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Microbiological and Proximate Analysis of Okpehe, a Locally Fermented Condiment

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Abstract

The high cost and harmful health effect of animal protein has directed interest towards several leguminous seed as potential sources of vegetable protein for human food. This study is therefore designed to determine the physicochemical, microbiological and nutritional quality of okpehe (*Prosopis africana*). Enumeration and identification of microorganisms in okpehe samples was carried out employing standard methods. The physiochemical, nutritional, anti-nutritional and mineral content of the okpehe samples were determined. The bacterial count in the okpehe samples ranged from 9.5×10^5 - 1.7×10^6cfu/g while for fungal count; 1×10^4 - 3×10^4cfu/g. Bacterial isolates were identified as *Bacillus subtilis, Bacillus licheniformis, Bacillus megaterium, Staphylococcus aureus* and *Escherichia coli* with their occurrence varying with sampling points. Fungal isolates were *Aspergillus niger*, *Mucor* sp. and *Paecilomyces* sp. The physicochemical parameters examined showed that pH values ranged from 6.7-7.8 and titratable acidity ranged from 0.27-0.50 g/L. The nutritional content ranges; moisture (4.74 - 8.64%), crude protein (31.76 - 40.28%), crude fibre (3.24 - 4.86%), carbohydrate (41.56 -51.63%), ash (5.31 - 6.23%) and lipids (7.34 - 11.45%) varied significantly with physical characteristics such as texture and various sampling points. Higher nutritional content was observed in softer samples with more offensive ammoniacal smell while harder samples appeared to have reduced nutritional quality. The soft okpehe samples contained high amounts of potassium, calcium, iron, copper, zinc and manganese although the drier samples had the highest potassium content (3.496 mg/g). Cadmium and lead were Below Detection Limits (BDL) in all the samples. Anti-nutritional factors such as alkaloids, steroids, flavonoids, resins and phenols were present. Saponin content was very high as compared to the other phytochemicals. This study demonstrates that okpehe possess immense microbiological and nutritional benefits.

Keywords: Okpehe; Microbial Load; Nutritional Content; Mineral Content; Anti-Nutritional Content

Introduction

In many countries in Africa including Nigeria, protein malnutrition is a major problem. The food diet of Nigerians is mostly from roots, tubers and cereals. The low protein content in the Nigerian diet contributes to the low nutrition security of the people [1]. Investigations carried out on methods of improving the protein and mineral content of food from plant sources have been reported to be successful. The high cost of animal protein has also directed interest towards several leguminous seed proteins as potential sources of vegetable protein for human food [2]. Most of the fermented vegetable proteins reported are from leguminous seeds [3]. Quite often, seeds that are used for fermentation are inedible in their raw unfermented or cooked state. The seeds of legumes may account for up to 80% dietary protein and be the only source of protein for some groups of people. Although fermented food condiments have constituted a significant proportion of the diet of many people, Nigerians exhibit an ambivalent attitude in terms of consumer tastes and preferences for such foods [4]. *Prosopis africana* commonly known as African mesquite but also known by different native Nigerian names such as Kiriya (Hausa), Kohi (Fulani), Sam chi lati (Nupe), Ayan (Yoruba), Kpaye (Tiv), Ubwa (Ibo) and Okpehe (Idoma) (Ogunshe et al., 2007) [3] is one of the lesser known legume seed crops which can be fermented and used as a food condiment known as okpehe [5]. Okpehe is used as a food condiment in Nigeria by the Idoma and Igala people of the middle belt region and some parts of the Eastern and Southern Nigeria. It adds variety and pleasure to the otherwise monotonous

traditional diet. It serves not only as a seasoning agent but also as a low-cost source of protein in the diet [6]. Okpehe can serve as a substitute for meat for low-income earners and can reduce protein-calorie malnutrition and essential fatty acid deficiencies [6].

