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Influence of Fermentation Duration and Starter Culture on Nutritional Quality and Bioactive Compounds of Fermented Peanut Products

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ABSTRACT: Peanut (*Arachis hypogaea* L.) is an important plant-based protein source containing substantial amounts of lipids, amino acids, phenolic compounds, tocopherols, and other bioactive constituents. However, the nutritional and functional utilization of peanuts is constrained by the presence of antinutritional compounds, limited mineral bioavailability, allergenic proteins, and susceptibility to oxidative deterioration. Fermentation has emerged as a promising biotechnological strategy to improve the nutritional quality, digestibility, functional properties, and bioactive potential of peanut-based products. This review critically evaluates the effects of fermentation duration and microbial starter cultures on nutrient transformation and bioactive compound formation in fermented peanuts. The review discusses the roles of lactic acid bacteria, *Bacillus* spp., filamentous fungi, and mixed-culture systems in modulating proteolysis, phenolic release, antioxidant activity, γ -aminobutyric acid (GABA) production, phytate degradation, and allergen reduction. Fermentation duration was found to exert dynamic and non-linear effects on nutritional quality, where intermediate fermentation stages generally produced the highest enhancement of digestibility, antioxidant activity, peptide formation, and mineral bioavailability. In contrast, prolonged fermentation frequently resulted in nutrient degradation, off-flavor formation, lipid oxidation, and sensory deterioration. Comparative analysis further revealed that different starter cultures possess distinct optimal fermentation windows and metabolic responses due to variations in enzymatic activity and substrate utilization. Mixed-culture fermentation demonstrated potential synergistic effects through sequential metabolism and broader bioactive compound production, although process standardization remains challenging. This review also highlights current limitations involving fermentation reproducibility, safety concerns, omics integration, and limited clinical validation. Future advances in precision fermentation, artificial intelligence-assisted optimization, engineered starter cultures, and multi-omics approaches may substantially improve the development of fermented peanut functional foods. Overall, controlled fermentation represents a highly promising approach for producing nutritionally enhanced and sustainable plant-based functional foods derived from peanuts.

INTRODUCTION

Peanut (*Arachis hypogaea* L.) is one of the most widely cultivated legume crops and serves as an important source of plant-derived protein, lipids, vitamins, minerals, and bioactive compounds in many developing and developed countries. Peanut kernels contain substantial amounts of protein and unsaturated fatty acids, particularly oleic and linoleic acids, which contribute to their nutritional and economic value. In addition, peanuts are recognized as a rich source of phenolic compounds, tocopherols, phytosterols, and resveratrol with potential antioxidant and health-promoting properties (Taheri et al., 2023).

The increasing demand for functional foods and sustainable plant-based nutrition has stimulated interest in improving the nutritional quality and biological functionality of legume-derived products, including peanuts. Compared with animal-based protein sources, peanuts offer advantages related to affordability, availability, and environmental sustainability. However, despite their nutritional potential, raw peanuts still exhibit several limitations associated with digestibility, antinutritional compounds, allergenicity, and storage stability (Makinde & Dauda, 2020).

Peanuts naturally contain antinutritional factors such as phytic acid, tannins, oxalates, and protease inhibitors that may reduce nutrient bioavailability and interfere with protein digestion. Phytate, for example, can chelate essential minerals including iron, zinc, calcium, and magnesium, thereby decreasing their intestinal absorption (Brouns, 2021). In addition, peanut allergenic proteins, particularly Ara h1, Ara h2, and Ara h3, remain major concerns for sensitive individuals because these proteins can induce severe immunological responses (Yu & Eghbali, 2025). The presence of these compounds limits the broader utilization of peanut-based products in functional food applications.

Fermentation has emerged as an effective bioprocessing strategy for improving the nutritional characteristics and functional properties of plant-based materials. Microbial fermentation can induce substantial biochemical transformations through the action of enzymes such as protease, phytase, lipase, cellulase, and β -glucosidase. These enzymatic activities contribute to protein hydrolysis, liberation of bound phenolic compounds, degradation of antinutritional factors, and formation of various bioactive metabolites (Kanimozhi et al., 2026). Fermentation may also improve sensory quality, shelf life, and digestibility of peanut-based products while enhancing their antioxidant potential and functional attributes.

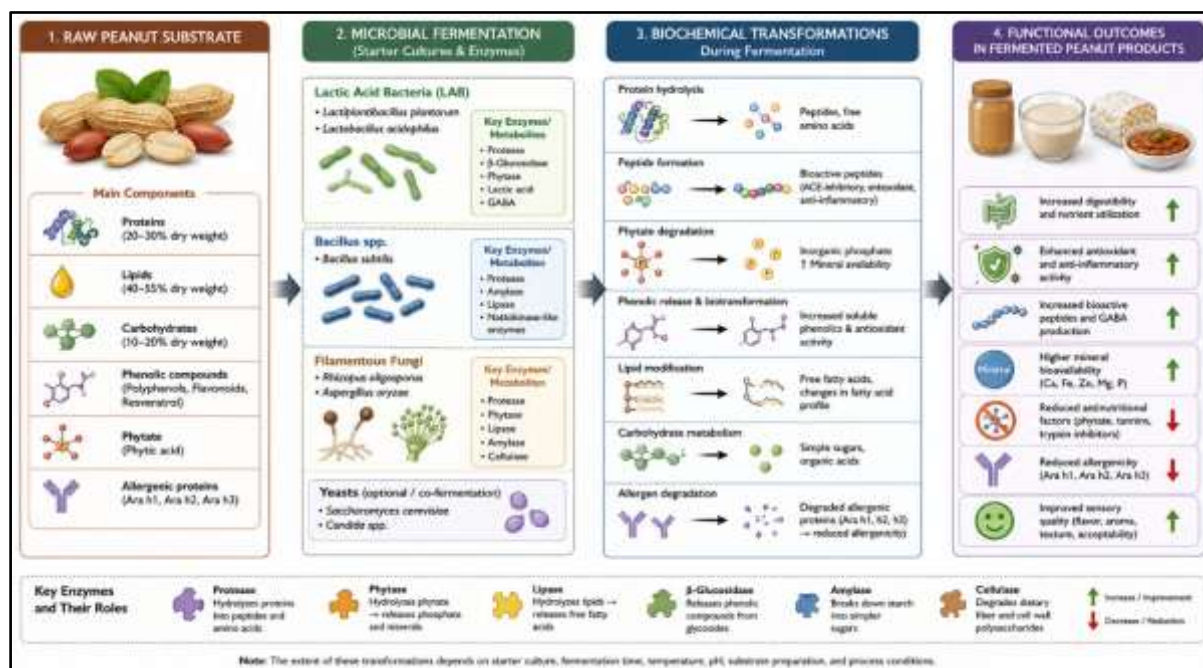
Several microbial groups have been applied in peanut fermentation systems, including lactic acid bacteria (LAB), *Bacillus* spp., yeasts, and filamentous fungi such as *Rhizopus* and *Aspergillus*. LAB fermentation is commonly associated with acidification, probiotic functionality, and γ -aminobutyric acid (GABA) production, whereas *Bacillus* fermentation is characterized by intensive proteolysis and peptide formation (Christensen et al., 2022). Meanwhile, fungal fermentation may promote extensive degradation of complex macromolecules and facilitate the release of phenolic compounds through extracellular enzymatic activity (Šelo et al., 2024). The metabolic responses generated during fermentation are therefore highly dependent on the type of starter culture used.

In addition to microbial diversity, fermentation duration represents another critical factor influencing the final nutritional and functional quality of fermented peanut products. During early fermentation stages, microbial adaptation and initial enzymatic hydrolysis generally occur with relatively limited biochemical modification. Intermediate fermentation periods often correspond with maximum microbial activity, increased antioxidant capacity, enhanced peptide production, and substantial reduction of antinutritional compounds (Wang et al., 2024). However, prolonged fermentation may lead to nutrient degradation, lipid oxidation, excessive acidification, texture deterioration, and formation of undesirable metabolites (Saritaş et al., 2024). Therefore, the relationship between fermentation duration and product quality is dynamic and non-linear.

Recent studies have reported various fermented peanut products, including peanut yogurt, fermented peanut beverages, peanut-based probiotic foods, fermented peanut paste, and tempeh-like products. These studies have demonstrated improvements in protein digestibility, antioxidant activity, mineral bioavailability, and bioactive compound formation following microbial fermentation (Borresen et al., 2012; Kanimozhi & Sukumar, 2025). Nevertheless, most previous studies have primarily focused on isolated fermentation systems, specific microbial strains, or individual biochemical parameters. Comparative evaluations regarding the interaction between fermentation duration and starter culture diversity in shaping nutritional and bioactive properties remain limited.

Previous review articles have broadly discussed legume fermentation, microbial biotechnology, and functional fermented foods. However, a comprehensive synthesis specifically addressing the combined effects of fermentation time and microbial

starter type on nutrient transformation and bioactive formation in fermented peanut products is still lacking. Understanding these interactions is important for optimizing fermentation strategies aimed at developing nutritionally enhanced and functionally superior peanut-based foods.



Therefore, this review critically evaluates the effects of fermentation duration and starter culture diversity on the nutritional quality, antinutritional compounds, phenolic release, antioxidant activity, and bioactive compound formation of fermented peanut products. This review also discusses the underlying biochemical mechanisms, current research limitations, and future perspectives for the development of functional fermented peanut foods.

PEANUT AS A FERMENTATION SUBSTRATE

Macronutrient composition

Peanut (*Arachis hypogaea* L.) is recognized as a nutrient-dense legume containing substantial amounts of proteins, lipids, carbohydrates, and essential amino acids, making it a suitable substrate for microbial fermentation (table 1).

Table 1. Nutritional composition and functional components of raw peanut kernels

Component	Reported composition / characteristics	Functional and nutritional relevance	References
Moisture	Approximately 3.8–7.9%	Influences storage stability and microbial susceptibility during fermentation	(Yu & Eghbali, 2025)
Protein	Approximately 19–32% dry weight	Major source of amino acids and precursor of bioactive peptides generated during fermentation	(Yu & Eghbali, 2025)
Lipid	Approximately 43–57%	Rich in unsaturated fatty acids, especially oleic and linoleic acids	(Yu & Eghbali, 2025)
Carbohydrate	Approximately 10–20%	Serves as carbon substrate for microbial metabolism during fermentation	(Yu & Eghbali, 2025)
Dietary fiber	Around 8.5%	Supports gastrointestinal health and may influence fermentation dynamics	(Yu & Eghbali, 2025)

Essential amino acids	Rich in leucine, arginine, glutamic acid, and phenylalanine	Important for nutritional quality and peptide biotransformation	(Davis & Dean, 2016)
Unsaturated fatty acids	Oleic acid (53–72%) and linoleic acid (13–26%) dominate lipid fraction	Associated with oxidative stability and cardiovascular benefits	(X. Liu et al., 2025)
Phenolic compounds	Includes phenolic acids, flavonoids, stilbenes, and procyanidins	Contribute to antioxidant and anti-inflammatory activities	(Çiftçi & Suna, 2022)
Resveratrol	Present in kernel and skin fractions	Bioactive compound with antioxidant and cardioprotective potential	(Yu & Eghbali, 2025)
Tocopherols	High γ -tocopherol content	Functions as lipid-soluble antioxidant protecting peanut oil stability	(X. Liu et al., 2025)
Minerals	Rich in Mg, P, K, Zn, and Fe	Important for metabolic and physiological functions	(X. Liu et al., 2025)
Phytate	Approximately 0.2–0.4%	Reduces mineral bioavailability but may exhibit antioxidant activity	(Kim et al., 2017)
Tannins and polyphenols	Mainly concentrated in peanut skin	Influence antioxidant capacity and sensory properties	(Çiftçi & Suna, 2022)
Allergenic proteins	Ara h1, Ara h2, and Ara h3 are major allergens	Important targets for fermentation-mediated allergen reduction	(Yu & Eghbali, 2025)

The chemical composition of peanuts may vary depending on cultivar, environmental conditions, postharvest handling, and processing methods; however, peanut kernels generally contain approximately 20–30% protein and 40–55% lipids on a dry weight basis (Singh et al., 2021). The high nutritional density of peanuts contributes not only to their dietary importance but also to their suitability for the production of functional fermented foods.

