harvested and dried seeds of *Prosopis africana* were purchased from the local markets in Ilorin, Kwara state and Benue State. Fermented samples were also purchased from the mammy markets in the Army Barracks, Ojoo, Ibadan, Oyo state and Oyingbo market in Lagos state.

Traditional preparation: Required quantity of harvested and dried seeds of *Prosopis africana* were soaked overnight and later boiled in a large earthen-ware pot for about 1-2 days with kerosene stove, during which the seed coats became soft and the seeds swollen. Seed coats were removed by either pounding the boiled seeds in a big mortar for faster removal of the seed coats or by pressing between fingertips.

The seeds coats were later decanted along with the washing water, leaving the clean seed cotyledons. The cotyledons were boiled for another 1-2 hours depending on the quantity of the seeds. The cotyledons were later drained through a sieve and wrapped with paw-paw leaves, traditional leaves or clean cement papers. The wrapped cotyledons were stacked together and then covered by nylon. These were then kept in an incubating unit for about 5-6 days to produce the fermented *Prospis* mash – *afiyo*, a strong-smelling mass of sticky brown cotyledons. The fermented cotyledons were covered by a whitish mucilaginous film produced during fermentation (Ogunshe, 1989).

Controlled preparation: The boiling step in the traditional process is long tedious and wasteful; it also requires more volume of water. This boiling by fuel (kerosene) was replaced by boiling in an autoclave in the laboratory. At each stage about 1000 g of *Prosopis africana* seeds were boiled at 121°C for 2 hours in an autoclave and later dehulled. The cotyledons were separated from the coats and later rinsed in sterile water, before boiling again in an autoclave for about 30 minutes to soften the cotyledons. The cotyledons were later drained through a sterile sieve and cooled to about 35°C before wrapping in paw-paw leaves already cleaned with alcohol. The wrapped cotyledons were then incubated in an incubating unit for 5-6 days to produce the usual fermented mash of *afiyo* (Ogunshe, 1989).

Isolation technique: The technique employed in isolating the microorganisms present in the fermented *afiyo* mash was the pour-plate method of Harrigan and McCance (1976) while the taxonomic tools used included those of Barritt (1936), Buchanan and Gibbons (1974) and Holding and Collee (1972). The isolations were done on plate count agar (PCA), nutrient agar (NA), MacConkey agar, eosin methylene blue agar, potato dextrose agar (PDA) and Sabouraud dextrose agar (SDA).

Physico-chemical analysis

pH determination: ph of the fermenting *afiyo* samples were determined using PYE Unicam pH meter (Model 9450).

Moisture content: Moisture content of the fermenting *afiyo* mash was according to the methods of A.O.A.C (1990).

Biochemical analyses: The fermenting *afiyo* samples collected at the different fermentation periods were dried in hot air oven at 80°C to a constant weight followed by grinding to powdered form for further analyses. The soluble sugars and free amino acids in the milled samples were extracted with 70 % ethanol water mixture (v/v) according to the method of Odibo *et al.* (1990).

Determination of sugars: The reducing sugars were determined using the modified Summer and Howell (1935) method. The total concentration was determined from a standard curve prepared using known concentration of maltose.
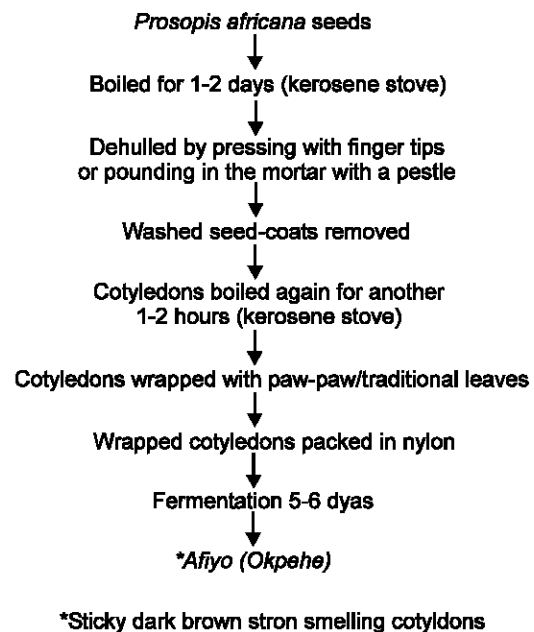
Prosopis africana seeds
↓
Boiled for 1-2 days (kerosene stove)
↓
Dehulled by pressing with finger tips
or pounding in the mortar with a pestle
↓
Washed seed-coats removed
↓
Cotyledons boiled again for another
1-2 hours (kerosene stove)
↓
Cotyledons wrapped with paw-paw/traditional leaves
↓
Wrapped cotyledons packed in nylon
↓
Fermentation 5-6 dyas
↓
Afiyo (Okpehe)

