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Valorization of peanut meal through solid-state fermentation: Enhancing protein functionality and reducing aflatoxins, allergenic and anti-nutritional factors

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ABSTRACT

The increasing demand for sustainable and nutritious food ingredients has intensified interest in the valorization of agro-industrial side streams. This study investigated the impact of solid-state fermentation (SSF) on the nutritional quality, safety, and techno-functional properties of peanut meal, with emphasis on relative increases in crude protein content on a dry matter basis, reduction of ELISA-detectable allergenic proteins, and improvements in aflatoxin safety. Selected bacterial and fungal strains (*Bacillus subtilis, Rhizopus oryzae,* and *Aspergillus oryzae*) were applied under strain-specific optimized SSF conditions to evaluate the overall effect of the bioprocess on peanut meal as a food ingredient. Thermal processing followed by solid-state fermentation resulted in a relative increase in crude protein content on a dry matter basis and promoted substantial reductions in total aflatoxins, a decrease in detectable allergenic proteins, and antinutritional factors when compared to the untreated peanut meal. In addition, SSF significantly improved key functional and physical properties, including swelling capacity, foaming ability, and oil-holding capacity. Taken together, these results demonstrate that solid-state fermentation is a simple and scalable bioprocess for converting low-value peanut by-products into nutritionally enhanced and functionally improved protein ingredients with improved safety-related attributes, suitable for food applications.

1. Introduction

Peanuts are among the most important oilseeds worldwide, valued for their high protein (26–38%) and oil (42–46%) contents, which support both nutritional and industrial applications (Lu et al., 2026; Li et al., 2023a). Peanut oil production generates >9 million tons of peanut meal annually, a protein-rich by-product containing approximately 47–55% protein (Hariharan et al., 2023). The peanut meal market was valued at approximately USD 255.5 million in 2022 and is projected to reach USD 291.7 million by 2029 (Tang et al., 2026).

Despite its nutritional potential, the direct use of peanut meal in food ingredient applications remains limited due to the presence of anti-nutritional factors, allergenic proteins, and mycotoxins, particularly aflatoxins (Li et al., 2023a; Zhao et al., 2023). Phytate is the most abundant antinutritional compound in peanut meal and negatively affects protein digestibility and mineral bioavailability by forming insoluble complexes with essential nutrients and amino acids. In addition, peanut allergenic proteins, including Ara h 1 to Ara h 9, can trigger severe immune responses even at trace concentrations. Aflatoxins, mainly produced by *Aspergillus* species during post-harvest handling and storage, are highly toxic and carcinogenic, representing one of the most critical safety barriers for the utilization of peanut by-products in food formulations (Hariharan et al., 2023; Zhao et al., 2023). Recent studies have further highlighted the severity of aflatoxin contamination in peanut meals and the challenges associated with achieving effective detoxification under food-oriented processing conditions (Tang et al., 2026; Soylemez et al., 2025). Overcoming these limitations is therefore essential to enable the safe and sustainable incorporation of peanut meal

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into food ingredient applications.

Aflatoxin B1 (AFB1) is considered the most toxic and chemically stable member of the aflatoxin group, which contributes to its persistence in food matrices and complicates effective detoxification strategies (Söylemez et al., 2025). Consequently, a wide range of physical, chemical, and biological approaches have been investigated for aflatoxin mitigation, including adsorption, chemical transformation, enzymatic degradation, and fermentation-based strategies. Each of these approaches presents specific advantages and limitations in terms of efficacy, safety, and applicability (Arimboor et al., 2024). Although highly efficient detoxification systems have been reported, many rely on complex materials, catalysts, or tightly controlled process conditions, which may limit their scalability and direct food ingredient applications (Tang et al., 2026). Within this framework, solid-state fermentation (SSF) has emerged as a promising biotechnological approach for the valorization of agro-industrial by-products. Operating on moist solid substrates without free-flowing water, SSF offers advantages such as low energy demand, reduced wastewater generation, and high enzymatic efficiency. Previous studies have demonstrated that SSF can effectively reduce antinutritional factors, including phytate, and contribute to the reduction of mycotoxins such as aflatoxins, particularly in oilseed meals and cereal by-products (Udomkun et al., 2017; Kumar et al., 2021; Zhao et al., 2023). In addition to improving safety, SSF can induce structural modifications in plant protein matrices, leading to enhanced techno-functional properties such as solubility, foaming capacity, and oil- or water-holding capacity, which are critical for ingredient performance in food systems (Chen et al., 2015; Hashempour-Baltork et al., 2020).

