

Full Length Research Paper

The microorganisms and compounds influencing the organoleptic properties of Ugba (fermented *Pentaclethra macrophylla* Benth. seeds)

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The microorganisms and compounds influencing the organoleptic properties of Ugba were studied. Ugba was produced by the fermentation of boiled and shredded seeds of African oil bean. The pure cultures of microorganisms responsible for Ugba fermentation were isolated from Ugba samples, produced by traditional method and were used in singles and in combination to ferment African oil bean shreds. The Ugba so fermented were analyzed organoleptically and sensory scores statistically analyzed using analysis of variance at $P < 0.5$. Compounds in the samples of oil extracted from unfermented African oil bean shreds and Ugba samples fermented for 72 h with the microorganisms (in singles and in combinations) were analyzed by gas chromatograph. The result showed that the following microorganisms were involved in the fermentation of Ugba: *Staphylococcus saprophyticus*, *Bacillus pumilus*, *Bacillus subtilis* and *Bacillus licheniformis*. The organoleptic tests showed that in all parameters tested, the sample fermented by mixed starter culture of *B. subtilis* and *B. licheniformis* was generally liked, which implied 'overall best'. Compounds found in unfermented Ugba were nineteen in number. Out of this number, eleven were found in unfermented samples, while eight were in the fermented Ugba. The best sample contained the following compounds: ethanol, ethyl stearate, ethyl oleate, ethyl linoleate, ethyl phenol and ethyl octanoate (their percentage concentration ranged from 2.69% for ethyl octanoate to 67.85% for ethanol). These compounds influenced the perceived Ugba flavor but had no direct influence on color and texture of Ugba sample.

Key words: African oil bean seed, Ugba, fermentation, microorganisms, starter culture, organoleptic characteristics, flavor compounds.

INTRODUCTION

Fermentation is a chemical change in food brought about by enzymes from living microorganisms (Uzogara et al., 1990). Fermented foods are prepared from plant and animal materials by processes in which microorganisms play important role in modifying the substrate physically, naturally and sensorily (Njoku and Okemadu, 1989). Some desirable changes in foods (especially legume based) which occur during fermentation include changes in texture and organoleptic characteristics (flavors, aroma and appearance or consistency); especially elimination of beany flavors, improvement in digestibility, enhancement

in the quality of the product, improved safety (absence of toxins and partial and/or complete elimination of nutritional factors), increased nutritional quality and reduced cooking time (Wang and Hesseltine, 1981; Beuchart, 1984). Many of the food fermentations are natural and/or controlled fermentation consisting of different species and genera of yeast, fungi and/or bacteria (Kuboye, 1985; Reddy et al., 1986). These microorganisms can cause desirable changes in various foods, which distinguish them from the ones that are responsible for undesirable changes, including bad flavor and spoilage (Kuboye, 1985). Flavor components are the primary contributors to product acceptability either desirable or undesirable, if fermentation is uncontrolled (Sathe et al., 1986). Identification and characterization of

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flavor compounds responsible for the final flavor may help in development of synthetic flavor, which may reduce product cost and improve product reproducibility. There is a relationship between flavor compounds and organoleptic qualities of fermented foods (Nunomura and Sasaki, 1986; Okafor and Uzuegbu, 1987).

Ugba, a fermented product of African oil bean seed is one of the common fermented legumes predominantly consumed by the Ibos and other smaller ethnic groups of South Eastern Nigeria. Many researches have been carried out on Ugba, which included nutrients and other biochemical changes in Ugba associated with microorganisms during fermentation (Enujiugha, 2003; Kolawole and Okonkwo, 1985; Njoku and Okemadu 1989). These works indicated that enzymes bring about the major changes in the principal chemical components observed during the natural fermentation of Ugba. In the starter culture evaluation, the microorganisms involved in Ugba fermentation were related to its organoleptic properties (aroma, taste, texture and overall acceptability) (Mbajunwa et al., 1998).

The organoleptic properties of Ugba have only been associated with microorganisms and biochemical changes during fermentation, but no effort has so far been made to relate these microorganisms and organoleptic properties to the compounds produced during Ugba fermentation. There is a relation between flavor compounds and organoleptic properties (Sathe et al., 1986; Nunomura and Sasaki, 1986) which may be used to control the manufacturing process of Ugba and improve reproducibility.

The objectives of the work are:

- i. Isolation and identification of microorganisms involved in fermentation of African oil bean seed.
- ii. Preparation of starter cultures which were used in production of Ugba.
- iii. Identification and characterization of flavor compounds produced by these microorganisms when used to ferment African oil bean shreds in singles and in combinations.

