COMPARATIVE ANALYSIS OF THE PROXIMATE AND AMINO ACID COMPOSITIONS OF "OGIRI" FROM SOYA BEANS (*GLYCINE MAX*), CASTOR OIL SEED (*RICINUSCOMMUNIS*) AND FROM MELON SEED(*COLOCYNTHIS VULGARIS*)

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ABSTRACT

Ogiri soya (OS), OgiriUgba (OU) and OgiriEgwusi (OE) fermented condiments were produced from Glycine max Riscinuscomminus and Citrullus vulgaris respectively and their proximate as well as their amino acid properties were analysed and compared. Quantitatively, crude protein and ash contents were highest in Ogiri soya had the highest with such values as 39.58±0.10% and 6.40±0.00% while OgiriUgba had the highest quantities of crude fat, 43.16±0.13%and 12.52±0.16% crude fibre. OgiriEgwusi had 10.55±0.93% carbohydrate. The three samples had high quantities of energy ranging from 2362.32KJ/100g in Ogiri soya to 2435.38 KJ/100g in Ogiriegwusi. Among the eighteen amino acids assayed, the highest concentrations obtained in g/100g unit, Aspartic acid 17.49±0.04, 11.53±0.00. 6.82±0.02 for OU, OS and OE respectively while their respective values for Glutamic acid were 16.05±0.05, 15.89±0.02 and 11.28±0.08 for OU, OS, and OE. Ogiri soya had higher values in terms of the essential amino acids while OE had higher values for arginine 6.80±0.38, Glycine 3.61 and Threonine. The use of Ogiri condiments from these substrates is recommended for consumers.

Keywords: *Soya Beans, Castor oil seed, Melon seed, Ogiri soya, Ogiriugba, Ogiriegwusi, Proximatecomposition, Amino acid profile*

INTRODUCTION

Ogiricondiment is of great importance to the "Igbos" (the South Eastern occupants of Nigeria). "Ogiri" also called "Ogiriisi" is a product of fermentation of such oil seeds as castor oil seed (*Ricinuscommunis*), melon seeds (*Citrullus vulgaris , Colocynthis vulgaris or other varieties*), fluted pumpkin seed (*Telferiaoccidentalis*), soy beans *(Glycine max)*, Ojinnaka and Ojimelukwe, (2012); Nzelu and Onyekwere (2017); Jideani and Okeke, 1991; Odibo and Ume (1989); Barber and Achinewhu, (1992)Akhuemonkhan and Badaru (2000) ; (Nzelu 2006 and 2007), Nwosu, and Ojimelukwe (2000); Omafuvbe*et al* (2004) and Dimejesi and Odibo(2017) among other raw materials. Castor oil bean,*Ricinuscommunis*, is a major oil seed belongs to Leguminousae family and has been known since ancient time. In the subtropical zones, its plant attains 11-13meters height even in the wild, Ojinnaka and Ojimelukwe (2013). It requires a temperature range of between 20^{o}C and 26^{o}C with low humidity throughout the

