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INTEGRATIVE ASSESSMENT OF NUTRITIONAL PROFILE, BIOACTIVE POTENTIAL, STRUCTURAL INTEGRITY, MICROBIAL VIABILITY, AND SENSORY QUALITY IN FLAXSEED-SUPPLEMENTED SUFU

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Abstract
The growing demand for functional, plant-based foods has renewed interest in fermented soy products such as Sufu*,* valued for their enhanced digestibility and nutraceutical potential. This study examined the effect of flaxseed (*Linum usitatissimum* L.) supplementation (0%, 5%, 10%, and 15%) on the nutritional profile, bioactive potential, structural attributes, microbial viability, and sensory quality of Sufu. Flaxseed-enriched formulations were prepared through traditional mold and lactic acid bacteria fermentation, followed by brining and aging. Flaxseed addition significantly improved the nutritional and bioactive characteristics. Enzymatic activity of protease and lipase increased notably in flaxseed-supplemented treatments, indicating improved protein and lipid metabolism during fermentation. Enhanced viability of lactic acid bacteria confirmed the probiotic potential of the product. Sensory evaluation identified the 10% flaxseed treatment (T2) as the most acceptable, combining favorable flavor, aroma, and texture. Overall, flaxseed supplementation particularly at 10–15% enhanced the structural stability, functional value, and sensory attributes of Sufu, supporting its development as a health-oriented, plant-based fermented food aligned with modern dietary and sustainability trends.
Keywords: Enhanced digestibility, Nutraceutical, Bioactive potential, Antioxidant activity, Functional food, Health-oriented, Sustainability trends

INTRODUCTION

Fermented foods have long been integral to traditional diets across cultures due to their enhanced nutritional value, digestibility, and sensory appeal compared to unfermented foods. Among these, Sufu also referred to as fermented tofu or Chinese cheese is a traditional Chinese soybean product that has gained recognition for its unique flavor, smooth cheese-like texture, and health-promoting effects (Xie & Gänzle, 2023). Fermentation improves the bioavailability of nutrients, increases the presence of health-promoting metabolites, and enhances the overall flavor profile of Sufu (Mao et al., 2023). Although Sufu originated in East Asia, it is now gaining global attention as a plant-based functional food (Rizzo, 2024). With growing consumer interest in functional and sustainable foods, incorporating plant-based ingredients such as flaxseed (*Linum usitatissimum* L.) rich in α-linolenic acid, lignans, dietary fiber, and plant proteins has emerged as a promising approach to improve the nutritional and health-promoting potential of traditional fermented soy products (Mueed et al., 2023). Integrating flaxseed into Sufu formulations presents an innovative strategy for developing enhanced functional fermented foods. While both Sufu and flaxseed have been individually studied for their nutritional and technological attributes, limited research has examined their combined effects (Liu et al., 2022). Flaxseed supplementation has the potential to improve Sufu's biochemical composition, structure, and functional properties, aligning with the growing global demand for clean-label, sustainable, and health-oriented foods (Shahada et al., 2024). Fermentation plays a critical role in enhancing the nutritional, functional, and sensory characteristics of soy-based foods (An et al., 2023). It reduces anti-nutritional factors such as phytic acid and trypsin inhibitors, thereby improving nutrient bioavailability (Di et al., 2024). Additionally, microbial metabolism during fermentation produces esters, alcohols, and short-chain fatty acids that enhance flavor complexity and consumer acceptance (Zhang et al., 2023). Fermentation also stimulates the generation of bioactive compounds, including antioxidant, antihypertensive, and immunomodulatory peptides (Singh & Negi, 2025), while increasing the availability of isoflavone aglycones with potential cancer-preventive activity (Alshehri et al., 2021). Furthermore, fermented foods contribute to gut microbiota balance, promoting gastrointestinal and metabolic health (Sawant et al., 2025; Qin et al., 2022). In recent years, the incorporation of functional ingredients into fermented foods has become a major focus of food innovation. Functional foods are those that provide health benefits beyond basic nutrition, often due to the presence of bioactive compounds such as antioxidants, omega-3 fatty acids, and phytochemicals (Agrawal et al., 2023). Plant-based ingredients like flaxseed have gained prominence due to their high content of α-linolenic acid (ALA), dietary fiber, protein, and lignans (Mueed et al., 2022; Mueed et al., 2023). These compounds are associated with reduced inflammation, improved cardiovascular function, and better metabolic regulation. Technologically, flaxseed mucilage and lipids improve product texture, moisture retention, and oxidative stability (Zhang et al., 2023). The integration of flaxseed into fermented soy products enhances their nutritional and functional properties (Cai et al., 2021), aligning with the shift toward sustainable and plant-based diets. Flaxseed's nutritional components—including omega-3 fatty acids, lignans, fiber, and proteins—have been associated with disease prevention and overall health improvement (Saidaiah et al., 2024). ALA, an essential fatty acid that must be obtained from the diet, has been shown to support heart health and reduce inflammation (Yuan et al., 2022). Lignans, particularly secoisolariciresinol diglucoside (SDG), act as phytoestrogens and antioxidants with potential protective effects against hormone-related cancers (Noreen et al., 2024). Dietary fiber from flaxseed helps lower cholesterol, regulate blood glucose, and promote gut health (Balaramnavar, 2021), while its high-quality plant protein contributes to tissue repair and enzymatic activity (Imran et al., 2024). The combined nutritional, structural, and bioactive attributes make flaxseed an effective functional ingredient for fortifying Sufu (Dzuvor et al., 2018). Recent studies have explored flaxseed incorporation into Sufu to create innovative functional foods that deliver enhanced nutritional, structural, and sensory qualities (Ali et al., 2024). The synergistic interaction between soy and flaxseed provides a balanced profile of proteins, essential fatty acids, and lignans, thereby improving antioxidant activity, structural integrity, and mouthfeel (Haque et al., 2024; Zhang et al., 2023). However, despite the individual health benefits of flaxseed and fermented soy products, research on their combined effects remains limited, indicating a gap in existing literature. Therefore, this study was conducted to evaluate the influence of flaxseed supplementation on the nutritional profile, bioactive potential, structural attributes, microbial viability, and sensory quality of Sufu. The work aimed to optimize fermentation conditions, analyze microbial dynamics and nutritional quality, assess biochemical composition and texture, and determine antimicrobial activity against selected pathogenic microorganisms. The findings contribute to a deeper understanding of flaxseed's role in improving the functional and nutritional properties of Sufu, supporting its development as a health-oriented, plant-based fermented food.

