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The effect of starter cultures on the pH, bacterial population and volatile flavour compounds in '*ugba*' during fermentation

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ABSTRACT: Traditionally, *ugba* of acceptable quality made from African oil bean (*Pentaclethra macrophylla* Benth*)* is characterized by its typical flavour and aroma. In this study, aroma profile and volatile flavour compounds released by spontaneously fermented *ugba* and five samples of *ugba* inoculated with starter cultures (A - *Bacillus subtilis*; B - *B. safensis*; C- Mixed culture of *B. subtilis and B. safensis*; D - Mixed culture of *B. subtilis, B. safensis, B. clausii, B. licheniformis, and B. xylanilyticus*; and E - *B. cereus sensu lato)* were monitored at 24 hour interval during 72 hours fermentation using solid-phase microextraction (SPME) and gas chromatography-mass spectrometry (GC-MS). Commercially prepared *ugba* is the control. The total heterotrophic bacterial count (THBC) of the samples were determined using Standard Microbiological Methods; the pH involved the use of standard method. Increase in the THBC and pH of all the samples of fermenting *ugba* were within the range of 1.4×10^4 to 1.7×10^{12} CFU/ml and 5.89 to 8.24, respectively. A total of 31 compounds which comprise of 7 ketones, 7 alcohols, 5 organic acids, 4 aldehydes, 2 pyrazines, 1 ester, 1 furan, 1 sulfur and 3 other compounds were identified as aroma compounds in the fermenting samples of *ugba*. Flavour compounds categorized as ester, furan and sulfur compound were consistently detected in all the samples of *ugba* prepared in the laboratory. *Ugba* prepared using starter cultures including the spontaneously fermented sample compare favourably with commercially produced *ugba* with regards to volatile flavour compounds.

Keywords: African oil bean, aroma profile, *Bacillus* sp., controlled fermentation, natural fermentation.

INTRODUCTION

Ugba or *ukpaka* is a very popular fermented product among the Igbo tribe in Nigeria (Kabuo et al*.,* 2013). The people consume the product as a snack, side dish or food condiment (Mbata and Orji, 2007; Ogbulie et al., 2014). *Ugba* is made from African oil bean (*Pentaclethra macrophilla* Bentham) derived from a tropical tree which belong to *Leguminosae* family (Ogueke et al., 2010; Okereke and Chuka, 2017).

African oil bean seeds typically has a bitter taste due to toxic alkaloids and saponins. After the seeds had been processed which usually involves fermentation, the product becomes non-toxic, nutritious and palatable (Enujiugha and Akanbi, 2008). Traditionally, the duration sliced African oil bean seeds are allowed to ferment, different types of leaves used in wrapping the fermenting sliced oil bean seeds and slight differences in processing

methods adopted by the processors are responsible for inconsistent quality of *ugba* (Kabuo et al., 2015; Nwokeleme and Ugwuanyi, 2015; Mbah et al*.,* 2018; Nwanagba et al*.,* 2020). In recent times, some researchers have successfully produced *ugba* using starter cultures. The product has some advantages compared with *ugba* produced by natural fermentation (Enujiugha et al*.,* 2008; Akpi et al., 2020). Some of the advantages of using starter cultures to prepare *ugba* include consistency in product quality and minimal risk of foodborne pathogens involvement during the fermentation process (Okorie and Olasupo, 2013). A study carried out by Sanni *et al*. (2002) reported that sensory attributes (consistency, taste and aroma) of *ugba* produced using a starter culture was better than *ugba* samples obtained from retail markets. Both traditional and improved methods of processing African oil bean seeds into *ugba* have been described by many researchers (Ogbulie *et al*., 2014; Okereke and Chuka, 2017; Nwokeleme and Ugwuanyi, 2015; Okorie and Olasupo, 2013; Onyekachi *et al*., 2021).

Microorganisms associated with natural fermentation of African oil bean seeds are *Bacillus, Leuconostoc, Staphylococcus, Enterobacter, Proteus, Lactobacillus plantarum* and *Escherichia coli.* These organisms are inoculated into the fermenting solid substrate by chance. *Bacillus* sp. predominates other microorganisms involved in the fermentation process of oil bean seeds after 24 hours till the process ends (Enujiugha et al., 2008; Ogueke et al., 2015). According to Onyekachi et al. (2021), *ugba* is produced as a result of *Bacillus pumilus, B. sphaericus, B. licheniformis* and *B. subtilis* involvement in the fermentation of African oil bean seeds. The activities of microorganisms and biochemical changes which occur during fermentation of African oil bean seeds slices influence the organoleptic properties of *ugba* (Kabuo et al., 2013). Utensils, air, water and leaves used in wrapping *ugba* are possible sources of microorganisms found in the product (Kabuo et al., 2015).

In many African and Asian countries, the sensorial attributes of many indigenous fermented food condiments such as *ugba* could be attributed to volatile compounds (Kabuo et al*.,* 2015; Parkouda et al., 2011; Olasupo et al., 2016). A study carried out by Kabuo et al. (2013) identified alcohols, esters and a phenol in unfermented *ugba* oil samples. After the samples were fermented, alcohols, esters, carbonyl-ketones were identified in the product. Microorganisms are responsible for elaborating the flavour components of various foods. According to Kabuo et al. (2013), the organoleptic qualities peculiar with fermented foods have a relationship with flavour compounds. The flavour of *ugba* is a mark of quality which influences consumer acceptability of the product (Nwokeleme and Ugwuanyi, 2015). Thus, it is necessary to identify the aroma compounds in a typical *ugba* undergoing fermentation process. The findings could provide useful information for researchers to develop synthetic *ugba* flavour*.* Consistent quality of *ugba* produced in commercial quantity using synthetic *ugba* flavour could be more acceptable by consumers than *ugba* prepared using the traditional processing methods.

A recent study carried out by Ohiri and Bassey (2017) reported the presence of volatile compounds in cooked unfermented and fermented African oil bean seeds. Nwokeleme and Ugwuanyi (2015) analyzed the volatile flavour compounds released during natural and controlled fermentation of African oil bean seeds using starter cultures. However, the amount of volatile aroma compounds in *ugba* monitored during fermentation process were not reported. In this work, the amount of aroma compounds released by naturally fermenting African oil been seeds slices, fermenting samples inoculated with single and mixed starter cultures were determined as well as the microbial population and pH of the fermenting samples.

MATERIALS AND METHODS

Sample collection

African oil beans seeds weighing about 2.5 Kg was purchased at Nkwo Ogwu market in Aboh Mbaise LGA, Imo state, Nigeria using sterile polythene bags. In the same market, 100 g of commercially prepared *ugba* wrapped with leaves was purchased using sterile polythene bags.

