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Study of the Volatile Compounds and Amino Acid Profile in *Bacillus* Fermented Castor Oil Bean Condiment

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Abstract

Bacillus subtilis was used as a monoculture starter for the production of three different fermented castor oil bean condiments: B_1 (0% NaCl/Lime), B_2 (2% NaCl), B_3 (3% Lime). The volatile components of the three samples were determined using Gas –Chromatography / Mass Spectrometry (GC-MS) while High Performance Liquid Chromatography (HPLC) was used in the study of the amino acid contents. A total of seventeen volatile constituents were identified in the fermented castor oil bean samples using GC-MS. The compounds identified were of various types: acids, esters, alcohols, furans, ketones and others. However, acids were found to be the dominant constituent group followed by esters. Results from the amino acid analysis shows the three fermented castor oil bean samples contained sufficient amount of amino acids. The essential amino acids were in the range of 42.22 – 54.17% for EAA_7 and 63.76 – 73.82% for EAA_{10} of total Free Amino Acids with the most abundant being tryptophan, isoleucine and phenyalanine. Bitter taste was observed as the predominant taste followed by sweet taste and tasteless amino acids. MSG-like taste was slight with B_1:1.624 µg/ml, B_2: 1.069 µg/ml and B_3: 0.881 µg/ml. It is suggested that the desirable taste of *ogiri* from castor oil bean is formed by interactions between the amino acid and volatile components analyzed in this study.

Keywords: *ogiri*, castor bean, volatile compounds, amino acid, *Bacillus subtilis*

1. Introduction

Ogiri is a traditionally fermented castor oil bean condiment which is widely consumed in eastern part of Nigeria as a protein-rich meat substitute. It is a product of alkaline fermentation of castor oil bean (*Ricinus communis*) which is similar in sensory attributes to African locust bean (*Parkia biglobosa*) daddawa (Ogabdu and Okagbue, 1988; Omafuvbe et al., 2002; Omafuvbe, 2006). *Ogiri* can also be produced from melon seeds (Ogueke & Nwagwu, 2007; David & Aderibigbe, 2010) and fluted pumpkin, *Telfaria occidentalis*, (Odibo et al., 1990; Omafuvbe & Oyedapo, 2000).

Obizoba and Atti (1991) studied the chemical properties of fluted pumpkin, as the mostly used food condiments in some parts of Nigeria. Mixed cultures of microbes are usually present in traditionally fermented *ogiri* condiment. It has been reported that bacteria in the genus *Bacillus* are responsible for the fermentation of these alkaline fermented food condiments. (Dajanta et al., 2011; Omafuvbe, 2006). Some studies have reported different *Bacillus* species (*B. subtilis, B. pumilus, B. brevis, B. macerana, B. polymyxa and B. licheniformis*) as being isolated from daddawa, kinema, thua nao and chungkukjang (Sarkar & Tamang, 1995; Omafuvbe Oyedapo, 2000; Chantawannakul et al., 2002; Lee et al., 2005; Chukeatirote et al., 2006). Of these *Bacillus* species, only *B. subtilis* is a monoculture starter that produces same sensory attributes with the traditionally fermented *ogiri* from castor oil bean seeds.

It is well known that proteolysis is the most principal and complex biochemical event occurring during the preparation of some legume based fermented condiments. The degradation products, amino acids, not only have a considerable influence on the nutritional values, but also contribute directly to the taste characteristics, in some cases serving indirectly as precursors of aromatic products (Kiers et al., 2000; Han et al., 2004). It is well established that the water-soluble fraction contains the majority of taste compounds such as salt and amino acids

produced during proteolysis (Kim & Lee, 2003). Consequently, quality indexes such as free amino acids of many legume based fermented foods have been reported (Omafuvbe et al., 2000; Kim & Lee, 2003; Han et al., 2004).

In the study of volatile constituents in some traditionally fermented condiments like *ogiri* from melon seeds and *daddawa* from locust bean and soybean, the identified compounds were aldehydes, alcohols, ketones, esters, pyrazines, alkanoic acids, alkanes, alkenes and others with aldehydes being the predominant group (Onyenekwe et al., 2012). Some volatile compounds in some Benninese condiments have also been studied (Azokpota et al., 2010, 2008). Ouoba et al. (2005) determined the profile of volatile compounds responsible for the aroma of soumbala produced spontaneously with pure and mixed cultures of *B.subtilis* and *B.pumilus*. The compounds identified were pyrazines, aldehydes, ketones, esters, alcohols, acids, alkanes, alkenes, amines, pyridines, benzenes, phenols, sulphurs, furans and other compounds. Parkouda et al. (2011) also studied the volatile compounds associated with baobab seeds fermentation for maari production and a total of 96 compounds were identified including esters, acids, alcohols and ketones.

