

Gut-friendly chickpea (*Cicer arietinum*): Germination as a sustainable approach to reduce flatogenic sugars

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Chickpea (*Cicer arietinum*), a widely consumed legume, is rich in proteins and dietary fiber but contains raffinose family oligosaccharides (RFOs), which cause gastrointestinal discomfort due to the absence of alpha galactosidase enzyme in humans. Traditional processing methods, such as germination, have been used to enhance the nutritional and functional properties of legumes. This study investigates the effect of germination on α -galactosidase activity and RFO reduction in two chickpea varieties-Desi (NBeG-49) and Kabuli (NBeG-119). Chickpeas were soaked, germinated for up to 72 h, and analyzed for moisture content, texture, enzyme activity, and RFO levels. The results showed that α -galactosidase activity peaked at 48 h, leading to a significant reduction in RFOs (100% after 72 h in Desi and 60 h in Kabuli). Germination also influenced texture by increasing seed hardness due to structural modifications. SEM analysis revealed increased porosity, facilitating enzymatic action. These findings reinforce germination as a cost-effective, traditional method to improve chickpea digestibility, reducing antinutritional factors while preserving its nutritional value. This approach aligns with indigenous knowledge practices and offers potential applications in functional food development.

Keywords: α -Galactosidase, Germination, HPLC, Hardness, Raffinose family oligosaccharides, SEM

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Chickpea (*Cicer arietinum*) is a widely used legume crop belonging to the Fabaceae family. Chickpea cultivation contributes to 15% of the total cultivation area of pulses, with the contribution of 18.09 million tonnes (dry seeds)¹. It is classified into two types: Kabuli chickpea, characterized by a large, beige colored seed coat, and Desi chickpea, a small, brown colored seed coat. Chickpea is a good source of protein (18.77%), and fibre (25.22%), along with water-soluble vitamins such as thiamine, niacin, and riboflavin, and minerals such as calcium, iron, and zinc². Despite its high nutritional value, the presence of anti-nutritional factors such as α -galactosides, phytic acid, trypsin inhibitors, and tannins limits its consumption. α -galactosides or Raffinose Family Oligosaccharides (RFOs) are sucrosyl derivatives, distinguished by the presence of α -(1 \rightarrow 6) linkage between the galactose and glucose moieties of

sucrose. These compounds are not digested in the human gastrointestinal tract because of the absence of α -galactosidase (α -Gal) enzyme and are fermented by the intestinal microbial flora to produce gases causing flatulence with symptoms of bloating, pain, nausea, cramps, diarrhoea, abdominal rumbling, and social discomfort. Raffinose, stachyose, verbascose, and ajugose are the major members of this group³.

The germination of pulses is one of the traditional processes that modifies the biochemical, nutritional, and physical characteristics. As the seeds absorb water during soaking, metabolism of the seed becomes activated with the production of enzymes such as amylase, invertase, α -Gal, proteases, peptidases, lipase, phytase, etc., and it continues during germination to enhance the process of emergence of radicle⁴. α -Gal acts upon α -galactosides to produce galactose and sucrose units, thereby reducing the flatogenic capacity⁵.

Chickpea, a legume crop, is valued for its protein, fiber, and mineral content. However, its consumption is limited due to flatogenic sugars (α -galactosides) that cause gastrointestinal discomfort. This study aims

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Abbreviations:

α -Gal: alpha-galactosidase, RFOs: Raffinose Family Oligosaccharides, DCP: Desi Chickpea, KCP: Kabuli Chickpea, p-NPG: p-nitrophenyl α -D galactopyranoside, SEM: Scanning Electron Microscope

to evaluate germination as a method to reduce α -galactosides along with its effects on moisture content and textural attributes in chickpea. The findings will help to optimize germination conditions to improve the digestibility and consumer acceptance of chickpea-based foods.

Materials and Methods

Materials

Two genotypes of chickpea, NBeG-49 (Desi chickpea-DCP) and Kabuli- NBeG-119 (Kabuli chickpea-KCP) were procured from RARS, Nandyal, Andhra Pradesh. Grains were sorted, packed under vacuum and stored under refrigerated conditions until the experiment. HPLC-grade chemicals were obtained from M/s SD Fine Chemicals, and standards were from Sigma Aldrich.

