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Effect of Fermentation Period on the Organic Acid and Amino Acid Contents of Ogiri from *Ricinus communis*

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Abstract: In this study, ogiri, a Nigerian fermented food condiment was prepared from castor oil bean using *Bacillus subtilis* as a monoculture starter for the production of three different fermented castor oil bean condiment samples: B_1 (0% NaCl/Lime), B_2 (2% NaCl), B_3 (3% Lime). Variations in the composition of the castor oil bean with fermentation >96 h period were evaluated for organic acids and amino acids using high performance liquid chromatography. Organic acids were detected in the fermented castor oil bean samples as fermentation period increased to 96 h. At 96 h fermentation higher values were recorded in most of the samples. Organic acids identified were oxalic, citric, tartaric, malic, succinic, lactic, formic, acetic, propionic and butyric acids. The production of these organic acids are undoubtedly the determining factor in which the shelf-life and safety of the final production depends while the inhibition of pathogenic and spoilage flora is also dependent on a rapid and adequate formation of these organic acids. The three fermented castor oil bean samples also contained sufficient amount of amino acids. Sample B_1 had the highest values in isoleucine, glycine and histidine while sample B_2 had the highest value in leucine content with 0.915 μg mL^{-1} at 96 h fermentation, closely followed by sample B_3 and B_1 with 0.798 and 0.205 μg mL^{-1}, respectively. The results of amino acid analysis indicated a high concentration of all amino acids at 96 h of fermentation which contributes to the flavour and aroma of the ogiri condiment.

Key words: Castor bean, ogiri, organic acid, amino acid, leucine, Nigeria

INTRODUCTION

Fermentation is one of the oldest methods of food preservation known to man. In Africa, the art of fermentation is widespread including the processing of fruits and other carbohydrate sources (Adewusi *et al*., 1991). Oil seeds such as African locust bean, melon seed, mesquite bean and soybean are also fermented to give condiments (Omafuvbe *et al*., 2004). The production of condiments is largely done on a traditional small-scale household basis under highly variable conditions (Odunfa, 1983). Condiments are known to contribute to the calorie and protein intake and are generously added to soups as low cost meat substitute by low-income families in parts of Nigeria (Eka, 1980).

Many proteinaceous seeds are fermented to make food condiments in West Africa. The seeds of African locust bean (*Parkia biglobosa*) are fermented to produce dawadawa or iru in Ghana and Nigeria, respectively. The seeds of melon (*Citrullus vulgaris*) are fermented to produce ogiri in Nigeria and Sierra Leone (Odunfa, 1985).

Fermentation markedly improves digestibility, nutritive value and flavour of raw seeds or the seeds cannot be consumed in their raw state. In Africa, fermented foods play a major role in the diet whereby many staple foods undergo fermentation before they are consumed (Kpikpi *et al*., 2009). Fermented food condiments enhance flavour and improve the protein content, essential amino acids and fatty acids of foods. Fermented foods contribute to food security in developing countries like Nigeria.

Organic acids occur in fermented products as a result of hydrolysis, biochemical metabolism and microbial activity (Andersson and Hedlund, 1983). In almost all fermentation involving *Bacillus* species, there is production of primary organic acids especially butyric acid (Moat, 1979). Organic acids affect stability, colour and flavour of the final product but their greatest role comes from their ability to stop or at least retard the growth of many potentially harmful microorganisms that cause spoilage in food products (Zotou *et al*., 2004). This

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study reports on the organic acid and amino acid content of the castor oil bean mash as fermentation period increases.

MATERIALS AND METHODS

The castor bean seeds *(Ricinus communis)* used in this research were purchased from a local market in Aba, Abia State, Nigeria.

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

Preparation of Ogiri from *Ricinus communis* using starter culture: The controlled fermentation of castor oil bean into ogiri was done using the method of Enujiugha (2009).

Determination of amino acids in fermenting castor bean using waters 616/626 HPLC: High Performance Liquid Chromatography (HPLC Water 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:

- Hydrolysis
- Derivatisation
- Separation of the derivatised amino acids
- 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. About 5 nmoles of the internal standard norleucine was added to the samples and then dried under a vacuum. The tube was again 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 h at 108°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 water 616/626 HPLC by reacting the five amino acids under basic situations with phenylisothiocyanate (i.e., PITC) to get Phenylthiocarbamyl (PTC) amino acid derivatives. The duration for this reaction was 45 min 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 umol) 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 (1.5 N) containing the PTC-amino acids were transferred to a narrow bore (Waters 616/626) HPLC System for separation.

Step 3 (The HPLC separation and quantization): The separation and quantization of the PTC-amino acids were done in reverse phase (18 silica column and the PTC chromophone were automatically and digitally detected at the wavelength of 254 nm. The elution of the whole amino acids in the samples took 30 min. 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 min) 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 and displayed on the system digitally.

