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Impact of acha, pigeon pea, and oyster mushroom flour blends on amino acid profiles: Economic and health benefit

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Abstract

Most Africans, especially the people of Nigeria, are diagnosed for reduced muscles mass, slow metabolism, and low immunity due to consumption of poor-quality proteins. In this research, an investigation on the possibilities of improving the amino acid contents, the protein value of composites flours of different blends of acha, pigeon pea and oyster mushroom was done. Four formulations were evaluated: sample A1P0M0 contained only acha, while samples A75P20M5 contained 75% acha, 20% pigeon peas, and 5% mushroom; sample A70P20M10 contained 70% acha, 20% pigeon peas, and 10% oyster mushroom, and sample A65P20M15 contained 65% acha, 20% pigeon peas and 15% oyster mushroom. For amino acids profile, Leucine levels increased from 9.40 % (A75P20M5) to 10.01 % (A65P20M15). Concentration of essential amino acid, amino acid score based on whole chicken egg amino acid and essential amino acid score increased as pigeon pea and oyster mushroom were increased with threonine ranging from 0.99 % (A1P0M0) to 1.13 % (A65P20M15). Protein quality improved, ranging from 3.53 % (sample A1P0M0) to 3.83 % (sample A65P20M15) for protein efficiency ratio. This study suggests best flour blends to optimize nutritional quality, economic and health benefit.

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INTRODUCTION

Acha (*Digitaria exilis*), commonly referred to as fonio, is a small-grain considered to have been cultivated in West Africa for thousands of years (1). It is enjoyed and valued for its nutritional benefits, including large quantities of essential and non-essential amino acids also micronutrients (2). Acha has some limitations in lysine content; lysine is an essential amino acid and is frequently low in cereals (3).

Pigeon pea (*Cajanus cajan*) is a perennial crop belonging to the family Leguminosae and is cultivated mainly in the tropical and subtropical parts worldwide (4). It is well endowed with proteins and essential amino acids, particularly lysine, which makes it a good complement to cereals such as acha. Introducing pigeon pea in food blends can increase protein quality only to a great degree, by addressing nutritional gaps, particularly in amino acid profiles. Pigeon pea is rich in essential amino acids such as lysine, which is often limited in cereals like acha. When combined, these foods complement each other's nutritional deficiencies, leading to a balanced amino acid profile (5).

The use of mushrooms, especially the oyster mushroom (*Pleurotus ostreatus*), is discovered to have nutritional value and be a cure for most diseases. It contains protein, vitamins, minerals, and bioactive compounds that are vital for our body. Oyster mushrooms are also rich in total sulfur-rich amino acids such as methionine and cysteine, which are normally low both in cereals, and pulses (6).

The principle of complementation has an impact on the amino acid profile score of the flour blend by combining ingredients with varying amino acid compositions to balance nutritional deficiencies. For example, blending acha that is limited in lysine with pigeon pea, which is lysine-rich, creates a more complete protein profile. Similarly, oyster mushrooms add sulfur amino acids like methionine and cysteine, which are often deficient in both cereals and legumes. This complementary effect enhances the overall protein quality and nutritional value of the blend (5).

Essential amino acids are rich in acha, lysine content is rich in pigeon peas, and sulfur amino acids are rich in oyster mushrooms (7). Uzodinma et al. (8) reported that pigeon pea blends significantly enhanced protein utilization can be attributed to the improved bio-accessibility of essential amino acids because pigeon peas are rich in lysine, an amino acid often deficient in cereals like acha, that supports weight and muscle mass maintenance. Similarly, Babarinde et al. (7) highlighted the benefits of such blends in enhancing essential amino acid profiles, crucial for dietary fortification.

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.

In conclusion, the combination of acha, pigeon pea, and mushroom in flour blends offers a viable approach for enhancing nutritional content of foods. This approach not only improves the amino acid profile but also contributes to better overall health outcomes. The study's goals focused on to determine the amino acid profile of flour blends composed of acha, pigeon pea, and oyster mushroom.

MATERIALS AND METHODS

Materials

Procurement of raw resources

Production of acha flour

The procedure outlined by Ubbor et al. (11) was adapted with minor adjustments to prepare the acha flour. Two kilograms of acha grains were sorted to remove stones, dirt, chaff and other extraneous matters before washing in pure tap water and drained in a plastic sieve. Acha grains were gently placed in a hot air oven at a temperature of 50 °C (6 h; Gallenkemp 300 Plus, England) in order to minimize microbial growth and for proper milling. After drying, the dried samples were ground with a Fritsch hammer mill (Fritsch Pulverisette 19 Mill, Fritsch GmbH, Germany) into a fine powder and passed through a 500 µm mesh sieve. The flour was sealed in an airtight polyethylene bag and was displayed at a room temperature of 23 ºC for other analysis.

