

Enhancement of Nutritional Quality and Antioxidant Activity Through Fermentation and Reduction of Antinutritional Compounds in Peanuts (*Arachis hypogaea* L.)

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Abstract. Peanuts are a nutrient-dense food with considerable potential for further utilisation. However, their nutritional value may be limited by antinutritional compounds, particularly phytic acid and tannins, which can interfere with nutrient absorption. This study investigated the effects of fermentation time on the nutritional composition, antioxidant capacity, and antinutritional content of peanut flour. Fermentation was performed with *Rhizopus oligosporus* at 1% (w/w). A completely randomised design was applied with three treatments: unfermented peanuts (0 h) and peanuts fermented for 48 and 72 h, with each treatment conducted in triplicate. The findings demonstrated that fermentation significantly improved the nutritional and functional properties of peanut flour. Protein content increased from 25.26% to 30.46%, while total phenolic content rose from 3.61 to 7.38 mg GAE/g. Antioxidant activity also improved, as reflected by the reduction in IC₅₀ value from 184.81 to 96.82 ppm. In contrast, phytic acid content declined from 0.81% to 0.33%, and tannin content decreased from 2.21 to 0.87 mg TAE/g. In conclusion, 72-hour fermentation was identified as the optimal condition for improving both nutritional quality and functional properties. Further studies on bioavailability and industrial application are recommended.

1 Introduction

Peanuts (*Arachis hypogaea* L.) are a nutrient-rich legume with strong potential as a functional food ingredient [1]. They provide key nutrients such as protein, healthy fats, and carbohydrates [2], along with bioactive compounds such as phenolics that contribute to antioxidant activity, making them valuable for creating food products that support better health [3].

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However, the existence of compounds that hinder nutrition, like phytic acid and tannins, restricts their best use [4]. Phytic acid can reduce the absorption of essential minerals, including iron and zinc, by binding to them and calcium, thereby reducing their bioavailability [5]. Tannins can attach themselves to proteins and digestive enzymes, potentially reducing the ability to digest proteins [6]. This indicates that despite their high nutritional value, peanuts are not fully utilised.

Fermentation is an effective processing technique to enhance the nutritional value and functional properties of food ingredients [7]. This mechanism includes the action of microorganisms that generate enzymes for hydrolysis, including proteases and phytases, which decompose complex substances into simpler versions that the body can absorb more easily [8]. This enzymatic activity enhances nutrient availability and improves protein quality [9]. In addition, fermentation can increase phenolic content by releasing bound compounds into free forms with higher antioxidant activity [3].

Previous research has demonstrated that fermentation can improve antioxidant activity and functional properties of foods derived from plant sources [10]. These improvements are associated with biochemical changes during fermentation, including increased total phenolics and other bioactive compounds [8]. Fermentation also reduces antinutritional compounds through enzymatic activity [11], particularly phytase, which degrades phytic acid [12].

However, most studies focus on individual aspects such as antioxidant activity or antinutrient reduction separately. Integrated studies evaluating the nutritional composition, antinutritional compounds, and antioxidant activity simultaneously remain limited. In addition, the influence of fermentation duration as a key factor in shaping the biochemical changes in food systems has not been extensively studied in a comprehensive manner [6]. This highlights a research gap that warrants further investigation to achieve a more holistic understanding. Consequently, this research aims to assess how the length of fermentation influences the outcomes of nutritional quality, antinutritional compounds, and antioxidant activity in peanuts to determine optimal fermentation conditions for producing functional food with improved nutritional and health value.

2 Materials and Method

2.1 Materials

Equipment used included a digestion unit, Kjeldahl distillation apparatus, Kjeldahl flasks, burettes, hot plate/water bath, retort stand and clamps, Soxhlet extractor, round-bottom flask, condenser, analytical balance, oven, desiccator, filter paper/thimbles, heating mantle, Erlenmeyer flasks, volumetric and measuring pipettes, volumetric flasks, beakers, UV-Vis spectrophotometer, cuvettes, micropipettes, test tubes, tube racks, vortex mixer, and centrifuge.

Every chemical utilized in this research was of a high analytical standard. The reagents used for proximate analysis included concentrated sulfuric acid (H_2SO_4), catalyst mixture (K_2SO_4 – CuSO_4 or selenium), sodium hydroxide (NaOH), boric acid (H_3BO_3), mixed indicator (methyl red and bromocresol green), and standard acid solution (0.1 N HCl or H_2SO_4). For fat analysis, organic solvents such as n-hexane or petroleum ether were used.