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:

$$\text{Extract (mg/mL)} = \text{Dilution factor x Peakheight intensity}$$

$$\text{Sample (mg/mL)} = \frac{\text{Extract (}\mu\text{g/mL) x Sample volumn}}{\text{Weight of sample}}$$

Determination of organic acids in fermenting castor bean using waters 616/626 HPLC: The samples were extracted and determined on waters 616/626 HPLC. The samples were extracted by weighing 25 g of the ground samples into a set of centrifuge tubes (50 mL) (Thermo Electron Corporation IEC Centra GP8 Model, USA). About 15 mL of extraction mixture, i.e. (ultrapure water-methanol) in the ratio of (150:50 v/v) was added to the set of the weighed samples. The sample solutions were covered and shaken on a mechanical shaker (Edmund Buhler model, USA) for 10 min and the samples were transferred to a set of centrifuge machine and centrifuged for 25 min at 5000 rpm.

The supernatants were sampled by further filtration with whatman No.2 filter paper. Further filtration was carried out for two more times and the whole filtrates were pooled together. Finally, the filtrates were centrifuged and filtered to a separate set of volumetric flask 100 mL each.

Determination of the analytes: The organic acids were determined on the waters 616/626 HPLC. For the analysis of the analytes, the HPLC used the following accessories:

- UV absorbance detector
- Wavelength of 215 nm

It was interphased with chromatographic separation moderator or converter software called shodex RSPaK Kc118 Model ion-exchange organic acid column (300×8 mm) size. This accessory made it possible to interpret the peak height intensity of the analyte in terms of concentration. The mobile phase was 0.15% phosphoric acid in ultrapure water (HPLC) grade. The flow rate for the chromatographic separations was 1.0 m min^{-1}. About 45 uL of the combined working standards of the analytes was injected into the HPLC to obtain the standard curve from the software interphase with the HPLC instrument.

The software stored the intensity of the peak height of the standard solutions. This was then used to interpret the peak height of the analytes of the unknown concentration.

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).

RESULTS AND DISCUSSION

Effect of fermentation period on the organic acid contents in fermented castor oil bean samples: Table 1-5 show the results for the organic acids found in the fermented castor oil bean samples. From the Table 1-5, it can be observed that there were fluctuations in most of the organic acids present in the samples monitored from 0-96 h fermentation period. But 96 h fermentation witnessed higher values for some of the organic acids

Table 1: Effects of fermentation period on the acetic and butyric acid contents (μg mL^{-1}) of fermented castor seed

Fermentation time	Samples (%) B_1 (0 NaCl/Lime)	B_2 (2 NaCl)	B_3 (3 Lime)	Mean
Acetic acid				
0.00	0.096	0.088	0.094	0.092
24.00	0.103	0.076	0.119	0.099
48.00	0.091	0.067	0.204	0.120
72.00	1.124	0.079	1.293	0.832
96.00	1.204	0.677	1.401	1.094
Mean	0.523	0.197	0.622	-
Butryic acid				
0.00	1.021	0.923	1.011	0.985
24.00	1.989	0.875	1.128	1.330
48.00	0.899	0.631	1.140	0.890
72.00	1.261	0.596	1.209	1.022
96.00	1.398	0.604	1.224	1.075
Mean	1.313	0.725	1.142	-

LSD (0.05%) ferm_time: Acetic acid = 0.05, Butyric acid = 0.06; LSD (0.05%) samples: Acetic acid = 0.04, Butyric acid = 0.04; LSD (0.05%) ferm_time x samples: Acetic acid = 0.10, Butyric acid = 0.10

Table 2: Effects of fermentation period on the citric and formic acid contents (μg mL^{-1}) of fermented castor seed

Fermentation time	Samples (%) B_1 (0 NaCl/Lime)	B_2 (2 NaCl)	B_3 (3 Lime)	Mean
Citric acid				
0.00	0.128	0.133	0.119	0.126
24.00	0.116	0.085	0.143	0.114
48.00	0.102	0.089	0.121	0.104
72.00	0.146	0.078	0.132	0.118
96.00	0.172	0.069	0.141	0.127
Mean	0.132	0.090	0.131	-
Formic acid				
0.00	0.795	0.669	0.788	0.751
24.00	0.811	0.693	0.936	0.813
48.00	0.712	0.753	0.988	0.818
72.00	0.949	0.811	1.013	0.924
96.00	1.164	0.732	1.029	0.975
Mean	0.886	0.732	0.951	-

LSD (0.05%) ferm_time: Citric acid = NS, Formic acid = NS; LSD (0.05%) samples: Citric acid = 0.01, Formic acid = 0.08; LSD (0.05%) ferm_time x samples: Citric acid = NS, Formic acid = NS

Table 3: Effects of fermentation period on the lactic and malic acid contents ($\mu g\ mL^{-1}$) of fermented castor seed