Okpehe is consumed as a food supplement eaten mixed with other foods like tapioca, boiled yam, boiled maize, fish and meat. These are usually mixed with palm oil, pepper and other additions like salt and akanwu (trona-sodium sesquin carbonate or potash) [7]. Okpehe is generously applied to soups as a flavor enhancer and consumed as a meat analogue especially by the poor people [8]. Fermented *Prosopis africana* seeds are rich in protein and may be used in exceptional cases as condiment for bread production [9]. The lack of standard quality control procedures during traditional production of okpehe necessitates the need for the microbial assessment of the condiment for pathogens and high microbial load. It is also observed that low income earners use this condiment as a replacement for macro nutrients especially protein thus there is the need to ascertain its nutritional composition. This study is therefore designed to determine the physicochemical, microbiological and nutritional quality of okpehe.

Materials and Methods

Study area

The study covered major towns in Kogi State, Nigeria namely Anyigba, Idah, Ankpa and Lokoja where okpehe is majorly consumed.

Collection of okpehe samples

A total of eight (8) okpehe samples were purchased from eight (8) separate markets in Lokoja, Anyigba, Idah and Ankpa towns. The samples were labeled accordingly and transported immediately in ice chest to the laboratory for analysis.

Determination of physicochemical parameters

The pH and titratable acidity of okpehe samples were determined according to the method of Balogun (2012) and Eze et al. (2014) [5,10] respectively.

Microbial assay

The total viable bacterial count in the samples was determined using standard plate count agar employing a suitable tenfold serial dilution of the samples in 0.1% peptone water using the pour plate technique as described by APHA (1985) [11]. The coliform count was determined using MacConkey agar incubated at 37^0C for 48 h. The fungal isolation and enumeration was carried out on Potato Dextrose Agar (PDA) incubated at 27 ± 1^0C for 72 h employing the method of Fawole and Oso (2001) [12]. The pure cultures of bacterial isolates were subjected to various morphological and biochemical characterization tests for the identification of the bacteria isolates with reference to Holt et al. (1994) [13]. The fungi were characterized based on colonial morphology and cellular morphology was identified as described by Cooper (1995) [14].

Identification of fungal isolates

The fungal isolates were identified based on colonial morphology like color of hyphae, shape and texture. The method of Fawole and Oso (2001) [12] was used for the microscopic examination of the fungal isolates using the wet mount technique. A drop of cotton blue lactophenol was placed on a slide. An inoculating loop was used to pick a mycelial strand and mixed with the lactophenol blue on the slide. The slide was examined under the X40 objective lens. The sexual and asexual reproductive structures like sporangia, conidia head, ascospores and vegetative mycelium. The observed characteristics were compared with the characteristics of reference organisms according to Cooper (1995) [14].

Proximate analysis of okpehe samples

- **Determination of moisture content:** The method according to James (1995) [15] was employed.
- **Determination of crude lipid:** Extraction of the fat and quantification was carried out using soxhlet apparatus as adopted by AOAC (2002) [16] using petroleum ether as solvent.
- **Determination of crude fibre content:** The method according to AOAC (2002) [16] was employed.
- **Determination of total ash content:** The method according to AOAC (2002) [16] was employed.
- **Determination of crude protein:** The Micro-Kjeldhal method adopted by AOAC (2002) [16] was employed.
- **Determination of carbohydrate content of okpehe samples:** The method according to AOAC (2002) [16] was employed. The carbohydrate or nitrogen free extract content of the samples was determined by the difference obtained by subtracting the sum of percentage ash, crude protein, crude fiber and ether extract from 100% on dry matter basis.

% Carbohydrate = 100 - (% ash + % crude protein + % ether extract + % crude fibre)

The déterminations were carried out in duplicates.

Qualitative determination of anti-nutritional factors in okpehe samples

The qualitative determination of ant nutritional factors such as resins, flavonoids, tannin, anthraquinones, saponins, phenol, alkaloids, terpenoids was carried out employing the method of Debela (2002) [17].

