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ORIGINAL ARTICLE

Volatile Compounds Determination by GC-MS and Identification of Dominant Microorganism at Varying Fermentation Period of Ogi Slurry

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Abstract

Volatile compounds (VOCs) and associated microbes of ogi at varying soaking and fermentation periods were detected with the aid of Gas Chromatography-Mass Spectrometry (GC-MS) and cultural methods, respectively. Thirty-five (35) VOCs were identified and classified into Carbonyl, Alcohol, Aldehydes, Esters and Organic acid. The average amount of volatile compounds of Ogi produced at varying period (16 and 24 h) varies significantly. Twelve (12) of the detected VOCs had higher concentration and the principal components analysis (PCA) revealed that few of the identified VOCs contributed significantly to the characteristic attributes of Ogi. The first principal components (PC1) had Eigen values of 15.33 (43.80%) and 17.84 (51%) while the second principal components (PC2) had Eigen values of 12.57 (35.90%) and 9.67 (27.60%), respectively. Presence of different species of *Candida* and Lactic Acid Bacteria (LAB) isolates at different fermentation levels (0 to 48 h) of Ogi was established. The population of LAB and yeast increased with rate of fermentation from 0 to 24 h but remained constant at 36 and 48th h of fermentation. The isolated LAB include *Pedicoccus spp*, *Leuconostoc spp*, *Lactobacillus acidophilus*, *Lactococcus spp* and other Lactobacillus spp. while the *Candida spp* present in the Ogi samples include; *Candida krusei*, *Candida glabrata*, *Candida parasilosis*, *Candida albicans*, *Candida tropicalis* and *Candida kefyr*. *Candida spps* exhibited a varying temperature for growth while growth of LAB ceased at temperature above 45^{o}C but not below 15^{o}C. Molecular identification further revealed the following LAB; *Lactobacillus pentosus* strains, *Lactobacillus fermentum* strains, *Pediococcus pentosaceus* strains and *Weissella confusa* strains, while dominant yeast were *Candida tropicalis* strains, *Candida auris* isolate, *Candida glabrata* strain, *Pichia kudriavzevii* culture, *Candida albicans* strain and *Issatchenkia orientalis* strain. This study revealed that LAB and Yeast are actively involved in the fermentation of ogi. The fermentation condition and the identified microorganism evidently contributed to the amount and types of VOCs detected.

Practical application

The consumers' acceptance of Ogi slurry is predominantly hinged on the aroma, flavour and taste. Sensory acceptability is indirectly or directly connected to the concentration of volatile compounds of the product. The processing conditions (fermentation periods) stand the chance of playing very important role in the types and concentration of these volatile compounds. The knowledge of the volatile compounds will be useful in monitoring Ogi's freshness and quality and subsequently may be of commercial interest, most especially with the identified Lactic Acid Bacteria and yeast and the detected volatile compounds. This information may be of commercial interest if the predominant volatile compounds detected in Ogi are to be synthesized artificially.

Keywords: Ogi, Volatile compounds(VOCs), Gas chromatography (GC), Lactic acid Bacteria, Yeast, Aroma.

1. Introduction

Ogi is a fermented cereal product; it is common and predominantly consumed in Nigeria, West African in various forms. The production involves soaking of cereal for 12-96 h, sieved and subsequently allowed to sediment for 12-48h depending on the degree of sourness desired by consumers (Ijabadeniyi, 2007; Bolaji *et al.*, 2011; Bolaji *et al.*, 2017). The sediment referred to as ogi, can be made into pap or agidi through the aid of heat application. It is an alternative weaning food source in West African countries Traditional and commercial manufacturing of ogi are still similar with some modifications (Bolaji *et al.*, 2011; Bolaji *et al.*, 2017).

Considering the growing population and economic situation of most African countries, commercial production of ogi may be necessary. Here, reduction in processing time and cost of production without compromising the quality is highly necessary (Bolaji *et al.*, 2017; Bolaji *et al.*, 2018). Often time, the pap consistency, flavour and taste are important to consumers (Ijabadeniyi, 2007; Bolaji *et al.*, 2017). According to some researchers, aroma and taste of ogi are important parameters used to determine freshness and quality of ogi or ogi-pap and these are important in determining consumers' acceptability (Teniola & Odunfa, 2002; Ijabadeniyi, 2007).

Literature abounds on the influence of fermentation process on aroma of food materials (Uzochukwu *et al.*, 1997; Jirovetz *et al.*, 2001; Nur *et al.*, 2012). Fermentation process was reported as a complex process and most times, complex organic and inorganic compounds are produced (Uzochukwu *et al.*, 1997; Jirovetz *et al.*, 2001). The fermentation time, microbial presence and activities were important factors reported widely in the literatures playing significant role in the aroma and flavour of food materials (Nur *et al.*, 2012; Jirovetz *et al.*, 2001). It has also been widely documented that some volatile compounds are indicator of freshness or spoilage (Lee & Ahn, 2009; Zhao *et al.*, 2011; Nur *et al.*, 2012; Jelen *et al.*, 2013). According to some researchers, the profile of volatile components of fermented food is dependent on the type of processing conditions, fermentation and drying (Shukla *et al.*, 2010).

Identification of volatiles compounds in food materials were attempted by many methods. However, considering the ease, sensitivity, reliability and sample preparation methods, GC and HPLC are the two mostly used recent techniques (Shukla *et al.*, 2010; Nur *et al.*, 2012). According to some researchers, volatile compounds may play an important role in the characterization of foods (Welke & Zini, 2011; Lee & Ahn, 2009). The presence of some volatile compounds may sometime indicate undesirable content or indicate the safety level of the food (Nur *et al.*, 2012; Lee &Ahn, 2009). According to some researchers, the information on volatile compounds from natural fermentation may be necessary to determine and monitor quality of food, processing and storage conditions (Nur *et al.*, 2012) The knowledge of volatile compounds in food materials were reported as relevant in determining the optimal acceptable flavour (Nur *et al.*, 2012; Lee & Ahn, 2009; Zhao *et al.*, 2011; Jelen *et al.*, 2013).

The literature is scanty on the potential volatile compounds present in ogi before and during fermentation and also, the corresponding dominant microorganism. There is likelihood of the unique flavour and aroma of ogi impacted by volatile compounds being affected by predominant lactic acid bacterial and some yeast as reported in the literature (Annan *et al.*, 2003; Ijabadeniyi, 2007; Lee & Ahn, 2009; Zhao *et al.*, 2011; Nur *et al.*, 2012;Jelen *et al.*, 2013). A number of organisms have been implicated in playing important role in the fermentation process and characterisation of aroma and flavour (Annan *et al.*, 2013; Ijabadeniyi, 2007).