At the end of this work, microorganisms/their combinations, which contribute to the organoleptic properties of Ugba and compounds produced, which contribute to Ugba flavor will be known. This will not only help to produce pure starter cultures of microorganisms responsible for organoleptic properties of Ugba, but will also help to produce Ugba with consistent organoleptic properties. The data from this investigation will be a step forward to production of synthetic Ugba flavors.

MATERIALS AND METHODS

The African oil bean seeds and the *Alchornea laxiflora* Benth. leaves (Akwukwo Ugba – the popular leaves for wrapping Ugba) were obtained from Mbaise, Imo State Nigeria. The seeds were sorted and washed in order to remove spoilt seeds, dust and extraneous materials from wholesome seeds.

Production of Ugba (fermented African oil bean seeds)

2 kg of raw African oil bean seeds were sorted to remove rotten seeds and extraneous materials and washed with clean water. The seeds were put in the pot and covered with water and boiled with occasional stirring for 45 min. The heating was discontinued and the seeds were removed in batches and defueled while hot. After dehulling, a local vegetable shredder (called Nkwoo in Igbo – a perforated piece of metal, which, when the seed is made to run over it at a certain angle, the seed came out in shreds) was used to shred the seeds. Then the shreds (shredded seeds) were poured into a covered pot of boiling water and stirred at 5 min intervals, for 30 min. The boiled shreds were poured into sterile sieve to drain out the hot liquor. Water was sprayed on the shreds to completely remove the hot liquor and to cool the shreds. Then the shreds were washed three times, drained of wash water, and steeped in distilled water in a pot and covered. The shreds were steeped for 10 h. At the end of steeping, the shreds were vigorously stirred and poured into a sterile sieve (which has been autoclaved at a temperature of 121°C and pressure of 16 psi) to completely drain the steep water from the shreds. Then the shreds were poured into a sterile sieve lined with steam heated and cooled akwukwo Ugba (*A. laxiflora* Benth. leaves) and covered with the leaves, and kept in a warm environment (temperature 34°C) to initiate fermentation.

After 5 h, the fermenting shreds were aseptically taken, weighed into 50 g portions and wrapped in sterile akwukwo Ugba. The wraps were packed in sieves lined and covered with the same leaves, put in sterile pot, kept in a warm environment and fermented for 3 days (72 h). Figure 1 shows the flow diagram of Ugba production via traditional procedure.

The unfermented sample and samples, fermented from 24 up to 72 h were aseptically taken with sterile spoon (the spoon was sterilized by dipping in ethanol and flamed on a Bunsen flame) and ground in a sterile porcelain mortar, and diluted with 45 ml 0.1% normal saline. This was serially diluted and plated out (using NA) using pour plate method. The plates were incubated at 30°C for 24 to 48 h. Thereafter, colony forming units per gramme (cfu/g) was calculated (Harrigan and McCance, 1976). Representative colonies were isolated, purified by repeated streaking. The cultures were identified using biochemical methods according to Bergey's Manual of Determinative Bacteriology (Buchanan and Gibbon, 1974). The Biological tests carried out include gram staining, sugar fermentation test, catalase tests and starch hydrolysis test.

The bacteria isolates for fermentation of Ugba were prepared by the aforementioned methods (Mbajunwa et al., 1998; Buchanan and Gibbon, 1974). The starter cultures were prepared as follows: The subcultures of the bacteria isolates were harvested with sterile water in screw-cap bottles and thoroughly mixed. The experimental procedure for Ugba preparation was followed as in traditional process except that the shreds of African oil bean samples were autoclaved for 121°C for 5 min at 16 psi and the pure cultures were inoculated into the autoclaved samples (Figure 1), via experimental procedure. Ten milliliters of the cultures containing 7.2 to 11.4×10^{10} cfu/mol was used to inoculate 100 g sterile African oil bean shreds in singles and in combinations, and aseptically wrapped in sterile akwukwo Ugba sterilized over a steam. The wrapped samples were kept in warm environment and fermented for 72 h. At the end of the 72 h fermentation, the samples were pasteurized using steam and allowed to cool.