Although the application of solid-state fermentation to peanut meals has been previously reported, existing studies have predominantly addressed isolated outcomes, such as aflatoxin reduction or improvements in nutritional composition, often relying on a single microbial strain or a limited analytical scope. Recent studies employing solid-state fermentation and co-fermentation strategies have demonstrated promising improvements in nutritional quality and aflatoxin mitigation in peanut meal; however, these investigations remain largely centered on specific endpoints, such as detoxification efficiency or protein composition, with limited integration of allergenicity and techno-functional performance relevant to food applications. Moreover, while modified peanut meal has been shown to exhibit improved interfacial and emulsifying properties in food systems, these functional enhancements are rarely evaluated in conjunction with safety-related parameters, such as mycotoxin reduction and allergenic protein content (Liu et al., 2024). Consequently, comparative evaluations integrating safety, allergenicity, compositional changes, and techno-functional performance under controlled SSF conditions remain scarce for peanut meal.

In this context, the present study investigates the effects of solid-state fermentation of peanut meal using selected bacterial and fungal strains (*Bacillus subtilis, Rhizopus oryzae*, and *Aspergillus oryzae*) chosen based on their documented enzymatic potential in plant-based substrates. Rather than aiming at direct strain-to-strain comparison, this work evaluates the overall impact of SSF on peanut meal under strain-specific optimized conditions. By integrating nutritional, functional, and safety-related analyses, this study provides a comprehensive and application-oriented assessment of SSF as a scalable strategy for converting peanut by-products into protein ingredients with improved safety-related attributes and enhanced functionality for food use.

2. Material and methods

2.1. Material

Peanut meal was kindly provided by CRAS Agroindustrial Ltda. (Itajú, São Paulo, Brazil). The bacterial strain *Bacillus subtilis* (MTCC 25,292) was obtained from Meteoric Biopharmaceuticals. *Aspergillus oryzae* and *Rhizopus* oryzae, were obtained as commercial food-grade fermentation cultures (koji and tempeh starters, respectively) from Kensho Sake (L'Ampolla, Tarragona, Spain). According to the supplier, spores were provided on a rice- and potato-based carrier. Cultures were used as received for solid-state fermentation experiments. All reagents and chemicals used in the analyses were of analytical grade.

2.2. Solid-state fermentation

Peanut meal was adjusted to an initial moisture content of 40% (w/w), which is within the typical range reported for fungal and bacterial solid-state fermentation on oilseed meals. Water activity (a_w) was not measured directly; instead, water availability was controlled through moisture adjustment. The substrate was sterilized at 121 °C for 15 min and cooled to room temperature prior to inoculation. The inoculum was prepared from commercially available lyophilized starter cultures. For *A. oryzae* and *R. oryzae*, the inoculum consisted of fungal spores (conidia), while the B. subtilis culture was supplied in spore form. After rehydration, the inoculum was applied at an estimated level of approximately 10^7 CFU per gram of dry substrate.

Fermentation conditions were selected based on strain-specific optimization reported in the literature and supported by preliminary assays. Fermentation with *A. oryzae* and *R. oryzae* was conducted at 30 °C for 48 h, whereas *B. subtilis* fermentation was carried out at 39 °C for 24 h. These conditions were not intended for direct strain-to-strain comparison, but rather to evaluate the overall impact of solid-state fermentation on peanut meal under optimized microbial growth conditions. Due to the solid-state nature of the system, pH evolution during fermentation was not directly monitored, as pH determination in SSF generally requires extraction procedures that may not accurately represent the local microenvironment of the substrate. At the end of fermentation, all samples were dried at 60 °C until reaching approximately 10% moisture content and subsequently milled prior to analysis. Non-fermented peanut meal subjected to the same thermal and drying conditions was used as the control.

To minimize experimental variability and improve reproducibility, all fermentations were carried out using a single batch of peanut meal and standardized operating conditions. Critical parameters, including substrate moisture content, inoculum level, incubation temperature, and fermentation time, were controlled for each system. Although experiments were conducted in independent runs, all procedures followed the same protocol, ensuring consistency across replicates.

2.3. Proximate composition

Proximate composition analyses of peanut meal and fermented samples were performed according to AOAC Official Methods: moisture (925.10), ash (923.03), and crude protein (920.87), determined by the Kjeldahl method using a nitrogen conversion factor of 6.25. Lipid content was determined following the method of Bligh and Dyer (1959). Carbohydrate content was calculated by difference.

2.4. Amino acid profile

The total amino acid composition of peanut meal and fermented samples was determined by an external accredited laboratory (CBO Análises Laboratoriais Ltda., Brazil) according to the method described by White et al. (1986), using pre-column derivatization and chromatographic analysis after acid hydrolysis. Tryptophan was determined separately following alkaline hydrolysis, as described by Lucas and Sotelo (1980). Results are expressed as percentage of amino acids on a dry matter basis.

