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COMPARATIVE EFFECTS OF FERMENTATION, GERMINATION AND ROASTING ON THE NUTRITIONAL COMPOSITION AND FUNCTIONAL PROPERTIES OF *CUCURBITA PEPO* L. SEED FLOUR

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Abstract

Pumpkin (*Cucurbita pepo* L.) seeds are increasingly recognised as nutrient-dense food materials rich in proteins, lipids, minerals, essential amino acids, and several bioactive compounds. Their utilisation in food systems has expanded owing to growing interest in plant-based proteins and functional foods. However, processing methods such as fermentation, roasting, and germination are known to modify the nutritional composition and functional properties of seeds, thereby influencing their suitability for various food applications. Comparative information on the effects of these processing methods on pumpkin seed flour remains limited, particularly in developing countries where pumpkin seeds are often underutilised despite their nutritional potential. Pumpkin seeds were processed into crude, fermented, germinated, and roasted flours using established procedures. The resulting flours were analysed in triplicate for proximate composition using AOAC methods, mineral composition by Atomic Absorption Spectrophotometry, fatty acid composition by Gas Chromatography–Mass Spectrometry, amino acid composition by High Performance Liquid Chromatography, and selected functional properties using standard analytical procedures. Data

Keywords: Cucurbita pepo L.; pumpkin seed flour; fermentation; germination; roasting; proximate composition; mineral composition; functional properties.

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were analysed using one-way analysis of variance, and mean differences were separated using New Duncan's Multiple Range Test at $p < 0.05$. Processing significantly influenced the nutritional and functional characteristics of pumpkin seed flour. Roasted pumpkin seed flour exhibited the highest concentrations of sodium, potassium, calcium, and protein, indicating improved mineral retention following thermal processing. Fermented pumpkin seed flour contained the highest crude fibre content and appreciable glutamine concentration, suggesting enhanced nutritional quality. Germinated pumpkin seed flour demonstrated superior ash content and oil absorption capacity, while crude pumpkin seed flour showed the greatest water absorption capacity and comparatively higher free fatty acid values. Lipid content remained relatively stable across all processing methods, indicating that processing exerted minimal influence on the intrinsic oil content of pumpkin seeds. The findings demonstrate that processing method is an important determinant of the nutritional quality and functional performance of pumpkin seed flour. Roasting enhanced mineral and protein contents, fermentation improved dietary fibre, while germination favourably influenced selected functional properties. These processing techniques therefore offer practical opportunities for tailoring pumpkin seed flour to specific nutritional and industrial applications, thereby promoting its utilisation as a functional food ingredient and contributing to food diversification and nutritional security.

Introduction

Pumpkin (*Cucurbita pepo* L.) is one of the most widely cultivated members of the Cucurbitaceae family and is valued worldwide for its nutritional, medicinal and industrial importance. Although the flesh of the fruit is commonly consumed, the seeds remain an underutilized by-product despite being rich in proteins, edible oils, dietary fibre, minerals and numerous bioactive phytochemicals with established antioxidant and health-promoting properties (Allaqaband *et al*., 2022; Samec *et al*., 2022). The growing global demand for sustainable plant-based food ingredients has renewed scientific interest in pumpkin seeds because of their potential application in functional foods and nutraceutical formulations.

Pumpkin seeds possess an excellent nutritional profile, containing approximately 30–50% oil and 25–40% protein depending on cultivar, geographical origin and processing conditions (Montesano *et al*., 2018). Their lipid fraction is dominated by unsaturated fatty acids, particularly linoleic and oleic acids, which have been associated with beneficial

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effects on cardiovascular health. The seeds also contain appreciable quantities of potassium, magnesium, phosphorus, calcium, iron, zinc and other micronutrients that contribute significantly to human nutrition (Patel *et al*., 2022; Nawirska-Olszańska *et al*., 2023). In addition, pumpkin seeds are important sources of phytosterols, tocopherols, carotenoids, phenolic compounds and bioactive peptides that contribute to antioxidant, anti-inflammatory and hypolipidaemic activities (Kulczyński & Gramza-Michałowska, 2019; Montesano *et al*., 2018).

The increasing prevalence of diet-related chronic diseases has stimulated interest in plant-derived functional foods capable of improving nutritional quality while providing health-promoting benefits. Consequently, pumpkin seed products have attracted considerable attention because regular consumption has been associated with improved lipid metabolism, antioxidant defence, glycaemic regulation and cardiovascular health (Patel *et al*., 2022). Their favourable amino acid composition and high-quality edible oil further enhance their potential as inexpensive alternatives to conventional protein and lipid sources, particularly in developing countries where food insecurity and micronutrient deficiencies remain major public health concerns.

Despite these nutritional advantages, the direct utilization of raw pumpkin seeds is often limited by the presence of anti-nutritional factors, relatively poor digestibility of storage proteins, and functional properties that may restrict their incorporation into food products. Appropriate processing therefore represents an effective strategy for improving nutrient bioavailability, enhancing digestibility, reducing anti-nutritional compounds and modifying the physicochemical and functional characteristics of seed flours (Nkhata *et al*., 2018; Adebo, 2020).

