PROXIMATE COMPOSITIONS, AMINO ACID PROFILES AND SENSORY EVALUATION OF ‘OKPA’ PREPARED AND ENRICHED WITH THREE LEAFY GREEN VEGETABLES

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ABSTRACT

Background: Under-nutrition is a global health problem that affects all age groups especially the vulnerable ones particularly young children.

Objectives: The study examined proximate compositions, amino acid profiles and sensory qualities of ‘Okpa’ prepared and enriched with fluted pumpkin, bitter leaf and scent leaf.

Materials and methods: The ‘Okpa’ was prepared by thoroughly mixing the flour with palm oil, uziza, pepper, salt, water and the three vegetables. Three samples labelled A (Okpa + scent leaf), B (Okpa + fluted pumpkin and C (Okpa + bitter leaf) were prepared. Proximate and amino acid analysis were done following standard procedures and twenty panelists evaluated their sensory qualities.

Results: This indicated that there were significant differences (P<0.05) in samples crude protein contents and values ranged from 12.38% (A)-15.10% (C). (Okpa + Bitter leaf) had highest ash content of 7.91% while (Okpa + Scent leaf) had the least (4.44%). Carbohydrate content of samples ranged from 47.73% (C)-56.12% (A). Total dietary fiber content of (Okpa + Scent leaf) was the highest (8.62%). Amino acid profiles, (Okpa + Bitter leaf) was significantly higher in leucine, lysine, isoleucine, phenylalanine, tryptophan, valine, histidine and threonine with values of 9.48, 5.21, 4.11, 4.81, 1.26, 4.41, 2.95 and 3.11g/100g, respectively. Sensory qualities of samples showed that (Okpa + Scent leaf) was accepted more than the others because it scored highest in colour, texture, taste and flavor while (Okpa + Bitter leaf) was the least accepted.

Conclusion: Addition of bitter leaf to Okpa improved nutrient content and could be used instead of the usual scent leaf and sometimes fluted pumpkin often used.

Keywords: proximate composition, amino acid, enrichment, vegetables.

INTRODUCTION

Food is intended to provide energy and nutrients to the body. But often, the nutrients provided are lacking in one micronutrient or the other. This is as a result of monotonous nature of most diets particularly in developing nations of the world including Nigeria. Nigerian diets are mainly based on staples such as cereals and roots. This predisposes many people to the triple burden of malnutrition (under-nutrition, over-nutrition and micronutrient deficiencies) [1]. Therefore, tackling malnutrition requires an adequate intake of energy and nutrients. Thus, providing an adequate diet requires a food-based approach to improve diet. No single food provides all the nutrients needed by the body, hence diet diversity is encouraged. However, when that is not possible, staple foods used in preparing different diets can be enriched or fortified with other foods abundant in the nutrients that are likely to be either entirely lacking or insufficient in amount.