Quantitative determination of anti-nutritional content in okpehe samples

Alkaloid content was determined using the method of Sowofara (1993) [18], saponins, oxalate, tannins and phenols were determined employing the method of Debela (2002) [17] while flavonoids were determined according to the method of Trease et al. (2002) [19].

Determination of mineral content of okpehe samples

The minerals copper (Cu), manganese (Mn), zinc (Zn), and iron (Fe) were determined via atomic absorption spectophotometry according to methods described by Balogun (2012) [5].

Determination of Potassium, Lead and Cadmium content in okpehe samples

The determination of K, Pb and Ca was carried out by flame photometry by methods adopted by Balogun (2012) [5].

Determination of vitamin content of okpehe samples

The method according to Eby (2014) [20] was employed for determination of vitamin A, C and E content.

Results

The physical characteristics of the okpehe samples were examined at the point of purchase. It was noted that softer samples where characterized by a strong ammoniacal smell and a rich, black colour and slightly harder samples possessed a milder ammoniacal smell and were brown - light brown in colour as shown in Table 1.

Sample	Sampling location	Colour	Smell	Texture
A	Ojogobi, Ankpa	Black	Strong ammoniacal smell	Soft
B	Otukpo, Ankpa	Brown	Strong ammoniacal smell	Soft
C	Garage market	Black	Strong ammoniacal smell	Soft
D	Unity square, Anyigba	Black	Strong ammoniacal smell	Soft
E	Ejah, Idah	Brown	Strong ammoniacal smell	Soft
F	Inikpi, Idah	Brown	Strong ammoniacal smell	Soft
G	Old market, Lokoja	Light brown	Mild ammoniacal smell	Hard
H	New market, Lokoja	Light Brown	Mild ammoniacal smell	Hard

Table 1: Physical characteristics of okpehe samples.

The pH of the okpehe samples ranged from 6.72 -7.83, with samples from new and old markets in Lokoja slightly acidic with corresponding high titratable acidity values than other samples analyzed. Samples from Anyigba garage and unity square markets were significantly alkaline, with pH values considerably higher than all the other samples as shown in Table 2.

Sample	Sampling location	pH	Titratable acidity (g/L)
A	Ojogobi, Ankpa	7.46	0.38
B	Otukpo, Ankpa	7.69	0.35
C	Garage market	7.83	0.27
D	Unity square, Anyigba	7.79	0.30
E	Ejah, Idah	7.15	0.43
F	Inikpi, Idah	7.34	0.40
G	Old market, Lokoja	6.72	0.50
H	New market, Lokoja	6.91	0.45

Table 2: Physicochemical parameters of okpehe samples.

Microbiological quality of okpehe samples

The total bacterial counts of the okpehe samples ranged from 9.5×10^5 - 1.8×10^6 cfu/g. The samples purchased from Anyigba and Ankpa

displayed significantly high bacteria counts, while the lowest counts were recorded in samples purchased from both markets in Lokoja. The total fungal count of okpehe samples analyzed ranged from $<1 \times 10^4$ - 4.0×10^4 cfu/g. The highest fungal count was recorded in samples from new market, Lokoja as shown in Table 3.

The identified bacterial species isolated from okpehe samples were *Bacillus subtilis*, *Bacillus megaterium*, *Bacillus licheniformis*, *Escherichia coli* and *Staphylococcus aureus* while the identified fungal isolates were *Aspergillus niger*, *Paecilomyces* sp. and *Mucor* sp. as shown in Table 4,5 respectively.

Samples	Sampling location	Total bacterial count (cfu/g)	Total fungal count (cfu/g)
A	Ojogobi, Ankpa	1.7×10^6	1.0× 104
B	Otukpo, Ankpa	1.5×10^6	1.0× 104
C	Garage market	1.6×10^6	3.0× 104
D	Unity square, Anyigba	1.8×10^6	1.0× 104
E	Ejah, Idah	1.2×10^6	2.0× 104
F	Inikpi, Idah	1.4×10^6	1.0× 104
G	Old market, Lokoja	9.5×10^5	3.0× 104
H	New market, Lokoja	1.1×10^6	4.0× 104

Table 3: Total fungal and bacterial count of okpehe samples.