growing season so as to obtain, maximum yields for which reason its cultivation is limited to the tropical areas of the developing world.Soya bean originated in eastern Asia and is still widely cultivated in China, Japan, Korea, and other countries but USA is the current largest producer of Soya beans, (Unilever 1975) Beddows (1988). Soya bean is able to thrive inextreme temperatures, from tropical Brazil and Nigeria, to the snows of Japan. In addition to its potential as a prime cash crop, it has attractive features nutritionally due to its high content of protein, fat, with poly unsaturation, minerals and low starch content.Soya beans (*Glycinemax* (L) Merrill) belonging to the plantate kingdom and processed into numerous products, (Chukeatirote 2015). Such products include kinema, thuanato, soy-daddawa and Ogiri, (Chukeatirote 2015, Omafuvbe*et al* 2002).“Ogiri” is prepared by traditional methods of uncontrolled solid substrate fermentation resulting in extensive hydrolysis of the protein and carbohydrate component,Achi (2005). At the same time, its quality is unpredictable as the varying environment and techniques used Ogueke*et al* (2013). However, the processor may modify some of the traditional steps. The production typically involve five steps namely (1) the boiling of the dehulled seeds, (2) cooling, (3) mashing of the softened seeds, (4) wrapping the substrates mash in natural leaves and (5) fermenting the mash at the prevailing temperature and humidity. Through the fermentation process, the anti-nutritional factors in the oil seeds are reduced or eliminated in the product, flavourous compounds are developed (Mensah, *et al.,* 1990; Manandhar (1995) and their characteristics ammonical taste enhances the tastes of foods, beverages and drugs containing them. The fermentation also improves digestibility and nutritive value of the raw samples, (Achi, 2005). It has been reported that Bacteria in genus Bacillus were responsible for the fermentation of these fermented food products, (Ojinnaka and Ojimelukwe, 2013). Through proteolytic activities the quality of the fermented product is enhanced with such attributes as improved protein quality, texture as well as characteristics aroma and taste. According to Alais and Linden (2009), flavour enhancing substances confer as a rule a new aroma while the development of strong odours seems to be the rule in foods where *Bacillus subtilis* dominates, (Dirar 1993).The micro-organisms predominant in the fermentation to produce “Ogiri” include *Bacillus* species, especially *B. subtilis, B.pumilus,B. brevis, B. macerana, B. polymyxa* and*B. licheniformis* (Omafuvbe, *et al* 2000 and 2004; Maureen Theodore *et al* (2013). Sarkar*et al*, (1997) indicted *Bacillus subtilis* as the most dominant naturally fermenting agents in Soybeans. Soya bean, *Glycine max,* has a prominent role in the world food and agriculture, mainly because of its ability to be used in many forms, (Ashok *et al*, 2010).Against the background of the significance and nutritional benefits of Ogiri, a traditional fermented condiment, the object of this study was to evaluate the proximate and amino acid profiles of “Ogiri soya”, “Ogiriugba” and “OgiriEgwusi” from fermented soya beans, castor oil seeds and melon seeds.

MATERIALS AND METHODS

Sources of Materials

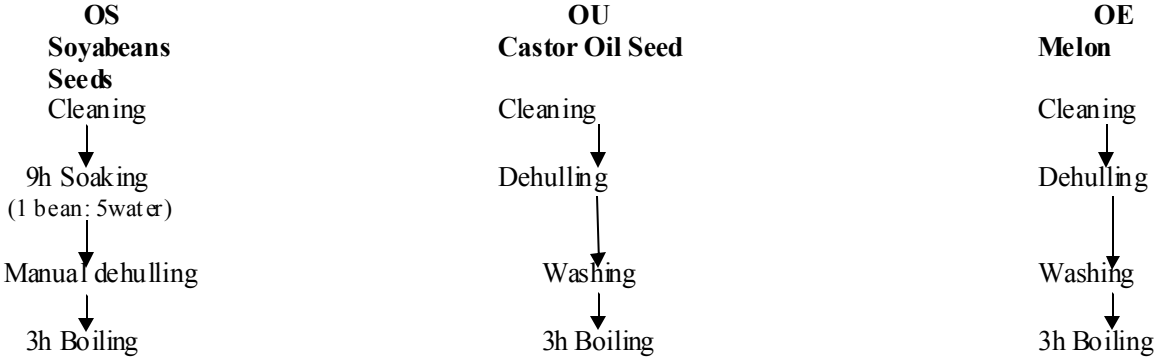
The raw materials soya bean (*Glycine max*), castor oil seed (*Ricinuscommunis*) and from melon seed (*Citrullus vulgaris*) were bought from Eke Oko market, Orumba North, Anambra State of Nigeria.

Production of Ogiri Soya bean

Soya bean seeds were cleaned by winnowing, sorting, and removal of pieces of string, stones and other extraneous materials as shown in Figure 1. The cleaned golden coloured seeds were soaked for 9h in excess potable water. The soaked beans were then removed into a stainless steel pot to which potable water had been added and the beans were boiled for 60mins. The boiled beans were then removed from the pot and cooled to about 30^0C, a manageable temperature for the manual removal of the beans' hulls. The dehulledbeans were wrapped with aluminum foil paper and boiled for 3h after which the wraps were pierced using tooth picks and, incubated by the fire side for the next 4 days. The wraps were turned from one side to the other thrice daily. On the fourth day, stainless steel hand grinder (Corona brand) was sterilized in boiling water and used to grind the fermented beans while the used aluminium foil paper was discarded. The ground soya beans, mash wrapped with aluminum foil paper for secondary fermentation. The proximate and amino acid profiles of the Ogiri Soya wereanalyzed on the fourth day of secondary fermentation. The results were recorded on Table 2.