Production of pigeon peas flour

Procedure of Arukwe at al. (12) was followed for the preparation of pigeon peas flour with slight changes. 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 grain was oven-dried (Gallenkemp, 300 Plus, England) at 60 °C for 8 h to reduce moisture content, enable efficient milling and suitable storage for 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.

Production of oyster mushroom flour

Slightly altered version Owheruo et al. (13) were used to prepare the oyster mushroom flour. The fresh oyster mushroom (1 kg) sample was sorted to remove dirt and debris then washed (in clean tap water), clean sharp knives was used to cut the oyster mushrooms into uniform pieces for even drying using oven (Gallenkemp, 300 Plus, England) at 60 °C for 6 min for faster drying, ground with a Fritsch hammer mill (Fritsch Pulverisette 19 Mill, Fritsch GmbH, Germany) into a fine powder, and sieved (500 µm mesh) to fine flour. The blended flour mixtures were thoroughly combined to ensure uniform distribution of ingredients and stored in a zip lock bag, at room temperature (23°C) until needed.

Preparation of composite flour:

Sample codes A1P0M0, A75P20M5, A70P20M10, and A65P20M15 represent different blends of flour with varying proportions of acha, pigeon pea, and oyster mushroom. Specifically:

• A1P0M0: 100% acha flour (control).

• A75P20M5: 75% acha, 20% pigeon pea, 5% oyster mushroom flour.

• A70P20M10: 70% acha, 20% pigeon pea, 10% oyster mushroom flour.

• A65P20M15: 65% acha, 20% pigeon pea, 15% oyster mushroom flour.

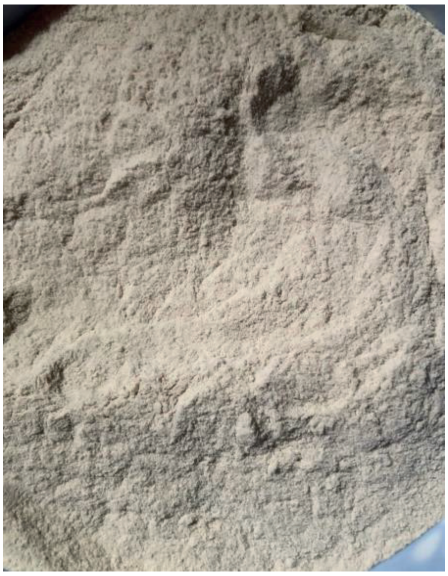

Plate 1. Acha flour

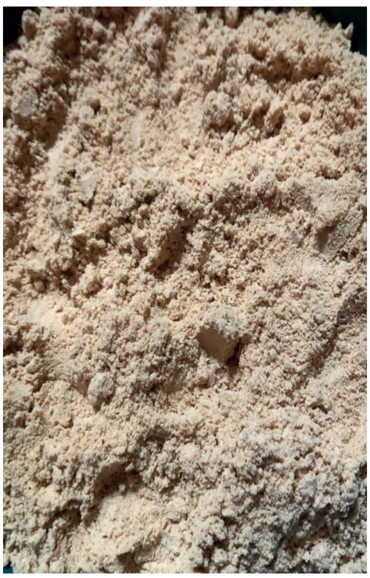

Plate 2. Oyster mushroom flour

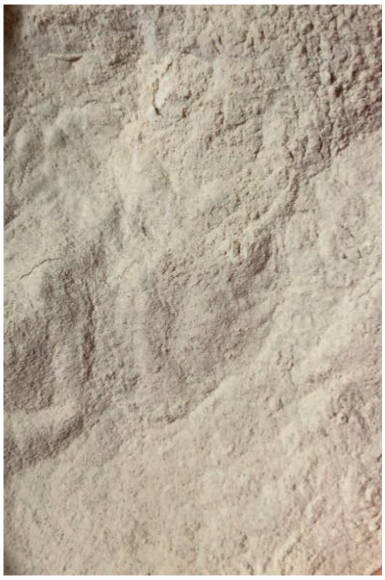

Plate 3. Pigeon pea flour

The flour blends were thoroughly blended using a Kenwood KW-3006 350W electric mixer (Kenwood Appliances, Uk). and stored in a zip-lock bag as are detailed in Table 1.

Methods

Protein quality determination

Amino acid profile

The determination of amino acid profile was done following (15), a defatted 20 mg sample was placed in a glass ampoule, 7 mL of 6 hydrochloric acid, were added into the ampoule and nitrogen passing through the solution was used to remove oxygen. The glass ampoule, flamed with a Bunsen burner and was then placed in an oven already set at 105±5°C for 22 h. The ampoule was cooled (23°C) and then cut open carefully at the top using a glass cutter. The contents were filtered using Whatman No. 1 filter paper to remove particulates and ensure a clear solution for analysis. Identification of amino acids was identified using technique of ion exchange chromatography on a multiple-sample amino acid analyzer in sequence analyser Technician (Technician Instruments Corporation, New York, USA). Tryptophan was excluded from this analysis, L-Norleucine (Sigma-Aldrich, USA) was used as the internal reference standard. The amino acid analyzer was calibrated with Norleucine at a known concentration of 5 μmol/mL, ensuring precision and consistency in quantifying the amino acids in the sample. This method allows for accurate determination of amino acid concentrations, expressed as a percentage of total protein content. The determination of the amino acid profile was conducted following established protocols. A defatted 20 mg sample was placed in a glass ampoule, and 7 mL of 6 N hydrochloric acid (HCl) was added. Oxygen was removed by passing nitrogen gas through the solution. The ampoule was sealed using a Bunsen burner flame and placed in an oven at 105 ± 5°C for 22 hours for hydrolysis.