Methods

One hundred grams of washed grains were soaked in a 10% hydrogen peroxide solution for 30 min for decontamination. Furthermore, it was washed with distilled water thoroughly to take out peroxide residue. It was soaked for 12 h in distilled water in a 1:10 (grain: water) ratio and subsequently spread on cheese cloth at 30°C in an incubator with frequent hydration (thrice a day) to allow germination. Fresh samples were analyzed for the enzyme assay, texture, and moisture and were then dried at 50°C for 24 h for the estimation of α -galactosides and microstructure analysis.

Estimation of moisture content

The moisture content of the seeds was measured by the gravimetric method⁶. Seeds (10-20 g) were dried at 105°C until constant weight was reached, and the percent moisture content was calculated.

Texture analysis or hardness measurement

The texture of the seeds, particularly the hardness, was measured using a Texture analyzer (TA. XT Plus, Stable Microsystems, UK). A cylindrical probe with 40 mm diameter, a load cell of 50 kg, 65% deformation strain, 1 mm/s pre-test, and 5 mm/s post-test speed with 0.5 mm/s cross-head speed, was used as test parameters. Ten seeds were chosen as representative, and hardness was calculated by using the texture profile curve and expressed in N (newtons)⁷.

Microstructural analysis using SEM images

The microstructural analysis was done in SEM-Scanning Electron Microscope (Quanta 200, Icon Analytical, FEI, US). A dried, ground sample was spread on carbon tape of stubs and gold and palladium plasma were coated on the samples to make them conductive. The SEM images were collected at an accelerating voltage of 10 kV and 500 and 1000X magnification⁸.

α -galactosidase enzyme assay

α -Gal activity was measured by hydrolyzing p-nitrophenyl α -D galactopyranoside (p-NPG) as explained by Katrolia *et al.*⁹, and samples were prepared in acetate buffer as described by Lien¹⁰. One gram of the grain sample was ground in a pestle and mortar and mixed with acetate buffer (0.2 M, pH 4.8), further shaken for 1 h at 20°C. The solution was subsequently centrifuged at 18300 \times g at 4°C for 40 min, and 0.1 mL of supernatant was mixed with 0.8 mL of acetate buffer and 0.1 mL of 2 mM p-NPG. The solution was mixed and incubated at 37°C for 15 min for enzyme activity. Then 3 mL of 0.2 M sodium carbonate was added to stop the reaction, and the absorbance was read at 405 nm using a UV-Visible spectrophotometer (UV-2550, Shimadzu, Japan). p-nitrophenol was used to prepare a regression curve for quantification. One unit of enzyme activity (1 U) is defined as the enzyme required to liberate 1 μ mole of p-nitrophenol from p-NPG per minute under specified assay conditions.

Estimation of α -galactosides or RFOs and sucrose

RFOs and sucrose content were analyzed with reverse phase- High Performance Liquid Chromatography (HPLC) method¹¹ with some modifications in flow rate and run time. Ground samples of 0.5-1 g were taken and mixed with 70% ethanol, and further kept on a wrist shaker for 30 min. Five millilitres of supernatant was taken after centrifugation at 1717 \times g for 30 min and mixed with 7 mL of acetonitrile. After 2 h of incubation at room temperature, the mixture was centrifuged at 3670 \times g for 30 min. A total of 10 mL of the supernatant was evaporated to dryness at 50°C in a rotary flash evaporator. It was then dissolved in 60% acetonitrile and filtered through a SEP-PAK cartridge (0.25 μ m, C₁₈, 3cc vac, 500 mg) before being injected into the HPLC system.

Chromatographic conditions: HPLC system

(Waters Corporation, USA) with dual pump, a manual injector equipped with 20 μ L sample loop and Refractive Index detector (Waters, 410) were used for the analysis. Carbohydrate column (Waters' carbohydrate column, 4.6 mm \times 250 mm, 4 μ m) and 60% acetonitrile were used as the mobile phase. An injection volume of 20 μ L, run time of 10 min and detector and column temperature of 40°C were maintained throughout the analysis. Raffinose, stachyose and sucrose standards were injected individually for identification and mixed standards were used for quantification. Empower 3 software was used for integration and data analysis.

