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Microgreens as a sustainable plant-based functional food to improve nutritional quality and bioactive potential of legumes

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Abstract

Legumes are recognized for their rich nutritional composition and proven health benefits, however, the superior nutritional quality attributed to their germinated stages requires systematic scientific validation. Therefore, this study evaluated and compared the nutritional profile, bioactive composition, and anti-nutritional factors in sprouts, microgreens, and boiled seeds of mung bean (*Vigna radiata* L.) and chickpea (*Cicer arietinum* L.). Nutritional quality was assessed by analyzing proximate composition, antioxidant capacity, mineral content, and anti-nutritional factors, while structural changes were evaluated using FTIR spectroscopy. Significant differences ($p < 0.05$) were observed across growth stages and processing methods. Microgreens exhibited the highest protein content (66% increase in mung bean and 72.4% in chickpea compared to raw seeds), whereas boiling led to protein reductions of 10% and 21.4%, respectively. Carbohydrate content declined significantly ($p < 0.05$) in sprouts and microgreens due to germination but increased in boiled seeds. Microgreens exhibited the highest antioxidant activity (DPPH: 76.58% in mung bean and 75.53% in chickpea) along with increased mineral content and a marked reduction in anti-nutritional factors such as tannins, saponins, polyphenols, and phytic acid. Fourier-Transform Infrared (FTIR) analysis confirmed structural modifications in carbohydrates, proteins, and lipids during sprouting and microgreen development. Overall microgreens demonstrate the superior nutritional and bioactive profiles, highlighting their potential as a functional food with greater health benefits than traditionally consumed boiled or sprouted legumes.

Highlights

- This study evaluated nutritional quality in mung bean and chickpea across raw seeds, boiled seeds, sprouts and microgreens.
- Microgreens of mung bean and chickpea showed superior nutritional quality over sprouts and boiled seeds.
- Microgreens enhanced protein, mineral bioavailability, and antioxidant capacity while reducing anti-nutritional factors.
- Chemometric analysis identified microgreens as the most nutrient dense stage, supporting their potential application in functional food development.



Keywords Legumes, Sprouts, Microgreens, Antioxidant capacity, Anti-nutritional factors, Functional foods, Health benefits

1 Introduction

A healthy diet plays a crucial role in maintaining overall health and preventing diseases. It provides essential nutrients in appropriate amounts while avoiding harmful components [1]. The rising global rates of nutrient deficiencies and non-communicable disease have shifted consumer focus toward healthier dietary choices [2]. Legumes are increasingly valued for their health-promoting properties serving as a vital source of protein, fiber, and essential nutrients. They can contribute up to one-third of daily protein requirements [3]. Chickpeas (*Cicer arietinum* L.) and mung beans (*Vigna radiata* L.) are among the most widely consumed legumes, particularly in India and other Asian countries. They are rich in macronutrients, such as proteins, carbohydrates, and fats, along with a range of important vitamins and minerals [4]. Chickpeas contain around 20% protein, 60% carbohydrates, and 5% fat, along with significant amount of dietary fiber, iron, magnesium, and B vitamins [5]. Mung beans have a similar nutritional profile, with slightly higher protein content (approximately 24%) and higher levels of micronutrients like vitamin C and potassium [6].

Despite their nutritional value, raw legumes also contain anti-nutritional factors (ANFs) such as phytic acid, tannins, saponins, and certain high-molecular-weight polyphenolic compounds, which can reduce nutrient bioavailability by forming complexes with proteins and minerals. Although polyphenols are primarily recognized for their antioxidant properties, condensed tannins and some non-tannin polyphenols in chickpea and mung bean have been reported to exert anti-nutritional effects when present in high concentrations [7]. The adverse effects of these ANFs can be mitigated by various processing techniques such as soaking, sprouting, and boiling which enhance nutritional availability and overall nutritional quality. Boiling, for instance, is a common cooking method to reduce the levels of tannins, phytic acid, and saponins, thus facilitating better mineral absorption, particularly of Ca, Fe, and Zn [8]. Boiling also improves protein digestibility by denaturing proteins and inactivating enzyme inhibitors, which enhances nutrient absorption [9]. Furthermore, boiling has a dual effect on antioxidants; while some antioxidants are degraded by heat, others become more available due to the breakdown of cell walls [10]. Although boiling may cause some loss of water-soluble vitamins like folic acid, it generally improves the nutrient profile by reducing antinutritional factors. Similarly, soaking is a simple and effective method to reduce antinutritional factors in legumes. For example, soaking in water for 12–16 h can significantly decrease phytic acid, tannins, and trypsin inhibitor levels, thereby improving protein digestibility and mineral bioavailability [11, 12]. Soaking in sodium bicarbonate solution is even more effective for reducing tannins and trypsin inhibitors [12, 13]. Additionally, soaking helps leach out oligosaccharides and other soluble antinutrients, making legumes more suitable for consumption [14].

