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Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

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Changes in the Nutrient Composition of *Okpehe* During Fermentation

M.A. Balogun[1] and G.P. Oyeyiola[2]
[1]Department of Home Economics and Food Science, University of Ilorin, Ilorin, Nigeria
[2]Department of Microbiology, University of Ilorin, Ilorin, Nigeria

Abstract: *Okpehe* is a condiment derived from the fermentation of *Prosopis africana* seeds. This study was based on the changes in total sugar, amino acids and minerals during *okpehe* production. The condiment was processed using three methods namely traditional, autoclave and oven and labeled samples A, B and C respectively. The mean values of the total sugar present in the samples ranged between 0.10 mg/g and 0.05 mg/g for sample A; 0.08 mg/g for sample B and 0.10 mg/g and 0.06 mg/g for sample C at the end of fermentation. The total amino acids increased steadily throughout the period of fermentation for the three samples. The amino acid analysis revealed that the samples contained useful amounts of essential amino acids. Calcium, iron and copper increased in small amounts while there was a reduction in the quantities of potassium and manganese as fermentation progressed. The amount of zinc was constant throughout the period of fermentation while lead and cadmium were not detected in all the samples.

Key words: *Prosopis africana* seeds, sugar, amino acids, minerals

INTRODUCTION

Propsopis africana (Guill. and Perr.) Taub belongs to the family Fabaceae and sub family Mimosoideae (USDA, 2011). It is a savannah tree, 12.2-18.3m (40-60 feet) high and up to 2.1 m (7 feet) in girth (Ogunshe *et al*., 2007). The trees of *P. africana* can be found growing wild in Northern (especially middle belt), eastern and southern parts of Nigeria (Aremu *et al*., 2006). *Prosopis africana* is one of the lesser-known legume seed crops used as food condiment that can serve as a substitute for meat for low-income earners and can reduce protein-calory malnutrition and essential fatty acid deficiencies (Oguntoyinbo *et al*., 2010).

Generally, fermentation results in increased nutritional value and wholesomeness over the starting material (Van Veen and Steinkraus, 1970). Fermentation plays an important role in the masking of undesirable odours and flavours while imparting desirable flavour to the finished product (Beuchat, 1976). Hesseltine and Wang (1979) reported that fermentation enhances digestibility of starting materials by breaking down complex protein structures to peptides and free amino acids.

The methods employed in the production of fermented condiments differ from one region to another depending on socioeconomic circumstances. Fermented 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). These condiments are produced by spontaneous fermentation carried out in people's homes using rudimentary utensils under varying hygienic conditions (Oguntoyinbo *et al*., 2010).

Okpehe, a fermented product of *P. africana* seeds has an important role to play in improving the nutritional quality of the traditional diets of Nigerians which often lack variety and consist of large quantities of staple food (cassava, yam, maize) with supplements of plantain, cocoyam, rice and beans depending on availability and season. The staple foods provide the calories but are poor in other nutrients like protein and minerals (Achi, 2005). The low protein and minerals in these diets contributes to low nutrition security of the people (Karim and Adekunle, 2010).

The Nigerian diets can be improved by complementing the staples with soups that are rich sources of protein and minerals. *Okpehe* can be used as a condiment in the preparation of such soups so as to enrich its nutrient content. This work was therefore aimed at studying the changes in total sugar, amino acids and mineral composition of *okpehe* during fermentation.

MATERIALS AND METHODS

Sources of fruits and Okpehe samples: Prosopis africana fruits and okpehe samples were collected as described earlier (Balogun and Oyeyiola, 2011). 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

Corresponding Author: M.A. Balogun, Department of Home Economics and Food Science, University of Ilorin, Ilorin, Nigeria

number UIH/472. *Okpehe* was produced in the laboratory using the autoclave and hot plate following the traditional method. The commercial *okpehe* was collected aseptically from the homes of local producers and transported to the laboratory in sterile polyethene bags under ice cubes.

Traditional Preparation of *Okpehe* from Prosopis africana seeds: *Okpehe* was prepared as described earlier (Balogun and Oyeyiola, 2011). 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 hrs. 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 *okpehe* was made into balls of 3-5 cm 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. The flow chart of the production of okpehe is as presented in Fig. 1.