2.5. Anti-nutritional compounds

2.5.1. Total phenolic compounds

Phenolic compounds were extracted by mixing 400 mg of sample with 1.1 mL of ethanol solution, followed by incubation at 60 °C for 1 h and centrifugation at 4000 × g for 10 min at 20 °C. Total phenolic content was determined according to Singleton et al. (1999). Phenolic concentration was calculated using Eqs. (1) and (2):

$$C = (b - abs_{760})/a \tag{1}$$

$$GAE = (C \times d \times V_1)/(MS \times 1000) \tag{2}$$

where C is the phenolic concentration in the extract (mg GAE L^{-1}), GAE is the phenolic concentration in the sample (mg GAE g^{-1} DM), b and a are the intercept and slope of the standard curve, abs_{760} is the absorbance at 760 nm, d is the dilution factor, V_l is the extraction volume, and MS is the dry mass of solids.

2.5.2. Tannins

Tannin content was determined following the method described by Sadh et al. (2018). The reaction mixture was kept in the dark for 30 min, and absorbance was measured at 760 nm. A standard curve of tannic acid (10–70 µg mL^{-1}) was used for quantification.

2.5.3. Trypsin inhibitory activity

Trypsin inhibitory activity was determined according to Çabuk et al., 2018. Absorbance was measured at 410 nm, and trypsin inhibitory activity was calculated based on the reduction of trypsin activity relative to a trypsin calibration curve, extract volume, and sample moisture content. Results were expressed as trypsin inhibitor units per milligram of dry sample (TIU/mg).

2.5.4. Phytic acid

Phytic acid extraction was performed following the protocol proposed by Olukomaiya et al. (2020). Phytate concentration in the extract and sample was calculated using Eqs. (3) and (4):

$$C = (b - abs500)/a \tag{3}$$

$$PA = (C \times d \times V_1)/(MS \times 1000) \tag{4}$$

where C is the phytate concentration in the extract (mg PAE L^{-1}), b and a are the intercept and slope of the standard curve, d is the dilution factor, V_l is the extraction volume, MS is the dry mass, and PA is the phytate concentration in the sample (mg PAE g^{-1} DM).

2.6. Peanut allergenicity

Total peanut allergenic proteins were quantified by an external ISO/IEC 17,025 accredited laboratory (Eurofins do Brasil) using a commercial High Sensitive Peanut ELISA Kit II (MioBS, 2021), according to the manufacturer's instructions. Results are expressed as µg of peanut allergenic protein equivalents per g of sample (µg/g, dry matter basis). The analytical working range of the assay was 10–20,000 µg/g; samples with concentrations above this range were reported as >20,000 µg/g (above the upper limit of quantification, ULOQ). The assay is based on a commercial ELISA method designed for high sensitivity detection of total peanut allergenic proteins, as specified by the manufacturer.

2.7. Aflatoxin content

Aflatoxins were extracted from 25 g of sample with 2.5 g of NaCl and 100 mL of methanol:water (8:2, v/v) and homogenized for 30 min. The extract was filtered, purified using an immunoaffinity column (Aflatest WB, Vicam), and analyzed by HPLC (Agilent 1260 Infinity) equipped with a Zorbax Eclipse Plus C18 column and a fluorescence detector (λ_ex = 362 nm; λ_em = 455 nm) after post-column derivatization with a Kobracell reactor. The mobile phase consisted of water:acetonitrile:methanol (6:2:3, v/v/v) containing 119 mg L^{-1} KBr and 350 µL L^{-1} of 4 M nitric acid. The limits of detection and quantification for total aflatoxins were 0.05 µg kg^{-1} and 0.17 µg kg^{-1}, respectively. A spiked blank sample (~10 µg kg^{-1} total aflatoxins) was used as a positive control to verify method performance (Stroka et al., 2000). In this study, only total aflatoxins (sum of aflatoxins B1, B2, G1, and G2) were reported; individual aflatoxin species were not quantified separately.

2.8. Physical properties

2.8.1. Bulk density

Bulk density was determined according to Oladele and Aina (2007) and expressed as the ratio between sample mass (g) and volume (mL), as an indicator of powder handling and potential implications for storage and processing.

2.8.2. Swelling capacity

Swelling capacity was determined according to Okaka and Potter (1977).

2.9. Functional properties

2.9.1. Emulsifying activity index and emulsion stability index

Emulsifying activity index (EAI) and emulsion stability index (ESI) were determined according to Gouvêa et al. (2023) using Eqs. (5) and (6):

$$EAI(m^2g^{-1}) = (2 \times 2.303 \times 100 \times A_0)/(c \times 0.25 \times 10,000) \tag{5}$$

$$ESI(\text{min}) = A_0/(A_0 - A_{10} \times 10) \tag{6}$$

where A_0 is the absorbance at time zero, A_{10} is the absorbance after 10 min, and c is the protein concentration (g mL^{-1}).