Volatile compounds of *Bacillus* fermented soybeans have been studied (Leejeerajumnean et al., 2001). The major volatile compounds in the soybeans were 3-hydroxybutane (acetoin), 2-methylbutanoic acid, pyrazines, dimethyly disulphide and 2-pentylfuran. They reported that the natto samples were devoid of aldehydes, aliphatic acids, esters and sulphur compounds whereas the thua nao samples contained a diversity of those volatile compounds (Leejeerajumnean et al., 2001).

Addition of 0-3% salt and 0-3% lime in the traditional fermentation of castor oil bean into ogiri have been reported to improve the organoleptic and physicochemical properties of the product (Ojimelukwe et al., 2011). This present study aims to quantify the volatile compounds and amino acid contents in fermented castor oil bean samples produced using *B.subtilis* as starter culture and its impact on their flavour and aroma.

2. Materials and Methods

2.1 Collection of Samples

The castor bean seeds *(Ricinus communis)* used in this research was purchased from New Aba market in Abia State, Nigeria. All reagents and media used in the research work were obtained from major commercial suppliers and they were all of analytical grade.

2.2 Organism

B.subtilis used as starter culture was previously isolated from traditional fermenting castor oil bean, *ogiri* and was maintained on nutrient agar slope in the refrigerator prior to use.

2.3 Preparation of 'Ogiri' from Ricinus Communis Using Starter Culture

The laboratory fermentation of castor bean was done using the method of Enujiugha (2009). Approximately 1 kg of castor bean seeds were cleaned and sorted to remove defective seeds and contaminants. The cleaned seeds were boiled for eight hours, dehulled, drained and boiled again for two hours. The boiled seeds were soaked for twelve hours, drained and mashed into *ogiri* paste and kept refrigerated for 24 h before addition of the starter culture. One hundred (100) g wet weight portions were put in sterile I L beakers each in three different portions. To one portion was added 2% NaCl, to another 3% lime while to the third portion, no additive was added. The three samples were sterilized in the autoclave at 121^{o}C for 15 minutes. The three portions were then inoculated with 2 ml each of the broth cultures of the *B. subtilis* isolate. The beakers were then covered with aluminum foil and kept in the incubator to ferment for 96h.

2.4 GC-MS Analysis of the Fermented Castor Oil Bean Samples

GC-MS analysis was carried out on a GC Clarus 500 Perkin Elmer system comprising a AOC-20i autosampler and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: column Elite-1 fused silica capillary column (30 x 0.25 mm ID x 1 µM df, composed of 100% Dimethylpolysiloxane), operating in electron impact mode at 70 eV; helium (99.999%) was used as carrier gas at a constant flow of 1 ml /min and an injection volume of 0.5 µI was employed (split ratio of 10:1) injector temperature 250ºC; ion-source temperature 280ºC. The oven temperature was programmed from 110ºC (isothermal for 2 min), with an increase of 10ºC/min, to 200ºC, then 5ıC/min to 280ºC, ending with a 9 min (isothermal at 280ºC). Mass spectra were taken at 70 eV; a scan interval of 0.5 seconds and fragments from 40 to 450 Da. Total GC running time of 36min.

2.5 Identification of Components

Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared

with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

2.6 Determination of Amino Acids in Fermenting Castor Bean using HPLC Waters Model 616/626

High Performance Liquid Chromatography (HPLC Waters Model 616/626) was used for the determination of the amino acid profile of fermenting castor bean samples. The sample preparation and determination were carried out in the following stages:

(i) Hydrolysis

(ii) Derivatisation

(iii) Separation of the derivatised amino acids

(iv) Data processing/interpretation and calculations of the final results

Step 1: Hydrolysis of the samples

A quantity of 0.5 g of the samples was weighed into a sterile furnace hydrolysis tube. 5 nmoles of the internal standard norleucine was added to the samples and then dried under a vacuum. The sample was placed in a vial containing 10.05 N HCl with a small quantity of phenol, thereby hydrolyzing the protein by the HCl vapours under vacuum. This stage of hydrolysis of the sample lasted between 20-23 hours at 108^{o}C. After the hydrolysis, samples were dissolved in ultra-pure water (HPLC grade) containing ethylene diamine tetraacetic acid (EDTA). The EDTA chelates the metals present in the samples. The hydrolysed samples were stored in HPLC amino acid analyzer bottles for further analytical operations.

Step 2: Derivatization

The hyrolysed samples were derivatised automatically on the Waters 616/626 HPLC by reacting the amino acids, under basic situations with phenylisothiocyanate (i.e. PITC) to get phenylthiocarbamyl (PTC) amino acid derivatives. The duration for this reaction was 45 minutes per sample, as calibrated on the instrument. A set of standard solutions of the amino acids were prepared from Pierce Reference standards H (1000 umol) into auto-sampler cups and they were also derivatised.

