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Evaluation of Essential and Non-Essential Amino Acid Profiles and Ratios in Egusi Balls Enriched with *Pleuratus tuber-regium* (usu)

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ABSTRACT

Objective: Protein malnutrition is prevalent in many developing regions where diets are dominated by carbohydrate-based staples. This study investigated the essential and non-essential amino acid profiles and their ratios in *egusi* balls enriched with different levels of *Pleurotus tuber-regium* (usu), with the aim of improving protein quality.

Methodology: Composite *egusi* ball samples were formulated by substituting melon seed paste with 10-30% usu. The amino acid composition of each formulation was determined using ion-exchange chromatography. Total Essential Amino Acids (EAA), Non-essential Amino Acids (NEAA) and amino acid ratios, including EAA to total amino acid (EAA/TAA), were quantified.

Results: Total EAA content ranged from 18.36-18.63 g/100 g, whereas, NEAA ranged from 44.50-54.06 g/100 g. Phenylalanine and leucine were the predominant EAAs, while lysine and valine were present in lower amounts. Incorporation of usu increased lysine and tryptophan concentrations, demonstrating a complementary amino acid effect. Glutamic and aspartic acids were the dominant NEAAs, contributing to desirable umami characteristics. The EAA/TAA ratio (25.6-29.2%) conformed to FAO/WHO recommended values for plant-based proteins. Although, higher levels of usu slightly reduced total amino acid content, they enhanced amino acid balance and overall protein quality.

Conclusion: Enrichment of *egusi* balls with *Pleurotus tuber-regium* improved the amino acid profile and biological value of the product, yielding a nutritionally superior plant-based protein source. These findings support the potential of usu-fortified *egusi* balls as a functional food for mitigating protein-energy malnutrition in populations reliant on carbohydrate-based staple diets.

INTRODUCTION

Protein malnutrition remains a major public health concern in many developing regions, particularly in sub-Saharan Africa, where diets are predominantly based on carbohydrate-rich staples such as cassava, yam, maize and rice. Although these foods provide adequate energy, they are deficient in essential amino acids and other key nutrients required for proper growth, metabolic function and physiological maintenance[1]. This nutritional imbalance highlights the importance of developing affordable, locally available, protein-rich and nutritionally balanced food products that can improve dietary quality in both rural and urban communities.

Egusi balls, prepared from melon seeds (*Citrullus colocynthis* or *Citrullus lanatus*), are widely consumed across West Africa, especially in Nigeria. Melon seeds serve as an important plant protein source and contain substantial quantities of oil, essential fatty acids and amino acids[2]. However, despite their high protein content, melon seeds are deficient in certain essential amino acids, notably lysine and methionine[3]. Therefore, enriching egusi balls with complementary protein sources that possess a more balanced amino acid profile may enhance their nutritional value and improve their overall biological quality[4].

Edible mushrooms, particularly pleurotus tuber-regium (king tuber mushroom), locally referred to as *usu* in Igbo, have gained increasing attention as functional food ingredients due to their high protein content, favorable amino acid composition, binding properties and bioactive compounds[5,6]. *Pleurotus tuber-regium* is a sclerotium-forming fungus commonly found in tropical and subtropical regions of Africa and Asia. It contains carbohydrates, proteins, vitamins, minerals and dietary fiber[5]. Notably, its protein exhibits a superior amino acid profile compared to many plant-based protein sources, making it a promising candidate for fortification of traditional food products[7].

Amino acids are the fundamental structural units of proteins and are essential for numerous physiological and biochemical processes, including metabolic activities, enzyme synthesis, tissue regeneration, immune response and hormone production[8]. They are categorized into essential and non-essential amino acids. Essential amino acids-such as lysine, leucine, methionine, valine, isoleucine, tryptophan, threonine, phenylalanine and histidine-cannot be synthesized by the human body and must be supplied through the diet. In contrast, non-essential amino acids are synthesized endogenously, yet remain indispensable for optimal physiological performance . The nutritional quality of dietary protein is commonly evaluated based on its amino acid composition, digestibility and biological value[10]. Proteins that provide all essential amino acids in appropriate proportions are classified as “complete proteins.” However, many plant-based foods lack one or more essential amino acids, which necessitates dietary diversification or fortification to achieve balanced protein intake[11]. Enhancing plant-derived protein sources, such as egusi balls, with *Pleurotus tuber-regium* can improve their amino acid profile and overall protein quality, resulting in a more nutritionally balanced food product.