*Sticky dark brown stron smelling cotyldons

Fig. 1: Flow sheet for traditional preparation of *afiyo*

Proteinase: The extracting buffer was 0.1M sodium hydrogen phosphate (pH 6.9). The assay method was according to that of Yong and Wood (1977). The method was noted to be useful in the analysis of proteinase in the presence of reducing sugars normally found in food substances. The enzyme activity was expressed in terms of BLN arbitrary unit.

Lipase: The extracting buffer was 0.1M sodium-acetate-acetic acid mixture (pH 5.7). The modified Yong and

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Wood (1977) assay method was employed. The unit of enzyme was the amount of enzyme which liberated 1.0 mg of oleic acid per minute.

Dehulled by pressing between fingertips

↓

Prosopis africane seeds

↓

Boiled 121°C for 2 hrs or more in an autoclave

↓

Dehulled by pressing between fingertips

↓

Hulls separated from the cotyledons and reboiled at 121oC for 30 min. in an autoclave

↓

Cotyledons washed with sterile water, drained through sterile sieve, and cooled to 35°C

↓

Cotyledons wrapped in sterile paw-paw leaves

↓

Wrapped cotyledons incubated at 35°C for 5-6 days

↓

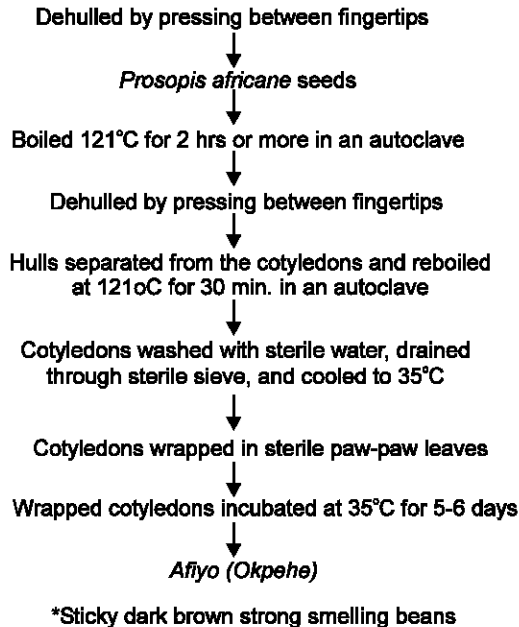*Afiyo (Okpehe)*

*Sticky dark brown strong smelling beans

Fig. 2: Flow sheet of controlled preparation of *afiyo*

Determination of free amino acids: The total free amino acids in the ethanolic extracts were determined by the ninhydrin calorimetric analysis method of Rosen (1957). The concentration of the amino acids was calculated from a standard curve based on known concentrations of leucine. Each determination was done in triplicates.

Enzyme assays
α- amylase: The extracting buffer was potassium hydrogen phosphate (pH 6.9). The assay method of Bernfield (1955) was used in the determination. The amount of reducing sugar was calculated from a standard curve prepared with known concentration of maltose.