These standards (0.0, 0.5, 1.0, 1.5, 2.0 µmol) were used to generate a calibration file that was used to determine the amino acid contents of the samples. After the derivatisation, a methanol solution (1.5N) containing the PTC-amino acids were transferred to a narrow bore (Waters 616/626) HPLC system for separation.

Step 3: The HPLC separation & Quantization

The separation and identification of amino acids were done in reverse phase C18 silica column and the analytes were detected at the wavelength of 254 nm. The elution of the whole amino acids in the samples took 30 minutes. The buffer system used for separation was 140 mM sodium acetate pH 5.50 as buffer A and 80% acetonitrile as buffer B. The program was run using a gradient of buffer A and buffer B concentration and ending with a 55% buffer B concentration at the end of the gradient.

Step 4: Data interpretation and calculations

The intensity of the chromatographic peaks areas were automatically and digitally identified and quantified using a Dionex chromeleon data analysis system which was attached to the waters 616/626 HPLC System. The calibration curve or file prepared from the average values of the retention times (in minutes) and areas (in Au) of the amino acids in 5 standards runs was used. Since a known amount of each amino acid in the standard loaded into the HPLC, a response factor (Au/pmol) was calculated by NAP 2 software that was inter-phased with the HPLC. This response factor was used to calculate the amount of each of the amino acid (in pmols) in the sample.

The amount of each amino acid in the sample was finally calculated by the software by dividing the intensity of the peak area of each (corrected for the differing molar absorptivities of the various amino acids) by the internal standard in the chromatogram and multiplying this by the total amount of internal standard added to the original sample.

After the picomole by the intensity of the height of each amino acid has been ascertained by the software, the data, the digital chromatographic software extrapolate back to 5 nmoles of the Internal standard (Norleucine), and displays for the total amount that was pipetted into the hydrolysis tube at the beginning of the analysis as follows:

Calculation:

$$\text{mg/ml (in Extract)} = \text{Dilution factor} \times \text{Peakheight intensity}$$

$$\frac{\text{mg}}{\text{ml}}(\text{in sample}) = \frac{\mu \frac{\text{g}}{\text{ml}} \text{ in sample} \times \text{sample volumn}}{\text{Wt. of sample}}$$

2.7 Free Amino Acid Grouping

Amino acids were grouped according to the taste characteristic as described by Tseng et al. (2005).

2.8 Statistical Analysis

Statistical analysis was done for each set of data obtained following the procedures of Steel and Torie (1984) for a Factorial Randomized Complete Block Design (Factorial RCBD) while GENSTAT discovery package (2006 edition) was used for the analysis of the data. Comparison of treatment means and significant differences between treatment means separated using Fisher's Least Significant Difference (LSD) as outlined by Gomez and Gomez (1984). Data from the contents of free amino acid group based on taste characteristics of *ogiri* samples were expressed as means $\pm$ standard deviations of triplicate observations. Analysis of variance was also performed by Duncan's multiple range test using the SPSS software.

3. Results and Discussion

3.1 Volatile Compounds in Fermented Castor Oil Bean Condiment Samples

The volatile compounds of the three fermented castor oil bean condiment samples analyzed using GC-MS are shown in Tables 1 and 2. The volatiles were identified quantitatively by comparing their retention time and mass spectra with the known components stored in the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The GC-MS chromatograms of the volatile constituents in fermented castor oil bean samples are displayed in Figure 1.

A total of seventeen volatile constituents were identified in the fermented castor oil bean condiment samples, while nine were identified in sample B_1 followed by eight each for samples B_2 and B_1. The seventeen volatiles are of various types; acids, esters, alcohol, furan, ketone and others (Table 1). Acids are the dominant constituent group from the result shown in Table 1 and constitute over 50% of total volatiles in the fermented castor oil bean condiment samples. This is contrary to the findings of Azokpota et al. (2008) who found pyrazines as the dominant constituent group in *afitin, iru* and *sonru* (three fermented soybean food condiments from Benin) and Dajanta et al. (2011) in thua nao, a Thai fermented soy product. Onyenekwe et al. (2012) reported aldehydes as the dominant volatile compound in three fermented food flavour enhncers; melon seed *ogiri*, soybean *daddawa* and locust bean *daddawa*. The major carboxylic acids in sample B_1 was found to be butanoic acid, 3-methyl, n-Hexadecanoic acids, oleic acid and ricinoleic acids. Among the three samples, a total of eight carboxylic acids were identified. n-Hexadecanoic acids were found in samples B_1 and B_3 at the same retention time of 21.158 minutes with concentration values 19.64 and 20.18 respectively. However, butanoic acid, 2-methyl, pentanoic acid, 4-methyl and oleic acids were found in the samples but at different retention times. Butanoic acid and 3-methylbutanoic acid, compounds with a sweaty odour have been determined as major aroma compounds in Korean soy sauces and barley bran sauces (Lee et al., 2006; Choi et al., 2007; Steinhaus & Schieberle, 2007)

Table 1. Percent composition of the different volatile compounds identified in the fermented castor oil bean condiment samples

		Samples		
S/N	**Compounds**	**B_1**	**B_2**	**B_3**
1	Esters	2.87	3.01	22.37
2	Alcohol	5.81	0.0	0.0
3	Furan	0.0	1.29	0.0
4	Acids	58.88	89.19	49.41
5	Ketone	0.0	0.0	12.49
6	Others	32.43	6.50	15.72

Where B_1: 0% NaCl/lime, B_2: 2% NaCl, B_3: 3% Lime.

