

Article

Effect of Germination on Antioxidant Capacity and Protein Characteristics of Chickpea (*Cicer arietinum* L.) with Implications for Food Processing Applications

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Featured Application

Germinated chickpea with enhanced antioxidant capacity and improved protein-related characteristics may be used as a value-added plant-based ingredient for food processing applications requiring higher protein solubility and reduced phytic acid.

Abstract

This study investigated the effects of germination on the antioxidant capacity and protein characteristics of chickpea (*Cicer arietinum* L.) with a focus on processing-induced biochemical and structural modifications relevant to food applications. Raw, soaked, and germinated chickpeas were comparatively analyzed to evaluate integrated changes in antioxidant activity, protein composition, amino acid profiles, and anti-nutritional factor. Antioxidant-related properties were assessed using total phenolic content (TPC), total flavonoid content (TFC), 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, Trolox equivalent antioxidant capacity (TEAC), and ferric-reducing antioxidant power (FRAP), while protein-related characteristics including soluble protein, amino acids, and sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) profiles were examined alongside phytic acid content. Germination significantly enhanced antioxidant capacity, with TPC increasing from 0.91 to 1.64 mg GAE/g, and DPPH, TEAC, and FRAP values also markedly elevated compared with raw samples. Soluble protein content increased from 72.79 to 82.86 mg/g, while phytic acid content decreased from 92.49 to 59.49 mg/g. Free amino acids, particularly alanine, histidine, and phenylalanine, showed substantial increases following germination. SDS–PAGE and densitometric analysis revealed a redistribution of protein fractions, characterized by a reduced intensity of high-molecular-weight protein bands and a relative increase in intermediate molecular weight proteins, indicating partial proteolysis and structural modification of storage protein. These findings demonstrate that germination acts as a biochemical processing step that enhances antioxidant potential, improves protein-related nutritional attributes, and reduces antinutritional factors, supporting the use of germinated chickpea as a functional plant-based ingredient for food processing applications.



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Keywords: chickpea; germination; antioxidant capacity; protein solubility; phytic acid

1. Introduction

As a globally significant pulse crop, the chickpea (*Cicer arietinum* L.) represents a nutrient-dense plant resource providing a complex matrix of carbohydrates, high-quality proteins, and essential micronutrients [1]. In particular, the protein fraction of chickpea has gained considerable research interest as a plant-based substitute for animal proteins due to its comparatively high protein content and well-balanced amino acid composition relative to other leguminous crops [2]. With the growing global demand for plant-based diets and environmentally sustainable food production, chickpea has emerged as a viable candidate raw material for the development of functional ingredients and value-added food products [3,4]. The physicochemical and functional properties of chickpea proteins in food systems are largely influenced by their storage globulin composition, particularly vicilin (7S) and legumin (11S), which serve as key structural determinants of their functionality [5,6].

Despite these nutritional advantages, the effective utilization of chickpea in food processing remains constrained by several intrinsic factors. The presence of anti-nutritional compounds such as phytic acid and trypsin inhibitors, restricts mineral bioavailability and protein digestibility [7]. Moreover, interactions between storage proteins and anti-nutritional compounds within the seed matrix may restrict protein extractability and functional performance during processing [5,6,8]. To overcome these limitations, various processing methods, including physical, biochemical, and thermal treatments, have been explored to modify protein structure and enhance nutritional and functional quality [7]. Among these methods, germination is considered a simple and cost-effective bioprocess that induces biochemical and structural changes in legume seeds without the use of external additives [2].

Germination involves the controlled hydration of seeds followed by sprouting, during which endogenous enzymes such as proteases, amylases, and phytases are activated [9,10]. These enzymes promote the enzymatic hydrolysis and mobilization of storage macromolecules, resulting in the release of low-molecular-weight proteins, peptides, free amino acids, and bioactive compounds [11,12]. Such enzymatic mobilization contributes to improved protein digestibility, a reduction in anti-nutritional factors, and an enhanced availability of bioactive compounds [13]. As a result, germination is widely recognized as an effective processing method for enhancing the nutritional quality of legumes through improved digestibility and reduced antinutritional factors [14].

Recent studies have demonstrated that germination significantly enhances the antioxidant capacity of legumes by promoting the release of bound phenolic compounds due to enzymatic hydrolysis and increasing the availability of bioactive constituents [13,15–17]. In chickpeas, germination has been reported to increase the total phenolic and flavonoid contents, leading to enhanced radical scavenging activity and reducing power [15,16]. Similar increases in antioxidant capacity have also been observed in other legumes, such as lentils and peas, suggesting that germination-induced enhancement of antioxidant properties is a common phenomenon among legume seeds [13,17]. These improvements are particularly relevant for food processing, as enhanced antioxidant capacity contributes to oxidative stability and the quality of plant-based food products [18]. However, despite the well-documented nutritional improvements associated with germination, limited information is available on the structural modifications of chickpea storage proteins and their relationship with physicochemical and functional properties during processing.