In recent years, sprouts and microgreens have gained attention for their superior nutritional profiles compared to raw or boiled seeds. Sprouting involves germinating seeds, which boosts the levels of vitamins, essential amino acids, and minerals. For example, sprouting increases the content of vitamin C, B vitamins, and carotenoids [15],

and reduces phytic acid levels, thereby enhancing the absorption of minerals like Fe and Ca. Additionally, sprouted legumes contain higher levels of phenolic compounds and flavonoids that help in reducing oxidative stress [16]. Sprouted mung beans provide high levels of essential amino acids, particularly lysine, which is commonly deficient in cereal-based diets, thereby improving the overall amino acid balance of plant-based foods [17].

Microgreens, harvested at a later stage of growth when the first true leaves have emerged, are particularly rich in vitamins, minerals, and antioxidants. Research indicates that microgreens may possess nutrient levels up to 40 times higher than fully matured plants [18]. They are exceptionally rich in vitamins C, E, and K, along with β -carotene and lutein, which are essential for maintaining healthy vision and skin [19]. The nutrient density of microgreens, along with their high concentrations of bioactive compounds like phenolic acids, flavonoids, and glucosinolates, makes them potent antioxidants with potential anti-inflammatory and anticancer properties [20, 21].

As legumes are well established as rich sources of essential nutrients, their alternative developmental stages, such as sprouts and microgreens, are expected to exhibit superior nutritional quality and enhanced health promoting properties. However, comparative studies evaluating different developmental stages of legumes alongside their commonly consumed forms remain limited. The quantitative comparison provides, reliable measures of nutritional value, supporting functional food development and enable dietary choices of consumers. Therefore, the present study aims to comparatively assess the nutritional composition, antioxidant potential, and antinutritional factors of chickpea and mung bean across different developmental stages and their conventional consumption forms based on the following hypotheses: (a) Sprouts and microgreens would significantly enhance antioxidant potential compared to raw and boiled seeds, (b) Boiling would reduce anti-nutritional factors but may also result in partial losses of heat-sensitive bioactive compounds, (c) Microgreens may exhibit superior nutritional and functional profiles compared to sprouts and boiled legumes due to advanced metabolic stage and active secondary metabolite biosynthesis.

2 Materials and methods

2.1 Sample collection and preparation

2.1.1 Raw mung bean and chickpea

Certified seeds of mung beans (*Vigna radiata* L. var. CARI Mung 2) and chickpea (*Cicer arietinum* L. var. Pusa JG 16) were purchased from the ICAR-Indian Agriculture Research Institute, New Delhi, India, an authorized national agricultural research organization. Seed samples were manually sorted to exclude damaged seeds and extraneous matters. For the control treatment, both mung bean and chickpea seeds were used without removing their seed coats. The seeds were ground into flour using a laboratory grinder (NIMA-8300 Burman, Germany) and stored in resealable food grade polyethylene pouches (thickness of 0.75 μm , the oxygen permeability of 160 $\text{mm}/100\text{ cm}^2$ and water vapour permeability of 0.27 $\text{g}/100\text{ cm}^2$ in 24 h at 37.8 $^\circ\text{C}$ and relative humidity of 90%) until further analysis.

2.1.2 Boiled mung beans and chickpea

For the boiled samples, mung beans and chickpeas were cooked in water at 100 $^\circ\text{C}$ on an electric induction heater until the seeds became soft, as confirmed by tactile assessment.