2. MATERIALS & METHODS

2.1 Materials and Reagents

Raw soybeans were locally sourced from Faisalabad, Pakistan, and manually cleaned to ensure uniform quality. Food-grade flaxseeds were obtained from a certified supplier and stored in airtight containers at room temperature. All chemicals and reagents, including solvents, acids, and analytical standards, were of analytical grade and purchased from Sigma-Aldrich.

2.2. Treatment Plan

Flaxseed was incorporated into Sufu at varying levels to examine its influence on the product's nutritional, structural, and sensory characteristics. Four formulations were prepared, each containing 100 g of Sufu with different proportions of flaxseed supplementation. The control sample (T0) contained no flaxseed, while the experimental treatments included 5% (T1), 10% (T2), and 15% (T3) flaxseed. This treatment plan provided a systematic basis for evaluating the progressive impact of flaxseed addition on the overall quality attributes of Sufu.

2.3. Preparation of Soybeans and Tofu for Sufu Production

Soybean seeds were cleaned, soaked in distilled water for 8–12 hours at 25–28 °C to enhance hydration, soften texture, and reduce anti-nutritional factors such as phytic acid and oligosaccharides, thereby improving digestibility and extraction efficiency (Kashif et al., 2025). The soaked beans were drained, rinsed, and ground with water (1:2 w/v) into a homogeneous slurry, which was cooked at 100 °C to inactivate trypsin inhibitors and denature proteins for better coagulation and microbial safety. The hot slurry was filtered through muslin cloth to separate soy milk from okara, and the milk was reheated (75–85 °C) and coagulated using magnesium chloride or calcium sulfate to form curds. These curds were molded and pressed under 0.3–0.5 MPa pressure for 15–30 minutes to achieve the desired firmness

(Kashif et al., 2025). The resulting tofu was cut into 2–3 cm cubes and placed on sterile bamboo mats for initial fermentation (pehtze preparation), ensuring uniform air circulation and microbial growth essential for Sufu development.

2.4. Fermentation and Aging of Flaxseed-Supplemented Sufu

Tofu cubes were inoculated with *Actinomucor elegans* or *Mucor racemosus*, fungi commonly used in sufu fermentation for their strong proteolytic and lipolytic activities (Xie et al., 2023). The cubes were incubated at 28–40 °C and 75–95% relative humidity, while ground flaxseed (5%, 10%, and 15%) was evenly sprinkled on the surface as a prebiotic and functional additive to enhance nutritional quality and microbial activity (Mueed et al., 2022). The primary fermentation lasted 3–5 days, during which mold mycelium developed, softening the texture and initiating protein and fat breakdown (Fang et al., 2024). Afterward, the cubes were lightly salted to draw out moisture and stabilize microbial growth before being transferred into sterilized jars containing a 10–15% salt brine. The brine, prepared by boiling and cooling distilled water with salt, ensured microbial safety and complete dissolution. Secondary fermentation was carried out at 10–15 °C for 1–3 months with periodic agitation to promote uniform maturation and flavor development (Zhao et al., 2016). Finally, the fermented cubes were aged for 3–6 months in either the same brine or a flavored medium (rice wine, chili, or sesame oil) to enhance umami taste and aroma, yielding a creamy-textured, fully matured Sufu (Cai et al., 2024).

2.5. Analytical Procedures

2.5.1. Proximate Analysis for Macronutrients

The proximate composition of Sufu samples was determined to evaluate nutritional variations resulting from flaxseed supplementation, following standard methods of the Association of Official Analytical Chemists (AACC, 2000). All analyses were performed in triplicate to ensure reproducibility. Moisture content was measured by oven-drying 5 g of homogenized sample at 105±2 °C until constant weight, calculated as Moisture (%) = ((Wi – Wf)/Wi) × 100, where Wi and Wf denote the initial and final sample weights. Ash content was determined by incinerating 3–5 g of dried sample in a muffle furnace at 550±25 °C for 4–6 hours and computed as Ash (%) = (Wa/Ws) × 100, with Wa representing ash weight and Ws the initial dry weight. Crude protein was analyzed using the Kjeldahl method, with total nitrogen multiplied by the factor 6.25 (Protein (%) = N × 6.25). Crude fat was quantified by Soxhlet extraction of 2–3 g dried sample using petroleum ether or hexane for 6–8 hours, calculated as Fat (%) = (Wf/Ws) × 100. Total carbohydrate was estimated by difference as Carbohydrate (%) = 100 – (M + A + P + F), where M, A, P, and F represent percentages of moisture, ash, protein, and fat, respectively. These proximate analyses provided insight into the nutritional alterations of Sufu caused by flaxseed enrichment and microbial fermentation across treatments (T0, T1, T2, T3).

