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Identification and quantification of headspace volatile constituents of okpehe, fermented *Prosopis africana* seeds

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Abstract

The volatile components of the fermented seeds of *Prosopis africana*, (okpehe), were determined. Traditional method of production was used. The volatile constituents were analyzed by Gas Chromatography using the PDMS-SPME head space technique and identification of volatiles was by comparing their retention time and mass spectra with those of the library. In all, there are about 51 volatile components with 8 alcohols, 15 aldehydes, 8 ketones, 2 acetates, 11 benzene derivatives, 4 alkanes, 2 alkenes and 4 others. The aldehydes constitutes the bulk of the volatiles followed by the pyrazines. Most of the identified compounds are known to have strong impact on the flavour and fragrance of fermented and roasted products.

Introduction

Okpehe, a fermented flavouring food condiment, most popular in the middle belt of Nigeria, is produced from *Prosopis africana*, a leguminous oil seed. It is a strong smelling mash of sticky dark brown seeds and produced in moist solid state fermentation by chance inoculation, supposedly by various species of microorganisms (Ogunshe, 1989).

Prosopis africana also known as African mesquite is of the genus *Prosopis* in the family of *Fabaceae* and of the order of *Fabales*. It can grow up to 20 m in height; has an open crown and slightly rounded buttresses, bark is very dark, scaly, slash, orange to red brown with white streaks. The pods are cylindrical, hard and shinny up to 1.5 – 3 cm compartmented with woody cells. The flowering of *Prosopis africana* occurs shortly before the onset of rains and seed matures in February to March containing some loose rafting seeds per pod and 7,500 – 8000 seed per kg (Keay, 1982).

The pods have a thick pericarp consisting of three layers: a hard woody exocarp, a pulpy mesocarp and a thin septate endocarp between the seeds. The pod is fleshy when immature, but dries at maturity leaving the seeds loose 'rattling'. Each pod contains about 10 seeds, each one in its own compartment (septum). The seeds are embedded within a pulpy matrix (mesocarp). The hard seed coat is impermeable to water. The mean thousand seed weight is 146 g. The seeds contain c. 2% neutral lipids (Keay, 1982).

Extraction of *Prosopis* seed is generally difficult because the seeds are imbedded in a pulpy mesocarp within a hard dry pod. Grinding mills have been used to remove the outer dry pod. The pods are then soaked in a 0.1 M solution of hydrochloric acid for 24 hours. The pods can then be washed in water for 1 hour and sun dried. The dried mass can then be pounded to separate the seeds from the coating. Traditionally the pods are immersed in boiling water and allowed to cool and remain soaked in water for 24 hours, after which the fruits are easily opened. The seeds are cleaned by robbing in between palms (Booth and Wickens, 1988).

Apart from the use of the fermented seeds as food condiments, the seeds are used as animal feed. The fermented products are known by different names by various ethnic groups in Nigeria - okpehe in Idoma, kiriya in Hausa, gbaaye in Tiv and okpiye in Igala. Although significant efforts had been made to understand the microbial and biochemical characteristics of fermented *Prosopis africana* seeds (Ogunshe *et al.*, 2007; Balogun and Oyeyiola, 2012) none to our knowledge had dwelt on the flavour constituents. Ammoniac odour has been found to be a common odour of most fermented leguminous products as a result of protein degradation, however each product has unique characteristic odour that makes it possible for one product to be differentiated form another apart from the texture and colour. This study was therefore designed to identify the volatile constituents of fermented *Prosopis africana* seeds

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Materials and Method

Sample collection

The harvested pod of *Prosopis africana* were picked from a farm in Otukpo, Benue State Nigeria. The hard pod was broken with mortar and pestle to collect the seeds. The seeds were sorted to remove stones and bad ones. The leaves used for fermentation were purchased from Keffi main market Nasarawa State.

Preparation of the cooked fermented sample of Prosopis Africana seed (okpehe)

Preparation of the fermented seeds was carried out as described by Ogunshe (1989). The cooked cotyledons were spread into a sterile nylons and fermented for 7 days in an incubator at 37^{o}C to produce a sticky mucilaginous light brown and odorous mash of Okpehe condiment.

Identification of volatile components of okpehe

The volatile constituents were analyzed by gas chromatography using the PDMS-SPME head space technique. To extract the volatile constituents, 5 g of the mashed fermented seeds were transferred into capped glass vial and SPME fiber–Polydimethylsyloxane-Divinylbenzene (PDMS-DVB) polymer was used for extraction. The extraction was held for 30 min at a temperature of 60^{o}C. The constituents of the extract were analysed using GC-MS by direct injection method in the split mode with (split ratio 20:1) under the following conditions; Hewlett – Packard 6890 GC equipped with a flame ionization detector (FID) and a quartz capillary column; 30 m x 0.25 nm x 0.25 nm, nitrogen was used as carrier gas, oven temperature 60^{o}C (initial) with oven programmed at 220^{o}C, detector temperature 300^{o}C, hydrogen pressure and compressed air were 28 psi and 35 psi, respectively. A digital integrator was then used to integrate the area of the signal from the detector. The integrated area, retention time and composition in mg/100 g were printed automatically at the end of each peak.