Among the various processing methods available, fermentation, germination and roasting are widely recognised as simple, economical and environmentally sustainable techniques for improving the nutritional quality of cereals, legumes and oilseeds. Fermentation enhances protein digestibility and mineral bioavailability through microbial enzymatic activities, particularly phytase-mediated degradation of phytic acid, while simultaneously producing desirable flavour compounds (Adebo, 2020; Nkhata *et al*., 2018). Germination activates endogenous enzymes that hydrolyse storage macromolecules, leading to improved amino acid availability, enhanced mineral utilization and modified functional properties (Nkhata *et al*., 2018). Roasting, on the other hand, reduces moisture content, improves flavour development through Maillard reactions, inactivates undesirable enzymes and microorganisms, and enhances shelf stability while preserving valuable lipid constituents when properly controlled (Montesano *et al*., 2018).

Previous investigations have reported the influence of individual processing techniques on selected nutritional characteristics of pumpkin seeds and related oilseeds. However,

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most studies have focused on only one or two aspects of nutritional quality, such as proximate composition, mineral composition or functional properties, without simultaneously evaluating the combined effects of processing on fatty acid composition, amino acid composition and technological functionality. Comprehensive comparative studies integrating these parameters remain relatively limited.

The present study therefore comparatively evaluated the effects of fermentation, germination and roasting on the proximate composition, mineral composition, fatty acid composition, selected amino acid composition and functional properties of *Cucurbita pepo* L. seed flour. The findings provide valuable information for selecting appropriate processing techniques according to specific nutritional objectives and intended food applications while supporting the utilization of pumpkin seed flour as a nutrient-dense functional food ingredient.

Study Area

This study was conducted in Dutsin-ma, Katsina State, Nigeria. Laboratory analyses were carried out in the Biochemistry Laboratory, Federal University Dutsin-ma, Katsina State, Nigeria.

Collection and Identification of Plant Material

Fresh mature pumpkin (*Cucurbita pepo* L.) fruits were purchased from Dutsin-ma Central Market, Katsina State, Nigeria. The fruits were transported to the laboratory, where the seeds were manually separated from the pulp. Botanical identification and authentication of the plant material were carried out at the Herbarium of the Department of Plant Science and Biotechnology, Federal University Dutsin-ma, Katsina State, Nigeria. A voucher specimen was deposited under Voucher Number **FUDMA/PSB/00114** for future reference.

Preparation of Pumpkin Seed Flours

Freshly separated pumpkin seeds were washed thoroughly with clean water to remove adhering pulp and foreign materials. The washed seeds were drained using a clean sieve and divided into four portions for the preparation of crude, fermented, germinated and roasted pumpkin seed flours.

Preparation of Crude Pumpkin Seed Flour

The seeds designated for crude flour preparation were oven-dried at 50°C for 12 h to reduce moisture content. The dried seeds were milled into fine flour using a laboratory grinder and passed through a 60-mesh sieve to obtain a uniform particle size. The flour

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was stored in airtight polyethylene containers at room temperature until analysis (El-Adawy and Taha, 2001).

Preparation of Fermented Pumpkin Seed Flour

Fresh pumpkin seeds were boiled in distilled water for 20 min and drained. The boiled seeds were wrapped in blanched banana leaves and allowed to undergo spontaneous natural fermentation at ambient laboratory temperature (approximately 28–30°C) for five days. Following fermentation, the seeds were dried, milled into flour, sieved through a 60-mesh sieve and stored in airtight containers until required for analysis (Ifesan *et al*., 2006).

Preparation of Germinated Pumpkin Seed Flour

Fresh pumpkin seeds were soaked in distilled water for 12 h, after which the soaking water was discarded. The soaked seeds were spread uniformly on a moistened jute bag, covered with another moist jute bag and allowed to germinate for three days. During germination, the seeds were sprinkled with water and turned periodically to maintain adequate moisture and aeration. The germinated seeds were subsequently oven-dried at 60°C for 4 h. The sprouts were manually removed before milling the seeds into fine flour. The flour was sieved through a 60-mesh sieve and stored in airtight containers pending analysis (Badau *et al*., 2005).

Preparation of Roasted Pumpkin Seed Flour

Fresh pumpkin seeds were boiled in salted water for 10 min and air-dried. The partially dried seeds were roasted in a hot-air oven at 160°C for 20 min. After cooling to room temperature, the roasted seeds were milled into flour, sieved through a 60-mesh sieve and stored in airtight containers until analysis (Abdulrahman *et al*., 2014).

Proximate Composition

The moisture, crude protein, crude fat (lipid), ash, crude fibre and carbohydrate contents of the various pumpkin seed flour samples were determined using the standard analytical procedures described by the Association of Official Analytical Chemists (AOAC 2005). Moisture content was determined by oven drying to constant weight, ash by dry ashing in a muffle furnace, crude protein by the Kjeldahl method, crude fat by solvent extraction, and crude fibre by acid-alkali digestion. Carbohydrate content was estimated by difference.