Statistical analysis

Statistical analysis was performed using Minitab (Ver. 18.1). The data were subjected to One-way ANOVA, and Tukey's test at $p \leq 0.05$ was used to assess the significance of the difference in data. Correlation among the factors such as germination time, enzyme activity, α -galactosides, moisture content, and hardness during germination was denoted by descriptive statistics, *i.e.*, Pearson's coefficient at $p \leq 0.05$ ⁷. The experiments in general were performed in triplicate, while in the case of hardness, 10 readings were taken, and data are represented as Average \pm SD.

Results and Discussion

Germination initiates with the water uptake of dry seeds when they are soaked in water, continues with the appearance of the radicle and subsequently ends with plumules. The first stage of germination is the imbibition stage, where fast uptake of water takes place, and the second stage is the plateau phase, where the enzymes start their activity in imbibed seeds. The last phase is post post-germination stage, where the emergence of the radicle and plumule takes place¹².

Changes in moisture content in chickpea during germination

The moisture content of grain affects the shelf life, texture, and acceptability of the food. The moisture content of the raw chickpea was 9.56 and 10.09% for DCP and KCP, respectively. Once the grain was soaked in water, the process of absorption of water initiated and after 12 h of soaking, it contained 48.01 and 49.57% of moisture. As germination continued, the moisture content rose from 51.31 (12 h) to 56.96% (72 h) in DCP and from 51.73 (12 h) to 58.47% (72 h) in KCP. Soaking and germination had significant effects ($p \leq 0.05$) on the moisture content of the seeds, as shown in Table 1. The present results are comparable with the results reported in mung bean^{13,14} and chickpea¹³.

As germination continues, the water requirement for the metabolic process is fulfilled by water from the surroundings. This may be the reason for the increase in the moisture content during germination. In addition, the number of cells within the seed is increased throughout germination, and cells need to be hydrated. Hence, water uptake during germination is increased¹⁵.

Changes in the texture and microstructure of chickpea during germination

In the present study, the hardness of the seed was used to represent the texture of the seeds. The raw grains of both types of chickpea were dry and hard initially, and subsequent to soaking, it enlarged and turned soft due to absorption of moisture. Soaking for 12 h reduced the hardness of the seed, and the value was found to be 58.39 N in DCP and 81.86 N in KCP. As the germination started, the seeds gained hardness gradually and increased from 61.46 N (12 h) to 96.99 N (72 h) in DCP and 82.23 N (12 h) to 188.59 N (72 h) in KCP, as represented in Table 1. A similar trend in mung beans during the initial germination period has been reported¹⁶.

The hardness of the seed increased despite the higher moisture content corresponding to germination

Table 1 — Changes in the moisture content, hardness, and enzyme activity of DCP and KCP during germination

Time (h)	Desi chickpea			Kabuli chickpea		
	Moisture content (%)	Hardness (N)	Enzyme activity (U/ 100 g)	Moisture content (%)	Hardness (N)	Enzyme activity (U/ 100 g)
0	48.01 \pm 0.97 ^d	58.39 \pm 2.36 ^c	168.43 \pm 4.99 ^d	49.57 \pm 0.42 ^g	81.86 \pm 5.44 ^d	128.53 \pm 5.42 ^e
12	50.98 \pm 0.12 ^c	61.46 \pm 4.56 ^b	193.05 \pm 6.32 ^c	51.73 \pm 0.36 ^f	82.23 \pm 5.17 ^d	166.57 \pm 3.31 ^d
24	51.67 \pm 0.96 ^c	63.21 \pm 6.95 ^b	205.31 \pm 7.9 ^c	53.4 \pm 0.45 ^e	93.36 \pm 9.02 ^d	169.3 \pm 6.29 ^{cd}
36	52.21 \pm 0.35 ^c	91.55 \pm 2.44 ^a	240.61 \pm 6.92 ^b	54.62 \pm 0.41 ^d	131.54 \pm 9.13 ^c	180.67 \pm 6.78 ^{bc}
48	53.59 \pm 0.42 ^{bc}	93.95 \pm 2.55 ^a	296.71 \pm 4.1 ^a	55.77 \pm 0.29 ^c	147.14 \pm 8.92 ^b	235.07 \pm 6.69 ^a
60	55.46 \pm 0.40 ^a	94.75 \pm 7.88 ^a	232.74 \pm 4.74 ^b	56.91 \pm 0.21 ^b	185.55 \pm 8.84 ^a	193.57 \pm 6.4 ^b
72	56.96 \pm 0.22 ^a	96.99 \pm 4.79 ^a	193.54 \pm 2.82 ^c	58.47 \pm 0.41 ^a	188.59 \pm 5.71 ^a	158.69 \pm 3.89 ^d