Beyond simple compositional shifts, germination induces significant structural modifications in storage proteins. The activation of endogenous proteases during germination leads to the partial hydrolysis of high-molecular-weight globulins into smaller peptide and protein fragments, thereby altering the solubility and interfacial functionality of the proteins [7,16]. Such protein structural modification is of particular importance for food

processing applications, as it influences key properties including the hydration, emulsification, and digestibility of plant-based ingredients [8,18]. Therefore, it is essential to understand germination-induced protein structural changes for evaluating the suitability of chickpeas as a functional material in food formulations.

Although the effects of germination on the individual nutritional components of chickpea have been reported, integrated investigations linking antioxidant capacity, protein structural characteristics, amino acid composition, and anti-nutritional factors under comparable processing conditions remain limited. From a food processing perspective, comparisons of raw, soaked, and germinated chickpeas that address both compositional changes and protein structural modifications have received limited attention. In particular, the relationship between germination-induced protein redistribution and its implications for functional performance in food systems has not been sufficiently elucidated. Therefore, this study evaluated the effects of germination on antioxidant properties, protein characteristics, amino acid composition, and phytic acid content by comparing raw, soaked, and germinated chickpeas. By integrating compositional and structural analyses, this study aims to provide mechanistic insight into processing-induced modifications in chickpea and to provide fundamental data for understanding processing-induced modifications in chickpea.

2. Materials and Methods

2.1. Materials

Dried chickpea (*Cicer arietinum* L.) was purchased from a local retail market in Changwon-si, Republic of Korea. The seeds were stored at room temperature until analysis. The samples utilized in this study were mature, post-harvest dried seeds suitable for food-grade applications.

2.2. Sample Preparation

Raw, soaked, and germinated chickpeas were prepared under germination conditions previously optimized using a three-factor Box–Behnken design (BBD) in our previous work [10]. Briefly, optimization was conducted to maximize antioxidant activities by evaluating the effects of soaking time, germination temperature, and duration, with model significance verified by ANOVA ($p < 0.5$). Based on the optimized parameters, chickpea seeds (250 g) were first surface-sterilized by immersion in 0.07% (v/v) sodium hypochlorite solution (1 L) for 30 min, subsequently washed with distilled water until the rinse water reached approximately pH 7, and then submerged in distilled water (1 L) for 6 h at room temperature. The soaked seeds were wrapped in a moist cotton cloth, placed on a sieve, and germinated at 30 °C for 6 days. Following preparation, all samples were freeze-dried, milled to powder, and stored at −70 °C prior to analyses. Freeze-drying was performed to minimize enzymatic degradation and preserve the stability of proteins and antioxidant compounds prior to analytical measurements.

2.3. Sample Extraction

Freeze-dried sample powders (1 g) were subjected to extraction using 70% (v/v) ethanol (5 mL) in a thermostatically controlled water bath at 65 °C for 3 h. Following extraction, the mixtures were centrifuged at $1628 \times g$ for 10 min, after which the resulting supernatants were carefully collected, filtered through a 0.45 µm membrane filter, and subsequently subjected to analytical measurements.

2.4. Determination of Antioxidant Properties

2.4.1. Total Phenolic Content (TPC)

The Folin–Ciocalteu colorimetric method was employed to determine the TPC of each sample [19]. Chickpea extract (2 μ L) was combined with 10% Folin–Ciocalteu reagent (Sigma-Aldrich, St. Louis, MO, USA) and 20% sodium carbonate solution and allowed to react at room temperature for 2 h. Absorbance was read at 765 nm, and results were expressed using a gallic acid calibration curve.

2.4.2. Total Flavonoid Content (TFC)

TFC was determined following the method described by Aung et al. [20]. A reaction mixture was prepared by combining 0.1 mL of extract with 1.25 mL of distilled water and 75 μ L of 5% NaNO₂ and allowed to stand for 6 min. Subsequently, 150 μ L of 10% AlCl₃ was introduced, and the reaction was allowed to proceed for a further 5 min, after which 0.5 mL of 1 N NaOH was added to terminate the reaction. The absorbance of the final solution was measured at 510 nm. (+)-Catechin hydrate was used to generate the standard curve.

2.4.3. DPPH Radical Scavenging Activity

DPPH scavenging activity was measured as described previously [10]. An aliquot of extract (5 μ L) was combined with 0.1 mM DPPH solution (Sigma-Aldrich, St. Louis, MO, USA) (195 μ L) and the mixture was allowed to react for 30 min under dark conditions at room temperature. The optical absorbance was subsequently measured at 515 nm, with Trolox serving as the reference standard for quantification.

2.4.4. Trolox Equivalent Antioxidant Capacity (TEAC)

TEAC was evaluated following the protocol described by Re et al. [21]. To generate the ABTS⁺ radical cation, 1.7 mM ABTS (Sigma-Aldrich, St. Louis, MO, USA) was reacted with 2.6 mM K₂S₂O₈ (Sigma-Aldrich, St. Louis, MO, USA), and the mixture was incubated in the dark at ambient temperature for 16 h. The prepared stock solution was then diluted with methanol until an absorbance value of 0.70 ± 0.02 was obtained at 734 nm. Subsequently, 40 μ L of each extract was added to the diluted ABTS⁺ solution, and the absorbance was recorded at 734 nm following a 7 min reaction period. TEAC values were calculated using Trolox as the reference standards.