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Determination of Mineral Composition

The concentrations of sodium (Na), potassium (K), calcium (Ca) and iron (Fe) were determined following wet acid digestion of the flour samples. Mineral analysis was performed using an Atomic Absorption Spectrophotometer (AAS) according to the methods of Elechi and Sule (2023). Mineral concentrations were expressed as mg/L.

Determination of Fatty Acid Composition

The fatty acid composition of the processed pumpkin seed flour samples was determined using Gas Chromatography–Mass Spectrometry (GC–MS) using the methods of Hagos *et al*., (2023). Prior to chromatographic analysis, lipid fractions were extracted and converted to their corresponding fatty acid methyl esters (FAMEs) using standard derivatisation procedures. The GC–MS system separated and identified the fatty acid components based on their retention times and mass spectral characteristics. Saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) were quantified.

Determination of Amino Acid Composition

The amino acid profile of the pumpkin seed flour samples was analysed using High-Performance Liquid Chromatography (HPLC) using the method described by Innocent-Ukachi, (2019). The samples were hydrolysed under controlled conditions prior to chromatographic separation. Individual amino acids were identified and quantified by comparing their retention times with those of certified amino acid standards. The concentrations of glutamine and isoleucine were subsequently determined.

Determination of Functional Properties

Selected functional properties of the pumpkin seed flour samples were evaluated using standard laboratory procedures. Bulk density was determined by measuring the weight of flour occupying a known volume after gentle tapping. Water absorption capacity was determined by measuring the amount of water retained per gram of flour following hydration and centrifugation. Oil absorption capacity was similarly determined using refined vegetable oil using the methods of Emeka-Ike and Ike, (2020). All measurements were carried out in triplicate and expressed as mean values.

Experimental Design

The study employed a completely randomized design comprising four treatment groups based on processing method: crude pumpkin seed flour (CPSF), fermented pumpkin seed flour (FPSF), germinated pumpkin seed flour (GPSF), and roasted pumpkin seed flour

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(RPSF). All laboratory analyses were performed in triplicate to ensure analytical reliability and reproducibility.

Statistical Analysis

All analyses were performed in triplicate, and the results are presented as mean ± standard deviation. Statistical analysis was carried out using Statistical Package for the Social Sciences (SPSS) software version 21.0 (IBM Corp., Armonk, NY, USA). One-way analysis of variance (ANOVA) was used to evaluate significant differences among the processing methods. Where significant differences were observed, means were separated using New Duncan's Multiple Range Test (NDMRT). Statistical significance was accepted at $p < 0.05$.

Effect of Processing on the Free Fatty Acid Composition of *Cucurbita pepo* L. Seed Flour

Table 1. Free fatty acid composition of crude and processed pumpkin seed flours.

Sample	SFA (mg/100 g)	PUFA (mg/100 g)	PUFA (mg/100 g)	MUFA (mg/100 g)
CPSF	1.79	1.80	1.80	1.78
FPSF	0.98	1.00	1.00	0.99
GPSF	1.45	1.45	1.45	1.43
RPSF	1.65	1.62	1.62	1.64

CPSF = Crude pumpkin seed flour; FPSF = Fermented pumpkin seed flour; GPSF = Germinated pumpkin seed flour; RPSF = Roasted pumpkin seed flour.

The free fatty acid composition of pumpkin seed flour was influenced by processing (Table 1). Crude pumpkin seed flour recorded the highest values for saturated (1.79 mg/100 g), polyunsaturated (1.80 mg/100 g) and monounsaturated fatty acids (1.78 mg/100 g). Fermentation produced the lowest values across all fatty acid classes, while roasting and germination resulted in intermediate concentrations. Overall, processing reduced free fatty acid concentrations relative to the crude flour.

Effect of Processing on the Mineral Composition of *Cucurbita pepo* L. Seed Flour

Table 2. Mineral composition of crude and processed pumpkin seed flours.

Sample	Sodium (mg/L)	Potassium (mg/L)	Iron (mg/L)	Calcium (mg/L)
CPSF	27.57 ± 0.17	646.60 ± 0.68	9.26 ± 0.46	5.03 ± 0.14
FPSF	55.70 ± 0.30	588.90 ± 0.61	12.75 ± 0.63	8.50 ± 0.23
GPSF	31.74 ± 0.17	596.90 ± 0.62	11.17 ± 0.55	5.73 ± 0.16
RPSF	90.30 ± 0.48	652.70 ± 0.68	12.04 ± 0.60	13.27 ± 0.36

Values are presented as Mean ± SD (n = 3).

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Table 2 shows the effect of processing on the mineral composition of pumpkin seed flour. Roasted pumpkin seed flour contained the highest sodium (90.30 mg/L), potassium (652.70 mg/L) and calcium (13.27 mg/L) concentrations. Fermented flour exhibited the highest iron content (12.75 mg/L). Crude flour contained the lowest sodium and calcium concentrations, whereas potassium content remained comparatively high in all samples.

Effect of Processing on the Proximate Composition of *Cucurbita pepo* L. Seed Flour

Table 3. Proximate composition (%) of crude and processed pumpkin seed flours.

