POTENTIAL OF EGUSI AND NERI SEEDS AS SOURCES OF POLYUNSATURATED FATTY ACIDS AND AMINO ACIDS FOR TABLE EGGS ENRICHMENT

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ABSTRACT
Egusi (Melon seeds) and Werewere (Neri seeds) are rich in proteins, oils, and excellent sources of amino acids. However, literature on the presence of essential polyunsaturated fatty acids (PUFA's) are not conclusive. The study objective was to ascertain the potential of these indigenous seeds as sources of polyunsaturated fatty acids (PUFA) and amino acids for egg enrichment. Proximate composition and amino acid profiling were obtained by AOAC procedures. The PUFAs were evaluated using the Gas Chromatography technique. Results showed higher crude fiber in Neri seeds (28.3%) than Egusi (7.6%). The moisture content was similar (Egusi 9.5% and Neri 9%). The ash content of Egusi was higher (3.5%) compared to Neri seeds (2.5%). The ether extract (crude fat) was high in Egusi (44%) than Neri (34%). The essential amino acids (arginine: 5.387%, 3.582%; methionine: 0.823% 0.650%; lysine:1.128%, 0.744%; threonine: 1.245%, 0.770%; isoleucine: 1.334%, 0.931%; leucine: 2.550%, 1.591%; valine: 1.537%, 1.078%; histidine: 0.867%, 0.591%; and phenylalanine: 1.795%, 1.260% in Egusi were higher as compared to that in Neri seeds. Both seeds were rich in linoleic acid, n: 6 PUFA but not in n: 3 PUFA. In conclusion, both seeds are good sources of omega-6 fatty acids and amino acids but not omega-3 fatty acids needed for egg enrichment.

Keywords*: Egusi, Werewere, proteins, fatty acids, amino acids, omega-3 eggs*

INTRODUCTION
Egusi (Melon seeds) and Werewere (Neri seeds) are highly nutritious plants in the watermelon family called *Colocynthis citrullus* L and *Citrullus lanatus* subspecies mucosospermus respectively. Egusi melon and Neri are a native of West Africa and are largely cultivated in Nigeria, Ghana, Northern Namibia, Sierra Leone, and Senegal. Both are members of the *cucurbitaceae* family and they belong to the tribe Benicaseae. *Cucurbit sp.* can thrive both in temperate and tropical countries. They belong to the economically most important vegetable crops worldwide (Paris, 2001). The primary part of the crops, which is the seed, is used in delicacies like soup and stew (sauce).

The Egusi seed is rich in oil and proteins just like soybean (*Glycine max)* comprising 50% oil and 35% protein (Sarwar *et al.,* 2013). However, the full profile of the oil and protein contents including essential fatty acids and amino acids are unknown or scantly reported. The seeds have both nutritional and cosmetic importance. Both Neri and melon seed are alleged to be used to prepare a meal for pregnant women in Africa for breastmilk yield purposes. The seeds contain vitamins C and B2, minerals, riboflavin, fat, carbohydrates, and protein (Lazos, 1986). Being

in the watermelon family Egusi could be a rich natural source of lycopene, a carotenoid of great interest because of its antioxidant capacity and potential health benefits (Edwards *et al*., 2003). Lycopene is protective against a growing list of cancer (Cho *et al*., 2004; Sahin *et al*., 2016). The melon seed is among the most popularly consumed plant foods that are oilseeds (Oluba *et al*., 2008).

Because both Neri and melon seeds are known to produce a lot of edible oils and usually used as rich food source to increase breast milk yield in local communities it is hypothesized that the oil component could have some level of omega-3 fatty acids, which will enhance the potential of omega-3 eggs development. Therefore, the seeds can make a considerable contribution towards attaining a balanced diet (Fokou *et al*., 2004) especially because of its high levels of crude protein and amino acids. In poultry, melon seeds in various processing forms have been used to feed broiler and reported that processing of melon seeds by cooking and fermentation made the protein in melon seed more available to the broiler chicks, and also removed some or all of the anti-nutritional factors in raw melon seed (Oloyede et al 2004). The objective of the study was to profile the polyunsaturated and amino acid contents and ascertain the availability of indigenous n:3 enriched seeds in Ghana as a potential substitute for flaxseed oil for eggs enrichment for omega-3 fatty acids eggs or designer eggs locally.

MATERIALS AND METHODS

Study Area

Seeds of Egusi (Melon seeds) and Werewere (Neri seeds) were obtained from the open market and transported to the Department of Animal Science, KNUST, Kumasi, which lies on 06o 41‟N and longitude 01o 33‟W with an elevation of 261m above ocean level with an ambient temperature between 23° to 31°C (Adomako, 2009; Meteorological Services Department, Kumasi, 2015).