2.4.5. Ferric-Reducing Antioxidant Power (FRAP)

FRAP was evaluated using the procedure reported by Benzie and Strain [22]. The FRAP working reagent was freshly prepared immediately before use by mixing 10 mM TPTZ (Sigma-Aldrich, St. Louis, MO, USA), 20 mM FeCl₃ (Sigma-Aldrich, St. Louis, MO, USA), and 0.3 M acetate buffer (pH 3.6) in a volumetric ratio of 1:1:10. A 10 μ L aliquot of each extract was then mixed with 40 μ L of the FRAP reagent and incubated at 37 °C for 10 min, after which absorbance was determined at 539 nm. A calibration curve was constructed using FeSO₄·7H₂O as the standard.

2.5. Soluble Protein Content

The quantification of soluble protein was performed using a modified Lowry method [23]. Briefly, 0.5 g of the chickpea sample was extracted with 0.2 M phosphate buffer (pH 7.5). The resulting mixture was centrifuged at $5455 \times g$ for 20 min, which was subsequently collected for further analysis. An aliquot (0.1 mL) was mixed with 2 N NaOH (0.1 mL) and heated at 100 °C for 10 min. After cooling, alkaline copper reagent (1 mL; 2% Na₂CO₃, 1% CuSO₄, and 2% sodium potassium tartrate mixed at 100:1:1, *v/v/v*) and Folin's reagent (0.1 mL) were added. After 45 min of reaction, absorbance was measured at 750 nm. Bovine serum albumin was used as the standard.

2.6. Total Phytic Acid Content

Phytic acid content was determined according to the method of Summo et al. with minor modifications [24]. Chickpea flour (0.5 g) was extracted with 2.4% (v/v) HCl (10 mL) by shaking at 200 rpm for 1 h, followed by centrifugation at $8000\times g$ for 20 min at 10 °C. Sodium chloride (0.05 g) was subsequently introduced into the supernatant, and the resulting mixture was kept at −20 °C for 20 min prior to a second centrifugation carried out under identical conditions. An aliquot of the supernatant (1.5 mL) was then mixed with Wade reagent (0.5 mL; 0.03% $\text{FeCl}_3\cdot 6\text{H}_2\text{O}$ and 0.3% sulfosalicylic acid) and centrifuged at $8000\times g$ for 10 min. Absorbance was measured at 500 nm, and sodium phytate was used to generate the calibration curve.

2.7. Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis (SDS–PAGE)

2.7.1. Protein Isolation

Protein isolates were prepared following the method of Tanger et al. [25]. Samples (5 g) were dispersed in distilled water (75 mL), and the pH was adjusted to 9.5 using 1 M NaOH. The suspension was stirred at 35 °C for 1 h and centrifuged at $4500\times g$ for 20 min at 4 °C. The supernatant was neutralized to pH 7.0 using 1 M HCl, freeze-dried, and used for SDS–PAGE.

2.7.2. SDS–PAGE

SDS–PAGE was conducted according to the protocol of Wang et al. [26] using a 4% stacking gel and a 12% resolving gel. Protein isolate (4 mg) was dissolved in distilled water to which $4\times$ Laemmli buffer and 10% (v/v) 2-mercaptoethanol were subsequently added. The resulting mixture was then denatured by heating at 100 °C for 5 min. A volume of 10 μL of each prepared sample was applied to each lane, and electrophoretic separation was performed at 80 V for 30 min, followed by 110 V for 90 min. After separation, gels were stained with Coomassie Brilliant Blue R-250 (Bio-Rad, Hercules, CA, USA) and compared using Precision Plus Protein™ Dual Xtra Standards.

The stained gels were scanned, and densitometric analysis was performed using ImageJ software 1.54g (National Institutes of Health, Bethesda, MD, USA). Band intensities were quantified, and the relative distribution of protein fractions across defined molecular weight ranges was calculated based on the proportion of band intensity within each range relative to the total lane intensity.

2.8. Free Amino Acid Composition

Free amino acids were analyzed according to the procedure by Muttucumaru et al. [27]. Chickpea samples (1 g) were extracted/hydrolyzed with 0.1 N HCl (5 mL), followed by centrifugation at $8000\times g$ for 15 min. The supernatants were filtered using a 0.45 μm membrane filter and analyzed using the Waters AccQ-Tag HPLC method (Waters Corporation, Milford, MA, USA) (PDA detector; AccQ-Tag column (Waters Corporation, Milford, MA, USA) at 37 °C). The mobile phase system consisted of acetate–phosphate buffer, acetonitrile, and water. Quantification was performed using calibration curves prepared with 18 amino acid standards (Waters Corporation, Milford, MA, USA).

2.9. Total Amino Acid Content

Total amino acids were determined using a ninhydrin-based colorimetric method described by Aung et al. [28]. Chickpea extracts were reacted with phosphate buffer (pH 8.9) and ninhydrin reagent (Sigma-Aldrich, St. Louis, MO, USA) containing tin(II) chloride and heated at 100 °C for 15 min. After cooling, the reaction volume was adjusted

to 25 mL with distilled water. Absorbance was measured at 570 nm, and total amino acids were quantified using L-theanine (Sigma-Aldrich, St. Louis, MO, USA) as the standard.