The values are Average \pm SD, and the values with the same superscript in a column are not significantly different at $p \leq 0.05$

time. This phenomenon is a result of biochemical and structural changes in the seed during germination. The accumulation of phenolic compounds such as tannins and lignin strengthens the cell wall and makes it firmer. This phenomenon is one of the key reasons for the increase in hardness. The uneven distribution of water during germination may increase the overall hardness of the seeds, especially the internal redistribution of water in the cell wall, which leads to a hard texture of the seeds¹⁷. Structural changes in protein and starch contents may also contribute to textural changes in seeds during germination¹⁸.

Figure 1 shows the porosity, surface roughness, and microfracture of chickpeas as seen in SEM images taken both before and after 60 h germination. Before

germination, the surface of the soaked chickpeas appeared compact, smooth, and had little porosity, as shown in (Fig. 1a & Fig. 1b). The irregularities in the structure were not so predominant, and uniformity existed. Water was absorbed as a result of the hydration process, which caused the components to swell and relax. Enzymatic diffusion was limited mainly by the maintenance of cellular integrity. SEM pictures taken after germination (Fig. 1c & Fig. 1d) showed fractures in the granular structure, rough surface textures, and increased porosity. It was dominated by irregular structures, and a lack of uniformity was found. It indicates the disruption of the integrity of the cell wall. These structural changes were associated with higher enzyme activity, facilitating hydrolysis of cell wall

