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ORIGINAL ARTICLE

Chemometric-based gamma-aminobutyric acid and amino acids profiling of fermented Okpeye using ultra-high-performance liquid chromatography diode-array detector (UHPLC-DAD)

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Abstract

Okpeye, a traditional West African fermented seasoning made from *Prosopis africana* seeds, is recognized for its flavour and nutritional benefits, especially its bioactive gamma-aminobutyric acid (GABA), which exhibits neuroprotective, anti-hypertensive, and anti-diabetic properties. This study applied an Ultra-High-Performance Liquid Chromatography Diode-Array Detector (UHPLC-DAD) and principal component analysis (PCA) to profile GABA and amino acids in four Okpeye samples (locally-sourced and laboratory-produced). Results showed significant variations in GABA content, ranging from 22.86 to 48.06 mg/100 g dry fermented Okpeye across samples. PCA analysis revealed two main components, explaining 83.39% of the variance, which highlighted leucine, lysine, and GABA as key differentiating metabolites. Additionally, a strong positive correlation was observed between GABA and its precursor, glutamic acid, underscoring the role of fermentation in enhancing nutritional quality. This study suggests optimizing fermentation conditions could improve GABA production, maximizing Okpeye's health benefits as a functional food.

Keywords Amino acid · Gamma-aminobutyric acid · High-performance liquid chromatography · Okpeye · Principal component analysis

Abbreviations

PCA	Principal component analysis
GABA	Gamma-aminobutyric acid
MSG	Monosodium glutamate
UHPLC-DAD	Ultra-High-performance liquid chromatography diode-array detector
ANOV	Analysis of variance
KMO	Kaiser–Meyer–Olkin

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Introduction

Fermented foods constitute an essential part of traditional diets worldwide, valued for their distinctive flavour, improved preservation, and enhanced nutritional and functional properties. The fermentation process, often mediated by complex microbial consortia, not only improves food safety and digestibility but also generates bioactive compounds with potential health benefits (Steinkraus 1997; Tamang et al. 2016). In West Africa, one such indigenous product is Okpeye, a condiment derived from the seeds of *Prosopis africana* (*African Mesquite*). It is widely consumed in Nigeria and neighboring countries, where its characteristic aroma and umami taste make it a significant cultural seasoning. Fermentation of Okpeye has been shown to enrich its biochemical profile, enhance protein

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digestibility, and improve its overall nutritional quality (Ugwuanyi 2016; Akpi et al. 2025).

Among the various metabolites formed during fermentation, gamma-aminobutyric acid (GABA) has gained increasing scientific and commercial interest. GABA is a non-protein amino acid synthesized by the enzymatic decarboxylation of glutamic acid through microbial glutamate decarboxylase activity (Tanamool et al. 2019). In humans, GABA serves as the primary inhibitory neurotransmitter in the central nervous system and is associated with various physiological functions, including the regulation of blood pressure, stress reduction, sleep enhancement, and neuroprotection (Phuong et al. 2021; Devi et al. 2023). Due to these health-promoting properties, the enrichment of foods with GABA is being increasingly pursued in the development of functional foods and nutraceuticals. Indeed, several studies on Asian and European fermented foods such as kimchi, soy products, and dairy cultures have demonstrated their potential as natural dietary sources of GABA (Komatsuzaki et al. 2005; Park et al. 2010).

However, African fermented foods remain comparatively underexplored in this regard. Research on Okpeye has primarily focused on its microbial ecology, proximate composition, sensory and nutritional properties (Akpi et al. 2020; Ezeocha et al. 2022). Yet, the production of GABA in Okpeye has not been systematically studied, nor has its relationship with amino acid composition been investigated. In particular, no work has examined whether GABA levels correlate with other metabolites. This understanding is crucial for elucidating the biochemical transformations that occur during fermentation and their contribution to nutritional quality. This knowledge gap restricts both the scientific understanding of Okpeye and its potential development as a health-promoting food ingredient.

To bridge this gap, the present study employs Ultra-High-Performance Liquid Chromatography coupled with a Diode-Array Detector (UHPLC-DAD) in combination with Principal Component Analysis (PCA) to comprehensively profile amino acids and quantify GABA in fermented Okpeye. While previous studies have reported GABA production in Asian and European fermented foods (Komatsuzaki et al. 2005; Wan-Mohtar et al. 2020), no comparable investigation has been conducted for African products. For Okpeye specifically, earlier research has described its microbial ecology and general nutritional properties (Ugwuanyi 2016; Ezeocha et al. 2022), but not its amino acid composition in relation to GABA. This study therefore makes two distinct contributions: (i) it provides the first systematic quantification of GABA in Okpeye using UHPLC-DAD, and (ii) it introduces a chemometric approach (PCA) to reveal the interrelationships between GABA and other amino acids. By integrating chromatographic and multivariate analyses, this work advances understanding of the biochemical basis of Okpeye and underscores its relevance both as a culturally significant food and as a promising functional ingredient with health-promoting applications.

Materials and methods

Sources of raw materials

Three fermented Okpeye seasonings were procured from markets in Orba, designated as Okpeye A, Opi designated as Okpeye B, and Uzouwani designated as Okpeye C, respectively, located within the Nsukka Local Government Area (LGA) of Enugu State, Nigeria. Additionally, one batch was produced at Universiti Kebangsaan Malaysia, Malaysia (UKM), designated as Okpeye D. The seeds used in this production were sourced from the Ogege market in the Nsukka Local Government Area of Enugu State. These Okpeye seeds underwent a thorough sorting process to eliminate debris, and all chemical reagents used in the study were of analytical grade.

Traditional production process of Okpeye

The process can vary slightly depending on the culture, a typical method in Southeastern Nigeria, as described by Akpi et al. (2020), involves boiling the seeds of *P. africana* for 6 h or until they become tender, and the cotyledons swell. The softened cotyledons were allowed to cool down at room temperature before it was extracted by pressing the seeds between the thumb and finger. After this, they were thoroughly washed, drained, and dried in a pot lined with banana leaves. Once dried, the cotyledons were arranged in a basket in thin layers, covered with more leaves, and weighed down with pebbles. Fermentation occurs over the next four days, causing the cotyledons to darken and develop a strong, pungent ammonia smell. After fermentation, the cotyledons were ground into a fine paste and shaped into balls. The Okpeye balls were sun-dried for 4 days until they were hard and turned black, which helped them develop a more aromatic and less pungent flavour.

Amino acid analysis

Stock solution

Standard amino acids containing 2.5 µmol/mL of L-aspartic acid (Asp), L-asparagine (Asn), L-serine (Ser), L-glutamine (Gln), L-arginine (Arg), L-glycine (Gly), L-threonine (Thr),

β-alanine (β-Ala), L-alanine (Ala), L-tryptophan (Trp), L-valine (Val), L-methionine (Met), L-phenylalanine (Phe), L-isoleucine (Ile), L-leucine (Leu), L-lysine (Lys), Proline (Pro), and 1.25 µmol/mL of L cystine were obtained from Waters (USA). A standard solution of L-Hydroxyproline (Hyp) and an internal standard solution (ISS) of L-Aminobutyric acid (AABA) were prepared at 2.5 µmol/mL each.

Amino acid profiling

Amino acid profiling was performed using the method described by Ismail et al. (2021) with minor modifications. Approximately 0.1–0.2 g of fermented Okpeye was subjected to acid hydrolysis using 5 mL of 6 N hydrochloric acid, and then incubated at 110 °C for 24 h. After the hydrolysis, the acid-hydrolyzed fermented Okpeye solution was mixed with 100 pmol/µL of α-aminobutyric acid (AABA), which served as the internal standard, diluted to 100 mL with distilled water, and then filtered through a 0.45 µm cellulose acetate membrane to form mixture A.