Carbohydrate analysis employed Luff–Schoorl reagents, including KI, $\text{Na}_2\text{S}_2\text{O}_3$ and, starch solution as an indicator, and standard iodine solution (I_2). Antioxidant assays utilized reagents such as DPPH, ABTS, TPTZ, FeCl_3 , Trolox standard, methanol or ethanol, potassium persulfate, and acetate buffer (pH 3.6). Total phenolic content was determined using Folin–Ciocalteu reagent, sodium carbonate (Na_2CO_3), and gallic acid were used as the standard reference. Phytic acid and tannin analyses were conducted using Wade reagent, phytic acid standard, and tannic acid standard.

2.2 Experimental design

This research employed a method of experimentation featuring a design that was entirely randomized with three replications. The treatments (fermentation) consisted of 0 h, 48 h and 72 h. Fermentation was conducted at 37°C under controlled conditions. All analyses were conducted at the Food Chemistry Laboratory, Universitas Muhammadiyah Gresik.

2.2.1 Preparation of fermented peanut samples

The primary raw material used in this study was Kancil variety peanuts obtained from Malang, sourced from the Indonesian Legumes and Tuber Crops Research Institute. Peanuts were sorted, weighed, soaked for 2 hours, and rinsed three times. The samples were boiled for 1 hour, cooled, and dehulled. Fermentation was carried out by adding 1% w/w *Rhizopus oligosporus*. The inoculated peanuts were packed in perforated plastic and subsequently incubated at 30°C for either 48 or 72 hours. The product obtained after fermentation (peanut tempeh) was then cut into smaller pieces for drying (Fig. 1).

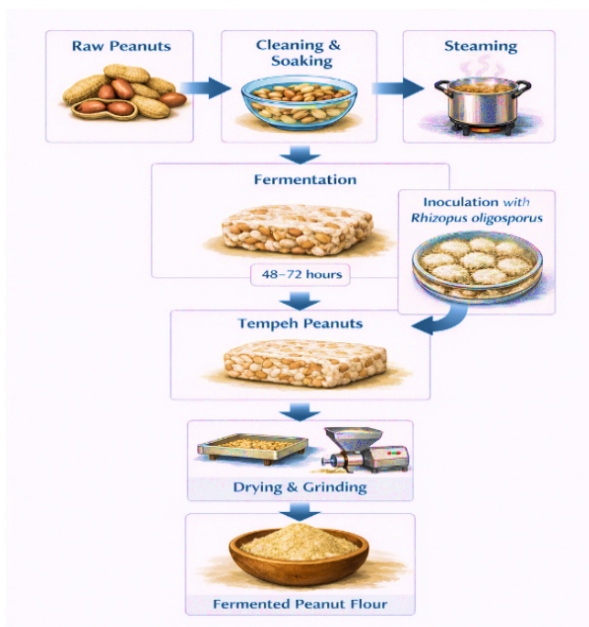


Fig 1. Schematic illustration of the production process of fermented peanut flour

2.2.2 Production of fermented peanut flour

The fermented peanuts (tempeh) were thinly sliced and dried in a food dehydrator at 50°C for 6 hours. The dehydrated specimens were pulverized into a fine powder and passed through an 80-mesh screen. The flour was vacuum-packed for further analysis.

2.2.3 Analytical methods

- a. Proximate Composition: The proximate composition of peanut flour was assessed following the established analytical methods set by AOAC International [13]. The Kjeldahl method was employed to assess protein levels, utilizing a conversion factor of 6.25. In contrast, fat levels were evaluated through Soxhlet extraction with petroleum ether, and the analysis of carbohydrate levels was carried out using the Luff–Schoorl method.
- b. Antioxidant [14]: Antioxidant effectiveness (AE) was assessed through the DPPH method, with absorbance measured at 519 nm. This method measures the capacity of a compound or extract to neutralize free radicals via electron-donating or hydrogen atom transfer mechanisms
- c. Total phenolics [14]: were measured utilizing the Folin–Ciocalteu spectrophotometric approach. The blue complex generated following the incorporation of carbonate (Na_2CO_3) was assessed at 765 nm through a UV–vis spectrophotometer
- d. Phytate content [15]: the phytate level was analyzed using the Wade colorimetric method, where the reduction in pink color intensity of the Fe^{3+} –Wade reagent complex was quantified at a wavelength of 500 nm utilizing a UV–vis spectrophotometer
- e. Tannin content [16]: The Folin–Ciocalteu technique was utilized to ascertain the value by evaluating the absorbance of the produced blue complex at a wavelength of 760 nm via a spectrophotometer.