The samples were then dried in a hot air oven at 55 °C for 24 h and ground into flour with the help of grinder (NIMA-8300 Burman, Germany). The resulting flour was then stored in similar resealable food grade polyethylene pouches as mentioned above for further analysis.

2.1.3 Sprouted mung beans and chickpea

Seeds were soaked in distilled water (1:3 w/v) at 24–25 °C for 12 h, drained and incubated in the dark on perforated aluminum trays for 48 h to allow germination. The sprouted samples were dried and converted into flour using the method mentioned in Sect. 2.1.2.

2.1.4 Mung beans and chickpea microgreens

Microgreens were grown in individual trays (40 × 35 × 6 cm) filled with cocopeat under controlled conditions (25–30 °C) in plant growth chamber. The microgreens were exposed to white light (200 μmol m⁻² s⁻¹) with a 12 h photoperiod and irrigated twice a day. Microgreens were harvested on day 8 (mung beans) and day 9 (chickpeas), when cotyledons were fully expanded. Three biological replicates were collected from each tray and were dried to convert into flour using the method mentioned in Sect. 2.1.2.

2.2 Proximate analysis

Protein, fat, crude fiber and ash were determined following the AOAC Official methods of Analysis [22]. The total carbohydrates were calculated by subtracting the sum of the moisture, crude protein, crude fat, and ash content percentages from 100.

$$\text{Carbohydrates (\%)} = 100 - (\% \text{ moisture} + \% \text{ crude protein} + \% \text{ crude fat} + \% \text{ ash}) \quad (1)$$

2.3 Bioactive components

2.3.1 Extraction of samples

Sample extraction followed the method of Umar et al. [23]. A 3 g sample was extracted with 50 mL of 50% (v/v) ethanol-water solution and shaken at 300 x g for 3 h at 25 °C. Extracts were centrifuged at 4000 x g for 10 min and the supernatant was stored at 4 °C until analysis.

2.3.2 Total phenolic content (TPC)

The TPC was determined using the Folin-Ciocalteu (FC) reagent method [24]. The assay involved combining 50 μL of extract with 250 μL FC reagent, 750 μL of 7% sodium carbonate solution and 3 mL distilled water. After 2 h incubation, absorbance was measured at 765 nm in a UV-visible spectrophotometer (Specord 200 Plus, Germany). The concentration was calculated using a standard curve of gallic acid, and expressed as milligrams of gallic acid equivalent per gram of dry weight (mg GAE/g).

2.3.3 Total flavonoid content (TFC)

The TFC was measured using protocol outlined by Kaur et al. [24]. The assay involved combining sample extract (250 μL) with deionized water (1.25 mL) and 5% sodium nitrite (75 μL) solution. After incubation for 6 min, 10% aluminum chloride (150 μL) was added and further incubated (5 min). The solution was then combined with 1 M sodium

hydroxide (0.5 mL) and ethanol (275 μ L) and adjusted to a final volume of 2.5 mL using deionized water. Absorbance was recorded at 510 nm with UV-visible spectrophotometer (Specord 200Plus, Germany). The flavonoid content was expressed in milligrams of quercetin equivalent per gram of dry weight (mg QE/g DWB).

2.3.4 Antioxidant activity (AOA)

The AOA was assessed using 2,2-diphenyl-1-picrylhydrazyl (DPPH) method [25]. To 100 μ L of extract, 4.0 mL of the DPPH solution (0.1 mM) was added and incubated for 30 min at room temperature in the dark. Absorbance was measured at 517 nm using a UV-visible spectrophotometer (Specord 200Plus, Germany). The AOA was reported as percentage of radical scavenging activity (% RSA).

$$\text{DPPH (\% inhibition)} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100 \quad (2)$$

2.4 Anti-nutritional factors

2.4.1 Polyphenols

The Prussian blue method [26] was used to measure total polyphenols in the samples. A 60 mg sample was shaken with methanol (3 mL, 1 min), filtered, and diluted using 50 mL distilled water. 0.1 M ferric chloride (3 mL) and 0.008 M potassium ferricyanide (3 mL) were combined with 1 mL of filtrate. After 10 min of incubation, absorbance was taken at 720 nm using UV-vis spectrophotometer (Specord 200Plus, Germany). Results were expressed as catechin equivalents using a standard curve.