A 10 µL aliquot of mixture A was derivatized using 70 µL of AccQ.Fluor borate buffer (Waters, Massachusetts, USA) to achieve a pH range of 8.2–10.0, and further reacted with 20 µL of pre-prepared AccQ.Fluor reagent (Waters, Massachusetts, USA). This reagent is composed of 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC) and AccQ.Fluor reagent diluent was used to derivatize primary and secondary amino acids. The resulting mixture (mixture B) was heated to 55 °C for 10 min to ensure complete derivatization before chromatographic analysis.

The chromatographic analysis was performed using a binary solvent system: solvent A, an aqueous solution of AccQ.Tag™ Eluent A concentrate (WAT052890) at a dilution of 1:10, and solvent B, a mixture of acetonitrile and de-ionized water (60:40). Both solvents were filtered using 0.45 µm membrane filters. One microliter of mixture B was injected into the Ultra-High-Performance Liquid Chromatography Diode-Array Detector (UHPLC-DAD) (Agilent, USA) and eluted using a gradient program. The separation was carried out on a Waters AccQ.Tag column (3.9 mm × 150 mm) at a 1 mL/min flow rate, maintained at 36 °C, with detection at 260 nm. The amino acid peaks were recorded on the chromatogram. A similar approach was carried out for the working standard of 17 amino acids.

The relative contents of amino acids and GABA in the samples were calculated by comparing their peak areas to those of the internal standard compounds.

Gamma-aminobutyric acid GABA analysis of fermented Okpeye by HPLC

Standard solution

The stock solution of GABA and 1-fluoro-2,4-dinitrobenzene (FDNB) was purchased from Sigma (USA). The stock solutions (1.0, 1.5, 2.0, 2.5, and 5.0 mg/L) of GABA were prepared with deionized water and stored at 4 °C before use.

A stock solution of FDNB (1%, w/v) was prepared by dissolving 1 g of FDNB in 100 mL of acetonitrile and stored at 4 °C before use.

Derivitization

A 1 g of the Okpeye sample was added to 10 mL of water and vortexed for 5 min, after which 1 mL of the Okpeye sample, 1 mL of 0.5 mol/L $NaHCO_3$ (pH 9.0), and 1 mL of FDNB (1%, w/v) reagent were added to a volumetric flask of the volume of 10 mL. The mixture was vortexed for 5 s and kept in the dark at 60 °C for 1 h. After cooling to room temperature, phosphate buffer (0.02mol/L, pH 7.0) was added to the scale line of the flask. After mixing, the solution was filtered through a 0.22mm membrane filter (Whatman, USA). A similar approach was carried out for the working standard of GABA.

GABA analysis

A modified version of the Lü et al. (2010) method was employed to assess the GABA content in fermented Okpeye samples. The derivative determination from the samples was performed using a Shimadzu high-performance liquid chromatography (HPLC) system (Japan) equipped with a UV spectrophotometric detector set at 360 nm. InertSustainSwift C18, a reversed-phase column paired with a C18 cartridge was used. The temperature of the column was maintained at 35 °C. The mobile phase comprised solvent A (50% acetonitrile) and solvent B (Phosphate buffer, 0.02 mol/L, pH 7.0). Before use, the column was conditioned and equilibrated with a 16% solution of solvent A and an 84% solution of solvent B. The gradient elution profile was 0 to 20 min: linear gradient from 16 to 100% solvent A, 20 to 26 min: linear gradient from 100% back to 16% solvent A, 26 to 30 min: maintained at 16% solvent A. A 0.22 µm membrane filter was used to filter the mobile phase and degassed before use. Each sample (5 µl) was injected into the chromatograph column. The chromatographic peak of the fermented Okpeye extract was compared against a calibration curve to determine the GABA content in the samples. The analysis was done in triplicate.

Dataset pre-processing

Data from each Okpeye sample were exposited into the dataset table using XLSTAT 2019 software (Addinsoft, Paris, France). Four Okpeye samples containing 17 amino acids and GABA were pre-processed. This pre-processing aimed to minimize variability within the dataset's amino acid and GABA levels. The tests performed during this stage included analysis of variance (ANOVA), box and whisker plots, the Kaiser–Meyer–Olkin (KMO) test, and principal component analysis (PCA).

Analysis of variance (ANOVA)

ANOVA was performed using Minitab 21.0 software (Minitab Inc., USA) to evaluate significant differences among amino acids and GABA in the Okpeye samples.

Outlier removal by box and whisker plot

Before conducting ANOVA and PCA, outliers in the dataset were identified and removed using box and whisker plots on a standardized dataset. The confidence interval for these plots was set at 95%. Skewness in the box and whisker plots was analyzed to assess whether the dataset required transformation. Data points exhibiting significantly different patterns for individual amino acids and GABA within each of the fermented Okpeye samples were flagged as outliers and removed accordingly.

Kaiser–Meyer–Olkin test

Before PCA, the Kaiser–Meyer–Olkin (KMO) test was used to ensure the dataset's adequacy. The adequacy of the dataset is crucial for determining whether a model can effectively extract latent variables. This study applied the KMO test with a significance level of $\alpha = 0.05$. The KMO values were categorized as follows: KMO < 0.5 = inadequate, 0.5–0.7 = mediocre, 0.7–0.8 = good, 0.8–0.9 = very good, and KMO > 0.9 = excellent. Only datasets with KMO values greater than 0.5 are considered acceptable for PCA (Lloret et al. 2017; Watkins 2018).

Correlation test

A Pearson correlation was performed to assess the strength and direction of the linear relationship between amino acids and GABA. In this study, correlation strengths were categorized as follows: weak for $|0.000| < R < |0.300|$, moderate for $|0.300| < R < |0.700|$, and strong for $|0.700| < R < |1.000|$.

Principal component analysis (PCA)

PCA based on Pearson correlation was applied to identify patterns in the dataset, assess the contribution of each amino acid and GABA in the fermented Okpeye samples, and explain the variance between intercorrelated amino acids and GABA. The dataset was transformed by PCA into smaller sets of new independent variables, called principal components (PCs), to reduce dimensionality ($p < 0.05$). Numbers of principal components (PCs) were selected based on a cumulative variability of 75% or more and an eigenvalue of 1 or greater (Taghizadeh et al. 2023). Amino acids and GABA with a factor loading (FL) of 0.750 or higher were considered to have strong factor loadings, while those with $0.500 < FL < 0.749$ were considered moderate, and those with $FL \leq 0.499$ were categorized as weak (Tan et al. 2023). A correlation biplot was used to visualize and interpret the contributions of amino acids and GABA to the group of fermented Okpeye samples.

Results and discussion

Outlier removal

Figure 1 presents the box and whisker plot for the fermented Okpeye samples. Outliers were identified for amino acids such as aspartic acid (Asp), threonine (Thr), proline (Pro), and valine (Val), with data points appearing outside the whiskers of the plot. Additionally, the dataset showed that glycine (Gly) and arginine (Arg) had median values equal to the mean. At the same time, amino acids like hydroxyproline (Hyp), serine (Ser), histidine (His), tyrosine (Tyr), methionine (Met), lysine (Lys), isoleucine (Ile), leucine (Leu), phenylalanine (Phe), and GABA displayed right-skewed distributions (median > mean). In contrast, Asp, glutamic acid (Glu), Thr, Pro, Val, and Ile exhibited left-skewed distributions, where the median was less than the mean. Also, outliers in Thr, Pro, and Val were observed beyond the box's whiskers. All outliers were amino acids with low concentrations, which were removed prior to principal component analysis (PCA).