2.5.2. Analysis of Micronutrients and Building Blocks of Macronutrients

The amino acid composition of Sufu samples was analyzed to assess the effect of fermentation and flaxseed supplementation on nutritional quality using High-Performance Liquid Chromatography (HPLC) and Ion-Exchange Chromatography (IEC) (Rutherfurd & Gilani, 2009; Bütikofer et al., 2007). Samples were hydrolyzed with 6N HCl at 110 °C for 22–24 hours under vacuum, with performic acid oxidation for sulfur-containing amino acids. Hydrolysates were neutralized, derivatized with OPA or FMOC-Cl, and analyzed on a C18 column using UV or fluorescence detection, while IEC used post-column ninhydrin derivatization at 570 nm and 440 nm. Essential and non-essential amino acids such as alanine, arginine, aspartic acid, cysteine, glutamic acid, glycine, proline, serine, and tyrosine were quantified (μmol/g dry weight) in triplicate for each treatment (T_0–T_3).

Fatty acid composition was determined to examine the effect of flaxseed addition on lipid enrichment, particularly α-linolenic acid (ALA, C18:3), using Gas Chromatography (GC) (Toishimanov et al., 2023). Lipids were extracted via Soxhlet using petroleum ether or hexane and converted to fatty acid methyl esters (FAMEs) through transesterification with methanolic KOH or BF_3-MeOH. FAMEs were analyzed using a capillary column (SP-2560 or BPX-70) with a flame ionization detector (FID) and helium or nitrogen as carrier gas. Fatty acids including palmitic (C16:0), stearic (C18:0), oleic (C18:1), linoleic (C18:2), and α-linolenic (C18:3) were identified and quantified using standard FAME mixtures from certified suppliers such as Sigma-Aldrich.

Vitamin content, including both water-soluble (B-complex, C) and fat-soluble (A, E, K) vitamins, was analyzed using HPLC and Atomic Absorption Spectroscopy (AAS) (Katsa et al., 2021). Water-soluble vitamins (B_1, B_2, B_3, B_6, B_9, C) were extracted via enzymatic or acid digestion, while fat-soluble vitamins (A, E, K) were extracted with hexane or ethanol after saponification. Separation was achieved on a C18 column with gradient elution of methanol, acetonitrile, and water, and detection used UV-Vis (254–280 nm) or fluorescence. Quantification was based on calibration with analytical-grade standards and expressed as mg/100 g dry weight. AAS was used to measure metal cofactors such as cobalt in vitamin B_{12} after wet digestion with HNO_3/H_2SO_4.

Mineral composition was evaluated following AOAC (2019) methods using AAS. About 0.5 g of dried Sufu was digested with nitric and perchloric acids, filtered, and diluted before analysis by Flame AAS (FAAS) or Graphite Furnace AAS (GFAAS). Essential minerals including calcium (Ca), iron (Fe), magnesium (Mg), zinc (Zn), potassium (K), sodium (Na), phosphorus (P), selenium (Se), and manganese (Mn) were quantified against calibration standards at specific wavelengths (e.g., Fe 248.3 nm, Mg 285.2 nm, Zn 213.9 nm) and expressed as mg/100 g dry weight.

2.5.3. Enzymatic Activity

The antimicrobial potential of Sufu samples was evaluated to determine their ability to inhibit foodborne pathogens and to assess the influence of fermentation and flaxseed supplementation. The study focused on *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella* spp., using agar disc diffusion and minimum bactericidal concentration (MBC) assays (Wong et al., 2023).

The agar disc diffusion method was performed following Hossain et al. (2022) and AOAC (2019). Aqueous and ethanolic Sufu extracts were tested against standardized bacterial suspensions (0.5 McFarland, ~1.5×10^8 CFU/mL) spread on Mueller-Hinton Agar plates. Sterile discs (6 mm) impregnated with 50 µL of extract were placed on the inoculated surfaces and incubated at 37°C for 18–24 hours. Zones of inhibition were measured in millimeters using a digital caliper. Positive (ampicillin or chloramphenicol) and negative (solvent-only) controls were included. Larger inhibition zones indicated stronger antimicrobial activity, providing qualitative evidence of the inhibitory potential of Sufu-derived compounds.

For quantitative evaluation, MBC values were determined using the broth microdilution method followed by plating (Wiegand et al., 2008; AOAC, 2019). Serial dilutions of Sufu extracts (0.5–64 mg/mL) were prepared in Mueller-Hinton Broth and inoculated with standardized bacterial suspensions (1×10^6 CFU/mL). After incubation at 37°C for 24 hours, samples from non-turbid wells were subcultured on nutrient agar. The MBC was recorded as the lowest concentration showing no bacterial colonies, indicating ≥99.9% kill rate.

2.5.4. Antimicrobial Activity

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2.5.5. Texture Profile Analysis (TPA)

Texture Profile Analysis (TPA) was performed to evaluate the textural properties of Sufu specifically hardness, cohesiveness, springiness, and chewiness using a texture analyzer (TA.XT Plus, Stable Micro Systems, UK) equipped with a 36 mm cylindrical probe. Uniform 20 mm cubes of Sufu were equilibrated to room temperature (25±1 °C) before analysis to ensure consistency (Ofosu et al., 2021). In TPA mode, samples underwent a double compression cycle (50% deformation) with 1.0 mm/s test speed and a 5 g trigger force to simulate mastication. Hardness was defined as the peak force during the first compression, cohesiveness as the ratio of the second to the first compression curve area, springiness as the recovery distance between compressions, and chewiness as the product of hardness, cohesiveness, and springiness (Pons & Fiszman, 1996).

2.5.6. Sensory Analysis

Data was expressed as mean ± standard deviation (Bower, 2000). Statistical comparisons were conducted using ANOVA at a 5% significance level ($P \leq 0.05$), and differences among treatment means were determined through Tukey's HSD post hoc test. Regression analysis was applied to examine correlations between key parameters. All statistical analyses were performed using Microsoft Excel and Minitab Statistical Software (version 20 or later, Minitab LLC, USA).

2.5.7. Statistical Analysis

The data obtained from compositional, functional, and structural analyses were presented as mean value±SD (Bower, 2000). Statistical analysis was performed at a significance level of $P \leq 0.05$, and significant differences between treatment means were identified using Tukey's HSD post hoc test. Regression analysis was also conducted to evaluate relationships between key variables. All statistical evaluations were carried out using Microsoft Excel and Minitab Statistical Software (version 20 or later, Minitab LLC, USA).

