

Effect of controlled temperature on the amino acid profile and protease activity of fermented bottle gourd seed

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Received 22nd January, 2019; Accepted 23rd July, 2019

ABSTRACT: The effect of controlled temperature on the amino-acid profile and protease activity of bottle gourd seed (BGS) as well as melon seed (MES) was studied. De-hulled seeds were boiled for 6 hours and fermented at 28°C, 35°C and 42°C for 96 hours. The amino-acid profile of raw, boiled and fermented BGS and MES were determined respectively. The protease activity of the seeds was as well determined at various stages of fermentation. The amino-acid profile of the samples showed high content of glutamic acid (152.1 to 158.8 mg/g for BGS and 145.4 to 166.7 mg/g for MES). Generally, the concentration of the respective amino-acids (essential and non-essential amino-acids) present in the seeds decreased with boiling but increased after fermentation. Fermentation was found to significantly ($p < 0.05$) affect the protease activity of the seeds. The magnitude of the effect was dependent on the seed type, fermentation time and temperature. An increase in fermentation temperature from 28 to 42°C led to an increase in the protease activity of the respective seeds (15.8 to 23.8 units/ml for BGS and 21.6 to 23.0 units/ml for MES) which invariably resulted to the increase in the concentration of the amino-acids present in the respective seeds thereby increasing the bioavailability of the proteins present in the seeds. Microorganisms isolated and identified in the fermenting mashes include; *Bacillus Subtilis*, *Bacillus cereals*, *Micrococcus luteus*, *Staphylococcus aureus* and *corynebacterium sp.* The results showed that fermented BGS could favourably substitute for fermented MES as a source of cheap and affordable dietary protein.

Keywords: Amino-acid, fermentation, gourd, melon, protease, temperature.

INTRODUCTION

Studies on the utilization of vegetable proteins had continued to attract attention globally due to increasing demand for cheap and affordable dietary proteins, particularly among the low income group. Projections based on the current trends indicate a gap between human population and protein supply (Vijayakumari et al., 1997), hence the need to examine unconventional legumes and oil seeds as alternative protein sources for the future (Onweluzo et al., 1994; Chau and Cheung, 1998; Fagbemi, 2007). This development has stimulated research on the utilization of *Lagenaria siceraria*, an indigenous underutilized oil rich seed as alternate protein source.

Bottle gourd (*Lagenaria siceraria*) belongs to *Cucurbitacea* family. The plant is an under-utilized legume cosmopolitan in most tropical and sub-tropical countries. The plant is believed to be a native of Africa and is commonly known as *Luddai* (Hausa). Its stem is prostrate, angular, ribbed, thick, brittle, hairy, up to 9 m long and produces no sap when cut. Leaves are simple, soft and hairy, up to 40 cm long and 40 cm broad. The seeds are edible and used in the preparation of local soups, fermented food product (ogiri), fried cake (robo) and pudding (igbalo or ugbaotiri). The seeds of *Lagenaria siceraria* are rich in dietary protein (Fokou et al., 2004).

Researchers have worked extensively on other species

of *Cucurbitacea* such as *Cococynthis citrullus*, *Citrullus vulgaris* and *Telfaira oceidentalis* (Fagbemi and Oshodi, 1991; Fagbemi et al., 2005; 2006; Fagbemi, 2007). Report on *Lagenaria siceraria* has been limited to its proximate and functional properties (Fokou et al., 2004). The bottle gourd seeds are high in nutrients but have not been used for culinary purposes (Ibeabuchi et al., 2019). In this study, fermentation was employed to utilize the inedible seeds in making edible condiments. Condiments are produced from spontaneous, uncontrolled fermentation of different substrates. Usually, fermentation variables (temperature, time, relative humidity, etc) are not strictly controlled resulting in irreproducible processes and products. Work on its amino-acid composition and amino acid profile is yet to have a wide horizon. Hence, the aim of this paper is to determine the effect of controlled temperature on the amino-acid profile and protease activity of fermented bottle gourd seed (BGS) and melon seed (MES), compare these properties and determine the extent to which fermented BGS can substitute for fermented MES as a good source of dietary protein.

