

RESEARCH ARTICLE/ARAŞTIRMA MAKALESİ

GERMINATION AND FERMENTATION PROCESSES ENHANCE ANTIOXIDANT CAPACITY AND BIOACTIVE COMPONENTS IN CHICKPEAS (*Cicer arietinum* L.)Şiir SARI¹, Selen MÜFTÜOĞLU^{2, *}, Tuğba İDUĞ³

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ABSTRACT

Aim: Chickpeas are valued for their high protein content, nutrient profile, and affordability. This study evaluates the effects of germination and fermentation on the antioxidant capacity and bioactive components of chickpeas.

Material and Method: Chickpeas sourced from Konya, Turkey, were sterilized using a 70% ethanol solution, then germinated in distilled water at 25°C for 96 hours. Fermentation was conducted in an orbital shaker at 37°C for 48 hours. Phenolic content was determined using the Folin-Ciocalteu method, with absorbance measured at 765 nm. Ash content was measured after incineration at 550°C. Fatty acid-rich extracts were analyzed using High-Performance Liquid Chromatography (HPLC). Data were collected in triplicate and analyzed using one-way ANOVA and post-hoc Tukey's HSD tests, revealing significant differences ($p<0.05$) in phenolic and ash content among raw, sprouted, and fermented samples.

Results: In our study, the antioxidant capacity of chickpeas was calculated using the α , α -diphenyl- β -picrylhydrazyl (DPPH) test, showing $4430 \pm 0601\%$ in raw chickpeas, $4101 \pm 0411\%$ in germinated chickpeas, and $3966 \pm 0201\%$ in fermented chickpeas. The lowest antioxidant capacity was observed in fermented chickpeas ($p<0.05$). The total phenolic content increased by 64% in germinated chickpeas and 29% in fermented chickpeas, with the highest phenolic content found in the germinated samples ($p<0.05$). Additionally, the ash content was measured at 3.04% in raw chickpeas, 2.72% in germinated chickpeas, and 2.05% in fermented chickpeas, with the lowest ash content observed in the fermented samples ($p<0.05$).

Conclusion: This study showed that germination and fermentation under optimum conditions decreased the antioxidant capacity, linoleic acid content, and ash content of chickpea seeds, but increased the total phenolic content.

Keywords: Chickpeas, Fermentation, Germination, Antioxidant levels, Bioactive components



***İletişim/Correspondence:** Selen MUFTUOĞLU, Assoc. Prof., Ankara Medipol University, Department of Nutrition and Dietetics, Faculty of Health Sciences, Ankara, Türkiye, **e-posta:** selen.muftuoglu@ankaramedipol.edu.tr

• **ORCID:** <https://orcid.org/0009-0005-7751-8224>

1.Şiir SARI, Master's degree, Ankara Medipol University, Faculty of Health Science, Ankara, Türkiye

2. Selen MUFTUOĞLU, Assoc. Prof., Ankara Medipol University, Department of Nutrition and Dietetics, Faculty of Health Sciences, Ankara, Türkiye • **ORCID:** <https://orcid.org/0009-0005-7751-8224>

3.Tuğba İDUĞ, Assis. Prof., İstanbul Medipol University, Faculty of Pharmacy, İstanbul, Türkiye.

• **ORCID:** <https://orcid.org/0000-0001-8241-647X>



INTRODUCTION

Legumes from the Leguminosae and Fabaceae families have provided numerous health benefits to humans for centuries due to their unique profiles. Rich in dietary fiber, protein, B vitamins, various minerals, and polyphenols, legumes form the foundation of traditional plant-based diets worldwide (1). Additionally, despite being rich in carbohydrates, legumes have lower glycemic indices compared to grains, leading to more favorable health effects (2).

One of the oldest and most widely consumed legumes globally is the chickpea (*Cicer arietinum* L.). With an average annual production of 20.5 million tons and cultivation on 13.7 million hectares worldwide, chickpeas are a significant crop (3). Chickpeas are rich in macro and micronutrients and contain various phenolic and bioactive compounds, making them a high-fiber source (e.g., phytosterols, saponins, and oligosaccharides) suitable for use as a functional food (4). Additionally, for individuals with limited access to protein sources or who follow specific diets (such as low-fat diets or vegetarianism), chickpeas are among the most affordable and high-quality protein sources (5).