This study was therefore undertaken to identify the volatile compounds impacted by fermentation process in ogi. The knowledge may also help in the subsequent synthesis of active compounds responsible for desired aroma and flavour, for possible industrial and commercial application.

2. Materials and Methods

2.1. Sample preparation

Twenty-five kilogram of yellow maize variety was obtained from Ikorodu market. About 10 kg of maize grains were divided into ten (10) equal parts of 1 kg each after foreign materials were carefully removed. These were divided into two (2) groups according to soaking period of 16 and 24 h, respectively and fermentation period of 0, 12, 24, 36 and 48 h, respectively (Bolaji *et al.*, 2017). Ogi samples were produced from soaked maize grains for respective periods in clean water, milled and sieved with 212 µm sieve. These samples of ogi were allowed to sediment and ferment accordingly at varying period. The volatile compounds of ogi at varying fermentation period of 0, 12, 24, 36 and 48 h were determined with the use of Gas Chromatography-Mass Spectrometry (GC-MS).

2.2. Determination of volatile compounds

Determination of volatile compounds in fermented ogi was achieved using GC-MS method as reported by Shukla *et al.* (2010) and Nur *et al.* (2012). The extraction of volatile compounds was determined by measuring 5 g of each sample and mixed with 20 mL of distilled water. This was filtered and centrifuged at 10,000 g for 30 min. All the volatile compounds of ogi were analysed without any prior treatment by using the gas chromatography (Model: Agilent Technologies 6890 HP chemstation Rev.A0901 (1206). The Headspace extraction of ogi volatile compounds was completed within 30min. at temperature of 60°C followed by GC separation elaborated with column oven initial temperature at 35°C at the rate of 5°C/min to reach 300°C. The samples were injected with 100:1 splitting for screening. Identification of the compounds was done by GC retention time against standards. The area of each peak was determined by ChemStation software (Agilent Technologies).

2.3. Microbial characterization

2.3.1. Isolation of lactic acid bacteria and Yeast

One milliliter (1 mL) of each sample was dispensed into tubes and the samples were processed for the isolation of lactic acid bacteria (LAB) and Isolation of Yeast. A teaspoon full of each samples were inoculated on PDA (Potato Dextrose agar) (Himedia) and MRS (de Mann Rogosa Sharpe agar) (Oxoid) and incubated anaerobically in anaerobic jar with AneroGen TM (Oxoid) at 37°C for 48 h.

Each isolate was sub cultured on PDA at 30°C for 48 h. After this, they were seeded on CHROMAgarTM Candida (Hi Chrome Candida Differential Agar Base) and incubated at 30°C for 48 h. The CHROMAgar™ allows selective yeast isolation, identifying colonies of *Candida albicans*, *Candida tropicalis* and *Candida krusei*, *Candida glabrata*, *Candida parasilopsis* by morphology and color reaction. The strains were identified according to the manufacturer's instructions, which define *Candida albicans* as green colonies, *Candida tropicalis* as steel blue colonies, *Candida krusei* colonies as showing rose color colonies, *Candida glabrata* as cream colonies and *Candida parasilopsis* as cream with mauve center colonies.

The Identification of Lactic acid bacteria: Identification of predominant bacterial colonies was done on the basis of morphological and biochemical characteristics (Mugula *et al.*, 2003). Isolates were further purified by streaking repeatedly on MRS agar plates, and the colony morphologies (color, shape and size) were examined. The LAB isolates were examined morphologically under the microscope. Colonies

with typical characters (such as pin pointed, whitish transparent etc.) were randomly selected from plates and tested for Gram-character, cell morphology, catalase activity and sugar fermentation. LAB are known to be Gram positive and the blue-purple color indicates the Gram positive nature of the bacteria.

2.3.2. Bacterial strains and yeast strain cultivations

Bacterial strains and yeast strain cultivations of fermented ogi were activated in ogi medium and MRS broth and PDA, respectively and then cultivated at 37°C for 10 h before further use. The ogi samples were subculture before DNA extraction was carried out on the isolates using the Zymo Fungal/Bacteria DNA extraction kit following manufacturing instructions. The purity and concentration of the extracted DNA was evaluated using a NANODROP (ND 1000) Spectrophotometer (Thermo Scientific, USA). All the samples showed a DNA yield between 5-25ng and the extracted DNA was optimally pure showing A260/A280 between 1.60-1.80.

2.3.3. PCR Amplification of the ITS gene and Lactic acid bacteria specific primers

Polymerase chain reaction was carried out to amplify the yeast using the primer pair ITS-1 and ITS-4 for yeast (Makun *et al.*, Yah, 2011). While for Lactic acid bacterial, BSF-8 TTGATCCTGGCTCAG) and BSR-534(ATTACCGCGGCTGCTGGC) were used (Reinhard *et al.*, 2005; Ayeni & Odumosu, 2016).

The PCR reaction was carried out using the Solis Biodyne 5X HOT FIREPol Blend Master mix. PCR was performed in 25 µl of a reaction mixture, and the reaction concentration was brought down from 5x concentration to 1x concentration containing 1x Blend Master mix buffer Buffer (Solis Biodyne), 1.5 mM $MgCl_2$, 200 µM of each deoxynucleoside triphosphates (dNTP)(Solis Biodyne), 25 pMol of each primer (BIOMERS, Germany), two (2) unit of Hot FIREPol DNA polymerase (Solis Biodyne). Additional Taq DNA polymerase was incorporated into the reaction mixture to make a final concentration of 2.5 units of Taq DNA polymerase, Proofreading Enzyme. About 2 µL of the extracted DNA, and sterile distilled water was used to make up the reaction mixture for ITS gene while 5 µL of the extracted DNA and sterile distilled water was used to make up the reaction mixture for Lactic acid bacteria.

Thermal cycling was conducted in an Eppendorf Vapo protect thermal cycler (Nexus Series) for an initial denaturation of 95°C for 15 min. This was followed by 35 amplification cycles of 30 sec. at 95°C; 1 min. at 58°C, 120 sec. at 72°C and lastly the extension step of 10 min. at 72°C. The amplification product was separated on a 1.5% agarose gel and electrophoresis at 80 V for 120 min. The DNA bands were visualized by ethidium bromide staining. 100bp DNA ladder was used as DNA molecular weight standard. All PCR products were purified with Exo sap and sent for sequencing.