After cooling, the ampoule was opened, and its contents were filtered. Amino acid identification was performed using ion exchange chromatography on a Technician Instruments Corporation amino acid analyzer (New York, USA). Norleucine served as the internal reference standard, and the amino acid content was quantified based on peak areas provided by the integrator. Results were expressed as a percentage of the total protein. Note: Tryptophan was excluded due to degradation in the acidic environment.

Nutritional parameters were determined on the basis of the amino acid profiles.

Amino acid score determination

Three different methods were used to calculate the amino acid scores

1. The scores are based on amino acid values when compared to those of a whole chicken egg (16)
2. The essential amino acid scoring pattern established by (10) is used to calculate the scores as reported by (9)

Essential amino acid index (EAAI)

Equation proposed by (17) was used to calculate the essential acid index.

Table 1. Proportions of acha, pigeon pea, and oyster mushroom flour blends

Sample code	Acha (%)	Pigeon pea (%)	Mushroom (%)
A1P0M0	100	0	0
A75P20M5	75	20	5
A70P20M10	70	20	10
A65P20M15	65	20	15

Key: A1P0M0= 100% acha flour (Control); A75P20M5=75% acha, 20% pigeon peas, 5% oyster mushroom flour; A70P20M10= 70% acha, 20% pigeon peas, 10% oyster mushroom flour; A65P20M15=65% acha, 20% pigeon peas, 15% oyster mushroom flour.

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

Where, (Phenyl x Vali x …………….) a is test sample; and (Phenyl x Vali x …………) b content of the sample amino acid is standard protein (%; casein), respectively…Equation 1

Determination of predicted protein efficiency (P-PER)

This was estimated as described by (18) in the equation:

P-PER ……………………..…...…...Equation 2

Determination of predicted biological value (BV)

This was calculated according to method described by (19) in the equation:BV…………………………………… Equation 3

Analysis of data

A completely randomized design (CRD) was used with triplicate replicates for this analysis and was subjected to statistical analysis through IBM SPSS Statistics 24.0 (Statistical Product for service solution) and Expert Software Version 11. The significant difference between means were determined using (ANOVA) and means separated using the Duncan Multiple Range test and significance was accepted at $p < 0.05$ as described by (20).

RESULTS AND DISCUSSIONS

Table 2 presents the amino acid composition of flour samples composed of various blends of acha, pigeon pea, and oyster mushroom. Each amino acid's concentration (%) is provided for four samples (A1P0M0 to A70P20M10) with different ingredient proportions, with means ±

Table 2. Amino acids profile (%) of flour blend samples

Amino acid (%)	A1P0M0	A75P20M5	A70P20M10	A65P20M15
Leucine	9.600±0.005 ^{b}	9.400±0.005 ^{a}	9.813±0.005 ^{c}	10.016±0.005^{d}
Lysine	3.870±0.005 ^{b}	3.450±0.005 ^{a}	4.310±0.005 ^{c}	4.610±0.005 ^{d}
Isoleucine	4.526±0.005 ^{c}	4.190±0.005 ^{a}	4.323±0.005 ^{b}	4.610±0.005 ^{d}
Phenylamine	5.070±0.005 ^{b}	4.170±0.005 ^{a}	5.230±0.005 ^{c}	5.590±0.005 ^{d}
Valine	5.780±0.005 ^{b}	5.610±0.005 ^{b}	4.976±0.005 ^{a}	5.170±0.005 ^{b}
Methionine	2.350±0.005 ^{b}	2.460±0.005 ^{c}	2.190±0.005 ^{a}	2.573±0.005 ^{d}
Proline	6.410±0.005 ^{b}	5.986±0.005 ^{a}	5.983±0.005 ^{a}	6.270±0.005 ^{ab}
Arginine	5.160±0.005 ^{c}	4.830±0.005 ^{b}	4.810±0.005 ^{a}	5.593±0.005 ^{d}
Tyrosine	3.440±0.005 ^{b}	3.110±0.005 ^{a}	3.110±0.005 ^{a}	3.790±0.005 ^{c}
Histidine	2.420±0.005 ^{a}	2.310±0.005 ^{a}	2.693±0.005 ^{a}	2.750±0.005 ^{a}
Cystine	2.256±0.005 ^{c}	2.060±0.005 ^{b}	1.950±0.005 ^{a}	2.480±0.005 ^{d}
Alanine	7.510±0.005 ^{d}	7.090±0.005 ^{b}	7.020±0.005 ^{a}	7.360±0.005 ^{c}
Glutamic acid	18.620±0.005 ^{d}	17.490±0.005 ^{a}	17.640±0.005 ^{b}	17.950±0.005 ^{c}
Glycine	3.713±0.005 ^{c}	3.400±0.005 ^{a}	3.520±0.005 ^{b}	3.870±0.005 ^{d}
Threonine	3.970±0.005 ^{a}	4.003±0.005 ^{b}	4.360±0.005 ^{c}	4.500±0.005 ^{d}
Serine	3.970±0.005 ^{a}	4.240±0.005 ^{b}	4.460±0.005 ^{c}	4.236±0.005 ^{d}
Aspartic acid	4.510±0.005 ^{a}	7.380±0.005 ^{b}	7.570±0.005 ^{c}	7.970±0.005 ^{d}
Tryptophan	2.050±0.005 ^{b}	1.940±0.005 ^{a}	2.210±0.005 ^{c}	2.370±0.005 ^{d}