Fermentation time	Samples (%) B_1 (0 NaCl/Lime)	B_2 (2 NaCl)	B_3 (3 Lime)	Mean
Lactic acid				
0.00	0.775	0.696	0.800	0.757
24.00	0.716	0.559	0.932	0.736
48.00	0.605	0.577	1.064	0.749
72.00	0.932	0.516	1.211	0.886
96.00	1.336	0.437	1.298	1.024
Mean	0.873	0.557	1.061	-
Malic acid				
0.00	1.129	1.092	1.205	1.142
24.00	1.096	0.931	1.206	1.078
48.00	0.985	0.692	1.329	1.002
72.00	1.295	0.702	1.428	1.142
96.00	1.596	0.659	1.662	1.306
Mean	1.220	0.815	1.366	-

LSD (0.05%) ferm_time: Lactic acid = 0.12, Malic acid = 0.07; LSD (0.05%) samples: Lactic acid = 0.09, Malic acid = 0.05; LSD (0.05%) ferm_time x samples: Lactic acid = 0.21, Malic acid = 0.12

Table 4: Effects of fermentation period on the oxalic and succinic acid contents ($\mu g\ mL^{-1}$) of fermented castor seed

Fermentation time	Samples (%) B_1 (0 NaCl/Lime)	B_2 (2 NaCl)	B_3 (3 Lime)	Mean
Oxalic acid				
0.00	0.049	0.048	0.051	0.049
24.00	0.051	0.039	0.067	0.052
48.00	0.043	0.039	0.084	0.055
72.00	0.067	0.041	0.097	0.068
96.00	0.088	0.037	0.058	0.061
Mean	0.059	0.040	0.071	-
Succinic acid				
0.00	0.656	0.591	0.649	0.632
24.00	0.691	0.507	0.871	0.690
48.00	0.501	0.384	0.985	0.623
72.00	0.815	0.270	1.056	0.714
96.00	0.986	0.249	1.065	0.767
Mean	0.730	0.400	0.925	-

LSD (0.05%) ferm_time: Oxalic acid = NS, Succinic acid = NS; LSD (0.05%) samples: Oxalic acid = 0.01, Succinic acid = 0.07; LSD (0.05%) ferm_time x samples: Oxalic acid = 0.01, Succinic acid = 0.16

present in the samples. The organic acids present in the fermented castor oil bean samples include: oxalic, citric, tartaric, malic, succinic, lactic, formic, acetic, propionic and butyric acids.

Table 1 shows the result acetic and butyric acids present in the samples. There were significant differences ($p<0.05$) in these two organic acids with regards to both the samples and fermentation time. The acetic acid content of sample B_1 increased as fermentation progressed and at 96 h fermentation, its value was 1.204 $\mu g\ mL^{-1}$ while those of B_2 and B_3 were 0.677 and 1.401 $\mu g\ mL^{-1}$, respectively.

The citric acid contents for the samples were higher for samples B_1 and B_3 with values 0.172 and 0.141 $\mu g\ mL^{-1}$ as shown in Table 2. While sample B_2 with 2% NaCl had 0.069 $\mu g\ mL^{-1}$. The same trend was recorded for lactic acid. Sample B_2 has the lowest value of 0.437 $\mu g\ mL^{-1}$ while B_1 and B_3 had citric acid values of 1.336 and 1.298 $\mu g\ mL^{-1}$ at the 96 h fermentation time. Organic acids occur in fermented products as a result of hydrolysis, biochemical metabolism and microbial activity. (Anderson and Hedlund, 1983). The metabolism of the carbohydrates in the raw seeds would have resulted in the accumulation of organic acids (Enujiugha, 2003). In almost all fermentation involving *Bacillus* species, there is production of primary organic acids, especially butyric acid (Moat, 1979).

Table 5: Effects of fermentation period on the tartaric and propionic acid contents ($\mu g\ mL^{-1}$) of fermented castor seed

Fermentation time	Samples (%) B_1 (0 NaCl/Lime)	B_2 (2 NaCl)	B_3 (3 Lime)	Mean
Tartaric acid				
0.00	0.103	0.101	0.107	0.103
24.00	0.099	0.063	0.146	0.102
48.00	0.061	0.056	0.079	0.065
72.00	0.131	0.059	0.086	0.092
96.00	0.156	0.051	0.097	0.101
Means	0.110	0.066	0.103	-
Propionic acid				
0.000	0.698	0.947	0.671	0.772
24.00	0.639	0.426	0.856	0.640
48.00	0.503	0.573	0.993	0.690
72.00	0.856	0.493	1.103	0.817
96.00	1.204	0.428	1.215	0.949
Mean	0.780	0.573	0.968	-

LSD (0.05%) ferm_time: Tartaric acid = 0.01, Propionic acid = 0.08; LSD (0.05%) samples: Tartaric acid = 0.01, Propionic acid = 0.06; LSD (0.05%) ferm_time x samples: Tartaric acid = 0.02, Propionic acid = 0.14