3. RESULTS

3.1. Proximate Composition

Analysis of variance revealed that flaxseed supplementation significantly influenced the proximate composition of Sufu. All parameters moisture, ash, protein, fat, and carbohydrates showed statistically significant differences among treatments. Increasing flaxseed concentration (5%, 10%, and 15%) led to a decrease in moisture (from 61.88±3.32 to 56.23±1.13) and carbohydrates (from 7.06±0.3 to 1.93±0.15), while ash (4.41±0.04 to 5.26±0.13), protein (17.69±1.06 to 19.67±0.34), and fat (12.81±1.00 to 17.92±0.28) contents increased progressively with higher flaxseed inclusion. The results are depicted in Table-1.

Table-1: Comparison of Means±SD for Proximate Composition of Sufu Samples Supplemented with Flaxseed (mg/100g dry weight)

Parameter	T0	T1	T2	T3
Moisture	61.88 ± 3.32^{a}	58.36 ± 0.99^{ab}	57.68 ± 0.69^{ab}	56.23 ± 1.13^{b}
Ash	4.41 ± 0.04^{c}	4.76 ± 0.15^{bc}	5.07 ± 0.22^{ab}	5.26 ± 0.13^{a}
Protein	17.69 ± 1.06^{b}	18.74 ± 0.51^{ab}	19.33 ± 0.44^{ab}	19.67 ± 0.34^{a}
Fat	12.81 ± 1.00^{c}	15.20 ± 0.59^{b}	16.13 ± 0.15^{b}	17.92 ± 0.28^{a}
Carbohydrate	7.06 ± 0.30^{a}	5.56 ± 0.59^{b}	3.57 ± 0.22^{c}	1.93 ± 0.15^{d}

3.2. Analysis of Micronutrients and Building Blocks of Macronutrients

Flaxseed supplementation significantly enhanced the nutritional composition of Sufu across all micronutrient and macronutrient building block categories. Essential amino acids, including histidine, leucine, lysine, methionine, and valine, increased progressively with higher flaxseed levels (5–15%), indicating improved protein quality. Non-essential amino acids such as alanine, glutamic acid, glycine, and tyrosine also rose, suggesting enhanced proteolysis and amino acid liberation during fermentation. The results of essential and non-essential amino acids are presented in Table-2.

Table-2: Comparison of Means±SD for Amino Acid Composition of Sufu Supplemented with Flaxseed (µmol/g dry weight)

Amino Acid	T0	T1	T2	T3
Histidine	2.55 ± 3.32^{c}	2.75 ± 0.99^{b}	2.84 ± 0.69^{ab}	2.97 ± 1.13^{a}
Isoleucine	5.19 ± 0.07^{c}	5.51 ± 0.07^{b}	5.67 ± 0.06^{ab}	5.81 ± 0.07^{a}
Leucine	7.73 ± 0.07^{d}	8.09 ± 0.08^{c}	8.36 ± 0.09^{b}	8.61 ± 0.04^{a}
Lysine	6.13 ± 0.09^{c}	6.34 ± 0.07^{b}	6.65 ± 0.07^{a}	6.81 ± 0.07^{a}
Methionine	1.56 ± 0.07^{c}	1.66 ± 0.05^{c}	1.82 ± 0.06^{b}	2.00 ± 0.07^{a}
Phenylalanine	2.55 ± 3.32^{c}	2.75 ± 0.99^{b}	2.84 ± 0.69^{ab}	2.97 ± 1.13^{a}
Threonine	5.19 ± 0.07^{c}	5.51 ± 0.07^{b}	5.67 ± 0.06^{ab}	5.81 ± 0.07^{a}
Tryptophan	7.73 ± 0.07^{d}	8.09 ± 0.08^{c}	8.36 ± 0.09^{b}	8.61 ± 0.04^{a}
Valine	6.13 ± 0.09^{c}	6.34 ± 0.07^{b}	6.65 ± 0.07^{a}	6.81 ± 0.07^{a}
Alanine	7.36 ± 0.06^{d}	7.59 ± 0.07^{c}	7.80 ± 0.06^{b}	8.05 ± 0.09^{a}
Arginine	6.25 ± 0.10^{c}	6.46 ± 0.11^{c}	6.74 ± 0.06^{b}	7.03 ± 0.14^{a}
Aspartic Acid	9.66 ± 0.08^{d}	10.04 ± 0.08^{c}	10.34 ± 0.09^{b}	10.62 ± 0.05^{a}
Cysteine	1.66 ± 0.08^{c}	1.78 ± 0.06^{bc}	1.92 ± 0.06^{ab}	2.04 ± 0.06^{a}
Glutamic Acid	12.49 ± 0.10^{d}	12.81 ± 0.08^{c}	13.24 ± 0.06^{b}	13.45 ± 0.09^{a}
Glycine	6.17 ± 0.51^{b}	6.39 ± 0.53^{ab}	7.00 ± 0.06^{ab}	7.22 ± 0.06^{a}
Proline	5.83 ± 0.09^{c}	6.09 ± 0.08^{b}	6.32 ± 0.10^{ab}	6.50 ± 0.08^{a}
Serine	5.23 ± 0.10^{c}	5.45 ± 0.08^{b}	5.69 ± 0.08^{a}	5.84 ± 0.06^{a}
Tyrosine	3.05 ± 0.10^{c}	3.17 ± 0.09^{bc}	3.33 ± 0.09^{ab}	3.47 ± 0.12^{a}