Peanut proteins mainly consist of globulins and albumins, with arachin and conarachin representing the dominant storage proteins (L. Wang et al., 2013). These proteins contain various essential amino acids, including leucine, valine, phenylalanine, and arginine, which are important for human nutrition and microbial metabolism during fermentation. Although peanuts are relatively deficient in sulfur-containing amino acids such as methionine, their overall amino acid composition still provides a favorable substrate for proteolytic microorganisms (Arya et al., 2015). During fermentation, microbial proteases hydrolyze complex proteins into peptides and free amino acids, thereby improving digestibility and generating bioactive compounds with potential antioxidant, antihypertensive, and immunomodulatory activities.

Lipids constitute the largest fraction of peanut kernels and are predominantly composed of unsaturated fatty acids, especially oleic acid and linoleic acid (Musa, 2010). These fatty acids contribute to oxidative stability, flavor formation, and nutritional quality. In fermentation systems, lipids may undergo enzymatic hydrolysis through microbial lipase activity, resulting in the release of free fatty acids and volatile compounds that influence aroma and sensory characteristics. However, excessive lipid degradation during prolonged fermentation may also promote rancidity and oxidative deterioration (Chandra et al., 2020).

Carbohydrates in peanuts are present mainly as soluble sugars, starch, and dietary fiber. Although peanut carbohydrate content is lower than that of many cereal grains, these compounds still provide important carbon sources for microbial growth and metabolism during fermentation (Wu et al., 2025). Fermentative microorganisms utilize carbohydrates to produce organic acids, alcohols, enzymes, and secondary metabolites that contribute to nutrient transformation and preservation. In addition, structural polysaccharides associated with peanut cell walls may be degraded by cellulolytic and hemicellulolytic enzymes, facilitating the release of bound phenolic compounds and improving substrate accessibility (Liu et al., 2020).

The nutritional composition of peanuts therefore provides a favorable biochemical environment for fermentation processes. The presence of proteins, lipids, carbohydrates, minerals, and endogenous bioactive compounds supports microbial growth

and enzymatic activity, allowing extensive biochemical transformation during fermentation. Consequently, peanut substrates can serve as promising raw materials for the development of fermented functional foods with enhanced nutritional and biological properties.

Bioactive compounds in peanut

Peanuts contain a wide range of bioactive compounds that contribute to their antioxidant potential and health-promoting properties. These compounds include phenolic acids, flavonoids, stilbenes, tocopherols, phytosterols, and other secondary metabolites that may exert anti-inflammatory, cardioprotective, antimicrobial, and antioxidant activities (Nunes et al., 2023). The concentration and composition of these compounds are influenced by peanut genotype, environmental conditions, processing techniques, and storage conditions.

Phenolic compounds represent one of the major classes of bioactive constituents in peanuts. These compounds occur in both free and bound forms and are distributed primarily in peanut skins and cotyledons (Cordeiro-Massironi et al., 2023). Common phenolic acids identified in peanuts include *p*-coumaric acid, ferulic acid, caffeic acid, and chlorogenic acid. Many of these phenolics are associated with cell wall structures and may become more bioavailable after microbial fermentation due to enzymatic degradation of polysaccharide matrices (Kumar et al., 2025).

Flavonoids such as catechin, quercetin, and anthocyanin derivatives are also present in peanut tissues and contribute significantly to antioxidant activity (Li et al., 2024). These compounds can neutralize reactive oxygen species and reduce oxidative stress, thereby potentially lowering the risk of chronic diseases. During fermentation, microbial enzymes including β -glucosidase and esterase may hydrolyze glycosidic bonds and release aglycone flavonoids with higher biological activity and bioavailability (Huynh et al., 2014).

Resveratrol, a stilbene phytoalexin widely associated with antioxidant and cardioprotective functions, is another important bioactive compound found in peanuts (Sales & Resurreccion, 2013). Although the concentration of resveratrol in peanuts is relatively lower than in grapes, its presence remains nutritionally relevant because fermentation processes may enhance its release and transformation into more bioactive derivatives. Microbial metabolism during fermentation can also induce the synthesis of additional phenolic metabolites with improved antioxidant capacity (Kumar et al., 2025).

Tocopherols, particularly γ -tocopherol, constitute another major antioxidant group in peanuts. Tocopherols function as lipid-soluble antioxidants that protect unsaturated fatty acids from oxidative degradation and contribute to oil stability during storage and processing (Fang et al., 2017). Fermentation may influence tocopherol stability depending on oxygen availability, microbial activity, and fermentation duration. Controlled fermentation conditions may preserve or even enhance antioxidant functionality, whereas prolonged fermentation could promote oxidative losses of sensitive compounds (Adebo, 2026).

Overall, peanuts provide a rich reservoir of bioactive compounds that can undergo substantial biochemical modification during fermentation. The interaction between microbial enzymes and endogenous phytochemicals plays a central role in determining the final antioxidant activity and functional quality of fermented peanut products.

Antinutritional compounds

Despite their nutritional advantages, peanuts also contain several antinutritional compounds that may interfere with nutrient utilization and reduce overall nutritional quality. The major antinutritional factors identified in peanuts include phytic acid, oxalates, tannins, and enzyme inhibitors such as trypsin inhibitors (Makinde & Dauda, 2020). These compounds are naturally synthesized by plants as protective or storage-related metabolites but may negatively affect digestibility and mineral absorption in humans.

Phytic acid, or myo-inositol hexakisphosphate, is considered one of the primary antinutritional compounds in legumes and oilseeds. In peanut kernels, phytate strongly chelates divalent minerals such as iron, zinc, calcium, and magnesium, thereby reducing their bioavailability in the gastrointestinal tract (S. Singh et al., 2022). The formation of insoluble phytate-mineral complexes limits intestinal absorption and may contribute to micronutrient deficiencies, particularly in populations relying heavily on plant-based diets. However, microbial fermentation can significantly reduce phytate levels through phytase-mediated hydrolysis, leading to increased mineral availability and improved nutritional quality (Sawant et al., 2025).

Oxalates are another group of antinutritional compounds capable of binding calcium and forming insoluble calcium oxalate complexes (Salgado et al., 2023). Excessive dietary oxalate intake may reduce calcium absorption and potentially increase the risk of kidney stone formation. Although oxalate concentrations in peanuts are generally lower than in certain leafy vegetables, their presence still contributes to mineral utilization limitations.

Tannins are polyphenolic compounds that may interact with proteins, digestive enzymes, and minerals. These interactions can reduce protein digestibility and impair nutrient absorption (Fontes-Candia et al., 2025). Tannins are primarily concentrated in peanut skins and may also contribute to bitterness and astringency. During fermentation, tannase-producing microorganisms may partially degrade tannins, resulting in improved palatability and digestibility (Jiménez et al., 2014).

Enzyme inhibitors, particularly trypsin inhibitors, can interfere with digestive proteases and reduce protein hydrolysis efficiency in the gastrointestinal system (De Lima et al., 2019). These inhibitors may impair amino acid utilization and decrease overall protein digestibility. Fermentation has been reported to reduce protease inhibitor activity through microbial enzymatic degradation and denaturation processes, thereby improving protein utilization efficiency (Yousefi et al., 2025).

Therefore, although antinutritional compounds present substantial nutritional challenges, microbial fermentation offers an effective strategy for mitigating their negative effects. Controlled fermentation systems may improve nutrient bioavailability, reduce antinutritional factors, and enhance the functional quality of peanut-derived foods.

Peanut allergenic proteins

Peanut allergy represents one of the most serious food allergies worldwide and is frequently associated with severe immunological reactions, including anaphylaxis (Burks, 2003). The allergenic potential of peanuts is mainly attributed to several storage proteins, among which Ara h1, Ara h2, and Ara h3 are considered the major allergenic proteins. These proteins exhibit high structural stability and resistance to thermal processing and gastrointestinal digestion, making them potent allergens in sensitive individuals.

Ara h1 is a vicilin-like seed storage glycoprotein belonging to the cupin superfamily and accounts for a substantial proportion of total peanut protein (Barre et al., 2005). Ara h2, categorized as a 2S albumin protein, is often regarded as one of the most clinically relevant peanut allergens because of its strong immunoglobulin E (IgE)-binding capacity and high resistance to proteolytic digestion (Kulis et al., 2012). Meanwhile, Ara h3 is a glycinin-like storage protein that also contributes significantly to allergenic responses in peanut-sensitive individuals.

The structural characteristics of these allergenic proteins, including compact folding patterns and disulfide bond stability, complicate their degradation during conventional thermal processing (Pekar et al., 2018). Consequently, alternative processing technologies capable of modifying allergenic epitopes have gained increasing attention in food biotechnology research.

Microbial fermentation has emerged as a promising strategy for reducing peanut allergenicity through enzymatic hydrolysis and structural modification of allergenic proteins. Proteolytic microorganisms such as lactic acid bacteria, *Bacillus subtilis*, and filamentous fungi can hydrolyze peanut proteins into smaller peptides with reduced IgE-binding affinity (C. Wang et al., 2025). Fermentation-induced acidification and enzymatic activity may alter protein conformation, disrupt allergenic epitopes, and reduce allergenic potential.

Several studies have reported partial degradation of Ara h1, Ara h2, and Ara h3 during fermentation processes, particularly under prolonged proteolytic conditions (Pi et al., 2019). However, the extent of allergen reduction is highly dependent on starter culture selection, fermentation duration, pH conditions, and enzymatic activity. Excessive fermentation may also negatively affect sensory quality and nutritional stability, highlighting the importance of optimizing fermentation parameters.

Although fermentation shows considerable potential for reducing peanut allergenicity, complete elimination of allergenic risk has not yet been consistently achieved. Further investigations integrating proteomics, immunological assays, and clinical validation are therefore required to better understand the mechanisms underlying allergen modification during peanut fermentation and to develop safer fermented peanut products for sensitive populations.

MICROBIAL STARTERS USED IN PEANUT FERMENTATION

Table 2. Microbial starter cultures commonly used in peanut fermentation and their metabolic characteristics

Starter culture	Representative microorganisms	Main enzymes or metabolites produced	Major effects on peanut substrate	Typical fermentation duration	Representative applications	References
Lactic acid bacteria (LAB)	<i>Lactiplantibacillus plantarum</i> , <i>Lactocaseibacillus casei</i> , <i>Lactobacillus acidophilus</i>	Lactic acid, protease, β -glucosidase, glutamate decarboxylase	Acidification, protein hydrolysis, enhancement of antioxidant activity, GABA production, reduction of antinutrients	12–48 h	Peanut yogurt, probiotic beverages, fermented peanut milk	(He et al., 2023)
<i>Bacillus</i> spp.	<i>Bacillus subtilis</i> , <i>Bacillus natto</i>	Protease, amylase, lipase, nattokinase-like enzymes	Extensive proteolysis, bioactive peptide formation, improved digestibility, alkaline fermentation characteristics	24–72 h	Peanut paste, fermented peanut protein products	(Peruzy et al., 2022)
<i>Rhizopus</i> spp.	<i>Rhizopus oligosporus</i> , <i>Rhizopus oryzae</i>	Protease, phytase, lipase	Reduction of phytate, increased mineral bioavailability, softening of texture, liberation of phenolic compounds	24–48 h	Peanut tempeh-like products	(Centeno & Carballo, 2023; Grana et al., 2023)
<i>Aspergillus</i> spp.	<i>Aspergillus oryzae</i> , <i>Aspergillus niger</i>	Amylase, protease, cellulase, phytase	Degradation of complex macromolecules, enhancement of soluble nutrients and phenolic compounds	24–96 h	Fermented peanut flour and seasoning products	(Çiftçi & Suna, 2022)
Yeasts	<i>Saccharomyces cerevisiae</i> , <i>Candida utilis</i>	Ethanol, esterase, volatile flavor compounds	Flavor enhancement, partial carbohydrate utilization, improvement of sensory quality	24–72 h	Fermented peanut beverages and mixed-culture fermentation	(Zhang et al., 2022)
Mixed-culture	LAB + yeast or LAB + fungi	Combined enzymatic	Synergistic nutrient transformation, enhanced	24–72 h	Functional fermented	(Qiao et al., 2022)

fermentation	combinations	activities	antioxidant activity, improved sensory characteristics		peanut foods	
Spontaneous fermentation	Indigenous mixed microbiota	Variable metabolites depending on microbial ecology	Natural acidification and flavor development, but inconsistent quality and safety	Variable	Traditional household peanut fermentation	(Centeno & Carballo, 2023)

SPONTANEOUS FERMENTATION

Spontaneous fermentation represents one of the oldest traditional fermentation approaches used in legume- and cereal-based foods. In this process, fermentation occurs naturally through the activity of indigenous microorganisms originating from raw materials, processing equipment, water, air, and surrounding environments (Ray et al., 2016). Peanut substrates subjected to spontaneous fermentation commonly harbor diverse microbial communities consisting of lactic acid bacteria, yeasts, filamentous fungi, and various environmental bacteria whose composition changes dynamically during fermentation.