Identification of Components

The qualitative identification of the different constituents was performed by the comparison of their retention times and mass spectra with those of the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

Results and Discussion

Using headspace analysis, a total of 51 constituents were identified. On weight basis the aldehydes are the dominant group. Table 1 shows the alkanolic constituents of okpehe, with the major alkanolic constituents been undecanol, octanol, ethanol, propanol, and dodecanol. The alkanols present in the condiment help prevent them from spoilage since alkanols are known to act as antifungal and prevent food spoilage. Most of these alkanols had been reported present in other fermented leguminous products (Dajanta *et al*., 2010; Onyenekwe *et al.*, 2012). From the result Okpehe contains 15 aldehydes, with hexanal, heptanal, 2-nonanal, nonanal, 2,4 decadienal, Decanal, dodecanal, 2,4 – nonadienal, 2-butyloctenal, 2, 4-decadienal been the major. The odour in the fermented food condiment could be due to the presence of these aldehydes. This is consistence with the previous work of Grosch (1982) that carbonyl compounds such as aldehyde and alcohol have strong impact on odour because of their sensitivity to olfactory receptors. Hexanal, the dominant aldehyde, is a key organoleptic element of green-note that is found in both fragrances and flavours (Schade *et al.*, 2003), such as traditional commercial shrimp paste kapi (Wittanalai *et al*., 2011) and fermented soybean, melon and locustbean (Onyenekwe *et al*., 2012). Hexanal is produced during advanced lipid oxidation, promoted by alkyl and alkoxyl radicals (Abdalla and Roozen, 1999).The effectiveness of hexanal as a metabolizable fungicide and enhancer of aroma production by its inter conversion to other aroma volatiles in minimally processed apples has been demonstrated (Song *et al.*, 1996; 1997).

Eight ketones were identified and quantified in the sample and they are acetophone, acetophenone, 2-nonadecanone, 3-ethylhexanone, 3-ethyloctanone, ethylnonanone, 2,5-hexadione and 3-ethythepentanone. Ketones are usually derived from lipid and amino acid degradation during microbial fermentation and have a high impact on food odour (Owens *et al*., 1997), ketones may contribute to the odour of Okpehe, this is in consistent with the work of Stephan and Steinhart (1999) that identified 17 ketones in soyabean lecithin.

The table shows the ester constituents of the condiment, the higher concentration of esters compared with the levels reported by Onyenekwe *et al*. (2012) in ogiri (fermented melon seed) and daddawa (fermented locustbean and soybean seeds) may be responsible for the 'fruitiness' associated with okpehe. The acetates of higher alcohols and the ethyl ester of fatty acids had been suggested to be the most

Table 1. Concentrations of volatile compounds (mg/100 g dry basis) in fermented *Prosopis africana* (Okpehe)

S/No	Retention time [min]	Name of constituents	mg/100 g
		Alkanols	
1	7.653	Ethanol	6.977
2	9.826	Propanol	0.492
3	10.792	1,2-Ethanediol	0.11
4	18.677	1-Octan-3-ol	5.357
5	18.773	Octanol	2.923
6	24.784	Dodecanol	4.041
7	25.257	Undecanol	5.277
8	31.356	3,7,11-Trimethyl-2,6,10-dodecatrien-1-ol	11.564
			36.741
		Acids and esters	
9	12.830	Ethylacetate	13.939
10	14.157	Ethylhexanoate	13.333
11	30.985	9,12-Octadecadienoic acid (Z,Z)	5.826
12	31.834	Octadecyl acetate	2.741
			35.839
		Alkanones(Ketones)	
13	18.125	Acetophone	2.662
14	18.394	Acetophenone	2.215
15	22.312	2-Nonadecanone	1.457
16	22.503	3-Ethylhexanone	1.022
17	22.832	3Ethylheptanone	2.682
18	23.217	3-Ethyloctanone	2.228
19	23.952	Ethylnonanone	2.949
20	26.933	2,5-Hexadione	2.099
			17.314
		Alkanals(Aldehydes)	
21	11.142	Pentanal	0.247
22	11.367	Hexanal	39.181
23	14.501	Benzaldehyde	3.993
24	15.027	Benzeneacetaldehyde	3.483
25	18.009	Heptanal	1.827
26	18.956	2-Nonenal	2.183
27	19.246	Nonanal	0.657
28	19.917	Decanal	0.62
29	20.611	2-Butyl-octenal	0.413
30	20.935	Dodecanal	0.27
31	21.982	2,4-Nonadienal	1.108
32	24.704	2,4-Decadienal	2.383
33	27.610	Hendecanal	0.123
34	28.435	2-Nonenal	1.357
35	29.215	2,4-Undecadienal	4.709
36	29.695	2,4,6-Dodecatrienal	4.209
			68.764
		Hydrocarbons	
37	19.784	Decene	1.131
38	21.435	Cyclotetradecane	0.487
39	25.799	Tetradecene	3.504
40	28.760	Cyclododecane	5.091
41	30.138	Cyclohexadecane	6.13
42	32.918	Hexacosane	8.358
			24.701
		Pyrazine	
43	16.115	2-Ethyl-6-methylpyrazine	4.399
44	16.224	2,6-Dimethylpyrazine	3.113
45	16.459	Trimethyl pyrazine	2.484
46	16.544	Tetramethyl pyrazine	9.797
47	16.924	2,5-Dimethylpyrazine	1.647
			21.44
		Benzenes	
48	13.832	Toluene	7.653
49	15.797	1,2-Dimethylbenzene	4.6
			12.253
		Others	
50	15.388	Dimethyldisulphide	12.021
51	30.811	2,6-Diphenylpyridine	7.842
			19.863