MATERIALS AND METHODS

Sample collection

The bottle gourd seeds (*Lagenaria siceraria*) were collected from the retail sellers of bottle gourd rinds at Ose market in Onitsha, Anambara state Nigeria who discard the seeds, design and sell the bottles or rind for decorations and musical instruments. The discarded seeds were willingly given to the researchers by the retail sellers since they are only interested in the rinds or bottles. The melon (egusi) seeds (*Colocynthis citrullus lanatus*) were purchased from Eke-ukwu, Owerri market in Imo state, Nigeria.

Equipment

The various equipment used for this study were obtained from Department of Food Science and Technology and Department of Soil and Animal Sciences, Dr. Wesly Braide Laboratory, Nekede, Reliable Research Laboratory services, Umuahia, and Department of Zoology Laboratory, University of Jos, Nigeria.

Chemical reagents

All chemical reagents used in this experiment were of analytical grade and as prescribed by the official methods of analysis.

Preparation of 'ogiri' samples

The traditional method of producing *ogiri* was used with

slight modifications. Dry and healthy bottle gourd seeds (*Lagenaria siceraria*) were cracked, de-hulled, washed, and boiled with 3:1 w/w volume of water for six hours until they became soft. The seed (400 g) was then wrapped with tender banana leaves which were briefly passed through flame. The wrap was then boiled for another 30 min in the boiling water. The sample was milled or mashed with mortar and pestle. The mashed sample was then divided into thirty portions of 10 g each and wrapped with the sterile plantain leaves. Ten wraps each were aseptically transferred into two different incubators preset at 35 and 42°C respectively while third batch (10 wraps) was covered properly to avoid contamination and kept on the laboratory bench. The samples were properly labeled and fermentation was allowed to progress for ninety-six hours (96h). The same procedure was followed for melon seed (*Colocynthis citrullus lanatus*). Samples were collected every 24 hours for analysis. The fermented samples were dried and stored in airtight containers for subsequent analysis. The procedure is represented in Figure 1.

Determination of amino acid profile

The amino acid profile in the known sample was determined using methods described Nwabueze (2007). The known sample was dried to constant weight, defatted, hydrolyzed, evaporated in a rotary evaporator and loaded into the Technicon Sequential Multi-sample amino acid analyzer (TSM). The following procedures were involved in the amino acid determination:

Defatting sample

A known weight of the dried sample was weighed into extraction thimble and the fat was extracted with chloroform/methanol (2:1 mixture) using soxhlet extraction apparatus as described by AOAC (1990), the extraction lasted for 15 hours.

Nitrogen determination

A small amount (200 mg) of ground sample was weighed, wrapped in whattman filter paper (No1) and put in the Kjeldhal digestion flask. Concentrated sulphuric acid (10 ml) was added, catalyst mixture (0.5 g) containing sodium sulphate (Na_2SO_4), copper sulphate (CuSO_4) and selenium oxide (SeO_2) in the ratio of 10:5:1 was added into the flask to facilitate digestion. Four pieces of anti-bumping granules were also added. The flask was then put in Kjeldhal apparatus for 3 hours until the liquid turned light green. The digested sample was cooled and diluted with distilled water to 100 ml in a standard volumetric flask. An Aliquot (10 ml) of the diluted solution with 10 ml of 45% sodium hydroxide was put into the Markham distillation apparatus and distilled into 10 ml of 2% boric acid