The oils extracted from unfermented African oil bean shreds and 72 h samples fermented with the starter cultures were used in determination of the compounds in Ugba. Diethyl ether was used as solvent because of its low boiling point (34°C) and the Soxhlet extraction method was used to extract the oils. These oils were put in vials and stored in a refrigerator for analysis. A Shimadzu Gas chromatograph instrument (model 17A) was used to determine the compounds in the Ugba oil samples using Nitrogen at a pressure of 16 psi as the carrier gas. The system was automated with its own

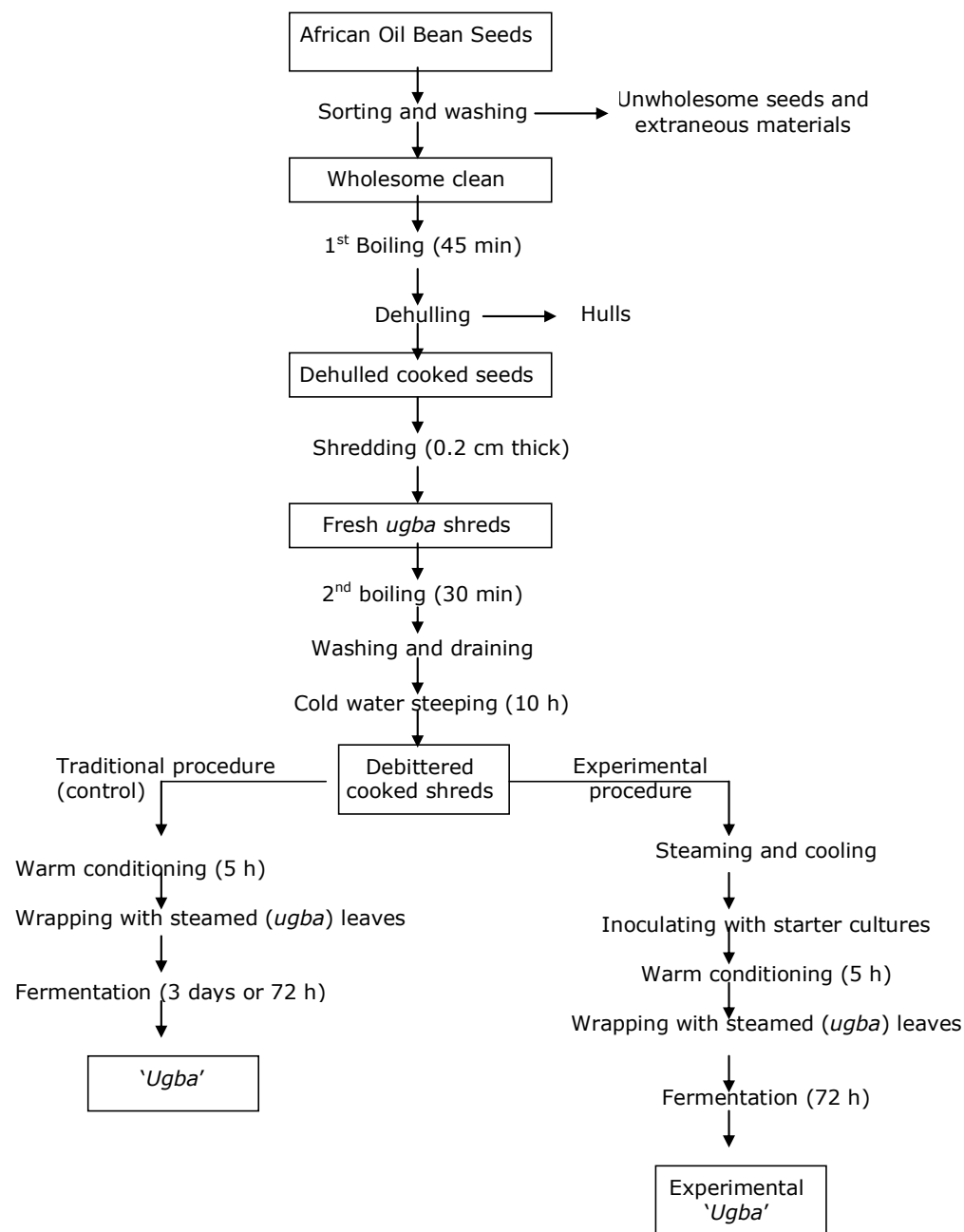


Figure 1. Flow diagram of 'Ugba' production.

workstation. Computer software was connected to the Gas chromatograph (GC), via the communication bus module. The instrument was set at an injector and detector temperatures of 240°C with programmed initial oven temperature of 50°C, increasing at 5°C per min for 20 min, and then 30°C per min for 20 min. The sample size injected into the system was 0.01µl. The internal standard used was Isopropanol. The calculations of the peak areas and retention times of compounds were carried out by the computer integrator coupled to the GC. The retention times of the compounds are matched with those of pure standards on the chromatograms obtained, to identify the various compounds in Ugba oil samples. The amount (in percentages) of each compound in a sample was calculated from the fractions of its peak area in

the total peak areas of all the identified compounds.