2.10. Statistical Analysis

All experiments were independently conducted in triplicate, and results are reported as the mean $\pm$ standard deviation. Statistical evaluation was carried out using XLSTAT 2024.3.0 (Addinsoft, Paris, France). Differences among treatment groups were assessed by one-way ANOVA, with post hoc comparisons performed using Duncan's multiple range test. A significance threshold of $p < 0.05$ was applied throughout, with higher levels of significance additionally denoted at $p < 0.01$ and $p < 0.001$.

3. Results and Discussions

3.1. Antioxidant-Related Bioactive Compounds and Scavenging Activities

To assess antioxidant-related bioactive compounds and radical scavenging activities, raw chickpea (RC), soaked chickpeas (SC), and germinated chickpeas (GC) were characterized using five parameters: total phenolic content (TPC), total flavonoid content (TFC), DPPH radical scavenging activity, Trolox equivalent antioxidant capacity (TEAC), and ferric-reducing antioxidant power (FRAP) (Table 1). In general, RC and SC displayed similar levels of antioxidant activity, while GC demonstrated markedly higher values relative to both RC and SC ($p < 0.05$ or $p < 0.001$, depending on the measured parameter).

Table 1. Influence of soaking and germination on the total phenolic, flavonoid contents, and antioxidant capacities of chickpea (*Cicer arietinum* L.).

Sample	TPC *** (mg GAE/g)	TFC * (mg CE/g)	DPPH * (μ M TE/g)	TEAC *** (mM TE/g)	FRAP *** (mg FeSO ₄ /g)
RC	0.91 $\pm$ 0.08 ^b	0.34 $\pm$ 0.01 ^b	2.18 $\pm$ 0.08 ^b	3.71 $\pm$ 0.07 ^b	0.09 $\pm$ 0.00 ^b
SC	0.82 $\pm$ 0.03 ^b	0.33 $\pm$ 0.00 ^b	2.33 $\pm$ 0.10 ^b	3.67 $\pm$ 0.12 ^b	0.09 $\pm$ 0.01 ^b
GC	1.64 $\pm$ 0.03 ^a	0.43 $\pm$ 0.03 ^a	3.05 $\pm$ 0.24 ^a	5.98 $\pm$ 0.01 ^a	0.38 $\pm$ 0.01 ^a

GAE, gallic acid equivalent; CE, catechin equivalent; TE, Trolox equivalent. Data are presented as mean $\pm$ standard deviation of triplicate measurements. Superscript letters within each column denote statistically distinct groups based on Duncan's multiple range test. Asterisks indicate levels of statistical significance: *, and *** correspond to $p < 0.05$, and $p < 0.001$, respectively.

Soaking had a limited effect on the antioxidant properties, as RC and SC did not differ significantly in TPC, TFC, DPPH, TEAC, or FRAP (Table 1). In contrast, germination markedly enhanced both the phenolic content and antioxidant activity. TPC increased from 0.91 $\pm$ 0.08 mg GAE/g (RC) to 1.64 $\pm$ 0.03 mg GAE/g (GC), and TEAC increased from 3.71 $\pm$ 0.07 mM TE/g (RC) to 5.98 $\pm$ 0.01 mM TE/g (GC) ($p < 0.001$). Notably, FRAP increased from 0.09 $\pm$ 0.00 mg FeSO₄/g (RC) to 0.38 $\pm$ 0.01 mg FeSO₄/g (GC), indicating a substantial improvement in reducing capacity after germination ($p < 0.001$) (Table 1). These results demonstrate that germination is more effective than soaking in improving antioxidant properties of chickpea, consistent with previous studies reporting increased phenolic content and antioxidant activity after chickpea germination [15,16]. Soaking alone did not markedly enhance antioxidant capacity, which may be attributed to the leaching of water-soluble phenolic compounds into the soaking medium and partial oxidative degradation during hydration.

The enhancement in antioxidant properties following germination may be associated with metabolic activation and improved extractability of phenolic compounds during sprouting. Germination can activate endogenous enzymes and promote the release of conjugated or bound phenolics into more extractable forms, thereby contributing to increased antioxidant activity [29]. Previous chickpea-based studies have similarly reported

that germination enhances antioxidant capacity through activation of phenylpropanoid metabolism and the enzymatic hydrolysis of cell wall bound phenolic compounds [18,30]. From a food processing perspective, germination can therefore be considered as a practical bioprocessing strategy rather than a simple hydration step, as sprouting induces enzymatic biotransformation, matrix softening, and the release of bioactive compounds relevant to functional ingredient development [31,32]. Therefore, the present results support the use of germination as a practical processing strategy for producing chickpea-based ingredients with improved antioxidant-related functionality for food applications. From a food processing perspective, germination represents a practical, low-input bioprocess that enhances antioxidant functionality and nutritional quality through endogenous enzymatic modification, without requiring chemical treatments or complex processing operations, thereby supporting its application in chickpea-based ingredient development.