Laboratory preparation using the traditional method
Using the autoclave: The method of Ogunshe *et al.* (2007) in the fermentation of *Prosopis africana* seeds was used with some modifications. 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 in an autoclave at 121°C for 2 hrs. 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°C for about 30 min 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 with 70% alcohol and rinsed with sterile water. The wrapped cotyledons were then incubated in an incubating unit for 3 days to produce the fermented mash of *okpehe*. The *okpehe* produced by this method was labeled and designated as sample B.

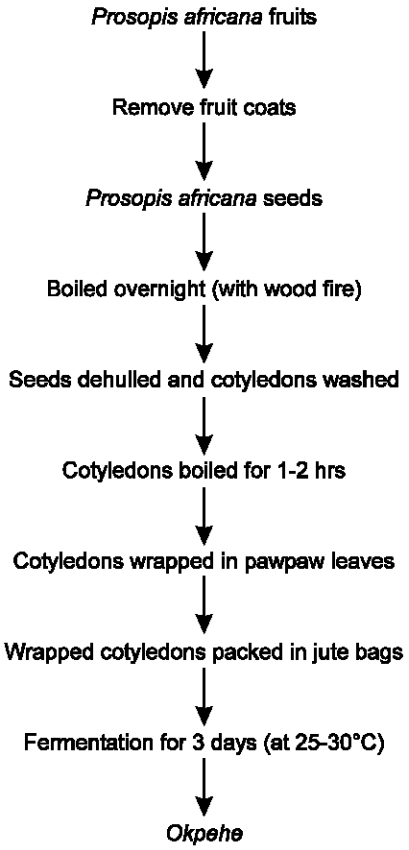

Fig. 1: Flow chart of the traditional method of production of *okpehe* from *Prosopis africana* seeds

Using the hot plate method: *Okpehe* was prepared as described earlier (Balogun and Oyeyiola, 2011). 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°C for 6 hrs 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 seed coats were later decanted along with the washing water, leaving the clean cotyledons. The clean cotyledons were rinsed with sterile water before putting them in another clean pot with small amount of water and cooked on the hot plate set at 60°C for 30 min. The cotyledons were later drained through a sterile sieve and wrapped with paw-paw leaves. The paw- paw leaves used had been disinfected with 70% alcohol and rinsed with sterile water prior to its usage. The wrapped cotyledons were covered with jute bags and then left 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, the time of wrapping was regarded as the zero hour and subsequently after every 24 hrs. The samples were stored at 4°C in a refridgerator prior to analysis.

Determination of amino acid profile using the amino acid analyzer: The amino acid profiles of the samples were determined using the method described by Speckman *et al*. (1958). The samples were dried to constant weight, defatted, hydrolyzed, evaporated in a rotary evaporator and loaded into the Technicon Sequential Multi-Sample Amino Acid Analyzer (TSM) Model DNA 0209.

Determination of mineral content: The mineral contents of samples were determined using the X-Ray Fluorescence (XRF) spectroscopy method. The type of technique that was used is the portable Energy Dispersive X-Ray Fluorescence (EDXRF) technique. The first stage was the sample preparation and this was done by pulverizing the samples to fine powdery form using an agate mortar. Pellets of the samples were formed using a carver model manual pelletizing machine at a pressure of 10-12 torr. The pelletized sample was inserted into the sample holder of the XRF system and was bombarded by the X-ray fluorescence produced from the X-ray tube source at a voltage of 25 KeV and current of 50 μA. The sample characteristic X-ray was detected by the solid state Si-Li detector and spectrum acquisition was done using ADMCA software. The spectrum analysis was done using the AXIL software which relates the peak areas into concentration values (Funtua, 1999).

Statistical analysis: Data obtained were subjected to one way analysis of variance (ANOVA) while means were separated by Duncan's Multiple Range Test (DMRT) (Duncan, 1965).

RESULTS AND DISCUSSION

The total sugar decreased as fermentation progressed for all the samples (Fig. 2). There was an increase in the total amino acids of the three *okpehe* samples from 80.26 g at zero hour to 82.64 g after 72 hours of fermentation (Table 1). The fermentation resulted in an increase in the non essential amino acids (50.45 g-55.56 g) (Table 2). Potassium, calcium, manganese, iron, copper and zinc were present in appreciable amounts while cadmium and lead were not detected in all the samples (Table 3).

Okpehe, with its characteristic appearance and aroma was produced from the spontaneous fermentation of *Prosopis africana* seeds. The mash became soft and dark with a strong ammoniacal odour after seventy two hours of fermentation.