Isolates Code	Cultural Characteristics	Gram Reaction	Spore Characteristics	Starch Hydrolysis	Motility	Citrate	Urease	Catalase	Oxidase	Indole	Sugar Fermentation					Probable Organism
											Glucose	Fructose	Maltose	Sucrose	Lactose	
Aoj1, Lom3, Lnm1, Iim1, Aot4, Aus2, Ijm3	Small and mucoid, circular, slightly raised, entire, opaque, creamy, dry.	Gram +ve rods	+	+	+	+	-	+	-	-	A	A	A	A	A	*Bacillus subtilis*
Aoj2, Aot2, Aus3, Agm2, Iim4, Lom2, Lnm2	Large, irregular, flat, lobate, opaque, whitish, smooth.	Gram +ve rods in clusters	+	+	+	+	-	+	-	-	A	AG	A	A	A	*Bacillus licheniformis*
Aoj3, Agm1, Aot5	Medium sized, circular, raised, translucent, light metallic green, smooth.	Gram -ve rods	-	-		-	-	+	+	+	AG	AG	AG	A	AG	*Escherichia coli*

Aoj4, Aot3, Aus1, Agm3, Ijm4, Ijm3, Lom1. Lnm3	Moderate, undulate (wavy), slightly raised, convex, opaque white, dry and slightly wrinkled.	Gram +ve rods	+	+	+	+	-	+	-	-	A	A	A	A	A	*Bacillus megaterium*
Aoj5, Aot1, Aus4, Ijm2, Iim2, Lom4, Lnm4	Moderate, Circular, Convex, Entire (smooth), Opaque, Pale yellow, glistening.	Gram +ve coccus in clusters	-	-	+	-	-	+	-	+	AG	A	A	A	A	*Staphylococcus aureus*

Table 4: Morphological and biochemical characteristics of bacterial isolates from okpehe samples.

Isolate code	Colour	Texture	Shape	Type of hyphae	Conidiophore	Sporangium	Collumella	Probable Organism
Agm1, Lom1, Lnm2	Black	Wrinkled	Round	non-septate	Non-branched	-	-	*Aspergillus niger*
Agm2, Ijm1, Lom2, Lnm1	Cotton white	Rough and loose	Rhizoid	non-septate	Non-branched	+	Well developed	*Mucor* sp.
Aus1, Ijm2, Lom3, Lnm3	Greenish brown	Smooth	Irregular	non-septate	Branched	-	-	*Paecilomyces* sp.

Table 5: Macroscopic and microscopic characteristics of fungal isolates from okpehe samples.

Bacillus species was the most predominant in the okpehe samples, followed closely by *S. aureus* and then *E. coli*, which is the least frequently occurring organism as shown in Table 6.

Sample	***B. subtilis*** **(cfu/g)**	***B. licheniformis*** **(cfu/g)**	***B. megaterium*** **(cfu/g)**	***E. coli*** **(cfu/g)**	***S. aureus*** **(cfu/g)**
A	4.7×10^5	3.5×10^5	3.7×10^5	2.1×10^5	3.0×10^5
B	4.3×10^5	3.6×10^5	4.1×10^5	1.6×10^5	1.9×10^5
C	5.1×10^5	4.4×10^5	4.7×10^5	1.0×10^4	2.0×10^5
D	5.3×10^5	4.3×10^5	3.9×10^5	2.4×10^5	3.0×10^5
E	4.7×10^5	2.5×10^5	2.6×10^5	1.0×10^4	2.7×10^5
F	5.4×10^5	3.3×10^5	3.9×10^5	1.0×10^4	2.0×10^5
G	3.8×10^5	2.3×10^5	1.9×10^5	1.0×10^4	1.5×10^5
H	4.3×10^5	3.2×10^5	2.9×10^5	1.0×10^4	1.5×10^5

Table 6: Total count of bacterial species in okpehe samples.