2.9.2. Foaming capacity and foaming stability

Foaming capacity (FC) and foaming stability (FS) were determined according to Gouvêa et al. (2023) using Eqs. (7) and (8):

$$FC(\%) = (V_1 - V_0)/V_0 \times 100 \tag{7}$$

$$FC(\%) = (V_1 - V_0)/V_0 \times 100 \tag{8}$$

where V_0 is the initial volume, V_1 is the volume after homogenization, and V_2 is the volume after standing for 10, 30, or 60 min at room temperature.

2.9.3. Water-holding capacity and oil-holding capacity

Water-holding capacity (WHC) and oil-holding capacity (OHC) were determined according to Gouvêa et al. (2023) using Eq. (9):

$$WHC \text{ or } OHC(gg^{-1}) = (M_1 - M_t - M_0)/M_0 \tag{9}$$

where M_1 is the mass of the tube containing the wet sample, M_0 is the initial sample mass, and M_t is the mass of the empty tube.

2.10. Statistical analysis

All fermentation experiments were conducted in independent runs under the same conditions. Analytical determinations were performed in triplicate, and results are expressed as mean ± standard deviation. Statistical analysis was conducted using SISVAR software. Statistical analyses were performed separately for each fermentation system, comparing fermented samples with their respective non-fermented control. Analysis of variance (ANOVA) followed by Tukey's test ($p \leq 0.05$) was applied within each system to evaluate the effect of solid-state fermentation. For allergenic protein content, only samples with ELISA results within the assay quantification range were included in the ANOVA; samples reported as >20,000 µg/g (above the ULOQ) were excluded from statistical comparisons and interpreted qualitatively.

3. Results and discussion

3.1. Effect of solid-state fermentation on peanut meal proximate composition

The proximate composition of peanut meal flour and fermented samples obtained with *Rhizopus oryzae, Bacillus subtilis*, and *Aspergillus oryzae* is presented in Table 1. Overall, solid-state fermentation resulted in a relative increase in crude protein content on a dry matter basis, accompanied by reductions in the relative contents of carbohydrates and lipids. On a compositional basis, crude protein content increased by approximately 7–14.5% relative to the non-fermented control, while lipid and carbohydrate contents decreased by up to 32% and 22%, respectively. These changes reflect compositional shifts driven by substrate utilization and concentration effects during fermentation, rather than an absolute gain in native peanut protein, and may also be influenced to a minor extent by inherent variability in the composition of the raw material. Taken together, the results support the potential of SSF as a practical approach to obtaining ingredients with higher crude protein content on a dry matter basis from peanut processing side streams.

Changes in proximate composition during SSF are primarily associated with microbial growth and metabolism, which require carbon and nitrogen sources and are mediated by extracellular enzymes that hydrolyze major macromolecules (Kumar et al., 2021). The reductions in carbohydrates and lipids are consistent with the secretion of carbohydrases and lipases, generating fermentable sugars and free fatty acids/glycerol that can be further metabolized. As these fractions decrease, the protein fraction becomes proportionally higher. In addition, the accumulation of microbial biomass contributes to increased crude protein, as microbial cells are protein-rich and become incorporated into the fermented matrix (Calvo-Lerma et al., 2022; Xiao et al., 2015; Dulf et al., 2016). The lipid reduction observed after fermentation is consistent with microbial substrate utilization and enzyme-mediated transformations occurring during microbial growth. Similar decreases in total lipids and medium- and long-chain fatty acids have been reported in plant-based matrices undergoing spontaneous microbial fermentation (Chen et al., 2024). Variations in the magnitude of lipid reduction across fermented samples are also expected, given that microbial metabolism and macromolecule degradation during fermentations are highly strain- and condition-dependent (Liu et al., 2024).

Increases in ash content may be associated with losses of organic matter and concentration of mineral components during fermentation (Terefe et al., 2021).

Similar compositional shifts, higher protein and ash and lower lipid and carbohydrate contents, have been reported for other oilseed meals subjected to SSF, including canola meal fermented with *Aspergillus* spp., soybean meal fermented with *Bacillus* spp. and lactic acid bacteria, and rapeseed meal fermented with filamentous fungi (Chi & Cho, 2016; Li et al., 2022; Shi et al., 2016).