Chickpeas consist of 19-20% protein by dry weight, with approximately 40% of the total protein content comprising essential amino acids. They contain 60-65% carbohydrates (including 40-45% starch, 15-20% dietary fiber, and 2-5% simple sugars), 5-6% fat (of which 0.6-0.7% is saturated fat, 1.5-2% is monounsaturated fat, and 2-2.5% is polyunsaturated fat), and 3-5% ash (6). Given this nutritional profile, particularly the high dietary fiber, low glycemic index, and healthy fats, studies have focused on its beneficial effects on chronic diseases such as obesity, diabetes, and cardiovascular diseases (7,8). Furthermore, chickpeas are considered a functional food due to their high concentrations of β -carotene, a significant carotenoid, and β -sitosterol among phytosterols (9). The



consumption of chickpeas is remarkably high, and given their significant health benefits, numerous studies have been conducted recently to maximize their efficiency. Particularly in recent years, with the increasing interest in additive-free, minimally processed, high-nutrient, and natural foods, methods such as fermentation and germination are frequently utilized (10).

Germination is an easy and economical method that enhances nutritional value. The germination of legume seeds involves a series of processes characterized by various metabolic activities within the seed, causing physico-chemical changes to produce simpler compounds from storage proteins and carbohydrates to support the growing embryo (11). It has been reported that the germination process increases the digestibility of fats, proteins, and carbohydrates in legumes, enriches vitamin content, enhances bioavailability, and reduces anti-nutritional factors (12). Additionally, germination has been reported to increase antioxidant capacity (11).

Studies have shown that fermentation is an effective technique for improving the nutritional quality of chickpeas and removing anti-nutritional factors (13,14). Fermentation is considered one of the best biological degradation processes for enhancing the nutritional profile of legumes by increasing essential nutrients and reducing anti-nutritional factors. This process, in turn, improves the *in vitro* digestibility of legumes (15). Based on all this information, this study aims to evaluate the changes in antioxidant capacity and bioactive components in chickpeas when subjected to germination and fermentation processes.

METHODS

The chickpea samples used in this study were sourced from a local producer in Konya, Turkey. The samples were obtained in a dry state and stored at 4°C in plastic containers to maintain their quality.



Germination

The chickpeas were carefully washed under running water to remove foreign materials, with close attention to water usage (16). To ensure sterilization and prevent microbial spoilage, the chickpeas were immersed in a 70% ethanol solution for 10 minutes. Subsequently, the sterilized chickpeas were rinsed in distilled water for 5 minutes to remove any residual ethanol and neutralize the grains. After being drained using a sieve the sterilized chickpeas were soaked in distilled water at a 1:3 ratio under optimal conditions ($25\pm1^{\circ}\text{C}$) in a dark environment for 24 hours. Following this soaking period, the chickpeas were drained again using a sieve and left to germinate for 96 hours in the same dark room and temperature environment. To ensure the germinating grains remained moist, distilled water was added every 12 hours using a wash bottle. Grains with a sprout length of 1 mm or more were considered germinated (Figure 1).

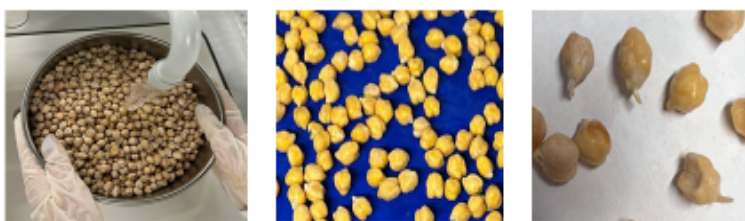


Figure 1. Preparation of chickpeas for the germination process and germinated chickpea seeds

Fermentation

The separated chickpea seeds were soaked in distilled water at a 1:3 ratio for 15 minutes for sterilization. Following sterilization, the chickpeas were drained, and fresh distilled water at a 1:3 ratio was added. To protect them from daylight, the Erlenmeyer flask was covered with aluminum foil. For natural fermentation by the microorganisms present on the seeds, the chickpeas were incubated in an orbital shaker (Unitron, Infors AG) at 37°C and 150 rpm for 48 hours. Before being subjected to analyses, the germinated and fermented chickpea seeds were arranged on trays lined with white drying paper, ensuring they did not touch each other. The



trays were then placed in an incubator set to 45°C (ILD-IN 55, Ankara, Turkey) for 2 hours to dry (Figure 2) (17).



