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Production of Soup Condiment (Ogiri ugu) from Fluted Pumpkin Seeds Using *Bacillus subtilis*.

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Abstract

The effect of fermentation variables on the production of a soup condiment from fluted pumpkin ("ogiri ugu") using Bacillus subtilis was studied with the view to improving the production and product quality. The variables studied were relative humidity (RH), temperature and pore size of wrapping material. Effect of variables on amino nitrogen, pH, peroxide value and free fatty acid was determined on 24h basis for 96h. Amino nitrogen progressively increased throughout the fermentation period with the highest value (8.32 mg N/g) being obtained from sample fermented at 85% RH, temperature 40°C and 70µm pore size of wrapping material. Daily amino nitrogen production indicated that fermentation attained its peak at 72h. pH increased into the alkaline range within 96h, the highest value (8.23) being from sample fermented at 65% RH, temperature 40°C and 70µm pore size of wrapping material. Peroxide values increased with fermentation time, however, the highest value (4.85 Meq/kg) obtained from the sample fermented at 85% RH, temperature 35°C and 90µm pore size of wrapper was far below the recommended value of 30 Meq/kg. Free fatty acid increased as fermentation progressed with the highest value (4.77 mg/g) being obtained from sample fermented at 65% RH, temperature 35°C and 50µm pore size of wrapper. Manipulation of the fermentation variables significantly improved amino nitrogen production ($p<0.05$). The suggested combination of variables for optimum fermentation, increased amino nitrogen production and low peroxide value is 75% RH, temperature 35°C and 70µm pore size of wrapper. This ultimately will improve product quality.

Key words: *Amino nitrogen; Bacillus subtilis; Fermentation variables; Ogiri ugu; Peroxide value.*

Introduction

Fermented food condiments have constituted a significant proportion of the diet of many people as they give pleasant aroma to soups and sauces in many countries, especially in Africa and India where protein calorie malnutrition is a major problem (Sarker *et al*., 1993). They also have a great potential as key protein and fatty acid sources, and are good sources of gross energy (Achinewhu *et al*., 1991; Omafuvbe *et al*., 2004). In Africa many proteinaceous oily seeds are fermented to produce food condiments (Sanni and Ogbonna, 1991; Baird-Parker, 1994), therefore these condiments are basic ingredients for food supplementation, and are named according to the substrate or raw materials employed (Odunfa, 1985) which include "ogiri ugu" produced from fluted pumpkin seeds (*Telferia occidentallis*) and "ogiri egusi" produced from melon seeds (*Citrullus vulgaris*). Previous studies have centred on ogiri egusi (Odunfa, 1982; Barimalaa *et al*., 1989; Abaelu *et al*., 1990; Barber and Achinewhu, 1992; Nwosu and Ojimelukwe, 2000; Omafuvbe *et al*., 2004; Ogueke and Nwagwu, 2007; David and Aderibigbe, 2010). Like most other indigenous fermented food products in Africa, ogiri ugu is associated with some problems. Its production is still a traditional family art done on household basis and its quality is as unpredictable as the varying environments and techniques used (Iwuoha and Eke, 1996). The fermentation process is purely natural with little control, thus many microorganisms whose functions are not necessary are involved. Various microorganisms have even isolated from fermenting seeds (Barber *et al*., 1989). Its packaging with leaves combined with moist nature of the product makes it look unhygienic and unappealing, thus distracting consumers from regular purchasing attitudes. It is envisaged that the microbiological safety of the condiment as well as its nutrition can be improved upon greatly by using starter cultures for the fermentation and also study effect of difference fermentation variables on the fermentation process. This work therefore aims at studying the effect of different fermentation variables on the fermentation using pure cultures of *Bacillus subtilis*.

Materials and Methods

Source of raw material

The fluted pumpkin fruits (*Telferia occidentallis*) used for the study were obtained from a market in Owerri, Imo State, Nigeria. The fruits were cut open and the seeds extracted, cleaned to remove the slimy coverings and stored at 4^{O}C until used for the study.

