Evaluation of Amino Acids and Fatty Acids Profile of A Complementary Food Developed From African Walnut and Fermented Maize Flour

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Abstract: *This work aimed at formulating and evaluating the fatty acids and amino acids profile of complementary food developed from African walnut and fermented maize flour. African walnut, turmeric roots and maize grains (Zea Mays) were processed separately into flour and formulated into blends containing 25 to 45% African walnuts. The most preferred blend was evaluated for amino acids and fatty acids profiles using standard methods. The fatty acids result revealed saturated fatty acids (14.25%) and total unsaturated fatty acids (85.75%). The results of amino acids ranged from 0.79-13.18%. Amino acids score of 51.4 was obtained for this complementary food. This show that the most preferred blend containing high essential amino acids and fatty acids required to support children growth.*
The study has shown that an acceptable nutrient-dense complementary food can be made from African walnut.

Keywords: Protein quality, African walnut, complementary food

INTRODUCTION

The development of high-protein foods of plant origin is essential in developing countries because of the shortage and high cost of animal protein. Consumption of such products plays a major role in combating malnutrition, which is a serious problem in the developing countries. Malnutrition in children is a major nutritional problem in African developing countries which leads to morbidity and mortality, retardation in physical growth and mental development, working capacity and increased risk of adult disease [6]. The growth of infants and young children in their first two years of life is very rapid. Breast feeding only will not be sufficient for the infant's nutritional requirement. After about six months of age, the infant needs complementary foods particularly food of adequate nutrient density [1].

Several researchers have worked to improve the nutrients in cereal based food by combining with legumes such as bambara nut, soya beans, African yam beans [11]. These are underutilized legumes and they are hard to cook. African walnut is an under-utilized nut that can be used to improve the nutritional composition of cereals. African walnuts cook faster, ready to eat and very rich in protein [8; 7; 13]. African walnut could be explored to improve the nutritional composition of fermented maize flour (*ogi)* for wider appeal.

African walnuts are largely underutilized in developing countries like Nigeria with high wastage in the farm due to short shelf life of the nut [9; 5]. Despite the economic importance of conophor nut, no commercial production and industrial utilization of the crop takes place in Nigeria. Based on this, complementary food can be developed from walnut and fermented maize flour for infants, children and elderly people. Natural spices such as ginger and turmeric are good antioxidants that prevent lipid oxidation in food product and extend its shelf life. These could be added to the formulated food. The quality of protein in foods is determined by its essential amino acids composition and digestibility and bioavailability of its amino acids, according to the Food and Agriculture Association and the World Health Organization (FAO/WHO). The objective of this study was therefore to develop a complementary food from African-walnut and fermented maize flour for wider appeal. The researcher also intends to evaluate the nutritional profiles (fatty acids and amino acids profiles) of the complementary food.

MATERIALS AND METHODS

Flour Preparation:

African walnut and maize grain (suwan1) and turmeric were purchased from Ibadan, Oyo State, Nigeria. ''Ogi'' flour was produced according to [2] by soaking maize grains in water for 72 hours. The steeping water was decanted and the softened kernel / maize were wet milled and sieved to remove the germs and hulls. The "ogi" slurry was allowed to ferment naturally in clean plastic bucket anaerobically for 48-72 hours and the excess water was squeezed out to give "Ogi" cake. The cake was dried in hot air oven at 60 ^{o}C for 12 hours and dry milled to produce "Ogi" flour which was packaged in clean polyethylene bag.

African walnut flour was produced according to the method of [1]. The nuts was sorted, and washed to remove adhering contaminants and then cooked for 30 minutes in sulphited water to the removal of the shell and then soaked in 0.2% potassium metabisulfite for 5mins to facilitate the deshelling process. The deshelled walnut was size reduced with a stainless steel kitchen

knife to increase surface area and then soaked to remove the bitter taste for 30mins and also blanched by adding into boiling water and allowed standing for 5mins before draining. This help to reduce the tannin content of the walnut. The blanched walnut will be dried in hot air oven at 60 ^{o}C for 5-10 hours and ground to produce walnut flour. Then walnut flour will be packaged in clean polyethylene bag. Tumeric powder was produced according to the method of [1]. The turmeric roots were washed in sterile water after peeling and then dried in air oven at 50 ^{0}C 5hrs and ground to powder and sieved using 0.25mm mesh screen and finally packaged.

Food formulation

The food samples were formulated with reference to FAO/WHO 2013; 2003 standards for infant/ children protein-energy requirement (10-15 g/ day). Different samples were prepared by combining 74.5%, 69.5%, 64.5%59.5% and 54.5% fermented maize flour, 25%, 30%, 35%, 40% and 45% African walnut flour with a constant 0.5% of Turmeric powder (Table 1). The flours were then made into porridges.