Table 1
Proximate composition of peanut meal and fermented peanut meal obtained by solid-state fermentation with *Rhizopus oryzae, Bacillus subtilis*, and *Aspergillus oryzae.*

Sample	*Protein (%)*	Carbohydrates (%)	*Lipids (%)*	Fibers (%)	Ash (%)
Peanut meal - control	50.6 ± 1.89	27.7 ± 1.29	10.1 ± 1.60	6.7 ± 1.01	4.8 ± 0.66
Peanut meal - *R. oryzae*	53.7 ± 1.90	25.1 ± 1.22	6.9 ± 1.15	9.0 ± 1.16	5.3 ± 0.66
Peanut meal - *B. subtilis*	53.8 ± 1.93	25.0 ± 1.27	9.3 ± 1.40	8.7 ± 1.10	5.2 ± 0.64
Peanut meal - A.oryzae	57.5 ± 1.89	20.8 ± 1.21	7.2 ± 1.15	9.1 ± 1.13	5.6 ± 0.66

3.2. Effect of solid-state fermentation on peanut meal amino acid composition

The essential and non-essential amino acid composition of peanut meal and fermented samples is shown in Table 2. Peanut meal presented a favourable profile of essential amino acids relative to FAO reference patterns, with high levels of aromatic amino acids (Phe and Tyr), which are important precursors for multiple biologically active compounds (Han et al., 2019). As commonly reported for peanut-derived proteins, lysine and sulfur-containing amino acids are among the main limiting amino acids (Li et al., 2023a; Ochoa-Rivas et al., 2017).

Amino acids in this study correspond to total amino acids determined after hydrolysis by an external laboratory method (including separate tryptophan determination), and the values in Table 2 are expressed as mg/g protein (i.e., normalized to protein content). Under this basis, a reduction in mg/g protein for several amino acids can occur even when crude protein (Kjeldahl N × 6.25) increases, because (i) Kjeldahl-based crude protein includes non-protein nitrogen and may be influenced by microbial metabolites; (ii) SSF can alter the relative contribution of microbial biomass (and its nitrogen-containing components) to the total nitrogen pool; and (iii) compositional shifts can occur due to selective consumption and synthesis of specific amino acids during microbial growth. Accordingly, the observed decreases in several amino acids likely reflect both metabolic utilization and a change in the nitrogen distribution of the fermented matrix, rather than a direct contradiction between crude protein and amino acid data (Knez et al., 2023; Adebo et al., 2022).

Reductions in specific amino acids, including Leu, Phe, and Val, were observed in fermented samples, reflecting their utilization through microbial metabolic pathways during solid-state fermentation. Amino acid utilization during fermentation can be associated with anabolic demands, deamination, and conversion into intermediates of central metabolism, as well as process-dependent effects (Li et al., 2023b; Shi et al., 2016; Yu et al., 2023; Zhao et al., 2017). In some fermented samples, increases in specific amino acids such as Lys, Met, Thr, Gly, and Cys were observed, indicating that SSF can modulate the amino acid profile depending on microbial metabolism and fermentation conditions (Li et al., 2023a; Shi et al., 2016; Yu et al., 2023; Zhao et al., 2017).

Table 2
Amino acid profile of peanut meal and fermented peanut meal obtained with *Rhizopus oryzae, Bacillus subtilis*, and *Aspergillus oryzae*. Values are expressed as mg/g protein. FAO reference pattern according to FAO (2013).

Amino acids	FAO Report (2013)	Peanut meal	Control	Fermented peanut meal		
				R. oryzae	*B. subtilis*	*A. oryzae*
His	**16**	17.13	16.60	16.95	13.75	15.69
Ile	**30**	31.47	32.60	25.70	27.32	30.11
Leu	**61**	60.16	60.86	50.28	47.86	54.45
Lys	**48**	37.05	34.97	33.52	34.98	40.03
Met		7.37	7.31	7.08	6.61	7.93
Phe		46.21	45.84	36.87	34.29	39.85
Trp	**6.6**	9.16	6.32	6.15	8.35	6.67
Thr	**25**	24.90	24.90	26.44	24.89	26.51
Val	**40**	36.85	37.34	33.52	32.02	36.24
Asp		95.41	99.78	95.16	73.79	86.91
Glu		158.55	163.60	146.74	121.48	138.66
Ser		40.24	42.68	37.06	33.59	38.41
Gly		54.78	55.13	54.19	41.25	55.18
Arg		94.81	92.67	70.20	58.30	70.14
Ala		34.26	35.17	38.36	31.50	34.80
Pro		34.06	34.38	30.17	25.06	27.95
Tyr		33.66	33.59	30.73	26.98	32.46
Cys		11.75	9.48	10.24	11.31	14.79
Met + Cys	**23**	19.12	16.79	17.32	17.92	22.72
Phe + Tyr	**41**	79.87	79.43	67.6	61.27	72.31
Total		1098.13	1099.96	985.84	883.41	1014.25

Overall, fermented peanut meal remained a relevant source of essential amino acids. In combination with complementary proteins (e.g., soybean, pea, or rice), it may support nutritionally balanced formulations while contributing to sustainable protein ingredient development.

