Online Publication Date: 20 December, 2011

Publisher: Asian Economic and Social Society

Microbiological and Chemical Changes During the Production of *Okpehe* from *Prosopis africana* Seeds

Balogun M.A (Corresponding author, Department of Home Economics & Food Science, University of Ilorin, Ilorin)

Oyeyiola G.P (Department of Microbiology, University of Ilorin, Ilorin, Nigeria)

Citation: Balogun M.A, Oyeyiola, G.P (2011): " Microbiological and Chemical Changes During the Production of *Okpehe* from *Prosopis africana* Seeds " Journal of Asian Scientific Research , Vol.1, No.8,pp.390-398.

Journal of Asian Scientific Research, Vol.1 (8), pp.390-398

Microbiological and Chemical Changes During the Production of *Okpehe* from *Prosopis africana* Seeds

Author (s)

Balogun, M.A.
Corresponding author, Department of Home Economics & Food Science, University of Ilorin, Ilorin, Nigeria
E-mail: balogun.ma@unilorin.edu.ng

Oyeyiola, G.P.
Department of Microbiology, University of Ilorin, Ilorin, Nigeria
E-mail: ganiyu@unilorin.edu.ng

Abstract

Two *okpehe* samples (B and C) were produced in the laboratory using the autoclave and hot plate while a third one (sample A) was obtained from local producers for comparison. Microorganisms associated with the production of *okpehe* from *Prosopis africana* seeds were *Bacillus subtilis, Bacillus licheniformis, Bacillus megaterium, Bacillus pumilus, Staphylococcus aureus, Escherichia coli* and *Saccaromyces cerevisiae*. These microorganisms increased in number as fermentation progressed. Only sample A had *Saccharomyces cerevisiae*. The temperature, pH and moisture content increased progressively with increase in period of fermentation for all the three samples. There was a significant increase ($p < 0.05$) in crude protein and crude fibre while the amount of crude fat, ash, carbohydrate and total solid decreased significantly ($p < 0.05$)till the end of fermentation for the three samples. Sample C contained the highest amounts of crude protein and fibre among the three samples.

Introduction

Prosopis (mesquite) is a genus of about 45 species of leguminous spiny pod-bearing trees and shrubs found in subtropical and tropical regions of America, Africa and southwest Asia (Anon, 2009). The trees of *P. africana* can be found growing wild in the Middle belt, North and some parts of Eastern and Southern parts of Nigeria. The indehiscent pods are palatable to man and animals (Aremu et al., 2006). The seeds are fermented into a traditional condiment, *okpehe* which can serve as a low-cost source of protein especially for the rural populace.

Fermentation of grains and seeds is said to result in increased nutritional value and wholesomeness over the starting material (Van Veen and Steinkraus, 1970). Fermentation also contributes to the masking of undesirable odours and flavours while imparting desirable flavour to the finished product (Beauchat, 1976). More importantly, fermentation has been reported to enhance digestibility of starting materials by breaking down complex protein structures to peptides and free amino acids (Hesseltine and Wang, 1979).

Food flavouring condiments are prepared by traditional methods of uncontrolled solid substrate fermentation resulting in extensive hydrolysis of the protein and carbohydrate components (Fetuga *et al.*, 1973; Eka, 1980). Production of these condiments is by spontaneous fermentation carried out in people's homes using rudimentary utensils under varying hygienic conditions (Oguntoyinbo *et al*., 2010). The methods employed in the production of fermented condiments differ from one region to another depending on existing traditional systems.

In Nigeria, most traditional diets often lack variety and consist of mainly staple foods with small amounts of other foods depending on season and availability. The staple foods provide the calories but are poor in other nutrients like protein and minerals (Achi, 2005). The diets of Nigerians are mostly from roots, tubers and cereals. The low protein in these diets contributes to low nutrition security of the people (Karim and Adekunle, 2010). Soups are the main sources of protein and minerals and one of the ways to improve the diet has been to improve the nutrient content of soups (Achi, 2005).

Although okpehe has long been used as a condiment by Nigerians especially the *Idomas* of Benue State (Northern Nigeria), the modification occurring during fermentation of *Prosopis africana seeds* is not fully understood. This study is therefore designed to carry out microbiological and proximate analysis on *okpehe* produced from *Prosopis africana* seeds.

Materials and Methods

Sources of Fruits and Okpehe Samples

The fruits of *Prosopis africana* were obtained from the Main Campus of the University of Ilorin, Ilorin, Nigeria and authenticated at the herbarium of the Plant Biology Department of the University with voucher specimen number UIH/472. *Okpehe* was produced in the laboratory using the autoclave and hot plate following the traditional method. The commercial *okpehe* sample was collected aseptically from the home of local producer and transported to the laboratory in sterile polyethene bag under ice cubes.