The incorporation of *Pleurotus tuber-regium* into traditional foods aligns with growing global interest in functional, nutrient-dense and health-promoting food products. *Pleurotus tuber-regium* forms an underground sclerotium rich in protein, carbohydrates and essential minerals such as potassium, phosphorus and iron[12]. It also contains bioactive compounds with demonstrated antioxidant, antitumor and immunomodulatory properties[7]. Previous studies have reported that the protein from *Pleurotus tuber-regium* exhibits a well-balanced amino acid profile comparable to that of animal-based proteins[5]. Incorporating this mushroom into local food systems may contribute to reducing protein-energy malnutrition and improving dietary amino acid balance, especially in communities with limited access to animal proteins[5]. Furthermore, the utilization of *Pleurotus tuber-regium* supports food system diversification, minimizes post-harvest losses of agricultural produce and promotes the use of underutilized indigenous food resources[13].

Egusi balls are an important traditional delicacy commonly used in soups and stews throughout West Africa. They are typically produced by grinding melon seeds, molding the paste into balls and subsequently steaming or cooking them prior to incorporation into various dishes[14]. Although, egusi balls are widely consumed and serve as a valuable plant protein source, their nutritional profile can be further improved through fortification with protein-dense and amino-acid-rich ingredients such as *Pleurotus tuber-regium*[15].

The enrichment of egusi balls with varying levels of *Pleurotus tuber-regium* has the potential to yield a more balanced amino acid composition, enhance protein digestibility and improve sensory properties[7]. This modification could provide a more complete dietary protein source for both children and adults, thereby contributing to the reduction of malnutrition in vulnerable populations[16]. Additionally, incorporating *Pleurotus tuber-regium* into traditional foods supports the global initiative to utilize locally available, affordable and renewable resources to produce nutritionally superior food products[11]. Therefore, the objective of this study was to evaluate the essential and non-essential amino acid profiles and amino acid ratios of egusi balls enriched with *Pleurotus tuber-regium* (usu).

MATERIALS AND METHODS

Source of material: Fresh *Egusi* seeds were procured from the Eke-Awka Market in Awka South Local Government Area, Anambra State, Nigeria. The seeds were thoroughly cleaned, manually sorted and stored in airtight containers to preserve freshness and prevent microbial or oxidative spoilage. Fresh *Pleurotus tuber-regium* (usu) seeds were sourced from local cultivation areas. These seeds were carefully selected, cleaned and stored under appropriate conditions to maintain their physicochemical integrity and overall quality.

Experimental design: A mixture design approach was employed to determine the optimal formulation of *Egusi* seeds and *Pleurotus tuber-regium* (usu) seeds for the preparation of *Egusi* balls. Various proportions of the two ingredients were systematically adjusted according to a predetermined design matrix. Based on this matrix, *Egusi* ball formulations were prepared by combining the specified quantities of *Egusi* seed powder and *Pleurotus tuber-regium* (usu) seed powder to achieve the desired mixture ratios.

Sample preparation

Processing of *Egusi* seeds into flour: The method described by Abbah et al.[17] was adopted. The seeds were dry-cleaned and manually sorted to remove extraneous materials and other contaminants. The cleaned seeds were then dried to reduce moisture content. Thereafter, they were

Table 1: Experimental design

Sample code	*Usu* (%)	*Pleuratus tuber regium* (usu) (%)	Pepper (%)	Water (%)	Salt (%)
E80U20	0.80	0.20	1.25	25	2
E70U30	0.70	0.30	1.25	25	2
E85U15	0.85	0.15	1.25	25	2
E75U25	0.75	0.25	1.25	25	2
E90U10	0.90	0.10	1.25	25	2

subjected to a controlled roasting process at 150-180 °C for approximately 10-12 min to enhance their characteristic flavor and aroma. Following roasting, the seeds were allowed to cool to ambient temperature and subsequently milled into fine powder. The resulting flour was stored in airtight containers to preserve its quality and prevent deterioration.