Isolation of Bacillus subtilis used for the study

Bacteria were isolated from various samples from three states of the consuming region of South Eastern Nigeria. Microbiological analyses were conducted immediately by suspending 10g of sample in 90mL sterile maximum recovery diluents (MRD Oxoid CM0733) and homogenized using a Stomacher (A. J. Seward, BA6021) for 1 to 2 min at normal speed. Ten-fold dilutions were then prepared and 0.1ml of suitable dilution was spread onto Nutrient agar (NA, Oxoid CM0003). Plates were incubated aerobically at 37^{0}C for 24 - 48 hours. Representative dominant colonies were picked and purified by streaking on same Nutrient agar. Stock cultures were kept in Nutrient broth containing 20% glycerol and stored at -20^{0}C.
The identification of the isolates was done using the methods described by Ouoba *et al.* (2008).

Before use the identified *Bacillus subtilis* were streaked on Tryptone Soy agar (BIOTEC) and incubated at 35^{O}C for 18h. The cultures were then washed by suspending the cells in 10mL of 0.1M potassium phosphate buffer (pH 7.0). This was followed by centrifugation at 14000xg for 5min. using an MSE centrifuge (Minor, UK) (Njoku *et al*., 1990).

Preparation and standardization of inocula

Cultures from 18h Tryptone Soy agar plates (BIOTECH) were examined for purity by microscopic examination of slides prepared from randomly selected colonies. A loopful of the 18h old culture was inoculated in a flask containing 50mL of Tryptone Soy broth (Oxoid) and was incubated aerobically for 48 h at 35°C. Cells from the broth culture were harvested and washed three times with 0.1 M potassium phosphate buffer by centrifugation at 14000xg for 5min. The cells were then aseptically re-suspended in 20mL sterile 0.1M potassium phosphate buffer (pH 7.0). The cell suspension was then standardized using a spectrophometer (Model S2100UV, UNICO, New Jersey, USA) at 550nm wavelength such that 1.0mL of the suspension contained about 2.0×10^{10} cells.

Fermentation of fluted pumpkin seeds using standardized broth cultures of B. subtilis

The seeds (40kg) were extracted from their pods and boiled for 2h in water to soften the seed coat and then cooled to 30^{O}C. The dark coloured water was discarded. The seed coats were removed to obtain the cotyledons. The cotyledons were washed and 1.5kg placed in a sterilized container. They were inoculated with 2.0mL of the standardized broth cultures and mixed. Approximately 750g were wrapped in polyethylene materials of different pore sizes (50µm, 70µm and 90µm). The 50µm and 70µm materials were made of low density polyethylene material while 90µm material was made of high density polyethylene material. The pore sizes were previously determined by Isu and Njoku (1998). The wrapped coltyledons were then put in an incubator set at the appropriate temperatures (see experimental design) and allowed to ferment for four days. The same process was repeated for all the variables as shown in the experimental design.

Experimental design: *A Box Behnken rotable response (Lawson and Madrigal, 1994) for k = 3 was employed to study the effects of* the independent variables on the fermentation process. The variables were of three levels (Table 1).

Table 1: The independent variables, their levels and Combination of the independent variables

Independent variables	Variable levels		
	-1	0	+ 1
Relative humidity (RH) % (X_1)	65	75	85
Temperature ^{O}C (X_2)	30	35	40
Pore size µm (X_3)	50	70	90

Table 2: Combination of the independent variables

Sample/Run	X_1	X_2	X_3
1	-1	-1	0
2	+1	-1	0
3	-1	+1	0
4	+1	+1	0
5	-1	0	-1
6	+1	0	-1
7	-1	0	+1
8	+1	0	+1
9	-1	-1	-1
10	0	+1	-1
11	0	-1	+1
12	+1	+1	+1
13	0	0	0 x 3

Generation of relative humidity (RH)

The various relative humidities were generated as follows:

i) 65% RH was generated by dissolving 50g of anhydrous $NaNO_3$ in 33.3mL of hot water.
ii) 75% RH was generated by dissolving 20g of NaCl in 50mL of hot water.
iii) 85% RH was generated by dissolving 25g of KCl in 50mL of hot water.

The salt solutions were put in the incubator to create the required relative humidity in the environment of fermentation.

The fermentations were monitored on 24 h basis for the amino nitrogen content, pH, free fatty acid content and peroxide value.

Determination of pH

The pH of each fermentation process was measured as follows. Five grammes of fermenting seeds was taken from the bulk and mashed in 45mL of sterile deionized water (Njoku *et al*., 1990). The pH was measured using a standardized (pH 4.0 – 9.0) digital pH meter (Jenway pH/MV/TEMPERATURE METER, Model 3510, Essex, UK) at ambient temperature (30-32°C).