Amino acid composition of fermented Okpeye seasoning

Amino acids, a crucial compound in the body, play a primary role in protein synthesis, metabolism, and immune response (He et al. 2024). Essential amino acids, including histidine, lysine, leucine, methionine, threonine, phenylalanine, valine, and isoleucine, were found in varying concentrations in the samples studied. Non-essential amino acids

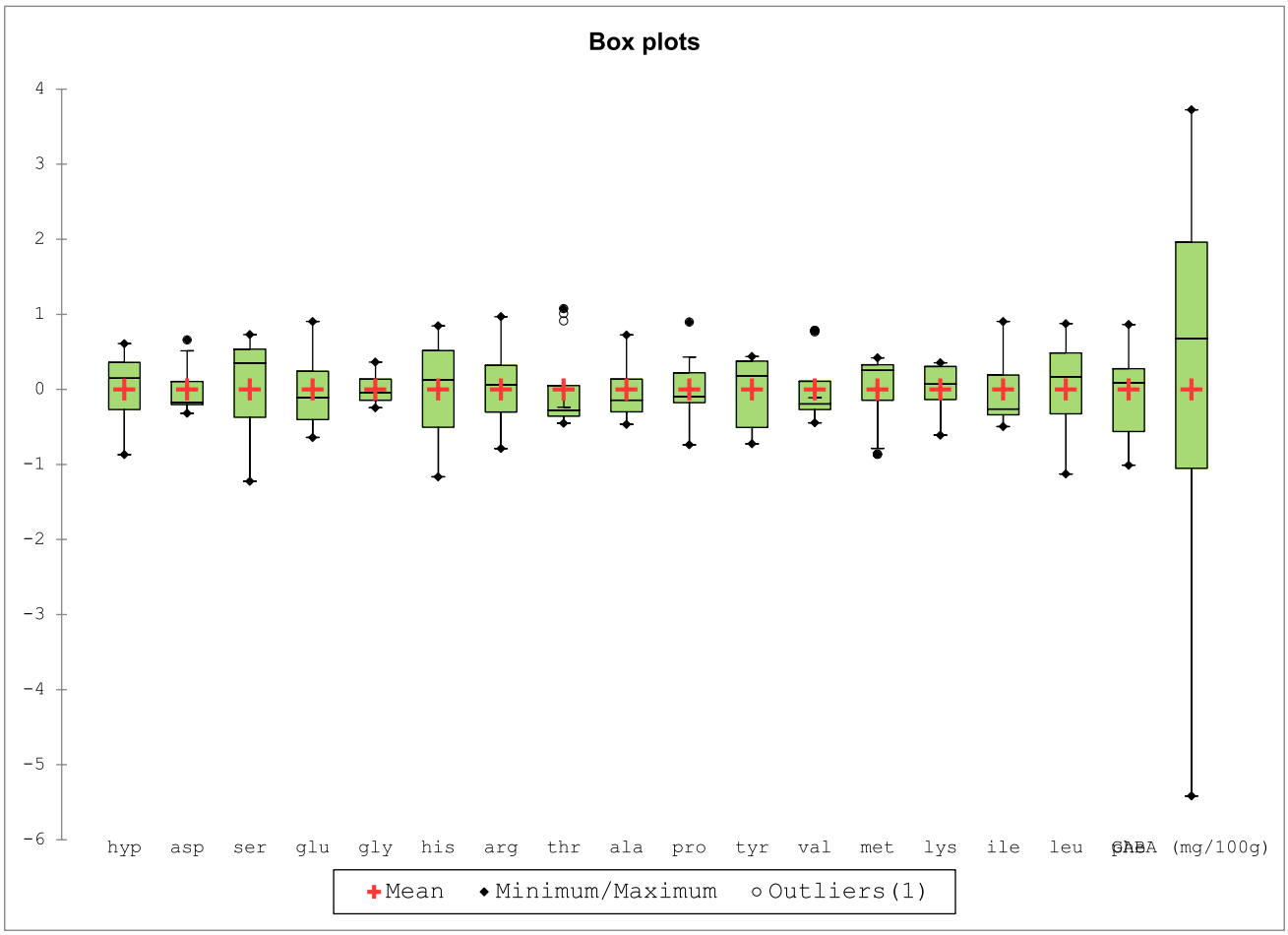


Fig. 1 Box-whisker plots of standardized amino acids and GABA of fermented Okpeye

like serine, hydroxyproline, glutamic acid, aspartic acid, arginine, tyrosine, proline, alanine, and glycine were also present, as outlined in Table 1. Among the essential amino acids, histidine exhibited the highest average concentration across the four Okpeye samples (Okpeye A—17.53 ± 0.38%, Okpeye B—18.42 ± 0.13%, Okpeye C—18.37 ± 0.18%, Okpeye D—17.64 ± 0.12%). Okpeye B and C showed no significant difference in histidine composition, but there was a significant difference between Okpeye A and D. Histidine cannot be synthesized in the human body; however, it has important physiological functions and is a dietary essential amino acid (Brosnan and Brosnan 2020). First, histidine is a precursor to the synthesis of histamine. Histamine is a bioactive substance that regulates vasomotor and blood flow regulation in the cardiovascular system (Yang et al. 2024).

Leucine, another essential amino acid necessary for muscle protein synthesis and tissue repair, had the highest concentration in Okpeye A (12.02 ± 0.38%), followed by Okpeye C (11.97 ± 0.14%), Okpeye B (11.78 ± 0.07%), and Okpeye D (10.94 ± 0.06%). Phenylalanine, involved in neurotransmitter production, was most abundant in Okpeye D (7.4 ± 0.05%) and ranged between 6.76 ± 0.37% and 6.8 ± 0.36% in the other samples. Valine, essential for muscle metabolism, was slightly higher in Okpeye D (5.03 ± 0.01%), with different samples showing similar concentrations (4.44 ± 0.05–4.54 ± 0.03%). Isoleucine, which contributes to muscle repair and energy regulation, peaked in Okpeye D (5.46 ± 0.00%) and significantly differed from other samples (4.71 ± 0.03–4.84 ± 0.08%).

Lysine, essential for calcium absorption and enzyme production, was most concentrated in Okpeye B (4.07 ± 0.01%), with slight variations in the other samples (Okpeye A—3.97 ± 0.03%, Okpeye C—4.01 ± 0.06%, Okpeye D—3.76 ± 0.02%). A deficiency in lysine can lead to issues such as fatigue, irritability, and reproductive complications (Oluwafemi et al. 2021). Methionine, a sulphur-containing amino acid involved in detoxification and liver health, had similar concentrations in Okpeye A, B, and C (approximately 4.7 ± 0.06%) but was significantly lower in Okpeye D (2.40 ± 0.05%). Methionine deficiency may lead to hair loss and skin problems (Zarkada et al. 1997). Threonine levels were relatively low in Okpeye A (1.55 ± 0.04%),