Modified assessment methods described by Okafor and Uzuegbu (1987), Ihekoronye and Ngoddy (1985) were used in the sensory evaluation of Ugba samples. Organoleptic quality of the 3 days fermented and pasteurized Ugba samples were evaluated subjectively by a set of 30 panelists drawn from the University community, made up of staff and students. The selection of the panelists from the university environment was for practical convenience. The panelists were untrained and made up of males and females who indicated having eaten Ugba as a meal. All the samples were presented at the same time and the identities of the samples were not revealed to the panelists. Each panelist was provided with sufficient privacy to ensure that his/her results

Table 1. Properties and bacterial isolates identified from fermenting African oil bean shreds (control – as presently consumed).

Isolates	Colony morphology	Cell characteristics	Size (mm)	Gram reaction	Biochemical tests						Probable organism	
					Catalase Test	Glucose	Starch	Sucrose	Fructose	Maltose		Mannitol
A	Whitish (creamy) convex, entire and smooth	Cocci in clusters	0.5	+ve cocci	+	+	+	+	-	+	+	<i>Staphylococcus saprophyticus</i>
B	Creamy convex	Long rods	0.5	+ve rods	+	+	-	+	+	+	+	<i>Bacillus pumilus</i>
C	Creamy, pin point and submerged in agar (when streaked – rough on agar surfaces)	Rods in singles	0.1	+ve rods	+	+	+	+	+	-	+	<i>Bacillus subtilis</i>
D	Whitish, flat entire	Rods in clusters	0.2	+ve rods	+	+	+	+	+	-	+	<i>Bacillus licheniformis</i>

+ Positive to fermentation, acid or gas forming; - Negative to fermentation, acid or gas forming.

would be arrived at, independently and without being influenced by other panelists. The quality parameters of Ugba evaluated were color, aroma/taste (flavor) texture and overall acceptability. A 9-point hedonic scale was used. The following instructions were given to the panelists:

- Indicate gender (male or female), age bracket.
- Testing for color: by visual inspection.
- Testing for aroma and taste (flavor): by sniffing two times or more of a spoonful of each sample provided, and then chewing the spoonful for 1 to 5 min. Enough water was provided and the panelist was free to swallow or spit out the sample and rinse his/her mouth after each test.
- Testing for texture: by touching and feeling some shreds of sample between the fingers.
- Scoring: the panelists were given rating based on 9-hedonic scale: 9 – liked extremely; 8 – liked very much; 7 – liked moderately; 6 – liked slightly; 5 – neither liked nor disliked; 4 – disliked slightly; 3 – disliked moderately; 2 – disliked very much; and 1 – disliked extremely.
- The panelists were to award a final score for overall acceptability.

The statistical analysis using a Completely Randomized Design (CRD) on a one-way classification model was used. Analysis of variance was done and the Friedman's Multiple Comparison test and Duncan Grouping Test at $P < 0.05$ was used to compare the differences and similarities between Ugba samples (American society of Brewing Chemists, 1987). The organoleptic data (especially for controls best and least accepted samples) were compared to the compounds identified.

RESULTS AND DISCUSSION

Table 1 shows the characteristics and microorganisms identified during traditional method of preparation of Ugba (control). They were *S. saprophyticus*, *B. pumilus*, *B. subtilis* and *B. licheniformis*. Only bacteria isolates were isolated in this work, which were in agreement with the observation made by Mgbajunwa et al. (1998) and Obeta (1983). Out of the micro-

organisms isolated by the workers, only *B. subtilis* and *S. saprophyticus* were isolated in this work.

The difference between my result and that of the former workers' could be due to the contamination of the wrapping leaves, which might have contributed since they usually do not sterilize the leaves.

Compounds identified in Ugba oil samples

Nineteen compounds were identified in the Ugba oil samples. Among the 19 compounds, eleven were naturally present in the unfermented sample (Table 2) and these include alcohols (butanol, phenyl alcohol and benzyl alcohol), esters (ethyl acetate, ethyl lactate, ethyl benzoate, ethyl heptadecanoate, ethyl stearate, ethyl oleate and ethyl linoleate), and a phenol (ethyl phenol). During the 72 h fermentation of Ugba, more

Table 2. Compounds identified in extracted Ugba oil samples for unfermented sample and samples fermented for 3 days with different cultures.