Production of *ugba*

The method described by Mbata and Orji (2007) and Balogun (2013) with some modifications was adopted in producing *ugba* using African oil bean seeds. Washing of African oil bean seeds was done using potable water. Afterwards, the oil bean seeds were pressure-cooked for 3 hours and manually dehulled by slicing the cotyledons into thin long slices. The sliced seeds of African oil bean were poured into a potable water and boiled for 30 minutes. Sliced African oil bean seeds was washed several times using potable water; used water was discarded severally. After washing was satisfactorily completed, the oil bean seeds slices were soaked in potable water for 6 hours and drip-dried before distributing 100 g portions into stomacher bags.

Inoculum preparation

Bacillus sp. was isolated from commercially prepared *ugba* (composite sample) using the method described by Orji et al. (2015) with slight modification. A porcelain mortar and pestle was sterilized using 70 % ethanol. Thereafter, it was

used to mash *ugba* into a paste. One gram (1 g) of *ugba* paste was transferred into 9 ml sterile peptone water. Thereafter, serial dilution of the stock solution was aseptically carried out until dilution 10^{-6} was reached. Pour plate method was used to inoculate 1 ml of dilution 10^{-4} and 10^{-5} into freshly prepared nutrient agar (NA) plates. The inoculated plates were incubated at 37°C for 24 hours. Repeated streaking of representative colonies were carried out in order to purify the isolates. The bacterial isolates were subjected to Gram's staining and biochemical tests. Identification of *Bacillus* spp was aided by Bergey's Manual of Determinative Bacteriology. Selection of *Bacillus* strains used as starter cultures for controlled fermentation of African oil bean slices was based on phenotypic and genotypic characterization of the isolates as well as their ability to produce enterotoxin (Ahaotu et al., 2013). The strains selected were maintained at 4°C on nutrient agar (NA; Oxoid CM003, Basingstoke, UK). From NA plates containing *Bacillus* strains incubated for 24 hours at 37°C, surface growth of the isolates were resuspended in 50 ml of sterile distilled water. The population of the microorganisms was estimated by optical density using a sensititre nephelometer (Trek Diagnostics 437R03N007, West Sussex, UK). Dilutions were made using maximum recovery diluent (MRD, Oxoid CM0733) to obtain an initial inoculum of *ca.* 10^4–10^5 cells ml $^{-1}$.

Controlled fermentation

African oil bean slices subjected to controlled fermentation was initially autoclaved for 25 minutes at 121°C. The starter cultures inoculated into the autoclaved samples of African oil bean slices labelled A, B, C, D and E comprise of (a) *Bacillus subtilis subsp. spizizenii* (b) *Bacillus safensis* (c) *B. subtilis subsp. spizizenii* and *B*. *safensis* (d) *B. clausii, Lysinibacillus xylanilyticus, B. licheniformis, B. subtilis subsp. spizizenii and B. safensis* and (e) *Bacillus cereus sensu lato*, respectively. Sample F was allowed to undergo spontaneous fermentation by incubating the sample at 37°C alongside Sample A- E. At 0, 24, 48 and 72 hours, samples of the fermenting solid substrates were aseptically collected for analyses.

Microbiological analysis

The total heterotrophic bacterial count of African oil bean slices undergoing fermentation process was monitored. At 24 hour intervals, 10 g of each sample was homogenized in 90 ml sterile maximum recovery diluent (MRD, Oxoid CM0733). Ten-fold dilutions was prepared using MRD and microorganisms enumerated on NA plates after 48 hours incubation at 37°C.

Determination of pH

The pH of all the samples of fermenting oil beans slices were determined using the method described by Eze et al. (2014). One gram (1 g) of each sample was ground using Benton electric blender (China) and dissolved in 9 ml of deionized water. Thereafter, pH of the dissolved sample was determined using pH meter (Hi-98107 pH, India) connected with glass electrode. The digital display observed after inserting the glass electrode into the solution was recorded as the pH value after it has stabilized.

Aroma profile using solid-phase microextraction (SPME)

A 50/30 µm divinylbenzene/carboxen^{tm} on polydimethylsiloxane fiber (Supelco, Bellefonte, PA) was used. It was conditioned at 250°C for 30 minutes before use. Chopped samples (10 g) were transferred into a 40 ml glass bottle fitted with a screw cap with polytetrafluoroethylene - lined septum. The stainless steel needle, housing the SPME fiber was placed through the hole and penetrated the liner. After equilibration at 37°C for 10 minutes in a thermostatic water bath, the fiber was exposed to the headspace above the sample for 30 minutes. This experiment was carried out on three separate occasions.

Gas chromatography-mass spectrometry (GC-MS)

After extraction, the SPME device was inserted into the injection port of an HP5972 GC-MS system (Agilent, Santa Clara, CA). The contents of the SPME fiber were desorbed for 3 min in a split/splitless injection port, held in splitless mode at 250°C, onto the front of an HP-5MS semivol fused silica capillary column (30 m × 0.25 m × 0.5 µm film thickness, Agilent). The front of the column had been twisted into a coil containing 5 small loops, which were cooled in solid carbon dioxide, contained within a 150-ml beaker. Immediately before desorption of the fiber, 0.1 µl of a standard (1000 ng µl^{-1} 1, 2-dichlorobenzene in methanol) was injected in splitless mode onto the GC column. During desorption, the oven was held at 50°C. The solid carbon dioxide was removed from the oven after desorption. The oven was maintained at 50°C for 1 minute. Then, the temperature was raised at 8°C min^{-1} to 240°C. Helium at 12.7 psi was used as the carrier gas resulting in a flow of 1.0 ml at 50°C. n-Alkanes (C_5-C_{25}) were analyzed under the same condition to obtain a linear retention index (LRI) value for the component. The mass spectrometer which operated in electron impact mode, scanned from m/z 20 to m/z 300 at 2.67 scans/s. Compounds were identified by first comparing their mass spectra with those

contained in the NIST/EPA/NIH Mass spectral database (MS Window version 2.0a, 2000) followed by comparing mass spectra and LRI value with those of authentic standards. Approximate quantities of the volatiles were estimated by comparison of their peak areas with that of 1, 2-dichlorobenzene standard obtained from the total ion chromatograms using a response factor of 1.

Statistical analysis

The quantitative data for each volatile aroma compound identified in the GC-MS analysis for each sample of fermenting *ugba* was compared with other samples using analysis of variance (ANOVA). For the aroma compounds exhibiting significant difference in the ANOVA, Fisher's least significant difference test was applied to determine which sample means differed significantly ($p<0.05$).