3.2. Contents of Soluble Protein and Phytic Acid

Soluble protein and phytic acid contents of RC, SC, and GC are presented in Figure 1. Soluble protein content significantly increased after soaking and germination ($p < 0.01$). RC showed the lowest soluble protein content (72.79 ± 1.68 mg/g), whereas SC (81.91 ± 1.32 mg/g) and GC (82.86 ± 1.61 mg/g) exhibited significantly higher values (Figure 1). However, no significant difference was observed between SC and GC, indicating that the increase in soluble protein occurred mainly during the soaking step, while additional germination under the present conditions resulted in only a marginal change.

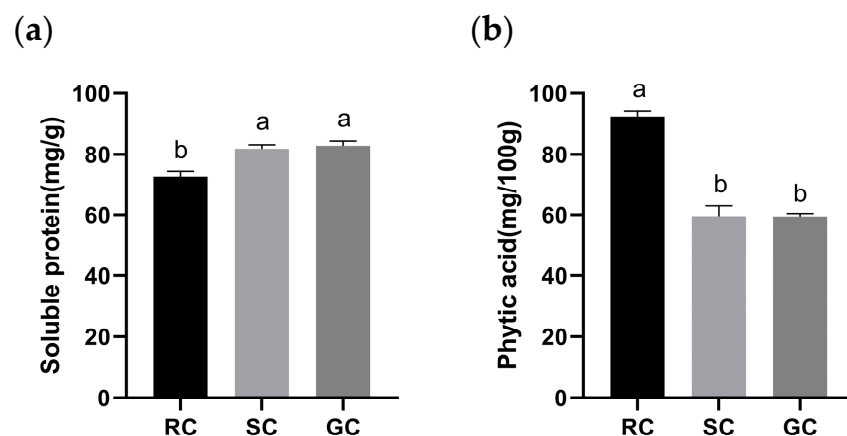


Figure 1. Soluble protein (a) and phytic acid contents (b) of chickpea (*Cicer arietinum* L.) subjected to soaking and germination. RC, raw chickpea; SC, soaked chickpea; GC, germinated chickpea. Different letters above the bars indicate significant differences ($p < 0.01$ and $p < 0.001$ for soluble protein and phytic acid, respectively).

Similar trends have been reported in chickpea, where hydration and early-stage sprouting promoted protein solubilization and extractability [33]. The observed increase is therefore more likely attributable to improved extractability rather than de novo protein synthesis. Hydration-induced swelling of the seed matrix and partial unfolding of storage proteins can enhance protein dispersibility and extraction efficiency [15]. In addition, germination-induced activation of endogenous proteases promotes the enzymatic breakdown of globulins into low-molecular-weight fragments. These fragments might enhance solubility, thereby increasing the total soluble protein content while maintaining overall protein integrity [17].

Phytic acid content showed a significant decrease after soaking and germination ($p < 0.001$). RC exhibited the highest phytic acid level (92.49 ± 1.80 mg/100 g), whereas SC (59.58 ± 3.47 mg/100 g) and GC (59.49 ± 0.93 mg/100 g) showed significantly lower

values (Figure 1). Notably, phytic acid decreased by approximately 36% in GC compared to RC, indicating that soaking and germination effectively reduced the phytate content. Since SC and GC were not significantly different, most of the reduction appeared to occur during soaking, with germination contributing limited additional reduction under the present conditions. This trend is supported by other studies showing that soaking conditions (e.g., solution composition and pH) can strongly influence phytate reduction through the leaching of soluble phytate and activation of endogenous phytase, potentially leading to a plateau effect before or during early germination [34].

The reduction in phytic acid after soaking and germination can be attributed to both the leaching of water-soluble phytate during soaking and the activation of endogenous phytase during hydration and germination, which hydrolyzes phytate into lower inositol phosphates and inorganic phosphate [17]. Similar decreases in phytic acid content during germination have been reported for chickpea and other pulses, supporting sprouting-based processing as an effective approach for reducing antinutritional factors [35]. Such phytate degradation is nutritionally important because it improves mineral bioavailability and reduces anti-nutritional interactions with proteins and digestive enzymes [6,31].

Taken together, the simultaneous increase in soluble protein content and decrease in phytic acid suggests that soaking and subsequent germination induced biochemical modifications by endogenous enzymes in chickpea, which may be associated with changes in protein structure. Therefore, an analysis of the molecular weight distribution of chickpea proteins during germination was conducted to further examine germination-induced alterations in protein profile and molecular weight distribution.

3.3. Changes in Molecular Weight Distribution of Chickpea Proteins

SDS-PAGE was performed to examine changes in the molecular weight distribution of chickpea proteins after soaking and germination (Figure 2). Overall, protein bands were mainly detected in the range of approximately 20–100 kDa across all samples. RC and SC exhibited similar electrophoretic patterns, with prominent bands distributed in the mid-molecular-weight region, particularly between 25 and 70 kDa. In GC, a comparable band distribution was observed; however, several bands in the higher-molecular-weight region (around ≥ 70 kDa) appeared relatively less intense, while bands in the lower-molecular-weight region (approximately 25–65 kDa) were comparatively more pronounced (Figure 2, Table 2).