The microbial ecology of spontaneous fermentation is strongly influenced by environmental conditions such as temperature, humidity, oxygen availability, substrate composition, and sanitation practices (Sawant et al., 2025). During the early stages, fast-growing microorganisms capable of utilizing readily available carbohydrates generally dominate the fermentation environment. As fermentation progresses, secondary microbial populations adapted to acidic or nutrient-limited conditions may become predominant, leading to complex biochemical transformations within the peanut matrix.

One of the main advantages of spontaneous fermentation is the development of unique sensory characteristics resulting from diverse microbial metabolism. Indigenous microorganisms may contribute to flavor complexity, organic acid production, proteolysis, and formation of volatile compounds that enhance product acceptability (Bai et al., 2025). In addition, spontaneous fermentation is relatively inexpensive and does not require specialized starter preparation, making it widely applicable in household-scale and traditional food systems.

However, spontaneous fermentation also presents substantial limitations related to process inconsistency and food safety. The absence of controlled starter cultures often results in significant variability in microbial composition, fermentation kinetics, nutritional outcomes, and sensory quality between production batches (Yee et al., 2025). Uncontrolled microbial growth may also increase the risk of contamination by spoilage microorganisms or pathogenic bacteria, particularly under inadequate hygienic conditions.

Safety concerns are particularly important in peanut fermentation because peanuts are susceptible to fungal contamination and mycotoxin production, including aflatoxins generated by *Aspergillus flavus* and related species (Norlia et al., 2019). Poorly controlled spontaneous fermentation may therefore compromise product safety and shelf stability. Consequently, although spontaneous fermentation remains culturally and economically important, modern food biotechnology increasingly emphasizes the use of controlled starter cultures to improve fermentation reproducibility, safety, and functional quality.

Lactic acid bacteria (LAB)

Lactic acid bacteria (LAB) are among the most extensively utilized microbial groups in food fermentation systems due to their safety status, probiotic potential, and ability to improve nutritional and sensory properties. In peanut fermentation, commonly applied LAB include *Lactobacillus*, *Lactiplantibacillus*, and *Pediococcus* species (Cheng et al., 2026). These microorganisms are capable of metabolizing carbohydrates into lactic acid and other metabolites that contribute to substrate preservation and biochemical transformation.

One of the primary effects of LAB fermentation is acidification. During carbohydrate metabolism, LAB produce lactic acid, resulting in a decrease in pH that inhibits spoilage microorganisms and pathogenic bacteria (Y. Wang et al., 2022). Acidification also influences protein structure, enzyme activity, and mineral solubility within the peanut matrix. Lower pH

conditions may facilitate protein denaturation and improve accessibility of substrates to microbial enzymes, thereby promoting nutrient transformation and digestibility enhancement.

LAB also exhibit substantial proteolytic activity through the secretion of extracellular and intracellular proteases capable of hydrolyzing peanut proteins into peptides and free amino acids (Toe et al., 2019). This proteolysis contributes to improved protein digestibility and formation of bioactive peptides with antioxidant, antimicrobial, antihypertensive, and immunomodulatory properties. The extent of proteolytic modification is highly strain-dependent and may vary according to fermentation duration and environmental conditions.

In addition to proteolysis, certain LAB strains are capable of producing γ -aminobutyric acid (GABA), an important bioactive metabolite associated with neurological and antihypertensive benefits (Iorizzo et al., 2023). GABA is synthesized through glutamate decarboxylase-mediated conversion of glutamate during microbial metabolism. Several studies have reported increased GABA concentrations in fermented plant-based foods inoculated with *Lactobacillus plantarum* and related LAB strains (Dhakal et al., 2012). The accumulation of GABA and other secondary metabolites contributes to the growing interest in LAB-fermented peanuts as functional food products.

Furthermore, LAB fermentation may improve antioxidant activity through the release of bound phenolic compounds and degradation of antinutritional factors such as phytate and tannins (O. A. Adebo & Medina-Meza, 2020). Enzymes such as β -glucosidase and phytase produced by certain LAB strains facilitate phenolic liberation and mineral bioavailability enhancement. Consequently, LAB fermentation is widely regarded as one of the most promising approaches for producing nutritionally improved and functionally enhanced peanut-based foods.

Bacillus fermentation

Species belonging to the genus *Bacillus*, particularly *Bacillus subtilis*, are widely used in alkaline fermentation systems involving legumes and protein-rich substrates. Unlike LAB fermentation, which is characterized by acid production, *Bacillus*-mediated fermentation generally results in alkaline conditions due to extensive protein degradation and ammonia formation during amino acid metabolism (Fernández & Zúñiga, 2006).

Bacillus subtilis possesses strong proteolytic capabilities and secretes various extracellular enzymes including proteases, amylases, and lipases that contribute to substantial biochemical transformation of peanut substrates (Rosazza et al., 2023). Proteolytic hydrolysis by *Bacillus* species generates peptides and free amino acids that improve digestibility and contribute to the formation of desirable flavor compounds. The released peptides may also exhibit antioxidant, antihypertensive, antimicrobial, and immunomodulatory activities depending on their amino acid composition and sequence (Kang et al., 2019).

One of the most important effects of *Bacillus* fermentation is the improvement of protein digestibility. The breakdown of complex storage proteins into smaller peptides reduces structural resistance to gastrointestinal enzymes and increases amino acid accessibility (M. He et al., 2025). This transformation is particularly important in plant-based substrates where native protein structures and antinutritional compounds may limit digestibility.

In addition to protein modification, *Bacillus* fermentation may influence lipid metabolism and flavor generation through lipolytic activity. Volatile compounds formed during amino acid and lipid degradation contribute to characteristic fermented and savory sensory properties commonly associated with alkaline fermented foods (Van Wyk, 2024). However, excessive fermentation may lead to undesirable ammonia-like odors, excessive softening, and quality deterioration.

Bacillus subtilis has also been associated with the production of functional metabolites including bioactive peptides and fibrinolytic enzymes such as nattokinase-like compounds (S et al., 2025). These metabolites have attracted interest because of their potential health-promoting properties. Nevertheless, optimization of fermentation conditions remains essential because excessive proteolysis and prolonged alkaline fermentation may negatively affect product stability and consumer acceptance.

Fungal fermentation

Filamentous fungi play important roles in traditional and industrial fermentation systems due to their extensive enzymatic capabilities and adaptability to solid-state fermentation conditions. In peanut fermentation, commonly utilized fungal genera include *Rhizopus*, *Aspergillus*, and *Monascus* (Mattison et al., 2024). These fungi produce a wide range of hydrolytic enzymes that facilitate nutrient transformation and bioactive compound release.

Rhizopus species, particularly *Rhizopus oligosporus*, are widely recognized for their application in tempeh fermentation and similar legume-based products. These fungi produce proteases, phytases, cellulases, and lipases that degrade complex macromolecules into smaller and more digestible compounds (Naeem et al., 2022). Phytase activity is especially important because it hydrolyzes phytate complexes and improves mineral bioavailability within fermented peanut substrates.

Aspergillus species such as *Aspergillus oryzae* and *Aspergillus niger* also exhibit strong enzymatic activities capable of degrading proteins, carbohydrates, and cell wall polysaccharides (De Vries & Visser, 2001). Enzymatic degradation of structural matrices may facilitate the release of bound phenolic compounds, thereby increasing antioxidant activity and functional properties. In addition, fungal fermentation may enhance the formation of flavor-active metabolites contributing to improved sensory quality.

Monascus species are increasingly investigated in food fermentation because of their ability to produce pigments and secondary metabolites with potential biological activities (Agboyibor et al., 2018). Certain *Monascus*-fermented products contain compounds associated with antioxidant and cholesterol-lowering effects. However, some strains may also produce citrinin, a nephrotoxic secondary metabolite, necessitating careful strain selection and process control.

A major advantage of fungal fermentation lies in its ability to liberate phenolic compounds through extracellular enzymatic degradation of plant cell wall structures. Enzymes such as cellulase, xylanase, esterase, and β -glucosidase facilitate the release of phenolics previously bound within the peanut matrix (Azkia et al., 2024). This process often leads to increased antioxidant activity and enhanced bioaccessibility of phytochemicals.

Despite these advantages, fungal fermentation requires strict control of environmental parameters including moisture, temperature, aeration, and contamination prevention. Inadequate process management may promote spoilage or toxin-producing fungi, thereby compromising food safety and product quality.

Mixed-culture fermentation

Mixed-culture fermentation involves the simultaneous or sequential utilization of multiple microorganisms within the same fermentation system. This approach has gained increasing attention because of its ability to generate synergistic metabolic interactions and more complex biochemical transformations compared with single-culture fermentation (add ref, 20xx).

In peanut fermentation, mixed cultures commonly combine LAB, yeasts, *Bacillus*, or filamentous fungi to exploit complementary enzymatic activities and metabolic pathways. For example, LAB may contribute acidification and phytate degradation, while fungi or *Bacillus* species provide strong proteolytic and cellulolytic activities (Fekri et al., 2019). Such synergistic interactions can improve nutrient digestibility, release bioactive compounds, and enhance flavor complexity simultaneously.

Co-fermentation systems may also improve microbial stability and fermentation efficiency. Sequential substrate utilization among different microorganisms allows more complete conversion of proteins, carbohydrates, and phenolic complexes into nutritionally valuable metabolites (Rowland et al., 2017). In some cases, one microbial group may produce metabolites that stimulate the growth or enzymatic activity of another microorganism, thereby enhancing overall fermentation performance.

Several studies have demonstrated that mixed-culture fermentation can increase antioxidant activity, peptide production, phenolic release, and sensory acceptability more effectively than monoculture systems (Zhao et al., 2021). The diversity of microbial enzymes generated during co-fermentation contributes to broader biochemical modification of peanut substrates and may improve the functional quality of final products.

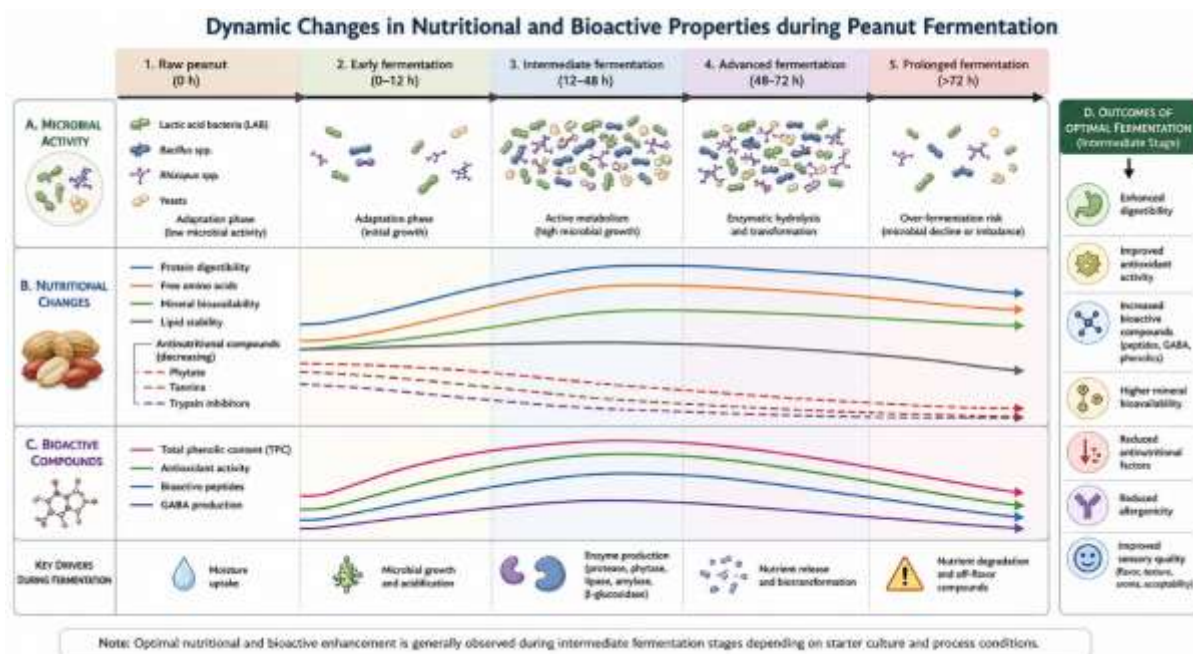
However, despite these advantages, mixed-culture fermentation also presents substantial challenges related to process standardization and reproducibility. Interactions among microorganisms are often highly dynamic and influenced by substrate composition, inoculum ratio, fermentation duration, oxygen availability, and environmental conditions (Solomon & Vishnu, 2025). Uncontrolled microbial competition may lead to inconsistent product quality and unpredictable metabolite production.