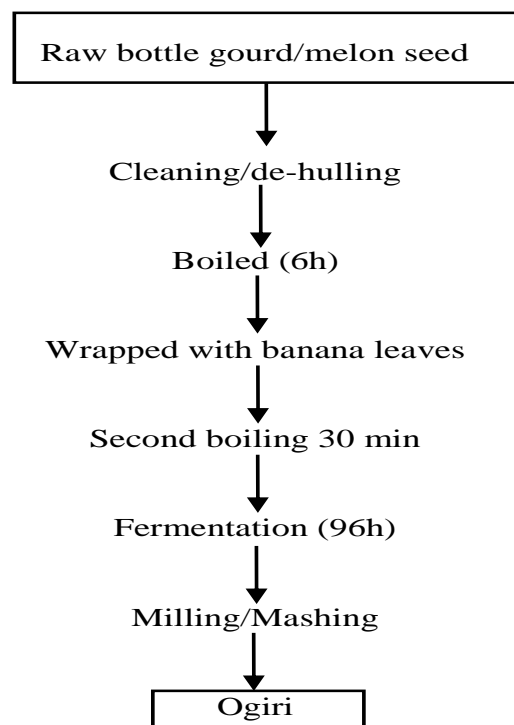


Figure 1. Production of 'ogiri' using bottle gourd and melon seed.

containing 4 drops of bromocresol green/methyl red indicator until about 70 ml of distillate was collected. The distillate was then titrated with standardized 0.01N hydrochloric acid to a grey coloured end point, the percentage nitrogen in the original sample was calculated using the formula:

$$\% \text{Nitrogen} = \frac{(a - b) \times 0.01 \times 14 \times V}{W \times C} \times 100$$

Where: a = Titre value of the digested sample, b = Titre value of blank sample, v = Volume after dilution (100 ml), w = Weight of dried sample, C = Aliquot of the sample used (100 ml) and 14 = Nitrogen constant in mg.

Hydrolysis of the sample

A known weight of the defatted sample was weighed into the ampoule (this is to avoid possible oxidation of some of the amino acids during hydrolysis e.g. methionine and cystine). The glass ampoule was then sealed with Bunsen burner flame and put in an oven pre-set at $105 \pm 5^\circ\text{C}$ for 22 hours. The ampoule was allowed to cool before being broken open at the tip and the content filtered to remove the humins. It should be noted that tryptophan is destroyed by 6N HCl during hydrolysis. The filtrate was then evaporated to dryness at 40°C under vacuum in a rotary evaporator. The residue was dissolved with 5 ml acetate

buffer (pH 2.0) and stored in plastic specimen bottles which were kept in the freezer.

Loading of the hydrolysates into TSM analyser

The amount loaded was 5 to 10 millilitres. This was dispensed into the cartridge of the analyser. The TSM analyser is designed to separate and analyse free acidic, neutral and basic amino acids of the hydrolysates. The period of an analysis lasted for 76 minutes.

Method of calculating amino acid values from the chromatogram peaks

The net height of each peak produced by the chart recorder of TSM (each representing an amino acid) was measured. The half-height of the peak on the chart was found and width of the peak on the half-height was accurately measured and recorded. Approximate area of each peak was then obtained by multiplying the height with the width at half-height of the peak of the standard mixture. This was calculated using the formula:

$$NE = \frac{\text{Area of Norcleucine Peak}}{\text{Area of each amino acid}}$$

A constant S was calculated for each amino acid in the standard mixture: $S_{\text{std}} = NE_{\text{std}} \times \text{Molecular weight} \times \mu\text{MAA}_{\text{std}}$

Where: S= Standard mixture constant for all amino acids, MAA = Micro mole amino acid of standard mixture and NE= Norcleucine equivalent.

Finally, the amount of each amino acid present in the sample was calculated in g/16gN or g/100g protein using the following formula: Concentration (g/100g protein) = $NH \times W @ NH/2 \times C$. And C is calculated as follows:

$$C = \frac{\text{Dilution} \times 16}{\text{Sample wt(g)} \times N\% \times 10 \times \text{vol.loaded} \times W(\text{nl}\mu)}$$

Where: NH= Net height, W= Width@ half height and nl μ = Norleucine

Preparation of extract for determination of proteolytic activities

The method of Adeyemo and Onilude (2013) was used: 1 ml of cold distilled water was added to 5 g of fermenting mash in a mortar and milled with clean pestle and transferred to 100 ml Erlenmeyer flask. An additional 10 ml of Tris-HCl buffer was added and thereafter the flask was stoppered and shaken for 15 minutes. The extract was then centrifuged at 2500 rpm for 10 minutes. The residue was re-suspended in 8 ml buffer subjected to the same

procedure. The supernatant was made up to 20 ml with the Tris-HCl buffer. This serves as the crude enzyme supernatant.