Table 2. Concentrations of volatile compounds in fermented castor oil bean condiments

				Area	Normalized (%)	
s/n	**Retention time (mins)**	**Compound**	**Formula**	**B_1**	**B_2**	**B_3**
		Acids				
1	3.200	Propanoic acid, 2-methyl	$C_4H_8O_2$	-	-	2.67
2	3.867	Butanoic acid, 3-methyl	$C_5H_{10}O_2$	-	39.56	-
3	4.008	Butanoic acid, 2-methyl	$C_5H_{10}O_2$	4.45	-	-
4	4.067	Butanoic acid, 2-methyl	$C_5H_{10}O_2$	-	-	11.20
5	5.292	Pentanoic acid,4-methyl	$C_6H_{12}O_2$	-	5.39	-
6	5.392	Pentanoic acid, 4-methyl	$C_6H_{12}O_2$	-	-	15.36
7	9.167	Benzenecarboxylic acid	$C_7H_6O_2$	-	4.96	-
8	21.158	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	-	19.64	20.18
9	21.175	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	17.14	-	-
10	22.667	Oleic acid	$C_{18}H_{34}O_2$	22.07	-	-
11	22.683	Oleic acid	$C_{18}H_{34}O_2$	-	19.64	-
12	24.250	Ricinoleic acid	$C_{18}H_{34}O_3$	15.22	-	-
		Esters				
13	12.292	4-nitrosophenyl-beta-phenylpropionate	$C_{15}H_{13}NO_3$	2.87	-	18.90
14	18.208	Cyclohexanecarboxylic acid,4-octyl ester	$C_{15}H_{28}O_2$	-	-	3.47
15	18.217	Cyclohexanecarboxylic acid,4-octyl ester	$C_{15}H_{28}O_2$	-	3.01	-
		Alcohol				
16	6.308	Glycerin	$C_3H_8O_3$	5.81	-	-
		Furan				
17	6.700	2(3H)- Furanone, dihydro-3-hydroxy-4,4-dimethyl	$C_6H_{10}O_3$	-	1.29	-
		Ketone				
18	22.692	Oxacyclotetradecan-2-one	$C_{13}H_{24}O_2$	-	-	12.49
		Others				
19	7.533	2-pyrrolidinone	C_4H_7NO	7.62	-	-
20	9.275	2-piperidinone	C_5H_9NO			15.72
21	9.283	2-piperidinone	C_5H_9NO	18.04	-	
22	10.517	3-oxo-4-phenylbutyronitrile	$C_{10}H_9NO$	-	6.50	-
23	25.883	9-octadecenamide	$C_{18}H_{35}NO$	6.77	-	-

Where B_1: 0% NaCl/lime, B_2: 2% NaCl, B_3: 3% Lime.

