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Enhancing protein quality in breakfast cereals with blends of acha, pigeon pea, and oyster mushrooms

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Abstract

This study focuses on enhancing the protein quality in breakfast cereal with acha (*Digitaria exilis*), pigeon pea (*Cajanus cajan)*, and oyster mushroom (*Pleurotus ostreatus*). Protein quality, concentration of essential amino acids, amino acid score with reference to whole chicken egg, and FAO/WHO standard were determined. A completely randomized design (CRD) was used for this analysis; the significant difference between means was determined (ANOVA) and separated using the Duncan multiple range test; the significance was accepted at $p < 0.05$. Four sample formulations were used: 100% acha (control) sample 101, blends of acha, pigeon pea, and oyster mushroom at different proportions of 75:20:5 (sample 102), 70:20:10 (sample 103), and 65:20:15 (sample 104). Protein efficiency ratio (P.E.R.), biological value (B.V.), essential amino acid index (EAAI), and percentage of EAAI (% EAAI) were determined for protein quality. (P.E.R.) values ranged between 2.93 (101), 2.82 % (102), 3.18 % (103) and 3.29 % (104). Essential, non-essential, acidic, neutral, sulfuric, aromatic, and their percentages were determined. For amino acid score, leucine levels showed values from 8.35% (101), 7.910% (102), 8.90 (103), and 9.31% (104) and sample 104 with the highest value and significantly difference ($p< 0.05$). These findings strongly suggest the potential of these blends to serve as sustainable, healthy dietary alternatives for diabetic people, as they substitute animal proteins, including providing nutrient-dense options such as improved amino acid balance.

INTRODUCTION

Protein quality is critical in diabetes type management to support muscle repair and maintain glucose levels, as well as enhance metabolic activities (1). Diabetes mellitus is a chronic disease characterized by elevated blood glucose levels, leading to damage in the heart, blood vessels, eyes, kidneys, and nerves. Over half a billion people worldwide, mostly of working age, are affected by this condition (2). Managing diabetes is expensive with complications and severe health impacts (3). High blood sugar causes oxidative stress, damaging pancreatic beta cells and disrupting insulin secretion (4). Foods that can help manage high blood sugar and oxidative stress are highly important. Low/moderate glycemic index functional foods most often consumed by diabetic patients, with high antioxidants and rich in dietary fibre, aid glucose control and protect the beta cells of the pancreas (5, 6). Such foods are recommended, specifically for diabetes treatment (2). Some foods assist in controlling blood glucose levels, weight, and boosting the brain exercises when consumed in breakfast (7).

Breakfast prevents diseases attributed to blood sugars and maintains the body fit (8). Combined with pigeon pea, acha (fonio) improves the content of essential amino acids, which helps introduce nutritional density into cereals for diabetes by helping muscles and regulating blood sugar levels (7). Cereal products made from acha and pigeon pea can be encouraged; nevertheless, it is vital to know that not all gluten-free cereals are characterized by low GI values. Low-GI diet refers to the rate at which glucose is ingested into the body's system and its circulatory system and is thus an important aspect of diabetes (9). The inclusion in cereals of low GI as well as good amino acid profiles: low GI cereals like acha and pigeon pea blends would ensure adequate intakes of the vital amino acids and a good glycemic index. Also, inclusion of oyster mushrooms enhances the nutritive quality specifically in amino acids, hence being appropriate for diabetics (10, 11).

Lysine, leucine, and threonine, which are important for protein synthesis, are highly present in pigeon pea. Acha contains methionine and cysteine and is comparatively high in fiber, which makes it low-glycemic. Both collectively help in the preservation of muscles and metabolic benefits (12, 13). Flavour and nutritive value are also boosted through the addition of oyster mushrooms; proteins as well as other substances such as antioxidants and polysaccharides are found in oyster mushrooms. It also raises the levels of glutamic acid, alanine, and aspartic acid, which enhance taste and immune function—a key factor for diabetics (10, 11). Enhancing the protein quality of breakfast cereals could aid in diabetes management by supporting muscle mass preservation and improving metabolic health. Bioactive compounds in pigeon pea and oyster mushroom offer additional health benefits (13). This research might lead to new functional foods that meet the nutritional needs of diabetic patients, providing a practical dietary solution.

Furthermore, the amino acid profiles of these blends have been shown to surpass those of their individual components in several key areas. For instance, the inclusion of pigeon peas and oyster mushrooms can address the lysine deficiency in acha and boost the overall nutritional quality of the flour. This makes such blends particularly valuable in regions where protein-energy malnutrition is prevalent and where dietary diversity is limited. A different criterion for determining amino acid scoring patterns was shown and obtained from the 14 standard reports. The objectives of the study were producing breakfast cereals from composite blends with acha, pigeon pea, and oyster mushroom and determining the amino acid profile.

MATERIALS AND METHODS

Materials

Source of Raw Materials

White acha grains (*Digitaria exilis*), white Pigeon pea (*Cajanus cajan*) was purchased from the international market in Abakaliki, Ebonyi State, while oyster mushroom (*Pleurotus ostreatus*) was gotten from the National Biotechnology Development Agency, South East Centre, government house, Ebonyi state. The chemicals for the analysis were of laboratory-grade quality, procured from Sigma-Aldrich, London, United Kingdom.

Production of Acha Flour

A modified version of the method described by (13) was applied to prepare the acha flour. Two kilograms of acha grains were sorted to remove stones, dirt, chaff, and other foreign materials. The cleaned grains were then washed with fresh tap water and drained using a perforated plastic container. The grains were dried in a hot air oven (Gallenkemp, 300 Plus, England) at 50°C for 6 hours to reduce moisture content, enable efficient milling, and suitable storage, minimizing microbial spoilage. After drying, the dried samples were milled (Fritsch Pulverisette 19 Mill, Fritsch GmbH, Germany) into a fine powder and sieved (500 µm mesh).