3.3. *Effect of solid-state fermentation on antinutritional, allergenic and aflatoxin content of peanut meal*

Peanut meal contains antinutritional factors that can impair protein quality and mineral bioavailability, including phytic acid, trypsin inhibitors, total phenolics, and tannins (Table 3). Total phenolics and tannins showed significant changes after solid-state fermentation when compared to the non-fermented control, depending on the fermentation system. Increases in phenolic content observed after SSF may be attributed to hydrolytic and oxidative enzyme systems that promote the release of bound phenolics from the matrix (Olukomaiya et al., 2020; Torres-León et al., 2019; Çabuk et al., 2018). Phenolic transformations during SSF can vary depending on microbial metabolism and process conditions, as microorganisms may either release bound phenolics or metabolize phenolic compounds during fermentation (Adebo & Medina-Meza, 2020; Mikulajová et al., 2024).

Phytic acid content was significantly reduced ($p < 0.05$) after solid-state fermentation in some fermentation systems when compared to the non-fermented control (Table 3). Phytate reduction during SSF is primarily associated with phytase activity, which hydrolyzes phytic acid and can improve mineral bioavailability (Azeez et al., 2022). The extent of phytate degradation is influenced by fermentation conditions, particularly pH, as phytase activity is often maximized around pH ~5 (Zhao et al., 2017). Although pH was not monitored in the present solid-state fermentation systems, it is important to note that pH determination in SSF typically relies on extraction-based methods, which may not accurately reflect the localized microenvironment within the solid matrix. Nevertheless, previous studies have shown that pH can vary during SSF depending on the microorganism, substrate, and process conditions, which may affect enzyme activity and substrate modification (Chen et al., 2023).

Trypsin inhibitor activity was markedly reduced in all fermented samples (92–94%), reinforcing the effect of the initial heat treatment (83%). No significant differences were observed among microorganisms. Previous work has indicated fermentation time as a key parameter for trypsin inhibitor reduction in peanut meal (Sadh et al., 2018), which may also contribute to the strong reductions observed here.

Peanut products are among the major causes of food allergies, often persistent and severe (Zhang et al., 2019; Pi et al., 2021). In this study, total ELISA-detectable peanut allergenic proteins decreased after solid-state fermentation in certain systems when compared to the non-fermented control, considering only samples with concentrations within the ELISA quantification range (Table 3). The observed reduction in detectable allergenic proteins can be attributed primarily to proteolysis by microbial proteases, which hydrolyze allergenic proteins into smaller peptides, and may alter IgE-reactive epitopes. Additional mechanisms may include matrix structural modifications and chemical changes occurring during fermentation (Pi et al., 2021; Yang et al., 2018; Shi et al., 2017). Samples with allergen levels above the upper limit of quantification were excluded from statistical analysis and interpreted qualitatively, indicating high allergen presence without allowing quantitative comparison. Overall, these results suggest that solid-state fermentation may reduce the levels of ELISA-detectable peanut allergenic proteins, although further studies are required to confirm the impact on immunoreactivity and allergenicity.

Aflatoxin contamination is a critical barrier for the utilization of peanut meals as food ingredients (Table 3). In this study, the heat treatment applied to the control sample reduced total aflatoxins from 41.8 to 16.2 μg/kg (approximately 61%), indicating that thermal processing was the primary contributor to aflatoxin reduction in the system. Subsequent solid-state fermentation with R. oryzae and A. oryzae promoted additional decreases in total aflatoxins relative to the heat-treated control, reaching 9.87 and 12.63 μg/kg, respectively, whereas fermentation with B. subtilis resulted in aflatoxin levels similar to the control (17.97 μg/kg). These findings suggest that SSF acts as a complementary step that can provide incremental improvements in aflatoxin safety rather than a standalone detoxification strategy. Microbial interactions with aflatoxins during SSF have been reported to involve physical binding to microbial biomass, adsorption to cell-wall components, and, in some cases, biotransformation or enzymatic degradation (Guan et al., 2021; Zhang et al., 2021). However, because only total aflatoxins (sum of B1, B2, G1, and G2) were quantified in this study, the data does not allow us to distinguish the fate of individual aflatoxin species or to attribute the observed reductions to specific detoxification mechanisms. Future work should include the separate quantification of individual aflatoxin species to better elucidate the mechanisms underlying aflatoxin reduction during solid-state fermentation. From a process perspective, the experiments were conducted at laboratory scale under static solid-state fermentation conditions without direct monitoring of pH or water activity, which defines the scope of the present study when considering process optimization or scale-up.