Determination of proteolytic activities

Protease activity was measured using the casein-digestion method of Ogbonna (2007). To 1 ml of 1% (w/v) casein solution pH 8.5, 1 ml of crude enzyme solution was added and incubated for 30 minutes at 40°C. The reaction was stopped by adding 3 ml of 5% trichloroacetic acid by centrifugation at 10000 g for 30 minutes and absorbance of amino acids and peptides released from casein by the proteases was read at 260 nm by a spectrophotometer. All protease activity assays were made in duplicate. Blanks were prepared in which 3 ml of trichloroacetic acid was added to the sample before incubation. One unit of protease activity was defined as the amount of enzyme that will release 100 mg of tyrosine under the specified conditions (pH 8.5, 40°C and 30 min).

Microbial analysis

Cultivation and Isolation of microorganisms

Pour plate method of Ibeabuchi et al. (2019) was adopted. Ten grams (10 g) of each mashed sample was weighed aseptically into 90 ml distilled water and serially diluted up to 10^{-8} . One mille litre (1 ml) each of 10^{-8} dilution was pipetted and spread on nutrient agar and potato dextrose agar plates respectively. This was done in duplicates. The nutrient agar plates were incubated at 37°C for 48 hours while potato dextrose agar plates were incubated at ambient temperature for 72 hours. After the period of incubation, the plates were examined for bacterial counts on nutrient agar plates while fungal counts were observed on potato dextrose agar plates.

Isolation of pure cultures and characterization of isolates

Discrete colonies were picked, sub-cultured and put in a slope. Pure cultures were obtained by restreaking the slope on nutrient agar and inoculated unto sterile nutrient agar slants in screw capped MacCartney bottles. These were incubated at 37°C for 24 hours and preserved at 4°C as stock cultures for further tests. The bacteria isolates were labelled A to E. The isolates were identified first by gram staining, then by biochemical reactions and sugar fermentation tests.

Experimental design

The experiment is a randomized complete block design with the following as sources of variation; Seed x 2,

Temperature x 3, and Time x 5. Thus, we have $2 \times 3 \times 5 = 30$ variables. Therefore, this work is a factorial design with 3 treatments.

Statistical analysis

Data obtained from the study was subjected to analysis of variance (ANOVA) according to the method described by Adesina and Bankole (2013) and run with SAS 9.2 on Windows. The means were separated using Fisher LSD and judged significantly different at 95% confidence level ($p \leq 0.05$).

RESULTS AND DISCUSSION

Amino acid profile of the raw, boiled and fermented bottle gourd seed (BGS) and melon seed (MES) samples

The result in Table 1 showed the amino acid (AA) contents of the raw, boiled and fermented bottle gourd seed (BGS) and melon seed (MES) samples. The most concentrated amino acid in fermented bottle gourd seed (BGS) was glutamic acid with the value of 159.8 mg/g crude protein while the least was methionine with value of 14.1 mg/g crude protein. Glutamic acid was also most abundant with the highest value of 166.7 mg/g crude protein in fermented MES while cystine was the least concentrated in MES with the lowest value of 11.9 mg/g crude protein.

A decrease in some amino acids was observed during boiling but later increased during fermentation (Table 1). This may be due to Amadori rearrangements that may go beyond the deoxy-ketosyl stage. It may also be due to formation of D-amino acids which results from high and prolonged heat treatment (Olaofe et al., 1994). This may be the case because the method used for amino acid analysis detects only L-amino acids from animal and plant proteins that do not produce racemisation (Adeyeye et al., 2010). The amino acids; lysine, histidine, arginine, valine, methionine, isoleucine, leucine, phenylalanine, glutamic acid, serine, glycine, alanine, cystine and tyrosine, for instance, were found to decrease following boiling but increased afterwards during fermentation. The increase in amino acid content with increased fermentation time is acceptable especially from the nutritional point of view. This is a positive development since it means increased digestibility and absorption (Nwabueze, 2007). During fermentation, protein is hydrolyzed to low molecular weight components such as peptides and amino acids due to the action of enzymes produced by bacteria (Kiers et al., 2000). The observed increase in amino acid agreed with Dajanta et al. (2011) who reported an increase in free amino acids, though prolonged fermentation resulted to losses in lysine and other essential amino acids. The increase in amino acid values showed that *ogiri* produced

Table 1. Amino acid profile of raw, boiled and fermented bottle gourd seed (BGS) and melon seed (MES) (mg/g crude protein).