Total fungal counts revealed higher counts of *A. niger* in samples from garage market - Anyigba and new market - Lokoja, while Mucor and *Paecilomyces species* occurred in frequently between samples from garage market- Anyigba and new and old markets, Lokoja. *Aspergillus niger* was the highest occurring fungi followed by *Mucor* sp. and *Paecilomyces* sp. occurring in relatively irregular patterns as shown in Table 7.

Sample	*Aspergillus niger*(cfu/g)	*Mucor sp.* (cfu/g)	*Paecilomyces sp.*(cfu/g)
A	-	-	-
B	-	-	-
C	2×10^4	1×10^4	-
D	1×10^4	-	-
E	1×10^4	-	1×10^4
F	-	-	-
G	1×10^4	1×10^4	1×10^4
H	2×10^4	1×10^4	1×10^4

Table 7: Total count of fungal species in okpehe samples.

Nutritional composition of okpehe samples

The protein and carbohydrate are the most abundant nutritive macro-elements, while lipids, ash and fiber occurred in minimal quantities (Table 8). The crude protein of okpehe samples ranged between 36.88% - 40.05% (Table 9).

Sample	Proximate Composition (% per g of sample)					
	Moisture Content	Ash Content	Lipid Content	Fibre Content	Protein Content	Carbohydrate Content
A	7.92 ± 0.10^b	5.85 ± 0.12^b	11.32 ± 0.32^a	4.67 ± 0.20^a	34.23 ± 0.01^d	47.06 ± 0.01^c
B	8.01 ± 0.04^a	5.72 ± 0.83^b	10.67 ± 0.01^b	4.52 ± 0.10^a	36.42 ± 0.02^c	46.45 ± 0.24^d
C	8.64 ± 0.05^a	5.31 ± 0.05^b	9.23 ± 0.05^c	4.23 ± 0.11^a	40.28 ± 0.33^a	41.56 ± 0.12^f
D	8.36 ± 0.13^a	5.65 ± 0.05^b	11.45 ± 0.01^a	4.86 ± 0.25^a	38.34 ± 0.15^b	44.32 ± 0.30^e
E	6.97 ± 0.03^c	6.23 ± 0.02^a	8.13 ± 0.01^d	3.24 ± 0.04^b	31.76 ± 0.2^f	47.54 ± 0.14^c
F	7.41 ± 0.03^b	5.92 ± 0.05^b	10.34 ± 0.01^b	3.82 ± 0.20^b	32.72 ± 0.01^e	47.62 ± 0.22^c
G	4.74 ± 0.02^e	6.09 ± 0.83^a	7.34 ± 0.05^e	3.58 ± 0.18^b	32.53 ± 0.01^e	51.63 ± 0.18^a
H	5.13 ± 0.24^d	5.85 ± 0.11^b	9.53 ± 0.05^c	3.74 ± 0.01^b	32.84 ± 0.22^e	49.21 ± 0.21^b

*Values are means of triplicate determination ± SD) Means in the same column with different superscripts are significantly different at ($p < 0.05$).

Table 8: Proximate composition of okpehe samples.

Alkaloids, steroids, flavonoids, saponins, resins and phenols were found to be present while anthraquinones, triterpenoids, tannins and oxalate were found to be absent from all samples analyzed (Table 9). The quantitative analysis of anti-nutritional factors in okpehe revealed the most abundant anti-nutritional factor to be saponin (Table 10), with concentration ranging between 0.72%- 1.10%. Alkaloids, steroids, flavonoids, resins and phenols were found in considerably low concentrations. Table 11 shows that potassium was the most abundant element (2.101mg/g -3.496 mg/g) in all samples analyzed. Copper, iron, calcium, manganese and zinc were found in relatively minute quantities while lead and cadmium were below detection limits in all okpehe samples.