The flour was sealed in an airtight polyethylene bag and kept at room temperature (23°C) for future use, as shown in Figure 4.

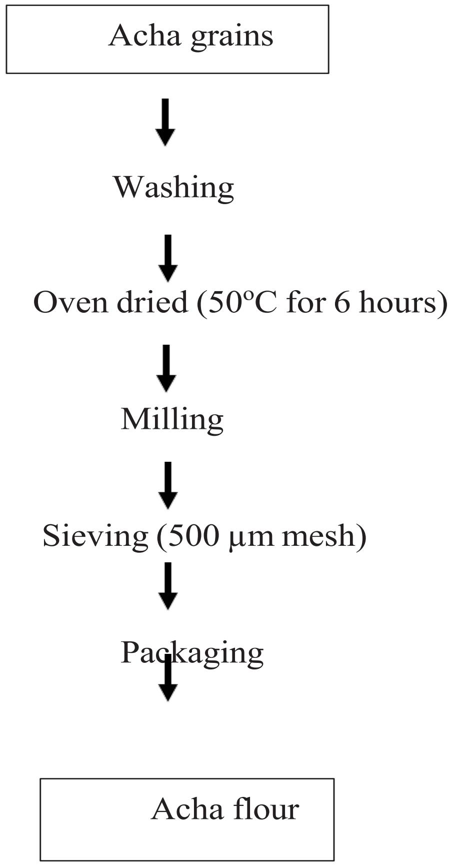

Figure 4. Flow chart for Acha flour production (13).

Production of Pigeon Peas Flour

Procedures of (15) were adopted for the production of pigeon pea flour with modifications. The pigeon peas (2 kg) were sorted; dirt, stones, and other foreign materials were removed, then washed in clean tap water and drained through a plastic perforated container, and blanched at 100°C for 10 minutes. It was then dehulled and toasted for 15 minutes at 150°C. The toasted

Figure 1

Plate 1. Acha grains

Figure 2

Plate 2. Fresh oyster mushroom

Figure 3

Plate 3. Pigeon pea grains

grain was oven-dried (Gallenkemp, 300 Plus, England) at 60°C for 8 hours to reduce moisture content, enable efficient milling, and suitable storage, minimizing microbial spoilage. The dried samples were milled (Fritsch Pulverisette 19 Mill, Fritsch GmbH, Germany) into a fine powder and sieved (500 µm mesh) to fine flour. The flour was sealed in an airtight polyethylene bag and kept at room temperature (23°C) for future use, as shown in Figure 5.

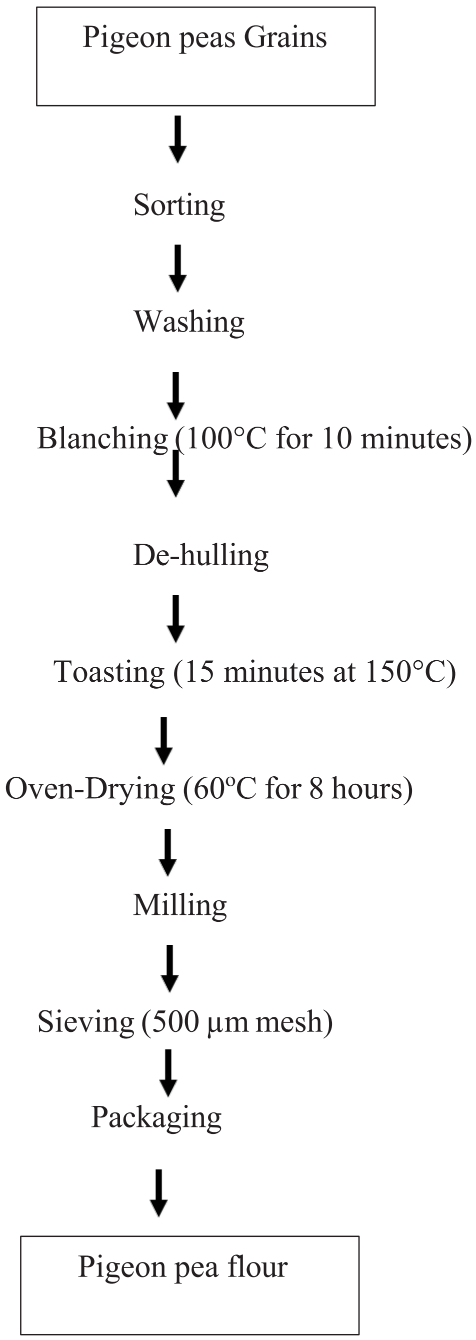

Figure 5. Flow chart for Pigeon pea flour production (15).

Preparation of Oyster Mushroom Flour

The approach of (16) with minor adjustments was used to prepare the oyster mushroom flour. The fresh oyster mushroom (8 kg) sample was sorted to remove dirt and debris, then washed (in clean tap water). Clean, sharp knives were used to cut the mushrooms into uniform pieces for even drying using an oven (Gallenkemp, 300 Plus, England) at 60°C for 8 hours for faster drying, then milled (Fritsch Pulverisette 19 Mill, Fritsch GmbH, Germany) into a fine powder and sieved (500 µm mesh) to fine flour. The flour was sealed in an airtight polyethylene bag and kept at room temperature (23°C) for future use, as shown in Figure 6.

