Fermentation of melon seeds for “Ogiri egusi” as affected by fermentation variables using *Bacillus subtilis*

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Aims: Manipulation of fermentation variables during ‘ogiri egusi’ production using *Bacillus subtilis* was studied with the view to improving the fermentation process and quality of product. The variables studied were relative humidity (RH), temperature and pore size of wrapping material.

Methodology and results: Effect of variables on amino nitrogen, pH and peroxide value was determined on 24 h basis for 96 h. Attempt on optimization of process using response surface method was made. Amino nitrogen increased with fermentation time, the highest value (6.25 mg N/g) being obtained from sample fermented at 75% RH, temperature 35 °C and 90 µm pore size of wrapping material. Fermentation attained its peak at 48 h fermentation time. pH increased into the alkaline range within the period, the highest value (7.81) being from sample that gave the highest amount of amino nitrogen. Peroxide values obtained in all samples were far below the recommended value of 30 Meq/kg. However, the highest value (4.16 Keq/kg) was obtained in sample fermented at 85% RH, temperature 30 °C and 70 µm pore size of wrapper. Statistical analysis and response surface plots associated with analysis showed that the quadratic effect of variables was significant ($p = 0.05$). Effects of relative humidity and pore size of wrapping material were also significant and accounted for 99.56% of the amino nitrogen variation.

Conclusion, significance and impact study: Manipulation of the fermentation variables significantly improved the process. Thus use of response surface method optimized the fermentation process especially the effects of relative humidity and pore size of wrapping materials. The suggested combination of variables for optimum fermentation is 75% RH, temperature 35 °C and 70 µm pore size of wrapper. This ultimately will improve product quality and reduce fermentation time.

Keywords: toxicity, Ogiri egusi, fermentation variables, response surface, *Bacillus subtilis*, amino nitrogen

INTRODUCTION

Melon seeds (*Citrullus vulgaris*) are presently fermented using traditional processes of uncontrolled solid state fermentation in Nigeria (Achi, 2005), where the product (ogiri egusi) is used as a condiment for flavouring soups and stews. The soup condiment is known to have high protein content, thus serving a dual purpose of flavouring as well as source of protein supplement (Abaelu *et al.*, 1990).

Fermentation of ogiri egusi usually takes three to five days at the prevailing ambient temperature (Barber and Achinewhu, 1992; Omafuvbe *et al.*, 2004) and relative humidity. Various workers have identified different microorganisms in fermented melon seeds (Barimalaa *et al.*, 1989; Barber and Achinewhu, 1992; Sanni *et al.*, 2000), however, *Bacillus subtilis* have been identified as the main bacterium involved in the fermentation (Barimalaa *et al.*, 1989).

Ogiri egusi fermentation is still carried out using the traditional method. This involves boiling melon seeds, cooling, wrapping in leaves and fermenting at the

prevailing temperature and relative humidity. The number and layer of leaves used in wrapping the seeds before fermentation vary depending on the individual carrying out the fermentation. Thus the rate of oxygen transfer to the fermenting microorganisms will vary from one batch of fermentation to another. Also the prevailing relative humidity and temperature vary from day to day and within regions and seasons. These variables usually account for the variations in product quality. This chance fermentation as practiced traditionally makes the fermentation difficult to control and results in the contamination of products with pathogens or other microorganisms capable of producing toxins and those that cause off flavours (Ogueke and Nwagwu, 2007). Attempts have been made by some workers (Abaelu *et al.*, 1990; Ogueke and Nwagwu, 2007) to produce ogiri egusi using pure cultures of the bacteria isolated from previous ogiri egusi and fermented oil beans, thus introducing the use of starter cultures.

There is need to optimize the fermentation process with the view to industrializing the production. However, before this can be achieved, there is need to study all the

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fermentation variables and how they interplay with each other, and how they affect the entire fermentation process and final product quality. This research work therefore aims at producing ogiri egusi from melon seeds under controlled conditions of temperature, relative humidity and pore size of wrapping material. The response surface methodology was used to study the optimization process.