2.2.4 Data analysis

The data were analysed using a one-way analysis of variance (ANOVA) at a 95% confidence level, followed by Duncan's Multiple Range Test (DMRT) to identify significant differences among treatments. Differences among treatments were denoted by letter annotations, with the same letter indicating no statistically significant difference.

3 Results and Discussion

The changes in the chemical composition of peanut flour during fermentation demonstrated a strong interrelationship among the observed parameters, reflecting the metabolic activity of microorganisms. Fermentation clearly altered nutritional components, antinutritional compounds, and antioxidant properties. The fermentation duration (48 and 72 hours) showed a pronounced effect, indicating intensified enzymatic and microbial metabolic activity over time, leading to the bioconversion of complex compounds into simpler forms that may enhance nutrient availability.

During extended fermentation, microorganisms utilized carbohydrates and lipids as energy sources, which relatively increased protein content and improved its quality through hydrolysis into peptides and amino acids [17]. Simultaneously, phenolic compounds and antioxidant activity increased as bound compounds were released from the cellular matrix, thereby improving the product’s functional properties [18]. Meanwhile, reductions in phytic acid and tannins were associated with enzymatic degradation, improving mineral availability and protein digestibility. The findings of this research are shown in **Table 1**.

Table 1. Chemical composition of peanut flour

Parameters	Treatment			P-value
	P1	P2	P3	
Protein (%)	25.26±0.69 ^c	28.56±0.586 ^b	30.46±1.04 ^a	0.00
Fat (%)	48.21±0.39 ^a	47.50±0.51 ^{ab}	46.80±0.25 ^b	0.02
Carbohydrate (%)	47.77±0.56 ^a	47.10±0.73 ^{ab}	46.43±0.49 ^b	0.09
Antioxidant /IC ₅₀ (ppm)	184.81±2.41 ^a	121,50±1.89 ^b	96,82±1.44 ^c	0.00
Total phenolics (mg GAE/g)	3.61±0.38 ^c	5.92±0.27 ^b	7.38±0.36 ^a	0.00
Phytic acid (%)	0.81±0.26 ^a	0.47±0.32 ^b	0.33±0.55 ^c	0.00
Tannins (mg TAE/g)	2.21±0.11 ^a	1.43±0.13 ^b	0.87±0.04 ^c	0.00

Notes: P1 (unfermented peanut flour); P2 (peanut flour fermented for 48 hours); P3 (peanut flour fermented for 72 hours)

3.1 Protein (%)

The results indicated that protein content increased significantly ($p < 0.05$) as the fermentation period was extended. rising from 25.26% to 30.46% after 72 h of fermentation. This indicates that fermentation plays a significant role in improving the nutritional quality of peanut flour protein. Biochemically, microorganisms produce protease enzymes during fermentation, which break down complex proteins into simpler peptides and amino acids [19], thereby enhancing protein availability and digestibility [8]. In addition, microbial biomass synthesis during fermentation may also contribute to the observed increase in total protein content [20]. The consistent increase in protein content across fermentation time highlights a positive shift in nutritional density, suggesting that fermentation not only modifies composition but also enhances the overall protein contribution of the final product.

The significant increase in protein content was accompanied by a decrease in fat and a tendency for carbohydrate reduction, suggesting that microorganisms utilize energy components such as lipids and carbohydrates as primary substrates for growth. This process results in a relative increase in the proportion of protein [3]. Furthermore, protease activity during fermentation promotes the breakdown of complex proteins into more digestible forms, improving overall protein quality [8]. Previous studies have also reported that fermentation of legumes can enhance protein quality by increasing the absorption and utilization of essential amino acids by the body [21].

The findings indicated a significant increase in protein levels ($p < 0.05$) with longer fermentation durations. This observation suggests that fermentation enhances the nutritional value of peanut flour. Throughout the fermentation process, microorganisms produce proteolytic enzymes that break down larger proteins into simpler peptides and amino acids [19], thereby improving protein bioavailability and digestibility [8]. Furthermore, the formation of microbial biomass during fermentation may contribute to the observed increase in total protein content [20].

Furthermore, fermentation can lower tannin and phytate levels, thereby enhancing protein digestibility and nutrient absorption. Reducing these compounds helps improve the biological value of the protein [5], in line with previous findings highlighting the combined effects of enzymatic activity and antinutrient reduction [22].