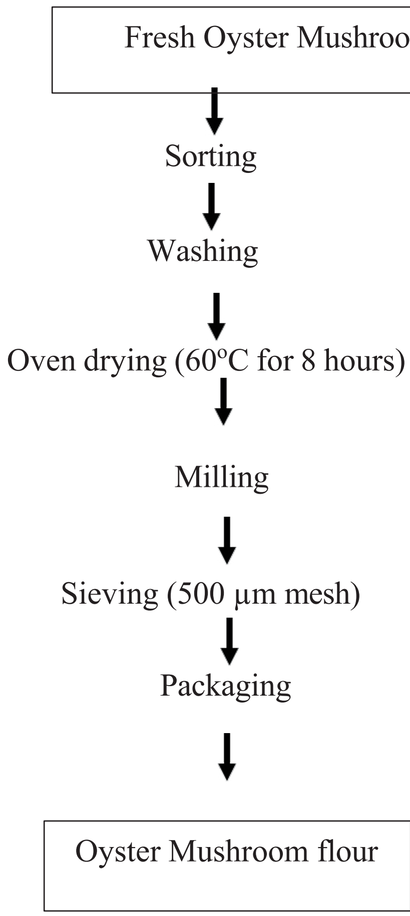

Figure 6. Flow chart for Oyster Mushroom flour production (16).

Pigeon Pea, and Oyster Mushroom Flour Blends

Sample codes 101, 102, 103, and 104 represent different blends of flour with varying proportions of acha, pigeon pea, and oyster mushroom. Specifically:

- 101: 100% acha flour (control).
- 102: 75% acha, 20% pigeon pea, 5% oyster mushroom flour.
- 103: 70% acha, 20% pigeon pea, 10% oyster mushroom flour.
- 104: 65% acha, 20% pigeon pea, 15% oyster mushroom flour.

The flour blends were thoroughly mixed using a Kenwood KW-3006 350W electric mixer (Kenwood Appliances, Uk). and stored in a zip-lock bag. The composite flour mixtures were chosen based on the results from previous research (17) and are detailed in Table 1.

Processing of Breakfast Cereal From Blends of Acha, Pigeon Pea and Oyster Mushroom

Breakfast cereals were produced from the flour blends, formulated as depicted in Figure 4, and prepared according to the method described by (18), with some modifications. Each blend consisted of 200 g of flour, which was hand-mixed in a stainless-steel bowl for about 3 minutes to ensure uniformity. 160 ml of water was added slowly, and the entire dough was mixed thoroughly for about 2 minutes to obtain uniform dough. The dough was shaped into flakes using a manual extruder and they were placed on a baking tray. The flakes were then toasted in an oven at 150°C for 35 minutes. After cooling to room temperature, the flaked breakfast cereals were removed from the pan, packaged in rigid plastic containers, and stored at ambient temperature for further analysis, as shown in Figure 7.

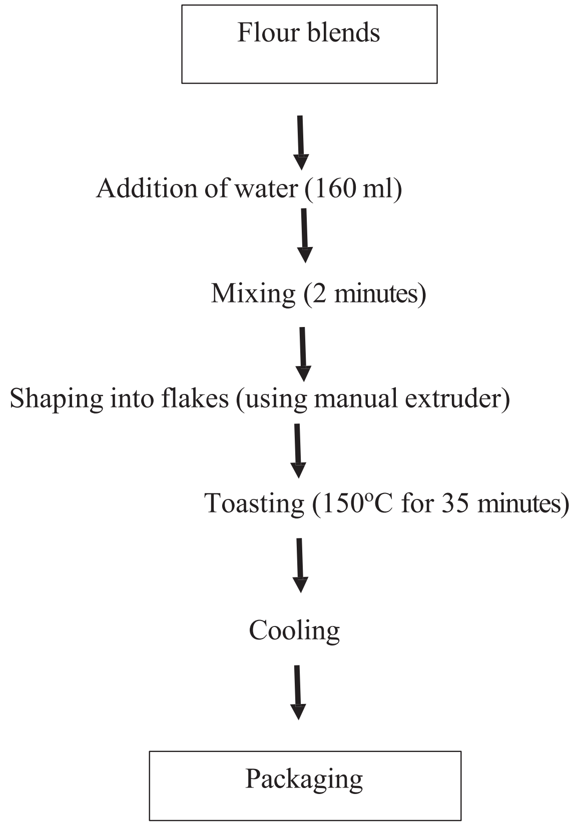

Fig. 7. Flow chart for the production of breakfast cereal (18) with slight modification.

METHODS

Protein Quality Determination

Amino acid analysis

The amino acid profile was assessed using the procedure outlined by (19). A defatted 20 mg sample was placed in a glass ampoule, and 7 ml of 6 normal hydrochloric acid were added. Nitrogen gas was used to purge the ampoule of

Table 1. Proportional composition of flour blends from acha, pigeon pea, and oyster mushroom

Sample Code	Acha (%)	Pigeon pea (%)	Oyster Mushroom (%)
101	100	0	0
102	75	20	5
103	70	20	10
104	65	20	15

Key: Sample Code 101= 100% acha flour (Control); 102=75% acha, 20% pigeon peas, 5% mushroom flour; 103= 70% acha, 20% pigeon peas, 10% mushroom flour; 104=65% acha, 20% pigeon peas, 15% mushroom flour.

oxygen. The ampoule was then sealed using a Bunsen burner flame and heated in an oven at 105±5°C for 22 hours. After cooling, the ampoule was carefully opened, and the contents were filtered. Amino acid analysis was conducted using ion-exchange chromatography with a Technician Sequential multi-sample amino acid analyzer (Technician Instruments Corporation, New York, USA). Tryptophan was not included in this analysis, and norleucine was used as the internal standard. The amino acid composition was determined from the standard area obtained from the integrator and expressed as percentages of the total protein. The determination of nutritional parameters was carried out by analyzing the amino acid profiles.