Acetic acid is both volatile and odourous, detectable as the smell of vinegar (Zotou *et al*., 2004). High concentration of acetic acid can result in an undesirable vinegar flavour in fermented dairy foods (Chick *et al*., 2001). Higher concentration of acetic acid can result in a pungent off-flavour. Harper and Collins (1992) reported an increased production of acids such as lactic acid and especially acetic acid accompanied with a decrease in pH during the production of furundu. Volatile fatty acids as well as traces of propionic acids were also detected. The production of organic acids is undoubtedly the determining factor on which the shelf life and the safety of the final product depends while the inhibition of pathogenic and spoilage flora is also dependent on a rapid and adequate formation of these organic acids (Lindgren and Dobrogosz, 1990; Olaoye *et al*., 2008). The antimicrobial effect of organic acids lies in the reduction of pH as well as the undissociated form of the molecules (Podolak *et al*., 1996).

Table 3 shows the result for lactic acid and malic acid. The lactic acid contents in sample B_1 (0% NaCl/Lime) decreased initially and then increased as the fermentation period progressed. The value at 96 h fermentation was

1.336 μg mL^{-1} as against 0.775 μg mL^{-1} at 0 h fermentation. Sample B_3 (3% lime) had lactic acid content that increased as fermentation period increased with lactic acid content of 1.298 μg mL^{-1} at 96 h fermentation. Organic acids affect stability, colour and flavour of the final product but their greatest role comes from their ability to stop or at least retard the growth of many potentially harmful microorganisms that cause spoilage in food products (Zotou *et al*., 2004). Biopreservation has gained increasing attention as a means of naturally controlling the shelf life and safety of food products (Olaoye and Onilude, 2009). Yasuda (2011) reported that glucose, organic acids, nucleotides and NaCl are involved in the taste components of tofuyo, a soybean fermented food, indigenous to Japan and also strongly suggested that the desirable taste of tofuyo is formed by interactions between these components. The production of these organic acids are undoubtedly the determining factor in which the shelf-life and safety of the final production depends while the inhibition of pathogenic and spoilage flora is also dependent on a rapid and adequate formation of these organic acids.

Effect of fermentation period on the amino acid profile of fermented castor oil bean samples: The graph of changes of the amino acid profiles of the samples with the fermentation time are shown in Fig. 1a-d and 2a-d. The three samples, B_1, B_2 and B_3 contained sufficient amount of amino acids. It was observed that there were fluctuations in some of the amino acid contents of the samples from 0-96 h fermentation. But the amino acid contents increased steadily before the 96 h fermentation. There was no significant difference in the phenylalanine contents of the samples as shown in Fig. 1a. Phenylalamine content of sample B_1 decreased from 0-48 h fermentation and increased from 72 h till 96 h fermentation with 1.503 μg mL^{-1}. Figure 1b shows there was significant difference in the between proline content and the fermentation period of all the samples. Samples B_2 and B_3 were significantly different ($p<0.05$) in proline content. Sample B_1 had higher values than B_2 and B_3 with 0.756 μg mL^{-1}. Also, Fig. 1c-d shows there was significant differences in the lysine and methionine contents as fermentation period progressed. At 48 h fermentation the sample containing 0%NaCl/Lime, B_1 had higher values than those of 2% NaCl, B_2 and 3% lime, B_3.

There were significant differences ($p<0.05$) in the graphs as shown in Fig. 2a-d. Sample B_1 had the highest values in isoleucine, glycine and histidine while sample B_2 had the highest value in leucine content