Production of OgiriUgba(OU) from Castor Oil Beans, and OgiriEgwusi(OE) from Melon Seeds:

The traditional method of processing "ogiri" was used. Two kilograms each of dehulled seeds were washed and boiled using stainless steel pots and with about 4 liters of potable water per sample. Cooking continued until the seeds were very tender as judged by the processor. Technically, effort was made to allow the water dry off avoiding the sticking of the seeds to the pot or the charring of the seeds. The cooked seeds were transferred to different sieves to aid cooling and the draining off of remaining water (if any). With clean and sterilized grinding machine, the very soft seeds were ground separately and the mash of each sample was wrapped separately. However about one teaspoon of heaped ash from burnt palm bunch was added and ground into the melon seed mash/paste so as to impart the gray colour to the OE. These wraps were kept by the fire side at night but taken out on stainless steel trays and allowed for incubation at the prevailing ambient temperatures in the environment. At this time of global warming, minimum average temperatures are usually around 30°C to 40°C in many parts of Nigeria. By the fourth day, the "ogiri" samples OU and OS were analysed for their proximate and amino acid compositions using the Benitez (1989) method.



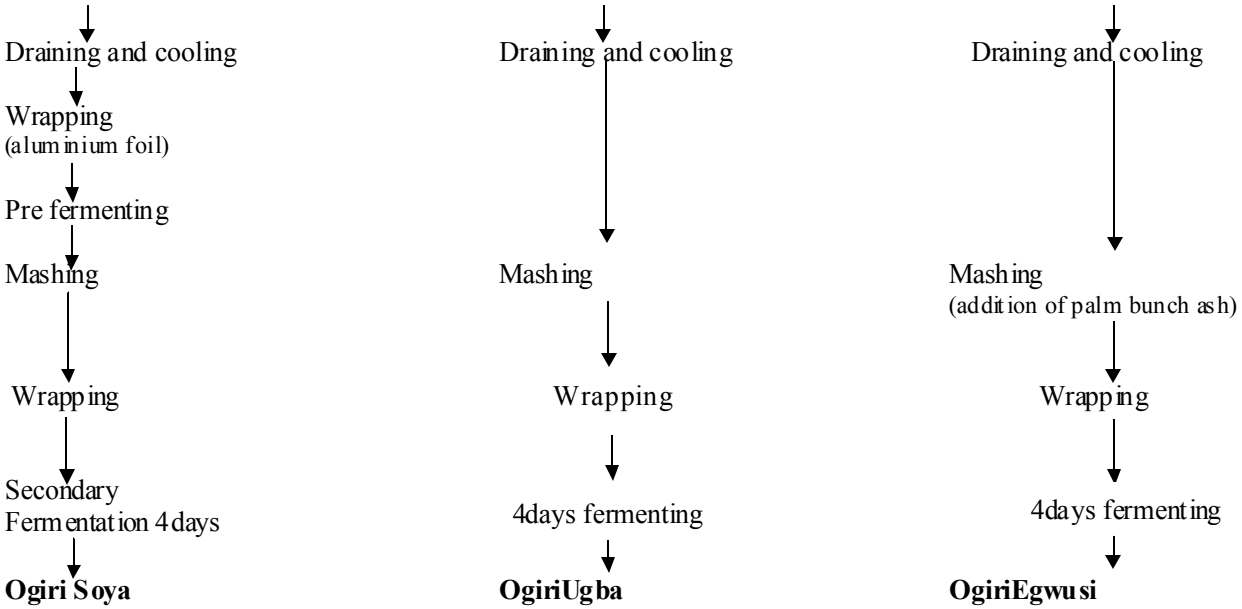


Fig. 1: Flow charts for production of Ogiri Soya, OgiriUgba and OgiriEgwusi

Proximate composition of the Ogiri Samples

The Proximate composition of the Ogiri Samples was determined using the standard methods of AOAC (2010). The energy values were calculated using the factors: 4.1 (for carbohydrates and protein); 9.4 for the fat (Fox and Cameron 1989) and the results were recorded on Table 1.