- Total Amino acid (TAA) = Add all amino acid

  Leu + Lys + Isoleu + Phen + Val + Meth + Pro + Arg +……+ Tryt …………... Equation 1
- Total non-essential amino acid (TNEAA) = Add all non-essential amino acid

  Alanine + Asparagine + Aspartic + Glu + Ser + Gly + Pro……………….........Equation 2
- Total essential amino acid (TEAA) = Add all essential amino acid

  Histi + Isoleu + Leu + Lys +Meth + Pheny + Threo + Try + Val ……..……….... Equation 3
- $\% \text{ TNEAA} = \frac{\text{Total non-essential amino acid}}{\text{Total Amino acid}} X \frac{100}{1}$……………………………..…..... Equation 4
- $\% \text{ TEAA with Histidine} = \frac{\text{Total essential amino acid with histidine}}{\text{Total non-essential amino acid}} X \frac{100}{1}$…….......... Equation 5
- % TEAA without Histidine

  $= \frac{\text{Total essential amino acid without histidine}}{\text{Total non-essential amino acid}} X \frac{100}{1}$…………………………….... Equation 6
- Total neutral amino acid (TNAA) = Add all neutral amino acid

  Ala + Gly + Val + Leu + Iso + Phe + Try + Meth + Pro + Ser + Thre + Cyst…Equation 7
- Total acidic amino acid (TAAA) = Aspartic Acid + Glutamic acid..…..........Equation 8
- % Total acidic amino acid

  $(\text{TAAA}) = \frac{\text{Aspartic Acid + Glutamic acid}}{\text{Total amino acid}} X \frac{100}{1}$…………………………….........Equation 9
- Total Sulphur amino acid

  (TSAA) = Methionine + Cystine…………………………………………...Equation 10
- % Total Sulphur amino acid

  $(\text{TSAA}) = \frac{\text{Methionine + Cystine}}{\text{Total amino acid}} X \frac{100}{1}$…………………………...…………..Equation 11
- Total basic Amino acid

  (TBAA) = Histidine + Lysine + Arginine…………………………..… ..…Equation 12
- $\% \text{ TBAA} = \frac{\text{Histidine + Lysine + Arginine}}{\text{Total amino acid}} X \frac{100}{1}$…………………………..……… Equation 13
- $\% \text{ Cystine in TSAA} = \frac{\text{Cystine}}{\text{Total Sulphuric acid}} X \frac{100}{1}$………………..… …...…..…....Equation 14
- Total aromatic amino acid (TArAA) = Phenylalanine + Tyrosine……..… ..Equation 15
- $\% \text{ TArAA} = \frac{\text{Phenylalanine + Tyrosine}}{\text{Total amino acid}} X \frac{100}{1}$………………..……………… .Equation 16

Table 2. Provisional amino acid scoring pattern reference pattern for whole chicken egg

Amino acid	Whole chicken egg (g/100g)
Lysine	6.2
Histidine	2.4
Arginine	6.1
Threonine	10.7
Serine	5.1
Glutamic acid	7.9
Proline	12.0
Glycine	3.8
Alanine	5.4
Cystine	1.8
Valine	7.5
Methionine	3.2
Isoleucine	5.6
Leucine	8.3
Tyrosine	4.0
Phenylalanine	5.1

(20)

- $\text{Amino acid score} = \frac{\text{mg of amino acid in 1g of test protein}}{\text{mg of amino acid in reference pattern}}$………………..… Equation 17

Table 3. Provisional amino acid scoring pattern reference pattern

Amino acid	mg per g of protein
Isoleucine	40
Leucine	70
Methionine + Cystine	55
Phenylalanine + Tyrosine	60
Threonine	40
Tryptophan	10
Valine	50
Total	360

(14)

$\text{Amino acid score} = \frac{\text{mg of amino acid in 1g of test protein}}{\text{mg of amino acid in reference pattern}} X \frac{100}{1}$……………………….... Equation 18

Determination of predicted protein efficiency (P-PER)

- This was estimated as described by (22) in the equation:

P-PER= 0.468 + 0.454 + Leu – 0.105 x Tyr …………….…....…...Equation 19

Determination of predicted biological value

- This was calculated according to method described by (23) in the equation:

BV= 1.09 x EAAI – 11.7……………………………………………….…...... Equation 20

Essential amino acid index (EAAI)

- Method of (24) was used to calculate the essential amino acid index by using the ratio of test protein to the reference protein for each nine essential amino acids. The reference protein for essential amino acids used was set by (25), as shown in table 4.

Table 4. Amino acid composition of casein

Phenylalanine	4.0
Isoleucine	4.3
Leucine	8.9
Lysine	6.5
Methionine	2.6
Threonine	4.3
Tryptophan	1.2
Valine	6.2
Arginine	2.5
Histidine	2.1
Cysteine	0.4
Tyrosine	4.2
Alanine	4.3
Glycine	3.4
Proline	13.4
Serine	6.8
Asparagine or Aspartic Acid (unspecified)	(6.2) 6.6
Aspartic Acid	2.8
Asparagine	(3.4)
Glutamine or Glutamic Acid (unspecified)	(18.6) 18.2
Glutamic Acid	(9.9)
Glutamine	(8.7)

$$EAAI = \sqrt[9]{\frac{(\text{Phenyl x Valine x Threo x Isoleu x Meth x Histi x Lys x Leu x Tryp})a}{(\text{Phenyl x Valine x Threo x Isoleu x Meth x Histi x Lys x Leu x Tryp})b}}$$

where, (Phenyl x Valine x …….) a is test sample; (Phenyl x Valine x...) b is standard protein casein (%), respectively…………………………….....……..... Equation 21

Casein is used as a reference protein due to the fact that it is a ''complete'' protein containing all of the nine essential amino acids in the appropriate proportions (26).