RESULTS AND DISCUSSION

The pH of all the samples of *ugba* monitored at 24 hours intervals throughout the period of fermentation were within the range 5.89 to 8.24 (Figure 1.) At 0 hour, pH of oil bean seeds slices inoculated with starter cultures were lower than the result recorded for the spontaneously fermented sample. This could be as a result of heating process during autoclaving of oil bean seeds slices before inoculum was added to the substrate which was not applicable to the spontaneously fermented sample. In contrast, Parkouda et al. (2009) reported that pH of *Hibiscus sabdariffa* increased after it was subjected to heat in the process of producing *bikalaga*. This could be attributed to the addition of alkalinizing leachete and leaching of acidic component in the cooking water. During 72 hours fermentation of all samples of *ugba*, there was an increase in pH. It is an indication of alkaline fermentation process. At 72 hours, the pH of Sample A is in agreement with the result reported by Enujiugha et al. (2008). Increase in pH recorded for all the samples of *ugba* is in agreement with a related study carried out by Nwanagba *et al*. (2020) which involved fermentation of African oil bean seeds by *Bacillus subtilis* used as a starter and *Lactobacillus fermentum* as adjunct. In a related study, Enujiugha et al. (2008) reported increase in pH during mixed culture fermentation of African oil bean seeds from 6.62 to 7.38. A similar result was reported for the fermenting samples inoculated with single starter culture (*Bacillus subtilis*, *B. licheniformis* and *Pseudomonas fluorescens*). According to Ogueke et al. (2015), increase in proteinase and deaminase activities associated with activities of fermenting microorganisms in *ugba* and other foods rich in protein could be responsible for the release of ammonia in high amounts. This assertion might be an explanation for the increase in pH of all the samples of *ugba* during the fermentation process. Although the amount of ammonia released in the fermenting samples of *ugba* were not determined, it should be noted that undesirable flavour and aroma of some foods could occur as a result of high level of ammonia nitrogen (Ogueke *et al*., 2015).

Depicted in Table 1 is the total heterotrophic bacterial count (THBC) of fermenting samples of *ugba* monitored at 24 hours intervals. Increase in THBC was recorded for all the samples of *ugba* during the period of fermentation. At 0 hour, spontaneously fermented *ugba* (Sample F) had the lowest bacterial count. This could be attributed to inoculum not introduced into the substrate. Enujiugha et al. (2008) reported that during single starter fermentation of African oil bean seeds by *Bacillus subtilis* which lasted for 72 hours, the microbial count of the substrate increased from 1.3×10^7 to 9.3×10^9 CFU/g. During fermentation, increase in microbial count from 3.0×10^6 to 1.5×10^9 CFU/g and 7.0×10^6 to 4.0×10^9 CFU/g was recorded in African oil bean seed slices inoculated with *Pseudomonas fluorescens* and *Bacillus licheniformis*, respectively. A similar result was recorded for the sample inoculated with mixed culture which increased from 7.0×10^6 to 1.72×10^{10} CFU/g. Although the starter cultures is at variance with the ones used in this study, there was increase in microbial count of African oil bean slices during the fermentation period (Enujiugha *et al*., 2008). At 72 hour, the highest bacterial count was recorded for Sample E (1.7×10^{12} CFU/ml) inoculated with *Bacillus cereus sensu lato*. This result is an indication that the microorganism is more adaptable to the substrate and environment than other *Bacillus* strains used in this study (Okanlawon et al., 2010).

The aroma profile during fermentation of *ugba* inoculated with starter cultures and spontaneously fermented sample monitored at 0, 24, 48 and 72 hours are shown in Tables 2, 3, 4 and 5, respectively. Also presented in Table 5 is the result recorded for the commercially produced *ugba*. Overall result shows that 31 aroma compounds belonging to various classes of compounds were identified in the fermenting samples (*ugba*). The classes of compounds are alcohols, aldehydes, organic acids, ketones, esters, pyrazines, furan, sulfur and others which comprise of limonene, antioxidant BHA and acetamide.

In Figure 2, the Bi- plot from principal component analysis, PC1 vs PC2 of relative concentration of volatile compounds showed three groups. Sample E and F are separated from the other samples along PC1 while E separates from F along PC2. Sample A is separated from the rest along PC2 because it is high in phenolics. There was also a time effect along PC1 where 72 hours samples were resolved from 48 hours samples, which were also resolved from 24 hours samples. These showed significant difference in the flavour component in terms of time.

A total of 31 flavour compounds which comprise of 7 ketones, 7 alcohols, 5 organic acids, 4 aldehydes, 2 pyrazines, 1 ester, 1 furan, 1 sulfur and 3 other flavour

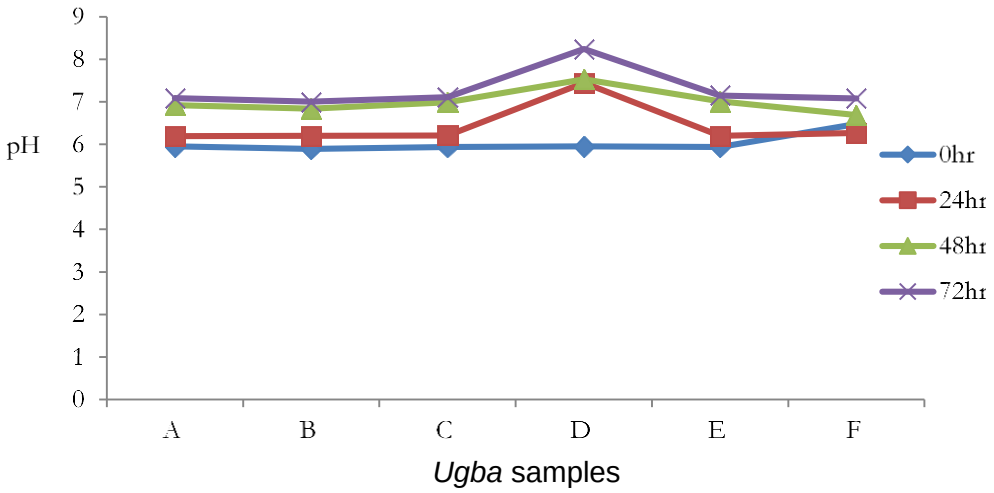

Figure 1. pH of the fermenting samples of *ugba* inoculated with starter cultures and spontaneously fermented *ugba*. **Key:** A, *Bacillus subtilis*; B, *Bacillus safensis*; C, Mixed culture of *Bacillus subtilis and Bacillus safensis*; D, Mixed culture of *Bacillus subtilis, Bacillus safensis, Bacillus clausii, Bacillus licheniformis, and Bacillus xylanilyticus*; E, *Bacillus cereus sensu lato*; F, Spontaneously fermented *ugba.*

Table 1. Total heterotrophic bacterial count (CFU/ml) of *ugba* inoculated with starter cultures during fermentation and spontaneously fermented *ugba*.