Amino nitrogen determination

The formol titration method (Pham and Del Rosario, 1983) was used for the determination of amino nitrogen in the samples. Three grammes of fermenting seeds was taken and mashed. Two grammes of the mashed seeds was placed in a conical flask, and then 0.5 mL of phenolphthalein (0.5%) and 0.4mL of neutral saturated potassium oxalate were added. The mixture was kept to stand for few minutes and this was neutralized with 0.1M NaOH to a standard pink color. Two milliliters of 40% formaldehyde solution was added and allowed to stand for few minutes (until mixture was colorless). This was then titrated with 0.1M NaOH to pink color. The titer value obtained was designated 'V_1'. A blank was run by titrating a mixture of 2mL of formaldehyde solution and 10mL distilled water with 0.1M NaOH to obtain 'V_2'. The amino nitrogen was calculated using the equation: % Amino Nitrogen = 1.7 (V_1 - V_2).

Determination of free fatty acid

One gramme of the fermenting cotyledons was measured into a conical flask. After this 25ml of diethyl ether and 25ml of alcohol were added. Phenolpthalein was added as indicator and titrated against 0.01N NaOH until a persistent pink colour was obtained. The free fatty acid was calculated as follows;

$$\text{Free fatty acid} = \frac{\text{Titre x 5.61}}{\text{Weight of sample}}$$

Determination of peroxide value

Fifteen grams of fermenting cotyledons was mashed and 5 g of each of the mashed seeds was placed into a 250mL glass – stoppered Erlenmeyer flask, and 30mL of solvent mixture (3 parts by volume of Glacial acetic acid + 2 parts by volume of Chloroform) was added. The flask was swirled to dissolve the mashed seeds in the solution. Five hundred microliters of saturated potassium iodide solution was added and the solution was allowed to stand (with occasional shaking) for 1 min, then 30mL of distilled water was added. The mixture was titrated by gradually adding 0.1N sodium thiosulphate and constantly shaking vigorously. The titration continued until the yellow color had almost disappeared. Five hundred microliters of starch indicator solution was added. The titration was continued, shaking the flask vigorously at near end point until the iodine from the chloroform layer was liberated. The sodium thiosulphate was then added drop wise until the blue color had just disappeared. The tubes were placed in boiling water to boil for

about 30 sec. A blank was also prepared at the same time. The peroxide value per 1000g of sample was calculated using the equation described below:

$$\text{Peroxide value} = \frac{(S - B) \times (N) \times (1000)}{W}$$

B = Titration of blank
S = Titration of sample
W = Weight of sample
N = Normality of sodium thiosulfate solution
All analyses were carried out in triplicate.

Statistical analysis

The data obtained from the study were analyzed statistically using Analysis of Variance (ANOVA). The means were separated using Fisher's Least Significant Difference (LSD). Microsoft Excel 2007 was used for the processing of the data.

Results and Discussion

Effect of fermentation variables on pH

The pH values obtained during fermentation are shown in table 3. pH of the samples increased throughout the period of fermentation. Sample 3 had the highest value of 8.23 while the least value of 7.48 was obtained in sample 12 at 96h fermentation. pH values of majority of the samples at 96h were well into the alkaline range (≥8.0). This is in agreement with the findings of other workers (Odunfa and Oyeyiola, 1985; Njoku and Okemadu, 1989; Ikenebomeh, *et al*., 1996) who also observed that fermentations of preteinous oily seeds usually result in increase in pH to the alkaline region. Increase in pH is as a result of the activities of the proteases and deaminase enzymes of the fermenting bacterium (*B.subtilis*) resulting in the release of ammonia which increases pH (Njoku and Okemadu, 1989).