Compounds	Unfermented sample	Control	Samples fermented with Bacteria cultures											Number of occurrence
			A	B	C	D	AC	AD	BD	BC	CD	BCD	ABCD	
Alcohols (7)														
Methanol		x					x		x				x	4
Ethanol		x	x						x	x	x	x	x	7
Buthanol	x		x								x			3
Benzyl alcohol	x													1
Phenyl alcohol	x										x			2
Furfuryl alcohol					x									1
Ethyl phenol	x		x	x	x	x	x	x				x	x	9
Esters (10)														
Ethyl acetate	x							x	x	x			x	5
Ethyl lactate	x								x	x				4
Ethyl benzoate	x	x		x	x	x				x				6
Ethyl heptadecanoate	x				x	x								3
Ethyl stearate	x							x			x	x		4
Ethyl oleate	x		x					x	x	x	x	x	x	8
Ethyl linoleate	x									x	x	x	x	5
Ethyl carbamate						x			x	x			x	4
Ethyl linoate									x					1
Ethyl octanoate		x	x	x	x						x	x		6
Carbonyls (2) (ketones)														
Methyl pentanone		x												1
Hexanone		x												1
Unidentified compounds		x							x	x				3

A – *Staphylococcus saprophyticus*; C – *Bacillus subtilis*; B – *Bacillus pumilus*; D – *Bacillus licheniformis*.

compounds were formed which included alcohols (methanol, ethanol and furfuryl alcohol), esters (ethyl carbamate, ethyl actonoate and ethyl linoate) and carbonyls–ketones (methyl pentanone and hexanone; found only in control sample). It was also observed that some

compounds occurred in both unfermented and fermented samples, while some compounds in fermented Ugba samples (control and mixed starter samples of *Staphylococcus saprophyticus* and *B. subtilis*; *s. saprophyticus* and *B. Licheniformis*; and *B. licheniformis*) were

unidentified (Table 2). The number of times these compounds occurred in various Ugba samples were also indicated in Table 2. The compound with the highest occurrence was ethyl phenol (9 times), followed by ethyl oleate (8 times) and ethanol (7 times). Some compounds

occurred only once (benzyl alcohol in unfermented sample; ethyl linoate in *S. saprophyticus* and *B. licheniformis* sample; and furfuryl alcohol in *B. subtilis* sample).

Organoleptic properties of Ugba fermented with the starter cultures of bacteria isolates

Table 3 shows the sensory attributes (color, flavor, texture and overall acceptability) of control and experimental samples fermented for 72 h.

Color

Of the eleven samples studied, the color of five of them were neither liked nor disliked (score approximately 5.0). Specifically, they were those samples fermented with the following starter cultures: *B. pumilus*; *B. licheniformis*; mixed cultures of *B. pumilus* and *B. subtilis*; *S. saprophyticus* and *B. subtilis*; and control (traditionally prepared Ugba). Five samples fermented with starter cultures of *S. saprophyticus*; *B. subtilis*, *S. saprophyticus* and *B. licheniformis*; *B. pumilus*, *B. subtilis* and *B. licheniformis* were slightly liked (scores approximately 6.0). Only the sample fermented with the four-mixed culture (*S. saprophyticus*, *B. pumilus*, *B. subtilis* and *B. licheniformis*) had color rated 'very much liked' (scored approximately 8.0). The sample fermented with *B. pumilus* and mixed culture *B. pumilus* and *B. subtilis* had the color rating significantly different ($P < 0.05$) from the rest of the samples. Definitely, the color rating (score > 5.5) of the control and other samples were significantly less acceptable than the color of sample fermented with the 4 mixed starters culture (score > 7.6).

Flavor (aroma/taste)

With regard to flavor, only two of the samples (*B. pumilus* and *S. saprophyticus* and *B. subtilis*) were "neither liked nor disliked" (scored approximately 5.0). This rating ('neither liked nor disliked') was significantly different (at $P < 0.05$) from the flavor which scored approximately 6.0 that is 'slightly liked' of other Ugba samples including the control. The sample fermented with mixed starter culture of *B. subtilis* and *B. licheniformis* had the highest score (6.4) which probably meant a better flavor than the rest of the samples.

Texture

Considering the texture of the samples, only two samples (*B. pumilus* and *B. subtilis*; *B. subtilis* and *B. licheniformis*) were "slightly liked" (scored approximately

6.0). The rest of the samples including the control were "neither liked nor disliked" (score < 5.5).

Overall acceptability

The samples fermented with single starter cultures of *B. pumilus*, and *B. licheniformis* were "neither accepted nor rejected" (scored approximately 5.0) while the sample fermented with mixed starter culture of *B. subtilis* and *B. licheniformis* received "moderate acceptance" (scored approximately 7.0). The rest of the samples were only "slightly accepted" (scored approximately 6.0). Significant differences exist between overall acceptability of mixed starter culture of *B. subtilis* and *B. licheniformis* sample and the acceptability of other samples (Table 3) by the sensory panel.