Sample	Time (Hours)			
	0	24	48	72
A	4.3×10^4	3.6×10^5	5.2×10^7	1.5×10^9
B	4.8×10^4	3.4×10^7	8.4×10^7	2.2×10^9
C	5.7×10^4	4.7×10^7	2.2×10^8	5.0×10^9
D	2.1×10^4	6.5×10^7	4.3×10^8	2.5×10^9
E	1.4×10^4	1.3×10^8	1.6×10^9	1.7×10^{12}
F	1.6×10^3	4.8×10^8	3.3×10^9	5.7×10^{10}

Key: A, *Bacillus subtilis*; B, *Bacillus safensis*; C, Mixed culture of *Bacillus subtilis and Bacillus safensis*; D, Mixed culture of *Bacillus subtilis, Bacillus safensis, Bacillus clausii, Bacillus licheniformis, and Bacillus xylanilyticus*; E, *Bacillus cereus sensu lato*; F, Spontaneously fermented *ugba.*

compounds were identified in all the samples of fermenting *ugba*. In a related study, Nwokeleme and Ugwuanyi (2015) reported that volatile compounds which comprise of one compound each of ketone, acid, aldehyde, phenol and furan; 10 hydrocarbons, 8 esters, 3 alcohols, 2 amines and 2 sulfur compounds totaling 30 compounds were detected in African oil bean seeds fermented by *Bacillus subtilis*. A total of 30 aroma compounds which comprise of 1 amine, 1 aldehyde, 1 furan, 1 lactone, 2 nitrogenous compounds, 2 ketones, 3 alcohols, 9 hydrocarbons and 10 esters were produced by the fermenting samples inoculated with *Bacillus megaterium* while the samples subjected to natural fermentation (mixed culture) released 36 volatile compounds (1 furan, 1 amine, 1 acid, 1 lactone, 1 thiophene, 2 ketones, 2 phenols, 5 alcohols, 10 esters, and 12 hydrocarbons).

Pyrazines are volatile six-membered heterocyclic compounds that contains nitrogen. They are found in so many bacteria, fungi, insects and plants. It is responsible for desirable aromas in a lot of foods and beverages (Yan et al., 2021). 2, 5 or 2, 6-dimethylpyrazine was detected in all the *ugba* samples throughout the fermentation period. 2, 5-dimetylpyrazine is a pyrazine extracted from natural sources. A roasted flavour is achieved using 2, 6-dimethyl pyrazine in meals, coffee, cocoa or potatoes (Mortzfeld et al., 2020). Pyrazines are often found in heated foods which gives a characteristic roasty or nutty flavour. They are part of volatile constituents present in some traditionally fermented condiments like *ogiri* from melon seeds and *daddawa* from locust bean and soybean. The dominant

Table 2. Flavour compounds (µl) obtained from *ugba* inoculated with starter cultures and spontaneously fermented *ugba* at 0 hour.

S/N	Compound name	Sample A	Sample B	Sample C	Sample D	Sample E	Sample F
Pyrazines							
1	2,5 or 2,6-dimethylpyrazine	2.3[a]	2.3[a]	2.3[a]	2.3[a]	2.3[a]	2.3[a]
2	3-Ethyl-2,5-dimethylpyrazine	0.0	0.0	0.0	0.0	0.0	0.0
Alcohols							
3	2-Methyl-1-propanol	0.0	0.0	0.0	0.0	0.0	0.0
4	Guaiacol	2.8[a]	2.8[a]	2.8[a]	2.8[a]	2.8[a]	2.8[a]
5	1-Hexanol	4.8[a]	4.8[a]	4.8[a]	4.8[a]	4.8[a]	4.8[a]
6	2-Ethyl-1-hexanol	22.5[a]	22.5[a]	22.5[a]	22.5[a]	22.5[a]	22.5[a]
7	2-(methylthio)ethanol	0.0	0.0	0.0	0.0	0.0	0.0
8	3-Methyl-1-butanol	0.0	0.0	0.0	0.0	0.0	0.0
9	2-Methyl-1-butanol	0.0	0.0	0.0	0.0	0.0	0.0
Acids							
10	2-Methylbutanoic acid	0.0	0.0	0.0	0.0	0.0	0.0
11	2-Methylpropanoic acid	0.0	0.0	0.0	0.0	0.0	0.0
12	3-Methylbutanoic acid	0.0	0.0	0.0	0.0	0.0	0.0
13	Butanoic acid	0.0	0.0	0.0	0.0	0.0	0.0
14	Acetic acid	20.8[a]	20.8[a]	20.8[a]	20.8[a]	20.8[a]	20.8[a]
Ketones							
15	2-Pentanone	3.4[a]	3.4[a]	3.4[a]	3.4[a]	3.4[a]	3.4[a]
16	5-Methyl-2-hexanone	0.0	0.0	0.0	0.0	0.0	0.0
17	Phenol	4.9[a]	4.9[a]	4.9[a]	4.9[a]	4.9[a]	4.9[a]
18	Acetoin	0.0	0.0	0.0	0.0	0.0	0.0
19	2-Heptanone	13.3[a]	13.3[a]	13.3[a]	13.3[a]	13.3[a]	13.3[a]
20	2-Butanone	92.8[a]	92.8[a]	92.8[a]	92.8[a]	92.8[a]	92.8[a]
21	6-Methyl-2-heptanone	0.0	0.0	0.0	0.0	0.0	0.0
Aldehydes							
22	Benzaldehyde	179.1[a]	179.1[a]	179.1[a]	179.1[a]	179.1[a]	179.1[a]
23	2-Methylbutanal	12.7[a]	12.7[a]	12.7[a]	12.7[a]	12.7[a]	12.7[a]
24	3-Methylbutanal	24.5[a]	24.5[a]	24.5[a]	24.5[a]	24.5[a]	24.5[a]
25	Phenylacetaldehyde	0.0	0.0	0.0	0.0	0.0	0.0
Ester							
26	2-ethylhexyl 2-propenoate	14.6[a]	14.6[a]	14.6[a]	14.6[a]	14.6[a]	14.6[a]
Furan							
27	2-Pentylfuran	33.0[a]	33.0[a]	33.0[a]	33.0[a]	33.0[a]	33.0[a]
Sulfur compound							
28	Dimethyl disulfide	10.8[a]	10.8[a]	10.8[a]	10.8[a]	10.8[a]	10.8[a]
Others							
29	Limonene	23.0[a]	23.0[a]	23.0[a]	23.0[a]	23.0[a]	23.0[a]
30	Antioxidant BHA	56.8[a]	56.8[a]	56.8[a]	56.8[a]	56.8[a]	56.8[a]
31	Acetamide	0.0	0.0	0.0	0.0	0.0	0.0