Sample	Anti-nutritional Factors									
	Alkaloids	Steroids	Flavoniods	Saponins	Resins	Anthraquinones	Phenols	Triterpeniods	Tannins	Oxalate
A	+	+	+	+	+	-	+	-	-	-

B	+	+	+	+	+	-	+	-	-	-
C	+	+	+	+	+	-	+	-	-	-
D	+	+	+	+	+	-	+	-	-	-
E	+	+	+	+	+	-	+	-	-	-
F	+	+	+	+	+	-	+	-	-	-
G	+	+	+	+	+	-	+	-	-	-
H	+	+	+	+	+	-	+	-	-	-

Table 9: Qualitative anti-nutritional screening of okpehe samples.

Sample	Phytochemical content (%)					
	Alkaloids	**Steroids**	**Flavonoids**	**Saponin**	**Resins**	**Phenols**
A	0.31	0.22	0.21	1.10	0.46	0.38
B	0.28	0.31	0.36	0.94	0.38	0.64
C	0.22	0.34	0.23	0.84	0.75	0.52
D	0.39	0.25	0.20	0.91	0.88	0.34
E	0.36	0.27	0.32	1.01	0.48	0.28
F	0.39	0.25	0.18	0.88	0.41	0.32
G	0.26	0.33	0.26	0.72	0.64	0.38
H	0.21	0.42	0.28	0.93	0.87	0.46

Table 10: Quantitative ant nutritional screening of okpehe samples.

Sample	Mineral (mg/g)							
	Potassium	**Cadmium**	**Copper**	**Calcium**	**Iron**	**Manganese**	**Zinc**	**Lead**
A	2.295	BDL	0.092	0.556	0.105	0.061	0.142	BDL
B	2.951	BDL	0.087	0.411	0.103	0.065	0.147	BDL
C	2.101	BDL	0.083	0.504	0.114	0.073	0.162	BDL
D	3.111	BDL	0.088	0.523	0.108	0.078	0.158	BDL
E	2.745	BDL	0.094	0.492	0.121	0.071	0.162	BDL
F	3.015	BDL	0.096	0.516	0.126	0.089	0.155	BDL
G	3.496	BDL	0.073	0.447	0.106	0.087	0.172	BDL
H	2.166	BDL	0.08	0.572	0.104	0.088	0.144	BDL
BDL- below detection limits								

Table 11: Mineral composition of okpehe samples.

The analysis for vitamin content of okpehe samples revealed minute quantities of Vitamin A and E with values ranging (0.001%-0.006%) and (0.001%-0.002%) respectively. Vitamin C was found to be below detection limits in all okpehe samples (Table 12).

Sample	Vitamin A (%)	Vitamin E (mg/ml)	Vitamin C (mg/ml)
A	0.006	0.002	BDL
B	0.002	0.001	BDL
C	0.004	0.002	BDL
D	0.001	0.002	BDL
E	0.003	0.001	BDL
F	0.005	0.002	BDL
G	0.003	0.001	BDL
H	0.001	0.001	BDL

Table 12: Vitamin content of okpehe samples.

Discussion

The okpehe samples with high moisture content as detected by texture had a stronger, more offensive ammonical smell, along with a characteristic rich, black color. The harder okpehe samples not only had slightly offensive smell, but also possessed a characteristic light brown color. The pH of the okpehe samples was mostly alkaline and this could be as a result of the elimination of the species responsible for acid fermentation during processing thus, encouraging a non-acid fermentation that is dominated by *Bacillus* species [4]. Odunfa (1985) [21] noted that the rise in pH during fermentation is due to the high proteinase activity of the microorganisms involved which ultimately results in the liberation of ammonia as is reported for some other fermenting protein foods such as natto (Hesseltine and Wang, 1967) [22], iru (Odunfa, 1985) [21], ugba (Oyeyiola, 2002) [23], and soumbala (Ouoba et al., 2007) [24].