Figure 2. Chickpeas undergoing natural fermentation in an orbital shaker incubator and subsequent drying of fermented chickpeas in the incubator

All samples were manually ground using a porcelain mortar and pestle, including the raw chickpeas. Each sample was weighed to 10 grams and subjected to maceration in methanol (200 mL) at room temperature for 72 hours to facilitate extraction. The solid-liquid heterogeneous extracts in the Erlenmeyer flasks were filtered into 250 mL Erlenmeyer flasks using a glass funnel and filter paper. To ensure no solid matter remained in the liquid extract, this process was repeated twice. The methanol was removed from the obtained extracts using a rotary evaporator. Each extract was placed separately into available flasks. Utilizing the principle of reducing pressure to lower the boiling point, methanol was evaporated from the extracts. By removing the natural water and methanol mixture present in the chickpeas, the pure extract of each chickpea seed was obtained. These steps were performed sequentially for all samples (Figure 3).

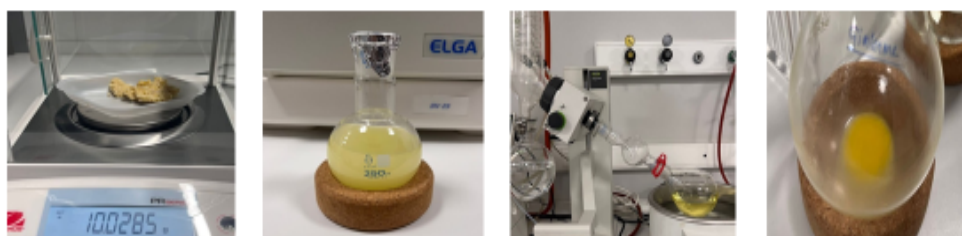


Figure 3. Soaking of 10 grams of ground samples in methanol, followed by filtration and



removal of methanol solution using a rotary evaporator

Chickpea extracts were prepared in tubes with quantities adjusted and selected based on previous studies using the dilution method. The extracts were dissolved in dimethyl sulfoxide (DMSO) to achieve a concentration of 10 mg/mL. The tubes were placed in an ultrasonic bath with their caps closed until the mixture became a homogeneous solution, free of visible particles. This ultrasonic treatment was repeated as necessary until all particles were completely dissolved.

α , α -diphenyl- β -picrylhydrazyl (DPPH) Method

The antioxidant capacity of the extracts was determined by their ability to scavenge DPPH radicals, following the method of Yanping Zou (19). The assay employed DPPH radicals. In a 96-well plate, 10 μ L of the extract solutions were added to each well using a multi-channel pipette (Figure 4). A 0.1 mM DPPH solution was prepared by dissolving 0.001 grams of DPPH in 25 mL of methanol, and 190 μ L of this solution was added to each well. The mixture was gently shaken and incubated in the dark at room temperature for 30 minutes. Absorbance was measured at 517 nm using a microplate reader against a DMSO blank. The procedure was repeated three times (18).

The DPPH radical scavenging activity was calculated using the following equation:

$$\text{Antioxidant activity (\%)} = [(A_0 - A_1) / A_0] \times 100$$

When A_0 is the absorbance of the control, A_1 is the absorbance of the extracts or standards. The inhibition percentage was calculated by plotting the percentage inhibition against the extract concentration. Gallic acid was used as the positive control. The results were expressed as percentage inhibition using the DPPH assay.



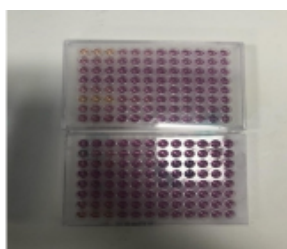


Figure 4. Solutions in 96-well plates prepared for the DPPH assay

Phenolic Content

The same dilution method used for the DPPH assay was applied. Four different dilutions prepared for the DPPH test (10 mg/mL, 2.5 mg/mL, 0.625 mg/mL, 0.15625 mg/mL) were also used for the determination of total phenolic content. In a 96-well plate, 5 μ L of each dilution was added to each well in triplicate using a multi-channel pipette. Subsequently, pre-prepared Folin-Ciocalteu reagent (0.2 N) and water were added. Specifically, 145 μ L of water and 25 μ L of Folin-Ciocalteu reagent were added to each well containing 5 μ L of the extract solution. After 6 minutes, 75 μ L of Na_2CO_3 was added. The reaction was allowed to proceed for 2 hours, and the absorbance was measured at 765 nm. A single well with gallic acid was used as the standard. Results were expressed as mg of gallic acid equivalents per gram of extract (mg GAE/g).