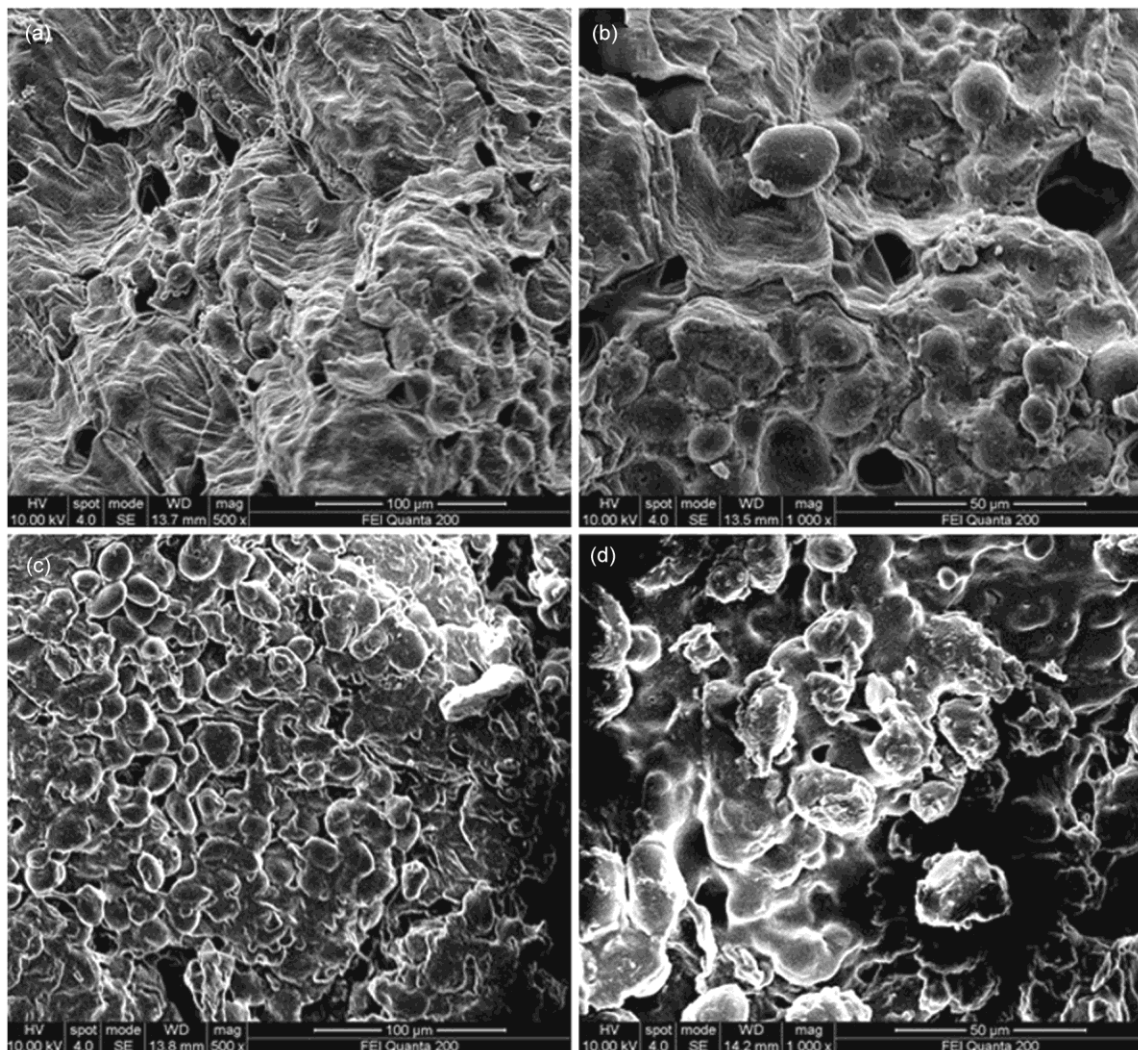


Fig. 1 — SEM images of Kabuli chickpea before and after germination- (a) before germination-500X, (b) before germination-1000X, (c) after 60 h germination-500X, and (d) after 60 h germination-1000X

polysaccharides and proteins⁸. The formation of porous networks and cavities allowed deeper water penetration, contributing to higher moisture content in germinated chickpeas, which is correlated with the present results.

α -galactosidase activity in chickpea during germination

The α -Gal enzyme activity was determined and revealed that, before soaking the raw seeds presented activity, hence it can be concluded that the enzyme pre-existed in the grains. Enzymes like α -Gal are typically stored in an inactive form during dormancy in grains and seeds, primarily due to the lack of water and the low metabolic state of the seeds. However, it becomes activated once it gains moisture during the imbibition period¹⁹. In this study, raw grains of DCP and KCP contained 87.9 and 81.94 U/100 g α -Gal activity. The activity was increased by 1.91 and 1.56 times after soaking in DCP and KCP, respectively. Similar effects were previously reported in blackgram soaking¹⁹. As the germination time increased, α -Gal activity improved up to a certain time period (Table 1). The α -Gal activity was increased from 168.43 (0 h) to 296.71 U/100 g (48 h) in DCP and from 128.53 (0 h) to 235.07 U/100 g (48 h) in KCP. The enzymatic activity decreased after 48 h to 232.74 U/100 g (DCP) and 193.57 U/100 g (KCP) in both chickpea samples. Similar results were reported for soybean^{10,20}.

In the initial stages, especially in the imbibition stage, metabolic activities are reactivated, and the activity is relatively low. However, in the next phase, it enhances the metabolic activity that necessitates additional energy, and the seed starts to break down the α -galactosides stored in cotyledons to produce sucrose and glucose with the help of α -Gal to support the growing metabolic activity. The activity continues until the radicle and plumule are established. As soon as the radicle and plumule emerge, the requirement of stored

oligosaccharides to produce energy decreases, because the seedlings become autotrophic and produce their own energy²¹. Hence, the activity of α -Gal declined after the radicle emergence phase in the DCP and KCP. Additionally, it may be due to galactose produced by the hydrolysis of raffinose, stachyose or verbascose, which can cause product inhibition effects by engaging the active sites of α -Gal²².

The study identifies a specific window during germination, when α -galactosidase activity reaches its peak. This insight into enzyme activity across different germination times is novel and could inform future strategies for enhancing enzyme levels in legumes. Also, α -Gal can be extracted, purified from plant and microbial sources and used for the removal of RFOs from legumes, in the beet sugar processing, and animal feed industries⁹. Hence, it is important to investigate α -Gal in germinating seeds to determine the maximum activity and stability.