2.4. Statistical Analysis

The concentrations of volatile compounds were analysed using SPSS 17. Analysis of variance was conducted on the data. The volatile compounds concentrations were subjected to principal component analysis (PCA) using Minitab 17 software.

3. Results and discussion

3.1. Volatile compounds

Thirty five (35) volatile compounds were detected in all the ogi samples as shown in Table 1. These compounds were identified as Acetaldehyde, Ethanol, Acetic acid, Propanol, 2-Methyl propanal, 2-Methy1 propanol, 2,3-Butanedione, 3-Methy1 butanal, 3-Methyl butanol, 2-Methyl butanol, Hexanal, Ethyl acetate, 2.3-Butanediol, Heptanol, Propyl acetate, Propanoic acid, Hexadecanoic acid, Isobutyl acetate, Ethy1 butyrate, Nonanal, 2-methyl-naphthalene, Ethyl lactate, 2-Methoxlphenol, E-2-Nonenal, 1-nethylnaphthalene, Ethyl isovalerate, Isoamyl acetate, 2-Methylbutyl acetate, Ethyl valerate, 2-Nonenal, Ethyl caproate, 2-Methoxy-4-vinylphenol, Ethy1 caprylate, Ethyl dec-9-enoate and Ethyl caprate. There was significant differences ($p<0.05$) in the amount of each volatile compound detected. The result revealed that soaking period of maize grains employed in this work had no significant effect ($p>0.05$) on the volatile compounds while varying fermentation period significantly affected ($p<0.05$) the volatile compounds..

Table 1 also shows the classification of these thirty five (35) volatile compounds into five broad classes of organic compounds namely: Carbonyl Compounds, Alcohols, Aldehydes, Acids and Esters. The respective percentage concentrations are as shown in Figure 1. The number of these volatile compounds were lower than values reported for fermented dough and yoghurt, respectively (Cheng, 2010; Annan *et al.*, 2003). The suggested characteristics are possible indication expected from the combination of their chemical compositions and may be considered as tentative association (Mariaca *et al.*, 2001; Cheng 2010).

Twelve (12) of the volatile compounds-Acetaldehyde, Ethanol, Acetic acid, Propanol, Hexanal, Ethyl acetate, Hexadecanoic acid, Nonanal, 2-methyl-naphthalene, Ethyl lactate, E-2.nonenal, and 1-methylnaphthalene-had higher and varying concentrations (Figure 1, 2 and 3) at all fermentation periods. The average amount of volatile compounds and the percent concentration of ogi produced from maize grains at varying periods (16 and 24 h) and varying fermentation period as shown in Figure 1 . Their magnitudes may contribute to the aroma and flavor of ogi at any particular time, a view supported by some researchers (Cheng, 2010; Annan *et al.*, 2003; Nur *et al.*, 2012) .

The box loading plot (Figure 3) of some volatile compounds with higher percent concentrations for ogi produced for both soaking period of 16 and 24 h, respectively, shows that acetaldehyde has a mean percent concentration of 9.49 and 10.98% between fermentation period of 0 and 12 h, respectively. Values reduced subsequently to 0.805 and 0.613% at 24 and 48 h, respectively for ogi produced from maize soaked at 16 and 24 h. The concentration of ethanol increased from 2.96 to 5.13% for ogi produced from soaking period of 16 h, while the least values were recorded at initial period (0 h). A more pronounced increase in percent concentration of ethanol (0.936 to 7.608%) was recorded at 0-36h and soaking period of 24 h. At 48 h of fermentation, this reduced to 1.27%. A regular pattern was not observed for Acetic acid, Propanol, Ethy lactate, Nonanal, 2-methyl-naphthalene, E-2-Nonenal, 1-Methylnaphthalen, Hexadecanoic acid, Hexanal and Ethyl acetate in

Table 1: Classification of identified volatile compounds in ogi produced from Maize grains at varying period (16 and 24 h) and varying fermentation period (0, 12, 24, 36 and 48 h)

S/N	DETECTED VOLATILE COMPOUNDS	CHARATERISTICS
1	***CARBONYL COMPOUNDS***	
	Vinylphenol	Colour of grape wine
	Ethyl caprylate	Waxy type odour
	Ethyl caprate	Waxy type of odour
	2-methyl-naphthalene	Unpleasant smell
	1-methylnaphthalene	Mothball odour
	2-3-Butanediol	Odourless
	Ethyl isovalerate	Strong odour
	2-methoxy-4	Pleasant, spicy odour
	Ethyl dec-a-enoate	Odourless
2	**ALDEHYDES**	
	Acetaldehyde	Fruity odour
	Nonanal	Fatty, citrus odour
	Hexanal	Herbal apple, green leafy
	E-2-Nonenal	Colourless, Aroma of aged beer
	2-Nonenal	Aroma of aged beer.
	3-Methy1 butanal	Malty flavour
	2-Methyl propanal	wet cereal odour
3	**ACID**	
	Propanoic acid	Sharp rancid odour
	Ethanol	Mild, ether
	Acetic acid	Distinctive sour taste and pungent smell
	Hexadecanoic acid	Nearly odorless
4	**ESTERS**	
	Ethyl acetate	Sweet smell, Solvent-like, fruity,
	Ethyl caproate	Fruity odour
	Ethyl lactate	Fruity
	Ethyl valerate	Pleasant aroma and taste
	Isobutyl acetate	Ester odour
	Propyl acetate	Odour of pear
	Ethyl 1 butyrate	Pinapple-like odour
	Isoamyl acetate	Fruity odour
	2-methyl butyl acetate	Pleasant odour
5	ALCOLHOL	
	2-Methyl1 propanol	Sweet
	Propanol	Sharp musty odour.
	Heptanol	Pleasant smell
	3-Methyl butanol	Choking alcohol odour
	2-Methyl butanol	Aroma of tuber
	2-Methoxlphenol	Sweet odour
	2-Methyl propanol	Buttery creamy