Concentration of essential compounds

This was estimated as described by (19) in the equation:

Amino acid score determination

These distinct methodologies were used to calculate the amino acid scores

1. The reference pattern for essential amino acid values when compared to those of a whole chicken egg was set by (20). Whole chicken egg is used because the protein is ideal and known to contain a biological value closely approaching 100% (13). Certain proteins may give an apparent score greater than 100%, but it is admitted that such a value cannot be used to alter the dietary protein requirement since intakes of N (nitrogen) should be less than required to meet the N requirements (21). The reference pattern for essential amino acid value is shown in Table 2.

2. The reference pattern for essential amino acids was set by (14) and used to determine the essential amino acid score of the breakfast cereal produced from blends of acha, pigeon pea, and

Table 5. Amino acid composition for breakfast cereals from blends of acha, pigeon pea and oyster mushroom

Amino acid	101 (%)	102 (%)	103 (%)	104 (%)
Leucine	$8.350^{b}\pm0.005$	$7.910^{a}\pm0.005$	$8.900^{c}\pm0.005$	$9.310^{d}\pm0.005$
Lysine	$3.450^{b}\pm0.005$	$3.230^{a}\pm0.005$	$3.740^{c}\pm0.005$	$4.370^{d}\pm0.005$
Isoleucine	$4.260^{b}\pm0.005$	$3.310^{a}\pm0.005$	$4.520^{c}\pm0.005$	$5.140^{d}\pm0.005$
Phenylanine	$4.610^{b}\pm0.005$	$3.720^{a}\pm0.005$	$4.520^{c}\pm0.005$	$5.140^{d}\pm0.005$
Valine	$3.860^{b}\pm0.005$	$3.360^{a}\pm0.005$	$4.240^{c}\pm0.005$	$4.740^{d}\pm0.005$
Methionine	$1.350^{b}\pm0.005$	$1.260^{a}\pm0.005$	$1.390^{c}\pm0.005$	$1.470^{d}\pm0.005$
Proline	$5.180^{b}\pm0.005$	$4.160^{a}\pm0.005$	$5.623^{c}\pm0.005$	$6.090^{d}\pm0.005$
Arginine	$4.823^{b}\pm0.005$	$3.530^{a}\pm0.005$	$4.990^{c}\pm0.005$	$5.850^{d}\pm0.005$
Tyrosine	$3.780^{b}\pm0.005$	$2.920^{a}\pm0.005$	$3.780^{c}\pm0.005$	$4.470^{d}\pm0.005$
Histidine	$2.620^{b}\pm0.005$	$1.920^{a}\pm0.005$	$2.910^{c}\pm0.005$	$3.350^{d}\pm0.005$
Cystine	$1.210^{b}\pm0.005$	$0.850^{a}\pm0.005$	$1.330^{c}\pm0.005$	$1.390^{d}\pm0.005$
Alanine	$4.130^{b}\pm0.005$	$3.603^{a}\pm0.005$	$4.360^{c}\pm0.005$	$4.550^{d}\pm0.005$
Glutamic acid	$14.310^{d}\pm0.005$	$10.290^{a}\pm0.005$	$13.470^{c}\pm0.005$	$12.890^{b}\pm0.005$
Glycine	$3.560^{c}\pm0.005$	$2.520^{a}\pm0.005$	$3.210^{b}\pm0.005$	$3.870^{d}\pm0.005$
Threonine	$3.220^{b}\pm0.005$	$2.860^{a}\pm0.005$	$3.500^{c}\pm0.005$	$3.750^{d}\pm0.005$
Serine	$3.700^{b}\pm0.005$	$3.003^{a}\pm0.005$	$3.810^{c}\pm0.005$	$4.160^{d}\pm0.005$
Aspartic acid	$6.820^{b}\pm0.005$	$5.640^{a}\pm0.005$	$7.320^{c}\pm0.005$	$8.003^{d}\pm0.005$
Tryptophan	$0.920^{b}\pm0.005$	$0.786^{a}\pm0.005$	$1.050^{c}\pm0.005$	$1.230^{d}\pm0.005$

Values are means ± standard deviation of triplicate determinations. Means with same superscripts in a row were not significantly different ($p > 0.05$). Key: 101= 100% acha; 102=75% acha 20% pigeon peas5% mushroom; 103= 70% acha, 20% pigeon peas, 10% mushroom; 104=65% acha, 20% pigeon peas, 15% mushroom.

oyster mushroom, shown in Table 3.

Analysis of Data

A completely randomized design (CRD) was used with three replicates for this analysis and results were subjected to statistical analysis using Statistical Product for service solution (IBM SPSS Statistics 24.0) and Expert Software Version 11. The significant difference between means were determined using (ANOVA). The means were separated using the Duncan Multiple Range test and significance was accepted at $p < 0.05$ as described by (27).

RESULTS AND DISCUSSION

Table 5 presents the amino acid composition of breakfast cereals made from various blends of acha, pigeon pea, and oyster mushroom. Each amino acid's concentration (%) is provided for four samples (101 to 104) at different proportions, the means ± standard deviation and significant differences with same superscript indicates no significant ($p > 0.05$) difference, respectively.

