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Effect of Fermentation on the Fatty Acid and Amino Acid Profile of Ofada Rice and Tropical Almond Seed

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ABSTRACT

Background and Objective: The demand for nutritious and healthy foods has increased, prompting research into processing methods that enhance nutritional quality. This study evaluated the effect of fermentation on the fatty acid and amino acid profiles of Ofada rice fortified with tropical almond seed blends. **Materials and Methods:** Ofada rice (Ire-Ekiti, Nigeria) and tropical almond seeds (FUTA campus, Akure) were cleaned, milled, and blended in proportions of 100:0, 90:10, 80:20, 70:30, 60:40, and 50:50. Samples were naturally fermented for 72 hrs. Fatty acid composition was analyzed using gas chromatography (GC-FID), and amino acid profiles were determined via standard chromatographic methods. Data were statistically analyzed to assess significant changes at $p \leq 0.05$. **Results:** Fermentation altered fatty acid composition significantly. Saturated fatty acids (SFAs) increased in the 70:30 and 50:50 blends (25.35%-25.70%), while Monounsaturated Fatty Acids (MUFAs), particularly oleic acid, rose from 7.50% to 12.17%. Polyunsaturated Fatty Acids (PUFAs), especially linoleic acid, increased from 2.96% to 6.14%. Essential amino acids, including leucine (6.54-7.80 g/100 g protein), lysine (4.16-4.37 g/100 g protein), and phenylalanine (3.06-4.64 g/100 g protein), were enhanced. Non-essential amino acids such as glutamic acid also increased (9.61-13.22 g/100 g protein). **Conclusion:** Fermentation of Ofada rice and tropical almond seed blends improves key macronutrients, including PUFAs and essential amino acids, highlighting its potential to enhance the nutritional quality of functional foods. Future studies could explore sensory acceptability and *in vivo* nutritional benefits.

KEYWORDS

Fermentation, Ofada rice, tropical almond seeds, fatty acids, amino acids, nutritional enhancement

INTRODUCTION

Fermentation is a traditional food processing technique that significantly influences the nutritional and biochemical composition of plant-based foods. In Nigeria, Ofada rice, a locally cultivated, unpolished rice variety, and tropical almond seeds (*Terminalia catappa*) are notable for their nutritional content and cultural importance. Recent research has focused on how fermentation alters the fatty acid and amino acid profiles of these foods, aiming to enhance their nutritional value and functional properties.

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Ofada rice, a staple in Southwestern Nigeria, is rich in carbohydrates and essential nutrients. Fermentation of Ofada rice has been shown to modify its physicochemical properties, including increases in moisture, fiber, fat, and protein contents, while reducing carbohydrate, ash, and anti-nutrient level. These changes are attributed to microbial activity during fermentation, which can enhance the rice's digestibility and nutritional profile[1]. Additionally, fermentation can influence the amino acid composition of rice, potentially increasing the availability of essential amino acids and improving protein quality[2].

Tropical almond seeds are underutilized despite their high oil and protein content. Fermentation of these seeds leads to significant biochemical transformations. Studies have identified the emergence of new compounds such as butyric acid and 2,3-butanediol during fermentation, indicating microbial biotransformation of original fatty acids[3]. Fermentation can alter the fatty acid profile, increasing the proportion of unsaturated fatty acids like oleic and linoleic acids, which are beneficial for cardiovascular health[4]. Moreover, fermentation can enhance the amino acid profile of tropical almond seeds, increasing the levels of essential amino acids and improving overall protein quality[5].

Fermentation also significantly influences the amino acid composition of plant-based foods, contributing to improved protein quality and nutritional value. During fermentation, proteolytic enzymes secreted by microorganisms break down complex proteins into simpler peptides and free amino acids, enhancing the bioavailability of essential amino acids such as lysine, leucine, methionine, and valine. These amino acids are vital for tissue repair, immune function, and overall metabolic health. Heo *et al.*[6] reported that fermentation of mixed grains with *Bacillus amyloliquefaciens* led to a marked increase in essential amino acids, particularly lysine and leucine, which are often limited in cereal-based diets. Similarly, Elhais *et al.*[7] highlighted the role of fermentation in enriching amino acid profiles in plant-based meat analogs, enhancing both their nutritional and functional properties. In another study, Owusu-Kwarteng *et al.*[8] emphasized that alkaline fermentation processes in plant-based foods can yield higher concentrations of health-promoting amino acids, thereby making such foods more suitable for addressing protein energy malnutrition in developing regions. Furthermore, Knez *et al.*[9] demonstrated that fermented legumes and vegetables not only had improved amino acid content but also showed enhanced antioxidant activity, which contributes to better health outcomes when included in regular diets. The present study, therefore, aims to investigate the effect of fermentation on the fatty and amino acid content of ofada rice and almond seed blend.