The fatty acid profile showed marked increases in both saturated (palmitic and stearic acids) and unsaturated fatty acids, particularly linoleic and α-linolenic acids, confirming enrichment with polyunsaturated fatty acids. Vitamin concentrations (A, C, E, K, and B-complex) improved significantly with higher flaxseed inclusion, while mineral content including calcium, iron, magnesium, zinc, potassium, phosphorus, and selenium also increased notably, reflecting enhanced nutrient density and bioavailability. The results are depicted in the Table-3.
The results (Table-4) revealed that flaxseed supplementation significantly increased the vitamin composition of sufu. Concentrations of both fat-soluble (A, E, K) and water-soluble (B-complex, C) vitamins rose with increasing flaxseed levels (5–15%). Vitamin A (retinol) increased from 0.37±0.06 (T0) to 0.56±0.02 μmol/g, vitamin C from 1.11±0.04 to 1.54±0.05 μmol/g, and vitamin E from 0.91±0.05 to 1.63±0.04 μmol/g. Similarly, vitamin K increased from 0.25±0.03 to 0.38±0.04 μmol/g, and thiamine (B1) from 0.34±0.04 to 0.50±0.06 μmol/g. Among other B-vitamins, riboflavin (B2), niacin (B3), pyridoxine (B6), and folate (B9) also showed progressive rises from T0 to T3 treatments.

Table-3: Vitamin and Fatty Acid Composition of Flaxseed-Supplemented Sufu (Mean ± SD, μmol/g dry weight)

Parameter	**T0**	**T1**	**T2**	**T3**
Palmitic Acid	142.36 ± 5.46^{c}	150.40 ± 2.38^{bc}	156.88 ± 2.59^{ab}	160.93 ± 1.40^{a}
Stearic Acid	76.42 ± 1.80^{b}	80.81 ± 1.55^{a}	82.11 ± 1.07^{a}	84.43 ± 1.29^{a}
Oleic Acid	159.66 ± 2.79^{d}	170.46 ± 2.50^{c}	178.16 ± 1.85^{b}	185.62 ± 1.69^{a}
Linoleic Acid	187.54 ± 1.46^{d}	203.65 ± 1.72^{c}	219.68 ± 2.03^{b}	233.65 ± 2.93^{a}
α-Linolenic Acid	67.11 ± 2.13^{d}	109.90 ± 2.75^{c}	154.12 ± 2.47^{b}	199.24 ± 2.39^{a}
Arachidonic Acid	17.52 ± 0.97^{c}	19.71 ± 0.47^{b}	20.65 ± 0.85^{ab}	22.05 ± 0.74^{a}
Vitamin A (Retinol)	0.37 ± 0.06^{c}	0.45 ± 0.04^{bc}	0.49 ± 0.04^{ab}	0.56 ± 0.02^{a}
Vitamin C (Ascorbic Acid)	1.11 ± 0.04^{d}	1.24 ± 0.05^{c}	1.38 ± 0.05^{b}	1.54 ± 0.05^{a}
Vitamin E (α-Tocopherol)	0.91 ± 0.05^{d}	1.21 ± 0.04^{c}	1.41 ± 0.03^{b}	1.63 ± 0.04^{a}
Vitamin K	0.25 ± 0.03^{b}	0.29 ± 0.03^{ab}	0.36 ± 0.04^{a}	0.38 ± 0.04^{a}
Vitamin B1 (Thiamine)	0.34 ± 0.04^{b}	0.40 ± 0.03^{ab}	0.46 ± 0.05^{ab}	0.50 ± 0.06^{a}
Vitamin B2 (Riboflavin)	0.30 ± 0.03^{b}	0.36 ± 0.03^{ab}	0.39 ± 0.04^{ab}	0.44 ± 0.05^{a}
Vitamin B3 (Niacin)	0.91 ± 0.06^{c}	1.00 ± 0.05^{bc}	1.12 ± 0.04^{ab}	1.20 ± 0.04^{a}
Vitamin B6	0.38 ± 0.03^{c}	0.43 ± 0.02^{bc}	0.49 ± 0.03^{ab}	0.53 ± 0.05^{a}
Folate (Vitamin B9)	0.34 ± 0.03^{b}	0.39 ± 0.04^{ab}	0.46 ± 0.06^{a}	0.49 ± 0.06^{a}

The results presented in Table-4 show that flaxseed supplementation led to a clear increase in the mineral composition of Sufu. All key macro- and micro-minerals, including calcium, iron, magnesium, zinc, potassium, phosphorus, sodium, selenium, and manganese, increased progressively with higher flaxseed levels.

Table-4: Comparison of Means±SD for Mineral Composition of Sufu Supplemented with Flaxseed (mg/100g dry weight)

Minerals	**T0**	**T1**	**T2**	**T3**
Calcium	135.14 ± 2.75^{c}	142.64 ± 2.77^{bc}	149.35 ± 3.29^{b}	158.72 ± 2.82^{a}
Iron	5.33 ± 0.11^{d}	5.83 ± 0.18^{c}	6.55 ± 0.11^{b}	7.09 ± 0.08^{a}
Magnesium	82.12 ± 2.38^{d}	93.20 ± 1.95^{c}	103.01 ± 1.61^{b}	111.01 ± 1.58^{a}
Zinc	2.76 ± 0.08^{d}	3.18 ± 0.08^{c}	3.62 ± 0.06^{b}	4.07 ± 0.11^{a}
Potassium	202.59 ± 0.82^{d}	218.57 ± 1.20^{c}	234.11 ± 2.63^{b}	250.12 ± 1.70^{a}
Phosphorus	184.54 ± 0.79^{d}	195.47 ± 0.57^{c}	208.48 ± 0.88^{b}	220.33 ± 1.07^{a}
Sodium	135.40 ± 1.37^{c}	137.61±0.80b^{c}	139.55 ± 0.82^{ab}	141.30 ± 0.88^{a}

Minerals	T0	T1	T2	T3
Selenium	0.79±0.07[c]	0.91±0.05b[c]	0.97±0.03[ab]	1.08±0.03[a]
Manganese	1.54±0.08[c]	1.71±0.04[b]	1.89±0.06[a]	2.01±0.05[a]

As shown in Table-5, flaxseed supplementation notably increased the enzymatic activity of Sufu, particularly in protease and lipase levels. Both enzymes exhibited a progressive rise with increasing flaxseed concentration, indicating that flaxseed enhanced overall enzymatic dynamics during fermentation.

