SOME QUALITY INDICES AND FATTY ACID PROFILE OF PURE-CULTURE FERMENTED UGBA (*Pentaclethra Macrophylla* Benth) CONDIMENT OIL EXTRACT

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Abstract
Effect of pure-culture fermentation on the quality indices and fatty acid profile of oil extract of African oil bean seed processed to ugba condiment was assessed by fermenting the seeds with a pure-culture of *Bacillus subtilis* for 5 days at 37^0C in an incubator. Some of the seeds were also traditionally fermented at ambient temperature (29 ± 2^0C) to serve as control. The condiment from each method was further processed by oil (soxhlet) extraction to obtain the crude oil extract. The extract was analysed for some quality indices namely density, free fatty acid, acid value, saponification value, peroxide value and iodine value. The extract was also saponified with KoH, esterified and further methylated to obtain the methyl esters of the fatty acids. The methyl/esters were separated and quantitized using a gas chromatography. A significant ($p<0.01$) increase was observed in free fatty acid, acid value, saponification value, peroxide value and iodine value of the oil extract of the condiments. The saturated fatty acids identified in the condiments were lauric, myristic, palmitic and stearic, while oleic and linoleic were the unsaturated fatty acids. The highest quantity of saturated fatty acid was identified in the pure-culture fermented condiment (48.26%); the least quantity of saturated fatty acid identified in the condiment was myristic acid (7.72%) while the highest quantity was stearic (26.66%). The highest quantity of unsaturated fatty acid (57.47%) was identified in the traditional fermented condiment, conferring a better quality (more heart-friendly) on the condiment.

Keywords: African oil bean seed; pure-culture, fermentation, indices, fatty acid, condiment.

Introduction
The African oil bean tree (*Pentaclethra macrophylla* Benth) is a perennial tree normally called the oil been tree, Congo acacia or Atta bean tree. It is a large, leguminous, woody plant that belongs to the family leguminosae. Its local name in Nigeria is *ugba*, *ukpaka* or *ukpakala* (Hutdrinson and Dalziel, 1954). It produces about 8 flat glossy brown, edible seeds per pod. The plant grows both as wild and cultivated types. The African oil bean seeds (AOBS) are inedible when raw and bitter until the final stage of fermentation (Ezedinma and Igbinosa, 1993).

The AOBS are a potential source of edible oils in view of their high oil content (Mbajunwa, 1995). Onwuliri *et al* (2004) reported that *ugba* lipids contain both saturated and unsaturated fatty acids of long chain lengths. However, according to Ikediobi (1981); Achinewhu, (1982) and Enujiugha, (1990), lenoleic acid is the major fatty acid in *ugba* followed by oleic acid.

Many studies have been done on the effect of fermentation period on the concentration of the fatty acids in *ugba* and observed no significant change due to processing and fermentation (Achinewhu, 1998, Onwuliri *et al* ; 2004). On the otherland, Enugiugha, (2003) reported a change in the fatty acid profile after AOBS fermentation (traditional). In spite of these studies no study has been done to assess the effect of fermentation method on quality indices and possible changes in the fatty acid profile of *ugba* oil after fermentation.

As AOBS is usually processed to delicacy (3 days fermentation) or condiment (5 days fermentation) employing the traditional fermentation method, this work was designed to assess the effect of pure-culture (experimental) fermentation method on some quality indices and fatty acid profile of the oil extract from the 5-day fermented soup condiment.

Materials and Method

Preparation of samples

The dry African oil bean seeds (AOBS) were obtained from Afor Oru market in Ahiazu Mbaise local government area of Imo State of Nigeria, and stored in air-tight polyethylene containers at ambient temperature (29 ± 2^0C). The seeds were cleaned, boiled, delulled cooked, sliced/pulverized and fermented for 5 days at ambient temperature to yield traditional fermented *ugba* slices (TFUS) (the control sample) and traditional fermented *ugba* condiment (TFUC), respectively.

Another portion was inoculated with a pure culture of *Bacillus subtilis* and allowed to ferment in an incubator at 37^0C for the same period to achieve pure-culture fermented *ugba* condiment (PFUC). The condiments were dried in an oven (Hot-box) at 60^0C to a constant weight and stored in air-tight non-transparent plastic containers for chemical analyses.