Maintaining microbial balance during mixed-culture fermentation therefore remains a critical challenge for industrial applications. Future research integrating microbial ecology, systems biology, and fermentation engineering is needed to optimize co-fermentation strategies and improve process reliability in fermented peanut products.

Effect of Fermentation Duration on Nutritional Quality

Early-stage fermentation (0–24 h)

The early stage of fermentation is characterized primarily by microbial adaptation to the peanut substrate and initiation of metabolic activity. During this period, microorganisms begin utilizing readily available nutrients, including soluble sugars and low-molecular-weight nitrogen compounds, to support cellular growth and metabolic stabilization (Malaysia et al., 2025). The efficiency of this adaptation phase is strongly influenced by starter culture type, inoculum concentration, substrate composition, temperature, and oxygen availability.



One of the earliest biochemical changes observed during fermentation is pH reduction, particularly in systems dominated by lactic acid bacteria (LAB). Organic acids produced through carbohydrate metabolism gradually lower the pH of the fermentation medium and create conditions that inhibit spoilage microorganisms and pathogenic bacteria ((Tang et al., 2023). Acidification also modifies the physicochemical properties of peanut proteins and cell wall components, thereby increasing substrate accessibility to microbial enzymes.

Although enzymatic activity begins during this stage, hydrolysis of proteins, lipids, and structural carbohydrates generally remains limited. Proteolytic degradation is still relatively moderate because microbial populations have not yet reached maximum metabolic activity. Consequently, improvements in digestibility, phenolic release, and bioactive compound formation are often minimal during the first 24 h of fermentation.

Table 3. Interaction model between starter culture and fermentation duration

Fermentation duration	Major biochemical changes	Nutritional effects	Changes in bioactive compounds	Potential limitations	Representative references
0–12 h (initial stage)	Microbial adaptation phase begins; slight acidification; initiation of enzymatic hydrolysis	Limited improvement in protein digestibility and nutrient availability	Minor increase in soluble phenolics and antioxidant activity	Bioactive enhancement generally still low	(He et al., 2023)
12–24 h	Increased	Initial reduction in	Increased total	Flavor	(Centeno &

	microbial growth and organic acid production	phytate and tannins; improved amino acid release	phenolic content and free radical scavenging activity	development may remain incomplete	Carballo, 2023)
24–48 h	Active proteolysis and carbohydrate metabolism	Significant improvement in protein digestibility and mineral bioavailability	Peak antioxidant activity frequently observed; increased bioactive peptide formation	Excess acidification may begin in LAB fermentation systems	(Grana et al., 2023)
48–72 h	Advanced enzymatic degradation and metabolic conversion	Further reduction of antinutritional compounds; accumulation of soluble peptides and amino acids	Elevated GABA, peptides, and phenolic derivatives depending on starter type	Risk of nutrient degradation and sensory deterioration increases	(Zhang et al., 2022)
>72 h (prolonged fermentation)	Over-fermentation and excessive metabolic activity may occur	Possible decline in nutritional quality due to oxidation or excessive proteolysis	Some antioxidant compounds may decrease because of prolonged exposure and degradation	Off-flavor formation, texture deterioration, lipid oxidation, contamination risk	(Qiao et al., 2022)

Nevertheless, the early fermentation phase remains critically important because it establishes the microbial and biochemical conditions necessary for subsequent nutrient transformation. The establishment of favorable pH conditions and microbial dominance determines the direction and efficiency of later fermentation stages.

Intermediate fermentation (24–72 h)

The intermediate stage of fermentation is generally considered the most metabolically active phase and is frequently associated with optimal nutritional and functional improvements in fermented peanut products. During this period, microbial populations reach high metabolic activity and secrete substantial quantities of hydrolytic enzymes including proteases, lipases, phytases, cellulases, and β -glucosidases (Zang et al., 2018).

Active proteolysis is one of the major biochemical events occurring during intermediate fermentation. Microbial proteases hydrolyze peanut storage proteins into peptides and free amino acids, thereby improving digestibility and increasing nitrogen availability (Yousefi et al., 2025). The accumulation of soluble peptides and amino acids may also contribute to flavor development and bioactive functionality. Several studies have reported increased concentrations of glutamic acid, leucine, valine, and other amino acids during this fermentation stage.

In addition to protein hydrolysis, antioxidant activity often increases substantially during intermediate fermentation. This enhancement is commonly associated with microbial degradation of plant cell wall matrices and liberation of bound phenolic compounds (P. Zhang et al., 2025). Enzymatic activities such as β -glucosidase and esterase facilitate the conversion of conjugated phenolics into more bioavailable and biologically active forms. Consequently, total phenolic content and radical scavenging activity frequently reach peak values during this period.

Intermediate fermentation may also promote the synthesis of secondary metabolites including γ -aminobutyric acid (GABA), bioactive peptides, and volatile flavor compounds depending on the microbial starter culture employed (Galli et al., 2021). The coordinated interaction between microbial growth and enzymatic activity during this phase often results in the most balanced improvement in nutritional quality, functional properties, and sensory characteristics.

Therefore, fermentation durations between approximately 24 and 72 h are frequently regarded as the optimal processing window for many peanut fermentation systems. However, the precise optimum duration remains highly dependent on microbial strain, fermentation conditions, and intended product characteristics.

Prolonged fermentation (>72 h)

Prolonged fermentation beyond 72 h may lead to excessive biochemical degradation and gradual deterioration of nutritional and sensory quality. Although microbial metabolism continues during this stage, beneficial effects observed during intermediate fermentation may decline because of over-hydrolysis, oxidative reactions, and accumulation of undesirable metabolites (Han et al., 2022).

One major concern associated with prolonged fermentation is nutrient degradation. Excessive proteolysis may result in overaccumulation of ammonia and degradation of valuable amino acids, thereby reducing nutritional efficiency and altering flavor profiles (H. Li et al., 2022). Similarly, prolonged microbial metabolism may degrade certain heat-sensitive or oxidation-sensitive bioactive compounds, leading to decreased antioxidant activity.

Off-flavor development is another common consequence of excessive fermentation. Extended proteolysis and lipid degradation may generate undesirable volatile compounds including ammonia, aldehydes, ketones, and short-chain fatty acids that negatively affect product acceptability (T. Wu et al., 2025). Alkaline fermentation systems involving *Bacillus* species are particularly susceptible to strong ammonia-like odors under prolonged fermentation conditions.

Over-acidification may also occur in LAB-dominated fermentation systems due to continuous organic acid accumulation. Excessively low pH conditions can negatively influence texture, palatability, and microbial stability while potentially inhibiting beneficial microbial activity (Atasoy et al., 2023). Furthermore, prolonged acidic environments may accelerate degradation of sensitive nutrients and pigments.

Lipid oxidation represents another important limitation associated with extended fermentation periods. Lipolytic activity and prolonged exposure to oxygen may increase the formation of lipid oxidation products, contributing to rancidity and reduced oxidative stability (Geng et al., 2023). Therefore, although extended fermentation may further reduce antinutritional compounds, excessive fermentation duration can compromise overall product quality and functional value.

PROTEIN DIGESTIBILITY CHANGES

Improvement in protein digestibility is one of the most significant nutritional benefits associated with peanut fermentation. During fermentation, microbial proteases hydrolyze complex storage proteins into smaller peptides and free amino acids that are more easily digested and absorbed in the gastrointestinal tract (Ter et al., 2023). Peptide formation occurs progressively throughout fermentation as microbial enzymes cleave peptide bonds within peanut proteins. This hydrolysis reduces protein molecular complexity and disrupts compact tertiary structures that normally limit enzymatic accessibility during digestion (Yao et al., 2026). The resulting peptides may possess various biological activities, including antioxidant, antihypertensive, antimicrobial, and immunomodulatory functions. Enzymatic hydrolysis during fermentation also contributes to the reduction of protease inhibitors such as trypsin inhibitors that interfere with digestive enzyme activity (Q. Wang et al., 2024). Degradation of these antinutritional compounds enhances the efficiency of gastrointestinal protein digestion and improves amino acid bioavailability. The extent of digestibility improvement is influenced by several factors including microbial starter type, proteolytic enzyme activity, fermentation duration, pH conditions, and substrate composition (Sharma et al., 2020). Fermentation systems involving *Bacillus subtilis* and filamentous fungi often exhibit particularly strong proteolytic capabilities because of extensive extracellular enzyme secretion. However, excessive proteolysis during prolonged fermentation may lead to nutrient losses and undesirable sensory changes. Therefore, optimization of fermentation conditions is essential to maximize digestibility improvement while maintaining product stability and acceptability.

Lipid transformation

Lipids constitute a major component of peanut kernels and undergo substantial biochemical transformation during fermentation. Microbial lipases hydrolyze triglycerides into free fatty acids and glycerol through lipolysis, thereby modifying the fatty acid profile and sensory characteristics of fermented peanut products (Chandra et al., 2020).

Controlled lipolysis may contribute positively to flavor development through the formation of volatile compounds including esters, aldehydes, ketones, and alcohols generated from fatty acid metabolism (Shahidi & Hossain, 2022). Certain microbial metabolites derived from lipid transformation may enhance aroma complexity and improve sensory quality in fermented products.

Fermentation may also alter the relative proportions of saturated and unsaturated fatty acids. Some microbial strains can selectively metabolize particular fatty acids or promote oxidative modification of unsaturated lipid fractions (Raclot & Oudart,

1999). These changes may influence oxidative stability, nutritional quality, and functional properties of fermented peanut products.

Despite potential benefits, excessive lipid degradation may increase the risk of rancidity and oxidative deterioration. Unsaturated fatty acids such as oleic and linoleic acids are particularly susceptible to oxidation during prolonged fermentation or inadequate storage conditions (Selvan et al., 2025). Lipid oxidation products may negatively affect flavor, nutritional value, and shelf life.

Therefore, the balance between beneficial lipid transformation and oxidative deterioration is strongly dependent on fermentation duration, oxygen availability, microbial activity, and environmental conditions. Appropriate fermentation control is necessary to maintain lipid stability while promoting desirable sensory and functional characteristics.

MINERAL BIOAVAILABILITY

Mineral bioavailability is significantly influenced by fermentation because microbial metabolism can reduce antinutritional compounds that interfere with mineral absorption. In peanut substrates, phytic acid represents the primary inhibitor of mineral bioavailability due to its strong chelating capacity toward divalent cations including iron (Fe), zinc (Zn), calcium (Ca), and magnesium (Mg) (Akond et al., 2011).