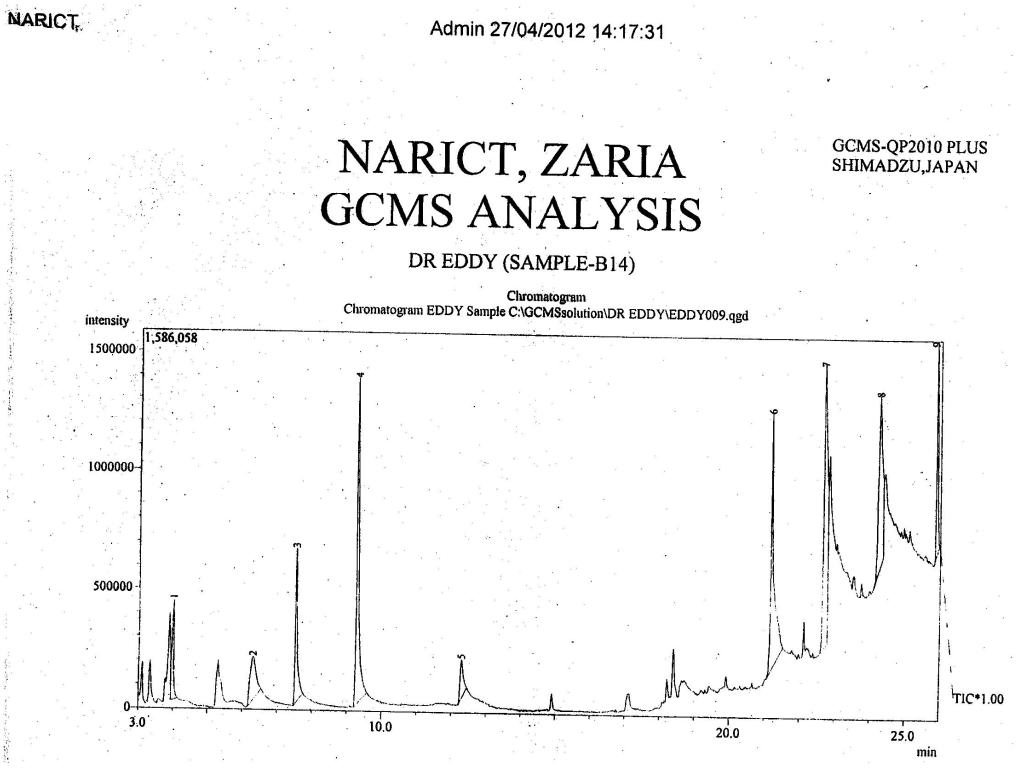
Esters constitute another major compounds of samples B_1 and B_3 (Table 1). The compound 4-nitrosophenyl-beta-phenylpropionate was found in samples B_1 and B_3 at the same retention time of 12.292 minutes while cyclohexanecarboxylic acid,4-octyl esters were found at different retention times in samples B_2 and B_3 having concentration of 3.01 and 3.47 respectively. Esters are mostly formed from the esterification of alcohols with fatty acids during the fermentation process (Sluis et al., 2001) many of which have attractive aroma. The esters are presumably the consequence of chemical reactions between microbial acidic and alcoholic metabolites (Leejeerajumnean et al., 2001), though it is also possible that the reactions may be catalysed by microbial esterases (Eskin, 1990). The importance of ester contributions toward food aroma is undisputed with

the fact that esters with low carbon atoms are highly volatile at ambient temperatures and the perception thresholds are ten times lower than their alcohol precursors (Izco & Torre, 2000; Nogueira et al., 2005). In addition to imparting a fruity floral character, esters can diminish or mask the sharpness of unpleasant FFA-derived notes. These esters are formed by esterification between the short-chain FFAs and the alcohols (Qin & Ding, 2007). Ester, mainly formed by esterification of carboxylic acids and alcohols was reported to determine the characteristic pleasant aromatic notes (Rodriguez-Bernaldo De Quiros et al., 2000; Klesk & Qian, 2003).

Furans have been reported as aroma constituents in some Asian sauces. Samples B_3 showed concentration of 1.29 of 2(3H)-Furanone, dihydro-3-hydroxy-4,-4-dimethyl. The compounds 4-HDMF (4-hydroxy-2,5-dimethyl-3(2H)-furanone) and 4-HEMF (4-hydroxy-2-ethyl-5-methyl-3(2H)-furanone) have been suggested as two key aroma constituents in Korean soy sauces, Japanese soy sauces and Japanese barley miso (Hayashida et al., 1998; Baek & Kim, 2004; Steinhaus & Schieberle, 2007).

Tables 1 and 2 show that ketones are also contained in sample B_3 with concentration 12.49. Ketones are usually derived from lipids and amino acids degradation during microbial fermentation and have high impact in food odour. Ketones contribute to the odour of the fermented castor oil bean condiment sample B_3. This is consistent with the work of Stephen and Steinhist (1999) and Dajanta et al. (2011). Table 2 shows the alcoholic constituent of sample B_1 with glycerin as the major alcoholic compound. Alcohol present in sample B_1 help prevent them from spoilage since alcohol are known to act as antifungal and prevent food spoilage (Onyenekwe et al., 2012).

Other volatile compounds that were identified were 2-pyrolidinone, 2-piperidinone, 3-oxo-4-phenylbutyronitrile and 9-octadecenamide. The compound 2-pyrrolidinone which is a nitrogen-containing compound was detected in sample B_1. It has been reported in Thai soy sauces (Wanakhachornkrai & Lertsiri, 2003) and Chinese soy sauces (Shu et al., 2010) as one of the volatile compounds that contribute to their aroma. These results support earlier studies that show the diversity of aroma volatile compounds in fermented food condiments such as Burkinabe soumbala, Ghanaian soy-dawadawa, Beninese *afitin, iru* and *sonru*, Japanese natto, Korean chungkukjang and Thai thua nao (Tanaka et al., 1998; Leejeerajumnean et al., 2001; Dakwa et al., 2005; Ouoba et al., 2005; Azokpota et al., 2008, 2010; Parkouda et al., 2011). For soumbala, Ouoba et al. (2005) revealed the presence of pyrazines, aldehydes, ketones, esters, alcohols, acids, alkanes, alkenes, benzenes, phenols, sulphurs, furans, pyridines and amines. Amines and pyridines were not found in *afitin, iru* and *sonru* (Azokpota et al., 2008). In natto and thua nao, ketones, acids and pyrazines were reported to be the major volatile compounds (Owens et al., 1997).