Determination of some Quality Indices of the crude oil of *ugba* condiments

Soxhlet extraction method was used to determine the oil yield of the unfermented African oil bean seed (UFAOBS), PFUC, TFUC and TFUS (AOAC, 1990). The crude oil extract of each sample was further analysed using the following parameters namely density, free fatty acid (FFA), acid value, saponification value, peroxide value and iodine value (AOCS 1986).

Derivatization of Fatty Acids to the Corresponding Methyl Esters and Chromatography

The crude oil extract of the condiments were saponified with KOH, methylated and subsequently esterified to obtain the methyl esters of the fatty acids. Gas liquid chromatography method was used to separate, purify and quantititize the individual fatty acids (AOAC, 1990).

Results and Discussion

The percentage crude oil (ether extract) crude oil contents of the condiments – PFUC, TFUC and TFUS, including the boiled, unfermented African oil bean seed (UFAOBS) are shown in Table 1. UF AOBS had the least oil content of 41.68% while PFUC and TFUC had 47.27% and 56.82%, respectively. TFUS had the highest content. However, no significant difference was observed in the condiments. The least increase as observed in PFUC could probably be

due to less extensive tissue brease down which occurred in the seed during fermentation because of sole fermenting activity of *Bacillus subtilis* unlike in traditional fermentation where there was a mixed culture of bacteria, leading to greater softening and ease of oil extraction.

Some Quality Indices of Oil Extract of *Ugba* Condiments

Density of the oil extracts of *ugba* condiments

The density of the crude oil extracting the ugba condiments (Table 2) indicated that this parameter remained unaffected by both fermentation and fermentation method. UFAOBS, PFUC and TFUC all had the same value of 0.897gcm^{-3} while TFUS had a value of 0.898gcm^{-3}.

Free Fatty Acid (FFA) of the Oil Extract of the *Ugba* Condiments

The result on FFA (Table 2) showed that UFAOBS had the least value (1.047%), while PFUC had the highest value (2.256%). TFUC and TFUS had 1.412% and 1.450%, respectively. A significant ($p<0.01$) difference was observed. Since FFA is an index of lipid hydrolyisis, the result indicated that fat hydrolysis (lipolysis) occurred most in PFUC. Iherekoronye and Ngoddy (1985) had reported that lipolytic enzymes occur in plants, animals and micro-organisms.

In their report, Winarno and Reddy (1986) established that extensive lipolysis does not occur in legume fermentation. In support of the above mothers, Njoku and Okemadu (1989) later observed a minimal participation of lipase in *pentaclethra macrophylla* during fermentation. Interestingly, low lipase activity in some fermented food has been considered desirable because of the problem of objectionable taste and development of rancidity (Odunfa, 1985b). FFA has been reported to contribute immensely to the flavour of many fermented vegetable proteins particularly condiments and seasonings (Odunfa and Adesomoju, 1985).

However, at 1.5% FFA (as linoleic), acidity begins to be noticeable with most oils. But according to Osueke (1998), combination of alkaline refining, bleaching and deodorization achieved a residual FFA level of as low as 0.03 – 0.5%.

Acid Value (AV) of the Oil Extract of the *Ugba* Condiments

Acid values of the condiments extracts followed the same trend as in FFA (Table 2). The result indicated that AVs of the oil extracts increased from 2.04 mg KoH/g (the least) in UFAOBS to 4.488 mg KoH/g in PFUC (the highest). TFUC and TFUS had 2.085 mg KoH/g and 2.876 mg KoH/g, respectively. AV is nulligrammes of potassium hydroxide necessary to neutralize the acidity of the oil. The result similarly indicated a higher degree of that hydrolysis in PFUC than in the traditional method.

Saponification Value (SV) of the Oil Extract of *Ugba* Condiments

The least value (59.86 mg KoH/g) was observed in UFAOBS whereas the PFUC had the highest value (86.64 mg KoH/g). TFUC and TFUS had 83.20 mg KoH/g and 70.13 mg KoH/g, respectively. A significant ($p<0.01$) difference was observed. SV is a measure of the mean molecular weight of the fatty acids present in the oil samples. Each fat, in principle acid within the limits of biological variation, a constant fatty acid composition (Iherekoronye and Ngoddy, 1985). This result suggests that more fatty acids are available in PFUC than in the traditional fermented condiment oil samples to combine with alkali. This further supports FFA and AV results that greater fat hydrolysis occurred in PFUC than in the rest oil samples.