α -galactosides or RFOs in chickpea during germination

The α -galactosides, such as raffinose, stachyose and sucrose, were determined by chromatography and chromatogram of α -galactosides of Kabuli chickpea at 0 h and 60 h are represented in as shown in (Fig. 2 & Fig. 3). The raw desi chickpea contained 0.54 g/100 g raffinose and 2.81 g/100 g stachyose, whereas the kabuli chickpea contained 0.57 g/100 g raffinose and 2.49 g/100 g stachyose. DCP and KCP contained 3.35 and 3.05 g/100 g of total RFOs, respectively. Soaking for 12 h reduced raffinose by 16.66% and stachyose by 18.86% in DCP and raffinose by 24.56% and stachyose by 11.64% in KCP, contributing to 18.51 and 13.77% reduction in total α -galactosides in DCP and KCP, respectively. Similarly, 56.3% reduction in soybean²³, 36.94% in cowpea²⁴, 65.7% in black gram dal¹⁹, and 6.8% reduction in soybean¹⁰ were reported.

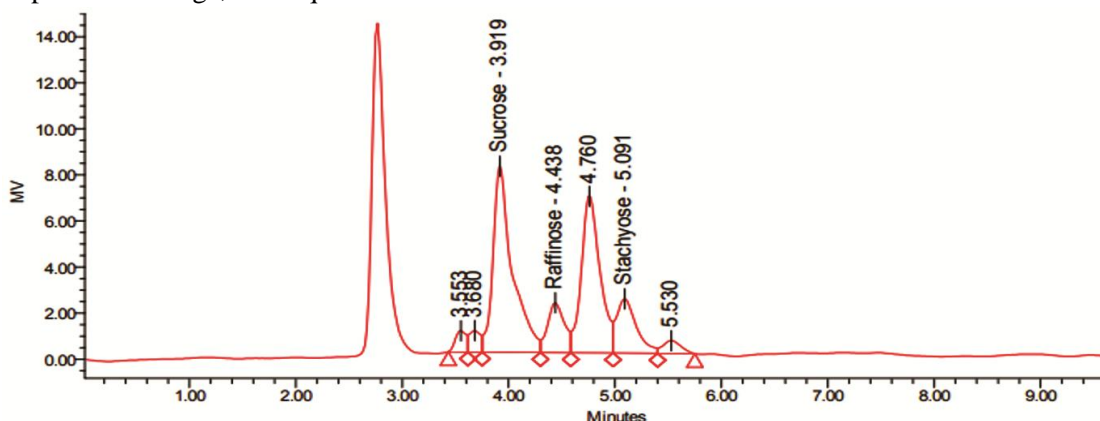


Fig. 2 — Chromatogram representing fresh Kabuli chickpea sample (0 h)

The reduction in α -galactosides may be due to leaching during soaking. When a chickpea is immersed, water is absorbed into it, and the α -galactosides dissolve and leach into the surrounding water^{23,24}. In addition, soaking activated the α -Gal in beans, which cleaves oligosaccharides into sucrose and galactose units to some extent¹⁹. Certainly, in the present study, α -Gal activity was increased by 1.91 (DCP) and 1.56 (KCP) times compared with that of raw seeds (Table 1).

In the present study, changes in oligosaccharides and sucrose content during germination were determined, and the statistical results (Table 2) revealed that the raffinose and stachyose contents of both chickpea cultivars were reduced significantly ($p \leq 0.05$). As the germination period was prolonged, considerable and successive diminution in α -galactosides was observed in the study. The maximum reduction was found after 72 h of germination in DCP, and 60 h in KCP corresponded to 100% reduction in total RFOs. Previous studies showed similar effects of reduction, *i.e.*, 85-90%

reduction in lentil²⁵, 76-80%²⁶ and 75-79%¹⁰ in soybean. The differences in reduction rate among the pulses are due to variation in the type of grains/beans and their composition, the structure of the seed coat, the amount of enzymes present, particularly α -Gal, and germination conditions and procedures.

Indeed, the sucrose content of the grains increased during germination for both the chickpea samples for a certain period of time, as shown in Table 2. The variations in sucrose content and the oligosaccharide content were contrasted with each other. The reason for the increase in sucrose and drop in oligosaccharides is that germination induced α -Gal catalyzed the dissociation of the bond between galactose and glucose moieties of galactosides to produce galactose and sucrose units²⁰. A similar increasing pattern was also mentioned for soybean seeds¹⁰.

As these α -galactosides cause flatulence in humans, the reduction of α -galactosides in beans and grains is favourable from a nutritional point of view.