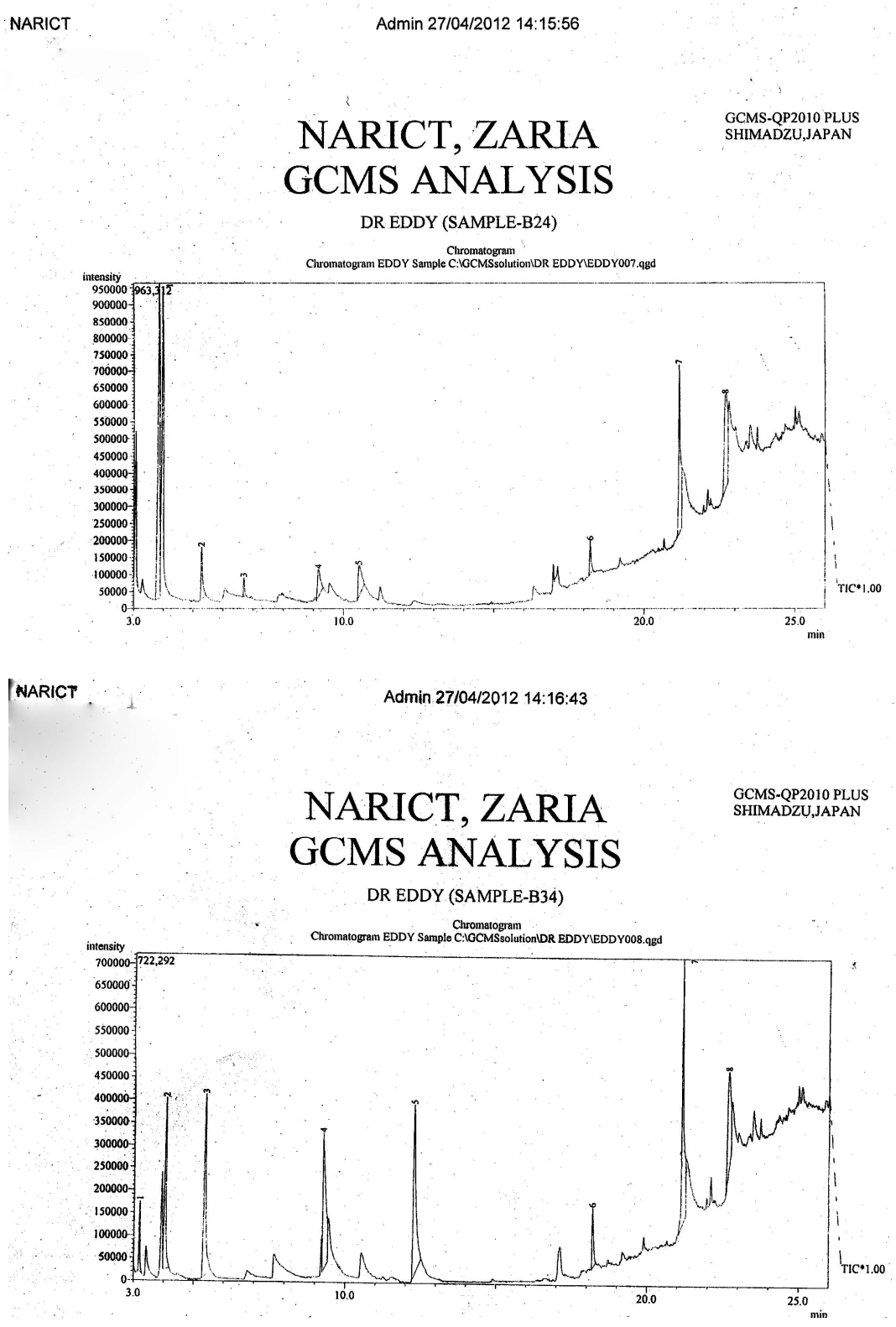


Figure 1. Chromatograms of the volatile compounds in fermented castor bean samples

3.2 Amino Acid Profiles in Fermented Castor Oil Bean Samples

Table 3. Free amino acid content of fermented castor bean products, (ogiri) at 96h fermentation (μg/ml wet sample)