3.4. *Effect of solid-state fermentation on peanut meal physical properties*

Bulk density and swelling capacity are presented in Table 4. Heat treatment (control) reduced bulk density, which may be associated with protein structural changes and matrix compaction. Fermentation partially mitigated these effects, likely due to enzymatic remodeling of proteins and cell-wall polysaccharides, increasing porosity and exposing hydrophilic groups that enhance water uptake (Chawla et al., 2017; Olukomaiya et al., 2020).

Bulk density influences handling and application: higher bulk density can be advantageous for compact formulations and packaging, whereas lower values may be preferred for lightweight products

Table 3
Antinutritional factors, allergenic proteins, and aflatoxin levels in peanut meal and fermented peanut meal obtained with *Rhizopus oryzae, Bacillus subtilis*, and *Aspergillus oryzae*.

Sample	Total phenolics (mg GAE/g)	Tannins (μg TAE/g)	Phytic acid (mg PA/g)	Trypsin inhibitor (TIU/mg)	Allergenic proteins (μg/g) *	Aflatoxin (μg/kg)
Peanut meal	5.00 ± 0.70^{cd}	2.50 ± 0.04^{cd}	22.20 ± 0.50^{a}	1.157 ± 0.01^{a}	>20,000 (above ULOQ)	41.80
Peanut meal – control	4.00 ± 0.03^{d}	2.34 ± 0.06^{d}	22.17 ± 0.37^{a}	0.196 ± 0.08^{b}	>20,000 (above ULOQ)	16.22
Peanut meal – *R. oryzae*	9.25 ± 0.28^{a}	3.15 ± 0.16^{ab}	19.60 ± 0.37^{cd}	0.073 ± 0.15^{d}	>20,000 (above ULOQ)	9.87
Peanut meal – *B. subtilis*	5.14 ± 0.02^{cd}	2.25 ± 0.10^{d}	21.40 ± 0.43^{ab}	0.087 ± 0.25^{cd}	17,000	17.97
Peanut meal – *A. oryzae*	7.87 ± 0.45^{b}	3.57 ± 0.13^{a}	19.97 ± 0.24^{bc}	0.079 ± 0.15^{d}	4200	12.63

Means with different lowercase letters within the same column are significantly different at $p < 0.05$ by Tukey test. * Values reported as >20,000 μg/g exceeded the upper limit of quantification (ULOQ) of the ELISA assay and were therefore treated as semi-quantitative indicators and excluded from statistical analysis.

Table 4
Physical properties of peanut meal and fermented peanut meal by *Rhizopus oryzae, Bacillus subtilis* and *Aspergillus oryzae.*

Sample	Bulk Density (g/cm3)	Swelling Capacity (ml)
Peanut meal	0.66 ± 0.2^{a}	2.50 ± 0.3^{ab}
Peanut meal - control	0.40 ± 0.3^{c}	1.75 ± 0.2^{d}
Peanut meal - *R. oryzae*	0.59 ± 0.2^{a}	2.50 ± 0.4^{ab}
Peanut meal - *B. subtilis*	0.46 ± 0.1^{b}	2.13 ± 0.1^{c}
Peanut meal - *A. oryzae*	0.45 ± 0.1^{b}	2.63 ± 0.3^{a}

Means with different lowercase within the same column are significantly different at $p < 0.05$ by Tukey test.

(Akapata & Akubor, 1999). The fermented peanut meals exhibited intermediate bulk densities, suggesting adequate compactness and flowability for incorporation into dry mixes and semi-solid matrices.

Swelling capacity followed a similar trend: heat treatment reduced swelling capacity, while fermentation partially restored this property (Table 4). This behaviour is consistent with microbial and enzymatic actions that improve water absorption through changes in protein and fibre structures (Olukomaiya et al., 2020; Zhao et al., 2017).

3.5. *Effect of solid-state fermentation on peanut meal functional properties*

Foaming and emulsifying properties, as well as water- and oil-holding capacities, are presented in Table 5. Solid-state fermentation modified the foaming behavior of peanut meal, with increases in foaming capacity and stability observed in some fermented samples, indicating protein structural changes and the generation of surface-active fractions that favor air incorporation and stabilization. However, the absence of improvements in other systems suggests that the extent and nature of proteolysis critically influence foam formation and stability.

Emulsifying properties were affected by both heat treatment and solid-state fermentation. Heat treatment influenced emulsifying capacity and stability, likely due to partial protein denaturation that enhances interfacial adsorption. Following SSF, emulsifying behavior varied among fermented systems, with some samples maintaining emulsifying capacity relative to the non-fermented material, while others showed reduced emulsion stability. These effects may reflect differences in proteolysis intensity, leading to the formation of low-molecular-weight peptides that modify the hydrophilic–hydrophobic balance and weaken interfacial film strength (Chawla et al., 2017; Xiao et al., 2015). Consistent with these observations, roasting-assisted aqueous ethanol extraction of peanut oil has also been reported to preserve or even enhance the foaming and emulsifying properties of peanut protein isolates and concentrates compared with commercial soy protein isolate (Lu et al., 2026). Taken together, these findings indicate that both upstream processing and solid-state fermentation can be strategically tuned to generate peanut protein ingredients with tailored techno-functional profiles for food applications.