Considering the significant differences between the control sample and the other samples analyzed, the control (traditionally prepared Ugba) sample (score 5.9) was only better in flavor than sample fermented with single starter culture *B. licheniformis* (score 4.9). This was therefore matched by most of the other samples in all parameters evaluated with the exception of sample fermented with mixed starter culture *B. subtilis* and *B. licheniformis*, which had a higher and better score. Considering the findings, the sample fermented with mixed starter culture *B. subtilis* and *B. licheniformis* was 'most accepted' among the samples studied including control. Thus, fermentation of Ugba with mixed starter culture *B. subtilis* and *B. licheniformis* for 72 h could be recommended as a new technology for a better and reproducible product. The sample fermented with single starter culture *B. licheniformis* was the 'least accepted' sample having the least scores in flavor, texture (major quality attributes of Ugba) and overall acceptability.

Effect of compounds on organoleptic properties of Ugba samples

Flavor

Table 4 shows the mean levels (%) of compounds and the overall acceptability mean scores of some Ugba samples with emphasis on the control, least and best accepted samples. The high concentrations of ethyl carbamate (27.25%) and ethyl phenol (63.14%) might have contributed to the poor acceptability of the sample fermented by *B. licheniformis*. This is because ethyl carbamate and ethyl phenol had been described as having the aromas of burnt wood, antiseptic and horse stable (Gawel, 2004).

Thus, high level of ethyl phenol and ethyl carbamate (urethane) in a food could affect its flavor and acceptability. Probably, the absence of ethanol might have contributed to the poor acceptance of Ugba sample fermented with *B. licheniformis*, considering that ethanol

Table 3. Mean sensory scores of organoleptic attributed of Ugba samples fermented for 72 h.

Parameters	Ugba samples fermented with different starter culture bacteria isolates											LSD
	<i>S. saprophyticus</i>	<i>B. subtilis</i>	<i>B. pumilus</i>	<i>B. licheniformis</i>	<i>S. saprophyticus</i> and <i>B. subtilis</i>	<i>S. saprophyticus</i> and <i>B. licheniformis</i>	Control	<i>B. pumilus</i> , <i>B. subtilis</i> and <i>B. licheniformis</i>	<i>B. pumilus</i> and <i>B. subtilis</i>	<i>B. subtilis</i> and <i>B. licheniformis</i>	<i>S. saprophyticus</i> , <i>B. pumilus</i> , <i>B. subtilis</i> and <i>B. licheniformis</i>	
Color	5.7 ^{bc}	5.6 ^{bc}	4.8 ^c	5.3 ^{bc}	5.4 ^{bc}	6.4 ^{abc}	5.2 ^{bc}	6.2 ^{abc}	4.8 ^{ab}	6.4 ^{abc}	7.6 ^a	1.664
Aroma/taste (flavor)	6.0 ^{ab}	5.7 ^{ab}	5.5 ^{abc}	4.9 ^c	5.4 ^{bc}	6.2 ^{ab}	5.9 ^{ab}	6.2 ^{ab}	6.3 ^{ab}	6.4 ^{ab}	5.7 ^{ab}	0.848
Texture	5.0 ^{bc}	5.0 ^{bc}	5.1 ^{bc}	4.8 ^c	4.8 ^c	5.4 ^{ab}	5.3 ^{abc}	5.3 ^{abc}	5.5 ^{ab}	5.8 ^a	5.2 ^{bc}	0.4438
Overall Acceptance	5.6 ^{cd}	5.9 ^{bcd}	5.3 ^{cd}	5.0 ^d	5.5 ^{cd}	6.0 ^{bcd}	5.7 ^{cd}	6.3 ^{abc}	5.9 ^{bcd}	7.0 ^a	5.9 ^{cd}	0.845

could help to mellow down the effect of ethyl phenol in the sample (Gawel, 2004). The absence of ethanol and ethyl stearate, ethyl oleate, ethyl linoleate, ethyl heptadecanoate and ethyl octanoate (esters, known for their fruity aromas) (Wikipedia, 2007), might have contributed to the poor flavor of *B. licheniformis* fermented sample, while their presence might have contributed to the acceptable flavor of the sample fermented by *B. subtilis* and *B. licheniformis* (best). There were significant differences between the least acceptable sample and the best in levels of ethyl phenol, but there was no significant difference between the traditionally fermented sample (control) and the most acceptable sample (*B. subtilis* and *B. licheniformis*) in ethanol content (Table 4).