Key: A, *Bacillus subtilis*; B, *Bacillus safensis*; C, Mixed culture of *Bacillus subtilis and Bacillus safensis*; D, Mixed culture of *Bacillus subtilis, Bacillus safensis, Bacillus clausii, Bacillus licheniformis, and Bacillus xylanilyticus*; E, *Bacillus cereus sensu lato*; F, Spontaneously fermented *ugba.* Means with the same superscript for each flavour compound across a row are not significantly different ($p > 0.05$).

group of volatile compounds in some Benninese condiments such as *Afitin, Iru* and *Sonru* are pyrazines (Azokpota et al., 2008). Ouoba et al. (2005) reported the profile of volatile compounds responsible for the aroma of soumbala fermented spontaneously by pure and mixed cultures of *B. subtilis* and *B. pumilus*. The compounds identified include pyrazines, aldehydes, ketones, esters, alcohols, acids, alkanes, alkenes, amines, pyridines,

Table 3. Flavour compounds (µl) obtained from *ugba* inoculated with starter cultures and spontaneously fermented *ugba* at 24 hours.

S/N	Compound name	Sample A	Sample B	Sample C	Sample D	Sample E	Sample F
Pyrazines							
1	2,5 or 2,6-dimethylpyrazine	2.2^{b}	2.1^{ab}	1.9^{a}	1.9^{a}	14.6^{d}	5.3^{c}
2	3-Ethyl-2,5-dimethylpyrazine	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.3^{b}	1.2^{c}
Alcohols							
3	2-Methyl-1-propanol	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	4.3^{b}
4	Guaiacol	2.1^{c}	2.3^{d}	2.6^{e}	2.7^{f}	1.7^{b}	1.1^{a}
5	1-Hexanol	4.2^{a}	10.1^{d}	13.1^{f}	9.1^{c}	8.6^{b}	11.4^{e}
6	2-Ethyl-1-hexanol	22.8^{f}	13.1^{d}	12.6^{c}	10.1^{b}	14.1^{e}	2.2^{a}
7	2-(methylthio)ethanol	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	1.8^{b}	3.6^{c}
8	3-Methyl-1-butanol	0.0^{a}	11.2^{e}	19.1^{f}	7.3^{c}	8.5^{d}	3.8^{b}
9	2-Methyl-1-butanol	0.0^{a}	0.5^{b}	3.0^{d}	0.0^{a}	0.5^{b}	2.7^{c}
Acids							
10	2-Methylbutanoic acid	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	4.1^{c}	1.0^{b}
11	2-Methylpropanoic acid	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	4.3^{b}
12	3-Methylbutanoic acid	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	50.3^{b}	116.3^{c}
13	Butanoic acid	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	7.8^{c}	4.9^{b}
14	Acetic acid	11.6^{c}	17.4^{e}	7.6^{b}	15.3^{d}	7.0^{a}	42.6^{f}
Ketones							
15	2-Pentanone	1.7^{e}	0.0^{a}	1.2^{c}	0.4^{b}	11.3^{f}	3.1^{d}
16	5-Methyl-2-hexanone	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	4.3^{b}	61.5^{c}
17	Phenol	4.9^{e}	2.2^{b}	4.0^{d}	2.5^{c}	2.2^{b}	2.0^{a}
18	Acetoin	2.13^{b}	40.6^{e}	60.5^{f}	21.4^{d}	0.0^{a}	4.7^{c}
19	2-Heptanone	8.8^{c}	7.9^{b}	8.8^{c}	7.4^{a}	22.4^{e}	9.6^{d}
20	2-Butanone	75.5^{d}	36.2^{c}	84.9^{e}	26.1^{a}	163.9^{f}	34.2^{b}
21	6-Methyl-2-heptanone	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	2.5^{c}	0.9^{b}
Aldehydes							
22	Benzaldehyde	100.1^{e}	101.5^{f}	92.6^{d}	92.3^{c}	15.3^{a}	18.1^{b}
23	2-Methylbutanal	5.9^{d}	2.5^{c}	0.0^{a}	0.0^{a}	6.7^{e}	2.2^{b}
24	3-Methylbutanal	9.8^{d}	5.0^{c}	0.0^{a}	0.0^{a}	14.4^{e}	1.0^{b}
25	Phenylacetaldehyde	0.2^{b}	0.5^{c}	0.0^{a}	0.0^{a}	7.2^{e}	5.1^{d}
Ester							
26	2-ethylhexyl 2-propenoate	14.9^{f}	9.3^{c}	11.4^{e}	9.0^{b}	11.0^{d}	2.2^{a}
Furan							
27	2-Pentylfuran	19.5^{d}	22.3^{f}	19.0^{c}	17.2^{a}	20.5^{e}	11.7^{b}
Sulfur compound							
28	Dimethyl disulfide	2.5^{c}	2.3^{b}	4.0^{d}	1.8^{a}	19.9^{f}	6.4^{e}
Others							
29	Limonene	9.3^{d}	5.6^{a}	6.4^{b}	35.4^{e}	40.5^{f}	8.5^{c}
30	Antioxidant BHA	42.5^{e}	32.0^{b}	40.2^{d}	38.7^{c}	51.0^{f}	17.6^{a}
31	Acetamide	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.8^{b}	0.0^{a}

Key: A, *Bacillus subtilis*; B, *Bacillus safensis*; C, Mixed culture of *Bacillus subtilis and Bacillus safensis*; D, Mixed culture of *Bacillus subtilis, Bacillus safensis, Bacillus clausii, Bacillus licheniformis, and Bacillus xylanilyticus*; E, *Bacillus cereus sensu lato*; F, Spontaneously fermented *ugba*. Means with the same superscript for each flavour compound across a row are not significantly different ($P > 0.05$).

benzenes, phenols, sulfurs and furans.