Table 1 Amino acid composition of fermented Okpeye seasoning

Amino acid	Composition (%)			
	Okpeye A (Orba)	Okpeye B (Opi)	Okpeye C (Uzouwani)	Okpeye D (UKM)
Essential amino acid				
Histidine	17.53 ± 0.38^{b}	18.42 ± 0.13^{a}	18.37 ± 0.18^{a}	17.64 ± 0.12^{b}
Leucine	12.02 ± 0.38^{a}	11.78 ± 0.07^{b}	11.97 ± 0.14^{b}	10.94 ± 0.06^{c}
Phenyl-alanine	6.76 ± 0.37^{b}	6.8 ± 0.37^{b}	6.87 ± 0.23^{b}	7.4 ± 0.05^{a}
Isoleucine	4.84 ± 0.08^{b}	4.71 ± 0.03^{b}	4.81 ± 0.05^{b}	5.46 ± 0.00^{a}
Methio-nine	4.68 ± 0.08^{a}	4.71 ± 0.03^{a}	4.71 ± 0.06^{a}	2.40 ± 0.05^{b}
Valine	4.48 ± 0.05^{b}	4.56 ± 0.04^{b}	4.54 ± 0.03^{b}	5.03 ± 0.01^{a}
Lysine	3.97 ± 0.03^{b}	4.07 ± 0.01^{a}	4.01 ± 0.06^{a}	3.76 ± 0.02^{b}
Threonine	1.55 ± 0.04^{c}	1.64 ± 0.03^{b}	1.59 ± 0.06^{c}	2.40 ± 0.05^{a}
Non-essential amino acid				
Serine	8.55 ± 0.17^{b}	8.88 ± 0.05^{b}	9.01 ± 0.08^{a}	7.70 ± 0.05^{c}
Arginine	4.21 ± 0.23^{a}	3.90 ± 0.04^{b}	4.17 ± 0.05^{a}	3.61 ± 0.06^{c}
Hydroxy-proline	3.89 ± 0.05^{b}	3.76 ± 0.07^{c}	4.02 ± 0.06^{a}	3.39 ± 0.05^{d}
Glutamic acid	3.85 ± 0.04^{d}	4.19 ± 0.02^{b}	4.01 ± 0.05^{c}	4.70 ± 0.02^{a}
Alanine	2.74 ± 0.07^{c}	2.77 ± 0.05^{c}	2.84 ± 0.03	3.19 ± 0.02^{a}
Aspartic acid	2.2 ± 0.03^{b}	2.15 ± 0.03^{c}	2.17 ± 0.01^{c}	2.44 ± 0.03^{a}
Tyrosine	1.96 ± 0.24^{b}	2.12 ± 0.05^{b}	2.2 ± 0.01^{a}	1.77 ± 0.02^{c}
Proline	1.21 ± 0.06^{b}	1.17 ± 0.02^{c}	1.23 ± 0.3^{b}	1.25 ± 0.30^{a}
Glycine	1.12 ± 0.03^{c}	1.15 ± 0.04^{b}	1.12 ± 0.01^{c}	1.20 ± 0.03^{a}
Total	85.56 ± 2.33^{a}	86.78 ± 1.08^{a}	83.63 ± 1.41^{a}	84.28 ± 0.94^{a}

Results are mean ± standard deviations of two replicate determinations. Mean values in the same row having different letters as superscripts are significantly different at the 5% level ($p < 0.05$)

Table 2 GABA concentration of fermented Okpeye

Sample	GABA content (mg/100 g of dry fermented Okpeye)
Okpeye A (Orba)	42.49 ± 1.77^{b}
Okpeye B (Opi)	39.30 ± 8.76^{ab}
Okpeye C (Uzouwani)	48.06 ± 6.24^{a}
Okpeye D (UKM)	22.86 ± 5.67^{b}

Results are mean ± standard deviation of triplicate determinations. Mean values in the same column having different letters as superscripts are significantly different at the 5% level ($P < 0.05$)

B (1.64 ± 0.03%), and C (1.59 ± 0.06%), compared to Okpeye D (2.40 ± 0.05%), which supports protein balance and immune function.

Among the non-essential amino acids, serine showed high concentrations, ranging from 7.70 ± 0.05% in Okpeye D to 9.01 ± 0.08% in Okpeye C. Serine plays a role in metabolism, cell proliferation, and the synthesis of biomolecules such as purines and pyrimidines. Glutamic acid, crucial for neurotransmission and energy production, varied slightly across the samples (3.85 ± 0.04–4.70 ± 0.02%). Arginine, essential for nitric oxide synthesis and immune function, ranged from 3.61 ± 0.06% (Okpeye D) to 4.21 ± 0.23% (Okpeye A). Hydroxyproline, a key component of collagen, ranged from 3.39 ± 0.05% (Okpeye D) to 4.02 ± 0.06% (Okpeye C), indicating that these fermented products may help support skin, bone, and connective tissue health.

Other non-essential amino acids, including proline, alanine, aspartic acid, tyrosine, and glycine, were in lower concentrations. These amino acids play a crucial role in various functions, including energy metabolism and the synthesis of neurotransmitters. For instance, glycine, found in concentrations ranging from 1.12 ± 0.03% to 1.20 ± 0.03%, is essential for collagen formation and maintaining healthy skin and joints. Alanine, essential for glucose metabolism, ranged from 2.74 ± 0.07% (Okpeye A) to 3.19 ± 0.06% (Okpeye D), while aspartic acid, which aids in the urea cycle and energy production, was found in lower amounts (2.15 ± 0.03–2.44 ± 0.03%).

Overall, the amino acid profile of fermented Okpeye demonstrates its richness in both essential and non-essential amino acids, positioning it as a valuable dietary supplement for promoting growth, tissue repair, and overall health. This fermented product's diverse range of amino acids enhances its nutritional value, making it a viable alternative protein source, especially in regions with limited access to traditional protein sources.

GABA analysis of fermented Okpeye

Gamma-aminobutyric acid (GABA), a non-protein amino acid, is a key inhibitory neurotransmitter in the mammalian central nervous system and is widely distributed across animals, plants, and microorganisms (Braga et al. 2024). GABA has been shown to exhibit numerous physiological benefits in humans, including antihypertensive, diuretic, and anti-diabetic effects (Devi et al. 2023). Despite these well-documented health benefits, there has been no prior research on producing GABA from fermented Okpeye, a traditional West African fermented food.

In this study, the GABA content of fermented Okpeye seasoning is presented in Table 2. The result shows that GABA concentrations in the fermented Okpeye samples varied from 22.86 ± 5.67 mg/100 g to 48.06 ± 6.24 mg/100 g of dry fermented Okpeye. While there were no statistically significant differences in GABA content between Okpeye A, B, and C, a significant difference was observed between Okpeye D and C. Specifically, Okpeye C had the highest GABA concentration, at 48.06 ± 6.24 mg/100 g, followed by Okpeye A (42.49 ± 1.77 mg/100 g), Okpeye B (39.30 ± 8.76 mg/100 g), and Okpeye D (22.86 ± 5.67 mg/100 g). It is well known that GABA is synthesized in plants by the enzyme glutamate decarboxylase, which is activated under stress

conditions such as low temperatures or reduced atmospheric oxygen levels. Normally, GABA is metabolized to succinic acid in plants, but when plants are subjected to stress, the metabolism of GABA is inhibited, causing it to accumulate. This accumulation of GABA in stressed plants could explain the presence of GABA in fermented Okpeye, which may be subjected to various environmental stresses during its production.

Another critical factor influencing GABA production is the concentration of glutamic acid in the food matrix. A high glutamate level is essential for increased GABA production, as it is the precursor for the synthesis of GABA. Samples with a higher glutamate content are likely to produce more GABA, especially when GABA-producing bacteria are present (Falah et al. 2024). Okpeye samples with lower levels of glutamate may exhibit reduced GABA production. This correlation between glutamate availability and GABA synthesis suggests that the nutritional composition of the starting materials and the microbial composition play a critical role in determining the final GABA content of fermented Okpeye products.

Kaiser–Meyer–Olkin test

The dataset's adequacy for analysis was evaluated using the Kaiser–Meyer–Olkin (KMO) test, which resulted in a value of 0.754. Various studies recommend KMO values above 0.6 for data reliability, including those on food forensics, such as gelatin (Ismail et al. 2021) and amino acid profiles (Taghizadeh et al. 2023). For gelatin sources, KMO values greater than 0.7 have been suggested as appropriate (Ismail et al. 2021). With a KMO value between 0.7 and 0.8, this dataset was deemed suitable for further principal component analysis (PCA).