Traditional Preparation of Okpehe from Prosopis africana seeds

The seeds of the *Prosopis africana* were removed by beating the fruits with a club on a concrete surface to break the tough fruit coat. One thousand grams of seeds were boiled overnight in a large earthen-ware pot with wood fire, during which the seed coats became soft and the seeds swollen. The seeds were allowed to cool. The seed coats were removed by pressing between fingertips. These coats were later decanted along with the washing water, leaving the clean seed cotyledons. The clean cotyledons were put in another pot with small amount of water and cooked for 1-2 hours. The cotyledons were later drained through sieve and wrapped with paw-paw leaves. The wrapped cotyledons were put in clean bowls covered with jute bags and then left for 3 days in an incubating unit during which natural fermentation occurred. After fermentation, the resultant product, which was brown in colour, was *okpehe*, a strong-smelling mass of sticky cotyledons. The fermented cotyledons were covered by a whitish mucilaginous film produced during fermentation (Ogunshe *et al.*, 2007). The *okpehe* was made into balls of 3 -5cm diameter, arranged in trays and dried for 1-2 days in the sun. The product became black after sun drying. The dried product was ground with mortar and pestle and it was ready to be sold by the local producers to the consumers. The *okpehe* produced by the local producer was designated as sample A

Prosopis africana fruits

↓

Remove fruit coats

↓

Prosopis africana seeds

↓

Boiled overnight (with wood fire)

↓

Seeds dehulled and cotyledons washed

↓

Cotyledons boiled for 1-2hrs

↓

Cotyledons wrapped in pawpaw leaves

↓

Wrapped cotyledons packed in jute bags

↓

Fermentation for 3 days (at 25-30^{0}C)

↓

Okpehe

Fig. 1. Flow chart for the traditional method of production of *okpehe* from *Prosopis africana* seeds

Laboratory Preparation Using the Traditional Method

Using the autoclave

The method of Ogunshe et al., (2007) was used with some modifications for the fermentation of *Prosopis africana* seeds. The seeds were removed by beating the fruits with a club on a concrete surface to break the tough fruit coat. One thousand grams of seeds were boiled in an autoclave at 121^{O}C for 2 hours. The seeds were allowed to cool. The seed coats were removed by pressing between fingertips. The cotyledons were separated from the coats and later rinsed in sterile water, before cooking on the hot plate set at 60^{0}C for about 30 minutes to soften the cotyledons. The cotyledons were later drained through a sterile sieve and cooled to room temperature before wrapping in paw-paw leaves already disinfected by swabbing with 70% alcohol, rinsed with sterile water and allowed to dry in sterile atmosphere. The wrapped cotyledons were then incubated at 25 - 30^{0}C for 3 days. The *okpehe* produced by this method was labeled and designated as sample B.

Using the hot plate method

The seeds of the *Prosopis africana* were removed by beating the fruits with a club on a concrete surface to break the tough fruit coat. One thousand grams of seeds were boiled at 100^0C for 6 hours in a stainless steel pot on a hot plate, during which the seed coats became soft and the seeds swollen. The seeds were allowed to cool. The seed coats were removed by pressing between fingertips. The cotyledons were separated from the seed coats and later rinsed with sterile distilled water. The clean cotyledons were put in another clean pot with small amount of water and cooked on the hot plate at 60^0C for 30minutes. The cotyledons were later drained through a sterile sieve and wrapped with paw-paw leaves. The paw- paw leaves used had been swabbed with 70% alcohol, rinsed with sterile water and allowed to dry in a sterile atmosphere prior to usage. The wrapped cotyledons were covered with jute bags and then left at 25 - 30^0C for 3 days in a disinfected laboratory cupboard during which natural fermentation occurred. The *okpehe* produced by this method was designated as sample C. For all the three samples A, B and C, the time of wrapping was regarded as the zero hour. The samples were stored at 4^0C in a refrigerator prior to analysis. *Isolation of Microorganisms*

The serial dilution method was used for the isolation of microorganisms from 1-g samples before and during fermentation. One gram of sample was added to 9ml of sterile distilled water and shaken to get 10^{-1} dilution. Then 1ml of this dilution was transferred to another 9ml of sterile distilled water, and shaken to obtain 10^{-2} dilution. Nutrient agar was used to isolate bacteria, while potato dextrose agar and yeast extract agar were used to isolate fungi. The spread plate method was used for the isolation. Plates were incubated at 37^oC for 24 – 48 hours for bacteria and at room temperature for 2-5 days for fungi. Colonies were counted for the different mixed culture plates. Representative colonies were differentiated on the basis of their morphology and then subcultured to obtain pure culture. Isolation of microorganisms was done at zero hour and subsequently after every 24 hours until the end of 72 hours. Isolation of microorganisms was done within 30 minutes of sample collection.