Peroxide Value (PV) of the Oil Extract of *Extract of Ugba* Condiments

The least PV was observed UFAOBS (5.16 meq/Kg) while TFUS had the highest value (18.68 meq/Kg). PFUC and TFU had 5.62 meq/Kg and 16.82 meq/Kg respectively. There was a significant ($p<0.01$) difference in the values peroxide value is an index of rate of deterioration of fat by oxidation peroxidation). (Iherekoronye and Ngoddy, 1985). As *ugba* oil is composed mainly of unsaturated fatty acids (poly unsaturated) (Ihediobi, 1981; Achinewhu, 1982; Enujiugha, 1990), the double bonds in the unsaturated fatty acids are prone to attack by molecular oxygen (auto-oxidations) high and molecular oxygen (photo-oxidation) and lipoxidation (enzymatic oxidation) (Iherkoronye and Ngoddy, 1985). This result indicated that the rate of oxidation is fastest in TFUS while it is slowest in PFUC.

Reports of Nout and Rombout (1990) and Ouoba *et al* (2003b) stated that the significant lipolysis of legumes yields predominantly oleic, linoleic and linolenic acids which have in the structures, one double bond, two double bonds and three double bonds, respectively. PFUC oil can be said to be the most stable (in terms of oxidation) of all the oil extracts of *ugba* condiments in view of the PV result. Since he fatty acid composition of African oil bean seeds (AOBS) oil was the same for all the samples prior to fermentation, pure-culture fermentation seemed to confer a higher shelf-stability on both the oil and food produced thereof.

Iodine Value (IV) of the Oil Extract of *Ugba* Condiments

Results on IV showed that UFAOBS had the least value (3.572 mg/100g) whereas the highest value (8.230 mg/100g) was observed in TFUC. PFUC and TFUS had 4.095 mg/100g and 4.793 mg/100g respectively. A significant ($p<0.01$) difference was observed in the values. The result showed that fermentation generally increases iodine value of *ugba* oil. Since iodine value is an index of total degree of unsaturation of the fatty acids in the oil (Iherkoronye and Ngoddy, 1985) the result suggests that method of fermentation of AOBS has a varying degree of increasing IV in the oil. The result implies that PFUC is the best in terms of possible shelf stability whereas TFUC is the least as it had the highest degree of unsaturation. Okafor *et al* (1991) had reported higher iodine value in fermented AOBS oil. The result also revealed that only UFAOBS oil remained liquid at room temperature, whereas the rest of the oil samples remained solid at room temperature. Enujiugha (2003) also corroborated this result by reporting an increase in unsaturation of the seed oil with fermentation.

Fatty Acid Profile of the *Ugba* Condiments

Gas chromatography of the fatty acids revealed that lacoric, myrostic, palmitic, stearic, oleic and linoleic acids were detected in the samples. Lacoric acid was only detected in UFAOBS. The result also indicated that total unsaturated fatty acids constituted 47.99% in UFAOBS, 51.71% in PFUC, and 57.47% both in TFUC and TFUS. Total detected saturated fatty acids were 51.99%, 48.20%, 39.24% and 39.24% in UFAOBS, PFUC, TFUC and TFUS, respectively. As earlier reported by Ikediobi, 1981; Achinewhu, 1982, Enujiugha 1990 and Onwuliri *et al* 2004), linoleic acid was the major fatty acid – 26.69% (UFAOBS), 38.51% (PFUC), 30.84% (TFUC) and 30.84% (TFUS), followed by oleic acid - 21.30% (UFAOBS), 13.20% (PFUC), 29.63% (TFUC) and 29.63% (TFUS); in AOBS.

On the contrary Onwuliri *et al* (2004) reported that the saturated fatty acids account for 25.2 to 26.5% of the fatty acids in *ugba* undergoing processing and fermentation (2 days) while the unsaturated fatty acids constitute between 73.5 and 74.7% with linoleic acid being more predominant ranging from 44.7 – 46.3%, whereas Enujiugha (2003) reported 9.84% of saturated and 90.06% of unsaturated fatty acids after three days of fermentation.