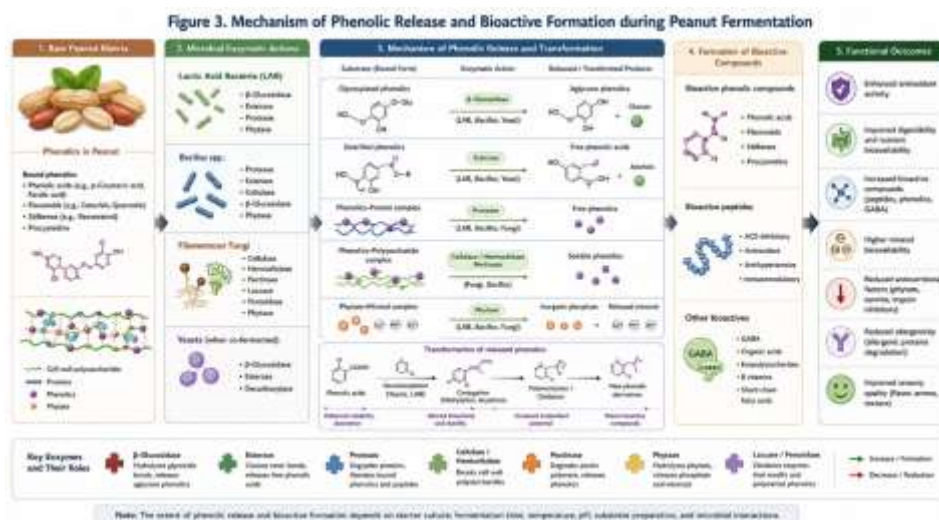
During fermentation, phytase-producing microorganisms hydrolyze phytate into lower-phosphorylated inositol derivatives and inorganic phosphate (Joudaki et al., 2023). This degradation weakens phytate-mineral interactions and increases the solubility and bioaccessibility of essential minerals. LAB, *Rhizopus*, and *Aspergillus* species are particularly recognized for their phytase activity and ability to reduce phytate concentrations during fermentation.

The reduction of phytate and related antinutritional compounds contributes to improved mineral absorption potential. Several studies have reported increased availability of iron, zinc, and calcium in fermented legume products compared with unfermented controls (Joudaki et al., 2023). Improved mineral bioavailability is particularly important in plant-based diets where micronutrient deficiencies remain common.

Fermentation-induced acidification may further enhance mineral solubility by lowering pH and promoting dissociation of mineral complexes (Proulx & Reddy, 2007). Organic acids generated during fermentation may also facilitate mineral absorption in the gastrointestinal tract.

However, the degree of mineral enhancement depends on fermentation duration, microbial strain specificity, initial phytate concentration, and processing conditions. Excessive fermentation may not necessarily provide additional mineral benefits and could potentially reduce product quality through over-acidification or nutrient degradation. Consequently, optimization of fermentation conditions remains essential for maximizing mineral bioavailability in fermented peanut products.

Effect of Starter Culture on Bioactive Compounds



Phenolic compounds

Phenolic compounds are among the most important bioactive constituents influenced by microbial fermentation in peanut-based substrates. The concentration, composition, and bioavailability of phenolics are strongly dependent on starter culture type because different microorganisms possess distinct enzymatic systems capable of modifying plant cell wall structures and releasing bound phytochemicals (P. Zhang et al., 2025).

Table 4 Comparative effects of different microbial starter cultures on nutritional quality and bioactive compounds in fermented peanut products

Starter culture	Main nutritional effects	Effects on bioactive compounds	Reduction of antinutritional factors	Sensory and functional characteristics	Major limitations	Representative references
Lactic acid bacteria (LAB)	Improved protein digestibility and amino acid availability through proteolysis	Increased total phenolics, antioxidant activity, and GABA production	Effective reduction of phytate and tannins via acidification and enzymatic activity	Produces mild acidic flavor and probiotic potential	Excessive acidification may reduce sensory acceptance during prolonged fermentation	(Z. He et al., 2023)
<i>Bacillus subtilis</i>	Strong proteolytic activity leading to high peptide and amino acid release	Promotes formation of bioactive peptides with antioxidant and ACE-inhibitory potential	Moderate degradation of phytate and protein inhibitors	Generates savory and alkaline flavor characteristics	Over-fermentation may produce ammonia-like odor and texture softening	(Peruzy et al., 2022)
<i>Rhizopus oligosporus</i>	Enhances protein digestibility and mineral availability through enzymatic hydrolysis	Increases free phenolic compounds and antioxidant activity	Significant phytate reduction due to phytase production	Produces compact texture similar to tempeh products	Requires controlled moisture and aeration conditions	(Grana et al., 2023b)
<i>Aspergillus oryzae</i>	Improves nutrient solubility and macromolecule degradation	Enhances phenolic liberation and enzymatic biotransformation	Effective degradation of phytate and complex carbohydrates	Produces desirable umami and fermented flavor notes	Potential contamination risk if fermentation is poorly controlled	(Peruzy et al., 2022)
Yeast (<i>Saccharomyces cerevisiae</i>)	Partial improvement in protein utilization and carbohydrate metabolism	Contributes to volatile bioactive metabolites and flavor compounds	Limited direct reduction of phytate compared with bacteria or fungi	Enhances aroma complexity and sensory acceptance	Lower proteolytic capacity compared with <i>Bacillus</i> or fungi	(Z. He et al., 2023)
Mixed-	Synergistic	Frequently	More efficient	Improved	Fermentation	(Peruzy et al.,

culture fermentation	enhancement of digestibility, soluble peptides, and nutrient transformation	produces the highest antioxidant activity and metabolite diversity	reduction of antinutritional compounds through combined enzymatic systems	flavor complexity and functional potential	control and reproducibility remain challenging	2022)
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In raw peanuts, a considerable proportion of phenolic compounds exists in bound forms associated with polysaccharides, proteins, and lignocellulosic matrices (De Camargo et al., 2017). These bound phenolics generally exhibit limited bioaccessibility because they are trapped within structural components of plant tissues. During fermentation, microbial enzymatic activity can disrupt these complexes and facilitate the liberation of soluble phenolic compounds.

Microbial enzymes such as β -glucosidase, cellulase, xylanase, esterase, and feruloyl esterase play important roles in phenolic release during fermentation (Azkia et al., 2024). LAB and filamentous fungi are particularly recognized for their ability to produce these enzymes and promote degradation of cell wall polysaccharides. Hydrolysis of glycosidic bonds may convert conjugated phenolics into aglycone forms with improved antioxidant activity and biological availability.

Different starter cultures may generate distinct phenolic profiles depending on their metabolic pathways and enzymatic specificity. LAB fermentation commonly increases total phenolic content through acidification-mediated matrix disruption and enzymatic hydrolysis, whereas fungal fermentation may induce more extensive degradation of structural polymers and release larger quantities of bound phenolics (Zhao et al., 2021). In addition, microbial metabolism may produce secondary phenolic derivatives with altered antioxidant potential and bioactivity.

The extent of phenolic enhancement is also influenced by fermentation duration, substrate composition, oxygen availability, and environmental conditions. Controlled fermentation systems capable of optimizing enzymatic hydrolysis while minimizing oxidative degradation are therefore important for maximizing phenolic retention and bioactive functionality in fermented peanut products.

Antioxidant activity

Antioxidant activity is one of the most frequently reported functional improvements associated with peanut fermentation. Changes in antioxidant capacity are strongly influenced by microbial starter culture because fermentation-induced biochemical transformations alter the concentration and composition of phenolics, peptides, vitamins, and other antioxidant compounds (Shao et al., 2025).

Several analytical assays are commonly used to evaluate antioxidant activity in fermented peanut products, including DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)), and FRAP (ferric reducing antioxidant power) assays. These methods assess different mechanisms of antioxidant action such as radical scavenging capacity, electron donation ability, and reducing potential (Shahidi & Zhong, 2015).

Fermentation generally increases antioxidant activity because microbial enzymes release bound phenolic compounds and generate bioactive peptides with radical scavenging properties. LAB fermentation is frequently associated with enhanced DPPH and ABTS radical scavenging activity due to increased soluble phenolic content and peptide formation (Shahidi & Zhong, 2015). Similarly, fungal fermentation involving *Rhizopus* or *Aspergillus* species may substantially improve FRAP values through extensive enzymatic degradation of structural matrices and liberation of antioxidant metabolites.

The relationship between fermentation duration and antioxidant activity is dynamic and non-linear. During early fermentation stages, antioxidant enhancement is often limited because microbial enzymatic activity remains relatively low (Saritaş et al., 2024). Antioxidant activity generally increases during intermediate fermentation periods when microbial growth and hydrolytic activity reach maximum levels. At this stage, liberation of phenolics, production of peptides, and synthesis of secondary metabolites contribute synergistically to enhanced antioxidant capacity.

However, prolonged fermentation may result in declining antioxidant activity because of oxidative degradation, over-metabolism of phenolic compounds, and instability of certain antioxidants under extended fermentation conditions. Therefore, optimization of fermentation duration is essential to maximize antioxidant potential while minimizing nutrient degradation.

BIOACTIVE PEPTIDES

Microbial fermentation can generate various bioactive peptides through enzymatic hydrolysis of peanut proteins. These peptides are typically encrypted within native protein sequences and become biologically active only after proteolytic cleavage by microbial enzymes (Cruz-Casas et al., 2021). The production and composition of bioactive peptides depend strongly on starter culture type, protease specificity, and fermentation duration.

One of the most extensively studied groups of bioactive peptides is angiotensin-converting enzyme (ACE) inhibitory peptides. These peptides may contribute to antihypertensive activity by inhibiting the conversion of angiotensin I into angiotensin II, a potent vasoconstrictor involved in blood pressure regulation (G. Li et al., 2004). Fermentation involving LAB and *Bacillus subtilis* has been reported to increase the generation of low-molecular-weight peptides exhibiting ACE inhibitory activity.

Antimicrobial peptides may also be produced during fermentation through microbial proteolysis. These peptides can inhibit the growth of spoilage microorganisms and pathogenic bacteria by disrupting cell membranes or interfering with microbial metabolic processes (Q. Zhang et al., 2021). Certain LAB strains are particularly recognized for their ability to produce bacteriocin-like compounds and antimicrobial metabolites during fermentation.

The formation of bioactive peptides is highly dependent on enzymatic hydrolysis conditions. Moderate fermentation periods often favor the accumulation of functional peptides, whereas excessive proteolysis during prolonged fermentation may further degrade peptides into free amino acids and reduce biological activity (Fabbri et al., 2024). Consequently, starter culture selection and fermentation optimization are critical factors influencing peptide functionality and nutritional quality.

In addition to antihypertensive and antimicrobial effects, fermented peanut peptides may exhibit antioxidant, anti-inflammatory, immunomodulatory, and metal-chelating activities depending on peptide sequence and amino acid composition. These multifunctional properties contribute to the growing interest in fermented peanut products as potential functional foods.

Reduction of antinutritional factors

One of the major advantages of microbial fermentation is its ability to reduce antinutritional compounds through enzymatic degradation and biochemical modification. The efficiency of antinutritional reduction depends largely on starter culture type because different microorganisms produce distinct hydrolytic enzymes capable of degrading phytate, tannins, oxalates, and enzyme inhibitors (Jeyakumar & Lawrence, 2021).

Phytase activity is particularly important in fermented peanut systems. Phytases hydrolyze phytic acid into lower-phosphorylated inositol derivatives and inorganic phosphate, thereby reducing mineral chelation and improving bioavailability of iron, zinc, calcium, and magnesium (Joudaki et al., 2023). LAB, *Rhizopus*, and *Aspergillus* species are frequently recognized for their phytase-producing capabilities.

Tannase activity also contributes significantly to nutritional improvement during fermentation. Tannases catalyze the hydrolysis of tannins into smaller phenolic compounds with lower protein-binding capacity (Mara et al., 2023). Reduction of tannin concentrations may improve protein digestibility, palatability, and mineral absorption while simultaneously increasing availability of antioxidant phenolics.

Fermentation may additionally reduce trypsin inhibitors and other enzyme inhibitors through microbial proteolytic degradation and denaturation processes (S. M. Singh et al., 2025). The decrease in inhibitor activity contributes to improved digestive efficiency and enhanced utilization of peanut proteins.

The extent of antinutritional reduction depends on fermentation duration, microbial population dynamics, environmental conditions, and substrate composition. Controlled fermentation systems employing phytase- and tannase-producing starter cultures are therefore considered promising strategies for improving the nutritional quality of peanut-derived foods.

Reduction in allergenicity

Reduction of peanut allergenicity through microbial fermentation has become an important research focus because peanut allergy remains a major public health concern worldwide. The allergenic properties of peanuts are mainly associated with storage proteins such as Ara h1, Ara h2, and Ara h3, which exhibit strong immunoglobulin E (IgE)-binding capacity and high structural stability (Pi et al., 2021).

Microbial fermentation may reduce allergenicity through enzymatic degradation of allergenic proteins and modification of immunoreactive epitopes. Proteolytic microorganisms including LAB, *Bacillus subtilis*, and filamentous fungi can hydrolyze Ara proteins into smaller peptide fragments with reduced IgE-binding affinity (Guzman et al., 2026). This peptide fragmentation alters protein structure and may disrupt conformational epitopes responsible for allergic reactions.