The fermentation of the samples resulted in decrease in its total sugars (Fig. 2). The 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 decrease in total sugars may be attributed to its utilization by the fermenting organisms.

There was a gradual increase in the total amino acids in the samples (Table 1) with sample C having the highest

Table 1: Amino acid composition (g/100 g protein) of samples

	0 hr			24 hrs			48 hrs			72 hrs		
Amino acid	A	B	C	A	B	C	A	B	C	A	B	C
Lysine	5.10^{b}	5.34^{c}	5.02^{a}	5.05^{b}	5.12^{c}	5.00^{a}	5.02^{a}	4.92^{a}	4.89^{a}	4.98^{b}	4.80^{a}	4.80^{a}
Histidine	1.69^{a}	1.76^{a}	1.70^{a}	1.63^{b}	1.52^{a}	1.61^{b}	1.60^{c}	1.51^{a}	1.57^{b}	1.50^{a}	1.49^{a}	1.50^{a}
Arginine	4.34^{a}	4.43^{b}	4.30^{a}	4.32^{b}	4.21^{a}	4.30^{b}	4.34^{b}	4.00^{a}	4.00^{a}	3.89^{b}	3.83^{a}	3.92^{b}
Aspartic acid	19.59^{c}	18.28^{a}	18.81^{b}	19.40^{c}	18.00^{a}	18.60^{b}	19.35^{c}	17.82^{a}	18.42^{b}	19.20^{c}	17.51^{a}	18.21^{b}
Threonine	2.61^{a}	3.00^{c}	2.72^{b}	2.49^{a}	2.78^{c}	2.62^{b}	2.47^{a}	2.68^{c}	2.57^{b}	2.38^{a}	2.61^{c}	2.54^{b}
Serine	3.87^{b}	3.59^{a}	3.91^{c}	3.82^{b}	3.20^{a}	3.85^{b}	3.75^{b}	3.59^{a}	3.75^{b}	3.51^{b}	3.15^{a}	3.60^{c}
Glutamic acid	12.20^{a}	12.48^{b}	13.04^{c}	14.00^{a}	15.52^{c}	15.32^{b}	16.21^{a}	17.26^{b}	17.89^{c}	18.64^{a}	19.52^{c}	19.34^{b}
Proline	3.40^{a}	3.68^{b}	3.42^{a}	3.38^{b}	3.68^{c}	3.30^{a}	3.40^{a}	3.70^{b}	3.40^{a}	3.40^{b}	3.70^{c}	3.34^{b}
Glycine	3.55^{b}	3.38^{a}	3.62^{c}	3.42^{b}	3.28^{a}	3.52^{c}	3.26^{a}	3.38^{b}	3.43^{c}	3.16^{a}	3.36^{b}	3.37^{b}
Alanine	4.20^{a}	5.17^{b}	4.18^{a}	4.15^{b}	4.42^{c}	4.06^{a}	4.02^{a}	3.36^{b}	4.01^{a}	3.98^{b}	4.47^{c}	3.92^{a}
Cystine	1.52^{b}	1.39^{a}	1.55^{b}	1.30^{a}	1.33^{a}	1.30^{a}	1.06^{a}	1.26^{b}	1.22^{b}	1.32^{c}	1.16^{a}	1.22^{b}
Valine	4.03^{b}	3.98^{a}	4.04^{b}	4.01^{b}	3.91^{a}	4.00^{b}	3.89^{a}	3.86^{a}	3.90^{a}	3.78^{b}	3.64^{a}	3.82^{b}
Methionine	0.76^{a}	0.83^{b}	0.78^{a}	0.60^{a}	0.69^{b}	0.71^{b}	0.55^{a}	0.65^{c}	0.60^{b}	0.60^{b}	0.55^{a}	0.60^{b}
Isoleucine	3.02^{b}	2.95^{a}	3.04^{b}	2.86^{b}	2.92^{b}	2.98^{c}	2.79^{a}	2.89^{b}	2.92^{c}	2.64^{a}	2.87^{b}	2.85^{b}
Leucine	5.16^{b}	5.00^{a}	5.16^{b}	4.92^{a}	4.89^{a}	5.04^{b}	4.89^{a}	4.89^{a}	4.91^{a}	4.64^{a}	4.62^{a}	4.84^{b}
Tyrosine	2.58^{b}	2.48^{a}	2.60^{b}	2.48^{b}	2.41^{a}	2.51^{b}	2.42^{b}	2.36^{a}	2.42^{b}	2.35^{b}	2.19^{a}	2.38^{b}
Phenylalanine	2.64^{a}	2.64^{a}	2.62^{a}	2.56^{ab}	2.58^{b}	2.52^{a}	2.46^{a}	2.56^{b}	2.46^{a}	2.30^{a}	2.40^{b}	2.39^{b}
Total	80.26^{a}	80.38^{b}	80.51^{c}	80.31^{a}	80.46^{b}	81.24^{c}	81.48^{a}	80.69^{b}	82.36^{c}	82.27^{a}	81.87^{b}	82.64^{c}