Leucine levels showed values from 8.35% (sample 101), 7.910% (sample 102), 8.90 (sample 103), 9.31% (sample 104) and sample 104 with the highest value, showing the nutritional benefit of adding pigeon pea and oyster mushroom, as supported by (23). Lysine concentrations ranged from 3.23% in sample 102 to 4.37% in sample 104, with all samples showing significant ($p < 0.05$) differences. This increase is particularly beneficial since lysine is often limited in cereals, aligning with findings by (5). Isoleucine levels improved significantly, rising from 3.31% in sample 102 to 5.14% in sample 104, supporting the findings of (28). Phenylalanine content increased between 3.72% (sample 102), 4.52% (sample 103) and 5.14% in sample 104, with the blend improvements is consistent with (23). Valine concentrations rose from 3.36% in sample 102, 4.24% in sample 103 and 4.74% in sample 104. Methionine levels showed a slight increase from 1.26% in sample 102 to 1.47% in sample 104, an essential amino acid often lacking in plant-based diets, as highlighted by (29). Proline content significantly rose from 4.16% in sample 102 to 6.09% in sample 104.

Arginine levels increased from 3.53% in sample 102 to 5.85% in sample 104. Tyrosine content increased from 2.92% in sample 102 to 4.47% in sample 104, further enhancing the nutritional profile of the blends (23). Histidine increased from 1.92% in sample 102 to 3.35% in sample 104, important for growth and tissue repair (30). Cystine levels rose from 0.85% in sample 102 to 1.39% in sample 104, enhancing the nutritional value by providing sulfur-containing amino acids. Alanine content increased from 3.60% in sample 102 to 4.55% in sample 104, supporting overall amino acid profile improvement (28). Glutamic acid showed the highest concentration among all amino acids, with values ranging from 10.29% in sample 102 to 14.31% in sample 101, essential for metabolic functions and neurotransmitter activity (29). Other amino acids such as Glycine, Threonine, Serine, Aspartic acid, and Tryptophan also showed increased concentrations, with the highest values generally in sample 104, crucial for various bodily functions (31).

Table 6 summarizes the various concentrations of essential compounds of breakfast cereal produced from blends of pigeon pea, acha, and oyster mushroom parameters (essential, non-essential, acidic, neutral, sulfuric, aromatic, and their percentages). Each sample (101 to 104) represents different proportions, with means ± standard deviation; the same superscript (a, b, c, d) indicates no significant difference ($p > 0.05$), respectively. Total amino acids (TAA) ranged from 64.640% (sample 102) to 89.590% (sample 104), showing substantial increases (23, 16). Total non-essential amino acids (TNEAA) range from 36.330% to 51.670%, indicating a positive impact of these ingredients (28).

Total essential amino acids with histidine (TEAA with His) increase from 28.360% in (102) to 38.320% in (104), enhancing the nutritional quality. Similarly, total essential amino acids without histidine (TEAA without His) show significant improvement, ranging from 26.440% to 34.970% (31). Percentage of total non-essential amino acids (% TNEAA) varies from 56.160% (102) to 59.290% (101), while percentage of total essential amino acids with histidine (% TEAA with His) ranges from 40.710% to 45.870%. Total neutral amino acids (TNAA) increase significantly, from 37.340% to 50.660%, indicating improved protein content according to (30).

Total acidic amino acids (TAAA) range from

15.930% (102) to 21.130% (101), highlighting enhanced metabolic functions. Total basic amino acids (TBAA) increase from 8.500% (102) to 13.570% (104), indicating enhanced nutritional profiles. Total sulfur amino acids (TSAA) range from 2.110% to 2.860%, and total aromatic amino acids range from 6.640% to 9.703%, both showing significant differences and reflecting improved health benefits (31).

Table 7 presents the amino acid composition of cereal blends from acha, pigeon pea, and oyster mushroom with reference to whole egg standards. This demonstrates their potential as plant-based protein sources; in sample 104, leucine (1.11%), lysine (0.70%), and isoleucine (0.92%) were enhanced. Leucine, thereby addressing protein synthesis and muscle maintenance, supports the research work of (31). Immunity function is built up with the addition of legumes, which

Table 6. Concentration of essential compounds of breakfast cereal produced from blends of pigeon pea, acha and oyster mushroom

Amino acid	101 (%)	102 (%)	103 (%)	104 (%)
Total Amino acid (TAA)	80.150^{b}±0.005	64.640^{a}±0.005	82.586^{c}±0.003	89.59^{d}±0.005
Total non-essential amino acid (TNEAA)	47.520^{b}±0.005	36.330^{a}±0.005	47.850^{c}±0.005	51.670^{d}±0.005
Total essential amino acid with Histi-dine (TEAA)	32.630^{b}±0.005	28.360^{a}±0.005	35.040^{c}±0.005	38.320^{d}±0.005
TEAA without Histidine	30.010^{b}±0.000	26.440^{a}±0.005	32.130^{c}±0.008	34.970^{d}±0.005
% TNEAA	59.290^{d}±0.005	56.160^{a}±0.005	57.730^{c}±0.005	57.712^{b}±0.005
% TEAA with Histidine	40.710^{a}±0.005	45.870^{d}±0.000	42.270^{b}±0.005	42.770^{c}±0.005
% TEAA without Histidine	37.440^{a}±0.005	42.760^{d}±0.005	38.760^{b}±0.005	39.030^{c}±0.005
Total neutral amino acid (TNAA)	44.350^{b}±0.005	37.340^{a}±0.005	46.680^{c}±0.005	50.660^{d}±0.005
Total acidic amino acid (TAAA)	21.130^{d}±0.005	15.930^{a}±0.005	20.790^{b}±0.005	20.890^{c}±0.005
% TAAA	26.360^{d}±0.005	24.760^{b}±0.008	25.080^{c}±0.005	23.320^{a}±0.005
Total basic Amino (TBAA)	10.890^{b}±0.005	8.500^{a}±0.005	11.400^{c}±0.005	13.570^{d}±0.005
% TBAA	17.610^{b}±0.005	13.750^{a}±0.005	14.040^{c}±0.005	16.370^{d}±0.005
Total Sulphur Amino acid (TSAA)	2.550^{b}±0.005	2.110^{a}±0.005	2.720^{c}±0.005	2.860^{d}±0.005
% (TSAA)	3.180^{a}±0.005	3.410^{c}±0.005	3.280^{b}±0.005	3.190^{a}±0.005
% Cystine in TSAA	47.436^{b}±0.018	40.280^{a}±0.005	48.900^{d}±0.005	48.600^{c}±0.005
Total aromatic amino acid	6.723^{a}±1.666	6.640^{a}±0.005	8.570^{b}±0.003	9.703^{b}±0.005
% TArAA	10.470^{c}±0.005	10.260^{a}±0.005	10.310^{b}±0.005	10.830^{d}±0.005