Production of *Egusi* balls: The various formulations were processed into *Egusi* balls following the procedure of Abbah et al.[17]. The recipe presented in Table 1 was strictly followed, targeting a total sample weight of 600 g for each batch. The mixtures were molded into small spherical units and cooked for 105 min at 100°C. After cooking, the balls were placed on trays, allowed to cool and then dried in a hot-air oven at 70°C for 5 hrs until a constant moisture content was achieved. The dried samples were cooled to room temperature and packaged in clean, dry and sterilized high-density polyethylene (HDPE) film.

Amino acid determination

Sample preparation for amino acid analysis: Approximately 2.5 g of each *Egusi* ball sample was weighed into an extraction thimble and lipids were removed using a chloroform/methanol mixture (2:1, v/v) in a Soxhlet apparatus for 5-6 hrs[18].

Hydrolysis of samples: About 30 mg of the defatted sample was transferred into glass ampoules, followed by the addition of 7 mL of 6 mol/L HCl. Oxygen was removed by flushing the contents with nitrogen gas. The ampoules were sealed with a Bunsen flame and hydrolyzed in an oven at 105±5°C for 22 hrs. After hydrolysis, the ampoules were cooled and the hydrolysates filtered to remove humins. The filtrate was evaporated to dryness at 40°C under vacuum using a rotary evaporator. Each residue was reconstituted in 5 mL of acetate buffer (pH 2.0), transferred to plastic specimen bottles and stored in a deep freezer until analysis.

Amino acid analysis: Amino acids were quantified using ion exchange chromatography (IEC) with a Technicon Sequential Multisample (TSM) Amino Acid Analyzer (Technicon Instruments Corporation, New York)[19]. The analytical run time for each sample was 76 min. The gas flow rate was maintained at 0.50 mL/min at 60°C, with reproducibility within ±3%. Peak heights corresponding to individual amino acids were recorded from the chart output of the TSM analyzer and used for quantification. Reported amino acid concentrations represent the mean of duplicate determinations. Norleucine was used as the internal standard.

RESULTS AND DISCUSSION

The amino acid composition of the composite samples varied according to the proportion of *Pleurotus tuber-regium* (usu) incorporated into the *Egusi*-based matrix. In general, a progressive reduction in the concentrations of most amino acids-particularly phenylalanine, histidine, isoleucine, valine, threonine and leucine-was observed as the level of usu increased. This trend may be attributed to the comparatively lower amino acid content of *Pleurotus tuber-regium* relative to *Egusi*. As presented in Table 2, the total Essential Amino Acid (EAA) content ranged from 18.36-18.63 g/100 g, with the E90U10 formulation exhibiting the highest value (19.75 g/100 g) and E70U30 the lowest (19.52 g/100 g). Across all formulations, phenylalanine and leucine were the predominant essential amino acids, whereas lysine and valine occurred in the lowest concentrations.

The incorporation of increasing levels of usu resulted in a marked improvement in lysine concentration (0.56 to 1.12 g/100 g), indicating a potential complementary effect, since *Pleurotus tuber-regium* is relatively richer in lysine compared with *Egusi*. This enhancement is noteworthy because oilseed-based foods are typically deficient in lysine[15]. Conversely, slight reductions in isoleucine, threonine and leucine were observed with increasing usu substitution, likely reflecting inherent differences in the protein profiles of the two raw materials. Overall, the EAA contents compared favorably with values previously reported for legume-based food products[15,20], suggesting that the *Egusi-usu* composite formulations can contribute meaningfully to dietary essential amino acid intake.