desirable compounds in miso products to enhance the aroma of the finished products and are responsible for the fruity tinge of freshly prepare miso (Giri *et al.*, 2010).

The major benzene derivative present in the food condiment include toluene, benzaldehyde, benzeneacetaldehyde, 1, 2 – dimethyl benzene, 2 – ethyl 5 – methyl pyrazine, 2, 6 – dimethyl pyrazine, trimethylpyrazine, tetramethylpyrazine 2,5–dimethyl pyrazine, 2, 6 – diphenyl pyrazine. This is consistent with previous work by Sogawara *et al.* (1985) who found 7 pyrazine derivatives in commercial and home – made natto. Lee and Ahn (2009) detected 9 pyrazine in commercial doenjang, while Onyenekwe *et al.* (2012) observed that pyrazines are the most dominant flavor constituents of daddawa (fermented locustbean seeds) after aldehydes and the major components been 2, 5 – dimethyl pyrazine, tetramethyl pyrazine and trimethyl pyrazine. Microbial fermentation of legumes is known to increase the free amino acids content by five folds (Odunfa, 1986) and some of these amino acids are precursors of pyrazines. Threonine could be the precursor of 2, 5 – dimethyl pyrazine while tetramethyl pyrazine can be produced from different amino acids example glycine, alanine, valine, isoleucine and leucine via Strecker degradation and deamination (Shu, 1998). Tetramethyl pyrazine is also derived from an interaction of acetoin (the bye product of maillard reaction) and ammonia (Larroche *et al.*, 1998). Pyrazines had been related to the sensory attributes of soy sauce (Lee and Ahn, 2009). Tetramethyl pyrazine, the most abundant heterocycline, reported to be the most abundant flavor compound in dark chocolate (Afoakwa *et al.*, 2009) was reported to exhibit milk-coffee-roasted-cooked notes. Apart from its sensory attributes, tetramethyl pyrazine has been shown to have antioxidant activity (Wang *et al.*, 2012). This coupled with the fungicidal and bactericidal activities of other constituents may be responsible for non-spoilage of this condiment no matter how long it is stored without refrigeration.

The major alkanes present in the condiment include cyclododecane, hexacosane and cyclotetradecane. While the major alcoholic constituents of the condiment include 1 – octanol., 3, 7, 11, - trimethyl – 2, 6, 10 – dodetrien – 1 – ol and 1, 2, – ethanediol with variation in concentration. Alcohols contribute to the flavour of the condiments. This is consistent with previous work where alcohol was reported in soy sauce, (Lee and Kwok, 1987), Miso (Ku *et al*., 2000) and Korea doenjang (Park *et al*., 1994) as important contributor of flavour. Hexanol and 1 – octen – 3 – ol arise from enzymatic oxidation of linoleic and linolenic acid (Tressl *et al.*, 1982).

Other volatile constituents present in the fermented food condiment include Dimethyl disulphide, a non-glucosinolate derived from sulphur-containing compounds, known for its toxic effect to a broad range of fungal and bacterial species. Dimethyl disulphide is one of the most important aroma compounds of black truffle (*Tuber melanosporum*) aroma (Culleré *et al*., 2010).

Conclusion

Okpehe like other condiments which has taste enhancing properties and serves as non meat substitute for low-income families in some parts of Nigeria requires a food processing technology that will meet the requirement and challenges of human

needs. Therefore, a more elaborate study to optimize the processing methods should be done. Also because the processing of Okpehe and other local condiments are still craft based remarkably, in many parts of Nigeria today, they are still made in traditional way. They often have a stigma attached to them due to the odour and they are often considered as food for the poor.

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