Color

The Ugba sample fermented with the 4-bacteria mixed culture of *S. saprophyticus*, *B. pumilus*, *B. subtilis* and *B. licheniformis* had an ethanol level of 89.91% and it did not seem that the compound influenced the color of the Ugba samples. This view was held because such compounds as

ethanol which could have helped in preservation and better color were both in the control and the sample fermented by *B. subtilis* and *B. licheniformis* in appreciable levels (63.71 and 67.89%, respectively) yet their sensory scores differed.

Texture

It was observed that there was no indication that any of the organic compounds identified had any marked influence on the texture of the Ugba samples studied. It is difficult to explain why the sample fermented by *B. subtilis* and *B. licheniformis* gave the best textures even with the same treatment as other samples. The texture of Ugba is usually affected by degree of maturity of the African oil bean seeds, degree of cooking and days of fermentation. Softness being enhanced by cooking and fermentation period. Most times, long fermentation resulted in very soft (unacceptable) product (Ogueke and Ariariatu, 2004).

Overall acceptability

The overall acceptability of the samples have

tended more towards flavor and texture, hence the Ugba sample fermented by the mixed starter culture *B. subtilis* and *B. licheniformis* had the highest overall acceptability rating (score = 7.0, that is, moderately accepted). In addition, the proportion of the flavor compounds in this sample may have affected its flavor and overall acceptability (Table 4). Furthermore, Figure 2 showed the chromatograms of the unfermented Ugba, control and the best Ugba sample.

Conclusion

The microorganisms responsible for fermentation of Ugba were bacteria: *S. saprophyticus*, *B. pumilus*, *B. subtilis* and *B. licheniformis*. To study Ugba production by these bacteria, isolates from traditional Ugba preparation were used as starter cultures (in singles and in combinations). Sensory evaluation results showed that tasters in all parameters (colour, flavour, texture and overall acceptability) generally liked the Ugba sample fermented with a mixed culture of *B. subtilis* and *B. licheniformis*. This implied that the sample was 'overall best'. A total of 19 flavors compounds were identified in Ugba which included 7