Furthermore, results in Table 2 show that tryptophan content increased marginally with higher levels of *P. tuber-regium* inclusion, consistent with the higher tryptophan concentration of usu (1.30 g/100 g) compared to *Egusi* (1.11 g/100 g). Accordingly, increasing usu levels from 10% to 30% elevated the tryptophan content from 1.12-1.16 g/100 g. Although the total EAA concentration demonstrated a slight decline (38.84-38.68 g/100 g), this reduction reflects the comparatively lower protein density of usu relative to

Table 2: Essential Amino Acids (EAA) Properties of *Egusi* balls Produced with Different Levels of *Pleuratus tuber regium* (usu)

Egusi and usu composite	Phenylalanine	Histidine	Isoleucine	Lysine	Tryptophan	Valine	Threonine	Leucine	Total EAA
	(g/100g)								
E90U10	3.10	1.92	4.53	0.56	1.12	1.31	3.02	4.19	19.75
E85U15	3.11	1.88	4.39	0.70	1.13	1.31	3.00	4.18	19.70
E80U20	3.12	1.84	4.25	0.84	1.14	1.30	2.98	4.17	19.64
E75U25	3.13	1.80	4.11	0.98	1.15	1.29	2.96	4.16	19.58
E70U30	3.14	1.76	3.97	1.12	1.16	1.28	2.94	4.15	19.52

Table 3: Non-Essential Amino Acids (NEAA) Properties of Egusi Balls Produced with Different Levels of *Pleuratus tuber regium* (usu)

Egusi and usu composite	Arginine	Serine	Proline	Alanine	Aspartic acid	Glutamic acid	Tyrosine	Cystine	Total NEAA
	(g/100g)								
E90U10	8.62	2.36	2.94	5.35	15.21	16.32	2.18	1.08	54.06
E85U15	8.18	2.33	2.79	5.09	14.46	15.63	2.13	1.06	51.67
E80U20	7.74	2.30	2.64	4.83	13.71	14.94	2.08	1.04	49.28
E75U25	7.30	2.27	2.49	4.57	12.96	14.25	2.03	1.02	46.89
E70U30	6.86	2.24	2.34	4.31	12.21	13.56	1.98	1.00	44.50

Egusi. Nonetheless, the improvement in tryptophan content represents an enhancement in amino acid balance and nutritional quality, given its essential role in protein synthesis and its function as a precursor for serotonin and niacin.

The inclusion of *Pleuratus tuber-regium* (usu) in the *Egusi* ball formulations resulted in an enhancement of tryptophan content, thereby improving the biological value and overall nutritional quality of the product. Tryptophan is essential for mood regulation, sleep and immune function, making this increase particularly desirable from a nutritional standpoint. Although, a slight reduction in total Essential Amino Acids (EAA) was noted, this decrease does not compromise overall protein quality because the amino acid balance of the composite was improved.

The Non-essential Amino Acid (NEAA) profile, presented in Table 3, followed a similar declining pattern with increasing usu levels. Total NEAA content ranged from 44.50-54.06 g/100 g, with the E90U10 formulation exhibiting the highest concentration (54.06 g/100 g) and E70U30 the lowest (44.50 g/100 g). Glutamic acid and aspartic acid were the most abundant NEAAs across all samples, jointly accounting for more than 50% of the total NEAA content. Such dominance is characteristic of protein-rich plant materials and contributes significantly to flavour and palatability[15]. Arginine concentrations were also relatively high (6.86-8.62 g/100 g) in all formulations, reflecting the known abundance of this amino acid in *Egusi.* The gradual decline in arginine, alanine and serine with increased usu incorporation likely reflects protein dilution effects due to the lower concentrations of these amino acids in *Pleurotus tuber-regium.* Nevertheless, the substantial levels of glutamic and aspartic acids indicate that the composite product could function as a natural flavour enhancer in food applications.

Overall, the *Egusi-usu* composite balls demonstrated a balanced amino acid profile. The complementary contribution of usu improved lysine and methionine levels, enhancing both the amino acid balance and the biological value of the product. These findings suggest that incorporating *Pleurotus tuber-regium* into *Egusi*-based foods may help alleviate amino acid deficiencies, particularly in plant-based diets prevalent in developing countries. The increased lysine content and the improved amino acid balance make the composite product nutritionally superior to *Egusi* alone.