Germination is known to induce a series of metabolic processes that not only reduce antinutritional

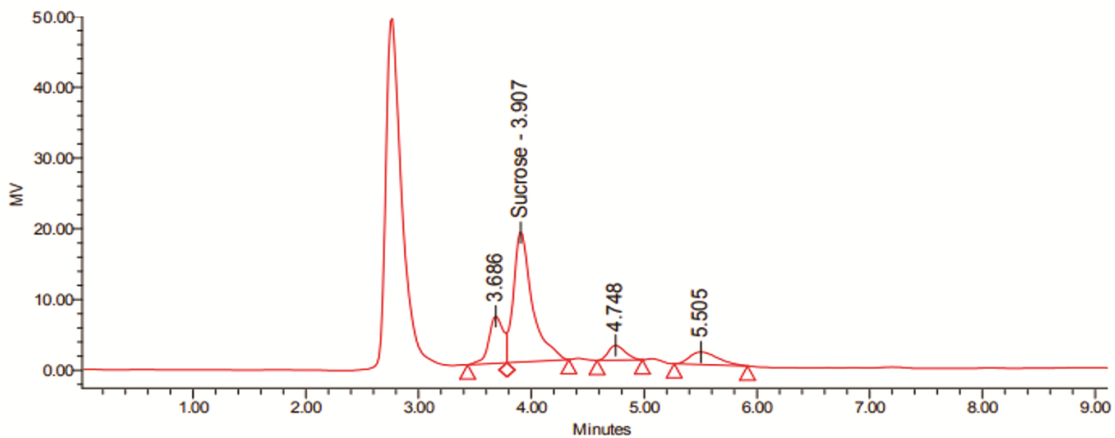


Fig. 3 — Chromatogram representing germinated Kabuli chickpea sample (60 h)

Table 2 — Changes in α -galactosides and sucrose content of DCP and KCP during germination

Time (h)	Desi chickpea				Kabuli chickpea			
	Raffinose	Stachyose	Total RFOs	Sucrose	Raffinose	Stachyose	Total RFOs	Sucrose
Raw	0.54±0.04 ^a	2.81±0.16 ^a	3.35±0.15 ^a	1.7±0.04 ^f	0.57±0.06 ^a	2.49±0.09 ^a	3.05±0.15 ^a	1.64±0.08 ^f
0	0.45±0.01 ^b	2.28±0.15 ^b	2.73±0.15 ^b	1.88±0.09 ^{ef}	0.43±0.03 ^b	2.2±0.07 ^b	2.63±0.07 ^b	2.24±0.06 ^c
12	0.39±0.01 ^b	1.77±0.18 ^c	2.16±0.19 ^c	2.14±0.08 ^c	0.22±0.03 ^c	1.21±0.15 ^c	1.44±0.12 ^c	2.48±0.09 ^d
24	0.24±0.02 ^c	1.09±0.12 ^d	1.32±0.13 ^d	2.57±0.11 ^d	0.15±0.02 ^{cd}	0.54±0.12 ^d	0.69±0.1 ^d	2.76±0.1 ^c
36	0.13±0.03 ^d	0.41±0.05 ^e	0.54±0.06 ^e	3.22±0.08 ^c	0.11±0.01 ^d	0.24±0.06 ^e	0.35±0.07 ^e	3.17±0.08 ^b
48	0±0 ^e	0.15±0.05 ^{ef}	0.15±0.05 ^f	3.87±0.12 ^b	0±0 ^e	0.13±0.04 ^{ef}	0.13±0.04 ^{ef}	3.61±0.06 ^a
60	0±0 ^e	0.08±0.03 ^f	0.08±0.03 ^f	4.8±0.18 ^a	0±0 ^e	0±0 ^f	0±0 ^f	3.55±0.07 ^a
72	0±0 ^e	0±0 ^f	0±0 ^f	4.67±0.08 ^a	0±0 ^e	0±0 ^f	0±0 ^f	3.5±0.01 ^a

The values are Average±SD, and the values with the same superscript in a column are not significantly different at $p \leq 0.05$

Table 3 — Correlation data for germination time, α -galactosidase activity, α -galactosides content (raffinose, stachyose, and total RFOs), sucrose, hardness, and moisture content in DCP and KCP