Identification of the Isolates

Each bacterial or yeast isolate was examined in pure culture and subjected to the conventional colonial morphology, cellular characteristics and biochemical characteristics determinations. After determining the characteristics of the isolates, reference was made to Buchanan and Gibbons, (1974) for the identification of each bacterial isolate, while Onions et al., (1981)and Fawole and Oso, (2004) were consulted for the identification of each yeast isolate.

Temperature, pH, moisture content and proximate analysis determinations

The temperature, pH, moisture content and proximate analysis were determined using the AOAC, (2002) methods. These parameters were determined at zero hour and subsequently after every 24 hours until the end of 72 hours.

Results

Different groups of microorganisms were isolated during the fermentation. The occurrence of these isolates in the three samples at the different stages of fermentation and their counts are shown in Tables 1, 2, 3 and 4. The isolated microorganisms were *Bacillus subtilis, Bacillus licheniformis, Bacillus megaterium, Bacillus pumilus, Staphylococcus aureus, Escherichia coli and Saccharomyces cerevisiae*. The variety of microorganisms present during the fermentation produced a whitish mucilaginous substance that covered and linked the individual light brown to dark brown coloured cotyledons. These microorganisms increased in number as the fermentation period increased.

There was an increase in the moisture content of the three *okpehe* samples from 45% at zero hour to 61% after 72 hours of fermentation. The fermentation was accompanied by a rise in pH (6.3 – 8.2) and temperature (27^0 – 35^0C) (Figs 2, 3 and 4). The crude protein increased significantly ($p < 0.05$) for the three samples as fermentation progressed (30.90% - 40.05%). There was a significant decrease in crude fat, ash, carbohydrate and total solids (Table5).

Discussion

The product of the spontaneous fermentation of *Prosopis africana* seeds is *okpehe* with its characteristic appearance and aroma. After fermentation for 72 hours, the mash became soft and dark and had a characteristic strong ammoniacal odour.

Table 1 Microbial counts of samples after zero hour of fermentation

Microorganisms Isolated	Sample A (cfu/g)	Sample B (cfu/g)	Sample C (cfu/g)
Bacillus subtilis	3.1×10^3	2.3×10^3	2.5×10^3
Bacillus licheniformis	2.3×10^3	3.2×10^3	3.3×10^3
Escherichia coli	5.2×10^1	3.3×10^1	3.4×10^1
Staphylococcus aureus	3.1×10^1	-	-
Saccharomyces cerevisiae	2.1×10^1	-	-

Sample A - *okpehe* produced from local producer

Sample B - *okpehe* produced using the autoclave

Sample C - *okpehe* produced using the hot plate

Table 2 Microbial counts of samples after twenty four hours of fermentation

Microorganisms Isolated	Sample A (cfu/g)	Sample B (cfu/g)	Sample C (cfu/g)
Bacillus subtilis	5.1×10^4	4.2×10^4	4.3×10^4
Bacillus licheniformis	4.3×10^4	4.2×10^4	4.2×10^4
Bacillus megaterium	2.0×10^1	1.6×10^1	2.1×10^1
Bacillus pumilus	-	2.1×10^1	2.1×10^1
Escherichia coli	3.4×10^2	2.8×10^2	2.9×10^2
Staphylococcus aureus	3.0×10^2	2.1×10^1	2.0×10^1
Saccharomyces cerevisiae	1.0×10^2	-	-

Table 3 Microbial counts of samples after forty eight hours of fermentation

Microorganisms Isolated	Sample A (cfu/g)	Sample B (cfu/g)	Sample C (cfu/g)
Bacillus subtilis	2.4×10^5	2.0×10^5	2.0×10^5
Bacillus licheniformis	1.5×10^5	1.4×10^5	1.5×10^5
Bacillus megaterium	2.4×10^2	1.3×10^2	1.3×10^2
Bacillus pumilus	-	1.5×10^2	2.0×10^2
Staphylococcus aureus	3.0×10^3	4.3×10^2	4.2×10^2
Saccharomyces cerevisiae	1.2×10^3	-	-