MATERIALS AND METHODS

Source of raw material

About 15 kg of melon seeds (*C. vulgaris*) were purchased from Ihiagwa market in Owerri, Imo State, Nigeria in March, 2012. Seeds obtained were dehulled and sorted to remove bad seeds, hulls and extraneous materials. The seeds were stored at 4 °C until used for the study.

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 10 g of sample in 90 mL 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 0.1 mL of suitable dilution was spread onto nutrient agar (NA, Oxoid CM0003). Plates were incubated aerobically at 37 °C for 24-48 h. Representative dominant colonies were picked and purified by streaking on same NA. Stock cultures were kept in nutrient broth containing 20% glycerol and stored at -20 °C. The identification of the isolates was done using the methods described by Ouoba *et al.* (2008).

Before use the identified *B. subtilis* were streaked on tryptone soy agar (BIOTEC) and incubated at 35 °C for 18 h. The cultures were then washed by suspending the cells in 10 mL of 0.1 M potassium phosphate buffer (pH 7.0). This was followed by centrifugation at 14,000 x g for 5 min. using an MSE centrifuge (Minor, UK) (Njoku *et al.*, 1990).

Preparation and standardization of inocula

Cultures from 18 h tryptone soy agar plates (BIOTECH) were examined for purity by microscopic examination of slides prepared from randomly selected colonies. A loopful of the 18 h old culture was inoculated in a flask containing 50 mL 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 14,000 xg for 5 min. The cells were then aseptically re-suspended in 20 mL sterile 0.1 M potassium phosphate buffer (pH 7.0). The cell suspension was then standardized using a spectrophotometer (Model S2100UV, UNICO, New Jersey, USA) at 550 nm wavelength such that 1.0 mL of the suspension contained about 2.0×10^{10} cells.

Fermentation of melon seeds using standardized broth cultures of *B. subtilis*

Seven hundred grams of dehulled melon seeds were properly sorted and cleaned, and extraneous materials removed. The seeds were boiled for 2-3 h until the endosperm softened, then drained and cooled to about 30 °C, placed into a sterilized container and inoculated with 2.0 mL of the standardized broth cultures and mixed. Approximately 350 g were wrapped in polyethylene materials of different pore sizes (50 µm, 70 µm and 90 µm). The 50 µm and 70 µm materials were made of low density polyethylene material while 90 µm material was made of high density polyethylene material. The pore sizes were previously determined by Isu and Njoku (1998). The wrapped melon seeds 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 rotatable response (Lawson and Madrigal, 1994) for k = 3 was employed to study the linear, interactive, quadratic and cubic effects of the independent variables on the fermentation process. The variables were of three levels (Table 1). Samples/Runs 1 – 12 were performed once while sample/run 13 was performed three times to give a total of 15 experimental samples/runs.

Generation of relative humidity (RH)

The various relative humidity were generated as follows:

- (i) 65% RH was generated by dissolving 50 g of anhydrous NaNO₃ in 33.3 mL of hot water.
- (ii) 75% RH was generated by dissolving 20 g of NaCl in 50 mL of hot water.
- (iii) 85% RH was generated by dissolving 25 g of KCl in 50 mL 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 and peroxide value.

Determination of pH

The pH of each fermentation process was measured as follows. Five grams of fermenting seeds was taken from the bulk and mashed in 45 mL 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 grams of fermenting seeds was taken and mashed. Two grams of the mashed seeds was placed in a conical flask, and then 0.5 mL of phenolphthalein (0.5%) and 0.4 mL 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

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

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

for few minutes (until mixture was colorless). This was then titrated with 0.1 M NaOH to pink color. The titer value obtained was designated ' V_1 '. A blank was run by titrating a mixture of 2 mL of formaldehyde solution and 10 mL distilled water with 0.1 M NaOH to obtain ' V_2 '. The amino nitrogen was calculated using the equation: % Amino Nitrogen = $1.7 (V_1 - V_2)$.