Varied amounts of aroma compounds (alcohols) were reported in fermenting samples of *ugba.* Among the alcohols detected, 2-methyl-1-propanol was absent in most of the samples. According to Nwokeleme and Ugwuanyi (2015), undesirable odour in soybean which manifested in the form of 'raw' or 'beany' flavour could be attributed to many alcohols. Three alcohols were detected

Table 4. Flavour compounds (µl) obtained from *ugba* inoculated with starter cultures and spontaneously fermented *ugba* at 48 hours

S/N	Compound name	Sample A	Sample B	Sample C	Sample D	Sample E	Sample F
Pyrazines							
1	2,5 or 2,6-dimethylpyrazine	3.0^{d}	2.1^{a}	2.7^{c}	2.5^{b}	7.6^{e}	27.7^{f}
2	3-Ethyl-2,5-dimethylpyrazine	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.3^{b}	14.7^{c}
Alcohols							
3	2-Methyl-1-propanol	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	10.3^{b}
4	Guaiacol	12.1^{d}	2.0^{b}	6.7^{c}	18.8^{e}	2.1^{b}	1.2^{a}
5	1-Hexanol	12.7^{c}	12.9^{d}	14.4^{f}	12.1^{b}	10.6^{a}	13.7^{e}
6	2-Ethyl-1-hexanol	24.1^{f}	7.1^{b}	9.8^{c}	10.8^{d}	13.0^{e}	3.3^{a}
7	2-(methylthio)ethanol	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.5^{b}	7.2^{c}
8	3-Methyl-1-butanol	10.6^{e}	8.8^{b}	9.6^{c}	10.1^{d}	7.5^{a}	14.8^{f}
9	2-Methyl-1-butanol	0.0^{a}	0.6^{b}	2.1^{d}	1.1^{c}	0.0^{a}	11.6^{e}
Acids							
10	2-Methylbutanoic acid	0.0^{a}	0.1^{a}	1.3^{c}	0.9^{b}	109.3^{e}	4.1^{d}
11	2-Methylpropanoic acid	0.0^{a}	0.0^{a}	1.9^{c}	1.4^{b}	93.4^{e}	61.4^{d}
12	3-Methylbutanoic acid	0.0^{a}	0.2^{b}	3.3^{d}	3.2^{c}	170.5^{f}	153.0^{e}
13	Butanoic acid	0.0^{a}	0.0^{a}	0.4^{b}	0.0^{a}	14.1^{d}	7.9^{c}
14	Acetic acid	28.68^{d}	6.2^{c}	2.5^{a}	5.4^{b}	37.6^{f}	36.5^{e}
Ketones							
15	2-Pentanone	0.0^{a}	0.0^{a}	3.2^{b}	11.4^{d}	16.9^{e}	8.0^{c}
16	5-Methyl-2-hexanone	0.0^{a}	0.0^{a}	0.3^{b}	0.0^{a}	9.9^{c}	89.9^{d}
17	Phenol	21.6^{e}	1.5^{b}	19.2^{d}	28.7^{f}	2.5^{c}	1.4 ^{a}
18	Acetoin	34.3^{e}	35.7^{f}	12.4^{c}	29.5^{d}	0.0^{a}	7.8^{b}
19	2-Heptanone	14.0^{d}	11.0^{a}	11.8^{c}	11.7^{b}	32.8^{f}	18.9^{e}
20	2-Butanone	95.4^{e}	26.2^{a}	46.9^{c}	37.4^{b}	278.1^{f}	50.9^{d}
21	6-Methyl-2-heptanone	0.0^{a}	0.0^{a}	0.3^{b}	0.0^{a}	2.2^{c}	4.0^{d}
Aldehydes							
22	Benzaldehyde	74.3^{f}	39.9^{e}	24.3^{d}	22.1^{c}	15.4^{a}	18.9^{b}
23	2-Methylbutanal	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	13.2^{c}	4.2^{b}
24	3-Methylbutanal	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	9.2^{c}	2.3^{b}
25	Phenylacetaldehyde	0.0^{a}	0.5^{a}	0.2^{a}	0.5^{b}	14.4^{d}	8.0^{c}
Ester							
26	2-ethylhexyl 2-propenoate	13.28^{f}	8.7^{c}	7.5^{b}	9.8^{d}	11.5^{e}	2.5^{a}
Furan							
27	2-Pentylfuran	24.0^{f}	15.5^{c}	15.1^{b}	17.9^{d}	19.9^{e}	9.1^{a}
Sulfur compound							
28	Dimethyl disulfide	6.1^{e}	1.5^{a}	3.8^{d}	2.1^{b}	19.3^{f}	3.5^{c}
Others							
29	Limonene	20.3^{e}	9.9^{a}	35.3^{f}	16.3^{c}	17.6^{d}	14.9^{b}
30	Antioxidant BHA	77.8^{f}	37.2^{c}	33.5^{b}	38.4^{d}	55.0^{e}	21.1^{a}
31	Acetamide	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.9^{b}	0.0^{a}

Key: A, *Bacillus subtilis*; B, *Bacillus safensis*; C, Mixed culture of *Bacillus subtilis and Bacillus safensis*; D, Mixed culture of *Bacillus subtilis, Bacillus safensis, Bacillus clausii, Bacillus licheniformis, and Bacillus xylanilyticus*; E, *Bacillus cereus sensu lato*; F, Spontaneously fermented *ugba*. Means with the same superscript for each flavour compound across a row are not significantly different ($P > 0.05$).

in *ugba* inoculated with starter cultures during the fermentation process. In this study, 7 flavour compounds categorized as alcohols were detected in *ugba*.

Among the flavour compounds (organic acids) detected in *ugba*, only acetic acid persisted in all the samples throughout the period of fermentation. According to Ojinnaka and Ojimelukwe (2013), butanoic acid and 3-methylbutanoic acid have a sweaty odour. Both compounds

Table 5. Flavour compounds (µl) obtained from *ugba* inoculated with starter cultures, spontaneously fermented *ugba* at 72 hours and commercially produced *ugba*.