Amino acid	BGS				MES			
	Raw	Boiled	Fermented	Mean	Raw	Boiled	Fermented	Mean
Essential Amino acids								
Lysine	40.5 ^a ± 0.12	40.0 ^a ± 0.29	42.1 ^a ± 0.52	40.87	36.2 ^b ± 0.06	31.1 ^c ± 0.29	44.2 ^a ± 0.57	37.40
Histidine	27.4 ^a ± 0.29	28.4 ^b ± 0.28	28.9 ^a ± 0.06	28.23	23.3 ^c ± 0.12	20.5 ^b ± 0.43	30.3 ^a ± 0.33	24.70
Threonine	43.6 ^a ± 0.37	38.7 ^c ± 0.31	45.5 ^b ± 0.14	42.60	40.0 ^a ± 0.21	35.9 ^a ± 0.00	40.9 ^a ± 0.06	38.93
Phenylalanine	53.2 ^b ± 0.02	52.3 ^c ± 0.16	54.8 ^a ± 0.36	53.43	43.2 ^b ± 0.84	39.1 ^c ± 0.23	45.8 ^a ± 0.02	42.70
Valine	48.1 ^a ± 0.05	40.3 ^b ± 0.03	49.1 ^c ± 0.12	45.83	35.2 ^b ± 0.39	31.5 ^c ± 0.09	38.7 ^a ± 0.06	35.13
Methionine	14.4 ^a ± 0.42	14.1 ^c ± 0.18	14.8 ^b ± 0.55	14.43	15.1 ^b ± 0.07	14.5 ^c ± 0.05	18.7 ^a ± 0.06	16.10
Isoleucine	34.9 ^a ± 0.26	35.5 ^a ± 0.00	41.0 ^a ± 0.49	37.13	45.6 ± 0.44	40.5 ^c ± 0.88	49.1 ^a ± 0.52	45.07
Leucine	71.5 ^b ± 0.11	71.4 ^b ± 0.35	76.5 ^a ± 1.20	73.13	68.3 ^b ± 0.66	59.0 ^c ± 0.08	61.9 ^b ± 0.11	63.07
Non-essential Amino acid								
Glutamic acid	152.1 ^b ± 0.13	153 ^c ± 0.23	159.8 ^a ± 0.2	154.97	145.4 ^c ± 0.56	152.3 ^b ± 0.05	166.7 ^a ± 0.27	154.80
Serine	39.6 ^a ± 0.06	30.9 ^c ± 0.66	33.1 ^b ± 0.02	34.34	33.4 ^b ± 0.22	30.1 ^c ± 0.09	39.1 ^a ± 0.39	34.20
Aspartic acid	103.6 ^a ± 0.05	100.5 ^b ± 0.14	103 ^a ± 0.12	102.37	99.6 ^b ± 0.73	94.3 ^c ± 0.44	108.9 ^a ± 0.21	100.93
Proline	34.6 ^a ± 0.12	33.6 ^b ± 0.22	35.6 ^b ± 0.03	34.67	31.5 ^b ± 0.55	28.5 ^c ± 0.23	36.6 ^a ± 0.05	32.20
Glycine	57.9 ^a ± 0.33	48.4 ^c ± 0.08	58.5 ^b ± 0.04	54.93	51.9 ^a ± 0.48	47.5 ^b ± 0.62	51.9 ^a ± 0.22	50.43
Alanine	41.0 ^a ± 0.22	38.0 ^b ± 0.08	40.3 ^a ± 0.53	39.77	33.4 ^{ab} ± 0.11	30.0 ^b ± 0.41	44.8 ^a ± 0.66	36.07
Cystine	15.7 ^a ± 0.18	15.2 ^b ± 0.51	15.9 ^a ± 0.19	15.60	14.6 ^b ± 0.33	11.9 ^c ± 0.28	16.5 ^a ± 0.67	14.33
Arginine	50.4 ^c ± 0.48	54.0 ^b ± 0.33	58.7 ^c ± 0.02	54.37	56.1 ^b ± 0.25	52.7 ^c ± 0.26	65.6 ^a ± 0.51	58.13
Tyrosine	36.5 ^a ± 0.07	28.6 ^c ± 0.11	30.2 ^b ± 0.21	31.77	31.8 ^b ± 0.23	30.2 ^c ± 0.08	34.9 ^a ± 0.36	32.30