Values are means ± standard deviation of triplicate determinations. Means with same superscripts in a row were not significantly different ($p > 0.05$). Key: 101= 100% acha; 102=75% acha 20% pigeon peas5% mushroom; 103= 70% acha, 20% pigeon peas, 10% mushroom; 104=65% acha, 20% pigeon peas, 15% mushroom.

are associated with lysine, thus supporting (32) and arginine (0.96%), having a role in insulin sensitivity in diabetic patients, as reported by (34). Essential sulfur amino acids such as methionine at 0.46% and cystine at 0.78% agree with (33) that help combat oxidative stress.

Despite this, these blends contain protein values below those seen in whole eggs; however, the blends have the correct amino acid composition, leading to a better-quality dietary protein. Consuming pulse-based proteins aids glycemic control of diabetes and muscle maintenance as they substitute animal proteins, including providing nutrient-dense options such as improved amino acid balance, enhanced digestibility, sustained nitrogen retention, and complementary proteins, which are in line with the dietary restrictions and health needs of diabetic patients (35). Plant proteins generally have lower saturated fat, supporting cardiovascular health, which is crucial for diabetics (36).

Table 8 presents the essential amino acid score of the breakfast cereal produced from blends of acha, pigeon pea, and oyster mushroom with reference to the (13) standard. The highest values for threonine (0.94%), isoleucine (1.29%), and leucine (1.33%) were recorded in sample 104, respectively, attributing to the inclusion of pigeon pea and oyster mushroom, which are rich in essential amino acids. These findings support the research by (37), that stated, cereal-legumes interactions for balanced amino acid profiling of foods.

Compared to the (13) reference standards, these

Table 7. Amino acid composition of cereal blends from acha, pigeon pea, and oyster mushroom with reference to whole egg standards

Amino acid	1O1 (%)	102 (%)	103 (%)	104 (%)
Leucine	0.100^{a}±0.005	0.950^{b}±0.005	1.060^{c}±0.005	1.110^{d}±0.005
Lysine	0.560^{b}±0.005	0.530^{a}±0.005	0.600^{c}±0.005	0.700^{c}±0.005
Isoleucine	0.760^{b}±0.005	0.590^{a}±0.005	0.810^{c}±0.005	0.920^{d}±0.005
Phenylamine	0.900^{b}±0.000	0.730^{a}±0.005	0.940^{c}±0.008	1.030^{d}±0.005
Valine	0.520^{b}±0.005	0.450^{a}±0.005	0.570^{c}±0.005	0.630^{d}±0.005
Methionine	0.420^{b}±0.005	0.390^{a}±0.000	0.430^{a}±0.005	0.460^{c}±0.005
Proline	1.360^{b}±0.005	1.100^{a}±0.005	1.470^{c}±0.005	1.610^{d}±0.005
Arginine	0.790^{b}±0.005	0.580^{a}±0.005	0.820^{c}±0.005	0.960^{d}±0.005
Histidine	1.090^{b}±0.005	0.800^{a}±0.005	1.210^{c}±0.005	1.400^{d}±0.005
Cystine	0.680^{b}±0.005	0.470^{a}±0.008	0.740^{c}±0.005	0.780^{d}±0.005
Alanine	0.770^{b}±0.005	0.670^{a}±0.005	0.810^{c}±0.005	0.850^{d}±0.028
Glutamic acid	1.190^{b}±0.005	0.860^{a}±0.005	1.120^{c}±0.005	1.07^{d}±0.005
Glycine	1.190^{c}±0.005	0.840^{a}±0.005	1.070^{b}±0.005	1.290^{d}±0.005
Threonine	0.630^{b}±0.008	0.556^{a}±0.005	0.690^{c}±0.005	0.740^{d}±0.005
Serine	0.470^{b}±0.005	0.380^{a}±0.005	0.480^{b}±0.005	0.496^{b}±0.005
Aspartic acid	0.630^{b}±0.005	0.520^{a}±0.005	0.680^{c}±0.061	0.740^{d}±0.003
Tyrosine	0.950^{b}±0.005	0.730^{a}±0.005	0.950^{b}±0.005	1.120^{c}±0.005

Values are means ± standard deviation of triplicate determinations. Means with same superscripts in a row were not significantly different ($p > 0.05$). Key: 101= 100% acha; 102=75% acha 20% pigeon peas5% mushroom; 103= 70% acha, 20% pigeon peas, 10% mushroom; 104=65% acha, 20% pigeon peas, 15% mushroom.

blends have lower values, but they have certain advantages for diabetic patients. Leucine is important for muscle protein synthesis and glucose metabolism, which are important for diabetics who are at risk of muscle protein loss (31). Lysine (0.80% in sample 104), aids immune function and enhances collagen formation, critical for wound healing in diabetic individuals. This agrees with findings by (38), that highlighted lysine's importance in improving immune response and tissue repair. Tryptophan (1.23% in sample 104) is crucial in diabetic aiding metabolic regulation, as reported by (39). Therefore, the present results show that cereal blends are functional, sustainable, and healthful substitutes for diabetic dietary control and dietary management.