Table 2. Relative distribution of protein fractions across molecular weight regions based on SDS-PAGE densitometric analysis.

MW Ranges	RC (%)	SC (%)	GC (%)
≥ 70 kDa	9.60	12.06	10.32
40–65 kDa	15.59	15.08	17.29
25–45 kDa	29.79	28.19	30.72
15–25 kDa	39.36	34.46	34.69
<15 kDa	5.66	10.21	6.98

Values represent the percentage of band intensity within each molecular weight range relative to total lane intensity.

To further examine these electrophoretic changes, densitometric analysis of the SDS-PAGE gels was performed using ImageJ software. The redistribution of protein fractions observed in GC (Figure 2) reflects the targeted proteolysis of major chickpea storage globulins. According to Tan et al. (2022), vicilin (7S) and legumin (11S) subunits are the predominant protein fractions in chickpea that form a physical barrier around starch granules, modulating nutrient accessibility [36]. The reduction in the intensity of high-molecular-weight bands (>7 kDa) and the concomitant increase in the 25–45 kDa range suggest the structural remodeling of these specific globulins. Specifically, the degradation of

the ~50–75 kDa vicilin and ~40 kDa acidic legumin subunits indicates that endogenous proteases mobilized these nitrogen reserves during germination, facilitating the transition of protein into more intermediate molecular weight polypeptides rather than extensive degradation. This targeted modification is consistent with the findings of Tan et al. (2022) [36], who mapped these subunits to the protein–starch matrix inherent to *Cicer arietinum* L.

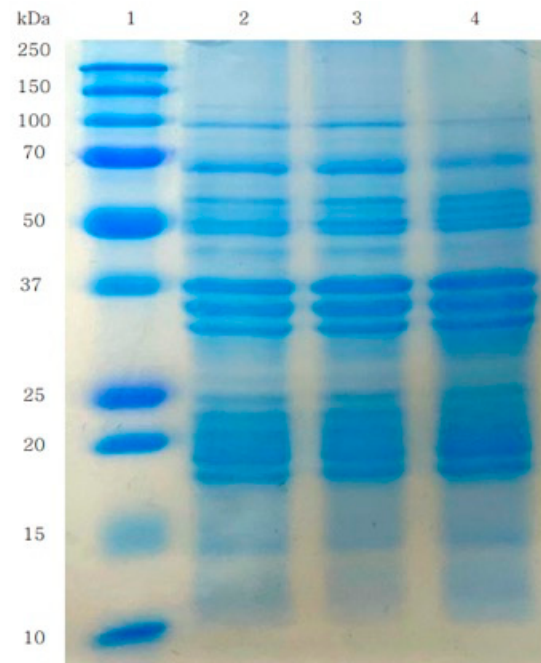


Figure 2. SDS–PAGE pattern of raw, soaked, and germinated chickpea. Lane 1, molecular weight standards; Lane 2, raw chickpea; Lane 3, soaked chickpea; Lane 4, germinated chickpea with optimal germination condition.

These observations are consistent with previous findings indicating that germination activates endogenous proteolytic enzymes, resulting in partial hydrolysis of storage proteins and altered electrophoretic patterns. Sofi et al. (2023) reported that germination time significantly influenced the physicochemical and electrophoretic properties of chickpea protein isolates, with band intensity changes reflecting protein degradation and structural modification during sprouting [15]. Similarly, Chandrasekaran and de Mejia (2022) demonstrated that germination led to decreased intensity of higher-molecular-weight protein bands and increased contribution of proteins below approximately 75 kDa depending on germination duration [37]. Germination-driven proteolysis and matrix softening have also been associated with enhanced protein extractability and the functional modification of legume flours [17,38].

Recent proteomic studies further support that major SDS–PAGE bands observed in chickpea seeds correspond primarily to vicilin (7S) and legumin (11S) storage proteins and their subunits, which undergo structural remodeling during germination [30,31]. Redistribution of protein fractions across molecular weight ranges has therefore been interpreted as a reflection of storage protein mobilization and partial proteolysis rather than nonspecific electrophoretic variation [30].

To support interpretation of the major SDS–PAGE bands, a representative gel band excised from RC (Lane 2) was additionally analyzed by MALDI–TOF/TOF (Table S1 and Figure S2). The obtained spectra indicated peptide signals consistent with typical chickpea seed storage proteins, including vicilin- and legumin-type globulins and albumin-related proteins. The representative MALDI–TOF/TOF spectra and identification summary are provided in the Supplementary Materials (Figure S2 and Table S1). The redistribution

of molecular weight fractions observed in the densitometric analysis can therefore be attributed to the modification and mobilization of these storage proteins during soaking and germination.

From a functional perspective, germination-induced proteolysis has been associated with changes in protein solubility, digestibility, and interfacial functionality in pulse-based food systems. Partial hydrolysis of storage proteins can generate intermediate poly peptides that contribute to improved functionality, whereas excessive degradation may lead to a loss of structural integrity [8,18]. The redistribution pattern observed in GC is therefore consistent with moderate structural modification rather than extensive protein breakdown.