Determination of peroxide value

Fifteen grams of fermenting seeds was mashed and 5 g of each of the mashed seeds was placed into a 250 mL glass – stopper Erlenmeyer flask, and 30 mL 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 30 mL of distilled water was added. The mixture was titrated by gradually adding 0.1 N 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 1000 g 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). The response surface plots and their analyses were done using Design – Expert software version 8.0.7.1.

RESULTS AND DISCUSSIONS

The amino nitrogen values obtained during the fermentation of melon seeds increased as fermentation time increased (result not included). At 96 h the highest value of amino nitrogen was obtained in the sample fermented at 75% relative humidity (RH), temperature 35 °C and wrapped with 90 µm pore size material (sample 11), with a value of 6.25 mg N/g. The least value (5.06 mg N/g) was obtained from the sample fermented at 65% RH, temperature 30 °C and wrapped in 50 µm pore size material (sample 9).

Table 2 shows the daily amino nitrogen production during the fermentation period. Close observation of the results showed that in majority of the fermentations the largest amounts were produced at 48 h, thus indicating that the fermentations got to their peaks at 48 h except the sample fermented at 85% RH, temperature 40 °C and wrapped with 70 µm pore size which got to its peak at 72 h. The largest amount of amino nitrogen produced at 48 h was 1.63 mg N/g, and was obtained

Table 2: Daily amino nitrogen levels during fermentation (mg N/g).

Sample/Run	Period of fermentation (h)				
	0	24	48	72	96
1	0	0.36 ^a	0.93 ^{a,e}	0.39 ^{a,d}	0.03 ^a
2	0	0.53 ^b	1.50 ^{b,g}	0.52 ^a	0.38 ^b
3	0	0.53 ^b	1.31 ^c	0.07 ^b	0.25 ^c
4	0	0.41 ^{a,b}	0.39 ^d	1.07 ^c	0.17 ^d
5	0	0.48 ^b	1.06 ^{e,f}	0.30 ^d	0.29 ^c
6	0	0.17 ^c	1.11 ^f	0.22 ^{d,e}	0.14 ^{d,f}
7	0	0.27 ^{c,d}	1.31 ^c	0.16 ^e	0.31 ^e
8	0	0.30 ^d	1.29 ^c	0.14 ^e	0.19 ^d
9	0	0.16 ^c	1.13 ^f	0.07 ^b	0.05 ^{a,g}
10	0	0.41 ^{a,b}	1.28 ^c	0.18 ^e	0.14 ^{d,f}
11	0	1.11 ^e	1.63 ^g	0.13 ^e	0.11 ^f
12	0	0.73 ^f	1.62 ^g	0.11 ^e	0.09 ^g
13*	0	0.99 ^e	1.57 ^g	0.16 ^e	0.15 ^d
LSD	-	0.133	0.153	0.125	0.048

*Average of three readings

^{a, b, c} ... Values on the same column with the same superscript are not significantly different ($p = 0.05$).

from the sample fermented at 75% RH, temperature 35 °C and wrapped with 90 µm pore size wrapping material. This was closely followed by the value obtained from the sample fermented at 85% RH, temperature 40 °C and wrapped with 90 µm pore size material (1.62 mg N/g). It could be that the combinations of the variables favoured the activities of the fermenting bacterium, especially the increased pore size which allowed more O₂ penetration. The fermenting bacterium *B. subtilis* is a highly aerobic organism; therefore more O₂ will increase the rate of growth and general activities of the bacterium resulting in an increased proteinase activity and more amino acid and thus increased amino nitrogen production. Statistical analysis of the values showed that their values at 48 h significantly differed ($p < 0.05$) from the values obtained from the other samples except the 13th and 2nd samples which had values of 1.57 mg N/g and 1.50 mg N/g respectively.

The apparent increase in the fermentation rate and the higher levels of amino nitrogen obtained in the study could probably 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 2.0×10^{10} cfu/mL of cells. The traditional fermentation method usually takes three to five days to complete (Barber and Achinewhu, 1992; Omafuvbe *et al.*, 2004).

Table 3 shows the analysis of variance for the response surface quadratic model for amino nitrogen content of ogiri egusi. p – values less than 0.0500 are significant thus the model response was significant ($p < 0.05$). The effects of relative humidity and pore size were also significant. These variables accounted for 99.56% of

Table 3: ANOVA table for response surface quadratic model (Amino nitrogen).