3.3. Enzymatic Activity

Flaxseed supplementation notably enhanced enzymatic activity in Sufu, particularly protease and lipase activities. Protease activity increased from 21.67±1.15 µmol/min/g in the control (T_0) to 34.56±0.93 µmol/min/g in T_3, while lipase activity rose from 18.6±1.18 µmol/min/g to 29.79±1.01 µmol/min/g. The increase followed a dose-dependent pattern, with higher flaxseed levels leading to greater enzymatic response. These results suggest that flaxseed not only improved the nutritional composition of Sufu but also enhanced enzymatic processes during fermentation, contributing to improved bioactivity and product quality.

3.4. Microbial Load

Flaxseed supplementation significantly reduced the microbial load in Sufu samples. Increasing flaxseed levels led to a consistent decline in *Escherichia coli*, *Staphylococcus aureus*, *Salmonella spp.*, and total viable bacteria. *E. coli* counts decreased from 1.88±0.13 $\log_{10}$ CFU/g in T_0 to 0.95±0.07 $\log_{10}$ CFU/g in T3, *S. aureus* from 1.96±0.08 to 0.88±0.12, *Salmonella spp.* from 1.57±0.11 to 0.85±0.10, and total viable bacteria from 2.13±0.11 to 1.17±0.11 $\log_{10}$ CFU/g. These reductions indicate that flaxseed addition improved microbial safety while maintaining product quality.

3.5. Texture Profile Analysis (TPA)

Flaxseed supplementation significantly affected the texture profile of Sufu. Hardness increased from 12.76±0.30 g in T0 to 18.24±1.04 g in T3, while chewiness rose from 43.08±2.11 g-mm to 67.99±2.13 g-mm. Cohesiveness (0.48±0.01 to 0.54±0.01) and springiness (6.97±0.31 mm to 7.01±0.19 mm) showed no significant differences. These results indicate that flaxseed addition strengthened the structure of Sufu, particularly improving firmness and chewiness without negatively affecting elasticity or cohesiveness.

Table-5: Comparison of Means±SD for Enzymatic, Microbial, and Textural Properties of Sufu Samples

Parameters	T0	T1	T2	T3
Protease (µmol/min/g)	21.67 ± 1.15[d]	26.57 ± 1.22[c]	30.59 ± 1.12[b]	34.56 ± 0.93[a]
Lipase (µmol/min/g)	18.60 ± 1.18[d]	21.69 ± 0.92[c]	25.61 ± 1.01[b]	29.79 ± 1.01[a]
Escherichia coli ($\log_{10}$ CFU/g)	1.88 ± 0.13[a]	1.53 ± 0.09[b]	1.27 ± 0.11[c]	0.95 ± 0.07[d]
Staphylococcus aureus ($\log_{10}$ CFU/g)	1.96 ± 0.08[a]	1.56 ± 0.07[b]	1.17 ± 0.08[c]	0.88 ± 0.12[d]
Salmonella spp. ($\log_{10}$ CFU/g)	1.57 ± 0.11[a]	1.34 ± 0.08[ab]	1.14 ± 0.09[b]	0.85 ± 0.10[c]
Total Viable Bacteria ($\log_{10}$ CFU/g)	2.13 ± 0.11[a]	1.78 ± 0.11[b]	1.43 ± 0.10[c]	1.17 ± 0.11[d]
Hardness (g)	12.76 ± 0.30[d]	14.66 ± 0.87[b]	15.89 ± 0.26[b]	18.24±1.04[a]
Cohesiveness (Ratio)	0.48 ± 0.01[a]	0.50 ± 0.04[a]	0.54 ± 0.01[a]	0.49±0.04[a]
Springiness (mm)	6.97 ± 0.31[a]	7.13 ± 0.18[a]	7.51 ± 0.31[a]	7.01±0.19[a]
Chewiness (g-mm)	43.08 ± 2.11[c]	52.27 ± 1.23[b]	68.50 ± 4.15[a]	67.99±2.13[a]

3.6. Sensory Evaluation

The sensory evaluation results, depicted in Figure 1, showed that flaxseed supplementation had a significant influence on all sensory parameters of Sufu, including color, flavor, taste, texture, and overall acceptability. The 10% flaxseed formulation (T2) received the highest scores for all attributes, making it the most preferred treatment among the tested levels.