Ash Determination

Ash refers to the inorganic residue remaining after incinerating all organic compounds in a food sample at high temperatures. This residue contains various minerals and trace elements such as magnesium, copper, iron, sodium, manganese, and potassium. To determine the ash content in raw chickpeas, sprouted chickpeas, and fermented chickpeas, 5 grams of each sample were taken and incinerated in an ash furnace at 550°C. Crucibles preheated to the same temperature as the furnace were brought to a constant weight, cooled to room temperature in a desiccator,



and their tare weights were recorded.

Approximately 5 grams of each sample were placed in porcelain crucibles and incinerated at 550°C until white ash was obtained. The white ash was then removed from the furnace and cooled in a desiccator.

The samples were weighed, and the ash content was calculated using the following equation:

$$\% \text{ Ash} = [(Tare + Ash) - Tare] / ((Tare + Sample) - Tare) * 100$$

Results are expressed as the percentage of ash.

High-Performance Liquid Chromatography (HPLC)

The method used by Gurassi et al. (20) was adapted with slight modifications. Seventy grams of chickpeas were weighed and soaked in 200 mL of methanol for 72 hours. After soaking, the mixture was filtered through standard filter paper and washed three times with methanol. The methanol was then removed using a rotary evaporator.

From the obtained material, four grams were weighed and boiled in 40 mL of 0.5 M NaOH at 100°C for 1 hour. The mixture was then extracted three times with 40 mL of petroleum ether. The petroleum ether phase from the first extraction was discarded, and the aqueous phase was adjusted to pH 2.9 with 1 M HCl. For the second extraction, the aqueous phase was extracted three times with 40 mL of petroleum ether, and the ether phase was removed under a nitrogen atmosphere. The resulting extract, rich in fatty acids, was dissolved in 100 mL of methanol. The solution was then brought up to 100 mL, further diluted at ratios of 1:10 and 1:100, and filtered through a 0.22 µm syringe filter before HPLC analysis. The system parameters for the study were determined in accordance with ISO 21748 (Guidance for the Use of Repeatability, Reproducibility, and Trueness Estimates in Measurement Uncertainty Estimation) and ISO



5725 (Accuracy of Measurement Methods and Results Package) standards, as well as the guidelines provided by Eurachem.

All data were collected in triplicate for each sample and reported as mean $\pm$ standard deviation. To assess the variability and reliability of the measurements, standard deviations were calculated. One-way analysis of variance (ANOVA) was performed to compare the phenolic content and ash content across different sample types (raw, sprouted, and fermented chickpeas). Post-hoc Tukey's HSD tests were conducted to identify significant differences between individual groups. A p-value of less than 0.05 was considered indicative of statistical significance. All statistical analyses were performed using statistical software such as SPSS (Version 22).

RESULTS

Table 1 shows the antioxidant capacity, total phenolic content, and ash content of chickpeas subjected to different processing methods. The antioxidant capacity of chickpeas using the DPPH test was calculated for raw (4430 ± 601), germinated (4101 ± 411), and fermented (3966 ± 201) states, with the lowest antioxidant capacity observed in fermented chickpeas ($p < 0.05$).



Table 1. Changes in antioxidant capacity, total phenolic and ash content in chickpea samples according to applied processes

Process	Antioxidant Activity (%)	Total Phenolic Content (mGAE/g)	Ash Content (%)
Raw	4430 ± 0601	44.073 ± 0.21	3.04 ± 1.7
Germination	4101 ± 0411	72.350 ± 1.57*	2.72 ± 1.1
Fermentation	3966 ± 0201*	57.523 ± 3.03	2.05 ± 0.4*

* $p < 0.05$

The total phenolic content of raw chickpeas was 44.073 ± 0.213 mg GAE/g, germinated chickpeas had 72.350 ± 1.574 mg GAE/g, and fermented chickpeas had 57.523 ± 3.034 mg GAE/g (Table 1). Additionally, an increase of 64% in total phenolic content was observed in germinated chickpeas and 29% in fermented chickpeas (Figure 5). Accordingly, the highest total phenolic content was obtained from chickpea samples subjected to germination ($p < 0.05$).