	Chickpea type	Germination time	Enzyme activity	Raffinose	Stachyose	Total RFOs	Sucrose	Texture	Moisture
Germination time	DCP	1							
	KCP	1							
Enzyme activity	DCP	0.448	1						
	KCP	0.493	1						
Raffinose	DCP	-0.955*	-0.676	1					
	KCP	-0.919*	-0.738	1					
Stachyose	DCP	-0.949*	-0.661	0.99*	1				
	KCP	-0.891*	-0.687	0.979*	1				
Total RFOs	DCP	-0.951*	-0.664	0.993*	1*	1			
	KCP	-0.898*	-0.697	0.985*	0.999*	1			
Sucrose	DCP	0.98*	0.468	-0.949*	-0.927*	-0.932*	1		
	KCP	0.942*	0.734	-0.949*	-0.92*	-0.928*	1		
Hardness	DCP	0.918*	0.639	-0.949*	-0.949*	-0.95*	0.921*	1	
	KCP	0.971*	0.435	-0.837*	-0.801*	-0.809*	0.918*	1	
Moisture	DCP	0.981*	0.376	-0.906*	-0.909*	-0.91*	0.949*	0.847*	1
	KCP	0.994*	0.524	-0.947*	-0.931*	-0.936*	0.943*	0.943*	1
	KCP	-0.959*	-0.397	0.795*	0.756*	0.764*	-0.9*	-0.989*	-0.923*

* denotes statistically significant correlation at $p \leq 0.05$

factors but also enhance the nutritional profile and bioavailability of grain components. During germination, the activation of hydrolytic enzymes such as amylases, proteases, and phytases leads to the breakdown of storage macromolecules into simpler, more digestible forms^{4,21}. This improves the digestibility of protein and starch through structural modification of seed reserves¹⁸. Furthermore, the activation of phytase enzymes during sprouting contributes to the hydrolysis of phytic acid, thereby enhancing mineral bioavailability^{5,8}. Germination has also been reported to cause inactivation of trypsin inhibitors, which further improves protein utilization¹⁴. These changes together contribute to better digestibility and nutrient absorption, making germinated legumes a more valuable food ingredient from both nutritional and functional perspectives.

Correlation data for chickpea during germination

Pearson's correlation coefficient is a descriptive statistic that is used to describe the strength and direction of the linear relationships among variables during germination. Table 3 represents the correlation data for germination time, α -Gal activity, α -galactoside content (raffinose, stachyose), sucrose content, hardness, and moisture in the DCP and KCP. Pearson's correlation coefficients illustrated the positive relationship of germination time with sucrose content (0.980, 0.942), hardness (0.918, 0.971), and moisture content (0.981, 0.941) with

$p \leq 0.05$, whereas negative associations with raffinose (-0.955, -0.919), and stachyose (-0.949, -0.891) with $p \leq 0.05$. Sucrose, as the product of enzymatic hydrolysis, increased as the activity of α -Gal increased. As germination continued, the moisture content increased because of the metabolic requirements of the seeds. Enzyme activity didn't show any correlation with the other variables due to its pattern of initial increase and subsequent reduction. The enzyme activity decreased once the substrate concentration became low or nil, and the product concentration became high. The sucrose content was negatively correlated with raffinose, stachyose, and total RFOs in both desi and kabuli chickpea, as it is a breakdown product of α -galactosides. This statistical approach provides a comprehensive, quantifiable understanding of the interrelation between factors during germination.

Conclusion

The current study highlights germination as a traditional and cost-effective method to enhance the digestibility of chickpeas by reducing raffinose family oligosaccharides (RFOs). By naturally enhancing enzymatic hydrolysis, germination improves chickpea digestibility, making it more suitable for human consumption. Additionally, germination-induced structural modifications influence texture and enzymatic activity, making chickpeas more suitable

for various food applications. The findings align with traditional food processing practices and highlight the potential of germination in functional food development. Future research can explore large-scale applications, enzyme extraction for industrial use, and the impact of germination on other bioactive components.

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Conflict of Interest

The authors of the present work declare that there is no conflict of interest associated with this manuscript. All the co-authors have gone through the manuscript, and they have given their consent to the corresponding author to handle the manuscript and to publish.

Author Contributions

P G H: Conceptualization, investigation, methodology, analysis, writing- original draft. D D W: Supervision, validation, data curation, writing-review & editing. A K: Methodology, analysis, writing-review & editing. A D S: Supervision, project administration, funding acquisition. All authors have read and approved the final version of this submission.

Ethics Approval

Not applicable.

Data Availability

The supporting data associated with the work will be made available on reasonable request to the corresponding author.

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