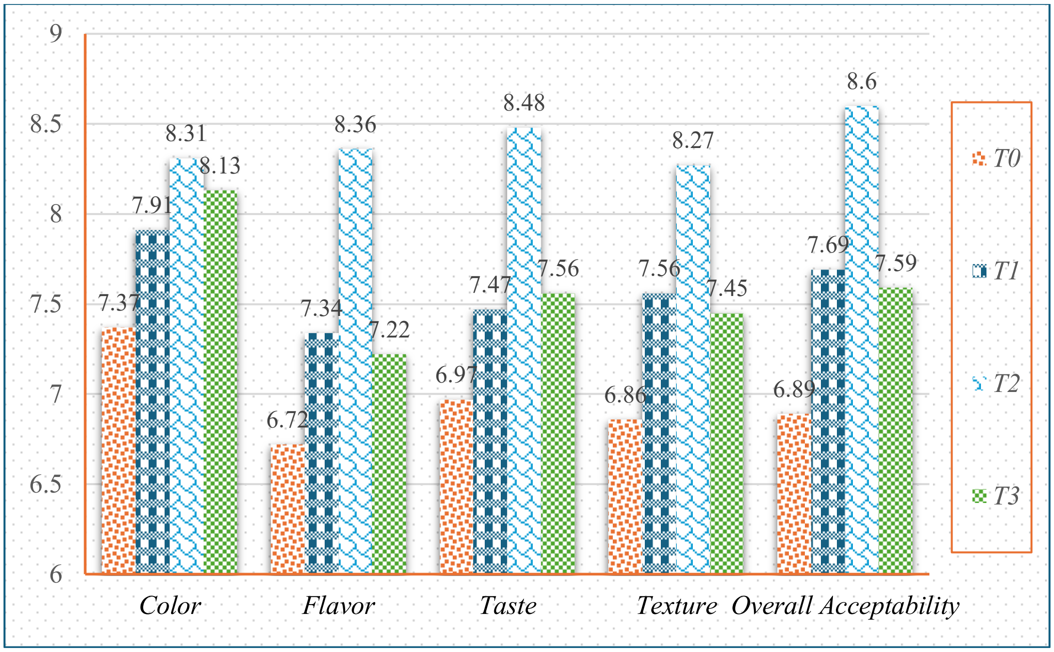

Figure-1: Comparison of Means for Sensory Scores of Sufu Supplemented with Flaxseed

4. DISCUSSION

The reduction in moisture may be linked to the hydrophobic and oil-rich nature of flaxseed, which limits water retention in the Sufu matrix (Tanislav et al., 2024), and to metabolic heat and enzyme activity during fermentation (dos Santos et al., 2004). The rise in ash content is attributed to flaxseed's mineral richness and improved mineral bioavailability due to phytate degradation during fermentation (Bernacchia et al., 2014; Jeyakumar & Lawrence, 2022). Enhanced protein levels reflect flaxseed's high-quality protein and fermentation-driven proteolysis releasing peptides and amino acids (Ren & Li, 2022). The increase in fat aligns with flaxseed's unsaturated fatty acid profile and microbial lipase activity enhancing lipid extraction (Chandra et al., 2020). Meanwhile, carbohydrate reduction results from microbial utilization during fermentation and flaxseed's lower carbohydrate content compared to soy (Zotta et al., 2017). Collectively, flaxseed fortification improved Sufu's nutritional density, functionality, and shelf stability by enriching proteins, minerals, and lipids while reducing moisture and fermentable sugars. The overall rise in amino acid content results from flaxseed's high-quality protein and fermentation-driven proteolysis, which enhance amino acid release and balance (Mueed et al., 2022; Lim & Abd Rahim, 2025). Lysine and methionine improvements demonstrate the complementary amino acid relationship between soy and flaxseed proteins (Tangyu et al., 2021; Sharma & Saini, 2022). The enhancement in non-essential amino acids reflects intensified microbial enzymatic activity and co-fermentation effects that stimulate protease production (Christensen et al., 2022; Guo et al., 2025).

Increased levels of polyunsaturated fatty acids, particularly α-linolenic acid, arise from flaxseed's intrinsic lipid profile and microbial lipase-mediated lipid transformation during fermentation (Wang et al., 2016; Fontes et al., 2023). The rise in fat-soluble and water-soluble vitamins can be attributed to flaxseed's antioxidant composition and microbial biosynthesis of vitamins such as K and B-complex (Capozzi et al., 2012; Zhao et al., 2021). Similarly, higher mineral concentrations are linked to flaxseed's inherent mineral richness and the breakdown of phytates by microbial phytases, improving mineral bioavailability (Adebo et al., 2022; Konietzny & Greiner, 2002). Overall, flaxseed fortification notably improved Sufu's biochemical, nutritional, and functional value enhancing amino acid, lipid, vitamin, and mineral profiles through synergistic effects of its nutrient composition and fermentation-induced enzymatic activity.

The observed rise in protease activity is attributed to the stimulation of proteolytic microorganisms such as *Bacillus subtilis*, which secrete extracellular enzymes to hydrolyze soy and flax proteins into peptides and amino acids (Cao et al., 2024). Flaxseed's rich protein content likely provided additional substrates, further promoting protease synthesis and activation (Necib et al., 2022). Similarly, the marked increase in lipase activity is linked to the lipid-rich composition of flaxseed, particularly its abundance of α-linolenic acid, which induces microbial lipase production and enhances triacylglycerol hydrolysis (Zan et al., 2025). These enzymatic reactions not only contribute to the development of desirable flavor and aroma but also generate bioactive peptides and lipid derivatives with antioxidant and antihypertensive potential (Daliri et al., 2019). The synergistic effect of flaxseed supplementation and microbial fermentation thus amplified enzyme activity, improving Sufu's digestibility, nutritional value, and overall functional quality. The decline in pathogenic and total bacterial counts can be attributed to flaxseed's bioactive compounds—particularly lignans, phenolics, omega-3 fatty acids, and dietary fibers—which possess strong antimicrobial properties

(Hu et al., 2024). During fermentation, beneficial microbes such as *Bacillus subtilis* and lactic acid bacteria produce organic acids, hydrogen peroxide, and bacteriocins, lowering pH and creating inhibitory conditions for spoilage organisms (Mokoena et al., 2021). The combined effect of flaxseed bioactive and fermentation-derived metabolites enhances Sufu's microbial stability and safety. Moreover, the antioxidant components of flaxseed reduce oxidative stress, limiting microbial proliferation (Odeh et al., 2021). Overall, flaxseed-enriched Sufu demonstrated improved hygiene, longer shelf life, and greater microbial safety, confirming flaxseed's dual role as a functional ingredient and natural bio preservative in fermented foods.