Table 3: pH values of the fermenting fluted pumpkin seeds

Sample/Run	Period of fermentation (hours)					
	0	24	48	72	96	LSD
1	$^{1}6.45^{a}$	$^{1,4}7.15^{b}$	$^{1,4}7.48^{b,c}$	$^{1,3}7.92^{c,d}$	$^{1}8.05^{d}$	0.50
2	$^{2,4}6.68^{a}$	$^{2,4}7.32^{b}$	$^{2,4}7.65^{b,c}$	$^{2,3}8.06^{c}$	$^{1,3}8.14^{c}$	0.47
3	$^{3}6.32^{a}$	$^{1}7.04^{b}$	$^{3,6}7.35^{b,c}$	$^{1,5}7.85^{c,d}$	$^{2}8.23^{d}$	0.57
4	$^{2}6.71^{a}$	$^{2}7.41^{b}$	$^{4}7.56^{b}$	$^{3}8.03^{c}$	$^{1,3}8.12^{c}$	0.44
5	$^{1}6.45^{a}$	$^{1,4}7.15^{b}$	$^{1,3}7.45^{b,c}$	$^{4}7.65^{c}$	$^{3}8.16^{d}$	0.49
6	$^{2,4}6.68^{a}$	$^{2}7.29^{b}$	$^{1,3}7.39^{b}$	$^{1,5}7.82^{c}$	$^{1}8.05^{c}$	0.41
7	$^{2,4}6.62^{a}$	$^{3}6.78^{a}$	$^{5}7.09^{a}$	$^{1,5}7.91^{c}$	$^{4}7.91^{c}$	0.59
8	$^{2,4}6.65^{a}$	$^{4}7.25^{b}$	$^{4}7.57^{b,c}$	$^{5}7.80^{c}$	$^{4}7.94^{c}$	0.40
9	$^{2,4}6.63^{a}$	$^{3,5}6.85^{a,b}$	$^{5}7.15^{b}$	$^{1,3}7.92^{c}$	$^{1,3}8.10^{c}$	0.51
10	$^{1}6.46^{a}$	$^{5}6.94^{b}$	$^{6}7.26^{b}$	$^{1}7.81^{c}$	$^{4}7.90^{c}$	0.47
11	$^{4}6.60^{a}$	$^{3}6.77^{a}$	$^{5}7.06^{a}$	$^{1,3}7.92^{b}$	$^{3}8.16^{b}$	0.54
12	$^{5}5.92^{a}$	$^{6}6.58^{b}$	$^{7}6.94^{b,c}$	$^{7}7.16^{c,d}$	$^{5}7.48^{d}$	0.47
13	$^{3}6.27^{a}$	$^{5}6.91^{b}$	$^{6}7.26^{b,c}$	$^{6}7.60^{c,d}$	$^{6}7.81^{d}$	0.47
LSD	0.10	0.12	0.10	0.11	0.09	

^{a,b,c}…. *Values on the same row with the same superscript are not significantly different (p = 0.05).*
^{1,2,3}…. *Values on the same column with the same superscript are not significantly different (p = 0.05).*

Effect of fermentation on free fatty acid

The level of free fatty acid produced during fermentation is shown in table 4. As fermentation progressed free fatty acid increased in all the samples. The highest value (4.77 mg/g) was obtained in sample 5 while the least value (3.41 mg/g) was obtained in sample 13. Such trend has been observed by Abaelu *et al.* (1990) during fermentation of melon seeds. The increase in free fatty acid is indicative of the production of lipases by the fermenting bacterium. This is compatible with what is known about fermentation by *Bacillus* species (Njoku and Okemadu, 1989; Abaelu *et al*., 1990). The result is also an indication of the fermentation of unsaturated fatty acids to saturated fatty acids. Such observations have been made by previous workers (Benchat and Worthington, 1974; Kuishi *et al.*, 1976; Abaelu *et al*., 1990). Generally values obtained at 72 and 96h were significantly different ($p<0.05$) from values obtained from previous days.

Table 4: Free fatty acid levels during fermentation of fluted pumpkin seeds (mg/g)