2.4.2 Tannin

Vanillin-HCl method [27] was used to quantify tannins content. The 1 g sample was extracted in 20 mL acidified methanol containing 1% HCl (20 min at 30 °C). The centrifuged extract (1 mL) was reacted with vanillin solution (5 mL) for 20 min. Absorbance was recorded at 500 nm and results were reported as catechin equivalents.

2.4.3 Saponin

Saponin content was determined with protocol adapted from Margier et al. [28]. A methanolic extract was prepared by dissolving 1 mg of sample with 5 mL 80% methanol. To 0.2 mL of extract, methanol (0.3 mL), vanillin (0.5 mL) and 72% sulfuric acid (5 mL) was added. Following incubation (60 °C for 10 min), absorbance was measured at 544 nm.

2.4.4 Phytate

Phytate content was measured using a protocol adapted from Dost & Tokul [29], with slight modifications. A 0.1 g fresh sample was extracted with 10 mL of 2.4% HCl in a mechanical shaker for 1 h. The extract (0.1 mL) was mixed with iron (III) chloride (2 mL) and water (0.9 mL), followed by incubation (2.5 h, 40 °C). Absorbance was recorded at 480 nm.

2.5 Minerals content

Mineral composition was analysed using the method described by Bhinder et al. [30]. One gram sample was heated at 600 °C and mixed with 1 N nitric acid (2.5 mL). The filtered mixture was made up to a final volume of 100 mL with Milli-Q water. The solution was analysed with the help of Atomic Absorption Spectrophotometer (iCE™ 3400, Agilent Technologies, USA).

2.6 Functional group analysis

Functional groups were analysed using a Fourier-Transform Infrared (FTIR) spectrophotometer (ALPHA Bruker, Germany). Freeze-dried samples were finely powdered and analyzed using the attenuated total reflectance (ATR) mode, with the sample dust placed directly onto the ZnSe crystal plate. Infrared spectra were recorded over a wavenumber range of 4000–500 cm⁻¹ at a spectral resolution of 4 cm⁻¹, with 32 scans accumulated per sample to improve the signal-to-noise ratio. The Origin Pro software was used to plot the obtained intensity-wavenumber data for further interpretation.

2.7 Statistical analysis

Experiments were conducted in a completely randomized design. Data were analysed using one-way analysis of variance (ANOVA) followed by Tukey's test (SPSS, IBM Corp., USA). Data visualization was performed using OriginPro 2017 (OriginLab, USA). Principal Component Analysis was conducted using R software (v4.3.3).

3 Result and discussion

3.1 Proximate composition

The changes in the proximate composition of mung bean and chickpea due to boiling and germination are summarized in Table 1. Germination significantly ($p < 0.05$) increased protein and ash content, while carbohydrate, fat and crude fiber levels decreased in both legumes. Specifically, protein content increased by 40% in sprouted mung bean, 66% in mung bean microgreens, 43% in chickpea sprouts, and 72.4% in chickpea microgreens compared to raw seeds. In contrast, boiling reduced protein content by 10% in mung bean and 21.4% in chickpea. There were no notable differences in fat content across all treatments in mung bean, but in chickpea, a decreasing trend was observed from raw seeds to microgreens. Similarly, ash content increased by 55% and

Table 1 Effect of boiling and germination (sprouts and microgreens) on the proximate composition of mung bean and chickpea seed