Values are means of triplicate determinations on dry weight basis; means within rows having different superscripts differ significantly ($p<0.05$). For what A, B and C stand for, see Fig. 2

Table 2: Changes in essential amino acids (g/100 g) of samples

	0 hrs			24 hrs			48 hrs			72 hrs		
Amino acids	A	B	C	A	B	C	A	B	C	A	B	C
Essential	29.35	29.93	29.38	28.44	28.62	28.78	28.01	27.96	27.82	26.71	26.81	27.26
Percentage	36.57	37.24	36.49	35.51	35.57	35.43	34.38	34.65	33.78	32.47	32.75	32.99
Non essential	50.91	50.45	51.13	51.87	51.84	52.46	53.47	52.73	54.54	55.56	55.06	55.38
Percentage	63.43	62.76	63.51	64.59	64.43	64.57	65.62	65.35	66.22	67.53	67.25	67.01

For what A, B and C stand for, see Fig. 2

Table 3: XRF analysis (mg/100 g) of samples

	0 hr			24 hrs			48 hrs			72 hrs		
Element	A	B	C	A	B	C	A	B	C	A	B	C
Potassium	224.9^{b}	210.1^{a}	312.9^{c}	210.2^{b}	190.2^{a}	311.1^{c}	200.1^{b}	182.3^{a}	295.1^{c}	183.1^{b}	164.2^{a}	229.5^{c}
Calcium	35.8^{a}	39.5^{b}	44.9^{c}	39.1^{a}	42.8^{b}	48.0^{c}	41.2^{a}	43.2^{b}	50.4^{c}	45.3^{b}	44.4^{a}	55.6^{c}
Manganese	4.8^{a}	6.1^{b}	15.2^{c}	4.7^{a}	6.0^{b}	10.2^{c}	4.4^{a}	5.8^{b}	7.8^{c}	4.2^{b}	5.6^{b}	6.1^{c}
Iron	10.1^{a}	10.2^{b}	10.1^{a}	10.1^{a}	10.3^{c}	10.2^{b}	10.2^{a}	10.3^{b}	10.3^{b}	10.2^{a}	10.7^{c}	10.5^{b}
Copper	8.7^{a}	9.1^{c}	8.9^{b}	8.8^{a}	9.1^{c}	8.9^{b}	9.1^{a}	9.2^{b}	9.1^{a}	9.3^{b}	9.2^{a}	9.2^{a}
Zinc	14.2^{a}	14.1^{b}	14.2^{a}	14.2^{a}	14.1^{b}	14.2^{a}	14.2^{a}	14.1^{b}	14.2^{a}	14.2^{a}	14.1^{b}	14.2^{a}
Lead	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
Cadmium	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D

Values are means of triplicate determinations on dry weight basis; means within rows with different superscripts differ significantly ($p<0.05$). For what A, B and C stand for, see Fig. 2. N.D - Not Detected

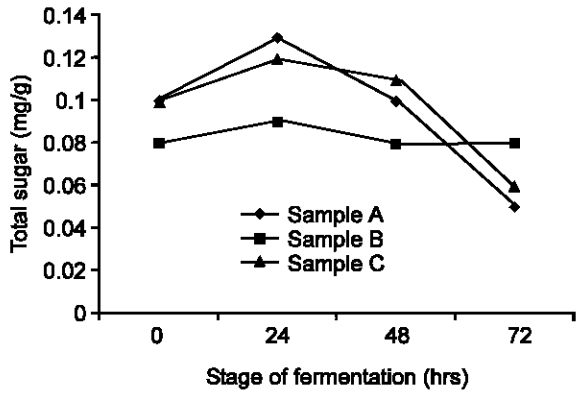

Fig. 2: Changes in total sugar content of *okpehe*. Sample A - okpehe obtained from local producer; Sample B - okpehe produced using the autoclave; Sample C - okpehe produced using the hot plate