ogi produced from maize grains soaked at 16 and 24 h, respectively. The highest percent concentration for propanol was obtained at soaking period of 16 and 24 h (6.03 and 7.39%) at fermentation period of 12 and 36h, respectively. The values recorded for Ethyl acetate (4.47-1.13%) at 24 h soaked period were significantly higher compared with values obtained for ogi produced from maize soaked at 16 h (4.11-7.71%). Ogi produced from maize soaked for 24 h had the highest value of Nonanal (10.95%) at 0 h fermentation. The ogi produced from maize grains soaked for 16 h also showed similar trends in all the fermentation periods with the highest values being recorded at 24 h of fermentation. The percent concentration for Hexanal at varying fermentation period of ogi ranged from 6.97-10.02% and 7.13 - 8.31% for ogi produced at 16 and 24 h soaking period, respectively. The result further revealed that relatively higher values of Hexadecanoic acid and Ethyl acetate were obtained compared with other volatile compounds. The percent concentration of Hexadecanoic acid and ethyl acetate were 14.75 and 20.91% (0 h), 19.89 and 24.94% (12 h), 20.18 and 14.87% (24 h), 14.19 and 26.25% (36 h) and 19.73 and 13.81% (48 h) for ogi produced at 16h soaking period of maize grains while 24.03 and 14.24% (0 h), 13.24 and 24.87% (12 h), 19.03 and 12.53% (24 h), 20.78 and 11.04% (36 h), 19.31 and 17.95 (48 h) for ogi produced at 24 h soaking period of maize grains.

The mean retention time for respective detected volatile compounds at varying period (16 and 24 h) and varying fermentation period (0, 12, 24, 36 and 48 h) are as shown in Table 2: These ranged from 9.67 to 25.52 min. Acetaldehyde was found in the ogi at both soaking periods and all the fermentation periods selected for the experiment. It appears to be the most volatile of the compounds detected. The retention time ranged from 9.73 to 9.87 min. and 7.41 to 9.75 min, respectively for ogi fermented between 0 to 48h, while Ethyl caprate was the last volatile compound recorded and ranged between 27.43-27.52 min. and 27.43-27.48 min, respectively. . Similar results were reported for retention time of volatile compounds detected in dried ogi (Bolaji *et al.*, 2020).

3.3. Principal components

The correlation plots of the first principal component (PC1) and second principal component (PC2) for ogi produced from both soaking period of 16 and 24 h are as shown in Figure 2 and Box loading Plot in Figure 3. The first principal component for ogi produced from maize soaked at 16 and 24 h had variance of eigen value 15.33 and 17.84% and accounts for 43.80 and 51% of the total variance while the second principal component had variance of 12.57 and 9.67% and account for 35.90 and 27.60% of the data variability. The third and fourth principal components had variances of 5.91 and 1.18 (16 h of soaking) and 4.07 and 3.41% (24 h of soaking) accounting for 16.9 and 3.4% (16 h of soaking) and 11.6 and 9.70% data variability, respectively. The fifth Principal components and above were less important. The principal components showed that few volatile compounds may have significant potential contribution and influence on the flavor and aroma of ogi produced at varying soaking and fermentation period. These were reflected in the Box loading plot shown in Figure 3.

3.4. Microbial identification

The presence of different species of yeast and Lactic Acid Bacteria isolates at different

fermentation period of Ogi under ambient temperature of (28±2°C) and steeping period of 24 h is as shown in Table 4. The population of LAB and yeast increased with rate of fermentation from 0 to 24 h however became constant at 36th and 48th h fermentation period. The Candida spp present in the samples include; *Candida krusei*, *Candida glabrata*, *Candida parasilosis*, *Candida albicans*, *Candida tropicalis* and *Candida kefyr*. The isolated LAB include Pedicoccus spp, Leuconostoc spp, *Lactobacillus acidophilus*, Lactococcus spp and Lactobacillus spp. The species of Candida exhibited a varying temperature for growth as