Desirable flavour components of the condiment are presumably developed after an alkaline pH is reached. Organic acids which may result from protein decomposition may contribute to the darkening of colour (Achi, 2005) [4]. The pH of okpehe being alkaline agrees with earlier reports of Achi (2005) [4], Oguntoyinbo *et al.* (2001) [6], Omafuvbe et al. (2003) [25] and Ogunshe et al. (2007) [3], all of who recorded alkaline pH in fermented food condiments from vegetable proteins. Most fermented vegetable-protein condiments are characterized by a very strong pungent smell. The increase in pH is generally due to the production of ammonia, which is characterized by the pungent smell of fermented condiments (Ogunshe et al., 2007) [3], this provides explanation for why slightly acidic samples G (samples from Lokoja, Old market) and H (Samples from Lokoja new market), possess only mild ammoniacal odor. Aremu (2006) [26] also reported that ammonia production might be due to protease deaminase enzymes produced by *Bacillus* isolates.

Microbial analysis of okpehe samples indicated bacterial counts range of 9.5×10^5 - 1.8×10^6 cfu/g and the predominance of *Bacillus* species. The presence of *Bacillus* species in the samples is expected since they have been found to be associated with fermented legume seeds for okpehe (Achi, 2005; Ogunshe et al., 2007) [3,4], dawadawa (Odunfa (1985) [21]; (Antai and Ibrahim, 1986) [27], iru (Oyeyiola, 2002) [23] and soumbala (Ouoba et al., 2007) [24]. *Bacillus* produces protease which is responsible for the breakdown of proteins and this contributes to the development of texture and flavor of fermented foods [28].

The presence of *Staphylococcus* species in the samples is also typical of the microflora of fermenting beans [27]. *Staphylococcus* species have been associated with fermenting foods of plant origin especially vegetable proteins [29]. The coagulase-negative *Staphylococcus* species were indicated in the samples and this could also be significant as they have been implicated as opportunistic pathogens [30].

Escherichia coli was present in three of the eight samples, A (samples purchased from Ojogbobi, Ankpa), B (samples purchased from Otupko, Ankpa) and D (samples purchased from Unity square, Anyigba). *E. coli* though fermentative and found in the air and soil, has been isolated from some fermentation of leguminous condiments [3]. *Enterobacter cloacae* were isolated by Achi (2005) [4] from okpehe, although no enterobacterium was isolated by Oyeyiola (2002) [23] in okpehe from local producers from Ilorin, Kwara State. This may also be indicative of fecal contamination as a result of mishandling of the product during production.

The higher bacterial load obtained in sample D (okpehe purchased from Unity square, Anyigba) may be attributed to the production procedure carried out for the sample along with the location of display and sale, which is a mini-market which also doubles as a motor-park garage, and a general meeting point for community functions, further emphasizing the need for appropriate health care and conscientious contact control precautions.

The presence of fungi in the samples; C, D, E, G and H may be due to contamination or the introduction of spores from the air during processing of the okpehe seeds. The incidence of a known toxigenic mould (*Aspergillus niger*), along with *Mucor* sp., and *Paecilomyces* sp., whose toxigenic status have not been clearly established also highlights the risk of mycotoxin contamination. The highest occurring fungal isolate was *A. niger* and studies have shown that this organism produces mycotoxin which is of health risk to the consumer after continuous accumulation [31]. The occurrence of *Paecilomyces* sp. and *Mucor* sp., may not pose any significant health challenge however, there is no known mycotoxin-associated risk. It was observed that none of the samples was moldy when purchased from the markets, yet they had fungal contamination, and this indicates that the okpehe samples may have been contaminated with the spores of the fungi, thus care should be taken to eliminate fungal contamination by proper handling and storage.