Amino Acid analysis:

The Amino Acid profile was determined by Benitez (1989) method. The Technicon Sequential Multiple (TSM) Amino Acid Analyzer (Technicon Instrument Corporation, New York) was used to determine the different amino acid composition of the three Ogiri samples. The amino acid values reported were the averages of three determinations.

Amino Acid profile

Two point zero grams of each "Ogiri" sample was weighed into the extraction thimble and the fat was extracted with chloroform/methanol (2:1 v/v) mixture, using soxhlet apparatus [AOAC 2005]. The extraction lasted for between 5-6 hrs. Thirty milligrams each of the defatted "Ogiri" samples was weighed into three separate glass ampoules. Seven millitres of 6M HCL was added to each ampoule and oxygen expelled by passing nitrogen gas into the samples. The glass ampoules were sealed with Bunsen flames and were placed into an oven at 105°C for 22hrs. The ampoules were allowed to cool, and filtered to remove the humins. The filtrates were then evaporated to dryness at 40°C, using a rotary drier under vacuum. Each residue was dissolved with 5ml acetate buffer (pH 2.0) and stored in a plastic specimen bottle, and lastly analysed for their amino acid contents. Tryptophan was determined by Maria et al (2004) method. The "ogiri" samples were individually hydrolysed with 4.2m sodium hydroxide. The known samples were each dried to constant weight, defatted, hydrolyzed,

evaporated in a rotary drier evaporator and loaded into the Applied BiosystemsPhenylthiohydration (PTH) Amino acid Analyser. Results were recorded on Table 2

Statistical Analysis

Data were collected on triplicate determinations and means ± standard deviations (SD) were computed. Data obtained were analyzed by one way analysis of variance (ANOVA) using the SPSS .version 17 (2014) statistical procedure and significance was accepted at 0.05 level of probability.

RESULT AND DISCUSSION

The result obtained from the analysis are recorded on Tables 1 and 2

Table 1: Proximate Composition of the OS,OU and OE Ogiri samples (Dry Matter)

Parameters	Ogiri Soya (OS)	OgiriUgba (OU)	OgiriEgwusi (OE)
Crude protein	39.58^{a} ± 0.10	32.71^{c}± 0.10	37.29^{b}±0.48
Fat	39.65^{c}± 0.36	43.16^{a}± 0.13	40.82^{b}± 0.22
Ash	6.40^{a}± 0.02	3.06^{c}± 0.04	4.20^{b}± 0.20
Crude fibre	4.36^{b}± 0.07	12.52^{a}± 0.36	4.44^{b}± 0.24
Carbohydrate	7.10^{b}± 0.21	7.24^{b}± 0.15	10.55^{a}± 0.93
Energy KJ/100g	2362.32KJ/100g	2391.90KJ/100g	2435.38KJ/100g

Values are means ± Standard deviation of triplicate determinations. Means with the different letter along a row showed significant difference ($p < 0.05$).

The result on Table 1, reveals that the crude protein content of the"Ogiri" samples ranged from 32.71±0.10% for OU through 37.29±0.48% for OE to 39.58±0.10% for OS. These crude protein concentrations agree with the results of Davis and aderibigbe (2010) where 24.60% to 32.00% protein was reported for fermented melon seeds, and with Nzelu, and Onyekwelu (2017) who reported a range of 24.78% to 35.08% for fermented castor oil seed. Legumes and oil seeds used for production of these condiments are usually high in proteins and fats. Besides, the microbial enzymes which catalyze the fermentation process, and consequently affect colour, flavour as well as texture are proteins, and also enhance the quality of these foods (Onwuka 2014). Fermented protein foods are used mainly for flavour enhancing condiments and gourmet food ingredients due to the meaty and appetite-stimulating flavour of protein hydrolysate which were formed during the fermentation, (Campbell-Platt 2009). The lipid content of the "Ogiri" condiments was also high and ranged between 39.65±0.36% and 43.16±0.13%. OU had the highest lipid content while the OS had the least content quantitatively. However these lipid contents agree with the fat contents reported by Nzelu and Onyekwelu (2014) for fermented castor oil seed product, and the 35.28% reported by Akinyele and Oloruntoba (2013), as well as with the range of 38.40% to 43.20% fat from fermented melon seeds reported by, David and Aderibgbe (2010). The fats supply energy for metabolic activities as well as the essential fatty acids for maintaining proper health.