Table 4 Microbial counts of samples after seventy two hours of fermentation

Microorganisms Isolated	Sample A (cfu/g)	Sample B (cfu/g)	Sample C (cfu/g)
Bacillus subtilis	2.8×10^6	2.3×10^6	2.4×10^6
Bacillus licheniformis	1.2×10^6	1.0×10^6	1.1×10^6
Bacillus megaterium	2.5×10^3	1.3×10^3	1.5×10^3
Bacillus pumilus	-	2.4×10^3	2.4×10^3
Staphylococcus aureus	3.2×10^4	3.2×10^3	3.3×10^3
Saccharomyces cerevisiae	3.3×10^3	-	-

Table 5 Proximate analysis of samples

Proximate composition (%)	0hr A	0hr B	0hr C	24hr A	24hr B	24hr C	48hr A	48hr B	48hr C	72hr A	72hr B	72hr C
Crude Protein	30.90[a]	32.68[a]	34.54[b]	32.42[a]	34.12[b]	35.98[c]	34.68[a]	36.33[b]	38.01[c]	36.88[a]	38.44[b]	40.05[c]
Crude Fat	9.32[a]	11.41[b]	11.49[c]	9.01[a]	11.40[b]	11.12[c]	8.62[a]	11.38[b]	10.81[c]	8.15[a]	11.35[b]	10.04[c]
Crude Fibre	2.85[a]	3.06[b]	3.16[c]	2.89[a]	3.25[b]	3.30[b]	2.92[a]	3.40[b]	3.52[c]	2.99[a]	3.57[b]	3.63[c]
Ash	4.89[a]	4.82[b]	4.86[c]	4.86[a]	4.82[b]	4.85[b]	4.82[a]	4.83[a]	4.84[a]	4.80[a]	4.84[b]	4.84[b]
Moisture Content	6.97[a]	7.41[b]	7.58[c]	7.34[a]	7.83[b]	7.92[c]	8.01[a]	8.21[b]	8.36[c]	8.12[a]	8.87[b]	9.46[c]
Nitrogen Free Extract (Carbohydrate)	52.04[a]	48.03[b]	45.95[c]	50.82[a]	46.40[b]	44.75[c]	48.96[a]	44.06[b]	42.82[c]	47.18[a]	41.80[b]	41.44[c]
Total Solid	93.03[a]	92.59[b]	92.42[c]	92.66[a]	92.17[a]	92.18[b]	91.79[a]	91.99[a]	91.64[a]	90.54[a]	91.13[b]	9188[c]

Values are means of triplicate determinations on dry weight basis; means within rows having different superscripts differ significantly ($p < 0.05$)