amount. This may be because of the mode of production of the condiment where some proteins might have been denatured in the samples (A and B) as a result of the high temperature employed. The result shows that the samples contained nutritionally useful quantities of most of the essential amino acids. Similar increases in the level of amino acids with fermentation have been reported in leguminous vegetable seeds (Ogunshe *et al*., 2007; Omafuvbe *et al*., 1999; 2000). This rapid increase in the total amino acids may be a reflection of the increased protease activity in the fermenting seeds (Ogunshe *et al*., 2007). Soluble low molecular weight peptides and amino acids that contribute to flavour are produced through the enzymatic breakdown of proteins (Odunfa, 1985; Ogbonna *et al*., 2001; Achi, 2005). Free amino acids increase but longer fermentation results in losses of lysine or other essential amino acids (Achi, 2005).

There was a significant increase in the non essential amino acids over the essential amino acids in all the samples. Similar increase in non essential amino acids over essential amino acids was reported by Muhammad and Oloyede (2009) in *Terminalia catappa* seed meal fermented by *Aspergillus niger*. This may be an indication that the non essential amino acids and nucleic acids have been synthesized at the expense of the essential amino acids, thus the reduction in the essential amino acids (Muhammad and Oloyede, 2009).

Potassium was found to be the most abundant mineral in all the samples although it decreased as fermentation progressed. The abundance of potassium is in close agreement with the observation of Aremu *et al*. (2006) that potassium was the most predominant mineral in Nigerian agricultural products. The appreciable high amount of potassium is good because the element helps in regulation of body fluids and maintenance of normal body pressure. It helps in controlling kidney failure, heart oddities and respiratory flaw (Anhwange, 2008). Aremu *et al*. (2006) also observed that potassium was the most abundant mineral in *Prosopis africana* flour. The work of Nda-Umar *et al*. (2008) gave a contrary result that calcium is the most abundant mineral in *okpehe*.

Calcium, which was found to be the second highest mineral increased with the period of fermentation for all the samples. Similar result was obtained by Aremu *et al*. (2006) on the flour of *P. africana*. The presence of calcium in this condiment is good because the element is needed for bone development and strong teeth.

Zinc was also found in an appreciable amount. The amount was constant throughout the period of fermentation for all the samples. Results obtained by other workers were higher; 90.70±0.03 mg/100 g by Nda-Umar *et al*. (2008) in *opehe* and 22.4 mg/100 g by Aremu *et al*. (2006) in *P. africana* flour. Zinc aids digestion and body functions.

The concentration of iron slightly increased as fermentation progressed for all the samples. Nda-Umar *et al*. (2008) also detected iron in moderate amounts (79.38 mg/100 g) in *opehe*. Lower amounts of iron (15.5±0.4 mg/100 g) was obtained by Aremu *et al*. (2006) in *P. africana* flour. Iron carries oxygen to the cells and is necessary for the production of energy, synthesis of collagen and the proper functioning of the immune system (Anhwange, 2008).

The amount of manganese detected in all the three samples was moderate. The values decreased as fermentation progressed. Observation by Aremu *et al*. (2006) showed that *P. africana* flour contained 36±0.4 mg/100 g of manganese. Manganese is known to aid formation of skeleton and cartilage of the body. Manganese dearth is scarce but could affect glucose tolerance, normal reproductive, skeletal and cartilage formation (Anhwange, 2008).

Copper was also detected in all the samples and it increased slightly as fermentation progressed. Copper (46.2±0.7 mg) was among the minerals present in *P. africana* flour (Aremu *et al*., 2006). Copper is a trace element that serves as a co-factor and is required for enzyme function.

Lead and cadmium were not detected in all the samples. Similar result was also obtained by Aremu *et al*. (2006) for *P. africana* flour. These elements are known to be toxic to the human body.

The result showed that sample C i.e. *okpehe* produced using the hot plate was better than the other two samples in terms of nutritional composition. This is because of the high temperature employed in the preparation of *okpehe* using the autoclave (sample B) and that obtained from the local producers (sample A) where some proteins might have been denatured. Too much energy, nutrient and time are usually wasted by the local producers before the end product is obtained The tedious boiling overnight by the local producers could be shortened using the hot plate set at 60-70°C so as to avoid loss of some essential nutrients.

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