^{abc}Means with the same superscript within the same row are not significantly different ($p \geq 0.05$).

from these seeds were good protein sources and exhibited favourable amino acid profiles. Other authors such as Dajanta et al. (2011) reported increase in values for some of the amino acids in the fermented castor seeds used as condiments. According to the authors, this could be due to synthesis of certain amino acids during fermentation.

The increase in concentrations of most amino acids observed in this work is in agreement with the report of other workers regarding condiments from oil seeds (Enujiugha 2000; Lee et al., 2005; Kpikpi et al., 2009; Dajanta et al., 2011). Sarkar et al. (1993) reported that inedible legumes are made edible during fermentation by extensive hydrolysis of nitrogenous compounds into amino acids. Thus, the increase in percentage protein could be due to either the release of protein from carbohydrate matrix or the generation of amino acids by proteolytic enzymes which increased the measurable nitrogen in the system (Ojinnaka and Ojmelukwe, 2012).

The amino acid profile revealed that among the amino acids, glutamic acid concentration was highest in both the BGS and MES. The glutamic acid (Glu) concentration for raw, boiled, and fermented BGS was 152.1, 153 and 159.8 mg/g crude protein respectively with a mean value of 154.97 mg/g crude protein while the glutamic acid values for MES were 145.4, 152.3 and 166.7 mg/g crude protein respectively for the raw, boiled and fermented seeds with a mean value of 154.8 mg/g crude protein. This was closely followed by aspartic acid with values of 103.6,

100.5, 103 mg/g crude protein for the raw, boiled and fermented BGS respectively with a mean value of 102.37 mg/g crude protein and 99.6, 94.3 and 108.9 mg/g crude protein for the raw, boiled and fermented MES respectively with a mean value of 100.93 mg/g crude protein. Several authors have reported that the presence of glutamic acid, aspartic acid, alanine, glycine and serine in most fermented condiments contribute to the savory flavor of the fermented condiment (Katsura, 1996; Norziah and Ching, 2000; Ouoba et al., 2003; and Aremu et al., 2006). The high content of glutamic acid in soy sauce was reported by Norziah and Ching (2000) where it contributed to the umami taste of the soy sauce and similar products. Davids et al. (2004), Adeyeye et al. (2007) and Khattab et al. (2009) reported that glutamic acid was the most important flavor enhancing amino acid.

Therefore, it could be that the strong savoury flavor and aroma of *ogiri* in foods is contributed by the high content of glutamic acid, aspartic acid and other amino acids.

The amino acid profile of fermented BGS and MES revealed high amounts of essential amino acids (EAA) with values ranging from 14.8 mg/g protein (methionine) to 76.5 mg/g protein (leucine) for BGS while that of the fermented MES ranged from 14.5 mg/g protein (methionine) to 68.9 mg/g protein (leucine). It could be seen that the highest values among EAA were obtained for leucine while the lowest were for methionine in both the BGS and MES. The range of values for the EAA is comparable to the values

reported by previous researchers (Igwe et al., 2012 and Ojinnaka and Ojmelukwe, 2012).

Protease activity of the fermenting BGS and MES as affected by seed type, temperature and period of fermentation

The results in Table 2 and Table 3 showed the effects of seed type and temperature of fermentation on protease activity of bottle gourd seeds (BGS) and melon seeds (MES) during fermentation, while the result in Figure 1 showed the effect of fermentation time on protease activity of 'ogiri' irrespective of the seed type and temperature of fermentation.