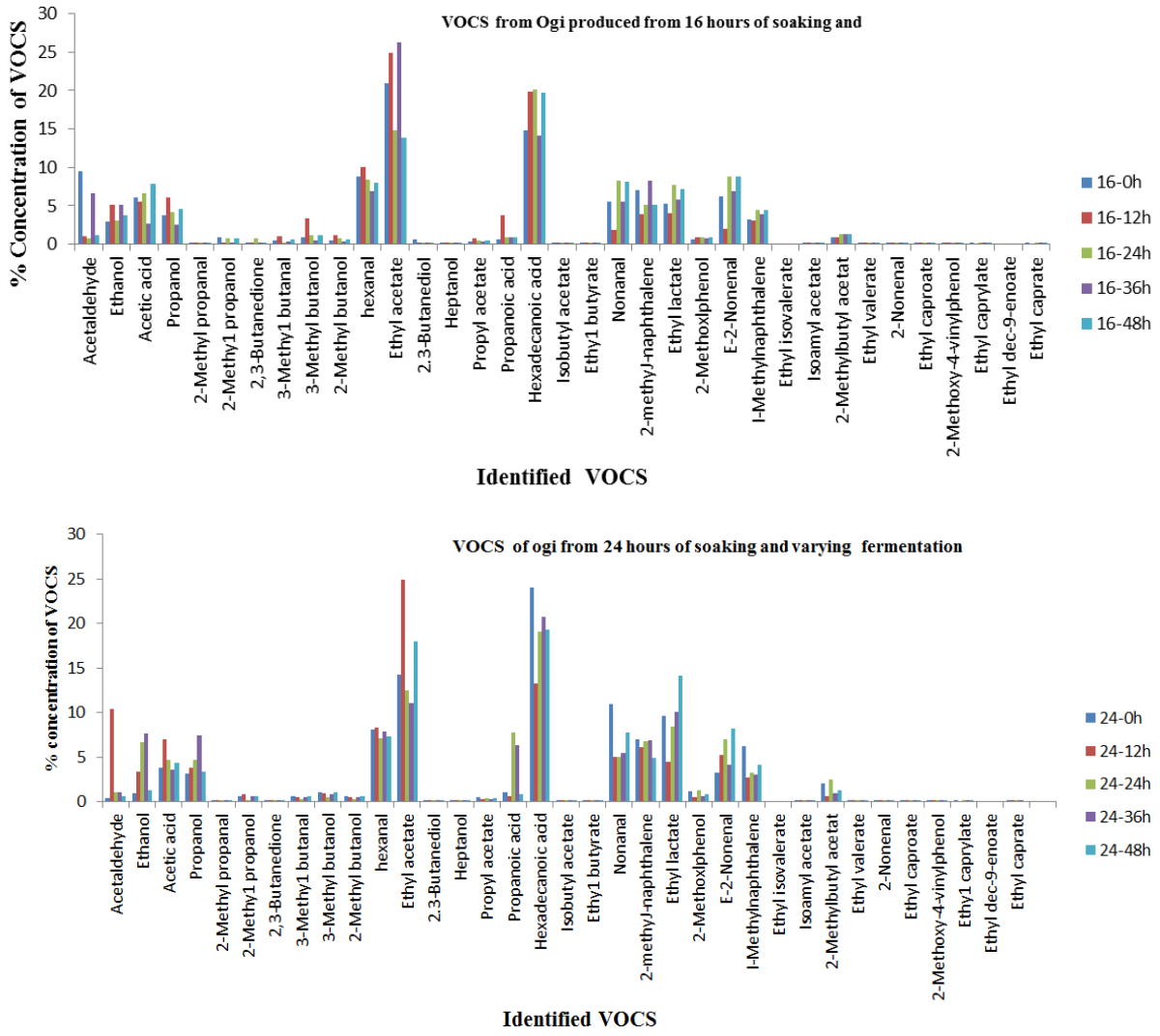

Figure 1: Percent concentration of volatile compound at varying soaking and fermentation of ogi produced from 16 and 24 h soaking of maize grains.

The mean percent concentration of the VOCs for respective soaking and fermentation period are indicative of the amount of volatile compound/area. The respective VOC concentrations are represented by colors for respective fermentation period in the Legend. The Zero (0) fermentation period is Navy blue, while fermentation period of 12, 24, 36 and 48 h are indicated for each VOCs by, Red, Green, Purple and Blue. This is applicable to bar chart of ogi produced from maize grains soaked for both 16 and 24 h, respectively.

Table 2: Detected volatile compounds and mean retention Time (min) of ogi produced from Maize grains at varying period (16 and 24h) and varying fermentation period (Fresh (0), 12, 24, 36 and 48 h)

VOLATILE COMPOUNDS	MAIZE GRAINS SOAKED FOR 16 H					MAIZE GRAINS SOAKED FOR 24 H				
	RETENTION TIME-RT (min).					RETENTION TIME-RT (min)				
	RT-Fresh	F12H	F24H	F36H	F48H	RT-Fresh	F12H	F24H	F36H	F48H
Acetaldehyde	9.68	9.86	9.74	9.87	9.73	9.75	9.67	9.64	9.75	9.74
Ethanol	10.64	10.59	10.64	10.38	10.64	10.36	10.65	10.50	10.37	10.64
Acetic acid	11.35	11.39	11.35	11.45	11.36	11.36	11.35	11.25	11.361	11.36
Propanol	12.82	12.84	12.82	12.83	12.82	12.83	12.82	11.83	12.83	12.84
2-Methyl propanal	13.54	13.44	13.54	13.53	13.54	13.55	13.54	13.55	13.55	13.54
2-Methy1 propanol	14.15	14.13	14.15	14.14	14.15	14.15	14.15	14.16	14.16	14.15
2,3-Butanedione	14.43	14.29	14.24	14.24	14.24	14.24	14.10	14.25	14.25	14.44
3-Methy1 butanal	15.03	14.92	15.04	15.02	15.04	15.05	15.04	15.03	15.05	15.04
3-Methyl butanol	15.39	15.38	15.39	15.40	15.39	15.39	15.39	15.40	15.40	15.39
2-Methyl butanol	15.62	15.67	15.62	15.62	15.62	15.62	15.62	15.62	15.63	15.62
Hexanal	16.04	15.98	16.03	16.04	16.04	16.04	16.04	16.06	16.04	16.04
Ethyl acetate	16.45	16.55	16.49	16.50	16.49	16.49	16.67	16.46	16.49	16.49
2.3-Butanediol	16.78	16.94	16.78	16.79	16.78	16.78	16.78	16.78	16.79	16.78
Heptanol	17.36	17.24	17.36	17.37	17.36	17.36	17.36	17.36	17.36	17.36
Propyl acetate	17.55	17.48	17.55	17.55	17.55	17.55	17.55	17.55	17.55	17.55
Propanoic acid	17.66	17.73	17.66	17.67	17.65	17.67	17.67	17.67	17.67	17.66
Hexadecanoic acid	18.05	17.98	18.05	18.60	18.05	18.05	18.05	18.05	18.05	18.05

Sobutyl acetate	18.49	18.55	18.49	18.49	18.494	18.49	18.49	18.50	18.49	18.49
Ethy1 butyrate	18.93	19.00	18.92	18.94	18.92	18.92	18.93	18.93	18.93	18.93
Nonanal	19.09	19.08	19.09	19.11	19.09	19.09	19.09	19.10	19.10	19.19
2-methyJ-naphthalene	19.53	19.42	19.52	19.54	19.52	18.52	19.52	19.521	19.52	19.52
Ethyl lactate	19.88	19.87	19.88	19.89	19.88	19.88	19.88	19.88	19.89	19.88
2-Methoxlphenol	20.19	20.21	20.19	20.19	20.19	20.19	20.19	20.19	20.19	20.19
E-2-Nonenal	20.47	20.55	20.47	20.61	20.47	20.59	20.46	20.48	20.47	20.47
I-nethylnaphthalene	20.79	20.66	20.78	20.79	20.79	20.78	20.79	20.66	20.79	20.79
Ethyl isovalerate	20.98	20.94	20.98	20.99	20.98	20.98	20.98	20.98	20.98	20.98
Isoamyl acetate	21.435	21.48	21.43	21.44	21.44	21.44	21.43	21.44	21.44	21.50
2-Methylbutyl acetat	22.07	21.99	22.06	22.07	22.06	22.06	22.06	22.07	22.07	22.07
Ethyl valerate	22.59	22.50	22.51	22.53	22.51	22.52	22.59	22.61	22.52	22.52
2-Nonenal	22.99	22.99	22.99	23.00	22.99	22.99	22.99	23.01	22.99	22.99
Ethyl caproate	23.66	23.61	23.62	23.63	23.62	23.62	23.61	23.63	23.63	23.62
2-Methoxy-4-vinylphenol	25.36	25.39	25.35	25.36	25.35	25.35	25.36	25.36	25.36	25.36
Ethy1 caprylate	26.50	26.69	26.50	26.51	26.50	26.50	26.635	26.51	26.51	26.51
Ethyl dec-9-enoate	26.95	26.99	27.06	26.95	27.07	26.95	26.95	26.97	27.06	27.06
Ethyl caprate	27.43	27.52	27.35	27.43	27.43	27.43	27.44	27.48	27.43	27.42