Correlation test

Pearson's correlation was used to measure the strength and direction of the linear relationship between amino acids and GABA in the dataset. The strength of these correlations was classified as weak ($|R|<0.300$), moderate ($0.300\leq|R|<0.700$), and strong ($0.700\leq|R|<1.000$). While some fields consider strong correlations as low as $|R|\geq0.500$ (Ismail et al. 2021; Taghizadeh et al. 2023), this study adhered to the classification (Table 3).

Among the 17 amino acids and GABA in the Okpeye samples, serine (Ser), valine (Val), methionine (Met), and isoleucine (Ile) exhibited 15 strong correlations each, followed closely by GABA, which had 14 strong correlations. This high degree of connectivity highlights the central role of GABA in the overall amino acid network. The strong relationship between GABA and several amino acids can be attributed to the microbial conversion of glutamate to GABA during fermentation, which in turn influences other pathways of amino acid metabolism. Lysine (Lys) showed 13 strong correlations, while glycine (Gly) and arginine (Arg) had 10 each. In contrast, histidine (His) displayed only one strong correlation, and proline (Pro) showed none, suggesting limited involvement of these amino acids in fermentation-driven transformations.

Beyond numerical strength, these correlations reveal important biochemical interactions. GABA was positively associated with glutamic acid, confirming its role as the metabolic precursor. Strong links with alanine, leucine, and valine suggest that GABA accumulation coincides with broader shifts in amino acid transamination and branched-chain amino acid metabolism, reflecting microbial regulation of fermentation pathways. Notably, several essential amino acids clustered with GABA, underscoring the dual nutritional significance of Okpeye as both a source of functional metabolites and a key dietary source of amino acids.

Comparable relationships have been reported in Asian fermented foods, such as Cheonggukjang (Park et al. 2010), but such findings have not been previously documented in African condiments. This positions Okpeye as a unique case where traditional fermentation enhances both neurotransmitter-related compounds and protein quality simultaneously. Taken together, the correlation data indicate that GABA is not an isolated metabolite, but part of an integrated amino acid network shaped by microbial activity. This provides valuable insight into the biochemical mechanisms underlying Okpeye fermentation and highlights its potential as a multifunctional food with applications in health promotion and dietary diversification).

Principal component analysis (PCA)

PCA was applied to explore patterns in the dataset by identifying amino acids with significant factor loadings and determining those contributing most to sample differentiation. Three principal components (PCs) with eigenvalues greater than 1 explained a cumulative variability of 90.23%. PC1 accounted for most of this variability (75.49%), followed by PC2 (7.90%). These two components alone explained 83.39% of the variance, which is consistent with established guidelines for food composition studies that recommend selecting components with eigenvalues greater than 1 (Taghizadeh et al. 2023). Amino acids and GABA showed strong factor loadings ($FL\geq|0.750|$) across the first two components. In PC1, Hydroxyproline (Hyp), Aspartic acid (Asp), Serine (Ser), Glutamic acid (Glu), Glycine (Gly), Arginine (Arg), Threonine (Thr), Alanine (Ala), Tyrosine (Tyr), Valine (Val), Methionine (Met), Lysine (Lys), Isoleucine (Ile), Leucine (Leu), Phenylalanine (Phe), and GABA

Table 3 Amino acids, correlation matrix and factor loading in fermented Okpeye

Amino acids[1]	Correlation Matrix, R[2,3,4,5,6]								
	Hyp (14)	Asp (14)	Ser (15)	Glu (14)	Gly (11)	His (1)	Arg (11)	Thr (14)	Ala (14)
Hyp	1	−0.87	0.86	−0.90	−0.75	0.35	0.85	−0.92	−0.83
Asp	−0.87	1	−0.93	0.84	0.70	−0.51	−0.67	0.95	0.93
Ser	0.86	−0.93	1	−0.79	−0.68	0.73	0.74	−0.90	-0.82
Glu	−0.91	0.84	−0.79	1	0.82	−0.17	−0.90	0.94	0.91
Gly	−0.75	0.70	−0.68	0.82	1	−0.22	−0.66	0.74	0.77
His	0.34	−0.51	0.73	−0.17	−0.22	1	0.24	−0.37	-0.26
Arg	0.85	−0.67	0.74	−0.90	−0.66	0.24	1	−0.84	−0.71
Thr	−0.92	0.95	−0.90	0.94	0.74	−0.37	−0.84	1	0.96
Ala	−0.83	0.93	−0.82	0.91	0.77	−0.26	−0.71	0.96	1
Pro	−0.17	0.08	−0.03	0.02	−0.15	0.10	−0.08	0.17	0.13
Tyr	0.74	−0.79	0.76	−0.56	−0.66	0.51	0.34	-0.68	−0.68
Val	−0.91	0.94	−0.87	0.96	0.81	−0.31	−0.80	0.98	0.98
Met	0.90	−0.97	0.92	−0.90	−0.79	0.46	0.74	−0.97	−0.95
Lys	0.80	−0.93	0.92	−0.74	−0.55	0.60	0.66	−0.91	−0.87
Ile	−0.84	0.96	−0.95	0.85	0.70	−0.56	−0.72	0.95	0.93
Leu	0.92	−0.89	0.78	−0.91	−0.81	0.17	0.71	−0.93	−0.94
Phe	−0.62	0.74	−0.79	0.73	0.49	−0.54	−0.75	0.77	0.69
GABA	0.97	−0.90	0.92	−0.90	−0.80	0.50	0.83	−0.93	−0.84

Amino acids[1]	Factor loading (FL)[7,8]										
	Pro (0)	Tyr (10)	Val (15)	Met (15)	Lys (13)	Ile (15)	Leu (14)	Phe (10)	GABA (10)	PC1	PC2
Hyp	−0.17	0.74	−0.91	0.90	0.80	−0.84	0.92	−0.62	0.97	-0.94^{a}	-0.15^{c}
Asp	0.08	−0.79	0.94	−0.97	−0.93	0.96	−0.89	0.74	−0.90	-0.97^{a}	-0.10^{c}
Ser	−0.03	0.76	−0.87	0.92	0.92	−0.95	0.77	−0.79	0.92	0.94^{a}	0.31^{c}
Glu	0.02	−0.56	0.96	−0.90	−0.74	0.85	−0.91	0.73	−0.90	-0.93^{a}	0.28^{c}
Gly	−0.15	−0.66	0.81	−0.79	−0.55	0.70	−0.81	0.49	−0.80	-0.80^{a}	0.21^{c}
His	0.10	0.51	−0.31	0.46	0.60	−0.56	0.17	−0.54	0.46	0.47^{c}	0.90^{b}
Arg	−0.08	0.34	−0.80	0.74	0.66	−0.72	0.71	−0.75	0.83	0.81^{a}	-0.18^{c}
Thr	0.17	−0.69	0.98	−0.97	−0.91	0.95	−0.93	0.77	−0.93	-0.99^{a}	0.01^{c}
Ala	0.13	−0.68	0.98	−0.95	−0.87	0.93	−0.94	0.69	−0.84	-0.94^{a}	0.17^{c}
Pro	1	−0.18	0.10	−0.13	−0.30	0.12	−0.22	−0.08	−0.05	-0.11^{c}	0.30^{c}
Tyr	−0.18	1	−0.70	0.80	0.71	−0.72	0.79	−0.34	0.79	0.77^{b}	0.12
Tyr	0.10	−0.71	1	−0.97	−0.86	0.94	−0.95	0.72	−0.91	-0.98^{a}	0.15^{c}
Met	-0.13	0.80	−0.97	1	0.91	−0.97	0.92	−0.75	0.93	0.99^{a}	0.09^{c}
Met	−0.30	0.71	−0.86	0.91	1	−0.96	0.79	−0.74	0.82	0.91^{a}	0.18^{c}
Ile	0.12	−0.72	0.94	−0.97	−0.96	1	−0.84	0.83	−0.87	-0.97^{a}	-0.16^{c}
Leu	−0.22	0.79	−0.95	0.93	0.79	−0.84	1	−0.52	0.90	0.93^{a}	-0.32^{c}
Phe	−0.08	−0.34	0.72	−0.75	−0.74	0.83	−0.52	1	−0.66	-0.77^{a}	-0.29^{c}
GABA	−0.05	0.79	−0.91	0.93	0.82	−0.87	0.90	−0.66	1	0.96^{a}	-0.02^{c}