The fat content of OE and oil in the study varied with the values (36.30% and 37.50% respectively) reported by Omafuvbe*et al* (2004). Some of these changes can be attributed to the soil, age, and the variety of the seeds. About 50%, 45% and a range of 44.80% to 53.40% oil have been reported as the oil content of the raw soya beans, castor oil seed and melon

seed by Beddows, (1988), Unilever (1975) and David and Aderigbe 2010. From records, the fat content of the fermented products are usually lower than that of the substrate. The ash content obtained on this study (2.8% to 4.44%) agreed with those reported by other researchers for similar products Nzelu and Onyekwelu (2017)

The crude fibre contents in the "Ogiri" samples ranged between 4.36±0.07% and 12.52±0.36% and 24.08% with the OU value being the highest. The crude fibre4.44±0.24% obtained in this study is lower than 15.6% and 11.59% reported by Omafuvbe et al (2004) and Akinyele and Oloruntoba (2013) respectively, for OE samples. Fibre is the non-starch polysaccharide carbohydrate portion of plants (i.e., celluose) that helps to maintain structural rigidity, Murano (2003). The three "Ogiri" samples have the potential to release a range of 2362.32KJ/100g to 2435.38KJ/100g to the diet when used.

Amino Acid profile of the Ogiri Samples

The amino acid profile of the soyabean "Ogiri soya", (OS), Castor oil seed "ogiri" (OU) and Melon seed "Ogiri" (OE) samples are shown in Table 2. Significant differences existed in the amino acid compositions of the samples except in isoleucine, methionine, proline and threonine contents of the "Ogiri" samples. The results reveal an increase in the concentrations of the essential amino acids in the "Ogiri" products which is in agreement with the reports of some other researchers regarding condiments from oil seeds (Ojinnaka and Ojimelukwe, 2012). Eighteen amino acids were detected. Nine essential amino acids (EAA) including Histidine and, nine other non-essentials (NEAA) were detected. Both essential (EAA) and non-essentials (NEAA) amino acids were present in various concentrations. Generally the EAAs, (designated (E) from the OS were quantitatively higher than the EAAs from OU and OE. For the neutral amino acids, Leucine had highest concentration, yielding 5.95% for OU, 7.00% for OE and 8.20% for OS, followed by Lysine where OE, OU and OS had 5.04%%, 6.04% and 6.36% respectively.For Histidine, the respective concentrations for the "Ogiri" samples were 2.04% OU, 3.13% for OE and 3.19% for OS. In this study, Tryptophan, cystine and methionine were the major limiting amino acids in the samples, a condition which is similar to "kinema", a fermented product form soyabeans, Sarkar*et al.* (1997). Besides, proteins from nut and seeds are rather low in tryptophan, Lee (1975)

Glutamic and Aspartic acids had the highest concentrations among all the amino acids. Glutamic acid is recognized as a flavor agent (Okeke and Elochukwu (2013). Its sodium salt, monosodium L-glutamate (MSG) has a sweet taste and has thus found application as a flavour enhancing salt in the food industry. Sweetness is a property, not only of sugars but also of lead acetate, saccharin, aspartame, sugar alcohols and other substances (Murano 2003). According to Ward (2010), the flavour enhancing properties of Sodium glutamate were discovered in Japan in the twentieth century. Production of glutamic acid and several other amino acids such as Lysine is carried out through a fermentation process using mutants of*Corynebacteriumglutamicum, (*Willey *et al* 2009*)*. According to Jeleń (2012), both Glutamic acid and Aspartic acid contribute to the Umami, (neither sweet, nor bitter, nor salty nor sour) tastes but described as savory and delicious sensation (Murano 2003; Onwuka 2014). Umami flavour enhancers are largely based on amino acids and nucleotides.