In addition, soaking and germination have been reported to increase the soluble protein and free amino acid contents in legumes, which may influence both flavor development and potential bioactivity [13]. Therefore, the protein redistribution observed in SDS-PAGE and densitometry may be accompanied by the increased availability of low-molecular-weight nitrogenous compounds, providing a biochemical basis for processing-induced functional and nutritional changes.

These findings indicate that soaking and germination induce the structural reorganization of chickpea storage proteins through partial proteolysis and molecular weight redistribution, highlighting the potential of germinated chickpea as a plant-based ingredient for food processing applications.

3.4. Free Amino Acid Composition

The free amino acid (FAA) compositions of RC, SC, and GC are shown in Table 3. Overall, soaking resulted in limited changes, whereas germination led to a marked accumulation of several FAAs, suggesting enhanced proteolysis and metabolic turnover during sprouting, which has been widely associated with the mobilization of storage proteins in legumes [16,38].

Table 3. Free amino acid compositions of raw, soaked, germinated chickpea (*Cicer arietinum* L.).

Amino Acids (mg/100 g)	RC	SC	GC
Alanine ***	0.82 ± 0.13 ^b	0.92 ± 0.05 ^b	4.70 ± 0.40 ^a
Arginine **	116.99 ± 4.80 ^a	128.06 ± 8.48 ^a	92.81 ± 4.58 ^b
Aspartic acid ***	1.04 ± 0.09 ^a	0.08 ± 0.03 ^c	0.65 ± 0.05 ^b
Cystine	0.11 ± 0.02 ^a	0.07 ± 0.01 ^a	ND
Glutamate	10.57 ± 0.21 ^a	10.30 ± 0.66 ^{ab}	10.61 ± 0.27 ^a
Glycine *	1.29 ± 0.10 ^b	1.45 ± 0.07 ^b	1.63 ± 0.11 ^a
Histidine ***	2.45 ± 0.02 ^b	3.34 ± 0.21 ^b	17.97 ± 0.97 ^a
Isoleucine ***	0.35 ± 0.02 ^b	0.48 ± 0.07 ^b	1.29 ± 0.07 ^a
Leucine ***	0.39 ± 0.01 ^c	0.90 ± 0.11 ^b	1.59 ± 0.06 ^a
Lysine *	1.68 ± 0.08 ^b	1.70 ± 0.10 ^b	2.02 ± 0.11 ^a
Methionine *	0.61 ± 0.03 ^c	0.87 ± 0.09 ^b	1.22 ± 0.08 ^a
Phenylalanine ***	0.53 ± 0.03 ^c	1.46 ± 0.01 ^b	3.09 ± 0.16 ^a
Proline **	5.35 ± 0.37 ^c	8.28 ± 0.86 ^b	10.74 ± 0.60 ^a
Serine ***	3.19 ± 0.04 ^b	3.31 ± 0.13 ^b	8.09 ± 0.12 ^a
Threonine ***	2.26 ± 0.04 ^c	2.26 ± 0.13 ^b	7.22 ± 0.12 ^a
Tyrosine ***	0.51 ± 0.02 ^c	1.34 ± 0.11 ^b	3.26 ± 0.20 ^a
Valine ***	0.53 ± 0.01 ^c	0.74 ± 0.09 ^b	2.57 ± 0.09 ^a

Data are presented as the mean ± standard deviation of triplicate measurements. Superscript letters within each column denote statistically distinct groups based on Duncan's multiple range test. Asterisks indicate levels of statistical significance: *, **, and *** correspond to $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. ND, not detected.

In SC, moderate increases were observed in several amino acids. For example, leucine increased from 0.39 ± 0.01 mg/100 g (RC) to 0.90 ± 0.11 mg/100 g (SC), and phenylalanine increased from 0.53 ± 0.03 mg/100 g (RC) to 1.46 ± 0.01 mg/100 g (SC) (Table 3). Proline and tyrosine also increased to 8.28 ± 0.86 mg/100 g and 1.34 ± 0.11 mg/100 g, respectively (Table 3). In contrast, GC showed a substantially higher FAA pool for multiple amino acids. Alanine increased to 4.70 ± 0.40 mg/100 g, and histidine exhibited a pronounced increase to 17.97 ± 0.97 mg/100 g compared with 0.82 ± 0.13 mg/100 g and 2.45 ± 0.02 mg/100 g in RC, respectively (Table 3). In addition, several amino acids relevant to nutritional quality and processing performance increased after germination, including threonine (7.22 ± 0.12 mg/100 g), serine (8.09 ± 0.12 mg/100 g), valine (2.57 ± 0.09 mg/100 g), and phenylalanine (3.09 ± 0.16 mg/100 g) (Table 3). Meanwhile, arginine decreased from 116.99 ± 4.80 mg/100 g (RC) to 92.81 ± 4.58 mg/100 g (GC), which may reflect its utilization as a nitrogen reserve during germination metabolism [39]. Cystine, detected in RC and SC, was not detected in GC, indicating a possible shift in sulfur amino acid availability during sprouting (Table 3).