[1]Hyp = L-Hydroxyproline, His = L-Histidine, Ser = L-Serine, Arg = L-Arginine, Gly = Glycine, Asp = L-Aspartic acid, Glu = L-Glutamic acid, Thr = L-Threonine, Ala = L-Alanine, Pro = L-Proline, Lys = L-Lysine, Tyr = L-Tyrosine, Met = L-Methionine, Val = L-Valine, Ile = L-Isoleucine, Leu = L-Leucine, Phe = L-Phenylalanine, GABA = Gama-aminobutyric acid

[2]Kaiser–Meyer–Olkin (KMO) value = 0.754

[3]$|0.000| < R < |0.300|$ = weak correlation, $|0.300| < R < |0.700|$ = moderate correlation and $|0.700| < R < |1.000|$ = strong correlation matrix

[4]Correlation matrix with the bold value indicated a strong correlation between amino acids

[5]Value in parenthesis of the amino acid indicated numbers of strong correlation matrix between amino acids

[6]Dataset of fermented Okpeye

[7]$^{a}FL \geq |0.750|$ = strong factor loading, $^{b}|0.500| < FL < |0.749|$ = moderate factor loading and $^{c}FL \leq |0.499|$ = weak factor loading in the principal component

[8]Factor loading with bold superscript indicated strong factor loading in the principal component

all contributed strongly. By contrast, in PC2, only Histidine (His) displayed a strong factor loading, while Histidine (His) and Proline (Pro) showed weak contributions in PC1. This indicates that most of the variation in amino acid composition is captured in the first axis, with only marginal contributions from secondary PCs.

The biplot of amino acids along F1 and F2 axes (Fig. 2) provides further insight into their interrelationships. Amino acids that clustered closely along F1, such as Hyp, Tyr, Arg, Leu, Ser, Lys, Met, and GABA, exhibited strong positive correlations, suggesting they are co-regulated during fermentation. Their dominance in PC1 highlights that these compounds, including essential amino acids and the bioactive metabolite GABA, are key drivers of biochemical differentiation among Okpeye samples. The tight clustering of GABA with glutamate-related amino acids such as Ser and Met supports the role of microbial glutamate decarboxylase activity in shaping metabolite patterns (Tanamool et al. 2019; Corsetti et al. 2024).

In contrast, amino acids such as Gly, Thr, Ala, Val, Asp, Phe, Ile, and Glu projected on the opposite side of F1, suggesting negative correlations with the GABA-associated group. This implies that increases in GABA, Ser, Lys, and Met may coincide with reductions in these amino acids, possibly reflecting competing microbial pathways or resource allocation during fermentation (Corsetti et al. 2024). For

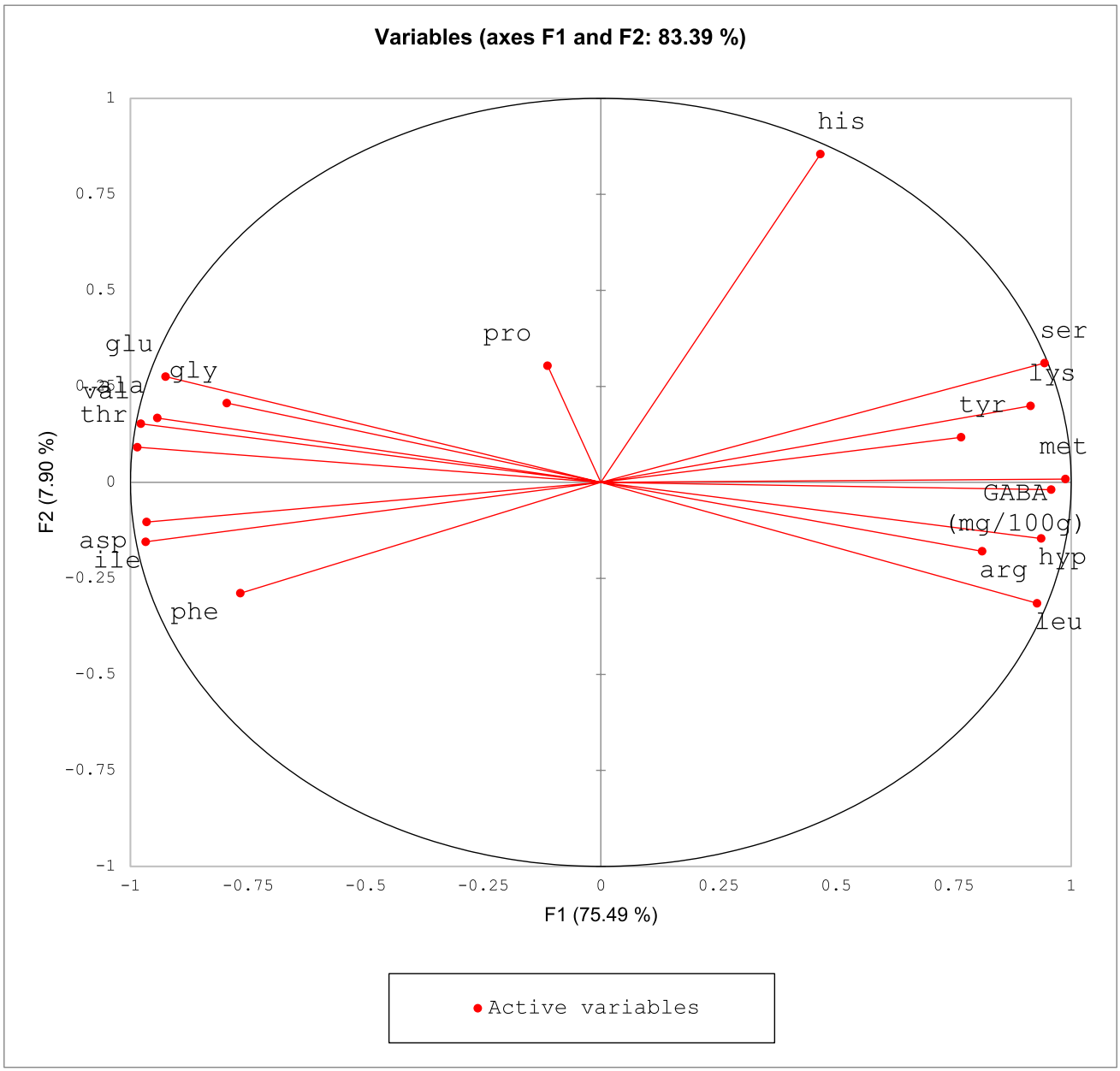


Fig. 2 Amino acid plot (axes F1 and F2: 83.39%)