Protease-mediated degradation of Ara proteins generally increases with fermentation duration because prolonged enzymatic exposure promotes deeper hydrolysis of allergenic proteins (Yu et al., 2020). Several studies have reported partial degradation of Ara h1 and Ara h3 during LAB and fungal fermentation systems, whereas Ara h2 often demonstrates greater resistance because of its compact structure and disulfide bond stability.

In addition to direct proteolysis, fermentation-induced acidification may alter protein conformation and increase susceptibility to enzymatic hydrolysis (Yousefi et al., 2025). Changes in protein folding and solubility may further reduce allergenic potential by modifying epitope accessibility.

Despite these promising findings, complete elimination of peanut allergenicity has not yet been consistently achieved through fermentation alone. Residual allergenic fragments may still retain immunoreactivity depending on fermentation conditions and microbial strain specificity (Yousefi et al., 2025). Therefore, further studies integrating proteomics, immunological assays, and clinical evaluation remain necessary to optimize fermentation strategies for hypoallergenic peanut product development.

Interaction Between Fermentation Time and Starter Type

The interaction between fermentation duration and microbial starter culture represents one of the most critical determinants influencing the nutritional, biochemical, and functional quality of fermented peanut products. Different microorganisms exhibit distinct metabolic pathways, enzymatic systems, growth kinetics, and substrate utilization patterns, resulting in variable nutrient transformation dynamics over time (Yang et al., 2021). Consequently, fermentation outcomes cannot be interpreted solely on the basis of starter culture or fermentation duration independently, but rather through their integrated interaction.

Figure 4. Interaction Model between Starter Culture and Fermentation Duration in Shaping Nutritional Quality and Bioactive Compounds of Fermented Peanut Products



Table 5. Current research gaps and future research opportunities in peanut fermentation studies

Research area	Current limitations	Scientific implications	Future research opportunities	Representative references
Fermentation time optimization	Most studies evaluate limited fermentation periods and use inconsistent experimental designs	Difficult to determine universal optimum fermentation duration across starter cultures	Development of kinetic and predictive fermentation models under standardized conditions	(Qiao et al., 2022)
Comparative starter culture studies	Many reports focus on single microorganisms without direct comparison among LAB, fungi, and Bacillus systems	Limits mechanistic understanding of starter-dependent metabolic pathways	Comparative multi-starter experiments using identical peanut substrates and process conditions	(Grana et al., 2023)
Bioactive compound characterization	Bioactive peptides and metabolites remain insufficiently identified at molecular level	Functional mechanisms and health claims remain partially speculative	Application of metabolomics, peptidomics, and proteomics approaches	(Z. He et al., 2023)
Allergenicity reduction	Limited evidence regarding long-term effectiveness of fermentation in degrading Ara allergens	Industrial application for hypoallergenic peanut products remains uncertain	Controlled clinical and immunological studies evaluating allergenic protein degradation	(Z. He et al., 2023)
Functional food development	Most studies remain laboratory-scale and rarely proceed to commercial application	Limited industrial translation of fermented peanut products	Scale-up studies focusing on stability, sensory quality, and consumer acceptance	(Z. He et al., 2023)
Safety and quality control	Insufficient monitoring of aflatoxin risk and microbial contamination during fermentation	Safety concerns may hinder commercialization and regulatory approval	Integration of HACCP systems and microbial safety validation during industrial fermentation	(Centeno & Carballo, 2023)
Mixed-culture fermentation systems	Synergistic microbial interactions remain poorly understood	Difficult to standardize product quality and metabolic outcomes	Systems biology approaches to evaluate microbial interactions and succession dynamics	(Centeno & Carballo, 2023)
Omics-based fermentation analysis	Genomic and metabolomic integration in peanut fermentation remains limited	Restricts mechanistic understanding of nutrient biotransformation	Multi-omics integration combining metagenomics, metabolomics, and transcriptomics	(Centeno & Carballo, 2023)
Artificial intelligence and precision fermentation	AI-assisted optimization is rarely applied in peanut fermentation systems	Fermentation efficiency and reproducibility remain variable	Application of machine learning for predicting fermentation outcomes and process optimization	(Qiao et al., 2022)
Underexplored peanut cultivars	Most studies use a limited number of commercial cultivars	Potential cultivar-dependent nutritional responses remain unknown	Evaluation of indigenous and tropical peanut varieties under controlled fermentation	(Qiao et al., 2022)

Microbial activity during fermentation evolves dynamically as environmental conditions, nutrient availability, pH, oxygen concentration, and metabolite accumulation continuously change throughout the process. The timing of enzymatic activation and substrate hydrolysis therefore differs substantially among LAB, *Bacillus*, fungal, and mixed-culture systems. Understanding these temporal-metabolic interactions is essential for optimizing functional quality while minimizing nutrient degradation and sensory deterioration.

COMPARATIVE METABOLIC RESPONSES

Different starter cultures induce distinct metabolic responses during peanut fermentation because of differences in enzymatic capacity, ecological adaptation, and metabolic end-products. LAB-dominated fermentation systems primarily emphasize carbohydrate metabolism and acidification, whereas *Bacillus* fermentation is characterized by extensive proteolysis and alkaline metabolite production (Grana et al., 2023). In contrast, filamentous fungi exhibit strong extracellular enzymatic activities capable of degrading complex structural polymers and releasing bound nutrients.

LAB fermentation commonly produces rapid pH reduction during the early and intermediate stages of fermentation through lactic acid production. Acidification enhances microbial stability and facilitates moderate proteolysis, phenolic release, and GABA synthesis (Aguirre-Garcia et al., 2024). LAB-associated metabolism generally favors production of soluble peptides, organic acids, and antioxidant metabolites under relatively short fermentation periods.

By comparison, *Bacillus subtilis* fermentation demonstrates stronger proteolytic activity and more extensive peptide generation because of secretion of extracellular alkaline proteases (Sun et al., 2023). This system often produces higher concentrations of free amino acids and bioactive peptides compared with LAB fermentation. However, prolonged alkaline fermentation may also increase ammonia formation and undesirable flavor development.

Fungal fermentation involving *Rhizopus* or *Aspergillus* species typically exhibits broader enzymatic diversity, including cellulases, hemicellulases, phytases, and β -glucosidases (El-Gendi et al., 2021). These enzymes facilitate degradation of structural polysaccharides and release of bound phenolics, thereby substantially enhancing antioxidant activity and mineral bioavailability. Fungal systems are therefore often more effective in modifying complex substrate matrices than purely bacterial fermentations.

The temporal dynamics of these metabolic responses differ considerably among microbial groups. LAB generally achieve peak acidification and moderate bioactive production earlier than *Bacillus* or fungal systems, whereas fungal fermentation often requires longer incubation periods to achieve maximum enzymatic hydrolysis and phenolic liberation.

Optimal fermentation windows

Each microbial starter culture possesses a distinct optimal fermentation window determined by growth kinetics, enzymatic activity, metabolite accumulation, and substrate transformation efficiency. Identification of these optimal fermentation periods is essential for maximizing nutritional enhancement while preventing excessive degradation or sensory deterioration.

LAB fermentation systems commonly achieve optimal functional and nutritional improvements within approximately 24–48 h of fermentation (Sharma et al., 2020). During this period, LAB populations generally reach high metabolic activity, resulting in efficient acidification, moderate proteolysis, increased antioxidant activity, and enhanced GABA production. Beyond this period, excessive acid accumulation may reduce sensory acceptability and contribute to nutrient instability.

In contrast, *Bacillus subtilis*-based fermentation often requires longer incubation periods, typically around 48–72 h, to achieve maximum proteolytic hydrolysis and peptide formation (Sharma et al., 2020). During intermediate and late fermentation stages, extracellular proteases secreted by *Bacillus* species actively degrade storage proteins into bioactive peptides and amino acids. However, prolonged fermentation beyond the optimal period may promote excessive ammonia production and undesirable alkaline odors.

Fungal fermentation systems may exhibit variable optimal durations depending on fungal species, substrate moisture, aeration, and enzymatic activity. *Rhizopus*-mediated fermentation often demonstrates substantial nutrient transformation and phytate degradation within 48–72 h, whereas certain *Aspergillus* systems may require longer incubation to maximize phenolic liberation and enzymatic hydrolysis (Sharma et al., 2020).

The existence of distinct fermentation windows highlights the importance of tailoring fermentation duration according to starter culture characteristics and desired product functionality. Failure to optimize fermentation time may reduce bioactive compound formation and increase the risk of nutrient degradation or quality deterioration.

Synergistic effects in mixed cultures

Mixed-culture fermentation systems may generate synergistic interactions that enhance nutrient transformation and bioactive compound production beyond the capabilities of single-culture fermentation. These synergistic effects arise from complementary metabolic activities among different microorganisms and sequential substrate utilization during fermentation (Feng et al., 2026).

Sequential metabolism represents one of the most important mechanisms underlying mixed-culture synergy. In many systems, LAB initiate fermentation through rapid carbohydrate metabolism and acidification, thereby creating environmental conditions favorable for subsequent microbial activity (A. Wang et al., 2026). Acidification may suppress undesirable microorganisms while increasing substrate accessibility for secondary fermentative organisms such as fungi or *Bacillus* species.

Subsequent microbial populations may then perform additional proteolysis, phenolic liberation, phytate degradation, and flavor compound synthesis. For example, fungal cellulases and β -glucosidases may release bound phenolics, while *Bacillus* proteases generate bioactive peptides and amino acids (A. Wang et al., 2026). The combination of these activities can substantially improve antioxidant activity, digestibility, mineral bioavailability, and sensory complexity.

Mixed-culture systems may also enhance microbial stability and fermentation efficiency through cooperative interactions. Certain microorganisms may produce metabolites or growth factors that stimulate enzymatic activity of companion cultures (W. Liu et al., 2025). Such interactions may accelerate substrate conversion and broaden the diversity of functional metabolites produced during fermentation.

However, synergistic benefits are highly dependent on inoculum ratio, fermentation sequence, environmental conditions, and microbial compatibility. Uncontrolled competition among microorganisms may reduce process reproducibility and compromise product quality. Therefore, optimization and standardization of mixed-culture systems remain important research priorities in functional peanut fermentation.

Trade-offs during prolonged fermentation

Although extended fermentation may further increase enzymatic hydrolysis and antinutritional reduction, prolonged fermentation frequently introduces important trade-offs affecting nutritional quality, functional stability, and sensory acceptability.

One major trade-off involves nutrient loss associated with excessive microbial metabolism. Prolonged proteolysis may lead to degradation of valuable peptides and amino acids into ammonia and secondary metabolites with lower nutritional value (Wallace, 1996). Similarly, extended fermentation may reduce concentrations of oxidation-sensitive compounds such as tocopherols and certain phenolics.

Sensory decline is another common consequence of excessive fermentation. Overproduction of organic acids, ammonia, aldehydes, ketones, and volatile fatty acids may negatively affect flavor, aroma, and texture (Van Wyk, 2024). LAB systems may become excessively acidic, whereas alkaline fermentation systems involving *Bacillus* species may develop strong ammonia-like odors under prolonged fermentation conditions.

Oxidation risk also increases during extended fermentation periods, particularly in lipid-rich substrates such as peanuts. Unsaturated fatty acids may undergo oxidative deterioration because of prolonged oxygen exposure and microbial lipolytic activity (Geng et al., 2023). Lipid oxidation products contribute to rancidity, reduced shelf life, and decreased nutritional quality.

Additionally, prolonged fermentation may alter microbial ecosystem stability and increase susceptibility to contamination if process control becomes inadequate (Z. Yao et al., 2025). Therefore, although longer fermentation periods may enhance certain functional properties, these benefits must be balanced against the risks of nutrient degradation, sensory deterioration, and oxidative instability.

Proposed mechanistic framework

Based on current evidence, a mechanistic framework can be proposed to explain the interaction between starter culture type, fermentation duration, nutrient transformation, and bioactive formation in fermented peanut systems.

In this framework, starter culture type serves as the primary determinant governing enzymatic activity profiles during fermentation. Different microorganisms produce distinct hydrolytic enzymes including proteases, phytases, lipases, cellulases, tannases, and β -glucosidases (Cevallos-Fernández et al., 2026). These enzymes initiate substrate-specific biochemical transformations affecting proteins, lipids, carbohydrates, phenolics, and antinutritional compounds.