Sensory evaluation:

The sensory attributes were evaluated on the complementary food for color, flavour, appearance, mouth feel, taste and overall acceptability using 9-point hedonic scale, where 1- dislike extremely and 9-like extremely. All the formulated blends were made separately into gruels (20% w/v) by making a smooth cold paste and gradually pouring same into boiling water. This was stirred vigorously until the entire mass became viscous. One (1) teaspoon of sugar was added to each sample to taste and serving to a panel of 50 judges consisting of staff and students of the University who were very familiar with the porridge. The data obtained were subjected to appropriate statistical analysis.

Fatty acids determination

Fatty acids profile was carried out according to [4]. The sample was defatted with petroleum ether using soxhlet apparatus. 50mg of the estimated fat content of the sample was saponified for five minutes at 96^{0}C with 3.4ml of 0.5M KOH in dry methanol. The mixture was neutralized by using 0.7M HCL. 3 ml of the 14% borontriflouride in methanol was added. The mixture was heated for 5 mins at the temperature of 90^{0}C to achieve complete methylation process. The fatty acid methyl esters were thrice extracted from the mixture with redistilled n-hexane. The content was concentrated to 1ml for gas chromatography analysis and 1 ul was injected into injection port GC.

Amino acids determination

The amino acids profile in the known sample was determined using method described by [12]. The known sample as dried constant weight was defatted using kjeldhal method (digestion, distillation, and titration), before amino acids analysis. A known weight of defatted sample was weighed into glass ampoule and hydrolyzed using 6N HCL and oxygen was expelled by passing nitrogen into the ampoule. The glass ampoule was then sealed with flame and oven dried at 105 ^{o}C for 22 h. The ampoule was cooled, the content was filtered with muslin filter and the filtrate was evaporated to dryness at 40 ^{o}C in a rotary evaporator. The residue was dissolved with 5ml of acetate buffer (pH 2.0) and stored in plastic bottle. About 5-10 microlitre of the hydrolysate was loaded into TMS Analyser to analyse the amino acids of the hydrolysate. The chromatogram peak produced was then used to calculate the amino acids.

Statistical Analysis:

Data generated were subjected to analysis of variance (ANOVA) using SPSS and treatment means that are significantly different were compared using the Duncan's multiple range test to separate the means with the aid of System Analysis System (SAS).

Table 1: Formulations of African-walnut based complementary food.

Blends	Fermented Maize Flour (%)	Walnut flour (%)	Turmeric(%)
A	100	-	-
B	74.5	25	0.5
C	69.5	30	0.5

D	64.5	35	0.5
E	59.5	40	0.5
F	54.5	45	0.5

KEY:

A(FMF:WF:T, 100:0 :0), B(FMF:WF:T,74.5:25:0.5),C(FMF:WF:T,69.5:30 :0.5)

D(FMF:WF:T, 64.5:35:0.5), E(FMF:WF:T,59.5:40:0.5),

F(FMF:WF:T 54.5:45 :0.5)

N:B :FMF-Fermented maize flour, WF- Walnut flour, T-Turmeric.

RESULTS AND DISCUSSION
Sensory evaluation of the formulated blends
The results of the sensory evaluation of gruels prepared from the food formulations are shown in Table 5. Out of 9, the colour scores of the experimental diet ranged from 7.7-7.9, while that of "ogi" and coerelac were 6.4 and 6.0 respectively. The aroma of the formulated blends ranged from 5.7 to 6.4, while that of "ogi" and cerelac were 6.3 and 7.5. The mouthfeel of the formulated blends ranged from 5.4 to 6.5, while that of "ogi" and cerelac were 7.5 and 7.0 respectively. Acceptability scores ranged from 5.5 to 6.5 in the formulated blends, while "ogi" (7.6) was the overall best in acceptability, followed by cerelac (7.5). Out of the formulated blends, the results showed that the blend C was the most preferred with the highest score of (6.5). This was followed closely by blend B (6.5), blend D (6.2), blend E (5.9) in that order. Sensory analysis results showed that the complementary food had highest value in term of color and generally acceptable by the panelists. While the result of control samples (*ogi* & cerelac) in term of aroma, taste and mouth feel were slightly higher when compared with the formulated blends The disparity between the overall acceptability of formulated food samples and that of cerelac and *ogi* could be due to the familiarity of the panelist with the *ogi* and cerelac over new formulated product.

Fatty acids profiles of the formulated blends
The fatty acids composition of the most preferred blend (blend C) of the complementary food showed that linoleic acid has the highest concentration (55.38%), followed by oleic acid (29.27%), palmitic acid (12.45%), stearic acid (1.8%), linolenic acid (0.87%) and palmitoleic acid (0.23%) respectively. The linoleic was the dominant fatty acid in the polyunsaturated fatty acid. For the monounsaturated fatty acid, oleic acid was the dominant fatty acid, while arachindonic acids and docohexanoic acids were not detected. The total mono unsaturated fatty acid (MUFA) was 29.5% while total poly unsaturated fatty acid (PUFA) was 56.25% (Table 4). For the saturated fatty acids (SFA), palmitic acid was the most dominant fatty acids, while stearic acid was the least. The total saturated fatty acid (14.25%) and total unsaturated (85.72%) well exceeded the WHO/FAO recommendation of 3.5, 44.8-52.9 respectively. Research study suggest that Mediterranean diet that is rich in mono-unsaturated fatty acids, and omega-3 fatty acids help to prevent coronary artery disease and strokes by favoring healthy blood lipid profile. Eating just as much as 25g each day provides about 90% of RDI (recommended daily intake). Essential fatty acids are important for brain and neutral tissue development of children. The evidence for abnormal development of children on a low intake of essential fatty acids in the western worlds is becoming clear. Malnourished children have low level of essential fatty acids, particularly n-3 poly unsaturated fatty acids [10].