S/N	Compound name	Sample A	Sample B	Sample C	Sample D	Sample E	Sample F	Sample G
Pyrazines								
1	2,5 or 2,6-dimethylpyrazine	8.5^{e}	5.5^{b}	8.1^{c}	8.3^{d}	78.5^{g}	77.7^{f}	1.15^{a}
2	3-Ethyl-2,5-dimethylpyrazine	0.0^{a}	0.3^{c}	0.1^{b}	0.3^{c}	0.7^{d}	43.9^{e}	0.0^{a}
Alcohols								
3	2-Methyl-1-propanol	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	24.0^{b}	0.0^{a}
4	Guaiacol	29.6^{f}	3.7^{c}	24.9^{e}	54.4^{g}	2.3^{b}	0.0^{a}	6.7^{d}
5	1-Hexanol	5.7^{a}	12.9^{d}	12.4^{d}	12.4^{d}	6.8^{b}	16.2^{e}	11.9^{c}
6	2-Ethyl-1-hexanol	21.5^{g}	12.7^{c}	14.8^{e}	14.9^{f}	13.6^{d}	2.2^{a}	3.88^{b}
7	2-(methylthio)ethanol	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.3^{b}	12.8^{c}	0.0^{a}
8	3-Methyl-1-butanol	1.4^{a}	10.8^{e}	10.4^{d}	8.4^{c}	4.7^{b}	33.4^{f}	36.28^{g}
9	2-Methyl-1-butanol	0.0^{a}	1.5^{b}	1.7^{c}	0.0^{a}	0.0^{a}	35.7^{e}	5.18^{d}
Acids								
10	2-Methylbutanoic acid	8.4^{b}	0.8^{a}	12.9^{d}	2.9^{d}	17.4^{e}	10.6^{c}	21.3^{f}
11	2-Methylpropanoic acid	8.1^{c}	6.4^{b}	10.8^{e}	0.0^{a}	65.6^{f}	94.9^{g}	8.7^{d}
12	3-Methylbutanoic acid	11.2^{a}	14.5^{c}	17.0^{d}	13.0^{b}	60.3^{e}	199.3^{g}	87.3^{f}
13	Butanoic acid	0.0^{a}	0.0^{a}	37.8^{e}	0.0^{a}	5.2^{c}	10.7^{d}	3.25^{b}
14	Acetic acid	17.8^{d}	2.5^{a}	12.7^{c}	5.5^{b}	29.7^{e}	46.9^{f}	1 09.7^{g}
Ketones								
15	2-Pentanone	1.3^{b}	1.2^{a}	5.5^{d}	5.1 ^{c}	27.2^{f}	12.3^{e}	70.6^{g}
16	5-Methyl-2-hexanone	0.9^{b}	7.0^{c}	0.9^{b}	0.9^{b}	30.6^{d}	98.2^{e}	0.0^{a}
17	Phenol	51.0^{e}	3.4^{c}	55.6^{f}	75.7^{g}	2.4^{b}	1.3^{a}	23.1^{d}
18	Acetoin	13.0^{e}	13.5^{f}	100.8^{g}	7.5^{c}	0.0^{a}	6.2^{b}	11.4^{d}
19	2-Heptanone	8.1^{a}	14.6^{d}	12.7^{b}	16.6^{c}	105.4^{g}	34.1^{e}	51.7 ^{f}
20	2-Butanone	44.7^{a}	94.3^{d}	84.4^{c}	127.3^{f}	257.0^{g}	111.0^{e}	55.4^{b}
21	6-Methyl-2-heptanone	1.3^{c}	0.6^{b}	1.4^{d}	2.6^{e}	11.9^{g}	9.2^{f}	0.0^{a}
Aldehydes								
22	Benzaldehyde	28.3^{f}	15.9^{c}	9.0^{a}	11.8^{b}	22.9^{e}	20.2^{d}	36.1^{g}
23	2-Methylbutanal	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	4.8^{c}	12.2^{d}	3.8^{b}
24	3-Methylbutanal	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	8.3^{c}	10.5^{d}	4.8^{b}
25	Phenylacetaldehyde	1.1^{c}	0.3^{b}	1.4^{d}	1.6^{e}	25.6^{g}	8.7^{f}	0.0^{a}
Ester								
26	2-ethylhexyl 2-propenoate	15.0^{g}	14.8^{f}	14.6^{e}	12.8^{d}	11.7^{c}	2.8^{b}	0.0^{a}
Furan								
27	2-Pentylfuran	27.1^{f}	22.6^{e}	16.8^{c}	20.2^{d}	16.6^{b}	9.1^{a}	66.3^{g}
Sulfur compound								
28	Dimethyl disulfide	2.5^{c}	4.0^{e}	2.3^{b}	3.1^{d}	14.9^{g}	7.6^{f}	0.73^{a}
Others								
29	Limonene	8.1^{b}	8.1^{b}	8.8^{d}	10.6^{e}	8.2^{c}	6.2^{a}	27.9^{f}
30	Antioxidant BHA	30.7^{c}	32.4^{d}	49.4^{f}	43.7^{e}	32.7^{d}	19.8^{b}	0.0^{a}
31	Acetamide	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	20.4^{b}	0.0^{a}	0.0^{a}

Key: A, *Bacillus subtilis*; B, *Bacillus safensis*; C, Mixed culture of *Bacillus subtilis and Bacillus safensis*; D, Mixed culture of *Bacillus subtilis, Bacillus safensis, Bacillus clausii, Bacillus licheniformis, and Bacillus xylanilyticus*; E, *Bacillus cereus sensu lato*; F, Spontaneously fermented *ugba*; G, commercially produced *ugba*. Means with the same superscript for each flavour compound across a row are not significantly different ($p > 0.05$).

were not detected in *ugba* at 0 hour. However, as fermentation of *ugba* progressed, varied amounts of butanoic acid and 3-methylbutanoic were detected.

Volatile flavour compounds categorized as aldehydes were detected in *ugba* undergoing fermentation. Among the aldehydes, only benzaldehyde was detected in all the samples. In a related study, Onyenekwe et al. (2012) reported that aldehydes constitute the predominant group