Fermentation duration subsequently regulates the extent and progression of these enzymatic reactions. Early fermentation stages primarily involve microbial adaptation and limited hydrolysis, whereas intermediate stages correspond to peak enzymatic activity and maximum nutrient transformation (Sharma et al., 2020). Prolonged fermentation may lead to over-hydrolysis, oxidative degradation, and accumulation of undesirable metabolites.

The interaction between enzymatic activity and fermentation time ultimately determines the degree of nutrient transformation and bioactive formation. Proteolysis generates peptides and amino acids, phytase activity improves mineral bioavailability, β -glucosidase releases phenolic compounds, and glutamate decarboxylase promotes GABA synthesis (Hu et al., 2024). These biochemical modifications collectively influence digestibility, antioxidant activity, allergenicity, sensory quality, and functional properties of fermented peanut products.

Therefore, optimization of fermented peanut systems requires integrated consideration of microbial starter selection, enzymatic functionality, and fermentation duration to maximize beneficial transformations while minimizing adverse effects. Future research combining metabolomics, proteomics, microbial ecology, and systems biology approaches may further refine this mechanistic framework and improve precision fermentation strategies for functional peanut food development.

Applications in Functional Food Development

The increasing global demand for sustainable, plant-based, and health-oriented foods has accelerated the development of fermented peanut products as functional food alternatives. Fermentation not only improves the nutritional quality and digestibility of peanuts but also enhances bioactive compound formation, sensory characteristics, and microbial stability (Sawant et al., 2025). Consequently, fermented peanut-based products have gained growing attention in both academic research and industrial food innovation.

The combination of high protein content, desirable lipid composition, and fermentation-induced functional metabolites makes peanuts a promising substrate for developing novel functional foods. Fermented peanut products may serve as alternatives to dairy-based foods while simultaneously providing antioxidant compounds, bioactive peptides, probiotics, and improved mineral bioavailability (Ali et al., 2021).

Fermented peanut beverages

Fermented peanut beverages have emerged as promising plant-based alternatives to conventional dairy drinks because of their favorable nutritional composition and lactose-free characteristics. These beverages are generally produced by extracting peanut slurry or milk-like emulsions followed by microbial fermentation using LAB, yeasts, or mixed cultures (Bulca & Büyükgümüş, 2024).

Fermentation substantially modifies the physicochemical and sensory properties of peanut beverages. LAB-mediated acidification improves microbial stability and contributes to characteristic fermented flavors, while enzymatic hydrolysis may reduce beany off-flavors commonly associated with plant-based beverages (Lee & Beuchat, 1991). In addition, fermentation may improve protein digestibility and increase concentrations of soluble peptides, amino acids, and phenolic compounds.

Several studies have reported enhanced antioxidant activity and increased GABA production in fermented peanut beverages inoculated with selected LAB strains (Hu et al., 2024). Fermentation may also improve emulsion stability and viscosity through microbial exopolysaccharide production, thereby enhancing mouthfeel and sensory acceptance.

Despite these advantages, formulation challenges remain important because peanut beverages possess distinct lipid compositions and protein structures compared with dairy milk. Optimization of starter cultures, fermentation duration,

stabilizers, and homogenization processes is therefore necessary to improve texture, flavor stability, and consumer acceptability.

PEANUT YOGURT ALTERNATIVES

Peanut-based yogurt alternatives represent one of the fastest-growing categories of fermented plant-based foods. These products are designed to mimic the texture, acidity, and sensory characteristics of conventional yogurt while providing lactose-free and vegan-friendly nutritional options (Bulca & Büyükgümüş, 2024).

Production of peanut yogurt alternatives typically involves fermentation of peanut milk using LAB such as *Lactobacillus*, *Lactiplantibacillus*, *Streptococcus thermophilus*, and *Pediococcus* species. During fermentation, microbial acidification promotes protein coagulation and gel formation, contributing to yogurt-like texture development (Bulca & Büyükgümüş, 2024).

Fermentation may significantly improve the functional properties of peanut yogurt alternatives through increased antioxidant activity, phenolic release, peptide formation, and GABA accumulation. Certain LAB strains also produce exopolysaccharides that improve viscosity, creaminess, and water-holding capacity (Saritaş et al., 2024). These microbial metabolites may partially compensate for the absence of dairy proteins and fats typically responsible for yogurt texture.

The nutritional value of peanut yogurt alternatives is often enhanced compared with unfermented peanut beverages because fermentation reduces phytate and enzyme inhibitor concentrations while improving digestibility and mineral bioavailability (Zhai et al., 2024). Furthermore, incorporation of probiotic cultures may provide additional health-promoting benefits.

However, sensory optimization remains a major challenge because peanut-derived products may exhibit flavor profiles and textural properties that differ substantially from conventional dairy yogurt. Balancing acidity, aroma, viscosity, and stability therefore remains critical for successful product development and market acceptance.

Fermented peanut paste

Fermented peanut paste is another promising application of peanut fermentation technology and is commonly produced through solid-state or semi-solid fermentation using LAB, *Bacillus*, or fungal cultures (Pi et al., 2021). Fermentation modifies the physicochemical properties of peanut paste and contributes to development of unique flavors, enhanced digestibility, and improved functional characteristics.

Proteolytic fermentation systems involving *Bacillus subtilis* are particularly effective in producing fermented peanut pastes with high concentrations of peptides and amino acids (S. Liu et al., 2024). These compounds contribute to savory flavor formation and may exhibit antioxidant or antihypertensive activities. Fungal fermentation systems may further enhance phenolic release and reduce antinutritional compounds through extensive extracellular enzymatic degradation.

Fermented peanut paste may also serve as a carrier for probiotics, bioactive peptides, and natural antioxidants. The semi-solid structure of peanut paste may provide protective effects for microbial survival and improve stability during storage. Nevertheless, prolonged fermentation may negatively affect texture and promote excessive softening, oil separation, or off-flavor development. Optimization of fermentation duration and starter culture selection is therefore essential to maintain product consistency and sensory quality.

Probiotic peanut products

Fermented peanut products have increasingly been explored as non-dairy probiotic carriers because peanuts provide proteins, lipids, and carbohydrates capable of supporting probiotic microbial growth and viability (Saizen et al., 2023). This application is particularly important for lactose-intolerant, vegan, and dairy-sensitive populations seeking alternative probiotic foods.

LAB strains such as *Lactiplantibacillus plantarum*, *Lactobacillus acidophilus*, and *Pediococcus pentosaceus* are commonly incorporated into peanut fermentation systems to produce probiotic beverages, yogurts, and fermented spreads (Doo et al., 2024). These microorganisms may contribute to gastrointestinal health, immune modulation, antimicrobial activity, and improved gut microbiota balance.

Fermentation may also improve probiotic stability through acidification and metabolite production that suppress spoilage microorganisms. Peanut proteins and lipids may provide protective matrices that enhance probiotic survival during storage and gastrointestinal transit (Voidarou et al., 2020).

In addition to probiotic viability, fermented peanut products may contain prebiotic components, peptides, and phenolic metabolites that interact synergistically with probiotic microorganisms. This combination may support the development of synbiotic food systems with enhanced functional properties.

However, maintaining adequate probiotic viability during processing and storage remains challenging because factors such as oxygen exposure, low pH, and lipid oxidation may reduce microbial survival. Therefore, strain selection, encapsulation technologies, and optimized fermentation conditions remain important areas of ongoing research.

Plant-based functional foods

The rapid expansion of plant-based food markets has increased interest in fermented peanut products as sustainable functional food ingredients and alternatives to animal-derived foods. Fermented peanuts provide a combination of high-quality plant proteins, unsaturated fatty acids, antioxidant compounds, and microbial metabolites that align with current consumer demand for health-oriented and environmentally sustainable products (Arya et al., 2015).

Fermentation may improve the nutritional and technological functionality of peanuts in various food applications including meat analogues, protein spreads, snack products, fermented condiments, and nutritional supplements. Enhanced digestibility, reduced antinutritional compounds, and improved bioactive content increase the value of fermented peanuts within plant-based dietary systems (Arya et al., 2015).

Moreover, fermentation may help address some of the sensory limitations commonly associated with plant-based products, including bitterness, beany flavors, and poor texture. Microbial metabolism can generate desirable volatile compounds, modify protein functionality, and improve mouthfeel characteristics (Arya et al., 2015).

The integration of fermentation technology with plant-based food innovation also supports sustainability objectives because peanuts possess lower environmental footprints compared with many animal-derived protein sources. Fermented peanut products may therefore contribute to the development of sustainable protein systems and functional food diversification.

Future research should focus on improving industrial scalability, sensory optimization, shelf stability, and clinical validation of health benefits associated with fermented peanut functional foods. Advanced approaches involving precision fermentation, metabolomics, and microbial engineering may further expand the commercial potential of fermented peanut-based products in global food systems.

Safety, Quality, and Industrial Challenges

Despite the growing potential of fermented peanut products as functional foods, several safety, quality, and industrial challenges remain important barriers to large-scale commercialization. These challenges include mycotoxin contamination, biogenic amine formation, microbial safety concerns, shelf-life instability, process standardization difficulties, and regulatory limitations. Effective management of these issues is essential to ensure product safety, consumer acceptance, and industrial feasibility. Because peanuts are highly susceptible to fungal contamination and lipid oxidation, fermentation systems require strict control of raw material quality, microbial inoculation, environmental conditions, and post-fermentation handling. Inadequate process control may compromise not only nutritional quality but also microbiological and toxicological safety.

Aflatoxin risk

Aflatoxin contamination remains one of the most serious safety concerns associated with peanuts and peanut-derived products. Aflatoxins are toxic secondary metabolites primarily produced by *Aspergillus flavus* and *Aspergillus parasiticus*, which commonly infect peanuts during cultivation, drying, storage, and processing (Yohannis et al., 2025). Among the various aflatoxin types, aflatoxin B1 is considered the most toxic because of its strong hepatotoxic, mutagenic, and carcinogenic properties.

Environmental factors such as high humidity, elevated temperature, insect damage, and poor storage conditions substantially increase the risk of fungal proliferation and aflatoxin formation in peanuts (Y. Yang et al., 2025). Fermentation alone does not necessarily eliminate aflatoxins completely, although certain microbial strains may partially degrade or adsorb these toxins during fermentation processes.

Several LAB and fungal strains have demonstrated potential aflatoxin-binding or detoxification capabilities through cell wall interactions, enzymatic degradation, or metabolite-mediated transformation (Aasa et al., 2026). However, the effectiveness of these mechanisms varies considerably depending on strain specificity, fermentation conditions, toxin concentration, and substrate composition.

Strict control of raw material quality therefore remains the primary strategy for minimizing aflatoxin risk in fermented peanut products. Implementation of good agricultural practices (GAP), appropriate drying procedures, hygienic storage systems, and regular toxin monitoring is essential for ensuring product safety.

Biogenic amines

Biogenic amines are nitrogen-containing compounds produced mainly through microbial decarboxylation of amino acids during fermentation. Common biogenic amines detected in fermented foods include histamine, tyramine, putrescine, cadaverine, and phenylethylamine (Spano et al., 2010). Excessive accumulation of these compounds may pose health risks including headaches, hypertension, allergic reactions, and gastrointestinal disturbances.

In peanut fermentation systems, biogenic amine formation is influenced by microbial strain composition, amino acid availability, pH, temperature, and fermentation duration (L. Liu et al., 2025). Proteolytic fermentation systems with high free amino acid concentrations may provide favorable substrates for decarboxylase-positive microorganisms capable of producing biogenic amines.

Certain LAB and *Bacillus* strains possess amino acid decarboxylase activity and may contribute to amine accumulation during prolonged fermentation. Inadequate fermentation control and contamination by undesirable microorganisms further increase this risk.

Selection of starter cultures lacking decarboxylase genes is therefore important for minimizing biogenic amine production. Additionally, optimization of fermentation duration, temperature control, sanitation, and rapid acidification may help suppress amine-producing microorganisms and improve product safety.

Microbial contamination

Microbial contamination remains a major challenge in fermented peanut production because peanuts provide nutrient-rich environments favorable for growth of spoilage microorganisms and opportunistic pathogens. Contamination may occur during raw material handling, soaking, grinding, fermentation, packaging, or storage processes (Sharma et al., 2020).