According to Yasuda *et al* (1994), it is well known that glutamic acid and aspartic

acid contribute to the pleasant Umami taste or savoury enhancement of foods.

Higher quantities of the rest of the EAAs were obtained from OS,Interestingly, OS had higherconcentrations in seven out of the EAAs and this was significant ($p < 0.05$). Methionine and Cystine (both sulphur containing amino acids)are limiting amino acids in plants and animal foods (Fox and Cameron 1989). All the "Ogiri" samples are from leguminous seeds and this could explain the lower concentrations of these amino acids. Methionine takes part in the synthesis of choline, component bile salts. Cysteine, Cystine and Methionine are all sulphur containing amino acids, and constitute the main source of sulphur in the diet. The body can make cysteine from methionine. Cystine is one of the main amino acids of insulin and is formed from cysteine in the body.

Table 1 also reveals that the "Ogiri" samples had energy values of between 2362.32KJ/100g and 2435.38KJ/100g.

Table 2.0: Amino Acid Profile of the Ogiri Samples (g/100g)

Amino Acid	Ogiri Soya (OS)	OgiriUgba (OU)	OgiriEgwusi (OE)
Leucine (LEU)	$8.20^{a} \pm 0.20$	$5.95^{c} \pm 0.05$	$7.00^{b} \pm 0.01$
Lysine (LYS)	$6.36^{a} \pm 0.14$	$6.04^{b} \pm 0.03$	$5.04^{c} \pm 0.04$
Isoleucine (ILEU)	$3.60^{a} \pm 0.70$	$3.50^{a} \pm 0.50$	$3.80^{a} \pm 0.20$
Phenylalanine (PHE)	$4.61^{a} \pm 0.30$	$3.72^{b} \pm 0.30$	$4.26^{a\,b} \pm 0.26$
Tryptophan (TRP)	$0.92^{a} \pm 0.02$	$0.84^{a} \pm 0.04$	$0.74^{a\,b} \pm 0.06$
Valine (VAL)	$4.74^{a} \pm 0.04$	$4.59^{a} \pm 0.50$	$3.33^{b} \pm 0.03$
Methionine (MET)	$1.07^{a} \pm 0.10$	$1.07^{a} \pm 0.07$	$1.34^{a} \pm 0.30$
Proline (PRO)	$3.45^{a} \pm 0.05$	$3.86^{a} \pm 0.46$	$3.96^{a} \pm 0.04$
Arginine (ARG)	$6.71^{a} \pm 0.40$	$5.16^{b} \pm 0.16$	$6.80^{a} \pm 0.38$
Tyrosine (TYR)	$3.27^{a\,b} \pm 0.50$	$2.49^{b} \pm 0.40$	$3.44^{a} \pm 0.30$
Histidine (HIST)	$3.19^{a} \pm 0.19$	$2.04^{b} \pm 0.04$	$3.13^{a} \pm 0.10$
Cystine (CYS)	$1.63^{b} \pm 0.03$	$2.06^{a} \pm 0.10$	$0.85^{c} \pm 0.05$
Alanine (ALA)	$4.44^{b} \pm 0.22$	$5.19^{a} \pm 0.19$	$3.72^{c} \pm 0.04$
Glutamic ACID (GLU)	$15.89^{b} \pm 0.02$	$16.05^{a} \pm 0.05$	$11.28^{c} \pm 0.08$
Glycine (GLY)	$3.37^{b} \pm 0.05$	$1.99^{c} \pm 0.11$	$3.61^{a} \pm 0.00$
Threonine (THRE)	$3.44^{a} \pm 0.40$	$3.45^{a} \pm 0.40$	$3.00^{a} \pm 0.06$
Serine (SER)	$5.54^{a} \pm 0.04$	$4.48^{b} \pm 0.08$	$3.40^{c} \pm 0.08$
Aspartic Acid	$11.53^{b} \pm 0.00$	$17.49^{a} \pm 0.40$	$6.82^{c} \pm 0.02$
Total Amino Acid (TAA)	91.96	89.97	75.52

Values are means ± Standard deviation of triplicate determinations. Means with the same letter along a row showed no significant difference ($p > 0.05$).