The increase in hardness and chewiness can be linked to flaxseed's high fiber and mucilage content, which enhances gel strength and water retention in the Sufu matrix (Zou et al., 2024). During fermentation, protein–polysaccharide and protein–protein interactions promoted by microbial enzymes further improve network stability and firmness (Gentile, 2020). The moderate rise in springiness, particularly at 10% flaxseed, reflects enhanced viscoelasticity due to mucilage's binding effects, while the slight decline at higher levels may be caused by excessive matrix saturation (Zhang et al., 2023). The optimal texture at 10% supplementation suggests a balance between elasticity and firmness, leading to improved mouthfeel and consumer acceptability (Pascua et al., 2013). Overall, flaxseed's structural and biochemical properties contribute to the textural enhancement of Sufu, supporting its role as a valuable functional ingredient in fermented soy products. The consistent increase in vitamin content with flaxseed addition may be attributed to its natural antioxidant and vitamin-rich composition, particularly β-carotene and tocopherols (Nazir et al., 2024; Yang et al., 2021). Fermentation further enhances bioavailability through enzymatic softening and microbial synthesis (Lim & Abd Rahim, 2025; Luo et al., 2023). Lignan-polyphenol complexes in flaxseed likely protected ascorbic acid and other labile vitamins from oxidative loss (Saleem et al., 2005). The increase in B-complex vitamins can also result from microbial biosynthesis by *Bacillus subtilis* and lactic acid bacteria during fermentation (Capozzi et al., 2012; Labuschagne & Divol, 2021). Moreover, flaxseed provided cofactors and nutrients that supported microbial metabolism and vitamin formation (Zhou et al., 2025; Kaur et al., 2018). Overall, flaxseed addition significantly enhanced the nutritional value of Sufu through both direct nutrient enrichment and fermentation-driven biosynthetic mechanisms.

The enhancement in mineral content can be attributed to the natural mineral richness of flaxseed and the fermentation process, which improves mineral bioavailability by breaking down anti-nutritional factors like phytates through microbial enzyme activity (Adebo et al., 2022; Konietzny & Greiner, 2002). Fermentative microorganisms such as *Bacillus* spp. contribute to this process by releasing bound minerals. The increased levels of key minerals, particularly calcium, iron, zinc, and selenium, suggest improvements in bone health, antioxidant capacity, and overall metabolic function (Manzoor et al., 2021; Guiotto et al., 2025). Overall, flaxseed fortification significantly enhanced the mineral and nutritional quality of Sufu by combining flaxseed's inherent mineral profile with fermentation-induced mineral liberation. The increase in protease and lipase activity suggests a synergistic effect between flaxseed bioactive and microbial metabolism during fermentation. Proteolytic microbes such as *Bacillus subtilis* likely contributed to this rise by producing extracellular enzymes that hydrolyze soybean and flaxseed proteins into peptides and amino acids, improving digestibility and bioactivity (Cao et al., 2024). Similarly, the lipid-rich composition of flaxseed—especially its α-linolenic acid content—stimulates microbial lipase activity, leading to the release of free fatty acids that enhance flavor and nutritional properties (Zan et al., 2025). Fermentation further stabilizes and activates these enzymes by optimizing pH and redox balance, thereby increasing nutrient availability and producing bioactive compounds with antioxidant and antihypertensive effects (Daliri et al., 2019; Necib et al., 2022). Overall, flaxseed fortification substantially improved enzymatic functionality in Sufu, contributing to enhanced texture, flavor, and nutritional quality.

Flaxseed supplementation notably improved the microbial safety and textural attributes of Sufu. The reduction in *E. coli*, *Staphylococcus aureus*, *Salmonella spp.*, and total viable counts suggests antimicrobial activity from flaxseed's phenolics, lignans, and omega-3 fatty acids, supported by fermentation metabolites like organic acids and bacteriocins that inhibit pathogens (Mokoena et al., 2021; Hu et al., 2024). Texturally, flaxseed increased hardness and chewiness due to its mucilage and fiber forming stronger protein–polysaccharide networks and improving water retention during fermentation (Zou et al., 2024; Gentile, 2020). Moderate inclusion (10%) provided optimal firmness and elasticity, enhancing mouthfeel and sensory quality (Pascua et al., 2013). Overall, flaxseed acted as both a functional nutrient enhancer and a natural bio preservative in fermented soy foods. The improved sensory performance of T2 can be attributed to the natural pigments and mild brown hue of flaxseed, which enhanced the color of Sufu (S. Zhang et al., 2023), while higher levels (T3) led to slight over-darkening (Serna-Saldivar, 2022). The flavor and taste enhancements up to 10% addition were likely due to Maillard reactions and nutty volatiles from flaxseed, though excessive supplementation caused mild bitterness linked to polyphenols (Mercier et al., 2014; Osakabe et al., 2024). Texture improvements were associated with flaxseed mucilage, oils, and fibers contributing to smoothness and a richer mouthfeel (Lorenc et al., 2022), while higher flaxseed levels introduced minor graininess (Zhang et al., 2023). Overall, moderate flaxseed addition (10%) provided the optimal balance of flavor, texture, and visual appeal, enhancing both the sensory quality and functional value of Sufu.

CONCLUSION

This study demonstrated that flaxseed supplementation significantly enhanced the nutritional, functional, and sensory properties of traditional Sufu. Incorporating flaxseed at levels of 5%, 10%, and 15% led to substantial improvements in protein, fat, mineral, and omega-3 (ALA) content, while reducing moisture and carbohydrate levels. Antioxidant potential and enzymatic activities (protease and lipase) were markedly elevated, indicating improved bioactivity and digestibility. The microbial analysis confirmed enhanced safety and probiotic growth, while sensory evaluation identified the 10% flaxseed formulation (T_2) as the most acceptable balance of taste, texture, and overall quality. Textural improvements, including higher hardness and chewiness, reflected better structural integrity due to flaxseed's mucilage and fiber components. Overall, flaxseed enrichment successfully transformed Sufu into a more nutrient-dense, functionally superior, and consumer-preferred fermented food. These findings establish flaxseed-supplemented Sufu as a promising plant-based functional product with potential health benefits and commercial application in sustainable food innovation.

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