	OGIRI	**SAMPLES**	
FAA	**B_1**	**B_2**	**B_3**
Lysine	0.725 ± 0.03^{a}	0.501 ± 0.01^{b}	0.377 ± 0.02^{c}
Argentine	0.739 ± 0.01^{a}	0.522 ± 0.04^{b}	0.409 ± 0.00^{c}
Histidine	1.005 ± 0.01^{a}	0.741 ± 0.07^{b}	0.532 ± 0.02^{c}
Methionine	1.098 ± 0.02^{a}	0.876 ± 0.05^{b}	0.619 ± 0.04^{c}
Isoleucine	1.382 ± 0.04^{a}	1.017 ± 0.08^{b}	0.856 ± 0.01^{c}
Tryptophan	1.778 ± 0.04^{a}	1.281 ± 0.07^{b}	0.997 ± 0.45^{c}
Threonine	0.861 ± 0.06^{a}	0.639 ± 0.07^{b}	0.498 ± 0.05^{c}
Proline	0.756 ± 0.04^{a}	0.519 ± 0.01^{b}	0.396 ± 0.01^{c}
Glutamine	0.442 ± 0.04^{a}	0.313 ± 0.01^{ab}	0.256 ± 0.13^{b}
Asparagine	0.324 ± 0.01^{a}	0.211 ± 0.01^{b}	0.203 ± 0.01^{b}
Glutamic acid	0.998 ± 0.10^{a}	0.578 ± 0.01^{b}	0.486 ± 0.03^{b}
Valine	0.996 ± 0.00^{a}	0.841 ± 0.00^{b}	0.673 ± 0.10^{c}
Phenylamine	1.503 ± 0.00^{a}	1.169 ± 0.43^{a}	0.988 ± 0.08^{a}
Aspartic acid	0.628 ± 0.04^{a}	0.491 ± 0.03^{b}	0.396 ± 0.01^{c}
Serine	0.508 ± 0.01^{a}	0.315 ± 0.01^{b}	0.192 ± 0.01^{c}
Glycine	0.814 ± 0.01^{a}	0.528 ± 0.03^{b}	0.448 ± 0.07^{b}
Tyrosine	0.598 ± 0.04^{a}	0.399 ± 0.04^{b}	0.215 ± 0.01^{c}
Alanine	0.433 ± 0.01^{a}	0.258 ± 0.04^{b}	0.189 ± 0.01^{c}
Cystine	0.571 ± 0.14^{a}	0.432 ± 0.08^{ab}	0.332 ± 0.01^{b}
Leucine	0.205 ± 0.01^{a}	0.915 ± 0.05^{b}	0.798 ± 0.00^{c}
*EAA7	6.908 ± 0.22^{a}	6.575 ± 1.36^{a}	5.341 ± 0.85^{a}
**EAA10	10.432 ± 0.18^{a}	9.129 ± 1.40^{ab}	7.279 ± 0.85^{b}

*EAA, essential amino acids were calculated according to the method of Lee *et al*. (1978); EAA7: Val+Leu+ le+Thr+Lys+Phe+Met;

**EAA10:EAA7+His+Arg+ Trp;

Where B_1=0%NaCl/Lime *Ogiri*; B_2= 2%NaCl *Ogiri*; B_3= 3% Lime *Ogiri*;

Data in the same row with different letters are significantly different ($p<0.05$).

Table 3 summarizes the profiles of free amino acid (FAA) in fermented castor seed samples "*ogiri"* after 96h fermentation. The amino acid with the highest content was found to be tryptophan and it occurred in all the samples. For sample B_1 (0% NaCl/Lime), isoleucine, phenylalanine, methionine, histidine and glutamic acid were found in higher quantities while for sample B_2 (containing 2% NaCl), isoleucine, phenylalanine, leucine were some of the abundant amino acid reported. In sample B_3 (containing 3% lime), phenylalanine, isoleucine and leucine were found to be abundant. However, the amino acid contents differed in all the samples with sample B_1 having higher contents of amino acid followed by B_2. Also observed in the compositions and quantities of the free amino acid in the fermented castor products are that the asparagine, glutamine and glycine contents in B_1 differed significantly from those of B_2 and B_3. Anosike and Egwuatu (1981) reported the presence of phenylalanine, tryptophan, tyrosine, serine, cysteine, glutamic acid and glutamine in traditional fermented castor seeds.

Table 3 also shows the profiles of essential amino acids (EAA7) and (EAA10). All the three fermented castor bean samples contained sufficient amounts of all FAA. The free amino acids can be classified in groups of either seven or ten amino acids (Lee et al., 1978). The EAA profiles of the fermented castor samples were in the range of 42.22 – 54.17% for EAA7 and 63.76 – 73.82% for EAA10 of total FAA with the most abundant being tryptophan followed by isoleucine and phenylalanine.

Apart from the flavouring attributes of some oil seed legumes, they contribute significantly to the intake of proteins, essential amino acids, fatty acids and B.vitamins particularly riboflavin (Fetuga et al., 1973; Campbell-Platt, 1980; Odunfa, 1983; Gernah et al., 2007). Odunfa (1983) and Sarkar et al. (1993) also reported that during fermentation, the inedible legumes are made edible by extensive hydrolysis of nitrogenous compounds into amino acids. Thus, the increase in percentage protein could be due to the release of proteins from carbohydrate matrix as well as the generation of free amino acids by proteolytic enzymes which increase the free measurable nitrogen in the system. Low protein intake or total absence has negative effect on body well being (Akobundu, 2008). Short-term protein deprivation has been shown to cause fine structural changes of the rat liver (Akobundu & Al-Bagdadi, 1982).