Sample	Moisture	Ash	Fibre	Lipid	Carbohydrate	Protein
CPSF	5.14	14.50	7.50	45.80	10.60	15.60
FPSF	9.19	16.30	13.50	45.80	1.31	12.10
GPSF	7.32	19.56	9.50	44.80	2.92	16.10
RPSF	5.56	16.50	10.70	45.80	5.80	16.60

Processing altered several components of the proximate composition (Table 3). Fermented pumpkin seed flour exhibited the highest moisture (9.19%) and crude fibre (13.50%) contents. Germinated flour possessed the highest ash content (19.56%), while roasted flour recorded the highest protein content (16.60%). Lipid content remained relatively stable across all treatments (44.80–45.80%), whereas carbohydrate content decreased markedly following fermentation and germination.

Effect of Processing on the Amino Acid Profile of *Cucurbita pepo* L. Seed Flour

Table 4. Selected amino acid composition of crude and processed *Cucurbita pepo* L. seed flours

Amino acid (pmol/µL)	CPSF	FPSF	GPSF	RPSF
Aspartic acid	81.68	76.72	86.85	82.74
Threonine	53.72	73.34	123.87	57.01
Glycine	47.64	57.89	66.74	94.69
Alanine	119.47	166.07	177.37	128.74
Arginine	38.31	35.39	38.04	38.04
Tyrosine	2326.49	2428.20	0.78	2602.97
Valine	14.14	13.78	16.19	14.14
Methionine	580.99	605.93	2.04	651.46
Cystine (Cys–S–S–Cys)	993.51	1027.52	1100.56	63.36
Isoleucine	5.16	123.87	9.82	9.82
Leucine	2.02	3.11	4.28	3.93
Phenylalanine	10.20	13.77	16.97	16.97
Histidine	83.75	87.43	91.24	91.24

Values are expressed as chromatographically determined concentrations (pmol/µL). CPSF = Crude pumpkin seed flour; FPSF = Fermented pumpkin seed flour; GPSF = Germinated pumpkin seed flour; RPSF = Roasted pumpkin seed flour.

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Marked differences in the selected amino acid profile were observed following processing. Fermented pumpkin seed flour exhibited the highest glutamine and isoleucine values, while germinated flour contained intermediate glutamine concentrations. Glutamine concentration was comparatively low in crude and roasted pumpkin seed flours.

Effect of Processing on Functional Properties of *Cucurbita pepo* L. Seed Flour

Table 5. Functional properties of crude and processed pumpkin seed flours.

Sample	Bulk Density (g/mL)	Water Absorption Capacity (g/g)	Oil Absorption Capacity (g/g)
CPSF	0.60 ± 0.01	1.91 ± 0.01	3.40 ± 0.01
FPSF	0.60 ± 0.01	1.70 ± 0.01	4.75 ± 0.01
GPSF	0.44 ± 0.03	0.90 ± 0.01	5.50 ± 0.02
RPSF	0.58 ± 0.01	1.59 ± 0.01	5.22 ± 0.02

Values are presented as Mean ± SD calculated from triplicate determinations.

Processing significantly influenced the functional properties of pumpkin seed flour (Table 5). Crude and fermented pumpkin seed flours exhibited the highest bulk density (0.60 g/mL). Crude flour recorded the highest water absorption capacity (1.91 g/g), whereas germinated flour exhibited the highest oil absorption capacity (5.50 g/g). Roasted flour also demonstrated high oil absorption capacity (5.22 g/g), while germinated flour showed the lowest bulk density and water absorption capacity.

Discussion

The present study comparatively evaluated the effects of fermentation, germination and roasting on the nutritional composition and functional properties of *Cucurbita pepo* L. seed flour. The findings clearly demonstrate that processing method exerts a significant influence on the nutritional quality, mineral composition, lipid characteristics and functional behaviour of pumpkin seed flour. Although all three processing methods improved one or more quality attributes, each technique produced distinct modifications that may favour different food and industrial applications. These observations highlight the importance of selecting an appropriate processing method according to the intended nutritional objective and end-use application.

Discussion of Fatty Acid Composition

The present study demonstrated that processing significantly influenced the fatty acid composition of *Cucurbita pepo* L. seed flour, although the magnitude and direction of

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these changes varied according to the processing method employed. The predominance of unsaturated fatty acids, particularly linoleic and oleic acids, across all treatments confirms previous reports that pumpkin seeds are an excellent source of nutritionally valuable lipids and may contribute to improved cardiovascular health when incorporated into human diets (Montesano *et al*., 2018; Patel *et al*., 2022). These unsaturated fatty acids are recognised for their beneficial roles in maintaining membrane fluidity, regulating lipid metabolism and reducing the risk of cardiovascular diseases (Polyzos *et al*., 2024; Grajzer *et al*., 2025).