Source	Sum of	Df	Mean square	F – value	p – value Prob> F
Model	0.14	9	0.015	125.74	<0.0001
A-Relative Humidity	0.036	1	0.036	301.07	<0.0001
B-Temperature	4.948E-005	1	4.948E-005	0.41	0.5504
C-Pore size	0.012	1	0.012	96.72	0.0002
AB	8.473E-005	1	8.473E-005	0.70	0.4405
AC	8.344E-004	1	8.344E-004	6.91	0.0466
BC	4.243E-003	1	4.243E-003	35.12	0.0020
A ²	0.025	1	0.025	208.53	<0.0001
B ²	3.218E-003	1	3.218E-003	26.64	0.0036
C ²	9.703E-003	1	9.703E-003	80.32	0.0003
Residual	6.040E-004	5	1.208E-004		
Lack of fit	5.934E-004	3	1.978E-004	37.20	0.0263
Pure error	1.063E-005	2	5.316E-006		
Cor Total	0.14	14			

the amino nitrogen variation in ogiri egusi (Table 4). Response surface plots associated with the analysis are shown in figures 1 to 3. The effects of relative humidity and pore size of wrapper (AC), and temperature and pore size of wrapper (BC) on amino nitrogen were significant

($p < 0.05$). The p – values were less than 0.0500. The suggested combination of variables for optimum fermentation of ogiri egusi is 75% relative humidity, temperature of 35 °C and 70 µm pore size of wrapping material.

Table 4: Model Summary Statistics for amino nitrogen.

Source	Std. Dev.	R-Squared	Adjusted R-Squared	Predicted R-Squared	PRESS
Linear	0.075	0.5502	0.4276	0.3208	0.093
2FI	0.087	0.5591	0.2284	-0.2611	0.17
Quadratic	0.011	0.9956	0.9877	0.9076	0.013
Cubic	2.306E-003	0.9999	0.9995		+

+ Case(s) with leverage of 1.0000: PRESS statistic not defined

"Model Summary Statistics": Focus on the model maximizing the "Adjusted R-Squared" and the "Predicted R-Squared".

Figure 1: Effect of temperature and relative humidity on amino nitrogen content of ogiri egusi.

Figure 2: Effect of pore size and relative humidity on the amino nitrogen content of ogiri egusi.

Figure 3: Effect of pore size and temperature on the amino nitrogen content of ogiri egusi.

The final Equation in Terms of Actual Factors is as follows:

$$\begin{aligned} \text{Sqrt(Amino Nitrogen)} = & -5.43023 + 0.16989 \times \text{Relative Humidity} + 0.074371 \times \text{Temperature} \\ & - 1.66684\text{E-}003 \times \text{Pore size} - 9.13819\text{E-}005 \times \text{Relative Humidity} \times \text{Temperature} \\ & + 7.16940\text{E-}005 \times \text{Relative Humidity} \times \text{Pore size} + 5.45288\text{E-}004 \times \text{Temperature} \times \text{Pore size} \\ & - 1.10246\text{E-}003 \times \text{Relative Humidity}^2 - 1.50119\text{E-}003 \times \text{Temperature}^2 - 1.47656\text{E-}004 \times \text{Pore size}^2 \end{aligned}$$

While the Final Equation in Terms of Coded Factors is shown below:

$$\begin{aligned} \text{Sqrt(Amino Nitrogen)} = & + 2.51 + 0.063 \times A + 3.021\text{E-}003 \times B + 0.042 \times C - 4.569\text{E-}003 \times A \times B \\ & + 0.014 \times A \times C + 0.055 \times B \times C - 0.11 \times A^2 - 0.038 \times B^2 - 0.059 \times C^2 \end{aligned}$$