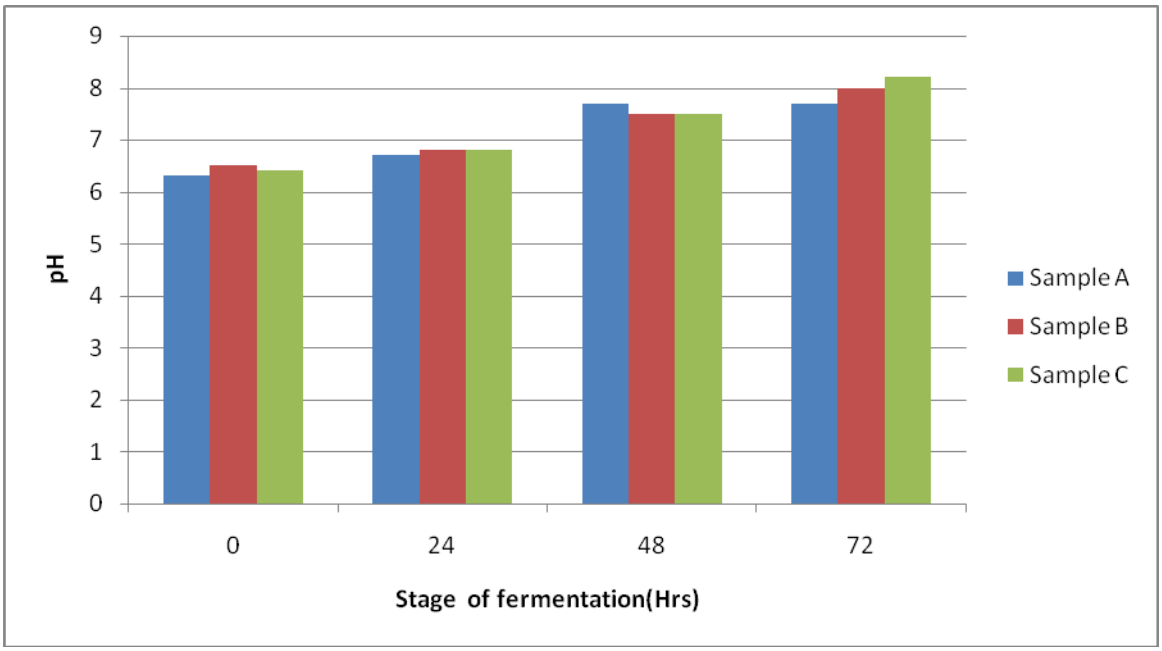

Fig.1 pH changes during the fermentation of *okpehe* samples

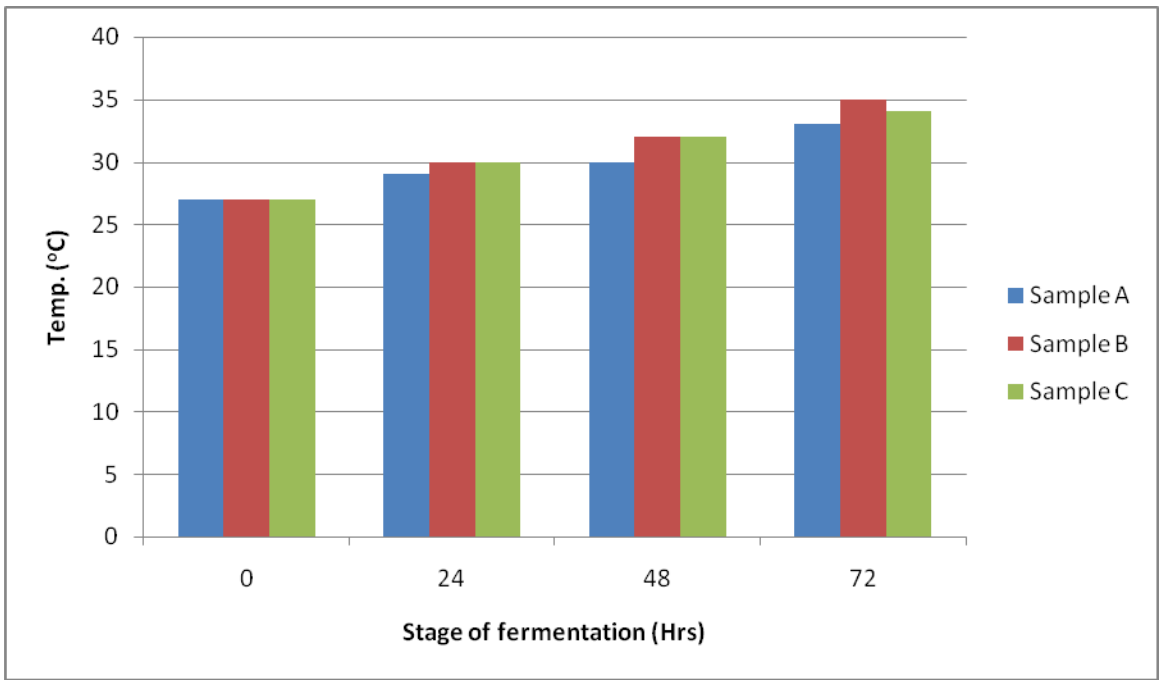

Fig.2: Temperature changes during the fermentation of *okpehe* samples

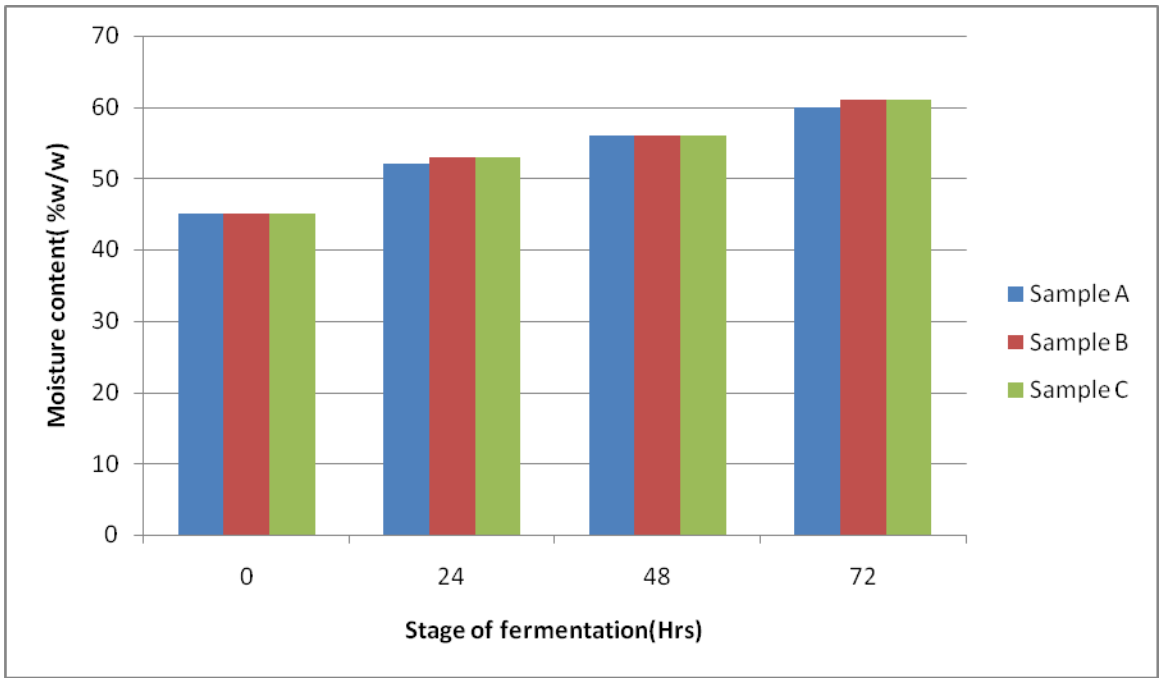

Fig.3: Changes in moisture content during the fermentation of *okpehe* samples

Higher microbial load was obtained in sample A than the other two samples. This may be attributed to the production procedure carried out for the samples. Aseptic techniques were observed during the production of *okpehe* in the laboratory while the local producers (sample A) might not pay too much attention to practising good hygiene.

The organisms involved in the samples fermentation would have been introduced by chance inoculation from the environment but the initial boiling would eliminate most of the surface microflora of the *Prosopis* seeds. Boiling of the seeds before fermentation has the effect of eliminating the species responsible for an acid fermentation and encouraging a non-acid fermentation that is dominated by Bacillus species (Achi, 1992).

The presence of Bacillus species in the samples is expected since they have been found to be associated with fermenting legume seeds for *okpehe* (Achi, 1992), *ugba* (Obeta, 1983), *dawadawa* (Odunfa, 1981; Antai and Ibrahim, 1986), *iru* (Oyeyiola, 1987) and castor oil seed (0dunfa, 1985) fermentation. The presence of these organisms, which are proteolytic, may lead to an increased proteinase activity, causing the breakdown of proteins.

The presence of Staphylococcus species in the samples was typical of the microflora of fermenting beans (Obeta, 1983; Antai and Ibrahim, 1986). Staphylococcus species have been associated with fermenting foods of plant origin especially vegetable proteins (Odunfa and Komolafe, 1989; Jideani and Okeke, 1991). The coagulase-negative staphylococcus species are non-pathogenic and safe organisms on vegetable proteins (Odunfa, 1981). These organisms may contribute to the flavour of the fermenting *okpehe* because of their lipolytic activity.