Values are means ± standard deviation of triplicate determinations. Means with same superscripts in a row were not significantly different ($p > 0.05$). Key: A1P0M0= 100% acha; A75P20M5=75% acha 20% pigeon peas5% oyster mushroom; A70P20M10= 70% acha, 20% pigeon peas, 10% oyster mushroom; A70P20M10=65% acha, 20% pigeon peas, 15% oyster mushroom.

standard deviation and significant differences indicated by different superscripts.

Leucine levels increased with higher oyster mushroom content, from 9.60 % (A1P0M0) to 10.02 % (A70P20M10). This trend demonstrates the nutritional benefit of adding pigeon pea and oyster mushroom, consistent with findings by Benitez (19), which highlight the enhancement of essential amino acids in blended cereal products. Lysine levels varied between 3.40 % (A75P20M5) to 4.31 % (A70P20M10), with significant differences ($p < 0.05$) between all samples. Increase in lysine is particularly beneficial, as lysine is often a deficient amino acid in grains. Krawecka et al. (21) made similar observations of increased lysine content as the legumes combined with cereals boosted the 'protein digestibility corrected amino acid score. Isoleucine showed significant difference ($p < 0.05$), with values increasing from 4.19 % (A75P20M5) to 4.61 % (A70P20M10) as evidenced by previous research emphasizing the complementary amino acid profiles of such blends (22). Phenylalanine content rose from 4.17 % (A75P20M5) to 5.59 % (A70P20M10). The increased levels of phenylalanine and other essential amino acids in the blends are consistent with findings by Benitez (19), which showed that combining grains with legumes and oyster mushrooms improves the amino acid profile significantly.

Valine concentrations increased from 5.61 % (A75P20M5) to 5.17 % (A70P20M10). Methionine levels increased slightly from 2.46 % (A75P20M5) to 2.57 % (A70P20M10). Methionine is another essential amino acid often lacking in plant-based diets, and its increase in these blends supports the nutritional adequacy of the product (23). Proline content showed a significant rise from 5.99 % (A75P20M5) to 6.27 % (A70P20M10). The inclusion of pigeon pea and oyster mushroom significantly enhances the proline content, which is beneficial for maintaining healthy skin and connective tissues. Arginine levels also increased, from 4.83 % (A75P20M5) to 5.59 % (A70P20M10). Arginine is essential for immune function and cardiovascular health, and its increase in these cereal blends aligns with research by Millward (9) on the benefits of legume and oyster mushroom fortification in cereals.

Tyrosine content rose from 3.11 % (A75P20M5) to 3.79 % (A70P20M10), further indicating the enhanced nutritional profile of the cereal blends. This improvement is consistent with findings by Sa et al. (24). Histidine increased from 2.31 % (A75P20M5) to 2.75 % (A65P20M15). Cystine levels rose from 2.06 % (A75P20M5) to 2.48 % (A65P20M15), enhancing the nutritional value of the cereals by providing a source of sulfur-containing amino acids, important for protein synthesis and metabolic functions (19).

Alanine content increased from 7.09 % (A75P20M5) to 7.36 % (A70P20M10). Glutamic acid showed the highest concentration among all amino acids, with values ranging from 17.49 % (A75P20M5) to 17.95 % (A1P0M0). Significant levels of glutamic acid are crucial for metabolic functions and neurotransmitter activity (24). Glycine, Threonine, Serine, Aspartic acid, and Tryptophan also showed increased concentrations across the samples, with the highest values generally observed (A70P20M10). These amino acids are essential for various bodily functions, including protein synthesis, immune response, and neurotransmission (23; 25).