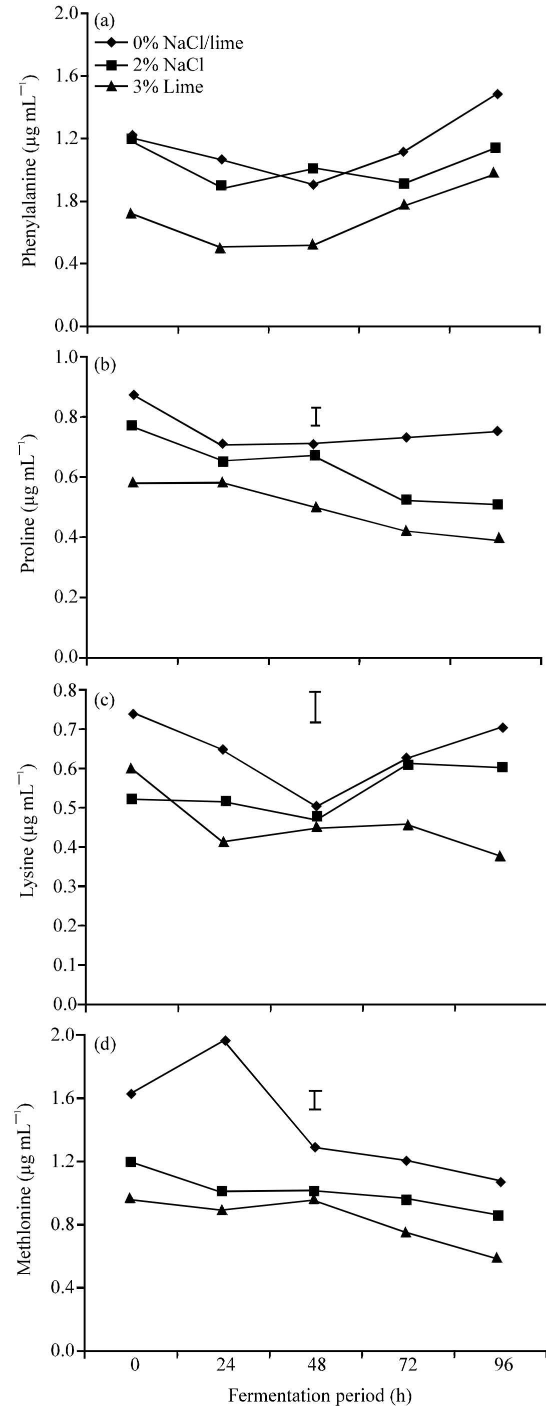

Fig. 1: Effect of fermentation period on; a) phenylalanine; b) proline; c) lysine and d) methionine contents of fermented bean samples

with 0.915 μg mL^{-1} at 96 h fermentation, closely followed by sample B_3 and B_1 with 0.798 and 0.205 μg mL^{-1}, respectively.

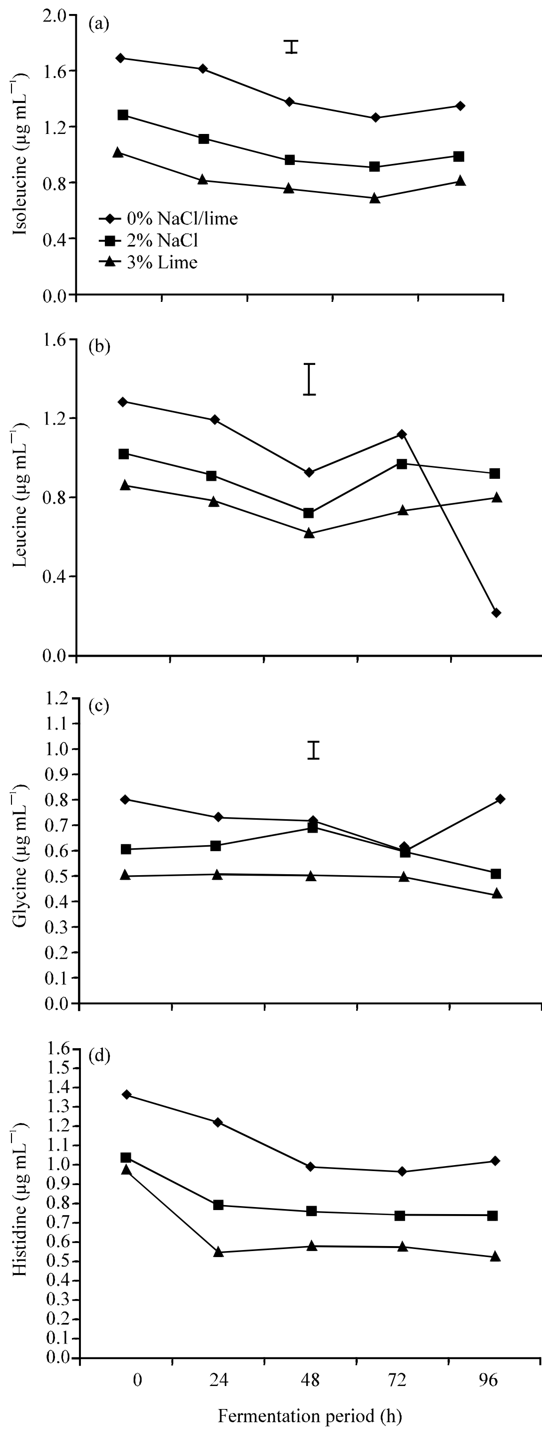

Fig. 2: Effect of fermentation period on; a) isoleucine; b) leucine; c) glycine and d) histidine contents of fermented bean samples