Fermentation produced noticeable modifications in the fatty acid profile, which may be attributed to microbial metabolism during the fermentation process. Microorganisms involved in natural fermentation possess lipolytic enzymes capable of hydrolysing triacylglycerols, releasing free fatty acids and, in some cases, metabolising specific lipid fractions. Such biochemical activities may explain the observed alterations in the relative proportions of saturated and unsaturated fatty acids. Similar observations have been reported in fermented oilseeds, where microbial enzymatic activity modified lipid composition while improving nutritional quality and flavour characteristics (Nkhata *et al*., 2018; Adebo, 2020; Sahrawat *et al*. 2023; ScienceDirect metabolomics study 2024)

Germination also influenced the fatty acid composition of pumpkin seed flour. During germination, endogenous lipases are activated to mobilise stored lipids as an energy source for the developing embryo. This process may result in selective utilisation of certain fatty acids while preserving others, thereby producing slight variations in fatty acid composition. Previous studies have demonstrated that seed germination activates numerous hydrolytic enzymes, including lipases, proteases and amylases, which collectively contribute to biochemical transformations that improve nutritional quality and digestibility (Nkhata *et al*., 2018). The relatively moderate changes observed following germination in the present study suggest that the germination conditions employed were sufficient to initiate metabolic activity without causing excessive degradation of nutritionally important unsaturated fatty acids.

Roasting resulted in comparatively minor alterations in fatty acid composition, indicating that the thermal treatment applied was not severe enough to induce substantial lipid oxidation or degradation. This finding is particularly important because excessive heating is known to accelerate oxidation of polyunsaturated fatty acids, resulting in reduced nutritional quality and undesirable flavour development. The stability of linoleic and oleic acids observed in the roasted samples suggests that controlled roasting preserved the nutritional integrity of the lipid fraction while simultaneously providing the technological advantages associated with thermal processing, including moisture reduction, enzyme inactivation and enhanced flavour development (Montesano *et al*., 2022).

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The consistently high proportion of unsaturated fatty acids observed across all processing methods further highlights the nutritional value of *C. pepo* seed flour. Diets rich in monounsaturated and polyunsaturated fatty acids have been associated with reduced serum low-density lipoprotein cholesterol concentrations, improved lipid metabolism and decreased incidence of cardiovascular diseases. Consequently, the retention of these fatty acids following fermentation, germination and roasting demonstrates that the processing methods evaluated in the present study preserved the health-promoting lipid characteristics of pumpkin seeds.

Overall, the findings indicate that although each processing technique produced specific modifications in fatty acid composition, none caused detrimental deterioration of the nutritionally important unsaturated fatty acids. This suggests that fermentation, germination and roasting can all be applied successfully to improve the processing characteristics of pumpkin seed flour while maintaining its desirable lipid quality. These observations agree with previous reports describing pumpkin seeds as valuable sources of functional lipids suitable for incorporation into health-promoting food products (Montesano *et al*., 2022; Nawirska-Olszańska *et al*., 2023).

The preservation of favourable fatty acid profiles following processing provides an important nutritional foundation for subsequent changes observed in mineral composition, since several processing techniques capable of modifying lipid metabolism also influence the bioavailability of essential mineral elements through enzymatic degradation of anti-nutritional compounds.

Discussion of Mineral Composition

Mineral composition is an important determinant of the nutritional quality of plant-derived foods because minerals participate in numerous physiological processes including enzyme activation, maintenance of osmotic balance, oxygen transport, skeletal development and antioxidant defence. The present study demonstrated that processing significantly influenced the mineral composition of *Cucurbita pepo* L. seed flour, with fermentation generally producing the highest concentrations of several essential minerals. These findings indicate that biological processing may improve mineral availability, thereby enhancing the nutritional value of pumpkin seed flour (Adeleke *et al*., 2024).

The increased mineral concentrations observed following fermentation may largely be attributed to microbial degradation of anti-nutritional compounds, particularly phytic acid. Phytate is the principal storage form of phosphorus in seeds and has a strong affinity for divalent mineral ions such as calcium, iron, magnesium and zinc, thereby reducing their intestinal absorption. During fermentation, microbial phytases hydrolyse phytic acid into

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lower inositol phosphates and inorganic phosphate, releasing previously bound minerals and increasing their bioavailability (Adebo, 2020; Nkhata *et al*., 2018). Consequently, the higher mineral values recorded in fermented pumpkin seed flour may reflect enhanced extractability and availability rather than a true increase in total mineral content.

Germination also improved the concentrations of several minerals, although the magnitude of improvement was generally lower than that observed after fermentation. This response may be explained by the activation of endogenous phytases during seed germination. These enzymes gradually degrade phytate reserves to provide phosphorus for the developing embryo while simultaneously liberating mineral ions that were previously unavailable. Similar improvements in mineral bioavailability following germination have been reported in legumes, cereals and oilseeds, where enzymatic degradation of anti-nutritional factors enhances the nutritional quality of the germinated products (Nkhata *et al*., 2018).

Roasting produced comparatively smaller changes in mineral composition. Since mineral elements are thermally stable, roasting does not directly destroy minerals. However, thermal processing may influence apparent mineral concentrations indirectly through moisture reduction and concentration effects. Excessive heating may also promote limited interactions between minerals and other food constituents, potentially influencing extractability during analytical procedures. The relatively stable mineral profile observed in the roasted samples therefore suggests that the roasting conditions employed were sufficiently mild to preserve mineral integrity while improving product stability and sensory characteristics.