3.2 Crude fat content (%)

Fat content decreased significantly ($p < 0.05$) during fermentation. The fat content of peanut flour decreased slightly but significantly during fermentation, from $48.21 \pm 0.39\%$ (P1) to $47.50 \pm 0.51\%$ (P2) and $46.80 \pm 0.25\%$ (P3) ($p = 0.02$). This decrease may be attributed to microbial activity, particularly the action of enzymes such as lipases that break down lipids into simpler compounds that are then used as energy by microorganisms. Longer fermentation may also promote lipid oxidation and transformation. Even so, the fat content remains relatively high, confirming that peanuts are naturally rich in lipids, while fermentation offers a practical way to modestly adjust fat levels without significantly reducing nutritional value.

This reduction indicates lipid degradation by microorganisms through the activity of lipase enzymes, which break down triglycerides into free fatty acids and glycerol. These compounds are subsequently utilized as energy sources by microorganisms [9]. In addition, some fatty acids may undergo oxidation during fermentation, contributing to the reduction in total fat content [23]. The declining trend in fat content reflects a systematic transformation of lipid components, indicating that fermentation promotes a redistribution of macronutrient composition toward a more balanced nutritional profile.

The decline in fat content may also be related to microbial metabolism, during which lipids are utilized as an energy source. This process is commonly observed in plant-based food fermentation, where microorganisms efficiently utilize available energy sources [3]. Previous studies have shown that fermentation can alter fatty acid profiles, including an increase in free fatty acids, which may influence sensory and nutritional properties [23].

Furthermore, lipid degradation during fermentation can improve oxidative stability by reducing lipid components susceptible to oxidation. This has a positive impact on shelf life and overall product quality [24]. Therefore, the decrease in fat content during fermentation not only affects nutritional composition but also contributes to product stability and quality.

3.3 Carbohydrates (%)

The amount of carbohydrates appeared to decline as fermentation advanced; however, this decrease did not reach statistical significance ($p > 0.05$). This decrease may be

explained by the use of carbohydrates as the main energy source for microbial growth during fermentation. Complex carbohydrates, including starch and oligosaccharides, are first broken down into simpler sugars by amylase enzymes and are then utilized by microorganisms in their metabolic processes [10]. The relatively stable yet slightly decreasing carbohydrate levels suggest that fermentation maintains energy-related components while subtly redirecting substrate utilization toward metabolic activities

In addition to serving as an energy source, carbohydrates also function as substrates for the formation of fermentation-derived metabolites, such as organic acids, ethanol, and gases [25]. Previous studies have also reported that fermentation can reduce oligosaccharides responsible for flatulence, thereby improving the physiological quality of food products [24].

Although not statistically significant, this trend reflects ongoing metabolic activity during fermentation. Variations in carbohydrate changes are influenced by the type of microorganisms and fermentation conditions [20]. Therefore, carbohydrate changes should be evaluated alongside other parameters to better understand fermentation dynamics.

3.4 Antioxidant activity (IC₅₀)

The IC₅₀ measurement demonstrated a notable reduction that was statistically significant ($p < 0.05$), indicating a significant rise in antioxidant activity during fermentation. A lower IC₅₀ value reflects a higher antioxidant capacity, as a lower concentration is required to inhibit free radicals. This improvement is mainly attributed to the release of phenolic compounds from their bound forms into more biologically active free forms [3]. The substantial reduction in IC₅₀ values confirms a progressive strengthening of antioxidant capacity, emphasizing the role of fermentation as a natural process for enhancing functional bioactivity.

Furthermore, the enhanced antioxidant activity was strongly linked to the increase in total phenolic content. This suggests that fermentation enhances the bioactivity of bioactive compounds by releasing and transforming bound phenolics into more active forms [26]. Enzymatic activities such as β -glucosidase play a role in breaking down plant cell wall matrices [27], thereby increasing the extractability of phenolic compounds [26]. Overall, increases in total phenolics and antioxidant activity serve as important indicators of improved functional properties in fermented products [2]. Consequently, fermentation plays a role not only in boosting the nutritional quality but also in increasing the functional benefits of food items.

3.5 Total phenolics (mg GAE/g)

Total overall phenolic content rose considerably ($p < 0.05$) with the lengthening of the fermentation period, suggesting that fermentation promotes the availability of bioactive compounds in food materials. During fermentation, microorganisms create enzymes that break down cell walls, which leads to the liberation of phenolic substances that were once attached to the plant's cellular structure [8]. The marked increase in total phenolic content demonstrates that fermentation effectively unlocks bioactive potential,

strengthening its contribution significantly to improving the functional characteristics of plant-derived substances.