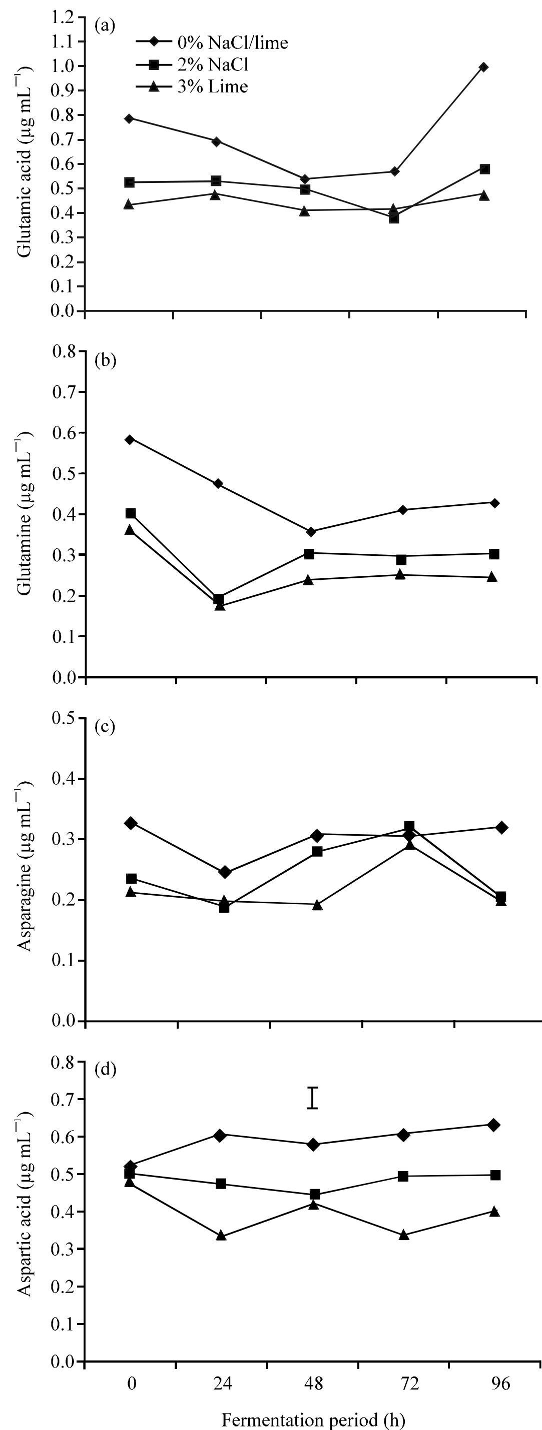

Fig. 3: Effect of fermentation period on; a) glutamic acid; b) glutamine; c) asparagine and d) aspartic acid contents of fermented bean samples

Figure 3a-d shows that glutamic acid content after 48 h fermentation increased steadily in sample B_1 after 48 h fermentation and was significantly different from samples B_2 and B_3. There was no significant difference in the glutamine content of the samples as shown in Fig. 3b. According to Anosike and Egwuatu (1980) glutamine

which is the amine of glutamic acid is one of the compounds that contribute to the characteristic flavour of the fermented castor oil seed, ogiri. It is therefore, suggested that glutamine would also increase in the product with increased period of fermentation. The arginine content in the samples differed significantly as fermentation period increased, Fig. 4b. There have been reports on the liberation of soluble amino acids during fermentation of vegetable seeds into condiments (Dulaney, 1967; Chattopadahay and Banerjee, 1973). Ogunshe *et al*. (2007) reported increased total free amino acids in the controlled fermentation of afiyo. Similar increases in the level of free amino acids with fermentation have been reported in other leguminous vegetable seeds (Omafuvbe *et al*., 1999, 2000). This rapid increase in the total free amino acids may be a reflection of the increased protease activity observed in the fermenting seeds. Odunfa (1985) and Campbell-Platt (1980) also noted a high level of proteolytic activity during dawadawa fermentation which culminated in the formation of peptides and amino acids (Fig. 5a-d).

Free amino acids such as glutamic acid and aspartic acid may contribute to the formation of flavour (Yasuda *et al*., 1994, 1995; Katsura, 1996). The data clearly indicated an increase in and the involvement of free amino acids after fermentation especially glutamic acid, alanine, aspartic acid, glycine and serine which may contribute to pleasant taste. It is well known that glutamic acid and aspartic acid contribute to the pleasant umami taste or savoury enhancement in foods (Yasuda *et al*., 1994).

Lioe *et al*. (2004, 2007) carried out chemical and sensory studies on savory fractions obtained from soy sauces and phenylalanine as well as NaCl and glutamic acid were identified in the fractions. A potential synergistic effect of umami among free glutamic acid, salt and phenylalanine (bitter amino acid) were observed.

Proteolysis has been reported as the main metabolic activity during the fementation of African locust bean which also contribute to the development of texture and flavour of fermented products (Ouoba *et al*., 2003; Olajuyigbe and Ajele, 2008). During soybean fermentation, protein will be hydrolysed to low molecula weight components such as peptides and amino acids due to the action of enzymes produced by bacteria (Kiers *et al*., 2000). These outcomes may be favourable to the flavour, absorbability, digestibility and functionality of bacterial douchi, traditional Chinese fermented soybean product used as seasoning. Glutamic acid is the most important flavour enhancing amino acid (Davids *et al*., 2004) and glycine and alanine give the sweet flavour (Norziah and Ching, 2000).