Results
The moisture content of the fermenting *afiyo* mash increased from 50.1 % at day 1 of fermentation to 67.4 % at the sixth day of fermentation while the pH of the fermenting *okpehe* was between 7.8 and 8.3. There was an increase in temperature of the fermenting mash from 35°C at day 1 to 39°C at day 3 before a slight drop in temperature to 37°C (Table 1).
The viable bacterial counts of the fermenting *afiyo* mash increased in population throughout the fermentation period. The highest viable counts were obtained at day 6 of fermentation. The aerobic counts were between 3.5 x 10^9 cfu ml^{-1} at day 1 and 4.8 x 10^{13} cfu ml^{-1} at day 6 while the anaerobic counts were between 1.2 x 10^7 cfu ml^{-1} at day 1 and 5.1 x 10^{11} cfu ml^{-1} at day 6 (Table 1). Only

bacteria were found associated with the traditional and controlled fermenting *Prosopis* cotyledons since no fungi was isolated on potato dextrose agar (PDA), yeast extract agar and Sabouraud dextrose agar (SDA).
The bacterial species isolated from the fermenting *Prosopis africana* cotyledons during the controlled fermentation of *afiyo*, a food seasoning agent, were characterized as *Bacillus licheniformis, B. megaterium B. pumilus, B. subtilis,* and the only non-sporing *Staphylococcus* species, with *Bacillus licheniformis* and *B. subtilis* being the most predominant. All the *Bacillus* species were proteolytic and were able to ferment the boiled *Prosopis africana* seeds into a condiment with the characteristic *afiyo* aroma which is a strong ammonia-like smell.
The biochemical changes and enzymatic activities in fermented *afiyo* mash were investigated in this study and it was found that there were fluctuations during the period of *afiyo* fermentation. There was an increase in the soluble reducing sugars during the first 2 days of fermentation (2.0-11.0 mg g^{-1}) followed by a subsequent decrease (Fig. 3). There were fluctuations in the total soluble sugars during the controlled fermentation of *afiyo*. The total soluble sugars increased from 9.2 mg g^{-1} to 13.4 mg g^{-1} in the first 24 hours of fermentation before a drop on the second day of fermentation. The subsequent peaks however were at days 3 and 6 of the fermentation (Fig. 4). The of total amino acids level was found to increase throughout the period of fermentation from 43.7 mg g^{-1} at the first day of fermentation to 70.0 mg g^{-1} at day 6 (Fig. 5).
Of the three extracellular enzymes assayed for, α-amylase activities showed unsteady peaks at days 1 and 3 while lipase also showed unsteady peaks at days 1 and 3 (Fig. 6 and 8). Only proteinase activity showed increase in enzymatic activities with increasing fermentation period from 0.92 mg g^{-1} dry wt. – 7.1 mg g^{-1} dry wt. during the *afiyo* fermentation (Fig. 7).
Organoleptic results (not shown) indicated *Bacillus licheniformis, B. subtilis* and *Bacillus megaterium* as producing the highest characteristic parameters of *afiyo*.

Discussion
In the proximate composition of fermenting *Prosopis africana* cotyledons in the laboratory into *okpehe* by Ogunshe (1989), the moisture contents were between 68.0 % at the first day of fermentation and 72.8 % after the fifth day of fermentation, while Oguntoyinbo *et al.* (2001) recorded moisture contents of 40.7 % - 60.0 % in *okpehe* samples from market sources. Similar results in the study of Omafuvbe *et al.* (2004) indicated that the moisture content of the processed African locust bean and melon seeds ranged between 51.9-56.7% and 43.0-44.1% respectively. In the present study there was also an increase in the moisture content of the fermenting *afiyo* mash.