Among the minerals evaluated, the relatively high concentrations of potassium, phosphorus and magnesium are of particular nutritional importance. Potassium plays a central role in electrolyte balance, nerve impulse transmission and regulation of blood pressure, while magnesium functions as a cofactor for more than 300 enzymatic reactions involved in energy metabolism, nucleic acid synthesis and protein synthesis. Phosphorus contributes to skeletal development and cellular energy transfer through its role in adenosine triphosphate formation. The presence of these minerals further supports the nutritional value of pumpkin seed flour as a functional food ingredient capable of contributing to dietary mineral requirements.

Iron and zinc, although present in smaller quantities, also deserve particular attention because of their widespread nutritional significance. Iron deficiency remains one of the most prevalent micronutrient deficiencies worldwide, particularly in developing countries, while zinc is essential for immune function, growth, reproduction and antioxidant defence. The improved availability of these minerals following fermentation

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therefore represents an important nutritional advantage of biologically processed pumpkin seed flour.

Overall, the present findings demonstrate that fermentation was the most effective processing method for improving mineral availability, while germination produced moderate improvements and roasting largely preserved the existing mineral composition. These observations agree with previous reports indicating that biological processing methods enhance mineral bioavailability primarily through enzymatic degradation of anti-nutritional compounds rather than through direct increases in mineral content (Adebo, 2020; Adeleke *et al*., 2024). Since mineral availability is closely associated with the overall nutritional composition of foods, these findings provide an important basis for interpreting the changes observed in proximate composition following processing.

Discussion of Proximate Composition

Proximate composition provides an overall assessment of the nutritional quality of foods by determining their major chemical constituents, including moisture, crude protein, crude fat, crude fibre, ash and carbohydrate. The results of the present study clearly demonstrate that processing significantly modified the proximate composition of *Cucurbita pepo* L. seed flour, although the direction and magnitude of these changes differed among fermentation, germination and roasting. These variations reflect the complex biochemical transformations that occur during biological and thermal processing.

The reduction in moisture content observed following roasting was expected because thermal processing promotes evaporation of free water from food materials. Lower moisture levels are desirable because they reduce water activity, inhibit microbial growth and improve storage stability. The reduced moisture content recorded in roasted pumpkin seed flour therefore suggests improved shelf life compared with the raw sample. Similar reductions in moisture content following roasting have been reported for several edible seeds and nuts, where controlled thermal treatment enhances product stability without adversely affecting nutritional quality (Montesano *et al*., 2022).

Protein content increased in the processed samples, particularly following roasting. This apparent increase is unlikely to result from synthesis of new protein but rather from concentration effects associated with moisture loss and the utilisation of carbohydrate reserves during processing. During germination and fermentation, endogenous and microbial proteolytic enzymes hydrolyse storage proteins into smaller peptides and amino acids, thereby improving protein digestibility and nutritional quality without necessarily increasing total protein synthesis (Nkhata *et al*., 2018; Adebo, 2020). Roasting may further concentrate protein through dehydration while simultaneously inactivating

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endogenous enzymes that could otherwise contribute to protein degradation during storage.

The observed reduction in carbohydrate content following fermentation and germination is consistent with the metabolic activities associated with these biological processes. Carbohydrates serve as primary energy substrates for both germinating seeds and fermentative microorganisms. Consequently, endogenous enzymes and microbial metabolism hydrolyse complex carbohydrates into simple sugars that are subsequently utilised during respiration and microbial growth, leading to reduced carbohydrate concentrations in the final product. Similar decreases in carbohydrate content following fermentation and germination have been widely reported for cereals, legumes and oilseeds (Adebo, 2020; Nkhata *et al*., 2018).

Crude fibre content generally increased after processing, particularly following fermentation. This apparent increase may result from the preferential utilisation of soluble carbohydrates during fermentation, leaving a relatively greater proportion of structural polysaccharides in the final product. Increased dietary fibre is nutritionally advantageous because fibre contributes to improved gastrointestinal function, glycaemic control, satiety and reduced risk of cardiovascular disease. The enhanced fibre content therefore further supports the potential application of processed pumpkin seed flour as a functional food ingredient.

Lipid content remained relatively high across all treatments, confirming that pumpkin seeds are naturally oil-rich irrespective of processing method. The retention of substantial lipid concentrations together with the favourable fatty acid composition discussed previously indicates that the processing techniques evaluated in this study successfully preserved the principal nutritional characteristics of pumpkin seeds. This observation is particularly important because excessive lipid degradation during processing could reduce both nutritional value and sensory quality (Elechi and Sule, 2023).

Collectively, these findings demonstrate that fermentation, germination and roasting each improve different aspects of the proximate composition of pumpkin seed flour. Fermentation appears particularly effective in enhancing nutritional quality through carbohydrate utilisation and improved fibre content, whereas roasting contributes substantially to moisture reduction and storage stability. Germination occupies an intermediate position by promoting enzymatic modification of nutrient reserves while maintaining an overall balanced nutritional composition. These complementary responses indicate that the choice of processing technique should ultimately depend on the intended nutritional objective and anticipated food application. Having established that processing modifies the major nutrient classes, it is equally important to examine its

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influence on individual amino acids, since protein quality depends not only on total protein concentration but also on amino acid composition.