Determination of Proximate Composition

Samples of the fresh seeds were used for proximate analysis following the procedures of the Association of Official and Analytical Collaboration International (AOAC International, 1995). In the analysis, 2 g of ground seeds from each of the two seeds were weighed and oven-dried at 135^{o}C until a constant weight was attained to determine the moisture content. The Soxhlet extraction method was used to determine the fat content. Kheldjal method was used to determine the crude protein fraction. Ash, the inorganic residue was obtained by burning a sample of the seed at 600^{o}C in a furnace. The crude fiber was determined by the digestion method. The individual amino acid composition of each seed was determined through collaborative and consultative work at the Evonik Africa (Pty) Limited laboratory in South Africa.

Fatty acids determination

To determine the presence of n:3 polyunsaturated fatty acids, the seeds were subjected to fatty acids extraction and identification at the lipid laboratory at the Department of Agriculture, Food and Nutritional Science; University of Alberta, Edmonton, Canada. In this analysis, 2 g of each seed was placed in a 50 ml falcon tube and digested using electronic ultrasonic homogenizer in the presence of 20 ml of hexane. The seed and its content were completely shredded and broken to release the oil content. The content was allowed to stand overnight at room temperature to allow the particles to settle. The oil content, which was part of the hexane, was gently taken out with a Pasteur pipette into a 1.5 ml micro-centrifuge tube leaving the slurry behind. The hexane was allowed to evaporate and the oil collected for analysis using the Gas Chromatography technique (Agilent Technologies, 6890N, Network GC Sys Jms-Q1000GC (A) Ultra Quad GC/MS). The total fatty acids profile including saturated, monounsaturated, and all polyunsaturated were analyzed. The n:3 fatty acids and n:6 fatty acids that were targeted included, α-linolenic acid (C18:3 n-3), docosatrienoic acid (C22:3 n-3), eicosapentaenoic acid (C20:5 n-3), docosapentaenoic acid (C22:5 n-3), docosahexaenoic acid (C22:6 n-3), linoleic acid (C18:2 n:6), γ-linolenic acid (C18:3 n:6), nonadecadienoic acid (C19:2 n:6), eicosadienoic acid (C20:2 n:6), dihomogamma-linolenic acid (C20:3 n:6), arachidonic acid (C20:4 n:6), docosadienoic acid (C22:2 n:6), docosatetraenoic acid (C22:4 n:6), docosapentaenoic acid (C22:5 n:6) and 11,14,17-eicosatrienoic acid (C20:3 n-

3). (De Oliveira *et al*., 2013) with the help of specific fatty acids standards.

Amino acids determination
The individual amino acid composition of each seed was determined through a collaborative and consultative work at the Evonik Africa (Pty) Limited laboratory in South Africa.

Data analysis
Data obtained were summarized using a Microsoft Excel spreadsheet, transformed to percentages, and compared.

RESULTS AND DISCUSSION

Proximate composition
Table 1 shows the proximate composition on a dry matter basis for Egusi and Neri. Both Egusi and Neri seeds were rich in oil, protein, and some other types of energy-rich fatty acids. Neri seeds recorded a high crude fiber content when compared with Egusi. This was because the seeds used were the readily available seeds on the market. Whereas Egusi seeds were dehulled and the Neri seeds were not. Similar quantities of water were recorded in Egusi (9.5%) and Neri (9%). The moisture content of the seeds falls within other recorded results of 7 – 10% (Arkroyed and Doughty, 2000). The ash content of Egusi in this study was high (3.5%) but is similar to previous results (Ojeih *et al*., 2008). Pomeranz and Clifton (1981) recommended that the ash content of seeds and tubers for animal feed formulation should be in the range of 1.5 to 2.5%. In the present study, the ash content of Neri was 2.5%. The crude protein value of both seeds compares favorably with plant protein-rich ingredients such as soybean, cowpeas, pigeon peas, and pumpkin with protein contents ranging between 23.1 and 33.0% (Olaofe et al 1994). This protein value of both seeds falls above or within the recommended daily allowance for children between 0 and 10 years (13.0 – 28.0 g/ day) (NRC, 1989). The Egusi seed is rich in oil and protein like soybean (*Glycine max)* comprising 50% oil and 35% protein (Oloyede et al 2004). Besides, Egusi seed meal has been noted to contain a high amount of protein to be used as animal feed (Wadhwa and Bakshi 2016). The ether extract (crude fat) content of 44% obtained for Egusi in this study agrees closely with that reported by Oluba et al (2008). The oil content of the Egusi appears very high compared to that obtained for soybean (23.5%) (Paul and Southgate 1987). With the high amount of crude fat obtained for Egusi in this study, Egusi melon is regarded as an oilseed (Sarwar et al 2013). However, the crude fat was lower in Neri compared to Egusi.