Protein-energy malnutrition (PEM) is one form of malnutrition and low energy intake is closely related to low consumption of proteins, vitamins and minerals. According to Food and Agriculture Organization [2], protein and energy shortage is a major challenge for public health in developing countries. Globally, 21% of children are stunted and 40% of all stunted children live in Africa. About 7% of children globally are wasted of which 27% live in Africa [3]. According to National Population Commission [4], prevalence of thinness among women of reproductive age and adolescent girls is 11% and 23%, respectively. Prevalence of stunting, underweight and wasting among children under 5 years is 37%, 29% and 18%, respectively. But the National Nutrition and Health Survey according to National Bureau of Statistics [5] reported a prevalence of 32.0%, 19.9%, 7.0% and 1.5% for stunting, underweight, global acute malnutrition and severe acute malnutrition among children 6-59months, respectively. Acute and severe malnutrition in women of reproductive age were put at 6.9% and 3.8%, respectively. The situation is precarious, hence to meet the sustainable development goal of zero hunger by 2030 and to end malnutrition in all its forms, adoption of diversified diets that are plant-based and nutrient-
Viagna subterranean is a legume which is identified by different names such as jugo beans (South Africa), nyimo beans (Zimbabwe), gurijiya or kwaruru (Hausa, Nigeria), Okpa (Igbo, Nigeria), epa roro (Yoruba, Nigeria) [6]. It is highly nutritious as the dried seeds contain carbohydrate (64.4%), protein (23.6%), fat (6.5%), fiber (5.5%), potassium (11.44-19.35mg), iron (4.9-48mg), sodium (2.9-12mg) and calcium (95.8-99mg/100g) [7]. Plant proteins play a potential role in global food security, providing 65% of the world’s supply of proteins with up to 15% of that coming from legumes and Bambara groundnut contributes 25% of the total protein. Sanusi et al. [8] reported that, white dried raw Bambara groundnut contains 20g of protein, 6.5g of fat, 58.8g of carbohydrate, 3.1g of ash per 100g. They also reported some minerals including calcium (50mg), iron (3.30mg), manganese (185mg), phosphorus (309mg), potassium (1370mg), sodium (28mg) and zinc (2.39mg). Only few (thiamin, riboflavlin and niacin) vitamins with values of 0.39mg, 0.12mg and 1.9mg, respectively were reported by the same authors. Bambara nut seeds are richer than peanuts in essential amino acids such as isoleucine, lysine, methionine, phenylalanine, threonine and valine [6]. Bambara groundnut has the following amino acid per 100g: tryptophan (192mg), lysine (114mg), methionine (312mg), phenylalanine (991mg), threonine (617mg), valine (937mg), leucine (1385mg) and isoleucine (776mg) [9]. Khan et al. [7], opined that due to its high protein content, Bambara groundnut has the potential to combat food insecurity and malnutrition in the future. It can also contribute up to 25% protein and serve as substitute for animal source protein because of its high cholesterol and high prices. Mbagwu et al. [10] reported that the nuts contain phytochemicals such as flavonoids and alkaloids. Rauf et al. [12] reported an abundance of the flavonoids epicatechin and catechin in raw and cooked red seed, respectively. Catechin and epicatechin can polymerize to form proanthocyanidins which exhibit antioxidant, cardioprotective, antitumour and neuroprotective properties [12]. According to Ndidi et al. [13], anti-nutrients such as phytate, oxalate, hydrogen cyanide and trypsin inhibitors exist in more than the allowable limit in raw Bambara groundnut which can greatly be reduced to a tolerable limit by steaming and roasting. Fermentation, germination, soaking, dehulling and cooking can effectively reduce anti-nutrients [14, 11]. In Nigeria, mature fresh Bambara groundnut is boiled and consumed as snack while mature dried seeds are either roasted and consumed as snack or milled into flour used to produce Okpa, a popular snack and food [15]. Okpa is growing in popularity and is eaten by many people across all ages. The commercial sellers prepare it without addition of vegetables and few who do usually add scent leaf. Vegetables are good sources of micronutrients and bitter, scent and fluted pumpkin leaves are sources of calcium, iron, magnesium, potassium, betacarotene, vitamin C. Scent leaf is particularly rich in beta-carotene and phytochemicals [8, 16]. Egediwe [17] reported that bitter leaf contains bioactive compounds including oxalate, phytate, tannins, flavonoids, cyanogenic glycosides and nutrients such as vitamin C, selenium, folate, betacarotene. Therefore enriching Okpa with these vegetables will improve its nutrient contents. The study was designed to produce Okpa with the vegetables and to evaluate its proximate compositions, amino acid profiles and sensory qualities.

MATERIALS AND METHODS

Study design: The experimental study was carried out at the Diet therapy Laboratory of the Department of Human Nutrition and Dietetics, Michael Okpara University of Agriculture Umudike.

(Okpa + Bitter leaf) collection: The Bambara groundnut was purchased from Eke-Ozizi market, Igbo Eze North Local Government of Enugu State. The bitter leaf, scent leaf and fluted pumpkin were bought from Ndioro market in Ikwuano Local Government Area of Abia State.