In addition to releasing bound compounds, fermentation can also induce structural transformations of phenolics into more biologically active forms, thereby improving antioxidant capacity [3]. Previous research has shown that fermentation can improve the bioaccessibility of phenolic compounds during digestion [28]. The increase in total phenolics is also linked to microbial activity involving phenolic-degrading and modifying enzymes, highlighting the important role of fermentation in improving functional quality [29].

3.6 Phytic acid (%)

The concentration of phytic acid decreased notably ($p < 0.05$) during fermentation, indicating the effectiveness of fermentation in reducing antinutritional compounds. Phytic acid is degraded by phytase enzymes produced by microorganisms into inositol and phosphate [6]. The reduction in phytic acid levels indicates a meaningful improvement in nutrient accessibility, underscoring the importance of fermentation in enhancing mineral bioavailability without additional processing.

This decrease holds nutritional significance as it enhances the body's ability to utilize crucial minerals, especially iron and zinc. With lower phytate concentrations, these minerals are more readily available for the body to absorb [11]. Phytic acid functions as a compound (substrate) that binds minerals; however, through the process of fermentation, it is broken down by phytase enzymes, which enhance the bioavailability of vital minerals (Fe and Zn) [6]. Previous studies have shown that fermentation is more effective at reducing phytate content compared to other processing methods [30]. Phytic acid binds to minerals, but during fermentation, it is broken down by phytase enzymes, which help make important minerals like iron and zinc easier for the body to use.

In addition, the reduction of phytic acid and tannins may contribute to improved protein utilization by minimizing interactions with proteins and digestive enzymes [5] [31]. The reduction of these antinutritional compounds not only improves nutritional value but also enhances the efficiency of nutrient utilization in the body.

3.7 Tannins (mg TAE/g)

Tannin levels declined significantly ($p < 0.05$) throughout fermentation, indicating that fermentation effectively reduces antinutritional compounds that interfere with protein digestibility. Tannins are degraded by tannase enzymes produced by microorganisms (5). In addition to enzymatic degradation, structural modifications of tannins during fermentation reduce their protein-binding capacity, thereby improving protein digestibility and nutritional quality [21]. Furthermore, reducing tannins enhances palatability and sensory quality by reducing astringency, making the fermented product more acceptable to consumers [31]. The decrease in tannin content reflects a reduction in inhibitory compounds, suggesting that fermentation contributes to a more favorable nutritional environment for protein utilization.

Furthermore, the interaction among parameters indicates that fermentation produces a synergistic effect. The reduction of carbohydrates as microbial energy sources contributes to metabolite production, while increasing functional compounds and reducing inhibitory substances that enhance antioxidant activity. At the same time, lipid degradation contributes to product stability and changes in nutritional profiles [9]. This confirms that fermentation is not merely a degradation process but a complex bioconversion process that enhances the value of food materials simultaneously.

Overall, the observed trends indicate that fermentation induces a coordinated shift in the chemical composition of peanut flour, reflecting a transition toward improved nutritional density and functional quality. The increase in protein content, accompanied by reductions in fat and slight changes in carbohydrates, suggests a reallocation of macronutrients driven by microbial metabolism. At the same time, the substantial enhancement in antioxidant activity, together with the rise in total phenolic content, highlights the ability of fermentation to unlock and intensify bioactive potential. Furthermore, the marked decline the reduction in antinutritional compounds, particularly phytic acid and tannins, indicates improved nutrient availability and more efficient nutrient utilization. Collectively, these changes demonstrate that fermentation acts not merely as a preservation technique but as an integrated bioprocess that simultaneously enhances both the nutritional value and functional properties of peanut-based products

4 Conclusion

Fermentation enhanced the overall quality of peanut flour, with the most pronounced improvements observed after 72 hours, appearing to be the most effective duration. The process supported higher protein availability while slightly lowering fat and carbohydrate levels, reflecting how microbes utilize nutrients during fermentation. It also enhanced antioxidant potential and reduced key antinutritional compounds, which may help improve mineral absorption and protein digestibility. Taken together, these changes suggest that fermentation is a practical and efficient way to upgrade peanut flour into a more functional and nutritionally beneficial ingredient. Future studies should focus on refining the process and assessing its safety, bioavailability, and consumer acceptance for wider use.

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