Table 1: Physico-chemical and proximate characteristics of the fermenting *afiyo* mash

Fermentation period	Temp. (^{0}C)	pH	moisture content (%)	aerobic (cfu ml^{-1})	anaerobic (cfu ml^{-1})
1	35.2	7.8	50.1	3.5×10^9	1.2×10^7
2	36.0	7.9	53.5	1.9×10^{10}	2.3×10^7
3	39.3	8.1	59.2	2.5×10^{11}	3.1×10^8
4	38.1	8.2	62.4	5.6×10^{11}	2.5×10^9
5	37.1	8.2	65.1	7.6×10^{12}	3.9×10^{10}
6	37.0	8.3	67.4	4.8×10^{13}	5.1×10^{11}

In the study of Omafuvbe *et al.* (2004) the pH values of fermenting African locust bean (*Parkia biglobosa*) was between 8.3-8.4 while the pH of fermenting melon seeds (*Citrullus vulgaris*) was between 7.2 and 7.9. Oguntoyinbo *et al.* (2001) recorded pH of 6.8-7.8 in *okpehe* samples from market sources while previous studies of Ogunshe (1989) recorded pH values of 7.5-8.2 in laboratory fermenting *okpehe* samples and pH of 7.0-9.0 in market *okpehe* samples. In the studies of Ogunshe *et al.* (2005), more of the *okpehe* (35.4%), samples had pH of 8.0. In this study however, the pH of the fermenting *okpehe* was between 7.8 and 8.3. The pH of the fermenting / fermented *afiyo* condiment being slightly alkaline agrees with earlier reports of Hesseltine (1965), Odunfa (1985), Ogbadu and Okagbue (1988), Ogunshe (1989, 2005) in which they all recorded a slightly alkaline to alkaline pH in fermented food condiments from vegetable proteins. All fermented condiments are characterized by very strong pungent smell. The increase in pH is generally due to the production of ammonia, which is characterized by the pungent smell of the fermented condiments. The production of ammonia and amines is quite common with the fermentation of vegetable proteins during the hydrolysis of protein (Torev, 1973; Whitaker, 1978), which leads to the distinctly ammoniacal smell of the fermented condiments. Hesseltine and Wang (1967) reported that during the short period of fermentation of *dawadawa*, heat was evolved and there was also an increase in pH due to the abundant production of ammonia during later stages of fermentation. This is a common feature of fermented vegetable proteins. He further stated that ammonia production might be due to the protease deaminanse enzymes produced by the *Bacillus* isolates.

The majority of the bacteria present in *afiyo* were aerobic or facultatively aerobic, since the viable counts in aerobic plates were quite higher than that of anaerobic plates probably due to the low oxygen tension in the fermenting *afiyo* mash. This agrees with oxygen-relationship test performed during the biochemical tests as indicated in Table 2.

B. subtilis and *Staphylococcus* species have been associated with fermenting foods of plant origin. *B. subtilis* has been associated with fermenting soybean for *natto* production (Hesseltine, 1965); wheatmilk mixture for *kishk* preparation (Morcos *et al.*, 1973) and fermenting rice in Ecuador (van Veen *et al.*, 1968). Most of the micro-organisms involved in the fermentation process of vegetable-proteins as reported by earlier workers, were predominantly *Bacillus spp.* – *B. subtilis, B. licheniformis, B. megaterium, B. firmus, Staphylococcus, Micrococcus* and some few Enterobacteria (Odunfa, 1981a,b). The most predominant microorganism isolated from the fermenting *okpehe* mash was *Bacillus subtilis* while other species were *B. licheniformis, B. megaterium, B. pumilus and staphylococcus*; although *Staphylococcus* did not appear to play any major role in the fermentation. The variety of bacteria growing in the fermenting beans during the fermentation produced a whitish mucilaginous substance that covered and linked the individual light brown to dark brown coloured cotyledons, however, no fungal species was isolated from the fermenting *afiyo* mash, and as suggested by Odunfa (1981) it is unlikely that the low oxygen tension in the fermenting mash would encourage the growth of fungi. The fact that fungi were not found in the controlled fermented *afiyo* makes it safe from the risk of mycotoxins.

The fermentation of *Prosopis africana* seeds resulted in substantial decrease in the total soluble sugars. This pattern of change in soluble sugar level have been reported in similar fermented condiments (Omafuvbe and Oyedapo, 2000; Omafuvbe *et al.*, 2000). From previous works, it has been found that oligosaccharides are present in the unfermented vegetable beans, but the quantity decreases during fermentation (Oyenuga, 1968).