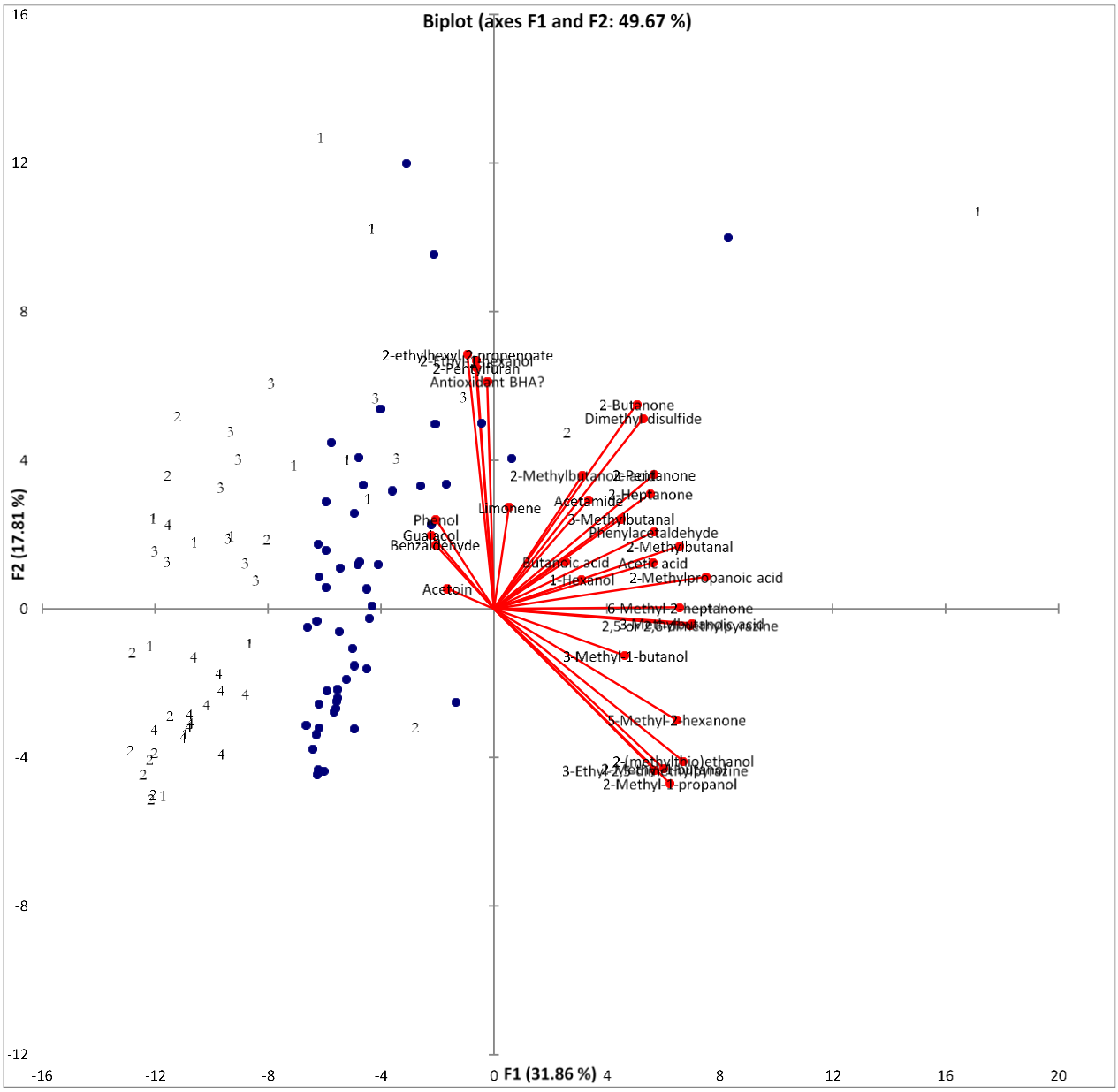

Figure 2. Bi-plot from principal component analysis (F1 *Vs* F2) of relative concentrations of volatile compounds from various samples of *ugba*. **Key:** A, *Bacillus subtilis*; B, *Bacillus safensis*; C, Mixed culture of *Bacillus subtilis and Bacillus safensis*; D: Mixed culture of *Bacillus subtilis, Bacillus safensis, Bacillus clausii, Bacillus licheniformis and Bacillus xylanilyticus*; E, *Bacillus cereus sensu lato*; F, Spontaneously fermented *ugba*

of flavour compounds in fermented condiments. It is not in agreement with the findings from this study which indicate that organic acids and ketones were predominant. Leejeerajumnean et al. (2001) reported that major volatile compounds found in *Bacillus* fermented soybeans were 3-hydroxybutane (acetoin), 2- methylbutanoic acid, pyrazines, dimethyl disulphide and 2-pentylfuran.

Among the flavour compounds (ketones) detected in the fermenting samples of *ugba*, only 2-heptanone and 2-butanone were detected in all the samples. Ojinnaka and Ojimelukwe (2013) reported that ketones which impact the odour of food are released during microbial fermentation which involves degradation of lipids and amino acids. During fermentation of *ugba*, phenol and other ketones were detected as flavour compounds. According to Nwokeleme and Ugwuanyi (2015), the presence of phenolic compounds in fermented foods is responsible for smoky and/brown odour.

2-ethylhexyl 2-propenoate is the only flavour compound referred as ester detected in *ugba*. Throughout the period of fermentation of all the samples of *ugba*, 2-ethylhexyl 2-propenoate was consistently detected. However, the flavour compound was not detected in the commercially produced *ugba*. The presence of esters in many fermented condiments of African origin have been reported (Adebiyi et al., 2021). According to Sluis et al. (2001), esters are mostly formed as a result of esterification of alcohol with fatty acid during fermentation. It is presumed that esters are formed as a result of chemical reaction which involves acidic microbial and non-alcoholic metabolites possibly catalyzed by microbial esterases. Esters are known to impart a fruity floral characteristic. This could reduce the unpleasant sharp FFA-derived notes (Leejeerajumnean et al., 2001; Eskin, 1990).

Among the flavour compounds categorized as aldehydes which were detected in *ugba*, phenylacetaldehyde was not detected in *ugba* at 0 hour. As fermentation progressed, it was detected in some samples. A similar result was recorded for other aldehydes which include benzaldehyde, 2-methylbutanal and 3-methylbutanal. In a related study, Nwokeleme and Ugwuanyi (2015) reported that aldehydes which include 2, 4-hexadienal and 14-heptadecenal were present in *ugba* fermented by *Bacillus subtilis* and *B. megaterium*. A lot of volatile aldehydes are derived from fatty acids and amino acids. Enzymatic transamination or oxidative deamination of amino acids generate metabolic by-products which might include aldehyde.

2-Pentylfuran is a flavour compound classified as furan. It was consistently detected in all samples of *ugba*. Furans are heterocyclic organic compounds which consist of five member aromatic ring with four carbon atoms and one oxygen. They are colourless, flammable and highly volatile liquid. Furans are aromatic compounds found in heat-treated food. Production of furans takes place by thermal degradation of natural food constituents (Moro et al., 2012). The result obtained from this study shows that commercially produced *ugba* contain higher amount of 2-pentylfuran than the laboratory-prepared *ugba*. This could be attributed to traditional method of cooking raw African oil bean overnight during preparation of *ugba* in commercial quantity.

A sulfur compound known as dimethyl disulfide was consistently detected in all the samples of *ugba*. In a related study, Nwokeleme and Ugwuanyi (2015) reported the presence of two sulfur compounds during production of *ugba* using pure culture. Various enzymatic reactions are associated with the release of some volatile sulfur compounds in food. This could be attributed to secondary metabolism of leucine and cysteine.

Although some flavour compounds were not detected in some fermenting samples of *ugba* at different intervals of monitoring, ester, furan and sulfur compound were consistently detected in laboratory-prepared *ugba* samples. This result is suggestive that 2-ethylhexyl 2-propenoate, 2-pentylfuran and dimethyl disulfide played an important role in the flavour of the final product (*ugba*). In a related study, Nwokeleme and Ugwuanyi (2015) reported that hydrocarbons, alcohols, esters, furan, phenols, ketones and amines were consistent volatile flavour compounds detected in *ugba* which could play some roles towards achieving the final flavour of the product.

Conclusion

Ugba produced using starter cultures and the spontaneously fermented sample share similarities with commercially prepared ugba with regards to volatile aroma compounds. During fermentation of *ugba,* there was increase in pH and heterotrophic bacterial count of all the samples. A total of 31 compounds which comprise of 7 ketones, 7 alcohols, 5 organic acids, 4 aldehydes, 2 pyrazines, 1 ester, 1 furan, 1 sulfur and 3 other flavour compounds were identified as aroma compounds in all the samples of *ugba*.

CONFLICT OF INTEREST

The authors confirm that they have no conflict of interest.

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