Table 3 presents the mean concentrations of various amino acids in flour samples composed of different blends of acha, pigeon pea, and oyster mushroom. Each sample (A1P0M0 to A70P20M10) represents varying proportions with means ± standard deviation and significant differences indicated by distinct superscripts. Incorporating pigeon pea and oyster mushroom into acha-based cereals significantly improves their amino acid content and overall nutritional value. Total Amino Acids (TAA) range from 93.09 % (A75P20M5) to 102.17 % (A70P20M10), with significant differences among samples ($p < 0.05$). Total Non-Essential Amino Acids (TNEAA) vary from 55.57 % (A75P20M5) to 59.82 % (A70P20M10), showing significant differences ($p < 0.05$). The higher TNEAA levels highlight the positive impact of these ingredients (19).

Total Essential Amino Acids with Histidine (TEAA with His) range from 37.52 % (A75P20M5) to 42.35 % (A70P20M10), with significant different ($p < 0.05$), demonstrating improved nutritional quality. Total Essential Amino Acids without Histidine (TEAA without His) range from 35.22 % to 39.60 %, also showing significant differences ($p < 0.05$) and confirming the beneficial impact on protein quality (25). Percentage of Total Non-Essential Amino Acids (% TNEAA) ranges from 59.69 % (A75P20M5) to 58.55 % (A75P20M5), with significant differences ($p < 0.05$), indicating a balanced nutritional composition. Percentage of Total Essential Amino Acids with Histidine (% TEAA with His) ranges from 40.31 % (A70P20M10) to 41.45 % (A75P20M5), showing significant differences ($p < 0.05$).

Total Neutral Amino Acids (TNAA) range from 56.02 % (A75P20M5) to 59.82 % (A65P20M15), with significant differences ($p < 0.05$). This indicates improved protein content and potential health benefits (18). Total Acidic Amino Acids (TAAA) range from 24.87 % (A75P20M5) to 24.78 % (A1P0M0), with significant differences ($p < 0.05$), highlighting improved metabolic and nutritional functions (25). Total Basic Amino Acids (TBAA) range from 10.57 % (A75P20M5) to 12.58 % (A70P20M10), with significant differences ($p < 0.05$). Total Sulphur Amino Acids (TSAA) range from 4.52 % (A75P20M5) to 5.05 % (A70P20M10), with significant differences ($p < 0.05$). Total Aromatic Amino Acids range from 7.27 % (A75P20M5) to 9.38 % (A70P20M10), with significant differences ($p < 0.05$), reflecting improved health benefits (19).

Table 4 presents the amino acid scores for flour samples composed of various blends of acha, pigeon pea, and oyster mushroom flours, compared against the amino acid profile of whole chicken egg. The scores are expressed as mean values with standard deviations, and significant differences are indicated by different superscripts. Different superscripts (a, b, c, d) reveal that the values are significantly different from each other at $p < 0.05$, while the same superscript indicates no significant difference.

Leucine content increases progressively with the

Table 3. Concentration of essential amino acid of flour blend samples

Amino acid	A1P0M0	A75P20M5	A70P20M10	A65P20M15
TAA	94.720±0.1.95 ^{b}	93.090±0.005 ^{a}	95.806±0.003 ^{c}	102.170±0.005 ^{d}
TNEAA	55.560±0.005 ^{a}	55.570±0.005 ^{a}	56.060±0.005 ^{b}	59.820±0.005 ^{c}
TEAA with His	39.363±0.005 ^{b}	37.520±0.005 ^{a}	39.740±0.005 ^{c}	42.350±0.005 ^{d}
TEAA without His	36.940±0.005 ^{b}	35.220±0.005 ^{a}	37.390±0.000 ^{c}	39.600±0.005 ^{d}
% TNEAA	58.530±0.005 ^{b}	59.686±0.005 ^{d}	58.510±0.005 ^{a}	58.550±0.005 ^{c}
% TEAA with His	41.670±0.005 ^{d}	40.310±0.005 ^{a}	41.480±0.005 ^{c}	41.450±0.005 ^{b}
% TEAA without His	38.910±0.005 ^{c}	37.830±0.005 ^{a}	39.760±0.005 ^{d}	38.630±0.005 ^{b}
TNAA	56.886±0.003 ^{c}	56.020±0.005 ^{a}	59.020±0.005 ^{b}	59.820±0.005 ^{d}
TAAA	23.130±0.005 ^{a}	24.870±0.005 ^{b}	25.210±0.005 ^{c}	24.780±0.005 ^{b}
% TAAA	24.370±0.005 ^{a}	26.710±0.005 ^{d}	26.310±0.005 ^{c}	25.373±0.005 ^{b}
TBAA	11.460±0.005 ^{b}	10.570±0.005 ^{a}	11.480±0.005 ^{c}	12.580±0.005 ^{d}
% TBAA	17.610±0.005 ^{d}	13.750±0.005 ^{a}	14.040±0.005 ^{b}	16.370±0.005 ^{c}
TSAA	4.586±0.005 ^{c}	4.520±0.005 ^{b}	4.130±0.005 ^{a}	5.050±0.005 ^{d}
% (TSAA)	4.840±0.005 ^{c}	4.863±0.003 ^{c}	4.310±0.005 ^{b}	4.190±0.005 ^{a}
% Cystine in TSAA	48.580±0.005 ^{c}	45.580±0.005 ^{a}	46.970±0.005 ^{b}	49.134±0.005 ^{d}
TarAA	8.500±0.005 ^{c}	7.270±0.005 ^{a}	8.330±0.005 ^{b}	9.380±0.005 ^{d}
% TarAA	8.960±0.005 ^{c}	7.810±0.005 ^{a}	8.686±0.003 ^{b}	9.810±0.005 ^{d}