Escherichia coli was also found to be present in the three samples at the beginning of fermentation but disappeared after 24 hours of fermentation. *E. coli* though fermentative and found in the air and soil, has been isolated from some fermentation (Ogunshe *et al.*, 2007). *Enterobacter cloacae* was isolated by Achi, (1992) from *okpehe*, although no enterobacterium was isolated by Oyeyiola (2002) in *okpehe* from local producers. These microorganisms did not survive until the end of fermentation, perhaps because of the modified environment, which had developed at the later stages of fermentation.

The involvement of a variety of microorganisms in spontaneous food fermentation is normal and does not render the product unsafe for human consumption, especially when none of the microorganisms is pathogenic to man (Oyeyiola 2002). The growth of microorganisms during the fermentation of o*kpehe* is likely to have a significant influence on the quality of the final product. The flavour of the final product can be attributed mainly to the activities of microorganisms, which grew in the mash.

The rise in pH which occurred during fermentation could be due to the high proteinase activity of the microorganisms involved (Odunfa, 1985), which ultimately resulted in the liberation of ammonia as was reported for some other fermenting protein foods such as *natto* (Hesseltine and Wang, 1967), *koji* (Yong and Wood, 1977), *iru* (Odunfa, 1985), *ugba* (Oyeyiola, 1989) and *kawal* (Dirar, 1993). During the period of the rise in pH, desirable flavour components of the condiment presumably were developed. Organic acids which may result from protein decomposition may contribute to the darkening of colour (Achi, 1992).

The rise in temperature indicates that the fermentation was exothermic with the changes being due to the metabolic activities of the microorganisms. The heat generated in the fermenting mash possibly provided the ideal temperature conditions for the optimal activity of the proteolytic enzymes (Odunfa, 1985).