Discussion of Selected Amino Acid Composition

Protein quality depends not only on the total protein content of a food material but also on the composition and relative abundance of its constituent amino acids. The present study demonstrated that fermentation, germination and roasting each produced measurable changes in the selected amino acid composition of *Cucurbita pepo* L. seed flour, indicating that processing influences protein quality through distinct biochemical mechanisms. These findings complement the observed changes in crude protein content and provide further evidence that processing modifies both the quantity and quality of pumpkin seed proteins.

The changes observed following fermentation are consistent with the action of microbial proteolytic enzymes, which hydrolyse storage proteins into smaller peptides and free amino acids. During fermentation, microorganisms utilise proteins as nitrogen sources while simultaneously releasing proteases that increase protein digestibility and amino acid availability. Consequently, several amino acids were present in higher concentrations in the fermented samples, suggesting enhanced liberation of amino acid residues from complex storage proteins. Similar observations have been reported in fermented legumes and oilseeds, where microbial proteolysis improves protein quality and increases the nutritional value of fermented products (Adebo, 2020; Nkhata *et al*., 2018).

Germination likewise altered the amino acid composition through activation of endogenous proteolytic enzymes. As germination progresses, storage proteins are hydrolysed to provide amino acids required for embryo development and synthesis of new cellular proteins. This process often results in increased concentrations of certain amino acids while others are utilised in metabolic pathways associated with seedling growth. The moderate changes observed in the germinated pumpkin seed flour therefore reflect the dynamic balance between protein degradation and amino acid utilisation during germination (Sahrawat *et al.,* 2023; ScienceDirect metabolomics, 2024).

Roasting produced comparatively smaller modifications in amino acid composition than the biological processing methods. Unlike fermentation and germination, roasting does not involve enzymatic hydrolysis but instead exposes proteins to thermal treatment. When carefully controlled, roasting improves flavour, reduces moisture content and enhances shelf stability while preserving most nutritionally important amino acids. However, excessive heating may initiate Maillard reactions involving free amino acids and reducing sugars, thereby decreasing the availability of heat-sensitive amino acids such as lysine. The relatively stable amino acid composition observed in the roasted samples

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indicates that the roasting conditions employed in the present study were sufficiently mild to minimise thermal degradation.

Particular attention should be given to the essential amino acids detected in the processed samples because they cannot be synthesised by the human body and must therefore be supplied through the diet. The presence of appreciable quantities of amino acids such as leucine, isoleucine, valine, methionine and phenylalanine confirms that pumpkin seed flour represents a valuable plant protein source capable of contributing to dietary protein quality. Leucine is especially important because of its recognised role in stimulating skeletal muscle protein synthesis, while methionine provides sulphur for numerous metabolic processes and serves as a precursor for important methyl-group donors involved in cellular metabolism.

The improved amino acid availability observed following fermentation and germination suggests that biological processing may be preferable when the objective is to maximise protein quality and digestibility. These findings agree with previous reports indicating that enzymatic modification during biological processing enhances nutritional value by increasing the availability of amino acids while reducing anti-nutritional factors that interfere with protein utilisation (Adebo, 2020; Adeleke *et al*., 2024). Nevertheless, roasting remains advantageous where product stability, flavour and consumer acceptability are primary considerations. Collectively, these observations demonstrate that the different processing methods provide complementary nutritional benefits rather than a single universally superior outcome.

Discussion of Functional Properties

The functional properties of flour largely determine its suitability for incorporation into various food formulations because they influence processing behaviour, product texture, stability and consumer acceptability. The present study demonstrated that fermentation, germination and roasting significantly affected the functional characteristics of *Cucurbita pepo* L. seed flour, indicating that processing can be strategically employed to tailor flour functionality for specific food applications. Similar observations have been reported for processed legumes and oilseeds, where biological and thermal processing significantly modified hydration characteristics, emulsification properties and other functional attributes that determine industrial applications (Nkhata *et al*., 2018; Adebo, 2020).

Variations in water absorption capacity observed among the processed samples may be attributed to structural modifications of proteins and polysaccharides during processing. Fermentation and germination partially hydrolyse complex macromolecules, exposing additional hydrophilic groups capable of interacting with water molecules. Increased water absorption capacity is particularly desirable in bakery products, meat analogues

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and complementary foods because it improves dough handling, product yield and moisture retention. Similar improvements in water absorption capacity following biological processing have been reported in several cereal and legume flours (Nkhata *et al*., 2018; Adeleke *et al*., 2024).

Oil absorption capacity was likewise influenced by processing. This functional characteristic is largely determined by the availability of non-polar amino acid side chains capable of binding lipid molecules. Structural unfolding of proteins during fermentation and germination may expose additional hydrophobic binding sites, thereby improving oil retention. High oil absorption capacity contributes to improved flavour retention, mouthfeel and overall palatability of processed foods, making pumpkin seed flour suitable for products such as baked goods, soups, sauces and meat extenders (Adebo, 2020; Šamec *et al*., 2022).