Values are means ± standard deviation of triplicate determinations. Means with same superscripts in a row were not significantly different ($p > 0.05$). Key: A1P0M0= 100% acha; A75P20M5=75% acha 20% pigeon peas5% oyster mushroom; A70P20M10= 70% acha, 20% pigeon peas, 10% oyster mushroom; A70P20M10=65% acha, 20% pigeon peas, 15% oyster mushroom. TAA = Total Amino acid; TNEAA = Total non-essential amino acid; TEAA = Total essential Amino acid with Histidine; TNAA = Total neutral amino acid; TBAA = Total basic Amino; TSAA = Total Sulphur Amino acid. TArAA = Total aromatic amino acid.

addition of pigeon pea and oyster mushroom. This significant increase ($p < 0.05$) highlights the effectiveness of blending acha with pigeon pea and oyster mushroom to enhance leucine levels, aligning with findings from recent studies on the amino acid enhancement properties of legumes and oyster mushrooms (18). Lysine content shows variability, with the highest value observed in A65P20M15 (0.69 %). The values range from 0.56 % (A75P20M5) to 0.700 % (A70P20M10), reflecting significant improvement in lysine content with increasing oyster mushroom content ($p < 0.05$).

Isoleucine scores range from 0.75 % (A75P20M5) to 0.84 % (A70P20M10), with a clear trend of enhancement as the proportion of pigeon pea and mushroom increases. This finding supports Songs et al. (19), who documented the high isoleucine content in mushroom and legume combinations. Phenylalanine content shows a significant increase ($p < 0.05$) from 0.82 % (A75P20M5) to 1.10 % (A70P20M10). Valine content ranges from 0.74 % (A75P20M5) to 0.75 % (A65P20M15), while methionine ranges from 0.77 % (A75P20M5) to 0.80 % (A65P20M15). Both amino acids show significant improvements ($p < 0.05$) with higher oyster mushroom content, corroborating findings from recent studies on nutritional enhancements through food blending (26).

Proline, arginine, and histidine scores show notable increases, with proline ranging from 0.05 % in Sample A75P20M5 to 1.72 % (A65P20M15), arginine from 0.79 % (A75P20M5) to 0.92 % (A65P20M15), and histidine from 0.96 % (A75P20M5) to 1.15 % (A70P20M10). Cystine and alanine scores are significantly higher in Sample A65P20M15 (1.32 %) for cystine and 1.40 mg/L for alanine), indicating effective sulfur amino acid enhancement through the blend (19). Glutamic acid, glycine, and threonine contents increase significantly with the addition of pigeon pea and oyster mushroom. Glutamic acid ranges from 0.86 % (A75P20M5) to 1.07 % (A70P20M10), glycine from 0.90 % (A75P20M5) to 1.07 % (A70P20M10), and threonine from 0.78

Table 4. Amino acid score from blends of acha, pigeon pea and oyster mushroom flours based on whole chicken egg amino acid.

Amino acid (%)	A1P0M0	A75P20M5	A70P20M10	A65P20M15
Leucine	1.150±0.005 a	1.200±0.005 a	1.136±0.141 a	1.200±0.005 a
Lysine	0.640±0.005 b	0.560±0.005 a	0.650±0.005 b	0.690±0.005 c
Isoleucine	0.800±0.005 c	0.7500±0.005 a	0.770±0.005 b	0.8400±0.005d
Phenylamine	0.990±0.000 b	0.816±0.005 a	1.030±0.008 c	1.100±0.005d
Valine	0.740±0.005 b	0.740±0.005 b	0.660±0.005 a	0.750±0.005 b
Methionine	0.730±0.005 b	0.770±0.005 c	0.690±0.005 a	0.800±0.005 d
Proline	1.650±0.005 a	0.050±0.005 a	3.060±0.005 d	1.720±0.005 c
Arginine	0.850±0.005 c	0.790±0.005 a	0.820±0.005 b	0.920±0.005 d
Histidine	1.020±0.005 c	0.960±0.005 a	0.980±0.005 b	1.150±0.005 d
Cystine	1.250±0.005 c	1.153±0.008 b	1.070±0.005 a	1.320±0.005 d
Alanine	1.400±0.005 b	1.320±0.005 a	1.300±0.005 a	1.403±0.028 b
Glutamic acid	1.190±0.005 d	0.860±0.005 a	1.120±0.005 c	1.070±0.005 b
Glycine	1.240±0.005 d	0.900±0.005 b	1.080±0.005 c	0.320±0.005 b
Threonine	0.776±0.008 a	0.780±0.005 a	0.850±0.005 b	0.880±0.005 c
Serine	0.570±0.005 b	0.540±0.005 b	0.570±0.005 b	0.600±0.005 a
Aspartic acid	0.730±0.005 a	0.690±0.005 b	0.766±0.061 a	0.743±0.003 a
Tyrosine	0.870±0.005 c	0.780±0.005 b	0.780±0.005 b	0.720±0.005 a