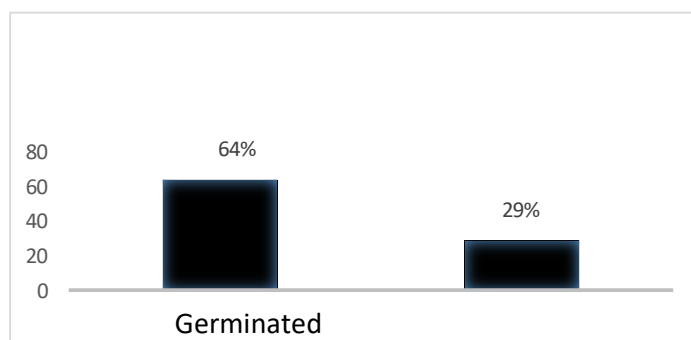


Figure 5. Percentage increase in total phenolic content in chickpea samples

On the other hand, the ash content was determined as follows: raw chickpeas had 3.04%, germinated chickpeas had 2.72%, and fermented chickpeas had 2.05%. According to this, the lowest ash content was found in fermented chickpea samples ($p < 0.05$) (Table 2).



Table 2. *Ash content in chickpea samples according to processing methods*

<i>Processing Method</i>	<i>Ash Content (%)</i>
Raw	3.04
Germinated	2.72
Fermented	2.05 *

* $p < 0.05$

Based on the HPLC analysis results, the linoleic acid content in the three samples (raw, germinated, and fermented) is presented in Table 3 and Figure 6. According to these results, the amount of linoleic acid per gram of chickpea is as follows: 29.404 mg in raw chickpeas, 11.744 mg in germinated chickpeas, and 54.010 mg in fermented chickpeas (Raw > Germinated > Fermented).

Table 3. Linoleic acid content per gram of chickpea based on HPLC analysis

Process	1st Peak Area	Concentration (ppm)	Concentration (mg/100 mL)	Extracted from 70 g Chickpea (mg)	Linoleic Acid per Gram of Chickpea (mg)	Linoleic Acid Percentage (g/g)
Raw	1.801.050	2.058	205.825	2.058.251.273	29.404	0.2940
Germination	688.801	822	82.206	822.061.929	11.744	0.1174
Fermentation	3.351.042	3.781	378.096	3.780.962.278	54.010	0.5401

* $p < 0.05$ 

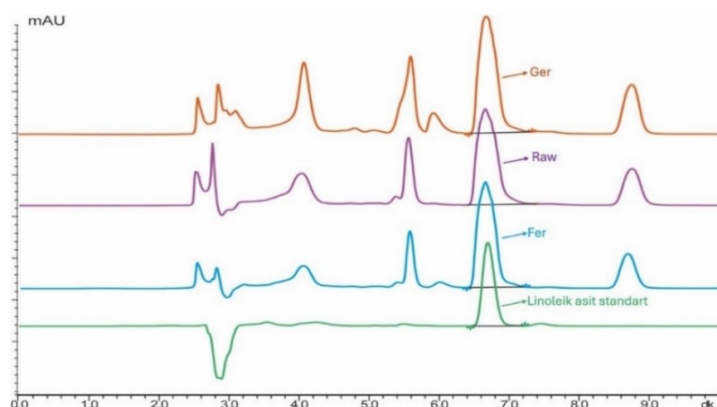


Figure 6. Comparison of the chromatograms of the samples

DISCUSSION

The total phenolic content and its composition are critical factors influencing antioxidant capacity. Numerous studies have investigated the specific phenolic compounds affecting the antioxidant capacity of foods and their extent (21-23). During germination, phenolic compounds can undergo polymerization, leading to significant structural changes in seeds. Dueñas et al. (24) reported that improvements in the phytochemical and nutritional quality of sprouted legumes are primarily due to changes in the composition of phenolic compounds and dietary fiber. Similarly, Gan et al. (25) found that the bound and soluble phenolic fractions in mung beans sprouted in a dark environment at 25°C changed daily, with phenolic content peaking on the 5th day.

Domínguez-Arispuro et al. (26) attributed the changes in total phenolic content after germination to the biosynthesis and release of phenolic compounds during this process. The continuous biosynthesis and release of phenolic compounds throughout the germination period explain the observed post-germination changes. Regarding fermentation, Juan and Chou (27) noted that the increase in phenolic content was due to the action of β -glucosidase and β -glucuronidase enzymes produced by *R. oligosporus*, which hydrolyze complex phenolics in legumes into soluble forms. Lin et al. (28) demonstrated that fermentation with two different



filamentous fungi significantly increased the total phenolic content and antioxidant activities in soybeans compared to non-fermented soybeans.