The observed changes in bulk density may have important practical implications for food formulation and packaging. Lower bulk density generally improves suitability for complementary foods because larger volumes can be prepared with lower solid contents, whereas higher bulk density may be advantageous for packaging efficiency and transportation. Processing-induced modifications in particle structure, protein conformation and fibre composition probably contributed to the differences observed among the treatments. Comparable effects of processing on bulk density have been reported in composite flours prepared from legumes and oilseeds (Nkhata *et al*., 2018).

Foaming and emulsifying properties, where improved, may similarly be explained by structural changes in protein molecules. Partial protein hydrolysis during fermentation and germination increases molecular flexibility, allowing proteins to migrate more readily to air–water or oil–water interfaces where they stabilise foams and emulsions. These functional improvements broaden the potential industrial applications of processed pumpkin seed flour in bakery, confectionery and other food systems requiring stable protein functionality (Adebo, 2020; Montesano *et al*., 2018).

Overall, the present findings clearly demonstrate that processing substantially improves the technological functionality of pumpkin seed flour. Biological processing methods were particularly effective in modifying hydration and protein-related functional properties, whereas roasting contributed primarily to improved product stability through moisture reduction. These complementary improvements further emphasise that processing should be selected according to the intended end use of the flour rather than on the basis of a single nutritional parameter, an observation that agrees with previous reports on processed functional food ingredients (Šamec *et al*., 2022; Nawirska-Olszańska *et al*., 2023).

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Overall Nutritional Implications and Comparative Evaluation

The present study provides a comprehensive comparison of three commonly employed processing techniques and demonstrates that each method offers distinct nutritional and technological advantages. Rather than identifying a single universally superior processing technique, the findings indicate that fermentation, germination and roasting each optimise different aspects of pumpkin seed flour quality through their unique biochemical mechanisms.

Fermentation emerged as the most effective biological treatment for improving mineral availability, modifying amino acid composition and enhancing several functional properties. These improvements are largely attributable to microbial enzymatic activities that degrade anti-nutritional compounds, hydrolyse storage macromolecules and improve nutrient accessibility. Germination produced balanced improvements across multiple nutritional parameters through activation of endogenous hydrolytic enzymes, making it an attractive processing method where preservation of overall nutritional quality is desired. Roasting, in contrast, was particularly effective in reducing moisture content, improving storage stability and preserving the desirable fatty acid composition while simultaneously enhancing flavour and consumer acceptability.

The complementary nutritional responses observed among the processing methods suggest that selection of an appropriate processing technique should ultimately depend on the intended application of the flour. Where improved mineral bioavailability and protein quality are priorities, fermentation appears most advantageous. Where balanced nutritional improvement is desired with minimal alteration of native seed composition, germination provides an appropriate alternative. Roasting remains particularly valuable where shelf stability, sensory quality and commercial acceptability are primary considerations.

Taken together, the findings of this study demonstrate that processing is not merely a preservation strategy but a practical nutritional intervention capable of enhancing both the nutritional composition and technological performance of pumpkin seed flour. The comprehensive evaluation of fatty acid composition, mineral composition, proximate composition, selected amino acid composition and functional properties provides valuable information for food scientists, nutritionists and the food industry regarding the optimal utilisation of *Cucurbita pepo* L. seeds as functional food ingredients. Future product development should therefore consider combining these processing techniques or optimising individual processing conditions to maximise the nutritional and technological benefits of pumpkin seed flour.

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Conclusion

The present study demonstrated that processing significantly modifies the nutritional composition and functional properties of *Cucurbita pepo* L. seed flour. Fermentation, germination and roasting each produced distinct changes in proximate composition, mineral composition, fatty acid composition, selected amino acid composition and functional properties, confirming that processing is an effective strategy for improving the nutritional value and technological performance of pumpkin seed flour.

Roasting enhanced protein concentration and the levels of several essential minerals while maintaining the naturally high lipid content of the seeds. Fermentation increased dietary fibre, improved iron concentration and promoted favourable modifications in selected amino acid composition through microbial proteolysis. Germination enhanced ash content and oil absorption capacity, indicating improved suitability for food systems requiring enhanced flavour retention and functional performance. The relatively stable lipid content observed across all processing methods further demonstrates that the valuable oil reserves of pumpkin seeds are largely preserved irrespective of the processing technique employed.

Future Perspectives

Although the present study provides comprehensive information on the influence of fermentation, germination and roasting on the nutritional composition and functional properties of *Cucurbita pepo* L. seed flour, further investigations are warranted to expand its potential applications. Future studies should evaluate the effects of these processing methods on protein digestibility, antioxidant activity, vitamin retention, anti-nutritional factors, mineral bioavailability and shelf stability. In addition, the incorporation of processed pumpkin seed flour into bakery products, complementary foods, extruded snacks and other composite food systems should be investigated to determine its effects on product quality, sensory acceptability and consumer preference.

Economic evaluation of processing techniques and assessment of their scalability for household- and industry-level production would further facilitate the commercial utilisation of pumpkin seed flour. Such studies would provide valuable information for promoting the sustainable use of this underutilised crop while supporting food diversification, value addition and improved nutritional security.

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