Potential contaminants include pathogenic bacteria such as *Salmonella*, *Escherichia coli*, *Staphylococcus aureus*, and toxin-producing fungi. Poor hygiene practices, inadequate equipment sanitation, and uncontrolled environmental conditions significantly increase contamination risks (Zavišić et al., 2024). Fermentation itself may improve microbial safety through acidification, competitive exclusion, bacteriocin production, and reduction of water activity. LAB fermentation is particularly effective in suppressing pathogenic microorganisms because of rapid organic acid production and antimicrobial metabolite formation (Bangar et al., 2022). Nevertheless, fermentation cannot fully compensate for poor raw material quality or inadequate sanitary conditions. Industrial-scale production therefore requires implementation of strict hygiene management systems including hazard analysis and critical control point (HACCP) protocols, microbial monitoring, sanitation procedures, and starter culture quality assurance. Controlled fermentation systems using standardized starter cultures generally provide higher microbiological safety and product consistency than spontaneous fermentation approaches.

Shelf-life issues

Shelf-life stability is another critical challenge in fermented peanut products because peanuts contain high concentrations of unsaturated fatty acids that are susceptible to oxidative deterioration. Lipid oxidation may result in rancidity, off-flavor formation, nutrient loss, and reduced consumer acceptability during storage (K. Liu et al., 2019).

Fermentation may influence shelf-life both positively and negatively depending on process conditions and microbial activity. Organic acids, bacteriocins, and antimicrobial metabolites produced during fermentation may improve microbial stability and inhibit spoilage organisms (Rathod et al., 2022). However, excessive lipolysis and prolonged oxygen exposure may accelerate oxidative degradation of lipids.

Texture instability, phase separation, viscosity changes, and microbial viability decline may also occur during storage of fermented peanut beverages and yogurt-like products (Terpilowski et al., 2023). These issues are particularly important in probiotic products where maintenance of viable microbial populations is necessary to preserve functional claims.

Packaging systems, oxygen control, refrigeration, antioxidant incorporation, and moisture management therefore play essential roles in maintaining product quality during storage. Future studies should prioritize shelf-life optimization and stability assessment under industrial distribution conditions.

Fermentation standardization

One of the major industrial limitations in fermented peanut production is the difficulty of achieving consistent fermentation performance and product quality. Fermentation outcomes are strongly influenced by microbial strain variability, inoculum concentration, substrate composition, temperature, pH, aeration, moisture content, and fermentation duration. Spontaneous fermentation systems often exhibit substantial batch-to-batch variation because microbial communities are uncontrolled and dynamically influenced by environmental factors. This variability may affect nutritional composition, bioactive formation, flavor development, safety, and shelf stability (Yee et al., 2025).

Standardization of fermentation processes requires controlled starter cultures with predictable metabolic properties and reproducible enzymatic activities. Optimization of fermentation parameters through process engineering, microbial selection, and real-time monitoring may improve consistency and industrial scalability.

Advanced approaches such as precision fermentation, metabolomics-guided optimization, artificial intelligence-assisted process control, and systems biology may further improve fermentation reproducibility and product standardization. However, industrial implementation of these technologies remains limited because of cost, technical complexity, and infrastructure requirements.

Research Gaps and Future Perspectives

Although substantial progress has been achieved in understanding peanut fermentation and its effects on nutritional and functional quality, several scientific and technological gaps remain unresolved. Most current studies are still conducted at laboratory scale and primarily focus on isolated biochemical parameters or specific microbial strains (Guzman et al., 2026). Comprehensive mechanistic understanding integrating microbial ecology, metabolite dynamics, process engineering, and clinical functionality remains limited.

Future research should therefore move beyond descriptive nutritional analyses toward systems-level investigations capable of explaining the complex interactions among microbial metabolism, fermentation duration, substrate composition, and bioactive compound formation. Advances in omics technologies, computational modeling, and microbial engineering provide promising opportunities for accelerating innovation in fermented peanut functional foods.

Omics-based fermentation studies

Application of omics technologies represents one of the most promising approaches for advancing mechanistic understanding of peanut fermentation systems. Current fermentation studies often rely on conventional physicochemical analyses that provide limited information regarding metabolic pathways, microbial interactions, and molecular transformations occurring during fermentation (Rocha & Silva, 2023).

Metabolomics may provide comprehensive profiling of metabolites generated throughout fermentation, including amino acids, peptides, organic acids, phenolics, volatile compounds, and secondary metabolites. This approach can improve understanding of how different starter cultures and fermentation durations influence metabolic trajectories and functional compound formation (Ji et al., 2025). Dynamic metabolite mapping may also facilitate identification of biomarkers associated with optimal fermentation stages and product quality.

Proteomics offers another valuable tool for investigating protein hydrolysis, peptide formation, allergen degradation, and enzymatic activity during fermentation. Proteomic analysis may help identify specific bioactive peptides and characterize structural modifications of allergenic proteins such as Ara h1, Ara h2, and Ara h3 (Rocha & Silva, 2023). Such information is important for developing hypoallergenic and functionally enhanced fermented peanut products.

Metagenomics can further improve understanding of microbial community dynamics and ecological interactions during spontaneous and mixed-culture fermentation. High-throughput sequencing approaches may reveal microbial succession patterns, functional gene expression, and metabolic capabilities associated with nutrient transformation and fermentation stability (Ji et al., 2025).

Integration of metabolomics, proteomics, metagenomics, and transcriptomics may therefore provide a more comprehensive systems-level understanding of peanut fermentation and support development of precision fermentation strategies.

PRECISION FERMENTATION

Precision fermentation represents an emerging frontier in food biotechnology and may substantially improve efficiency, reproducibility, and functional optimization of fermented peanut products. Traditional fermentation systems frequently exhibit variability because microbial activity and nutrient transformation are influenced by dynamic environmental and biological factors (Siddiqui et al., 2023).

Artificial intelligence (AI) and machine learning approaches may help optimize fermentation parameters including temperature, pH, inoculum ratio, aeration, moisture, and fermentation duration. Predictive algorithms trained on fermentation datasets could identify optimal process conditions for maximizing antioxidant activity, peptide formation, GABA production, or sensory quality (Z. Wang et al., 2025; Hernandez-Tenorio et al., 2025).

Predictive fermentation models may also facilitate real-time monitoring and process control during industrial production. Integration of biosensors, automated fermentation systems, and computational analysis may improve consistency and reduce risks associated with contamination, over-fermentation, and nutrient degradation (Ma et al., 2025).

Furthermore, digital fermentation platforms integrating metabolomic and microbial data may enable more accurate prediction of fermentation outcomes under different starter culture combinations and substrate conditions. Such technologies may significantly accelerate development of customized functional peanut products with targeted nutritional profiles.

Despite these opportunities, application of precision fermentation in peanut systems remains limited and requires multidisciplinary collaboration among microbiologists, food technologists, computational scientists, and process engineers.

Engineering starter cultures

Engineering and selection of specialized starter cultures provide another promising strategy for improving fermented peanut functionality and process efficiency. Conventional fermentation systems often rely on naturally occurring strains with variable metabolic performance and limited control over metabolite production (Obayomi et al., 2026).

Targeted starter culture engineering may enhance production of desirable compounds such as antioxidant peptides, GABA, phenolic metabolites, vitamins, and exopolysaccharides. Microbial strains could potentially be optimized for specific enzymatic activities including protease, phytase, tannase, β -glucosidase, and glutamate decarboxylase production (Hernandez-Tenorio et al., 2025). Selective breeding, adaptive evolution, and metabolic engineering approaches may also improve microbial tolerance to acidic environments, oxidative stress, and substrate variability. Such modifications may enhance fermentation stability, shorten processing time, and increase functional metabolite yield. In addition, engineered microbial consortia may improve synergistic metabolism in mixed-culture systems. Controlled microbial interactions could facilitate sequential nutrient transformation and broader bioactive compound diversity. However, regulatory acceptance and consumer perception of engineered microorganisms remain important challenges. Safety evaluation, genetic stability assessment, and regulatory compliance will therefore be essential considerations in future starter culture development.

Underexplored peanut varieties

Most existing fermentation studies utilize a limited number of commercial peanut cultivars, despite the substantial genetic and biochemical diversity present among global peanut varieties. Variations in protein composition, lipid profile, phenolic content, mineral concentration, and antinutritional compounds may significantly influence fermentation behavior and functional outcomes (Yousefi et al., 2025).

Local and tropical peanut cultivars remain particularly underexplored in fermentation research. Many indigenous varieties cultivated in tropical regions possess unique nutritional characteristics and phytochemical profiles that may affect microbial metabolism and bioactive formation during fermentation (Yousefi et al., 2025).

Differences in seed structure, phenolic localization, and fatty acid composition may alter enzymatic accessibility, fermentation kinetics, and antioxidant responses. Certain local cultivars may also exhibit greater suitability for probiotic fermentation or enhanced release of bioactive compounds.

Evaluation of cultivar-dependent fermentation responses may therefore support development of region-specific functional foods and improve utilization of local agricultural biodiversity. Such approaches may also contribute to sustainable food systems and value-added utilization of underutilized peanut germplasm. Future studies should integrate agronomic, nutritional, metabolomic, and fermentation analyses to identify peanut cultivars with superior functional fermentation potential.

Clinical validation

Although numerous in vitro and laboratory-scale studies have demonstrated nutritional and functional improvements in fermented peanut products, clinical validation remains extremely limited. Most current evidence regarding antioxidant activity, antihypertensive effects, probiotic functionality, and allergenicity reduction is based primarily on biochemical assays, cell culture studies, or animal experiments (Sampsel et al., 2025).

Human clinical studies are necessary to verify the physiological relevance and bioavailability of fermentation-derived bioactive compounds. Important aspects requiring validation include gastrointestinal digestion, peptide absorption, microbiota modulation, immune response, metabolic effects, and long-term safety (Bruce et al., 2025).

Clinical evaluation is particularly important for assessing potential reductions in peanut allergenicity following fermentation. Although several studies have reported structural degradation of allergenic proteins, direct evidence demonstrating reduced allergic responses in humans remains insufficient.

Similarly, probiotic claims associated with fermented peanut products require validation through controlled intervention studies evaluating microbial survival, colonization, and health outcomes. Without adequate clinical evidence, many functional claims remain speculative and difficult to translate into regulatory approval or commercial application. Future research should therefore prioritize well-designed human studies integrating nutritional biomarkers, metabolomics, microbiome analysis, and immunological assessment to strengthen scientific evidence supporting fermented peanut functional foods.

CONCLUSION

Fermentation represents a highly promising biotechnological approach for improving the nutritional quality, functional properties, and bioactive potential of peanut-based foods. The present review demonstrates that both starter culture selection and fermentation duration play central roles in determining the final biochemical and nutritional characteristics of fermented peanut products.

Different microbial starters exhibit distinct metabolic and enzymatic profiles that influence protein hydrolysis, phenolic liberation, peptide formation, mineral bioavailability, antioxidant activity, and allergen degradation. LAB-based systems generally favor rapid acidification, moderate proteolysis, and GABA production, whereas *Bacillus* fermentation promotes extensive peptide generation and alkaline metabolism. Filamentous fungi contribute broader enzymatic degradation and enhanced release of bound phenolic compounds.

The effects of fermentation duration are highly dynamic and non-linear. Early fermentation stages primarily involve microbial adaptation and limited hydrolysis, while intermediate fermentation periods commonly correspond with optimal nutritional enhancement and bioactive formation. In contrast, prolonged fermentation may lead to nutrient degradation, excessive acidification or alkalization, lipid oxidation, off-flavor development, and reduced sensory quality.

Importantly, the interaction between starter culture type and fermentation time determines the balance between beneficial biochemical transformation and undesirable quality deterioration. Controlled fermentation systems using selected starter cultures therefore provide greater potential for achieving reproducible functional properties, improved safety, and optimized nutritional outcomes compared with spontaneous fermentation approaches.

Despite substantial progress, several important research gaps remain, including limited omics-based mechanistic studies, insufficient clinical validation, lack of fermentation standardization, and underutilization of diverse peanut cultivars. Future integration of precision fermentation, systems biology, artificial intelligence, and engineered starter cultures may further accelerate development of next-generation fermented peanut functional foods. Overall, fermented peanut products represent a promising category of sustainable plant-based functional foods with significant potential for future nutritional innovation, industrial application, and health-oriented food development.

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