Protease activity of 'ogiri' as affected by the seed type

The mean protease activity (22.67 unit/ml) during MES fermentation was significantly ($p < 0.05$) higher than the protease activity (20.27 unit/ml) observed in BGS fermentation (Table 2). This result could connote a high amino acid release as observed above. It was observed that the relative increase in amino acid during fermentation corresponded with increase in activity of protease. This is however not unusual since a similar pattern had been observed by Okpalla et al. (2012): these authors reported a mean protease activity of 25.4 unit/ml during fermentation of melon seeds (*Citrullus vulgaris*). The activity of protease increases the digestibility of the product by breaking down the complex proteins to simpler peptides and amino acids that can be utilized or digested in the body (Ogueke and Nwagwu, 2007). Protease activity has been reported to be abundant in the fermentation of similar protein rich foods (Omafuvbe et al., 2002; 2004).

Effect of fermentation time on protease activity of 'ogiri' irrespective of the seed type and temperature of fermentation

As fermentation progressed, protease activity increased from 23.33 to 32 unit/ml within 24 to 48 hours but dropped to 24 unit/ml at 96 hours (Figure 2). It was observed that at 0 hour of fermentation, there was no protease activity but protease activity increased rapidly within 48 hours irrespective of the temperature but dropped after 72 hours of fermentation. This could be either due to depletion of the necessary nutrients needed to sustain the microbial population growth or accumulation of metabolites in the system or over-population. As a result, the protease activity reduced. The increase in the fermentation rate could probably be due to increase in microbial cell density per unit of fermenting mash as reported by Isu and Ofuya (2000). Similar trend of protease activity was observed on the fermented melon seeds (Table 2). Dike and Odunfa, (2001) reported high protease activity, which, in turn

Table 2. Protease activity of 'ogiri' as affected by the seed type and temperature of fermentation.

Seed	T°C	Protease activity (unit/ml)
BGS	28	15.8± 9.09
	35	21.2± 12.05
	42	23.8± 13.77
	Mean	20.27 ^b ±11.45
MES	28	21.6± 13.39
	35	23.4± 13.89
	42	23.0± 14.23
	Mean	22.67 ^a ±12.84
LSD		2.06

^{abc}Means with the same superscript within the same column are not significantly different ($p \geq 0.05$). T°C = Temperature.

Table 3. Effect of temperature of fermentation on protease activity of 'ogiri' irrespective of the seed type and fermentation time.

Temperature (T°C)	Protease activity (unit/ml)
28	18.7 ^b ± 11.22
35	22.3 ^a ± 12.31
42	23.4 ^a ± 13.21
LSD	2.52

^{abc}Means with the same superscript within the same column are not significantly different ($P \geq 0.05$).

increased the free available amino acid content of the fermented product. The positive effect of fermentation in amino acid content is important from the nutritional point of view as it would increase digestibility and absorption (Ojinnaka and Ojmelukwe, 2012).

Effect of fermentation temperature on protease activity of 'ogiri' irrespective of the seed type and fermentation time

Protease activity was significantly higher ($p > 0.05$) in samples fermented at 35°C (22.3 unit/ml) and 42°C (23.4 unit/ml) but lowest in the sample fermented at 28°C (18.7 unit/ml) (Table 3). Ogueke and Nwagwu (2007) reported increase in protease activities during fermentation of melon seeds which corresponded to the increase in amino acids such as glutamic acid. There have been reports on the liberation of soluble amino acids during fermentation of vegetable seeds into condiments. Ogunshe et al. (2007) reported increased amino acids in the controlled fermentation of *afiyo* (a food condiment). Increase in the level of free amino acids with fermentation has been reported in other leguminous vegetable seeds (Omafuvbe et al., 2000). Isu and Ofuya 2000 also noted a high level of