**Values are means of two replicates. RT- Fresh- unfermented fresh ogi, F12H- fermentation for 12 h, F24H- fermentation for 24 h, F36H- fermentation for 36 h, and F48H- fermentation for 48 h.*

Table 3: Identified lactic acid bacterial and yeast from ogi produced from Maize grains at varying period (16 and 24h) and varying fermentation period (0, 12, 24, 36 and 48 h)

SAMPLES (hours)	LAB (Ogi produced from sample soaked for 16th h	LAB (Ogi Produced From Sample Soaked for 24th H	Yeast (Ogi produced from sample soaked for 16th h	Yeast (Ogi Produced From Sample Soaked for 24th h
0	*Lactobacillus lactis*	*Lactobacillus lactis*	*Candida parasilopsis* *Candida tropicalis*	*Candida krussei* *Candida glabrata* *Candida parasilopsis* *Candida krusei* *Candida albicans*
12	*Lactobacillus fermentus*	*Pedicoccus spp* *Lactobacillus spp*	*Candida glabrata* *Candida albicans* *Candida krusei*	*Candida tropicalis* *Candida glabrata* *Candida krusei* *Candida albicans* *Candida tropicalis*
24	*Lactococcus* spp *Lactobacillus fermentus*	*Leuconostocspp*	*Candida glabrata* *Candida albicans* *Candida tropicalis*	*Candida tropicalis* *Candida kefyr* *Candida krusei*
36	*Lactobacillus casei* *Lactobacillus Plantarum*	*Lactobacillus spp*	*Candida tropicalis* *Candida krusei*	*Candida krusei* *Candida parasilopsis*
48	*Lactobacillus* spp	*Lactobacillus acidophilus* *Lactococcusspp*	*Candida parasilopsis* *Candida tropicalis*	*Candida glabrata* *Candida parasilopsis*

well as different colors. The results of this study revealed that LAB and Yeast are actively involved in the fermentation of ogi. Although some of the identified organisms (*Escherichia coli*, Salmonela species and Klebsiella species) are known to be pathogenic, their presence were eradicated as fermentation period increased. A view supported by Ijabadeniyi (2007) who reported that the presence of Lactobacillus spp in ogi may provide a good source of bacteriocins.

causing oligosaccharides. Also, some researchers reported that Some dominant Lactic acid bacteria and yeasts were detected as shown in Table 5 across all the fermentation period. The lactic acid bacteria identified can be categorised into *Lactobacillus pentosus* strains, *Lactobacillus fermentum* strains, *Pediococcus pentosaceus* strains, *Enterococcus faecium* strains and *Weissella confusa* strains while the dominant yeast detected were *Candida tropicalis* strains, *Candida tropicalis*, *Candida glabrata* strain,

Table 4: Detected predominant lactic acid bacterial and yeast after Sub culturing, DNA, PCR and Sequencing of ogi produced from Maize grains at varying period

S/N	LACTIC ACID BACTERIA	YEAST
1	*Lactobacillus pentosus* strain CE56.28.1	*Candida tropicalis* strain CMC 1978
2	*Lactobacillus pentosus* strain CE56.19	*Candida tropicalis* culture CBS:2313
3	*Lactobacillus plantarum* strain 28.19 E	*Candida tropicalis* strain 36-28B
4	*Lactobacillus plantarum* strain NWAFU1572	*Candida tropicalis* isolate CTR1258
5	*Pediococcus acidilactici* strain AA106	*Candida albicans* strain MGR 9
6	*Pediococcus pentosaceus* strain OO1	*Candida auris* isolate 4356
7	*Lactobacillus fermentum* strain CAU:235	*Candida glabrata* strain DMic 154894
8	*Lactobacillus fermentum* strain YLc37C	*Candida glabrata* isolate Cg107-A
9	*Lactobacillus fermentum* strain BB101	*Pichia kudriavzevii* culture CBS:2065
10	*Enterococcus faecium* strain AA109	*Issatchenkia orientalis* strain M129
11	*Weissella confusa* strain JBA12	
12	*Weissella confusa* partial	

According to Kohajdova & Karovicva (2007), the nutritional effects of Lactic acid bacteria fermentation may have some nutritional impact such as degradation of anti-nutritional factors and increase mineral bio-availability, improvement of protein digestibility of tannin-rich cereals and degradation of flatulence-

Pichia kudriavzevii culture, *Candida albicans* strain and *Issatchenkia orientalis* strain. Previous researches have reported the detection of some of the organisms reported in this study (Omemu, 2011; Omemu *et al.*, 2007; Kogno *et al.*, 2017). Omemu *et al.*(2007), established the significance and influence of yeast and lactic acid bacteria in