Supporting these findings, a study conducted in Muğla found that the total phenolic content of barley increased by 28.17% after germination (23). Sáez et al. (29) reported that fermentation was superior to soaking, germination, and cooking in enhancing the techno-functional properties of chickpea flour, producing doughs with 81% more total phenolic content and 64% more antioxidant activity compared to controls. Consistent with the literature (23-29), our study found significant increases in the total phenolic content of chickpea samples, with a 64% increase in germinated chickpeas and a 29% increase in fermented chickpeas (Figure 5). This increase post-germination is thought to be due to the *de novo* biosynthesis of phenols occurring on the embryonic axis of the chickpea seed.

In this study, the ash content of raw chickpeas, sprouted chickpeas, and fermented chickpeas was found to be 3.04%, 2.72%, and 2.05%, respectively. Similar results have been reported in the literature. For example, Ferreira et al. (30) found the ash content of chickpeas after 48 hours of germination to decrease from 3.3% in raw chickpeas to 3.0%. Elobuiké et al. (31) observed a reduction in ash content in mung beans during germination. Xu et al. (32) also noted a significant decrease in ash content in lentils post-germination, and Cornejo et al. (33) reported a reduction in ash content in brown rice after germination (raw: 2.85g/100g; germinated: 2.35g/100g). Similarly, Uppal and Bains (34) found that the ash content of mung beans increased with longer germination periods, reaching 8.8% after 20 hours. Adejuyitan et al. (35) reported a reduction in the ash content of tiger nut flour from 2.72% in the raw form to 2.37% after fermentation. The reduction in ash content observed during fermentation might be due to the utilization of essential salts by fermentation metabolites. Consistent with other studies, our findings indicate that the ash content decreases in chickpeas after germination and fermentation



(Table 2), possibly due to the leaching of minerals into the soaking water before germination.

Changes in linoleic acid content in legume seeds following fermentation and germination processes have been infrequently analyzed in scientific literature. To provide a comprehensive understanding, this study also includes a comparison of changes in fatty acid profiles in plant-based beverages post-fermentation and germination. Criste et al. (36) observed an increase in stearic acid content across all seed varieties after the germination process. It is suggested that germination and fermentation lead to a reduction in protein and fat content. The same study noted a significant presence of oleic acid in chickpeas, with values increasing post-germination but decreasing after fermentation with *L. plantarum*. Palmitoleic acid showed similar levels across all species and increased after fermentation. In the study by Criste et al. (36), high levels of linoleic acid (37.26 ± 2.24 mg/100 mL) were detected in beverages made from sprouted chickpeas, and these levels increased with fermentation. Among legumes, chickpeas had the highest linoleic acid content, which significantly increased with fermentation. Similarly, lupin seed beverage showed a significant increase in linoleic acid content after fermentation with *L. plantarum*. Conversely, linoleic acid content decreased in oat beverages, a popular plant-based milk, after fermentation.

In our study, we compared the linoleic acid content of germinated, fermented, and raw chickpeas. The linoleic acid content per gram of chickpeas was found to be 2.9404 mg in raw chickpeas, 1.1744 mg in sprouted chickpeas, and 0.5401 mg in fermented chickpeas (Raw > sprouted > fermented) (Table 3). The observed decreases in fatty acid content and linoleic acid may result from bacterial utilization, conversion to alternative metabolites, and interactions with other microorganisms.



CONCLUSIONS

Fermentation and germination processes significantly alter the linoleic acid content, total phenolic content, and antioxidant capacity of chickpeas compared to their raw form. With the increasing global interest in natural and additive-free foods, sprouted and fermented chickpeas offer a valuable alternative for consumers. Although a decrease in antioxidant capacity was observed post-germination and fermentation, this may be due to the type of chickpea and the reactions occurring during the process. Optimal germination conditions lead to the highest levels of total phenolic content, enhancing the functional properties and health benefits of chickpeas. Similar increases were noted in fermented chickpeas, making these processes beneficial for improving nutritional value.

Given the cost-effectiveness and simplicity of germination, its adoption can be widely promoted. The natural fermentation of foods, depending on the type and duration, also offers significant benefits. The inclusion of sprouted and fermented foods in markets could positively impact the Turkish economy. Despite a slight decrease in linoleic acid content following these processes, further research on the fatty acid profiles and health benefits of sprouted and fermented chickpeas grown in Turkey is recommended to fully understand and maximize their potential.

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Author contributions: Conception and design of the study: ŞS, SM, Tİ. Acquisition of data: ŞS, Tİ. Analysis and interpretation of data: ŞS, SM, Tİ. Drafting the article: ŞS, SM, Tİ. Critical revising: ŞS, SM. Final approval: ŞS, SM.

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