The increase in crude protein is in agreement with the work of Gernah *et al*., (2005) where the crude protein of *dawadawa* increased from 25% to 30.50% after fermentation for 72 hours. The high proteinase activity may be due to the high protein content of Prosopis seeds. The fresh (unboiled and unfermented) cotyledons of *Prosopis africana* contain 30.12% crude protein.

The progressive decrease in crude fat during the fermentation for all the three samples is desirable because it will result in the breakdown of fat into simpler substances which will enhance the digestibility of the product in human body.

The slight increase crude fibre may be due to the utilization of other nutrients by the fermenting organisms because they cannot degrade the cellulose and hemicellulose present in the samples. Crude fibre consists largely of cellulose and hemicellulose.

The ash content observed is an indication that *okpehe* samples are rich in minerals. Similar increase in ash content was reported by Gernah *et al*., (2005) after 72 hours of fermentation of *iru*.

The sharp decrease in carbohydrate content of the samples may be due to its utilization by the isolated organisms during fermentation. Bacillus species are important sources of amylases therefore, the high recovery rates of these organisms from the fermentation may account for their high amylase activity (Ogunshe *et al*., 2007) thereby leading to a reduction in the carbohydrate as fermentation progressed.

There was a corresponding reduction in the percent total solids, as it was observed with the carbohydrate content. This is probably due to carbohydrate utilization by the fermenting organisms.

The fermentation of the samples resulted in a decrease in its total sugars. The pattern of change in soluble sugar level have been reported in similar fermented condiments (Omafuvbre and Oyedapo, 2000; Omafuvbre *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 decrease in total sugars may be attributed to its utilization by the fermenting organisms.

The result showed that out the three samples compared, sample C was the best in terms of the microbiology and nutritional composition. Too much energy, nutrient and time are usually wasted by the local producers (sample A) before the end product is obtained The tedious boiling overnight by the local producers could be shortened using the hot plate set at 60^0 -70^0C and avoiding loss of some essential nutrients. The local producers should be encouraged on practicing good personal and environmental hygiene so as to produce a product with less contaminant.

References

Achi, O.K. (1992) "Microorganisms Associated with Fermentation of *Prosopis africana* Seeds for Production of *Okpiye"*. Journal of Plant Foods and Human Nutrition Vol. 4, No.2, pp.279-304.

Achi, O.K. (2005) "Traditional Fermented Protein Condiments in Nigeria". African Journal of Biotechnology Vol. 4, No.13, pp.1612-1621.

Anon (2009) www.wikipedia.Akee.html. 10/09/08.

Antai, S.P. and Ibrahim, M.H. (1986) "Microorganisms Associated with African Locust Bean (*Parkia filicoidea Welw*) Fermentation for *Dawadawa* Production". Journal of Applied Bacteriology Vol.61, pp.145-148.

AOAC (2002) Official Methods of Analysis 16th Edition. Association of Official Analytical Chemists: Washington D.C.

Aremu, M.O., Olonisakin, A., Atolaye, B.O. and Ogbu, C.F. (2006) "Some Nutritional and Functional Studies of *Prosopis africana"*. Electronic Journal of Environment, Agriculture and Food Chemistry Vol. 5, No.6, pp.1640-1648.

Beuchat, L.R. (1976) "Fungal Fermentation of Peanut Press Cake". Economic Botany Vol. 30, pp. 227-234.

Buchanan, R.E. and Gibbons, N.E. (1974) Bergey's Manual of Determinative Bacteriology. 8th Edn. Williams and Wilkins: Baltimore.

Dirar, H.A. (1993) The Indigenous Fermented Foods of the Sudan. CAB International: Oxon.

Eka, O.U. (1980) "Effect of Fermentation on the Nutrient Status of Locust Beans". Food Chemistry Vol.5, pp.305-308.

Fawole and Oso, (2004) Laboratory Manual of Microbiology. Spectrum Books Ltd: Ibadan.

Fetuga, B.L., Babatunde, G.M. and Oyenuga, V.A. (1973) "Protein Quality of Some Nigerian Foodstuffs 1: Chemical Assay of Nutrients and Amino Acid Composition". Journal of Science, Food and Agriculture Vol. 24, pp.523-525.

Gernah, D. I., Inyang, C.U. and Ezeora, N.I. (2005) "Effect of Incubation Materials on Fermentation of African Locust Beans (Parkia biglobosa) in the Production of 'dawadawa'". Nigerian Food Journal Vol. 23, pp.166-173.