Values are means ± standard deviation of triplicate determinations. Means with same superscripts in a row were not significantly different ($p > 0.05$). Key: A1P0M0= 100% acha; A75P20M5=75% acha 20% pigeon peas5% oyster mushroom; A70P20M10= 70% acha, 20% pigeon peas, 10% oyster mushroom; A70P20M10=65% acha, 20% pigeon peas, 15% oyster mushroom.

% (A75P20M5) to 0.880 % (A70P20M10). Serine, aspartic acid, and tyrosine scores increase with higher oyster mushroom content.

Table 5 presents the essential amino acid scores for flour samples produced from blends of acha, pigeon pea, and oyster mushroom flours, based on the Ubbor et al. (11) standard. The values are given in % and include the means and standard deviations for each blend. Different superscripts (a, b, c, d) indicate that the values are significantly different from each other at $p < 0.05$, while the same superscript indicates no significant difference. Threonine content varies significantly among the samples, from 0.720 % (A75P20M5) to 0.13 % (A65P20M15). The increase in threonine with higher oyster mushroom content is significant ($p < 0.05$). Isoleucine scores range from 1.05 % (A75P20M5) to 1.16 % (A70P20M10). The mean isoleucine content increases with higher oyster mushroom content, reflecting the findings of previous researcher (19)

Leucine content also shows a significant increase across the samples, from 1.34 % (A75P20M5) to 1.43 % in Sample A70P20M10. This enhancement is consistent with studies that report the benefits of pigeon pea and oyster mushroom supplementation in improving the leucine content in food blends (24). Lysine scores range from 0.63 % in Sample A75P20M5 to 0.77 % in Sample A70P20M10. The observed increases are significant and suggest that pigeon pea, known for its high lysine content, effectively boosts lysine levels when included in the blend (21). Sulphur amino acids (Methionine + Cystine) content ranges from 1.29 % (A75P20M5) to 1.44 % (A70P20M10). There is significant increase in these amino acids, especially in Sample A65P20M15.

Total aromatic amino acids (Phenylalanine + Tyrosine + Tryptophan) show significant variability, from 1.78 % (A75P20M5) to 1.76 % (A70P20M10), with a notable decrease (A65P20M15) to 1.96 %. Tryptophan content increases significantly, from 1.94 % in Sample A75P20M5 to 2.37 % (A70P20M10). This significant enhancement underscores the potential of combining cereals and legumes with oyster mushrooms to boost tryptophan levels, which are critical for serotonin synthesis (27).

Valine content ranges from 1.12 % (A75P20M5) to 1.14 % (A70P20M10). The observed increases are consistent with studies of (25).

Table 6 reveals the protein quality of flour samples composed of different blends of acha, pigeon pea, and oyster mushroom. Values are in %, including means and standard deviations for each blend. P.E.R values vary from 3.47 (A75P20M5) to 3.83 (A70P20M10). The highest P.E.R (A70P20M10) indicates that the blend

Table 5. Essential amino acid score from blends of acha, pigeon pea and oyster mushroom flours based on FAO/WHO (1973) standard

Amino acid (%)	A1P0M0	A75P20M5	A70P20M10	A65P20M15
Threonine	0.986±0.003 ^{b}	0.720±0.005 ^{a}	1.090±0.005 ^{c}	1.130±0.005 ^{d}
Isoleucine	1.110±0.005 ^{b}	1.050±0.005 ^{a}	1.800±0.005 ^{d}	1.160±0.005 ^{c}
Leucine	1.370±0.005 ^{b}	1.340±0.005 ^{a}	1.400±0.005 ^{c}	1.430±0.005 ^{d}
Lysine	0.766±0.005 ^{b}	0.630±0.005 ^{a}	0.730±0.005 ^{ab}	0.770±0.005 ^{b}
Sulphur amino acid (Met + Cystine)	1.310±0.005 ^{c}	1.290±0.005 ^{b}	1.180±0.005 ^{a}	1.440±0.005 ^{d}
Total Aromatic amino acid (Pheny + Tyr + Try)	1.850±0.005 ^{c}	1.780±0.005 ^{b}	1.760±0.005 ^{a}	1.960±0.005 ^{d}
Tryptophan	2.050±0.005 ^{b}	1.940±0.005 ^{a}	2.210±0.005 ^{c}	2.370±0.005 ^{d}
Valine	1.120±0.005 ^{a}	1.120±0.005 ^{a}	4.970±0.005 ^{c}	1.140±0.005 ^{c}