Amino acids composition of the formulated blends
The amino acids of African walnut ranged from 9.85g/100g for glutamic acid and 0.39g/100g for glycine, while that of blend C (most preferred blend) ranged from 13.18g/100g for glutamic acid to 0.79g/100g for cystine (Table 3). Table 3 showed that raw African walnut flour and the most preferred blend (blend C) contained high essential amino acids required to support infants and

children growth. Out of all the amino acids found in *tetracarpidium conophorum* and blend C, glutamic acid contains the highest concentration, followed by aspartic acid, arginine, leucine, valine, lysine, proline, alanine, isoleucine and phenylalanine respectively while glycine (0.53g/100g) was the least concentration in the raw African walnut, and the most preferred blend C (0.79g/100g). Lysine has the limiting amino acids score (51.4) for this complementary food.
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Table 2: Sensory attributes of formulated blends compared with a commercial Complementary food (cerelac) and "ogi".

Blends	Colour	Aroma	Taste	Appearance	Mouthfeel	Overall acceptability
A	6.4^b	7.5^a	7.3^a	7.5^a	7.5^a	7.6^a
B	7.9^a	6.4^b	6.3^b	6.5^b	6.7^b	6.5^b
C	7.9^a	6.3^b	6.3^b	6.5^b	6.5^b	6.5^b
D	7.8^a	6.2^{bc}	6.2^{bc}	6.3^{bc}	6.3^c	6.2^{cd}
E	7.8^a	6.0^c	6.0^c	6.2^c	5.9^d	5.9^d
F	7.7^a	5.7^d	5.5^d	6.0^d	5.4^e	5.5^e
*CC	6.0^c	6.3^b	6.5^{ab}	6.0^d	7.0^{ab}	7.5^{ab}

KEY:

A (FMF:WF:T, 100:0:0), B (FMF:WF:T, 74.5:25:0.5), C (FMF:WF:T, 69.5:30:0.5)

D (FMF:WF:T, 64.5:35:0.5) E (FMF:WF:T, 59.5:40:0.5), F (FMF:WF:T, 54.5:45:0.5)

*CC (Commercial complementary food-cerelac)

FMF-Fermented maize flour, WF- Walnut flour, T-Turmeric

Table 3: Amino acids Composition of *Tetracarpidium conophorum* flour and the most preferred formulated complementary food with FAO ref value.

Parameters	Tetracarpidium Conophorum flour	Blend C	FAO ref value*
Lysine	3.24	2.16	4.2
Histidine	1.55	2.14	NA
Arginine	7.08	4.66	NA
Aspatic acid	7.85	5.21	NA
Threonine	2.57	2.32	2.8
Serine	2.60	3.2	NA
Glutamic acid	9.85	13.18	NA
Proline	3.05	6.21	NA

Glycine	0.39	1.66	NA
Alanine	2.89	2.43	NA
Cystine	0.53	0.79	2.0
Valine	3.65	3.16	4.2
Methionine	1.02	1.46	2.2
Isoleucine	2.98	3.01	4.2
Leucine	5.13	4.18	4.2
Tyrosine	0.64	1.59	2.8
Phenylanaline	2.95	3.71	2.8
Tryptophan	ND	ND	ND

ND: Not defined

Table 4: Fatty acids composition (%) of the most preferred complementary food (blend C).

Fatty acids	%
Palmitic acid (C16:0)	12.45
Pamitoleic acid (C16:1)	0.23
Stearic acid (C18:0)	1.8
Oleic acid (C18:1)	29.27
Linoleic acid (C18:2)	55.38
Linolenic acid (C18:3)	0.87
Saturated fatty acids (SFA)	
Palmitic acid (C16.0)	12.45
Stearic acid (C18:0)	1.8
Total	14.25
Poly unsaturated fatty acids (PUFA)	
Linoleic acid (C18:2)	55.38
Linolenic acid (C18:3)	0.87
Total	56.25
Mono unsaturated fatty acid (MUFA)	
Oleic acid (C18:1)	29.27
Pamitoleic acid (C16:1)	0.23
Total	29.50

Conclusion

The study has shown that an acceptable nutrient-dense complementary food can be made from African walnut. The fortification increased the nutritive values of the product. The Formulated African walnut based complementary food can therefore be considered suitable for preventing proteins-energy malnutrition in children.

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