MATERIALS AND METHODS

Study area and duration: Ofada rice was obtained from Ire, Ekiti State, Nigeria (Longitude 7°44' 19" N and Latitude 5°23' 47" E) between July and November, 2022, and mature tropical almond fruits (*Terminalia catappa*) were harvested from trees located within the Federal University of Technology, Akure (FUTA) campus between February and March, 2023.

Collection of samples: Ofada rice (*Oryza glaberrima*) was purchased from a local market in Ire-Ekiti, Ekiti State, Nigeria. Mature tropical almond fruits (*Terminalia catappa*) were harvested from trees located within the Federal University of Technology, Akure (FUTA) Campus.

Preparation of Ofada rice and tropical almond seed samples: The Ofada rice was manually sorted to remove stones, husks, and other extraneous materials. It was then washed thoroughly, air-dried, and milled into fine flour using a laboratory-grade grinder.

Tropical almond fruits were manually depulped, and the extracted seeds were sun-dried to minimize moisture content and prevent rancidity. The dried seeds were manually cracked along the suture lines to obtain the brown, spindle-shaped kernels. These kernels were oven-dried at 100°C for 1 hour, and milled into fine powder, and stored in airtight containers at room temperature until further analysis.

Formulation of blends: Powdered Ofada rice and tropical almond seed flour were mixed in various proportions by weight to obtain six experimental formulations: 100:0, 90:10, 80:20, 70:30, 60:40 and 50:50. Each blend was homogenized thoroughly to ensure uniform distribution of components.

Fermentation of samples: Fermentation was conducted using a natural solid-state fermentation process. For each formulation, 1 kg of the sample was soaked in sterile distilled water at a ratio of 3:1 (water:sample, w/v) in sterile plastic containers. The samples were allowed to ferment spontaneously at ambient temperature (27±2°C) for 72 hours (3 days). After fermentation, samples were dewatered, oven-dried at 60°C, and then milled before subsequent analysis[10].

Fatty acid profile determination: The fatty acid composition of raw and fermented sample blends was analyzed using Gas Chromatography equipped with a Flame Ionization Detector (GC-FID, Alliance Instruments, Switzerland)[11]. Fatty acids were extracted as methyl esters (FAMEs) using standard procedures before GC analysis.

Amino acid profile determination: Amino acid composition was determined by hydrolyzing the samples, followed by chromatographic separation and quantification, using the method described by Weber[12]. Results were expressed in mg/100 g sample, covering both essential and non-essential amino acids.

Statistical analysis: Data gathered was analyzed using Two-way Analysis of Variance (ANOVA) and the means were compared using Duncan's New Multiple range test at $p<0.05$ level of significance (SPSS version 26).

Table 1: Fatty acid composition of unfermented and fermented rice and tropical almond seeds

Fatty acid %/samples	A	R	RASF 50:50	RASF 70:30
Saturated fatty acid (SFA)				
Caproic acid methyl ester (C6:0)	0.000	0.000	0.000	0.000
Caprylic acid methyl ester (C8:0)	0.000	0.000	0.000	0.000
Butyric acid methyl ester (C4:0)	0.000	0.000	0.000	0.000
Capric acid methyl ester (C10;0)	0.000±0.00	0.000±0.00	0.0013±0.0003	0.0027±0.0003
Tridecanoic acid methyl ester (C13:0)	0.000±0.00	0.000±0.00	0.003±0.00	0.005±0.0005
Myristic acid methyl ester (C14:0)	0.259±0.004	0.171±0.001	1.245±0.03	1.352±0.001
cis-10-pentadecanoic acid methyl ester (C15:0)	0.0000	0.0000	0.0000	0.0000
Palmitoleic acid methyl (C16:1)	0.583±0.001	0.384±0.001	1.261±0.001	1.193±0.001
Heptadecanoic acid methyl ester (C17:0)	0.000±0.00	0.000±0.00	0.037±0.001	0.062±0.001
Stearic acid methyl ester (C18:0)	1.053±0.001	1.521±0.002	3.123±0.001	3.076±0.001
Lauric acid methyl ester (C12:0)	4.011±0.001	2.542±0.001	3.786±0.002	2.063±0.001
Palmitic acid methyl ester (C16:0)	8.672±0.001	5.126±0.001	15.914±0.002	17.946±0.002
Behenic acid methyl ester (C22:0)	0.0000	0.000	0.0000	0.0000
SFA	14.578	9.744	25.3973	25.6997
Monounsaturated fatty acid (MUFA)				
Oleic acid methyl ester (C18:1)	2.876±0.001	3.357±0.001	10.850±0.001	12.163±0.001
cis-11-eicosenoic acid methyl ester (C20:0)	0.001±0.0003	0.003±0.001	0.002±0.001	0.002±0.001
Heneicosanoic acid methyl ester (C24:0)	0.000±0.00	0.002±0.001	0.025±0.001	0.002±0.003
MUFA	2.877	3.362	10.877	12.167
Polyunsaturated fatty acid (PUFA)				
Linoleic acid methyl ester (C18:2) n-6	2.973±0.001	2.965±0.001	6.142±0.001	5.646±0.001
Arachidic acid methyl ester (C20.4) n-6	0.199±0.001	0.325±0.001	0.197±0.002	0.364±0.001
g-linolenic acid methyl ester (C18:3) n-3	0.007±0.001	0.004±0.001	0.019±0.001	0.031±0.001
x-linolenic acid methyl ester (C18:3) n-3	0.005±0.001	0.013±0.001	0.139±0.001	0.199±0.001
cis-11-14-eicosadienoic acid methyl ester (C20:2)	0.000±0.000	0.000±0.000	0.002±0.001	0.002±0.0003
PUFA	3.184	3.307	6.499	6.242
MUFA/PUFA	0.903	1.017	1.674	1.949
PUFA/SFA	0.218	0.339	0.256	0.243
(PUFA+MUFA)/SFA	0.416	0.684	0.684	0.716