Values are means ± standard deviation of triplicate determinations. Means with same superscripts in a row were not significantly different ($p > 0.05$). Key: A1P0M0= 100% acha; A75P20M5=75% acha 20% pigeon peas5% oyster mushroom; A70P20M10= 70% acha, 20% pigeon peas, 10% oyster mushroom; A70P20M10=65% acha, 20% pigeon peas, 15% oyster mushroom.

with the highest proportion of oyster mushroom (15 %) facilitates the most efficient protein utilization. The Biological Value (B.V) of the flour blends ranges from 95.12 (A75P20M5) to 110.30 (A70P20M10). The B.V measures the proportions of absorbed protein incorporated into the body. The significantly higher B.V (A70P20M10), which includes the highest percentage of oyster mushroom, suggests enhanced protein quality. This finding concurs with previous studies where it was established that the addition of mushrooms enhances nutritional value of proteins (10).

The EAAI values range from 0.98 (A75P20M5) to 1.12 (A70P20M10). EAAI assesses protein quality based on the presence of essential amino acids. Highest EAAI in Sample A70P20M10 confirms superior amino acid profile of the cereal blend with the highest oyster mushroom content. This is backed up by research that shows that fortification of oyster mushroom improves the nutritional value of food stuffs (19). % EAAI mirrors the trend seen in EAAI, ranging from 98.00 % (A75P20M5) to 112.00 % (A70P20M10). This percentage provides a relative measure of the protein's adequacy in meeting human dietary needs. The highest % EAAI (A70P20M10) corroborates the enhanced nutritional value provided by higher oyster mushroom content (24)

The data indicate that increasing the proportion of oyster mushroom in the cereal blend significantly enhances the protein quality, as evidenced by improvements in P.E.R, B.V, EAAI, and % EAAI. These findings are supported by recent literature, which highlights the beneficial effects of oyster mushroom supplementation in boosting the nutritional quality of food products.

CONCLUSIONS

Incorporation of blends obtained from acha, pigeon pea, and oyster mushroom in different proportions into flour significantly increased their amino acid profile and protein quality hence it improves dietary value particularly in places where protein malnutrition is prevalent. These blends provide more balanced intake of amino acids necessary for growth, maintenance of muscles as well as overall health. Different cereal grains blended with protein sources such as mushrooms and pigeon peas increase consumption options other than relying on one food source reducing vulnerability to food deficits or poor diet diversity in areas affected by inadequate food supply. According to results of this research, most of the parameters improved significantly, especially in the sample A65P20M15. It shows that blending is potentially sustainable and provide healthy dietary alternatives.

These findings strongly suggest that these blends can be developed into functional foods or dietary supplements from catering to targeted populations such as sportspersons, the elderly, potentially sustainable and healthy dietary alternatives for people with specific nutritional needs. This would also generate economically viable options for local farmers and communities. The crops can also be included in sustainable agricultural systems for local economic improvement and maintenance of biodiversity. Improved protein quality and amino acid profiles can play a role in solving public health problems

Table 6. Protein quality of flour bends of acha, pigeon and oyster mushroom

Parameters	A1P0M0	A75P20M5	A70P20M10	A65P20M15
P.E.R	3.530±0.005 [b]	3.470±0.005 [a]	3.660±0.005 [c]	3.830±0.005 [d]
B.V	107.110±0.005 [c]	95.120±0.005 [a]	100.52±0.005 [b]	110.300±0.005 [d]
EEAI	1.090±0.005 [c]	0.980±0.005 [a]	1.030±0.005 [b]	1.120±0.005 [d]
% EEAI	109.003±0.000 [c]	98.003±0.005 [a]	103.000±0.008 [b]	112.003±0.003 [d]

Values are means ± standard deviation of triplicate determinations. Means with same superscripts in a row were not significantly ($p > 0.05$) different ($p > 0.05$). Key: A1P0M0= 100% acha; A75P20M5=75% acha 20% pigeon peas5% oyster mushroom; A70P20M10= 70% acha, 20% pigeon peas, 10% oyster mushroom; A70P20M10=65% acha, 20% pigeon peas, 15% oyster mushroom. P.E.R = Protein efficiency ratio, B.V = Biological Value, EEAI = Essential amino acid index related with protein-energy malnutrition and solve the widespread problem of micronutrient deficiency, especially in developing countries.

Conflict of Interest

There was no conflict of interest between authors.

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