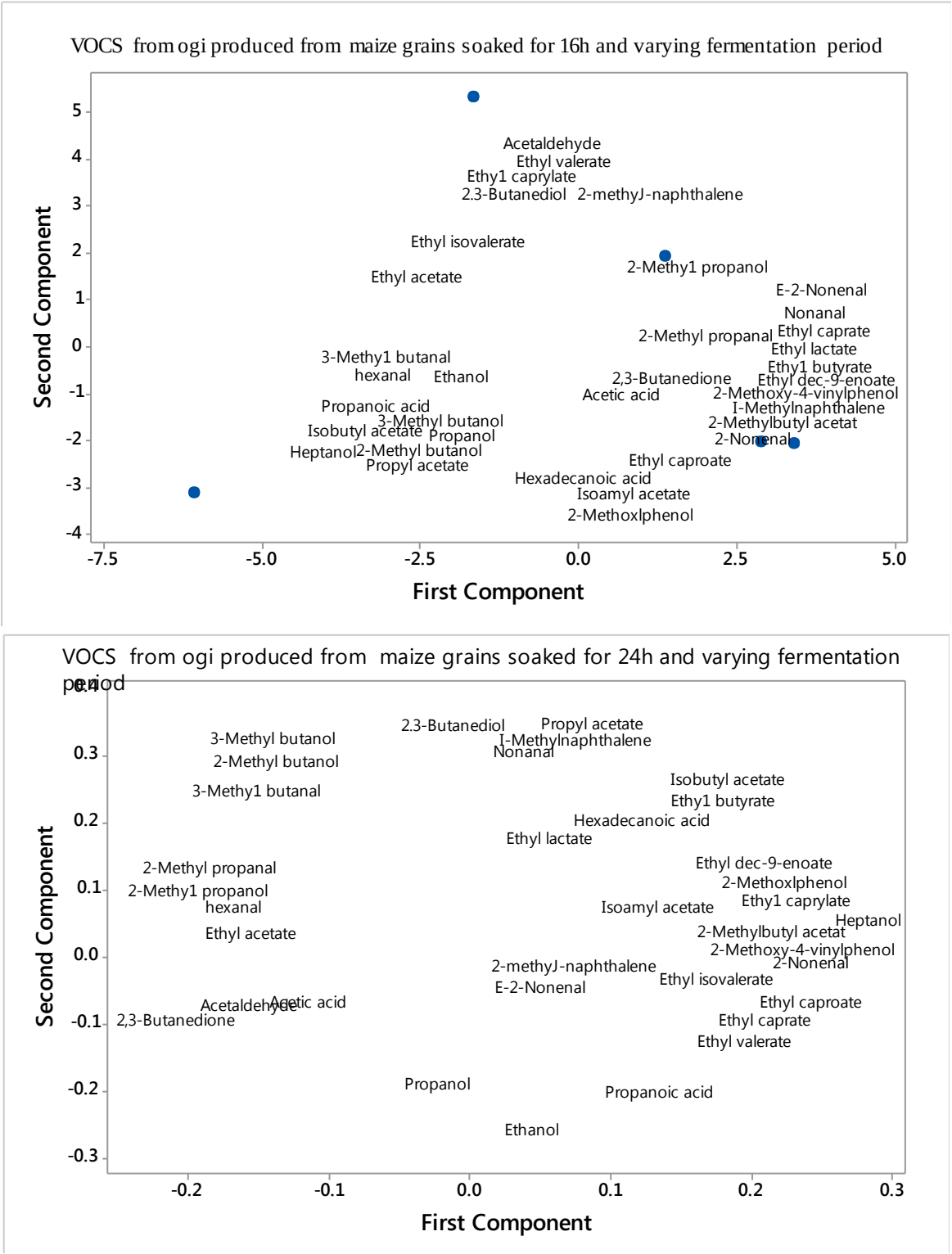

Figure 2: Plot of Correlation coefficients between the first PC_1 and PC_2 for detected volatile compounds of ogi produced from 16 and 24 hours soaking of maize grains.

These graphics are based on the first and second principal components. They represent the linear correlation coefficient between the component and each VOC. On the first component axis, VOCs on the right of the graphics are positively correlated, while VOCs on the left are negatively correlated. For second component axis, VOCs on the top of the graphics are positively correlated, while VOCs on the bottom are negatively correlated. The first PC1 had eigen values of 15.33 (43.80%) and 17.84 (51%) while the second PC had variance of 12.57 (35.90%) and 9.67 (27.60%), respectively.

the fermentation process of ogi. The possible role of microorganisms in the aroma of fermented product, ogi especially, were reported in the literature (Teniola & Odunfa, 2002; Jespersen, 2003). Omemu *et al.* (2007), reported the role of yeasts in the breaking down of starch into simple sugar, making them available to other microorganism to act on. Contrary to lactic acid bacteria and yeast identified in the fermentation of ogi which is in accordance with previous work, -this study provides the first documented evidence of the *Weissella confusa* strain, *Candida glabrata* strain, *Pichia kudriavzevii* culture, and *Issatchenkia orientalis* strain in ogi produced from soaked maize grain at varying fermentation. The active role of *Lactobacillus pentosus* in the fermentation process of cheese texture and addition of flavor through proteolysis and amino acid catabolic systems was reported by Han *et al.* (2013). Pan *et al*. (2013), reported that Lactobacillus pentosus, played significant role in the flavor of yoghurt and production of potential volatile compounds (Ethanol, 2,3-Butanedione and Acetic acid). The influence of Lactobacillus plantarum on volatile compound of cereal based substrate was reported by Salmerona *et al.* (2009). According to Kaseleht *et al*. (2011), volatile compounds differed significantly with varying lactic acid bacteria. For instance, Annan *et al.* (2003) reported, *Lactobacillus fermentum* aided significant production of acetic acid while Houngbédji *et al.* (2018), also reported *Lactobacillus fermentum* and *Pichia kudriavzevii* as the predominant lactic acid bacteria and yeast found during spontaneous fermentation.

According to Houngbédji *et al.* (2018), the type of cereal and processing condition may play vital role in the species of lactic acid bacteria and yeast in a fermentation process. Muyanja *et al.* (2012) stated *Weissella confusa* strains aided higher production of lactate and ethyl alcohol. Franciso *et al.* (2008) reported the effective role of yeast in the production of higher fatty acids and esters and as well adding noticeable aroma and flavor to wine.

Fatty acids and amino acids were implicated as the precursor of many volatile aldehydes (Cheng, 2010). According to the findings of Annan *et al.* (2003), fifty one (51) volatile compounds were detected by GC-sniffing as contributing to the aroma of the different fermented dough samples. It was also reported by these researchers that spontaneously fermented maize was characterized by higher levels of carbonyl compounds, while Lactobacillus fermentum addition recorded the high concentration of acetic acid. The alcohols productions were linked with *Saccharomyces cerevisiae* and increasing levels of esters with fermentation time. According to researched literatures, Ethyl acetate was connected with fruity flavour (Cheng, 2010). There may be possibility of continuous oxidation of ethanol to acetic acid and the esterification of acid in this work as reported to strongly influence the accumulation of ethyl acetate (Apostolopoulou *et al.*, 2005). The production of Ethyl lactate was linked with lactic acid bacteria (Apostolopoulou *et al.*, 2005). Some studies reported that lactic acid bacteria were responsible in acetic acid production through heterofermentation (Annan *et al.*, 2003). According to Borse *et al.* (2007), esters were reported to be present with increased ethanol content during fermentation.