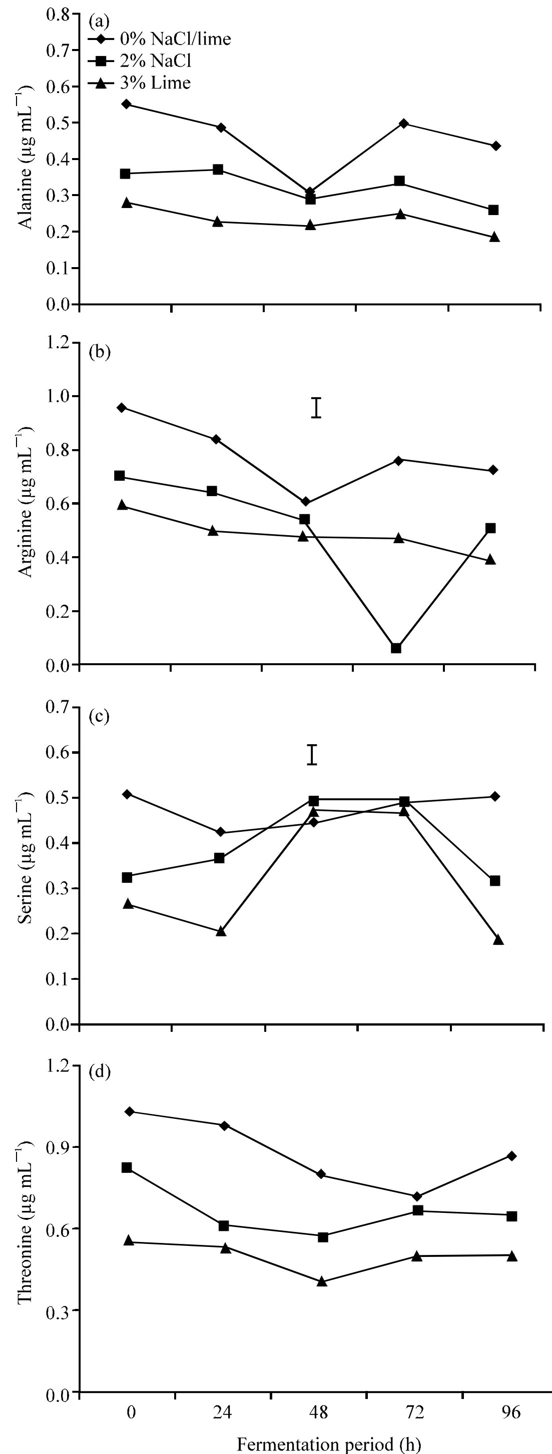

Fig. 4: Effect of fermentation period on; a) alanine; b) arginine; c) serine and d) threonine contents of fermented bean samples

Fermentation of castor oil seeds have been shown to markedly increase the levels of free amino acids, fatty