Sample preparation: The Bambara groundnut seeds were sorted, soaked for 48hours. The soaked seeds were washed twice with water, DE hulled and oven dried at a temperature of 40°C. The dried seeds were milled into fine flour and sieved. The flour was stored in air tight container until needed [18]. Figure 1 shows the flow chart for the preparation of Bambara groundnut flour. The three vegetables were thoroughly washed and separately sliced into tiny pieces.
Preparation of Okpa: The method described by Adumanya [18] was followed in the preparation of the Okpa. The recipe for the preparation are listed in Table 1. The Okpa flour was sieved with a 0.2mm mesh into a big bowl, salt, ground pepper and uziza (Piper guineese) were added and thoroughly mixed. Palm oil was added and thoroughly mixed into the flour until an even yellow colour was obtained. Then, 750ml of lukewarm water was added till there were no lumps. The remaining 250ml of lukewarm water was added to the mixture. The entire mixture was divided into three equal parts and labelled A, B and C and 75g of fresh scent, fluted pumpkin and bitter leaves were added to samples A, B and C, respectively. The banana leaves were softened over fire, removed from there stalks, washed and drained of water. Thereafter, the banana leaves were used to wrap the Okpa which were carefully labelled, placed into a pot containing boiling water. The pot was covered and allowed to cook in medium heat for at least 30-40 minutes. The samples were allowed to cool and packaged into different containers till they were needed for analysis.

Table 1: List of Ingredients for the preparation of Okpa

| Ingredients         | Quantity       |
|---------------------|----------------|
| Bambara nut flour   | 1kg            |
| Pepper              | 35g            |
| Uziza               | 35g            |
| Palm oil            | 750ml          |
| Salt                | 3tbsp          |
| Water               | 1000ml (1L)    |

Source: Adumanya et al., (2012).

Chemical analysis: The proximate composition (moisture, ash, fat, crude protein, dietary fiber and carbohydrate contents) were all done using the method described by the Association of Official Analytical Chemist (AOAC) [19]. Carbohydrate content was calculated by nitrogen free extractive method which was given as a difference between 100 and the sum total of other proximate components, That is, % carbohydrate= 100-% (moisture+ ash+ fat+ dietary fiber+ protein contents). Amino acid profiles of the Okpa samples were determined by ion exchange chromatography which involves hydrolysis of sample protein for free amino acid analysis. Sodium or lithium buffers were prepared for separation of the amino acids by Ion Exchange Chromatography (IEC). The elute from the ion exchange column was passed through a teflon coil placed in a boiling water bath. Prior to that, the column effluent was mixed with reduced ninhydrin reagent dissolved in acetate buffer. The ninhydrin reacts with amino acids forming a dye complex. The absorption was determined in a flow photometer and registered on a chart. The area under the peaks corresponds to the amounts of amino acids present in the sample [20].

Sensory evaluation: Twenty staff and students selected from the department of Human Nutrition and Dietetics evaluated the taste, flavor, colour and texture of the three samples prepared. The staff were technical and technologist. A seven-point hedonic scale was used for the evaluation with 7 as the highest while 1 was the lowest.

Statistical analysis: This was done using SPSS version 23. Results from the chemical analysis and sensory evaluation were subjected to Analysis of Variance (ANOVA) presented as means plus or minus standard deviation and Duncan Multiple Range Test was used to separate the means. Significance differences were judged at P less than or equal to 0.05.

RESULTS

Table 2 shows the proximate composition of the samples. The result showed that Okpa + Bitter leaf contained the highest moisture (52.72%), protein (15.10%) and ash (7.91%). The carbohydrate contents of the samples ranged from 47.73% (C)-56.12% (A). There was no significant difference (P>0.05) in the fat contents of sample B (Okpa + fluted pumpkin) (24.86%) and C (Okpa + bitter leaf) (24.59%). The energy values of the samples significantly differed from one another and ranged from 1787.36kilojoules (C)-1996.15kilojoules (B).