Each value is expressed as Mean±Standard error (n = 3). Values with different superscripts within a row are significantly different at ($p\leq0.05$), ASF: Almond seed fermented, seed, A: Almond R: Rice and RASF: Rice and almond seed fermented

Table 2: Amino acid composition of unfermented and fermented rice and almond seeds (g/100 g protein)

Amino acid	RUN	AUN	RSF 50:50	RASF 70:30
Leucine	6.54±0.02	7.39±0.05	7.80±0.03	7.19±0.01
Lysine	4.33±0.01	4.23±0.13	4.37±0.01	4.16±0.01
Isoleucine	3.74±0.01	3.52±0.01	4.08±0.03	3.64±0.02
Phenylalanine	4.21±0.03	3.06±0.10	4.64±0.03	4.30±0.03
Tryptophan	1.26±0.01	1.38±0.02	1.30±0.01	1.22±0.01
Valine	4.12±0.02	4.45±0.03	4.32±0.02	4.24±0.00
Methionine	2.24±0.09	2.12±0.01	2.37±0.01	2.30±0.00
Proline	2.49±0.03	3.65±0.01	3.60±0.03	2.50±0.61
Arginine	5.03±0.02	8.11±0.06	7.18±0.02	6.84±0.02
Tyrosine	3.36±0.05	3.62±0.01	4.14±0.01	3.78±0.01
Histidine	2.81±0.01	2.73±0.03	3.05±0.01	2.84±0.02
Cystine	1.24±0.02	2.09±0.02	1.67±0.02	1.54±0.02
Alanine	4.14±0.02	4.50±0.01	4.44±0.02	4.19±0.01
Glutamic acid	9.61±0.04	13.22±0.08	10.13±0.07	10.33±0.02
Glycine	3.84±0.02	3.66±0.31	3.93±0.02	3.76±0.02
Threonine	3.70±0.01	2.56±0.03	3.83±0.03	3.36±0.03
Serine	3.17±0.03	3.32±0.02	3.46±0.02	3.23±0.07
Aspartic acid	6.84±0.06	7.54±0.01	7.41±0.02	7.09±0.03

Each value is expressed as mean±standard error (n = 3). Values with different superscript within a row are significantly different at ($p\leq 0.05$), ASF: Almond seed fermented, ASUN: Almond seed unfermented, RUN: Rice unfermented and RASF: Rice and almond seed fermented

RESULTS

Fatty acid composition of unfermented and fermented rice and almond seeds: The fatty acid profile of the samples showed that the total saturated fatty acids increased in the fermented Rice and Almond Seed blend (RASB) 70:30, and 50:50 (25.35-25.70), and was typically lower in the unfermented rice and almond seeds as presented in Table 1.

The monounsaturated fatty acids increased after fermentation of the rice and almond seeds, ranging from 7.50% to 12.17% compared to the unfermented samples. The highest being the Oleic acid methyl ester component as shown in Table 1. The summation of the polyunsaturated fatty acids ranged from 3.19% to 6.50%. The linoleic acid methyl ester constituent was higher, ranging from 2.96% to 6.14% as presented in Table 1.