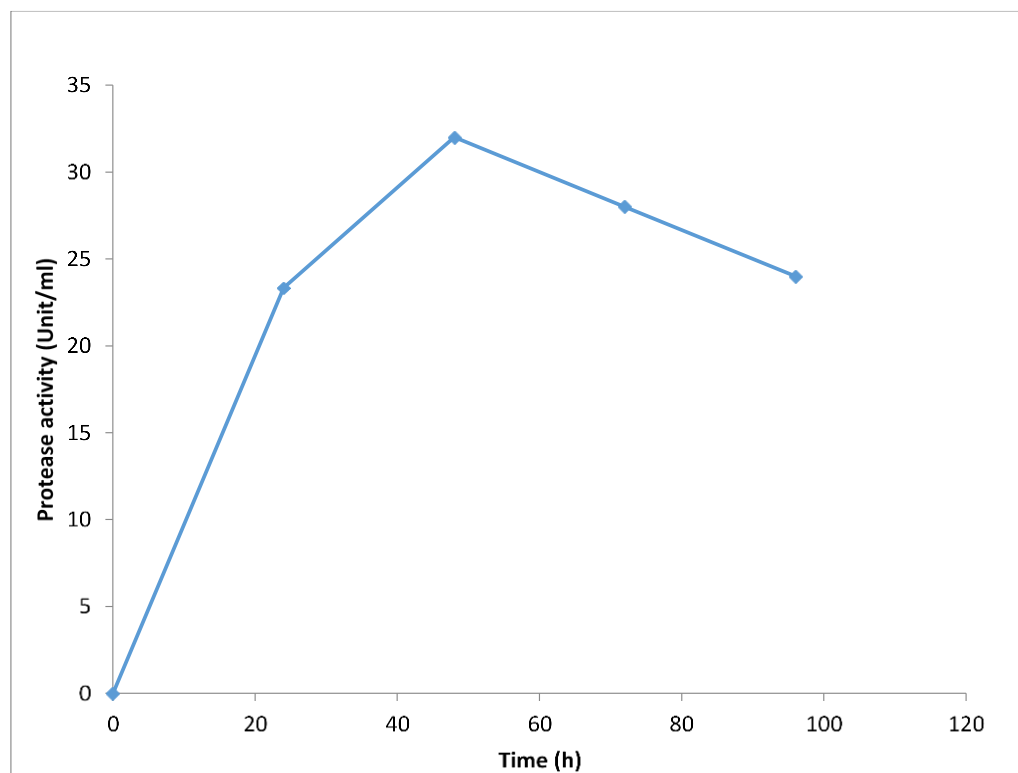


Figure 2. Effect of fermentation time on protease activity of 'ogiri' irrespective of the seed type and temperature of fermentation.

proteolytic activity during *dawadawa* fermentation, which culminated in the formation of peptides and amino acids.

The increase in protease activity at higher temperature may be due to an increased microbial activity, cellular growth and multiplication (Isu and Ofuya, 2000). As a result, all the processes dependent on the microbial enzyme activities also increased. This result implies that fermentation progressed at a faster rate at higher temperatures (35°C and 42°C). This result corresponded with the softening of the fermenting mash, which was faster at higher fermentation temperature.

Microorganisms isolated and identified during fermentation of bottle gourd and melon seeds

Five gram (5 g) positive gours and cocci were isolated from both bottle gourd and melon seeds. They were identified as follows; *Bacillus Subtilis*, *Bacillus cereals*, *Micrococcus luteus*, *Staphylococcus aureus* and *corynebacterium sp.* Ogunshe et al. (2007) observed that *Bacillus* Species occurred most consistently and predominated the fermentation of *Albizia sammon* into aisa condiment. Babalola and Oluwamodupe (2012) also observed that *Bacillus Sp* was the most fermenting microorganisms while Mbajunwa et al. (1998) isolated *Bacillus sp* and *Staphylococcus saprophyticus* from fermenting ugwa samples.

Conclusion

The findings from this work showed that protease activities during fermentation of bottle gourd and melon seeds were greatly influenced by seed type, fermentation temperature and time. Therefore, controlling these variables will help the product commercialization since this will make reproducible products possible. Increase in amino acids contents of the fermented samples showed that poor families can rely on the product for both flavor enhancements of their dishes as well as a supply of essential amino acids. As such, *ogiri* made from fermented BGS can be a cheap and affordable source of dietary protein most especially for these low income group.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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