The role of microbial activities in the fermentation of ogi have been reported by some researchers (Ijabadeniyi, 2007; Omemu *et al.*, 2007). According to Ijabadeniyi (2007),

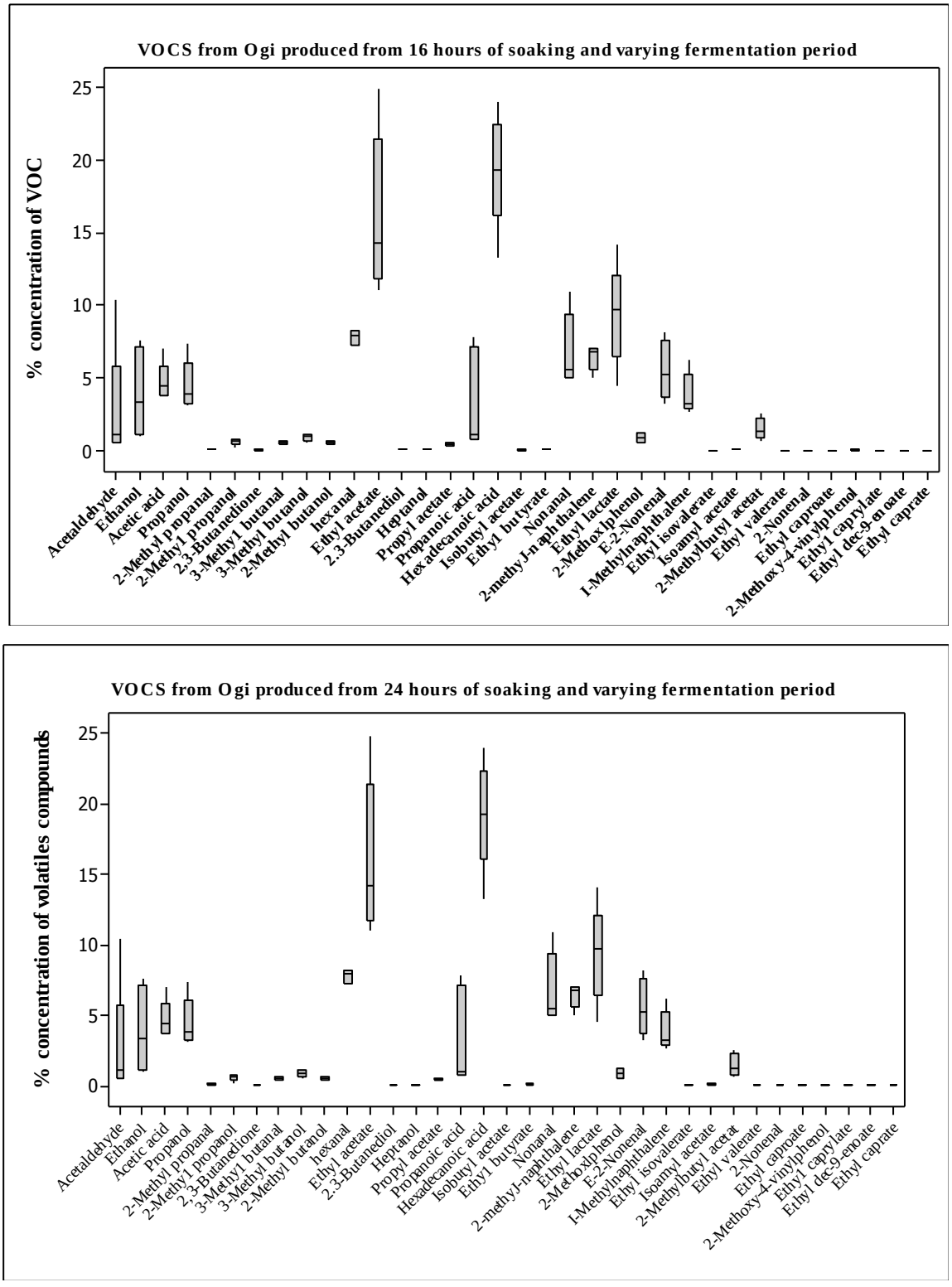

Figure 3: Box loading Plot of volatile compounds with large percent concentration in ogi produced from 16 and 24 hours soaking of maize grains.

As indicated by Box loading Plot, Twelve (12) of the volatile compounds- Acetaldehyde, Ethanol, Acetetic acid, Propanol, Hexanal , Ethyacetate, Hexadecanoic acid, Nonanal, 2-methyl-naphthalene, Ethyl lactate, E-2.nonenal, and I-methylnaphthalene-had noticeable higher and varying concentration at all fermentation period.

Aspergillus niger, Penicillium sp., *Mucor mucedo*, *Rhizopus stolonifer*, Corynebacterium sp., *Lactobacillus plantarum*, *Lactobacillus fermentum*, *Leuconostoc mesenteroides*, Clostridium bifermentans and *Staphylococcus aureus* and a yeast: *Saccharomyces cerevisiae* were isolated and characterized during primary fermentation while only *Lactobacillus plantarum*, *Lactobacillus fermentum* and *Saccharomyces cerevisiae* were isolated during secondary fermentation. Also, Annan *et al.* (2003) reported some esters and alcohols and the role of Lactic acid bacteria in their production. The esters, ethyl acetate, isoamyl acetate, ethyl lactate and alcohols (1-propanol, 2-methyl-propanol and 3-methyl-butanol,) were reported in higher concentrations in maize dough samples compared with values obtained in this work (Annan *et al.*, 2003). It was reported that some alcohols may be responsible for producing desirable flavour (Afoakwa *et al.*, 2008). Increased fermentation time and temperature were reported to contribute significantly to volatile compounds formation in food (Rodriguez-Campos *et al.*, 2012) and this was consistent with findings in this work

4. Conclusion

The fermentation period of ogi had a significant effect than the soaking period of maize grains on the profile of volatile compounds. Thirty five (35) volatile compounds were detected and twelve (12) of them were in higher concentration in the ogi slurry. The principal components showed that few volatile compounds may have contributed significantly to the aroma of ogi produced at varying soaking and fermentation period. These few volatile compounds may have significantly impacted the flavor, aroma and taste of ogi. It was obvious that fermentation process, conditions and the dominant lactic acid bacterial and yeast present greatly influenced the volatile compounds and the flavor of ogi. Weissella confusa strains, Candida glabrata strain, Pichia kudriavzevii culture, and Issatchenkia orientalis strain were identified among other dominant Lactic acid bacterial and yeast present in the ogi contrary to previous work. This study revealed that LAB and yeast are actively involved in the fermentation of Ogi and probably creates its unique aroma and sour taste.

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Conflict of interest

The authors declare that there are not conflicts of interest.

Ethics

This Study does not involve Human or Animal Testing.

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