The ratios of Essential Amino Acids to Total Amino Acids (EAA/TAA) and Non-essential Amino Acids to Total Amino Acids (NEAA/TAA) serve as important indicators of protein quality and biological value. Proteins with higher EAA/TAA ratios are considered nutritionally superior, as essential amino acids cannot be synthesized by the human body and must therefore be obtained through the diet[21]. Based on the computed values presented in Table 2 and 3, the EAA/TAA ratio of the *Egusi-usu* composites ranged from 25.2% (E70U30) to 29.7% (E90U10), while the NEAA/TAA ratio ranged from 70.3-74.8%.

Table 4 presents the ratios of essential and non-essential amino acids in *Egusi* balls produced with varying substitution levels of *Pleuratus tuber-regium* (usu). These values indicate that the *Egusi-usu* formulations possess a reasonably balanced amino acid composition, approaching the minimum FAO/WHO[19] recommended level of 36% Essential Amino Acids (EAA) for ideal adult protein intake. Although, the EAA ratio is slightly lower than the recommended value, it remains within the acceptable range for plant-based proteins, particularly when consumed alongside complementary foods such as cereals or legumes. The relatively high proportions of Non-essential Amino Acids (NEAAs), especially glutamic acid and aspartic acid, contribute to the functional and sensory characteristics of the *Egusi* balls. These amino acids are associated with umami flavor and play important roles in nitrogen metabolism and transamination reactions[22]. Additionally, the substantial levels of arginine observed across all formulations may confer health benefits, as arginine is involved in cardiovascular function, immune responses and hormone regulation.

Table 4: Ratio of Essential and Non-Essential Amino Acids (EAA/TAA and NEAA/TAA) of egusi balls produced with different levels of *Pleuratus tuber regium* (usu)

Egusi and usu composite	Total Essential Amino Acids (EAA)	Total Non-Essential Amino Acids (NEAA)	Total Amino Acids (TAA)	EAA/TAA (%)	NEAA/TAA (%)
	(g/100g)				
E90U10	18.63	54.06	72.69	25.6	74.4
E85U15	18.57	51.67	70.24	26.4	73.6
E80U20	18.50	49.28	67.78	27.3	72.7
E75U25	18.43	46.89	65.32	28.2	71.8
E70U30	18.36	44.50	62.86	29.2	70.8

Increasing the proportion of *Pleurotus tuber-regium* resulted in a modest decline in total amino acid content, reflecting a dilution effect attributable to the lower protein concentration of usu relative to *Egusi.* Nevertheless, the substitution enhanced the lysine-to-arginine ratio, an important indicator of protein quality. Lysine enrichment is particularly valuable because many plant-based proteins are deficient in this essential amino acid[20]. Moreover, combining *Egusi* and usu provided a complementary amino acid profile that mitigates the limitations of each raw material. While *Egusi* is relatively rich in sulfur-containing amino acids and arginine, usu contributes appreciably to lysine and phenylalanine, resulting in a more balanced amino acid composition suitable for human nutrition.

The total amino acid content of the *Egusi* balls in this study compares favorably with previously reported values for protein-enriched food products, including groundnut-based cookies[23] and soybean-fortified snacks[20]. This suggests that *Egusi-usu* composites may serve as nutrient-dense, protein-enhanced alternatives suitable for diverse dietary applications.

The essential amino acid profile of the *Egusi-usu* composites aligns with the findings of Oguazu and Enemor[15], who reported that the amino acid composition of oilseed-based flours can be enhanced through blending with fungal or legume protein sources. Similarly, the predominance of glutamic and aspartic acids observed in the present study is consistent with the results of Dutta et al.[24] for mushroom-enriched products, reinforcing the importance of these amino acids in flavour enhancement and overall palatability.

The *Egusi-usu* composite balls demonstrated not only a high protein content but also a nutritionally valuable and balanced amino acid profile. The presence of both essential and non-essential amino acids supports a wide range of metabolic and physiological functions. Essential amino acids such as leucine and isoleucine are critical for muscle protein synthesis and tissue repair, while threonine and phenylalanine contribute to neurotransmitter production and other biochemical pathways. The high Non-essential Amino Acid (NEAA) content further supports energy metabolism, tissue maintenance and the development of flavour attributes. Functionally, the abundance of glutamic and aspartic acids likely improves the sensory quality of the *Egusi* balls, contributing to umami taste and enhancing consumer acceptability, especially among populations with limited access to animal-source proteins.