The crude protein content of the okpehe samples was very high and this may be attributed to the role of *Bacillus subtilis* during fermentation as they produce protease that act on the legume seed to release more proteins [32]. The protein content values were observed to vary with increasing moisture content suggesting an association between both proximate factors. The lipid content was low which may be considered desirable, since high amounts of fatty acids in foods can cause rancidity thereby making the food to taste sour. However, the values varied indiscriminately and may have been influenced by factors in the production process unique to individual producers.

The crude fiber content was low. Locust beans are known to contain oligosaccharides that cause flatulence therefore the presence of the crude fiber may be responsible for the digestibility of okpehe. The ash content of the okpehe samples is an indication that it is rich in minerals although the values may be influenced mildly by a wide range of factors including length of fermentation time.

The varying carbohydrate content of the samples may be due to the hydrolytic effect of microbial amylase converting the carbohydrate into sugars easily utilizable by the isolated organisms during fermentation, samples with a higher *Bacillus sp.* load witnessed a rapid decline in carbohydrate levels. *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) [3] thereby leading to a reduction in the carbohydrate. However, these results also suggest okpehe as a good source of carbohydrate, and an even better one since it is balanced out by its high protein content.

Okpehe being of legume origin is known to contain anti-nutrients. Anti-nutritional screening of the samples revealed the presence of alkaloids, flavonoids, saponins, resins, steroids and phenols while tannins, oxalate, anthraquinones and triterpenoids were below detection levels in all samples analyzed. The toxic effects of tannins have been eliminated in this study due to their absence in the anti-nutritional screening conducted. The okpehe samples examined did not contain oxalates, anthraquinones and triterpenoids. Phenols have been shown to demonstrate antimicrobial properties [33], saponins have been implicated in causing bloating and also reducing protein digestibility due to the formation of sparingly digestible saponin-protein complexes [34,35], nevertheless they have been demonstrated to have antifungal properties, and their presence may have assisted in preventing the proliferation of fungi [36]. The variation in saponins content in the various samples may be attributed to the structural differences in their sapogenin fractions [37]. Alkaloids possess anti-diarrheal and anti-dysenteric properties. Flavonoids, resins, and steroids may either be beneficial and or detrimental [38]. These factors may however not perform significant functions since they occur in inconsiderable amounts in all samples analyzed.

Potassium was found to be the most abundant mineral in all the samples varying as well with increasing moisture content. The abundance of potassium in the okpehe samples is in close agreement with the observation of Aremu et al. (2006) [26], that potassium was the most predominant mineral in 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 [39]. Aremu et al. (2006) [26] in related literature also observed that potassium was the most abundant mineral in *Prosopis africana* flour. The work of Nda-Umar et al. (2008) [40] however gave a contrary result that calcium is the most abundant mineral in okpehe (Balogun, 2012) [5].

Calcium was found to be the second highest mineral present in all analyzed samples. 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 and this is desirable as it is known to aid digestion and body functions. Moderate amounts of iron were detected in the okpehe samples. 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 [39]. The amounts of manganese detected in the samples were relatively moderate. 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 [39].

Copper was also detected in all the samples analyzed. Copper is a trace element that serves as a co-factor and is required for enzyme function. Lead and cadmium were on the other hand not detected in all the samples similar to results obtained by Balogun (2012) [5]. This is preferred since the elements are known to be toxic to the human body. The analysis of vitamins in okpehe samples revealed barely significant quantities of vitamin A and E and a complete absence of vitamin C. This may however not be characteristic of the *Prosopis africana* or other legumes for that matter.

Conclusion

This study detected the incidence of potential pathogenic organisms including both bacteria and fungi which may have contaminated the food during processing, sale display or storage therefore, critical control points and good manufacturing practices should be deployed during the processing of okpehe. Okpehe has high nutritive value and thus could be a better alternative with added advantage of reduced health risk over the consumption of seasoning salts and animal protein. It is recommended that starter cultures be developed and made available commercially so that industrial/large scale production of okpehe having consistent quality with good export potential can be realizable.

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