Table 2: Proximate composition of Okpa

| Samples                         | (Okpa + Scent leaf) | Sample B                     | (Okpa + Fluted pumpkin leaf) | (Okpa + Bitter leaf) |
|---------------------------------|---------------------|------------------------------|------------------------------|----------------------|
| Moisture content (%)            | 51.14b ± 0.16       | 50.77b ± 0.11                | 52.72b ± 0.34                |
| Crude protein (%)               | 12.38a ± 0.02       | 13.37b ± 0.13                | 15.10b ± 0.08                |
| Fat (%)                         | 20.92a ± 0.14       | 24.86a ± 0.07                | 24.59b ± 0.49                |
| Ash (%)                         | 4.44c ± 0.07        | 5.12c ± 0.15                 | 7.91c ± 0.08                 |
| Carbohydrate (%)                | 56.12a ± 0.01       | 50.09b ± 0.01                | 47.73c ± 0.01                |
| Energy Value (KJ)               | 1932.20b ± 0.01     | 1996.15c ± 15                | 1787.36d ± 0.01              |

Values of mean ± standard deviation of duplicate samples

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The dietary fiber composition of Okpa is shown in Table 3. The total dietary fiber (g/100g) of the samples ranged from 7.06 (C) to 8.62 (A). Sample B (Okpa + Fluted pumpkin leaf) had the highest (5.24) insoluble dietary fiber while C (Okpa + Bitter leaf) had the least (3.01). There was no significant difference (P>0.05) between the soluble dietary fiber contents of sample B (Okpa + Fluted pumpkin leaf) (3.01) and C (Okpa + Bitter leaf) (3.05).

Table 3: Dietary fiber composition of Okpa

| Samples | Sample A (Okpa + Scent leaf) | Sample B (Okpa + Fluted pumpkin leaf) | Sample C (Okpa + Bitter leaf) |
|---------|-----------------------------|-------------------------------------|-------------------------------|
| Insoluble (g/100g) | 5.00 ± 0.00 | 5.24 ± 0.01 | 4.01 ± 0.01 |
| Soluble (g/100g) | 3.62 ± 0.01 | 3.01 ± 0.01 | 3.05 ± 0.01 |
| Total dietary fiber (g/100g) | 8.62 ± 0.03 | 8.25 ± 0.06 | 7.06 ± 0.21 |

Values of mean ± standard deviation of duplicate samples

The amino acid profiles of the samples are presented in Table 4. Generally, there were significant differences (P<0.05) in the amino acid profiles of the three samples. Okpa + Bitter leaf was significantly higher in all the amino acids except methionine, glycine and serine. This was closely followed by sample B (Okpa + fluted pumpkin leaf) except for the amino acids valine, cysteine and serine. However, sample A (Okpa + Scent leaf) consistently had the lowest values for most of the amino acids except for valine, tyrosine and cysteine where it had the second highest values and serine whose value was the highest (4.21).