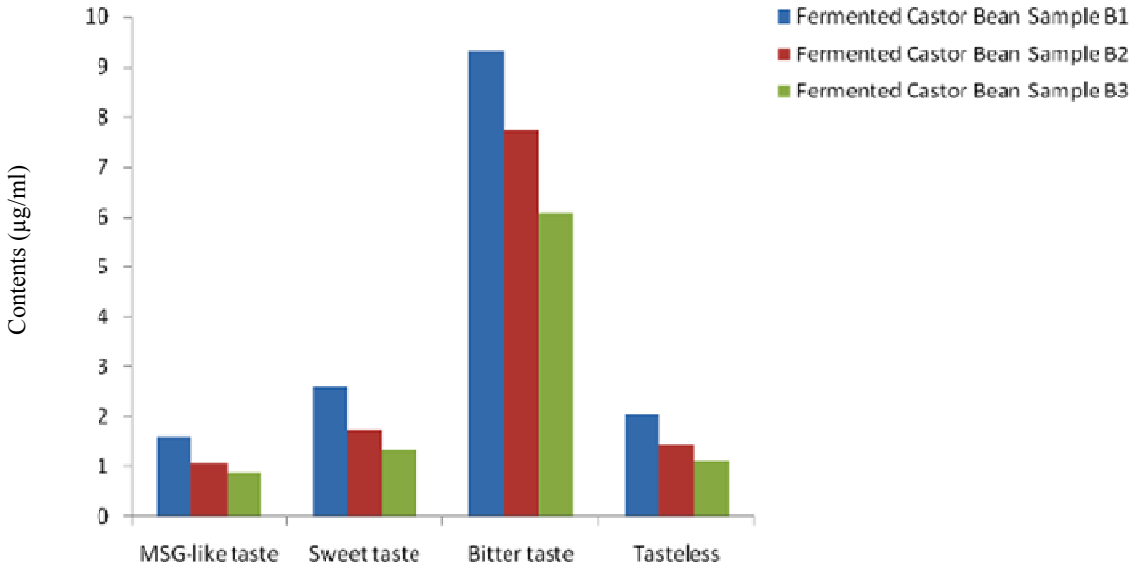


Figure 2. Free amino acid contents based on taste characteristics of fermented castor oil bean

The results in Figure 2 shows the free amino acid groupings based on their taste characteristics as described by Tseng et al. (2005). The most abundant tasty FAA class of the *ogiri* samples was the bitter FAA. This was observed in all the samples with sample B_1 having the highest content of 9.304 µg/ml followed by samples B_2 and B_3 with 7.761 µg/ml and 6.087 µg/ml respectively. Qin and Ding (2007) reported high concentrations of the bitter FAA class in Chinese douchina especially at ripening state. Also Dajanta et al. (2011) reported similar findings in thua nao, soy-fermented condiment of Thai origin.

The bitter taste was then followed by sweet taste and tasteless amino acid. The MSG-like free amino acids in the three samples were slight with B_1 having 1.624 µg/ml followed by B_2 with 1.069 µg/ml and B3 0.881 µg/ml. The 2% NaCl and 3% lime additives added to samples B_2 and B_3 affected the level of the MSG-like taste since sample B_1 had higher content. The bitter free amino acid was found much higher than MSG-like and sweet free amino acids in fermented castor bean samples. It has been known that enzymatic hydrolysis of proteins frequently leads to the production of a bitter taste due to the presence of strongly hydrophobic bitter peptides arising as natural degradation products of the proteolytic reaction (Kukman et al., 1995). The phenomenon may be explained also by the fact that the final characteristic taste of *ogiri* was definitely determined by the balance and interaction between different taste components (Yanfang & Wenyi, 2009). The change of unpleasant bitterness, derived from bitter free amino acids, may be attributed to the diminishing or masking effect of saltiness, umami taste, sourness and sweetness (Kim & Lee, 2003). The final characteristic taste of the fermented

castor bean samples, *ogiri*, could be determined also by the balance and interaction among the different taste components which corroborates with the observation of Qin and Ding (2007).

4. Conclusion

The volatile compounds identified have strong impact on the flavour and aroma of the fermented castor oil bean samples. The different *ogiri* samples in this study were found to be good protein sources based on the profiles of the amino acids. The addition of 2% NaCl and 3% lime did not affect the quality of *ogiri* from *Bacillus* fermented castor oil bean. The free amino acids detected in the samples are also important contributors to the taste of the *ogiri* samples. From the results the final characteristic taste of *ogiri* depends on the balance and interaction between the different taste components analyzed.

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