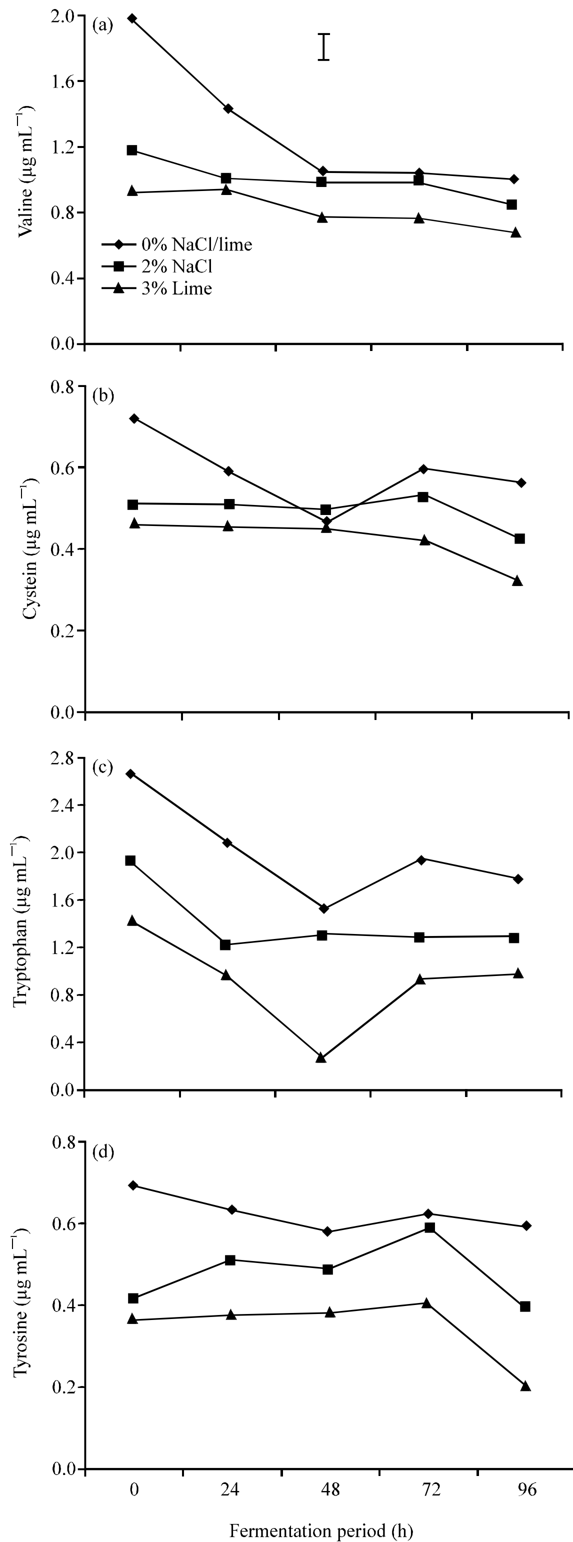

Fig. 5: Effect of fermentation period on; a) valine; b) cysteine; c) tryptophan and d) tyrosine contents of fermented bean samples

acids and iodine (Anosike and Egwuatu, 1980). Extensive hydrolysis of seed protein into amino acids hence, making the beans more digestible as well as higher in amino acids and B vitamins was reported in the fermented samples of iru than the unfermented product (Eka, 1980). Similarly, a remarkable feature of the changes during the fermentation of ogiri was the increased activity of proteinases as well as the amount of amino acids (Odunfa, 1985).

Amino acids are known to play a major role in the taste and flavour development of foods (Kpikpi *et al*., 2009). Specifically glutamic acid contributed to the umami taste of soy sauce and similar products (Halpern, 2000). The results of amino acid analysis indicated a high concentration of all amino acids especially at 96 h of fermentation. Thus, the flavour and aroma of ogiri may be due to the production of fatty acids and amino acids, especially glutamic acid during the fermentation process.

Soluble low molecular weight peptides and amino acids that contribute to flavour are produced through enzymatic breakdown of proteins (Njoku and Okemadu, 1989; Ogbonna *et al*., 2001; Ouoba *et al*., 2003). Amino acids produced because of protein metabolism are responsible for the gradual pH increase and leveling off towards pH 7.5-8.0 (Barimalaa *et al*., 1994; Achi, 1992; Barber and Achinewhu, 1992; Sarkar *et al*., 1997). Eka (1980) reported that daddawa is low in the essential amino acids leucine, isoleucine and phenylalanine. The deficiency of daddawa in some of the essential amino acids detracts from the value of daddawa as a source of high quality protein. However, daddawa is not consumed alone but added to soup and other vegetables as a flavouring agent (Dakare *et al*., 2011).

Salt may have enhanced the production and activity of protease enzyme since the level of protease activity in 1% salted daddawa was significantly higher than values obtained for salt free daddawa (Omafuvbe, 2006). About 1 and 0.4% NaCl have been reported to give maximum protease activity and enhanced protease production, respectively in *Bacillus clausii* (Joo and Chang, 2005). Also, NaCl has been reported to enhance the hydrolysis of protein in sufu (fermented soybean curd) to a large extent resulting in increased level of free amino acids (Han *et al*., 2003).

Popoola *et al*. (2005) reported increases in values for some of the amino acids in the fermented seeds of *C. altissimum* for use as condiments. Of particular mention were lysine, tyrosine and valine. The profile of amino acids in ogiri is comparable to those recommended by FAO/WHO. The proteolytic activities of bacteria isolates involved in the fermentation of the seeds are of assistance in improving the digestibility of ogiri. The significant increase in concentrations of the essential

amino acids in the final product is in agreement with the reports of other researchers regarding condiments from oil seeds (Eka, 1980; Kpikpi *et al*., 2009).

The advantages of using HPLC in the analysis of organic acids are speed, accuracy and precision (Fernandez-Garcia and McGregor, 1994). The production of organic acids is undoubtedly the determining factor on which the shelf life and the safety of the final product depend while the inhibition of pathogenic and spoilage flora is also dependent on a rapid and adequate formation of these organic acids (Lindgren and Dobrogosz, 1990). Amino acids are known to play a major role in the taste and flavour development of foods (Kpikpi *et al*., 2009). Specifically glutamic acid contributed to the umami taste of soy sauce and similar products (Halpern, 2000).

CONCLUSION

The results of amino acid analysis indicated a high concentration of all amino acids especially at 96 h of fermentation. Thus, the flavour and aroma of ogiri may be due to the production of fatty acids and amino acids, especially glutamic acid during the fermentation process.

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