Hesseltine, C.W. and Wang, H.L. (1967) "Traditional Fermented Foods". Biotechnology Bioengineering Vol. 9, pp.275-288.

Hesseltine, C.W. and Wang, H.L. (1979) Fermented Foods. Chem Ind Vol.12, pp.393-399.

Jideani, I.A.O. and Okeke, C.R. (1991) "Comparative Study of Microorganisms and Sensory Attributes of Condiments from the Fermentation of Different Seeds". Plant Foods and Human Nutrition Vol.41, pp. 27-34.

Karim, O.R. and Adekunle, O.A. (2010) "Food and Nutrition Security: A Conflict Prevention Strategy in Nigeria" in Dynamics of Peace Processes by I.O.

Albert and I.O. Oloyede, Eds,. Centre for Peace and Strategies Studies: Ilorin, pp.513-522.

Obeta, J.A.N. (1983) "A Note on Microorganisms Associated with the Fermentation of Seeds of the African Oil Bean Seed (*Pentaclethra macrophylla*)". Journal of Applied Bacteriology Vol. 54, pp. 433-435.

Odunfa, S.A. (1981) "Microorganisms and Amino acid Composition of *Ogiri*- a Food Condiment from Fermented Melon Seeds". *Die Nahrung* Vol. 25, pp. 811-816.

Odunfa, S.A. (1983) "Carbohydrate Changes in Fermenting Locust Bean During *Iru* Preparation". Qualitas Plantarum Plant Foods for Human Nutrition Vol.32, pp. 3-10.

Odunfa, S.A (1985) "Biochemical Changes in Fermenting African Locust Bean (*Parkia biglobosa*) During *Iru* Fermentation". Journal of Food Technology Vol. 20, pp. 295-303.

Odunfa, S.A. and Komolafe, O.B. (1989) "Nutritional Characteristics of Staphylococcus Species from Fermenting African Locust Bean (Parkia biglobosa)". Die Nahrung Vol. 33, pp. 607-615.

Ogunshe, A.A.O., Omotosho, M.O. and Ayanshina, A. D.V. (2007) "Microbial Studies and Biochemical Characteristics of Controlled Fermented Afiyo- a Nigerian Fermented Food Condiment from Prosopis africana (Guill and Perr.) Taub". Parkistan Journal of Nutrition Vol. 6, No.6, pp.620-627.

Oguntoyinbo, F. A., Huch Melanie, Cho Gyu-Sung, Schillinger Ulrich, Holzapfel, Wilhelm H., Sanni, A.I. and Franz, Charles M. A.P. (2010) "Diversity of Bacillus Species Isolated from *Okpehe*, a Traditional Fermented Soup Condiment from Nigeria". Journal of Food Protection Vol.73, No.5, pp. 870-878.

Omafuvbe, B.O. and Oyedapo, O.O. (2000) "Observed Biochemical Changes During Natural Fermentation of African Oil Bean (Pentaclethra macrophylla) Seeds". Nigerian Journal of Nutrition and Science Vol. 21, pp.19-23.

Omafuvbe, B.O., Shonukan, O.O. and Abiose, S.H. (2000) "Microbiological and Biochemical Changes in the Fermentation of Soybean for Soy-daddawa- Nigerian Food Condiment". Food Microbiology Vol. 17, pp. 469-474.

Onions, A.H.S., Allsopp, D and Eggins, H.O.W. (1981) Edward Arnolds (Publishers) Ltd: London.

Oyenuga, V.A. (1968). Nigeria Foods and Feeding Stuffs; their Chemistry and Nutritive Value. 3rd edn, Ibadan University Press, Ibadan: Nigeria.

Oyeyiola, G.P. (1987) Microbiological and Chemical Analysis of Fermenting *Parkia* Seeds to Produce a Local Spice. M.Phil Dissertation, University of Ilorin, Ilorin.

Oyeyiola, G.P. (2002). "Fermentation of the Seeds of *Prosopis africana* to Produce a Condiment". NISEB Journal Vol. 2, No. 2,pp.127-129.

Oyeyiola, G.P. (1989) "Microbiology of the Fermentation of Locally Produced *Ugba"*. Ecology of Food and Nutrition Vol.23, pp.287-291.

Van Veen, A.G. and Steinkraus, K.H. (1970). "Nutritional Value and Wholesomeness of Fermented Foods". Journal of Agriculture, Food and Chemistry Vol.18, pp. 576-578.

Yong, F.M. and Wood, B.J.B. (1977) "Biochemical Changes in Experimental Soy Sauce Kofi". Journal of Food Technology Vol.12, pp. 163-175.