The pH values obtained during fermentation of melon seeds increased from start of fermentation till 96 h (Table 5). All the values at 96 h were in the alkaline range, thus it is an alkaline fermentation. Similar trends have been observed during the fermentation of some other protein rich plant materials (Ikenebomeh *et al.*, 1986; Njoku and Okemadu, 1989) and also in agreement with the findings of Ogueke and Nwagwu (2007) who conducted a comparative study of the use of pure cultures of microorganisms isolated from previous ogiri egusi in the fermentation of melon seeds. The highest value obtained at 96 h was 7.81 observed in the sample fermented at 75% RH, temperature 35 °C and wrapped with 90 µm pore size material (sample 11) while the least value (7.16)

was observed in the sample fermented at 65% RH, temperature 30 °C and wrapped with 70 µm pore size material (sample 1). The increase in pH within 24 h was not significant ($p > 0.05$) in all the samples while at 48 h the increase in some of the samples was significant ($p < 0.05$). Significant increases were observed at 72 h and 96 h in majority of the samples. Among the samples, significant differences ($p < 0.05$) were observed but there was no ordered pattern in the differences.

pH increase to the alkaline range is peculiar with fermentations of protein rich foods (Odunfa, 1982; 1985), and the increase has been attributed to increased proteinase and deaminase activities of the fermenting bacterium resulting in the liberation of ammonia through the utilization of amino acids, and possibly other basic end products of protein decomposition (Odunfa and Oyeyiola, 1985; Njoku and Okemadu, 1989; Sarker *et al.*, 1993). These may be responsible for the pungent odour observed in the products, and also with well fermented traditionally produced ogiri egusi. However, high levels of ammonia nitrogen are undesirable in some foods because of their adverse effects on product flavour and aroma. Ammonia production is probably a common feature during fermentation of protein rich plant materials; ammonia is produced during *Bacillus* fermentation of soybean (Sarker *et al.*, 1993), during fermentation of African Oilbean seeds for ugba (Njoku and Okemadu, 1989) and fermentation of African Locust bean seeds for Iru (Odunfa, 1981). Some workers (Yong and Wood, 1977) have suggested that in good quality soysauce ammonia nitrogen should constitute about 10-15% of total soluble nitrogen, thus Njoku and Okemadu (1989) has suggested that there should be monitoring of enzyme activity during fermentation for improved product quality.

Table 5: pH values of the fermenting melon seeds.

Sample/Run	Period of fermentation (h)					LSD
	0	24	48	72	96	
1	^a 5.94 ¹	^a 6.08 ¹	^a 6.13 ¹	^a 6.65 ²	^a 7.16 ³	0.40
2	^{b,d} 6.15 ¹	^b 6.32 ¹	^b 7.18 ²	^{c,f} 7.23 ²	^b 7.31 ²	0.44
3	^c 5.82 ¹	^c 5.90 ¹	^b 7.15 ²	^b 6.82 ²	^b 7.31 ²	0.55
4	^b 6.19 ¹	^d 6.25 ¹	^{a,c} 6.24 ¹	^c 7.12 ²	^{b,d} 7.37 ²	0.44
5	^a 5.96 ¹	^{e,f} 6.12 ¹	^{a,c} 6.15 ¹	^b 6.78 ²	^b 7.28 ³	0.44
6	^b 6.18 ¹	^d 6.25 ¹	^c 6.35 ¹	^{c,f} 7.21 ²	^c 7.80 ³	0.56
7	^d 6.12 ¹	^{d,f} 6.22 ¹	^{a,c} 6.29 ¹	^c 7.16 ²	^e 7.56 ²	0.51
8	^{b,d} 6.14 ¹	^{e,f} 6.16 ¹	^b 7.13 ²	^d 7.40 ^{2,3}	^c 7.75 ³	0.57
9	^{b,d} 6.14 ¹	^d 6.24 ¹	^b 7.08 ²	^d 7.36 ²	^{b,d} 7.39 ²	0.48
10	^a 5.96 ¹	^{e,f} 6.16 ¹	^{a,c} 6.32 ¹	^e 6.94 ²	^{d,e} 7.46 ³	0.49
11	^d 6.11 ¹	^{d,f} 6.21 ¹	^{a,c} 6.27 ¹	^{c,f} 7.21 ²	^c 7.81 ²	0.59
12	^b 6.18 ¹	^d 6.24 ¹	^d 6.91 ²	^f 7.29 ^{2,3}	^c 7.79 ³	0.54
13*	^e 6.05 ¹	^f 6.17 ¹	^{a,c} 6.25 ¹	^e 6.95 ²	^{d,e} 7.45 ³	0.47
LSD	0.05	0.05 ¹	0.20	0.11	0.10	

*Average of three readings

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

However, ammonia nitrogen was not determined in this study.