Table 4: Amino acid profiles of Okpa

| Amino acid (g/100g) | Sample A (Okpa + Scent leaf) | Sample B (Okpa + Fluted pumpkin leaf) | Sample C (Okpa + Bitter leaf) |
|---------------------|------------------------------|-------------------------------------|-------------------------------|
| Leucine | 8.44 ± 0.01 | 9.40 ± 0.00 | 9.84 ± 0.01 |
| Lysine | 4.50 ± 0.00 | 4.91 ± 0.01 | 5.21 ± 0.01 |
| Isoleucine | 3.80 ± 0.00 | 3.81 ± 0.01 | 4.11 ± 0.01 |
| Phenylalanine | 3.72 ± 0.00 | 4.44 ± 0.01 | 4.81 ± 0.01 |
| Tryptophan | 0.82 ± 0.01 | 1.11 ± 0.01 | 1.26 ± 0.00 |
| Valine | 4.00 ± 0.00 | 3.94 ± 0.00 | 4.41 ± 0.01 |
| Methionine | 1.93 ± 0.01 | 2.33 ± 0.01 | 2.15 ± 0.01 |
| Proline | 3.66 ± 0.01 | 3.87 ± 0.01 | 4.27 ± 0.01 |
| Arginine | 4.65 ± 0.01 | 5.17 ± 0.01 | 5.94 ± 0.01 |
| Tyrosine | 3.11 ± 0.01 | 3.44 ± 0.00 | 3.45 ± 0.01 |
| Histidine | 2.24 ± 0.01 | 2.82 ± 0.01 | 2.95 ± 0.01 |
| Cystine | 1.34 ± 0.01 | 1.22 ± 0.01 | 1.41 ± 0.01 |
| Alanine | 3.26 ± 0.00 | 3.72 ± 0.01 | 4.03 ± 0.01 |
| Glutamic acid | 12.57 ± 0.00 | 13.03 ± 0.01 | 13.86 ± 0.01 |
| Glycine | 2.62 ± 0.01 | 3.48 ± 0.01 | 3.01 ± 0.01 |
| Threonine | 2.30 ± 0.00 | 2.81 ± 0.01 | 3.11 ± 0.01 |
| Serine | 4.21 ± 0.00 | 3.25 ± 0.01 | 4.06 ± 0.08 |
| Aspartic acid | 8.50 ± 0.00 | 9.22 ± 0.01 | 9.87 ± 0.01 |

Values of mean ± standard deviation of duplicate samples

Table 5 shows the sensory evaluation values of the samples. From the results, the texture of Sample A (Okpa + Scent leaf) was more liked by the panelists. There were significant differences in the textures of the three samples with values of 6.45, 6.05 and 5.40 for samples A, B and C, respectively. The tastes of the samples did not differ for samples A and B while C had the least taste of 4.45. The general acceptability of the samples indicated that Sample A (Okpa + Scent leaf) was more accepted than the rest but it did not differ significantly from B (Okpa + Fluted pumpkin leaf) but did from C (Okpa + Bitter leaf) which had the least score (4.65).
Dietary fibers are portions of plant foods that are resistant to human digestive enzymes. They provide many health benefits which includes reducing risks for coronary heart diseases, stroke, hypertension, diabetes, obesity. They also improve blood glucose control [22, 23, 24]. The vegetables used in the three samples contributed to the notable significant differences in total dietary fiber contents of the samples. However, the value reported for Sample A (Okpa + Scent leaf) was lower compared to the 11.30g reported by Adumanya et al. [18]. This may be due to differences in processing methods or quantities of the vegetables. In the present study, the Bambara groundnut seeds were De hulled and according to Oyeyinka et al. and Adebiyi et al. [25, 26], removal of testa reduces dietary fiber contents.

The rich amino acid contents of Bambara groundnut complemented by that present in the vegetables, particularly bitter leaf makes the samples produced to be potential alternatives for animal source protein. Additionally, the samples met the recommended daily intake stated by World Health Organization/ Food and Agriculture Organization/ United Nations University (WHO/FAO/UNU) [27] for histidine (1.9g), isoleucine (2.8g), leucine (6.6g), methionine (2.2g), phenylalanine (2.8g), threonine (3.4g), tryptophan (1.0g) and valine (3.5g). Tryptophan has been found to be the limiting amino acid in Bambara groundnut [28, 29]. That may be the reason why its values were relatively low in the samples and yet differed significantly probably because of the different vegetables used in their preparations. Generally, the higher protein content of bitter leaf contributed to the significantly higher amino acids reported in Sample C (Okpa + Bitter leaf).