The reducing sugar level however, increased with increasing period of fermentation. This finding was similarly reported in fermentation of African locust bean in the production of *iru* but fluctuated in fermentation of melon seeds in *ogiri* production (Omafuvbe *et al.*, 2004). The increased level of reducing sugar might be produced from the hydrolysis of oligosaccharides present in the fermenting cotyledons (Odunfa, 1983), which is a reflection of the activities of α-amylase in the fermenting cotyledons. This is however not unusual since similar pattern have been reported in similarly fermented seeds (Odunfa, 1983; Sanni, 1993; Omafuvbe *et al.*, 2002). The changes in the reducing sugar content

A study to process-optimize and to develop starter cultures for *afiyo* production

Table 2: Microscopic, biochemical and physiological characteristics of associated bacterial species isolated from *afiyo*

Characterization Tests	Bacterial strains						
	BL	BS1	BM	BP	BS2	SA	EC
G r a m ' reaction	+	+	+	+	+	+	-
Catalase	+	+	+	+	+	+	+
Casein hydrolysis	+	+	+	+	+	+	-
Starch hydrolysis	+	+	+	-	+	+	-
Gelatin liquefaction	+	+	+	+	+	-	-
Methyl red	+	+	-	-	-	-	+
Voges-Proskaeuer	+	+	-	+	-	+	-
Citrate utilization	+	+	+	+	+	-	-
Nitrate reduction	+	+	+	+	+	+	+
Lipolytic activity	+	+	+	+	+	-	-
Motility	+	+	+	+	+	-	+
Oxidase	-	-	-	-	-	-	-
KCN	+	+	+	+	+	-	-
H_2S production	-	-	-	-	-	-	-
Oxygen relationship	FA	FA	FA	FA	FA	FA	ND
Oxidative-fermentative	O	O	O	O	O	O/F	ND
Anaerobic growth	+	-	-	-	+	-	-
Growth in 5 % NaCl	+	+	+	+	+	+	+
Growth in 10 % NaCl	+	-	+	+	+	-	-
Sugar fermentation							
Fructose	A	A	A	A	A	A	A/G
Galactose	A	A	A	A	-	A	-
Glucose	A	A	A	A	A	A/G	A/G
Lactose	A	A	A	A	A	A	A/G
Maltose	A	A	A	A	A	A	A/G
Mannitol	A	A	A	A	A	A	A/G
Sucrose	A	A	A	A	A	A	A
Xylose	A	A	A	A	A	A	-
Growth at different temperatures-							
10°C	-	-	-	-	+	+	ND
15°C	+	+	+	+	+	+	ND
25°C	+	+	+	+	+	+	ND
30°C	3+	3+	3+	3+	3+	3+	ND
35°C	3+	3+	3+	3+	3+	3+	ND
40°C	2+	2+	2+	2+	2+	2+	ND
45°C	2+	2+	2+	2+	2+	2+	ND
50°C	+	+	+	+	+	+	ND
55°C	-	-	-	-	-	-	ND
Total no of strains	28	20	23	13	19	09	03

Keys: - = negative; + = positive; A = acid production; FA = facultative anaerobe; O = oxidative reaction; O/F = oxidative and fermentative reaction; + = slightly positive; 2+ = positive; 3+ = highly positive; ND Not determined. BL = *B. licheniformis*; BS1 = *B. subtilis*; BM = *B. megaterium*; BP = *B. pumilus*; BS2 = *B. subtilis*; SA = *Staphylococcus aureus*; EC = *Escherichia coli*.

of the fermenting *afiyo* mash in this study were quite significant.
Another significant biochemical change as reported by Dulaney (1967), Chattopadahay and Banerjee (1973) is the liberation of soluble amino acids during fermentation of vegetable seeds into condiments. In this study the level of total free amino acids increased in the controlled

fermentation of *afiyo*. Similar increases in the level of free amino acids with fermentation have been reported in other leguminous vegetable seeds (Omafuvbe *et al.*, 1999, 2000). This rapid increase in the total free amino acids may be a reflection of the increased protease activity observed in the fermenting seeds.
Proteinase activity has been reported to be abundant in