Sample/Run	Period of fermentation (hours)					
	0	24	48	72	96	LSD
1	[1]1.76[a]	[1,3,4]2.27[a]	[1]2.94[b]	[1,5]3.24[b,c]	[1,4]3.74[c]	0.61
2	[2]1.85[a]	[2,3]2.38[a,b]	[2,4]3.08[b]	[2,3]4.18[c]	[2,3]4.69[c]	0.93
3	[2]1.83[a]	[3,6]2.32[a,b]	[2]3.06[b]	[2]4.05[c]	[2,3]4.56[c]	0.89
4	[2]1.85[a]	[1,3]2.35[a,b]	[2,4]3.08[b]	[2]4.06[c]	[2]4.50[c]	0.87
5	[2]1.84[a]	[2,3]2.38[a,b]	[2]3.06[b]	[3]4.27[c]	[3]4.77[c]	0.96
6	[1]1.74[a]	[4,5]2.24[a]	[1]2.95[b]	[1]3.17[b]	[1]3.85[c]	0.64
7	[3]1.67[a]	[5]2.16[a]	[3]2.84[b]	[1]3.18[b,c]	[4]3.59[c]	0.60
8	[4]1.91[a]	[6]2.42[a,b]	[4]3.16[b]	[2,3]4.15[c]	[2,3]4.60[c]	0.88
9	[2]1.82[a]	[1,3]2.35[a,b]	[2]3.02[b]	[4]3.85[c]	[1]3.90[c]	0.71
10	[2]1.81[a]	[7]1.96[a]	[5]2.65[b]	[5]3.44[c]	[4]3.42[c]	0.60
11	[1]1.74[a]	[8]2.79[b]	[6]3.42[b,c]	[4]3.81[c]	[1]3.94[c]	0.70
12	[3]1.69[a]	[7]1.95[a]	[5]2.65[b]	[1]3.06[b,c]	[4]3.56[c]	0.60
13	[4]1.91[a]	[5]2.17[a]	[1,3]2.88[b]	[1]3.12[b,c]	[4]3.41[c]	0.50
LSD	0.04	0.10	0.09	0.21	0.23	

[a,b,c]…. *Values on the same row with the same superscript are not significantly different (p = 0.05).*
[1,2,3]…. *Values on the same column with the same superscript are not significantly different (p = 0.05).*

Peroxide value

Table 5 shows the peroxide values obtained during fermentation. The values increased throughout the period of fermentation. The least value (3.65 Meq/kg) was obtained in sample 8 while the highest value (4.85 Meq/kg) was obtained in sample 2. However, the highest value was not near the threshold value of 30 Meq/kg of oil in oil rich foods (Gotoh and Wada, 2006). Since the peroxide value is used as a measure of the extent to which rancidity can occur (Ihekoronye and Ngoddy, 1985) the lower values obtained in the samples showed that the chances of the product going rancid is very low and thus cannot introduce off flavours to soups and stews prepared with the condiment.

Table 5: Peroxide values obtained during the fermentation of fluted pumpkin seeds (Meq/kg)

Sample/Run	Period of fermentation (hours)					
	0	24	48	72	96	LSD
1	[1,4]2.82[a]	[1]3.04[a]	[1,5,7]3.45[b]	[1,5]3.61[b]	[1]4.14[c]	0.40
2	[2]2.94[a]	[2]3.26[a,b]	[2,4]3.62[b]	[2]4.35[c]	[2]4.85[c]	0.61
3	[1]2.85[a]	[3,7]3.41[b]	[3]3.81[c]	[3]4.12[c]	[3,4,6]3.82[c]	0.38
4	[3,6]3.12[a]	[3,6]3.34[a]	[2]3.65[b]	[1,4]3.71[b,c]	[3]3.90[c]	0.24
5	[2]2.94[a]	[4]3.17[a]	[4]3.58[b]	[4,6]3.78[b,c]	[1]4.16[c]	0.38
6	[1]2.85[a]	[4]3.12[a]	[5]3.51[b]	[4,6]3.77[b]	[3,4]3.81[b]	0.33
7	[4]2.76[a]	[5]2.90[a]	[1,6,7]3.42[b]	[1,5]3.62[b]	[4,5]3.71[b]	0.33
8	[4]2.79[a]	[5]2.94[a]	[6]3.38[b]	[5]3.58[b]	[5]3.65[b]	0.30
9	[3]3.06[a]	[6]3.28[a]	[2]3.65[b]	[6]3.83[b]	[3,6]3.92[b]	0.29
10	[5]2.65[a]	[5]2.90[a]	[1,6]3.41[b]	[5]3.67[b]	[4]3.75[b]	0.38
11	[6]3.20[a]	[7]3.45[a,b]	[2]3.60[b,c]	[6]3.85[c,d]	[1]4.06[d]	0.26
12	[2]2.92[a]	[4]3.18[a]	[7]3.48[b]	[5]3.60[b,c]	[4]3.79[c]	0.27
13	[3]3.04[a]	[8]3.59[b]	[2]3.64[b]	[6]3.86[b,c]	[6]3.96[c]	0.28
LSD	0.07	0.10	0.06	0.10	0.14	