The increase in free amino acids after germination can be explained by activation of endogenous proteases and peptidases, which hydrolyze storage proteins such as vicilin and legumin into smaller peptides and free amino acids to support seedling growth [8,18]. During germination, liberated amino acids are not only metabolic substrates but also participate in biosynthetic pathways associated with energy production, enzyme synthesis, and cellular differentiation, resulting in dynamic redistribution of the FAA profile [13,30]. In particular, the accumulation of alanine, histidine, and branched-chain amino acids suggests enhanced protein turnover and active nitrogen remobilization, which are typical biochemical events during early seed germination [32]. From a nutritional perspective, the increase in essential amino acids such as threonine, valine, and leucine may contribute to the improved protein quality and digestibility of germinated chickpea products [31].

Thus, these results support that germination under optimized conditions promoted the release and accumulation of FAAs, consistent with recent evidence that chickpea germination modifies protein structure and functionality through enzymatic activity and that germinated legume flours may provide improved nutritional and functional attributes for food applications [16,38].

3.5. Total Amino Acid Content

Total amino acid (TAA) contents are summarized in Figure 3. TAA significantly increased across processing stages ($p < 0.001$), with GC exhibiting the highest value (445.05 ± 1.15 mg/100 g), followed by SC (380.07 ± 0.90 mg/100 g) and RC (310.81 ± 0.25 mg/100 g) (Figure 3). The increase in TAA after germination is consistent with the overall improvement in amino acid availability and protein-related functionality reported for chickpea and other legumes after sprouting [16,38].

When interpreted together with the FAA (Table 2), the results suggest that germination not only increased the overall amino acid-related content but also shifted amino acids toward a more readily available free form. This pattern aligns with previous reports showing that germination induces protein modification and may contribute to improved nutritional quality and processing suitability of chickpea-based ingredients [16,40].

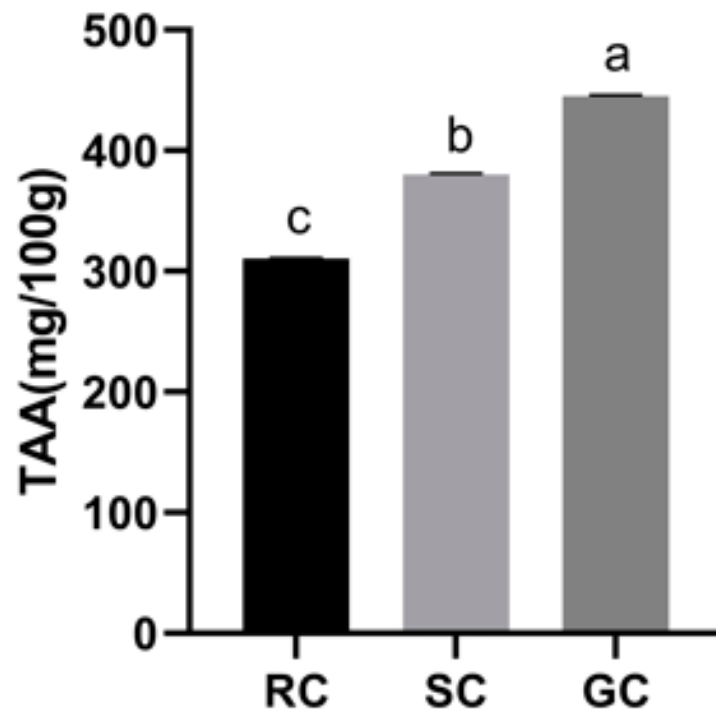


Figure 3. Total amino acid content of raw, soaked, germinated chickpeas. RC, raw chickpea; SC, soaked chickpea; GC, germinated chickpea; Different letters above the bars indicate significant differences ($p < 0.05$).

4. Conclusions

This study demonstrated that soaking and germination induced significant biochemical and structural modifications in chickpea (*Cicer arietinum* L.), influencing antioxidant properties, protein characteristics, amino acid composition, and antinutritional factors. Germination significantly enhanced antioxidant capacity and promoted changes in soluble protein and free amino acid contents while reducing phytic acid levels, indicating improved nutritional quality and potential bioavailability.

Electrophoretic and densitometric analyses revealed that germination led to a redistribution of protein fractions toward lower intermediate molecular weight ranges, reflecting the partial proteolysis and structural reorganization of storage proteins. These changes suggest the activation of endogenous enzymatic systems during germination and the subsequent mobilization of storage macromolecules.

Such coordinated transformations are closely associated with improved protein solubility, digestibility, and functional behavior relevant to food processing. The balance between protein modification, amino acid liberation, and anti-nutrient reduction indicates that germination acts as an effective bioprocessing strategy for enhancing both the nutritional and functional properties of chickpea.

Overall, the findings provide integrated evidence that germination promotes structural and compositional transitions within chickpea seeds and supports the utilization of germinated chickpea as a functional plant-based ingredient for food processing and product development.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/app16052477/s1>, Figure S1: Densitometric profiles of SDS–PAGE protein bands from raw, soaked, and germinated chickpeas. Figure S2: MALDI–TOF/TOF spectra of a representative protein band excised from SDS–PAGE (RC, Lane 2). Table S1: Peptide profiles used for protein identification of SDS–PAGE bands by MALDI–TOF/TOF analysis.

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