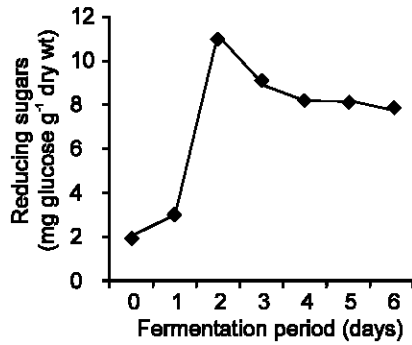A study to process-optimize and to develop starter cultures for *afiyo* production

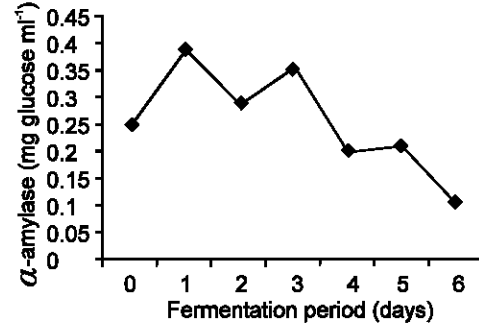Fig. 3: Reducing sugars activities during the fermentation of *afiyo*
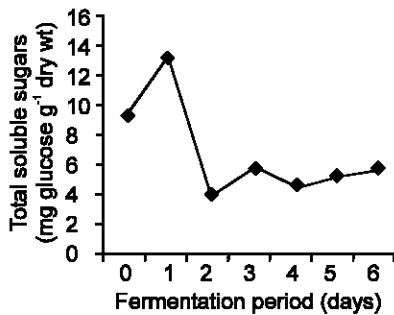

Fig. 4: Total sugars activities during the fermentation of *afiyo*

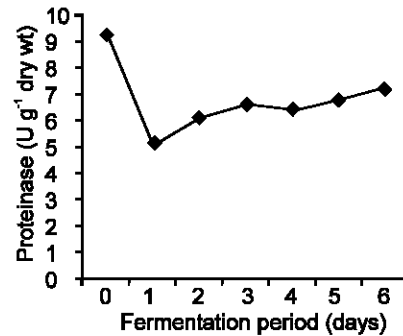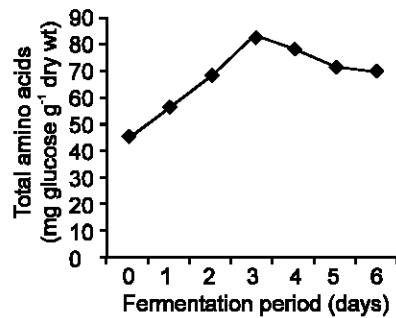Fig. 5: Total amino acids activities during *afiyo* fermentation

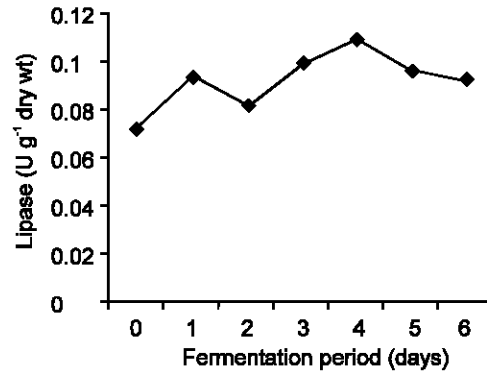

Fig. 6: α-amylase activities during *afiyo* fermentation

Fig. 7: Proteinase activities during *afiyo* fermentation

Fig. 8: Lipase activities during *afiyo* fermentation

the fermentation of similar protein rich foods (Omafuvbe *et al.*, 2002). This is probably due to a consistently active proteinase activity resulting in rapid amino-acid production. The high proteinase activity observed in this study may also due to the high protein content of *Prosopis* seeds. Protein in *Prosopis* has been reported to constitute about 60% or more of the seed kernel's weight (Felker and Bandurski, 1977). In the fermentation of other protein-rich seeds, proteinases have also been found to be abundant. This is true of the fermentation of soybean to produce the Japanese *miso*, soy-sauce *tofu*, a Chinese soy-bean curd, *tempe*, an Indonesian soybean fermented food (Odunfa, 1985) and *okpehe*