Heat treatment reduced water-holding capacity, likely due to protein aggregation and reduced exposure of hydrophilic groups. Fermentation partially restored water-holding capacity in some systems, which is consistent with proteolytic modifications that expose polar residues and increase water-binding sites within the protein matrix. Oil-holding capacity increased after fermentation in certain systems, possibly due to enhanced exposure of hydrophobic domains and matrix restructuring. Such improvements in oil-holding capacity are relevant for applications such as plant-based meat analogs, where fat retention contributes to texture and sensory attributes. Overall, these results demonstrate that solid-state fermentation can be used to modulate the techno-functional properties of peanut meal, with outcomes strongly dependent on the microbial strain and process conditions.

4. Conclusion

The growing demand for sustainable and nutritious food sources highlights the need for developing novel, low-cost plant-based protein ingredients from agro-industrial side streams, with improved quality and functionality. These results support the potential of SSF-treated peanut meal as a functional ingredient for food applications. Solid-state fermentation promoted a relative increase in crude protein content on a dry matter basis, associated with compositional shifts resulting from carbohydrate and lipid depletion and the incorporation of microbial biomass. Thermal processing accounted for most of the reduction in total aflatoxins, while subsequent solid-state fermentation provided additional, incremental decreases and contributed to reductions in ELISA-detectable allergenic proteins and antinutritional factors. Together, these biochemical modifications improved the techno-functional properties of the material, particularly its foaming and oil-holding capacity, broadening its potential applications in food systems.

Beyond these improvements, SSF stands out as a low-cost, sustainable, and easily scalable process that can be adapted to agro-industrial by-products. The resulting fermented peanut ingredient shows promising characteristics for use in plant-based meat analogs and other high-value protein foods. Future work will focus on exploring its performance in model formulations to assess its sensory, textural, and nutritional functionality in complex food matrices.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this manuscript, the authors used ChatGPT (OpenAI) to assist with language editing, text clarity, and organization of scientific content. The use of this tool was limited to improving readability and consistency of the text. All scientific interpretations, data analysis, and conclusions are the sole responsibility of the authors, who reviewed and edited the content to ensure accuracy and originality.

Table 5
Functional properties of peanut meal and fermented peanut meal by *Rhizopus oryzae, Bacillus subtilis* and *Aspergillus oryzae*.

Sample	Foaming Capacity (%)	Foaming Stability (%)	Emulsifying Capacity (%)	Emulsifying Stability (%)	Water-holding Capacity (g/g)	Oil-holding capacity (g/g)
Peanut meal	8 ± 0.2^{c}	4 ± 0.2^{c}	40 ± 0.6^{b}	20 ± 0.1^{b}	3.90 ± 0.01^{a}	1.68 ± 0.09^{c}
Peanut meal - control	8 ± 0.4^{c}	6 ± 0.1^{bc}	50 ± 0.7^{a}	40 ± 0.3^{a}	3.05 ± 0.04^{d}	1.80 ± 0.06^{bc}
Peanut meal - *R. oryzae*	20 ± 0.9^{a}	18 ± 0.4^{a}	40 ± 0.6^{b}	20 ± 0.2^{b}	3.37 ± 0.08^{c}	1.69 ± 0.02^{c}
Peanut meal - *B. subtilis*	4 ± 0.2^{d}	0 ± 0.0^{d}	30 ± 0.2^{c}	0 ± 0.0^{c}	3.70 ± 0.05^{ab}	2.26 ± 0.03^{a}
Peanut meal - *A. oryzae*	12 ± 0.2^{b}	8 ± 0.2^{bc}	30 ± 0.7^{c}	0 ± 0.0^{c}	3.28 ± 0.04^{c}	2.26 ± 0.06^{a}

All the data are expressed as means ± SD of three replicates. Means with different lowercase within the same column are significantly different at $p < 0.05$ by Tukey test.

CRediT authorship contribution statement

Liege de Souza Jacinto: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Cristiane Grella Miranda:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Adriana Raquel Persson da Silva:** Methodology, Formal analysis. **Vitória dos Santos Dionísio:** Methodology, Investigation, Formal analysis. **Tamara Santos de Oliveira:** Methodology, Formal analysis. **Beatriz Thie Iamanaka:** Methodology, Formal analysis. **Ana Carla Kawazoe Sato:** Methodology, Formal analysis. **Paula Speranza:** Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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