Amino acids: Results of the amino acid analysis of the fermented and unfermented rice and tropical almond seeds revealed a varying increase in the amino acid content after fermentation for leucine, lysine, isoleucine, phenylalanine, tryptophan, valine, methionine, histidine, and threonine (essential amino acids) ranging from 1.22-8.17 g/100 g protein. Tryptophan and histidine were the least occurring amino acids (1.22-3.14 g/100 g protein). While the amino acid content in the non-essential amino acids ranged from (1.24-13.22 g/100 g protein) as presented in Table 2. Glutamic acid was present in the highest concentration (9.61-13.22 g/100 g protein).

DISCUSSION

Fermentation of rice and almond seed blends significantly enhanced the fatty acid composition, notably increasing saturated fatty acids (SFA) in the fermented samples (25.40% and 25.70%) compared to unfermented almond seed (14.58%) and rice (9.74%). This increase is likely due to microbial hydrolysis of complex lipids and de novo synthesis of SFAs during fermentation[2,13]. Similar results have been reported in fermented cereal legume and nut based products[14].

Monounsaturated fatty acids (MUFA), especially oleic acid (C18:1), increased significantly post fermentation, with values of 12.16% and 10.85% in RASF 70:30 and 50:50, respectively, compared to 2.88% and 3.36% in unfermented samples. This aligns with findings that fermentation enhances oleic acid through microbial enzymatic activity[15-17]. Oleic acid enrichment improves the nutritional profile, given its known cardiovascular benefits[18].

Polyunsaturated Fatty Acids (PUFA), particularly linoleic acid (C18:2), also increased, indicating improved health value, as observed in fermented cereal nut formulations[12]. The increase in PUFA and omega-3 fatty acids reflects microbial biotransformation of lipid substrates[19,20].

Fat quality indices such as MUFA/PUFA and (MUFA+PUFA)/SFA improved in fermented blends, suggesting enhanced lipid health benefits despite a lower PUFA/SFA ratio due to increased SFAs[21,22]. This supports earlier reports that fermentation can positively modulate lipid quality in food matrice[23].

Fermentation markedly improved the amino acid profile across all blends. Leucine increased from 6.54 g/100 g protein in RUN to 7.80 g in RSF 50:50, while RASF 70:30 maintained a relatively high value (7.19 g), consistent with Zhang *et al.*[2], who observed leucine enhancement in fermented grain blends. Arginine content rose significantly in AUN (8.11 g), with notable levels also in RSF 50:50 (7.18 g) and RASF 70:30 (6.84 g), in line with Adebo and Medina-Meza[20], who linked fermentation to increased arginine in legumes.

Phenylalanine was highest in RSF 50:50 (4.64 g), followed by RASF 70:30 (4.30 g), surpassing RUN (4.21 g), similar to trends reported by Selvan *et al.*[23] in fermented rice-nut mixtures. Glutamic acid, a flavour-enhancing amino acid, peaked in AUN (13.22 g), with RSF and RASF also showing elevated values (10.13 and 10.33 g, respectively), aligning with Arya *et al.*[16].

Cystine, a sulphur-containing amino acid, increased from 1.24 g in RUN to 2.09 g in AUN, supporting Sura *et al.*[22], who noted cystine enrichment through microbial proteolysis. Similarly, threonine rose to 3.83 g in RSF 50:50, compared to 3.70 g in RUN and 2.56 g in AUN, demonstrating synergistic improvement via rice-almond fermentation[18].

CONCLUSION

The fermentation of Ofada rice and tropical almond seed blends significantly influenced their nutritional profiles, particularly in terms of fatty acid and amino acid composition. The observed increase in saturated, monounsaturated, and polyunsaturated fatty acids highlights the role of microbial metabolism in modifying lipid structures, thereby enhancing the functional and nutritional quality of the food product. Similarly, the elevated levels of essential amino acids post-fermentation reflect improved protein quality and potential bioavailability, which are critical for human health. These findings support the potential of fermentation as a natural and effective biotechnological tool for enriching the nutritional value of plant-based food formulations. This has promising implications for the development of functional foods and dietary interventions targeted at improving nutritional status, especially in resource-limited settings.

SIGNIFICANCE STATEMENT

This study demonstrates that fermenting Ofada rice fortified with tropical almond seeds significantly enhances nutritional quality by increasing essential amino acids and polyunsaturated fatty acids. These improvements suggest a promising strategy for developing functional foods that support health and nutrition. Incorporating fermentation into food processing can provide nutrient-dense, plant-based alternatives with potential benefits for dietary protein and heart-healthy fats.

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