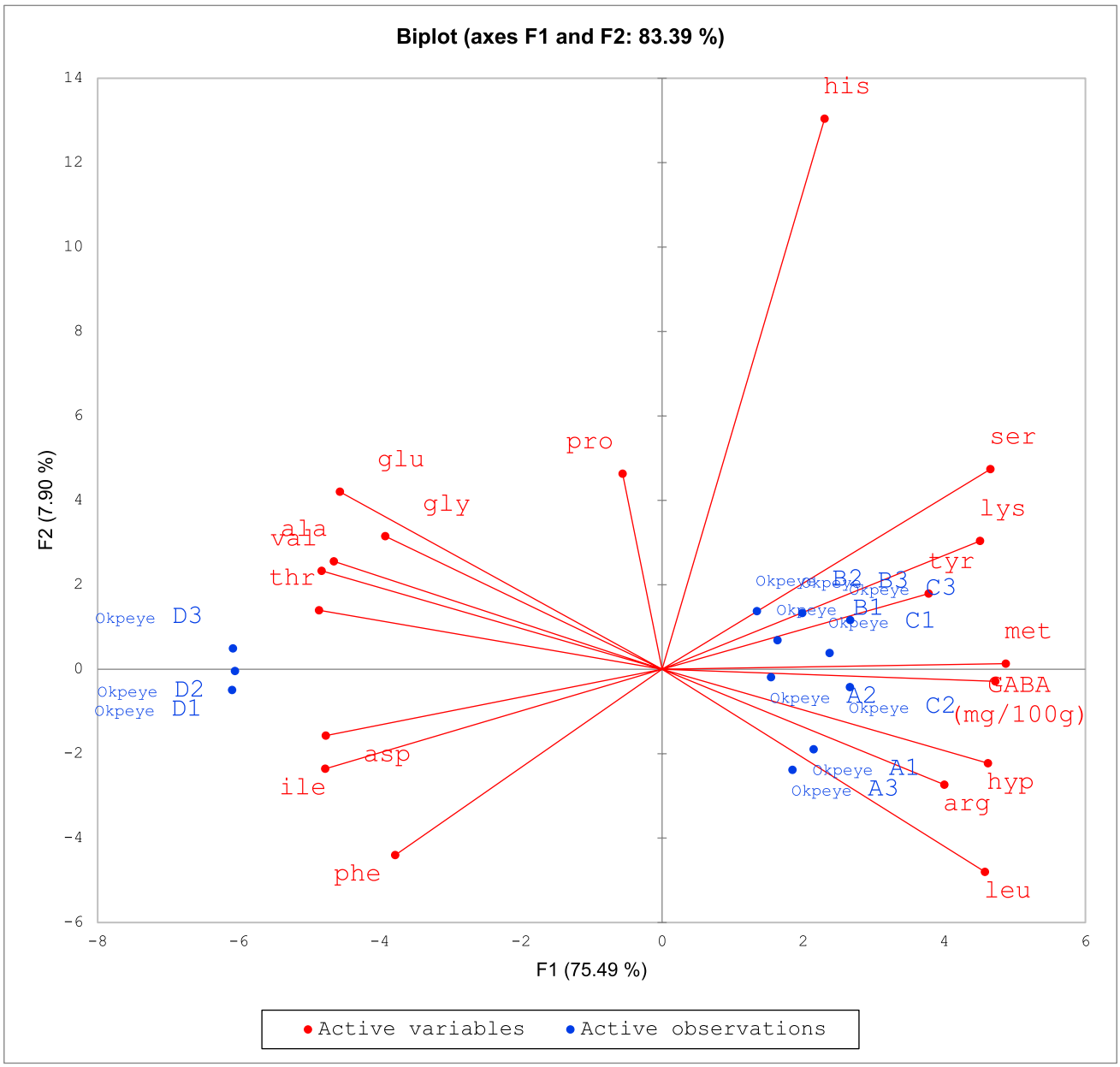


Fig. 3 Correlation biplot of factor loading of amino acids on fermented Okpeye

example, as glutamate is consumed for GABA biosynthesis, other pathways involving Asp or Phe may be downregulated, reflecting differential microbial priorities. Such trade-offs are consistent with findings in other fermented foods where amino acid networks shift dynamically in response to microbial activity (Park et al. 2010).

Although PC2 explained a smaller proportion of variance (7.90%), it provided complementary insights. Proline (Pro) contributed uniquely to PC2, indicating that distinct metabolic processes regulate it. Proline is often associated with stress response and structural protein synthesis, particularly collagen-related metabolism (Karna et al. 2020). The negative correlation of Leu and Arg along F2 relative to Pro may indicate divergent metabolic roles, possibly reflecting shifts between growth-related amino acid metabolism and stress-related pathways. Amino acids with vectors at approximately 90° (His, Pro against Hyp, Glu, Leu, Arg) can be considered uncorrelated (Ismail et al. 2021), further emphasizing the complexity of metabolic interactions in Okpeye.

The biplot distinguishing Okpeye varieties (Fig. 3) showed clear separation based on amino acid composition. Okpeye A was characterized by high levels of Leu, Arg, Hyp, Met, and GABA, suggesting a strong nutritional

potential due to the presence of essential amino acids and a bioactive neurotransmitter-linked metabolite. Okpeye B exhibited elevated Ser, Lys, Tyr, Met, and GABA, which are associated with savoury or umami taste profiles (Yamaguchi & Ninomiya 2000). Okpeye C clustered near B, sharing high levels of GABA, Met, Lys, Ser, and Hyp, but with reduced levels of Thr, Val, Ala, Asp, and Ile, suggesting a similar nutritional profile with subtle compositional differences. In contrast, Okpeye D was distinct, with higher Asp, Ile, Thr, Val, and Ala but lower GABA, Met, Hyp, Lys, and Ser, suggesting a comparatively milder flavour and lower nutritional density.

Taken together, the PCA results highlight that fermentation of Okpeye not only enhances the accumulation of GABA but also reorganizes amino acid networks in ways that influence both nutritional quality and sensory characteristics. The clustering of GABA with essential amino acids, such as Leu and Lys, suggests a synergistic enrichment of functional and dietary compounds. However, this is the first evidence of such interactions in an African fermented condiment, underscoring the novelty of the present work. While PCA provides valuable insights into overall patterns, it cannot explain causal mechanisms, which require complementary approaches such as pathway-specific microbial studies. Nonetheless, the multivariate perspective offered here provides a robust framework for understanding how microbial fermentation drives nutritional and functional enhancement in Okpeye.

Conclusion

This study explored the gamma-aminobutyric acid (GABA) content and amino acid profile of fermented Okpeye. The findings highlight the significant influence of fermentation conditions on the nutritional quality of Okpeye, particularly its GABA content. GABA concentrations, ranging from 22.86 mg/100 g to 48.06 mg/100 g, indicate that Okpeye is a valuable source of this bioactive compound, which is well known for its neuroprotective and therapeutic benefits. Additionally, the amino acid composition, particularly essential amino acids such as leucine, lysine, and glutamic acid, underscores Okpeye's potential as a functional food, contributing to protein synthesis, metabolic health, and neurological function.

Principal component analysis (PCA) provided critical insights into the biochemical variation between different Okpeye samples. PCA revealed distinct clusters driven by differences in GABA and amino acid content, with specific amino acids, such as leucine and lysine, playing key roles in differentiating the samples. This chemometric approach confirmed the variation in nutritional profiles due to fermentation. It demonstrated the strong correlation between glutamic acid and GABA, suggesting that optimizing fermentation can further enhance GABA production.

Author contributions All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Akpi Uchenna Kalu, Muhamad Shirwan Abdullah Sani, and Nurul Aqilah Mohd Zaini. The first draft of the manuscript was written by Akpi Uchenna Kalu and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials All data will be made available on request.