[a,b,c]……*values on the same row with different superscript are significantly different (p = 0.05).*
[1,2,3]…. *Values on the same column with the same superscript are not significantly different (p = 0.05).*

Amino Nitrogen

Table 6 shows the amino nitrogen produced during the fermentation period. The values progressively increased throughout the period of fermentation. The highest value (8.32 mg N/g) at the end of fermentation was obtained in sample 4 while the least value (7.17 mg N/g) was obtained in sample 7. Increased amino nitrogen is due to the breakdown of proteins to amino acids by proteases. The apparent increase in the levels of amino nitrogen could be due to increased cell density per unit of fermenting mash as has been suggested by Isu and Ofuya (2000) and enhanced cell wall permeability and metabolism. In this study the fermenting mash was

inoculated with broth cultures containing about 2.0 x 10^{10} cfu/ml of cells. The fermentation takes about 3-5 days to complete (Iwuoha and Eke, 1996).

Statistical analysis showed that values obtained from start of fermentation to 48h were not significantly different ($p>0.05$). However, values at 72 and 96h were significantly different from previous days but were not significantly different from each other. This indicates that the fermentation got to its peak at 72h.

The pungent odour usually observed in well fermented ogiri ugu is believed to be due to liberation of ammonia by deaminase activity. Ammonia nitrogen has been reported to be desirable at certain levels (10 – 15%) in some foods (Yong and Woods, 1977), however, at higher levels they may introduce off flavours into the product. Thus Njoku and Okemadu (1989) have suggested that there should be monitoring of enzyme activity during fermentation for improved product quality. In this work however, ammonia nitrogen was not determined.

Table 6: Amino nitrogen production during fermentation of fluted pumpkin seeds (mg N/g)

Sample/Run	Period of fermentation (hours)					
	0	24	48	72	96	LSD
1	$^{2}3.76^{a}$	$^{2}3.95^{a}$	$^{1}4.92^{a}$	$^{2}6.45^{b}$	$^{1}7.60^{b}$	1.29
2	$^{1,4}3.47^{a}$	$^{1}3.71^{a}$	$^{1}4.95^{a}$	$^{1}6.81^{b}$	$^{2,7}7.95^{b}$	1.52
3	$^{1,2}3.62^{a}$	$^{1,2}3.83^{a}$	$^{1}4.91^{a}$	$^{1}6.93^{b}$	$^{2}7.81^{b}$	1.46
4	$^{3,5}4.40^{a}$	$^{3}4.58^{a}$	$^{2,4}5.71^{a}$	$^{3}7.17^{b}$	$^{3,5}8.32^{b}$	1.31
5	$^{4}3.44^{a}$	$^{1}3.67^{a}$	$^{3}4.72^{a}$	$^{1}6.84^{b}$	$^{4,7}8.16^{b}$	1.60
6	$^{3,6}4.34^{a}$	$^{3}4.54^{a}$	$^{2}5.62^{a}$	$^{4,6}7.81^{b}$	$^{5}8.27^{b}$	1.42
7	$^{2}3.71^{a}$	$^{2}3.92^{a}$	$^{1}4.95^{a}$	$^{1}6.95^{b}$	$^{6}7.17^{b}$	1.28
8	$^{5}4.56^{a}$	$^{4}4.78^{a}$	$^{4}5.82^{a}$	$^{4}7.84^{b}$	$^{4,7}8.05^{b}$	1.29
9	$^{6}4.17^{a}$	$^{5}4.35^{a}$	$^{5,6}5.31^{a}$	$^{5,6}7.45^{b}$	$^{4}8.18^{b}$	1.42
10	$^{2,7}3.82^{a}$	$^{6}4.12^{a}$	$^{5}5.18^{a}$	$^{5,6}7.41^{b}$	$^{2}7.85^{b}$	1.45
11	$^{3}4.25^{a}$	$^{3}4.49^{a}$	$^{2}5.60^{a}$	$^{5,6}7.57^{b}$	$^{5}8.24^{b}$	1.40
12	$^{7}3.95^{a}$	$^{6}4.15^{a,b}$	$^{6}5.44^{a}$	$^{6}7.61^{c}$	$^{2}7.92^{c}$	1.46
13	$^{2}3.84^{a}$	$^{6}4.08^{a}$	$^{5}5.17^{a}$	$^{5,6}7.45^{b}$	$^{2}7.85^{b}$	1.46
LSD	0.17	0.16	0.16	0.20	0.15	

^{a,b,c}……values on the same row with different superscript are significantly different (p = 0.05).
^{1,2,3}…. Values on the same column with the same superscript are not significantly different (p = 0.05).