The result revealed that myristic acid decreased from 11.33% in UFAOBS to 7.72% in PFUC but was not detected both in TFUC and TFUS. There was an agreement with Enujiugha (2003) who reported that myristic acid was not detected after two days of fermentation of AOBS but about 0.23% was later detected after three days. This disappearance of mystic acid in the traditional samples could be attributed to lipases in the mixed culture of bacteria that fermented the seed, whereas the concentration as detected in PFU could be the residual since *Bacillus subtilis* is sparingly lipolytic. Njoku and Okemadu (1989) observed minimal participation of lipase in *Pentaclethra macrophylla* during *ugba* production.

Palmitic acid increased from 12.42% in UFAOBS to 13.85% in PFUC and 16.96% both in TFUC and TFUS. This result agreed with Enujiugha (2003) whose report indicated in increase in palmitic acid after 3 days of fermentation. Stearic acid increased from 18.96% in UFAOBS to 26.66% in PFUC and 22.55% both in TFUC and TFUS. In contrast, Enujiugha (2003) and Onwuliri *et al* (2004) reported a decrease in stearic acid after 2 – 3 days of fermentation.

In unsaturated fatty acids, while oleic acid decreased from 21.30% in UFAOBS to 13.20% in PFUC increased to 26.63% both in TFUC and TFUS; linoleic acid increased from 26.69% in UFAOBS to 38.51% in PFUC and 30.84% both in TFUC and TFUS. Whereas the result on oleic acid agreed with both Enujiugha (2003) and Onwuliri *et al* (2004) on PFUC; it, however disagreed with the authors on the traditional method (TFUC and TFUS).

However, the result on linoleic acid agreed with Enujiugha (2003) and Onwuliri *et al* (2004) who observed an increase percentage linoleic acid. The former author attributed the increase to increased hydrolysis of the glycorides. This observed increased in the saturated fatty acids (palnutic and stearic acids in this work could have led to the increased saturation of the crude oil, which made the oils of PFUC, TFUC and TFUS harden up and remain semi-solid at ambient temperature. It follows, therefore, that the observed increases in oleic (TFUC and TFUS) and linoleic acids in the condiments could not increase the level of liquidity of the crude oils.

Conclusion

Pure-culture fermentation of African oil bean seeds to produce condiment oil that was not significantly different from the traditional method. On the quality indices determined the oil sample differed significantly in free fatty acid, acid value, saponification value, peroxide value and iodine value. Pure-culture fermentation yielded crude oil that was most prone to lipolysis; led to the greatest increase in saturation, but was the least prone to detesuration by oxidation. Hence, the oil from the traditional fermented *ugba* condiment is more heart-friendly than the oil from pure-culture method.

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Table 1: Some Quality Indices of the Oil Extract of Ugba Condiments

	Sample			
Index	**UFAOBS**	**PFUC**	**TFUS**	**LSD**
Density (g/cm^3)	0.897	0.897	0.898	ND
FFA (%linoleic)	1.047^{c}±0.001	2.256^{a}±0.003	1.450^{b}±0.001	0.086
AV	2.048^{c}±0.002	4.488^{a}±0.001	2.878^{b}±0.001	0.166
SV (Mg KoH/g sample)	59.86^{c}±0.05	86.64^{a}±0.02	70.13^{b}±0.02	0.055
PV (Meq/Kg sample)	5.16^{b}±0.06	5.62^{b}±0.02	18.68^{a}±0.36	10.99
IV (Mg/100g sample)	3.572^{b}±0.07	4.095^{a}±0.05	4.793^{a}±0.003	0.052

Data are mean of three replications ± standard deviation (SD)

UFAOBS	=	Unfermented African Oil Bean Seeds
PFUC	=	Pure-Culture Fermented Ugba Condiment
TFUS	=	Traditional Fermented Ugba Slices
LSD	=	Least Significant Difference
ND	=	Not Determined
FFA	=	Free Fatty Acid
AV	=	Acid Value
SV	=	Saponification Value
PV	=	Peroxide Value
IV	=	Iodine Value