Declarations

Conflict of interest All the authors declare that they have no conflict of interest.

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References

Akpi UK, Nnamchi CI, Ugwuanyi JO (2020) Development of starter culture for the production of African condiments and seasoning agents. Adv Microbiol 10(12):599–622. https://doi.org/10.4236/aim.2020.1012044

Akpi UK, Wan-Mohtar WAAQI, Noor WSAWM, Mohd Zaini NA (2025) Assessment of GABA enrichment and probiotic potential in fermented Okpeye by *Bacillus amyloliquefaciens* AUK. J Food Sci Technol 62(4):1–12. https://doi.org/10.1007/s13197-025-06223-0

Braga JD, Thongngam M, Kumrungsee T (2024) Gamma-aminobutyric acid as a potential postbiotic mediator in the gut–brain axis. NPJ Sci Food 8(1):16. https://doi.org/10.1038/s41538-024-00253-2

Brosnan ME, Brosnan JT (2020) Histidine metabolism and function. J Nutr 150(9):2570S-2575S. https://doi.org/10.1093/jn/nxaa079

Corsetti G, Pasini E, Scarabelli TM, Romano C, Singh A, Scarabelli CC, Dioguardi FS (2024) Importance of energy, dietary protein sources, and amino acid composition in the regulation of

metabolism: an indissoluble dynamic combination for life. Nutrients 16(15):2417. https://doi.org/10.3390/nu16152417

Devi PB, Rajapuram DR, Jayamanohar J, Verma M, Kavitake D, Avany BAM (2023) Gamma-aminobutyric acid (GABA) production by potential probiotic strains of indigenous fermented foods origin and RSM-based production optimization. LWT 176:114511. https://doi.org/10.1016/j.lwt.2023.114511

Ezeocha CV, Ugwuja JL, Onyeabor CS (2022) Evaluation of Indigenous Okpeye (*Prosopis africana*) processing conditions in Nsukka LGA, Enugu, Nigeria and its effect on the quality of the fermented seasoning. Niger Agric J 53(1):191–199

Falah F, Vasiee A, Tabatabaei-Yazdi F, Moradi S, Sabahi S (2024) Optimization of γ-aminobutyric acid (GABA) production by *Lactobacillus* spp. from agro-food waste. Biomass Convers Biorefin 14(3):3425–3437. https://doi.org/10.1007/s13399-022-02361-z

He Y, Qin H, Wen J, Wang L, Cao W, Fan S (2024) Characterization of amino acid composition, nutritional value, and taste of fruits from different *Actinidia arguta* resources. J Food Qual 2024:1005194. https://doi.org/10.1155/2024/1005194

Ismail AM, Sani MSA, Azid A, Zaki NNM, Arshad S, Tukiran NA, Abidin SASZ, Samsudin MS, Ismail A (2021) Food forensics on gelatine source via ultra-high-performance liquid chromatography diode-array detector and principal component analysis. SN Appl Sci 3(1):79. https://doi.org/10.1007/s42452-020-04061-7

Karna E, Szoka L, Huynh TYL, Palka JA (2020) Proline-dependent regulation of collagen metabolism. Cell Mol Life Sci 77:1911–1918. https://doi.org/10.1007/s00018-019-03363-3

Komatsuzaki N, Shima J, Kawamoto S, Momose H, Kimura T (2005) Production of γ-aminobutyric acid (GABA) by *Lactobacillus paracasei* isolated from traditional fermented foods. Food Microbiol 22(6):497–504. https://doi.org/10.1016/j.fm.2005.01.002

Lloret S, Ferreres A, Hernández A, Tomás I (2017) The exploratory factor analysis of items: guided analysis based on empirical data and software. An Psicol 33(2):417–432. https://doi.org/10.6018/analesps.33.2.270211

Lü Y, Zhang H, Meng X, Wang L, Guo X (2010) A validated HPLC method for the determination of GABA by pre-column derivatization with 2,4-dinitrofluorodinitrobenzene and its application to plant GAD activity study. Anal Lett 43(17):2663–2671. https://doi.org/10.1080/00032711003763558

Oluwafemi RA, Agubosi OCP, Alagbe JO (2021) Proximate, minerals, vitamins and amino acid composition of *Prosopis africana* (African mesquite) seed oil. Asian J Adv Res 5(1):1011–1017

Park MK, Cho IH, Lee S, Choi HK, Kwon DY, Kim YS (2010) Metabolite profiling of Cheonggukjang, a fermented soybean paste, during fermentation by gas chromatography-mass spectrometry and principal component analysis. Food Chem 122(4):1313–1319. https://doi.org/10.1016/j.foodchem.2010.03.095

Phuong TNT, Jang SH, Rijal S, Jung WK, Kim J, Park SJ, Han SK (2021) GABA- and glycine-mimetic responses of linalool on the substantia gelatinosa of the trigeminal subnucleus caudalis in juvenile mice: pain management through linalool-mediated inhibitory neurotransmission. Am J Chin Med 49(6):1437–1448. https://doi.org/10.1142/S0192415X21500671

Steinkraus KH (1997) Classification of fermented foods: worldwide review of household fermentation techniques. Food Control 8(5–6):311–317. https://doi.org/10.1016/S0956-7135(97)00050-9

Taghizadeh H, Emamgholipour S, Hosseinkhani S, Arjmand B, Rezaei N, Dilmaghani-Marand A (2023) The association between acylcarnitine and amino acids profile and metabolic syndrome and its components in Iranian adults: data from STEPs 2016. Front Endocrinol 14:1058952. https://doi.org/10.3389/fendo.2023.1058952

Tamang JP, Watanabe K, Holzapfel WH (2016) Diversity of microorganisms in global fermented foods and beverages. Front Microbiol 7:377. https://doi.org/10.3389/fmicb.2016.00377

Tan E, Julmohammad NB, Koh WY, Abdullah MS, Rasti B (2023) Application of ATR-FTIR incorporated with multivariate data analysis for discrimination and quantification of urea as an adulterant in UHT milk. Foods 12(15):2855. https://doi.org/10.3390/foods12152855

Tanamool V, Hongsachart P, Soemphol W (2019) Screening and characterization of gamma-aminobutyric acid (GABA) producing lactic acid bacteria isolated from Thai fermented fish (Plaa-som) and application in Thai fermented vegetables (Som-pak). Food Sci Technol 40:483–490. https://doi.org/10.1590/fst.05419

Ugwuanyi JO (2016) Microbial Technology & Food Security: Microorganisms Put Safe Food on Our Tables.

Wan-Mohtar WAAQI, Sohedein MNA, Ibrahim MF, Ab Kadir S, Suan OP, Weng Loen AW, Ilham Z (2020) Isolation, identification, and optimization of γ-aminobutyric acid (GABA)-producing *Bacillus cereus* strain KBC from a commercial soy sauce moromi in submerged-liquid fermentation. Processes 8(6):652

Watkins MW (2018) Exploratory factor analysis: a guide to best practice. J Black Psychol 44(3):219–246. https://doi.org/10.1177/0095798418771807

Yamaguchi S, Ninomiya K (2000) Umami and food palatability. J Nutr 130(4):921S-926S. https://doi.org/10.1093/jn/130.4.921S

Yang ZH, Huang LF, Wang YS, Chang CC (2024) Turn-off enzyme activity of histidine-rich peptides for the detection of lysozyme. Microchim Acta 191(6):1–10. https://doi.org/10.1007/s00604-024-06388-z

Zarkada CG, Voldeng HD, Vu UK (1997) Determination of the protein quality of three new northern adapted cultivars of common and microtype soybeans by amino acids analysis. J Agric Food Chem 45(4):1161–1168. https://doi.org/10.1021/jf9604201

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