Nti [30] asserted that removal of the highly pigmented seed coats by some pre-treatment such as De hulling, roasting, germination and fermentation have been shown to improve the appearance, texture and taste of Bambara groundnut products. Also the challenge of long cooking time, particularly of dried seeds can be overcome and more so, if the seeds are milled. However, despite those processing methods, our samples’ general acceptability scores for samples A and B were all higher than the highest (5.10) reported by Adumanya et al. [18]. This could be due to the addition of uziza to the samples of the present study which was not added to the study under comparison. Specifically, in the present study, all the sensory attributes of colour, taste, flavor and texture scored than that for the study by Adumanya et al. [18] except for flavor of sample D which was the highest (6.90).

| Sensory attributes | Sample A (Okpa + Scent leaf) | Sample B (Okpa + Fluted pumpkin leaf) | Sample C (Okpa + Bitter leaf) |
|--------------------|-----------------------------|--------------------------------------|-------------------------------|
| Colour             | 6.75±± 0.44                 | 6.25±± 1.07                          | 5.20±± 1.54                   |
| Texture            | 6.45±± 0.68                 | 6.05±± 1.19                          | 5.40±± 1.14                   |
| Taste              | 6.70±± 0.47                 | 6.40±± 0.88                          | 4.45±± 1.79                   |
| Flavour            | 6.45±± 0.68                 | 5.95±± 1.05                          | 4.45±± 1.79                   |
| General acceptability | 6.55±± 0.51               | 6.40±± 1.23                          | 4.65±± 1.84                   |

Values of mean ± standard deviation of duplicate samples

Superscript a–c Mean with similar super script in each column are not significantly different (P>0.05)

DISCUSSION

Moisture content as used in this work is the quantity of water present in the edible portion of the samples prepared. The higher moisture content noted in Sample C (Okpa + bitter leaf) could be as a result of the addition of bitter leaf to it. Usually, bitter leaf has water even if you are washing it without additional water to it. That could probably have increased the water contents in the Okpa. Thus, the high protein and ash contents reported in Sample C (Okpa + Bitter leaf) could also be attributed to the addition of bitter leaf to it. Oboh (20) reported that bitter leaf contains 33.3% protein and 11.7% ash. The significant differences noted in the protein and ash contents could be due to the different vegetables used in enriching the samples. Sanusi et al. [8] reported that fresh scent and fluted pumpkin leaves contain 3.15g and 3.57g of protein and 1.49g and 2.27g of ash, respectively. The similarities noted in the fat contents of samples B (Okpa + Fluted pumpkin leaf) and C (Okpa + bitter leaf) may also be attributable to the vegetables used as scent leaf was reported to have 0.64g of fat by the same authors and that might have caused the lowest fat content noted in Sample A (Okpa + Scent leaf). The significant differences noted in carbohydrate and energy values of the samples was in congruent to the differences noted in the nutrients that contribute energy to diets. That is carbohydrate, protein and fat which all differed among the samples.
This could be due to the fact that the two vegetables (fluted pumpkin and scent leaves) were added to the sample. Generally, all the samples were accepted by the panelists as none was rated below 3.5 which is half of the overall score.

CONCLUSION
The authors deduced that addition of green leafy vegetables, particularly bitter leaf to Okpa, a snack and food consumed by all age categories not only improved the nutrient contents (protein, ash and dietary fiber) but also enhanced the sensory appeal. Bitter leaf in particular, in addition to the other vegetables reduce the hardness and dryness of Okpa as they increased the moisture contents. The study recommends that further chemical analysis be carried out on the samples to ascertain the specific minerals especially those of public health importance (iron, zinc, iodine, calcium) and vitamins (pro-vitamin A, vitamins D, C Bα, B12 and folic acid), as well as anti-nutrients in the samples.

Informed Consent: The twenty panelists were informed of the objectives of the study, they gave their oral consents and willingly tasted the products.

Declaration of interest: No conflict of interest existed between the authors.

Availability of data and materials: The materials and data are available.

Author’s contribution: Author AIC designed, supervised, wrote and interpreted the results. The second author PIS conceptualized the topic, did the analysis and searched for some of the literatures.

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