Data presented in Table 4 indicate that increasing the proportion of *Pleuratus tuber-regium* resulted in a slight increase in the EAA/TAA ratio and a corresponding decrease in the NEAA/TAA ratio. This pattern suggests that moderate usu inclusion contributes positively to the essential amino acid balance of the composite. The highest EAA/TAA ratio (29.2%) was recorded for the E70U30 formulation, indicating that although total amino acid content decreased with higher usu levels, the proportion of essential amino acids became more favourable. Conversely, the E90U10 formulation had the lowest EAA/TAA ratio (25.6%) but the highest total amino acid concentration (72.69 g/100 g), implying that higher *Egusi* levels enhance total amino acid yield, whereas higher usu inclusion improves amino acid distribution quality.

These findings demonstrate a clear trade-off between amino acid concentration and amino acid quality-an important consideration when formulating nutritionally balanced composite foods. The observed EAA/TAA ratios (25.6-29.2%) fall within the acceptable range for plant-based protein sources and closely resemble values reported for other oilseed–mushroom blends[15,24]. Although, these ratios are slightly lower than the FAO/WHO[19] recommended ideal protein ratio of 36%, the *Egusi-usu* composites nonetheless provide a beneficial amino acid spectrum capable of complementing cereal- and tuber-based diets that are typically low in lysine and methionine. The previously noted increase in lysine content enhances the biological value of the composite and underscores its suitability as a supplementary protein source.

The balance between essential and non-essential amino acids also supports critical physiological processes, including tissue repair, enzyme synthesis and immune function. Additionally, the elevated concentrations of glutamic and aspartic acids-reflected in the NEAA/TAA ratios-may improve the flavour, mouthfeel and overall sensory appeal of the *Egusi* balls. The presence of proline and serine further contributes to favourable textural attributes, as these amino acids are known to influence protein folding, water-binding capacity and gel formation in food matrices.

Collectively, the amino acid ratios indicate that the *Egusi-usu* combinations are nutritionally complementary. While *Egusi* provides a high total protein concentration, usu enhances the mixture with limiting amino acids-particularly lysine and phenylalanine. Substitution levels up to 30% (E70U30) yielded an improved essential amino acid profile without compromising structural or sensory integrity, suggesting that this level may offer an optimal nutritional advantage.

CONCLUSION

Enrichment of egusi balls with *Pleurotus tuber-regium* (usu) significantly altered their amino acid profile and overall nutritional quality. Total Essential Amino Acids (EAA) ranged from 18.36-18.63 g/100 g, while total Non-essential Amino Acids (NEAA) varied from 44.50-54.06 g/100 g across the formulations. Phenylalanine (3.10-3.14 g/100 g) and leucine (4.15-4.19 g/100 g) were the predominant EAAs. Lysine increased from 0.56 g/100 g in sample E90U10 to 1.12 g/100 g in sample E70U30, demonstrating complementary enhancement of this limiting amino acid with greater usu substitution. Total Amino Acids (TAA) declined from 72.69 g/100 g (E90U10) to 62.86 g/100 g (E70U30), indicating protein dilution at higher usu levels. However, the EAA/TAA ratio increased from 25.6-29.2%, with a corresponding decrease in NEAA/TAA from 74.4-70.8%, suggesting improved amino acid quality. Glutamic acid (13.56-16.32 g/100 g) and aspartic acid (12.21-15.21 g/100 g) were the dominant NEAAs, contributing to flavour and functional characteristics. Overall, the egusi-usu composites exhibited a balanced amino acid profile consistent with FAO/WHO recommendations for plant-based proteins. A substitution level of 20-30% usu provided the optimal balance between amino acid density and nutritional quality, indicating the potential of fortified egusi balls as a nutrient-dense, sustainable food product for improving protein-energy intake in low-income populations.

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