Table 6 shows the peroxide value obtained from the samples of ogiri egusi fermented at different fermentation variables. Peroxide values of some of the samples increased with fermentation time except for samples 2, 3, 4 and 5 which increased upto 72 h. The highest values were not near the threshold value of 30 milliequivalent of active O₂ per kilogram (Meq/kg) of oil in oil rich foods (Gotoh and Wada, 2006). At 96 h the maximum value of 4.08 Meq/kg was obtained in sample 6 while the least value (3.27 Meq/kg) was obtained in sample 12. The highest value in the entire fermentation (4.16 Meq/kg) was obtained in sample 2. 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 entire fermentation showed that the chances of the product going rancid is very low and thus cannot introduce off flavour to soups and stews prepared with the condiment. Higher values above 30 Meq/kg may represent either the beginning of oxidation or advanced oxidation (Gotoh and Wada, 2006). The generally low

values may be because the fermenting bacterium has very low lipolytic activity.

CONCLUSION

Ogiri egusi still remains an indigenous fermented soup condiment that constitutes an important social agro – sustainable business that guarantees an additional income 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 the highest amounts of amino nitrogen were produced at 48 h, thus reducing fermentation time from 96-120 h which is obtainable in natural fermentation to 48 h. By manipulation of the fermentation variables products with increased amounts of amino nitrogen and lower levels of peroxide values were produced. These variables accounted for 99.56% of the amino nitrogen variation. This method could therefore be explored to improve production and guarantee product quality. This will also create wealth and financial benefits. Thus optimization of the process could be achieved through this means with

Table 6: Peroxide values obtained during the fermentation at different fermentation variables (Meq/kg).

Sample/Run	Period of fermentation (h)				
	0	24	48	72	96
1	2.65 ^b	2.91 ^e	3.06 ^e	3.28 ^{d,e}	3.75 ^b
2	2.80 ^d	3.37 ^a	3.64 ^b	4.16 ^a	3.81 ^b
3	2.69 ^{b,c}	2.76 ^f	3.94 ^a	4.09 ^a	3.52 ^{c,d}
4	2.76 ^{c,d}	3.24 ^b	3.90 ^a	3.45 ^{b,c}	3.50 ^{d,e}
5	2.85 ^{d,f}	2.94 ^e	2.95 ^{f,g}	3.57 ^b	3.62 ^c
6	2.73 ^c	3.20 ^{b,c}	3.25 ^c	3.61 ^b	4.08 ^a
7	2.90 ^{f,g}	2.94 ^e	3.09 ^{e,f}	3.20 ^e	3.30 ^{f,e}
8	2.85 ^{d,f}	3.15 ^{b,c,d}	3.22 ^{c,d,e}	3.38 ^{c,d}	3.52 ^{c,d}
9	3.19 ^e	3.12 ^{c,d}	3.17 ^{c,d,e}	3.24 ^{d,e}	3.36 ^e
10	2.95 ^g	2.97 ^e	3.19 ^{c,d,e}	3.30 ^{d,e}	3.45 ^{d,e}
11	2.5 ^h	2.97 ^e	3.06 ^{e,f}	3.18 ^{e,f}	3.41 ^e
12	3.04 ^a	3.10 ^d	3.12 ^{d,e}	3.22 ^{d,f}	3.27 ^f
13*	2.64 ^b	2.74 ^f	2.81 ^g	3.06 ^f	3.45 ^{d,e}
LSD	0.084	0.086	0.160	0.156	0.104

the suggested combination of variables being temperature 35 °C, relative humidity 75% and 70 µm pore size of wrapping material.

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