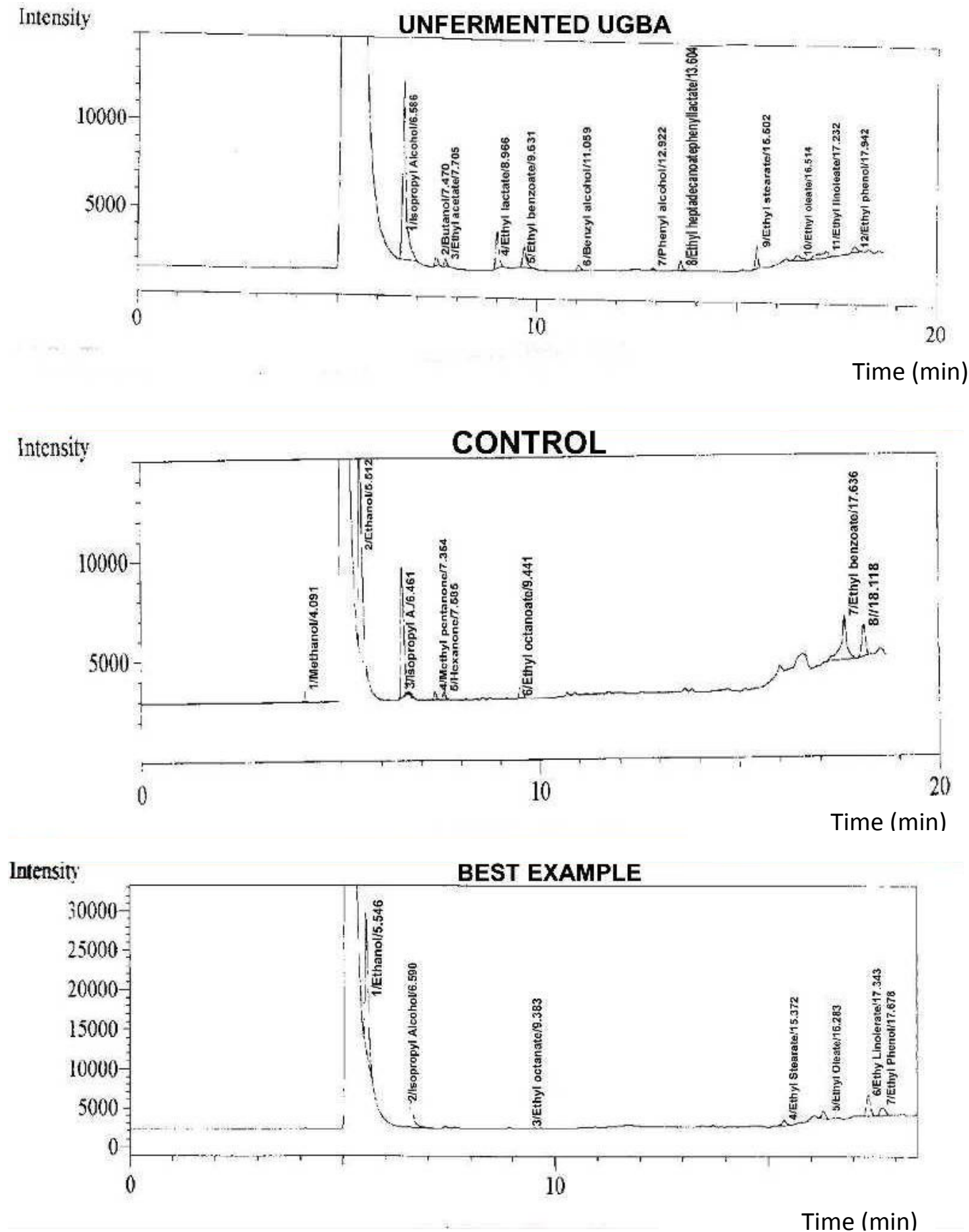


Figure 2. Chromatograms of flavor compounds for unfermented Ugba, control and best sample fermented for 72 h.

Table 4. Concentrations (%) of compounds that might affect the perceived flavours of some selected Ugba samples (control, best and least).

Overall acceptability/ compounds	■Control	Fermented Ugba samples									
		●CD	A	BC	BCD	ABCD	B	C	◆D	AD	AC
Acceptability	5.7 ^{bcd}	7.0 ^a	5.6 ^{bcd}	5.9 ^{bcd}	6.3 ^{abc}	5.9 ^{bcd}	5.3 ^{cd}	5.9 ^{bcd}	5.0 ^d	6.0 ^{bcd}	5.5 ^{bcd}
Ethanol	63.710 ^d	67.890 ^d	73.510 ^b	29.390 ^f	87.690 ^a	89.910 ^a	-	-	-	-	-
Methanol	1.38	-	-	-	-	-	-	-	-	-	-
Ethyl octanoate	4.720 ^a	2.690 ^e	1.610 ^e	7.750 ^c	-	-	32.600 ^a	28.680 ^b	-	-	-
Ethyl heptadecanoate	-	-	-	-	-	-	-	-	6.800	-	-
Ethyl cabamate	-	-	-	-	-	-	-	-	27.23	-	-
Ethyl phenol	-	6.940 ^e	21.910 ^c	-	-	-	64.420 ^a	16.820 ^d	63.140 ^a	40.700 ^b	39.640 ^b
Ethyl benzoate	16.180 ^b	-	18.400 ^a	-	-	-	3.00 ^d	5.280 ^c	2.830 ^d	-	-
Ethyl linoleate	-	14.140 ^b	-	24.430 ^a	-	9.200 ^c	-	-	-	-	-
Ethyl sterate	-	3.660 ^c	-	-	-	-	15.150 ^b	-	-	-	25.210 ^a
Ethyl oleate	-	4.700 ^{de}	3.020 ^{ef}	5.750 ^{cd}	0.840 ^f	-	-	-	-	13.880 ^b	16.340 ^a
Ethyl pentanon	1.57	-	-	-	-	-	-	-	-	-	-
Hexanone	1.65	-	-	-	-	-	-	-	-	-	-

Means with the same letters across the row are not significantly different at 95% probability level ($P \leq 0.05$). ● Best Ugba sample ◆ Least Ugba sample ■ Control. A - *Staphylococcus saprophyticus*; B - *Bacillus pumulis*; C - *Bacillus subtilis*; D - *Bacillus licheniformis*.

alcohols, 10 esters and 2 carbonyls: eleven of these compounds were found in unfermented sample, while 8 were mainly in fermented samples. The 'overall best' sample contained the following compounds: ethanol, ethyl stearate, ethyl oleate, ethyl linoleate, ethyl phenol and ethyl octanoate, and their percentage concentrations ranged from 2.69% for ethyl octanoate to 67.85% for ethanol. It was therefore concluded that these compounds produced during fermentation of African oil bean shreds influence the organoleptic properties of Ugba samples produced by starter cultures and traditional (normal) fermentation.

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