Results of the daily amino nitrogen production are shown in table 7. The daily amino nitrogen increased and got to its peak at 72h, still indicating that the fermentation got to its peak at 72h. Statistical analysis showed that the values at 72h were significantly different ($p<0.05$) from other values obtained during the fermentation. The highest value at 72h was obtained from sample 13 (2.28 mg N/g) while the least value (1.46 mg N/g) was obtained from sample 4.

Table 7: Daily amino nitrogen levels during fermentation (mg N/g)

Sample/Run	Period of fermentation (hours)					
	0	24	48	72	96	LSD
1	0	$^{2,3}0.19^{a}$	$^{1}0.97^{b}$	$^{1}1.53^{c}$	$^{1}1.15^{b,c}$	0.50
2	0	$^{1,4,6}0.23^{a}$	$^{2}1.25^{b}$	$^{2,6}1.86^{c}$	$^{1}1.14^{b}$	0.60
3	0	$^{1,2,4}0.21^{a}$	$^{3,4,5}1.08^{b}$	$^{3,5,6}2.02^{c}$	$^{2}0.88^{a,b}$	0.67
4	0	$^{3}0.18^{a}$	$^{3,5}1.13^{b}$	$^{1}1.46^{b}$	$^{1}1.15^{b}$	0.49
5	0	$^{1,4,6}0.23^{a}$	$^{2}1.28^{b}$	$^{3,4,5}2.12^{c}$	$^{1}1.32^{b}$	0.69
6	0	$^{2,4}0.20^{a}$	$^{3,4,5}1.08^{b}$	$^{4,5,7}2.19^{c}$	$^{3,6,7}0.46^{a,b}$	0.79
7	0	$^{1,2,4}0.21^{a}$	$^{4}1.03^{b}$	$^{3,6}2.00^{c}$	$^{4,6,7}0.22^{a}$	0.76
8	0	$^{4,6}0.22^{a}$	$^{4,5}1.04^{b}$	$^{3,5}2.02^{c}$	$^{4,6}0.21^{a}$	0.76
9	0	$^{3}0.18^{a}$	$^{1}0.96^{b}$	$^{4,5}2.14^{c}$	$^{2,5}0.73^{a,b}$	0.74
10	0	$^{5}0.30^{a}$	$^{4}1.06^{a}$	$^{4,7}2.23^{b}$	$^{3,6,7}0.44^{a}$	0.78
11	0	$^{6}0.24^{a}$	$^{3,5}1.11^{b}$	$^{6}1.97^{c}$	$^{5}0.67^{a,b}$	0.66
12	0	$^{2,4}0.20^{a}$	$^{2}1.29^{b}$	$^{4}2.17^{c}$	$^{6,7}0.31^{a}$	0.82
13	0	$^{6}0.24^{a}$	$^{5}1.09^{b}$	$^{7}2.28^{c}$	$^{7}0.40^{a,b}$	0.83
LSD	-	0.02	0.05	0.12	0.18	

[a,b,c]…. Values on the same row with the same superscript are not significantly different (p = 0.05).
[1,2,3]…. Values on the same column with the same superscript are not significantly different (p = 0.05).

Conclusion

Ogiri ugu still remains an indigenous fermented soup condiment that constitutes an important social agro – sustainable business that guarantees an additional in come to families. They are also rich in protein thus serving a dual purpose of flavouring and source of protein supplement. Daily amino nitrogen production showed that fermentation got to its peak at 72h. Sample 13 with fermentation variables of 75% RH, 35^{O}C and 70 µm pore size of wrapping material had the highest value. Thus fermentation at this combination of variables is recommended for optimum fermentation. By manipulation of the fermentation variables products with increased amounts of amino nitrogen and lower levels of peroxide values were produced. Such starters could be explored to improve production and guarantee product quality. This will also create wealth and financial benefits.

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