Amino acid profile
From tables 2 and 3 it can be seen that when both seed samples were standardized to a dry matter content of 88% they contained nine (9) of the essential amino acids. These were arginine (5.387%, 3.582%) recording the highest value, methionine (0.823% 0.650%), lysine (1.128%, 0.744%), threonine (1.245%, 0.770%), isoleucine (1.334%, 0.931%), leucine (2.550%, 1.591%), valine (1.537%, 1.078%), histidine (0.867%, 0.591%), and phenylalanine (1.795%, 1.260%) for Egusi and Neri respectively. It can be seen that Egusi recorded higher values for the essential amino acids than Neri did. Both seeds also contained considerable quantities of other nonessential amino acids like alanine, aspartic acid, and glutamic acid. Other conditional amino acids including cysteine, serine, glycine, and proline were observed and were higher in Egusi compared to Neri. Conditional amino acids are usually not essential except in times of stress and illness where they can be converted to important amino acids for utilization of the body. The results show that arginine, glutamic acid, and aspartic acid were the three most abundant amino acids in Egusi and Neri. In another study, it was reported that the total amino acid of Egusi is 33.3% and that of Neri is 22.7% (Aremu *et al*.,

Table 1: Proximate Analysis of Egusi and Neri Seeds

Seeds	Moisture (%)	Ash (%)	Crude Protein (%)	Fat (%)	Crude Fibre (%)	NFE (%)
Egusi	9.50	3.50	35.00	44.00	7.55	0.45
Neri	9.00	2.50	25.9	34.00	28.33	0.27

2006). The results show that Egusi and Neri are rich in amino acids especially arginine, isoleucine, and leucine. The high nutrient profile of Egusi, especially amino acids compares favorably with known protein-rich foods such as soybean, cowpeas, pigeon peas, and pumpkin (Ojieh *et al*., 2008).

Fatty acid profile

Table 4 shows the fatty acid composition of Egusi and Neri. It can be seen that palmitic acid (10.65%, 16.03%) heptadecanoic acid (6.18%, 6.565), stearic acid (10.10%, 10.855), oleic acid (11.77%, 12.42%), and linoleic acid (61.18%, 54.03%) are the principal fatty acids in Egusi and Neri respectively with arachidonic acid (0.11%) for both Egusi and Neri. The linoleic acid content of Egusi and Neri in this study is close to that reported by Oluba *et al*. (2008). Findings from these results show that both Egusi and Neri are richer in linoleic acid than animal fat, which is richer in oleic acid (29% – 48%) (NRC, 1994). Younis *et al*. (2000) worked on *Cucurba pepo* seed, which is in the same family as Egusi and Neri, and reported similar results. They found that the seed contains mostly palmitic, oleic, stearic, and linoleic acids with linoleic acids being the most. The results also show that Egusi and Neri contained no n:3 PUFAs, which was highly unexpected because of their abundant use for nursing mothers to increase their breast milk yield in some West African communities. However, they were rich in n:6 PUFA. Egusi seed contained a total PUFA of 61.29% while 54.14% was recorded for Neri, which is mostly linoleic acid (essential fat). Using these seeds in poultry feed will not meet the intended of objective of n-3 enrichment of egg but will increase

Table 2: Total contents of Amino acids after hydrolysis of protein Egusi (Melon seed)

Amino Acid	Content (%)[a]	AA (%) in CP	Content (As is)
Methionine*	0.823	2.201	0.849
Cysteine	0.551	1.475	0.569
Methionine + Cysteine	1.374	3.675	1.418
Lysine*	1.128	3.017	1.164
Threonine*	1.245	3.331	1.285
Arginine*	5.387	14.406	5.558
Isoleucine*	1.334	3.567	1.376
Leucine*	2.550	6.820	2.631
Valine*	1.537	4.111	1.586
Histidine*	0.867	2.320	0.895
Phenylalanine*	1.795	4.800	1.852
Glycine	1.760	4.707	1.816
Serine	1.523	4.072	1.571
Proline	1.267	3.388	1.307
Alanine	1.563	4.181	1.613
Aspartic acid	3.011	8.053	3.107
Glutamic acid	6.448	17.245	6.653
Total (without NH_3)	32.789	87.693	33.832
Ammonia	0.559	1.496	0.577
Total	33.348	89.189	34.409

**Essential amino acid; [a]Figures standardized to a dry matter content of 88%; AA = Amino acid; Dry matter (%): 90.80; CP = Crude protein; CP (%): 37.39; CP (% as is): 38.58.*

the n-6 levels that are already high in diets of Africans. The study results imply that using Neri and Melon seeds as sources of n-3 fatty acids in enrichment of eggs is untenable.