a Nigerian seasoning condiment (Sanni, 1993). *Bacillus* species are important sources of proteases, therefore the high recovery rates of *Bacillus* species from the fermenting mash may account for the high protease activities observed in this study.

The increased level of reducing sugar may be a reflection of the activities of α-amylase, one of the extracellular enzymes produced by the associated bacteria in the fermenting cotyledons and which hydrolyses the oligosaccharides (stachyose, raffinose and sucrose) and other complex sugars during fermentation into various components like glucose, dextrin and fructose. The amylase activity in the

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fermenting *afiyo* was fairly low although it was very significant in the first 24 hours of fermentation. According to Forgarty *et al.* (1974), *Bacillus* species are important sources of proteases and amylases, therefore, the high recovery rates of *Bacillus* species from the fermenting mash may account for the high proteases and amylases activities observed in the fermenting *afiyo* this study.

There was fluctuation in lipase activity throughout the fermentation period; however, the unsteady increase in lipase activity is probably attributed to increase in pH during this protein-based fermentation. A similar result was obtained by Odunfa (1981a,b). This increase in pH has been shown to enhance lipase activity in *Bacillus* and *Staphylococcus* species (Johnson and Snyaa, 1974). The lipase activity in the fermenting *afiyo* was fairly low as compared to other enzymatic parameters during the controlled fermentation of *afiyo*. The decrease in lipase activity may be due to lipase denaturing by increase in proteolytic activity during the fermentation of *afiyo* (Johnson and Snyaa, 1974). The source of lipase activity in this fermentation may be attributed to the *Staphylococcus* species, in which lipolytic activities are well known. This agrees with the earlier reports of Frankline and Sharpe (1963), Vadehra and Harmon (1969) and Mates and Sudakenvitz (1973). The lipase activity in the fermenting *afiyo* mash was however very low when compared with other vegetable proteins like *tempe* (Steinkraus *et al.*, 1979), *iru* and *natto* (Odunfa, 1985; Kiuchi *et al.*, 1976). This low lipase activity has been reported by (Odunfa, 1984) to be desirable, since high amounts of fatty-acids in foods can cause rancidity thereby making the food taste sour.

Determination of the ability of the different associated bacterial isolates to ferment *Prosopis* seeds showed that only the predominant *Bacillus* species played active roles in the *afiyo* fermentation with production of the characteristic *afiyo* aroma. *B. licheniformis* and *B. subtilis* produced the strongest characteristic *afiyo* aroma while the *Staphylococcus* species lacked the typical aroma of *afiyo* probably due to its extremely low ammonia production.

Despite the awareness of science in the society, the production of *afiyo* still remains the traditional family art practiced in homes with rudimentary utensils. Consequently, the production has not increased substantially and in fact, its non-popularity, especially among the growing urban population is as a result of importation of some foreign soup flavouring condiments. The indigenous fermented foods if properly developed, have a strong potential of increasing food production, improving the nutritional status of the rural population and decreasing food imports. It is essential to develop the industrial production of indigenous fermented food seasoning agents like *afiyo* so as to improve the nutritional status of the rural-urban population and reduce malnutrition, especially among children. In this study, information is provided on the nature and process of controlled *afiyo* fermentation; microorganisms associated with the fermentation and effects of the fermentation on some biochemical and physiological factors. The information obtained will provide relevant background data for the much-needed process-optimization of the Nigerian indigenous condiments such as *afiyo*. Further work to process-optimize and to develop starter cultures for *afiyo* production are going on in our laboratories.

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