Table 9 illustrates the protein quality of breakfast cereals made from various blends of acha,

Table 8. Essential amino acid score of the breakfast cereal produced from blends of acha, pigeon pea and oyster mushroom with reference to FAO/WHO (1973) standard

Amino acid	1O1 (%)	102 (%)	103 (%)	104 (%)
Threonine	0.810^{b}±0.005	0.720^{a}±0.005	0.880^{c}±0.005	0.940^{d}±0.005
Isoleucine	1.070^{b}±0.005	0.830^{a}±0.005	1.130^{c}±0.005	1.290^{d}±0.005
Leucine	1.190^{b}±0.000	1.130^{a}±0.005	1.270^{c}±0.008	1.330^{d}±0.005
Lysine	0.630^{b}±0.005	0.593^{a}±0.005	0.680^{c}±0.005	0.800^{d}±0.005
Sulphur Amino Acid (Met + Cystine)	0.730^{b}±0.005	0.6000^{a}±0.000	0.780^{c}±0.005	0.820^{d}±0.005
Total Aromatic amino acid (Pheny + Tyr +Try)	1.550^{c}±0.005	1.180^{b}±0.005	1.600^{c}±0.005	0.780^{a}±0.005
Tryptophane	0.920^{b}±0.005	0.790^{a}±0.005	1.050^{c}±0.005	1.230^{d}±0.005
Valine	0.780^{b}±0.005	0.670^{a}±0.005	0.850^{c}±0.005	0.900^{d}±0.005

Values are means ± standard deviation of triplicate determinations. Means with same superscripts in a row were not significantly different ($p > 0.05$). Key: 101= 100% acha; 102=75% acha 20% pigeon peas5% mushroom; 103= 70% acha, 20% pigeon peas, 10% mushroom; 104=65% acha, 20% pigeon peas, 15% mushroom.

Table 9. Protein quality of breakfast cereal produced from blends of acha, pigeon and oyster mushroom

Parameters	101 (%)	102 (%)	103 (%)	104 (%)
Predicted protein efficiency (P-PER)	2.930^{b}±0.005	2.820^{a}±0.005	3.180^{c}±0.005	3.290^{d}±0.005
Biological Value (B.V)	75.500^{b}±0.005	63.510^{a}±0.005	82.040^{c}±0.005	92.940^{d}±0.005
EEAI	0.800^{b}±0.005	0.690^{a}±0.005	0.860^{c}±0.005	0.960^{d}±0.005
% EEAI	80.000^{b}±0.000	69.000^{a}±0.005	86.000^{c}±0.008	96.000^{d}±0.005

Values are means ± standard deviation of triplicate determinations. Means with same superscripts in a row were not significantly different ($p > 0.05$). Key: 101= 100% acha; 102=75% acha 20% pigeon peas5% mushroom; 103= 70% acha, 20% pigeon peas, 10% mushroom; 104=65% acha, 20% pigeon peas, 15% mushroom.

pigeon pea, and oyster mushroom, which were evaluated using protein efficiency ratio (P.E.R.), biological value (B.V.), essential amino acid index (EAAI), and percentage of EAAI (% EAAI). The means with the same superscripts in a row were not significantly different ($p > 0.05$), while different superscripts (a, b, c, d) indicate that the values are significantly different. Protein efficiency ratio (P.E.R.) values range between 2.93 (101), 2.82 % (102), 3.18 % (103) and 3.29 % (104). The highest P.E.R. in sample 104 indicates the most efficient protein utilization, aligning with studies showing mushrooms' positive effects on protein efficiency due to their high-quality protein and balanced amino acid profile (16). The B.V. of the cereal blend ranged from 63.51% (102) to 92.94% (104). The higher B.V. (104), with the most oyster mushrooms, suggests enhanced quality (24). EAAI values range from 0.69% (102) to 0.96% (104). The highest EAAI in sample 104 confirms the superior amino acid profile of the blend with the highest oyster mushroom content. This supported findings that oyster mushroom fortification enhances food nutritional quality. The % EAAI ranges from 69.0% in sample 102 to 96.00% (104). This measure reflects the protein's adequacy for human dietary needs, with the highest % EAAI (104) corroborating the enhanced nutritional value provided by higher oyster mushroom content (16).

CONCLUSION

The amino acid composition analysis of breakfast cereals was made from blends of acha, pigeon pea, and oyster mushroom blend. According to results of this research, most of the parameters improved significantly, especially the sample 104, which contains the highest levels of leucine (9.31%), lysine (4.37%), and isoleucine (5.14%). These amino acids are vital for protein synthesis, muscle repair, and metabolic regulation and particularly beneficial for diabetic patients.

The blends also showed enhancements in arginine (5.85%) for insulin sensitivity and cystine (1.39%) for antioxidant defenses. Such compositions make these cereal blends promising functional foods for managing diabetes, supporting muscle maintenance, and promoting overall health. The addition of pigeon pea and oyster mushroom complements amino acid deficiencies in acha, offering a plant-based alternative with enhanced nutritional value. These findings strongly suggest that these blends are potentially sustainable and healthy dietary alternatives for diabetic people.

Conflict of Interest

There was no conflict of interest between the authors during the course of research work.

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