Fatty acid profile

Table 4 shows the fatty acid composition of Egusi and Neri. It can be seen that palmitic acid (10.65%, 16.03%) heptadecanoic acid (6.18%, 6.565), stearic acid (10.10%, 10.855), oleic acid (11.77%, 12.42%), and linoleic acid (61.18%, 54.03%) are the principal fatty acids in Egusi and Neri respectively with arachidonic acid (0.11%) for both Egusi and Neri. The linoleic acid content of Egusi and Neri in this study is close to that reported by Oluba *et al*. (2008). Findings from these results show that both Egusi and Neri are richer in linoleic acid than animal fat, which is richer in oleic acid (29% – 48%) (NRC, 1994). Younis *et al*. (2000) worked on *Cucurba pepo* seed, which is in the same family as Egusi and Neri, and reported similar results. They found that the seed contains mostly palmitic, oleic, stearic, and linoleic acids with linoleic acids being the most. The results also show that Egusi and Neri contained no n:3 PUFAs, which was highly unexpected because of their abundant use for nursing mothers to increase their breast milk yield in some West African communities. However, they were rich in n:6 PUFA. Egusi seed contained a total PUFA of 61.29% while 54.14% was recorded for Neri, which is mostly linoleic acid (essential fat). Using these seeds in poultry feed will not meet the intended of objective of n-3 enrichment of egg but will increase the n-6 levels that are already high in diets of

Table 3: Total contents of Amino acids after hydrolysis of protein Werewere (Neri)

Amino Acid	Content (%)[a]	AA (%) in CP	Content (As is)
Methionine*	0.650	2.426	0.677
Cysteine	0.350	1.308	0.365
Methionine+ Cysteine	1.000	3.733	1.042
Lysine*	0.744	2.777	0.775
Threonine*	0.770	2.874	0.802
Arginine*	3.582	13.368	3.731
Isoleucine*	0.931	3.475	0.970
Leucine *	1.591	5.937	1.657
Valine *	1.078	4.024	1.123
Histidine*	0.591	2.207	0.616
Phenylalanine*	1.260	4.704	1.313
Glycine	1.428	5.331	1.488
Serine	1.101	4.110	1.147
Proline	0.823	3.071	0.857
Alanine	1.096	4.092	1.142
Aspartic acid	2.028	7.571	2.113
Glutamic acid	4.314	16.102	4.494
Total (without NH_3)	22.338	83.375	23.270
Ammonia	0.389	1.451	0.405
Total	**22.727**	**84.826**	**23.675**

**Essential amino acids; [a]Figures standardized to a dry matter content of 88%; AA = Amino acid; Dry matter (%): 91.67; CP = Crude protein; CP (%): 26.79; CP (% as is): 27.91.*

Table 4: Fatty acid Composition of Egusi and Neri seeds

SEEDS (%)	Methyl Palmitate C16:0	Methyl Heptadecanoate C17:0	Methyl Stearate C18:0	Methyl Oleate C18:1	Methyl Linoleate C18:2	Methyl Arachidate C20:0
Egusi	10.65	6.18	10.10	11.77	61.18	0.11
Neri	16.03	6.56	10.85	12.42	54.03	0.11

Africans. The study results imply that using Neri and Melon seeds as sources of n-3 fatty acids in enrichment of eggs is untenable.

CONCLUSION AND RECOMMENDATIONS

Based on the results, Egusi and Neri seeds did not appear to be a good alternative for omega-3 PUFA enrichment substances in poultry eggs. Both seeds were richer in other saturated fatty acids, rich in protein, and rich in all the essential amino acids. In addition, other non-essential and conditional amino acids were observed. The two seeds could serve as protein and energy sources rather than omega-3 PUFA sources.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest regarding the submission of this article.

ACKNOWLEDGMENTS

The authors express heartfelt appreciation to Grand Challenges Canada, Saving Brains, the VOA farms, Kumasi for supporting infrastructure, the Department of Animal Science, KNUST, the Department of Agriculture Food and Nutritional Science, and Evonik Africa for supporting the laboratory analysis. Particular thanks to Mr. Prince Manu-Barfo of Adisio (formerly of Evonik).

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