Table 2 summarizes the total (protein: bound plus free) amino acid profile of the soyabean Ogiri soya, (OS), Castor oil seed Ogiri (OU) and Melon seed Ogiri (OE).

The proximate composition of the Ogiri sample (see Table1) reveal that the increase in the concentrations of the essential amino acids in the Ogiri products is in agreement with the reports of some other researchers regarding condiments from oil seeds Ojinnaka and Ojimelukwe (2012). Eighteen amino acids were detected by the Benitez (1989) method. Nine essential amino acids (EAA) including Histidine and, nine other non-essentials (NEAA) were detected. Both essential (EAA) and non-essentials (NEAA) amino acids were present in various concentrations. Generally the EAAs, (designated (E) from the OS were

quantitatively higher than the EAAs from OU and OE (see Table 1). For the neutral amino acids, Leu had highest concentration, yielding 5.95% for OU, 7.00% for OE and 8.20% for OS, followed by Lysine where OE, OU and OS had 5.04%%, 6.04% and 6.36% respectively.ForHistidine, the respective concentrations for the Ogiri samples were 2.04% OU, 3.13% for OE and 3.19% for OS. Going by Lee's classification, adopted by Sarkar*et al* (1997), where the amino acids obtained were grouped into acidic, basic and the amino acids in this study resulted to 29.81%, 37.28%, 23.97% of acidic; 17.68%, 14.72%, 19.82% of basic; and 8.55%, 6.40%, 10.20% of aromatics OS, OU and OE Ogiri samples respectively. Sarkar*et al* (1997) reported the acidic, basic and aromatic acid from their kinema, a product from fermented soya beans, as 20.8%, 15.1% and 13.0%. In this study, tryptophan, cystine and methionine were the major limiting amino acids in the samples, a condition which is similar to "kinema", a fermented product form soyabeans, Sarkar*et al* (1997), and general for legumes.

Glutamic and Aspartic acids had the highest concentrations among all the amino acids. Glutamic acid is recognized as a flavor agent (Okeke and Elochukwu (2013). Its sodium salt, monosodium glutamate (MSG) has a sweet taste and has thus found application as a flavourenhancing salt in the food industry. According toCampbell-Platt (2009) and Ward (2010), the flavour enhancing properties of Sodium glutamate was discovered in Japan in the twentieth century (in the 1950's) and a fermentation process for its production by *Corynebacteriumglutamicum* currently supplies large quantities of the salt in the world market annually through morden biotechnology. According to Jeleń (2012), both Glutamic acid and Aspartic acid contribute to the Umami, (neither sweet, nor bitter, nor salty nor sour) tastes.

Higher quantities of the rest of the EAAs were obtained from OS,Interestingly, OS had higherconcentrations in seven out of the EAAs and this is significant (P>0.05). Methionine and Cystine(both sulphur containing amino acids)are limiting amino acids in plants and animal foods (Fox and Cameron 1989). All the Ogiri samples are from leguminous seeds and this could explain the lower concentrations of these amino acids. These are sulphur containing amino acids and sulphur is a component of nails and hairs. Methionine takes part in the synthesis of choline, component bile salts. Cysteine, Cystine and Methionine are all sulphur containing amino acids, and constitute the main source of sulphur in the diet. The body can make cysteine from methionine. Cystine is one of the main amino acids of insulin and is formed from cysteine in the body.

CONCLUSION

The results from the study have shown that "Ogiri" from the three raw materials (substrates) have high contents of protein, fats and energy values. "Ogiri" soya yeilds results in additionally better amino acids profile especially when the essential amino acids are considered. The higher contents of Glutamic and Aspartic acids in the amino acids' composition of "OgiriUgba" (OU), "Ogiri"egwusi (OE) and "Ogiri"soya(OS) explain the flavourous nature of "Ogiri" samples, especially now that Umami tastes have been linked to "Ogiri" condiments. Furthermore the "Ogiri" condiments would contribute to energy and protein intake as well as nutrition of the consumers. By this, they would and the thus contribute to the food security in developing countries.

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