Treatments	Protein (%)	Fat (%)	Ash (%)	Crude fiber (%)	Carbohydrate (%)
Mung bean					
Raw (control)	22.86 ± 0.45 ^b	2.64 ± 0.44 ^a	3.01 ± 0.34 ^{ab}	5.57 ± 0.15 ^b	55.04 ± 1.19 ^c
Boiled	20.59 ± 0.28 ^a	2.14 ± 0.24 ^a	2.66 ± 0.30 ^a	5.30 ± 0.35 ^b	57.76 ± 1.29 ^c
Sprouts	32.07 ± 0.31 ^c	2.11 ± 0.78 ^a	3.62 ± 0.18 ^b	4.00 ± 0.22 ^a	46.41 ± 1.50 ^b
Microgreen	58.07 ± 0.35 ^d	2.05 ± 0.32 ^a	4.67 ± 0.24 ^c	4.11 ± 0.17 ^a	24.51 ± 0.79 ^a
Chickpea					
Raw (control)	19.47 ± 0.13 ^b	5.12 ± 0.50 ^c	2.98 ± 0.25 ^a	4.62 ± 0.86 ^d	58.45 ± 1.47 ^c
Boiled	15.30 ± 0.08 ^a	4.66 ± 0.39 ^{bc}	2.24 ± 0.47 ^a	3.87 ± 0.12 ^a	63.38 ± 1.42 ^d
Sprouts	37.94 ± 0.46 ^c	4.05 ± 0.14 ^b	2.84 ± 0.50 ^a	4.50 ± 0.06 ^b	41.06 ± 1.19 ^b
Microgreen	53.56 ± 0.35 ^d	3.18 ± 0.09 ^a	4.34 ± 0.33 ^b	4.56 ± 0.86 ^c	27.69 ± 1.33 ^a

All the values are in triplicate ($n = 3$) and represented by mean ± SE. Means with different letters are significantly different at $p < 0.05$ using Tukey's test. n number of treatments, SE standard error

45.6% in mung bean and chickpea microgreens, respectively as compared to raw seeds. Crude fiber content decreased across all ontogenic stages in both legumes compared to raw seeds, although no significant differences were detected. Carbohydrate content was significantly lower ($p < 0.05$) in sprouts and microgreens than in raw seeds, as carbohydrates are metabolised for energy during germination [31]. It was decreased by 15.67% and 55.51% in mung bean sprouts and microgreens, respectively. Similarly, in chickpea it was decreased by 29.75% in sprouts and 52.62% in microgreens. In both the legumes, the carbohydrate content was increased after boiling. This observed increase in carbohydrate content after boiling are in agreement with the findings of Oboh et al. [32], who reported similar results in roasted yellow and white maize. They suggested that roasting may cause reductions in protein, fat, ash, and moisture thereby an increase in calculated carbohydrate content.

In raw seeds, proteins are primarily stored in the cotyledons. During germination, enzymatic hydrolysis enhances protein levels and bioavailability [6, 33]. Similarly, Inyang et al. [34] also reported that the subsequent enhancement in protein levels may result from enzymatic activity that breaks down seed storage compounds, mobilizing nutrients for new tissue synthesis. Similar findings have been reported in microgreens belonging to the legume family, which exhibited higher protein content compared to raw seeds [35]. On the other side, boiling reduced protein content likely due to protein denaturation and loss of nitrogenous compounds through volatilization [36]. The reduced ash content in boiled legumes may result from the loss of nutrients and minerals into the cooking water [36, 37]. Increased ash content in sprouts and microgreens may be attributed to enhanced phytase activity during germination, which improves bioavailability [38, 39]. The observation is consistent with previous studies reporting an increase in ash content following germination in legumes [7]. The differences in fiber levels during boiling in chickpea can be attributed to the thermal process as discussed by Liao et al. [40].

3.2 Total phenolic content

The effect of different treatments on TPC in mung bean and chickpea is given in Table 2. In mung bean samples, TPC ranged from 3.95 ± 0.60 to 4.69 ± 0.32 mg GAE/g, with no significant ($p < 0.05$) variation in phenolic content across the treatments. Similarly, TPC content in chickpea samples varies from 3.73 ± 0.17 to 5.89 ± 0.22 mg GAE/g. Boiled chickpea seeds showed a 16% reduction ($p < 0.05$) in phenolic content compared to raw seeds. Germination increased TPC in chickpeas, with sprouts and microgreens showing rises of 16% and 33%, respectively, compared to the raw seeds. These findings are consistent with those reported for soaked faba beans and other Indian pulses [41]. The

Table 2 Effect of boiling and germination (sprouting and microgreens) on total phenol content, total flavonoid content and DPPH radical scavenging activity in mung bean and chickpea

Treatments	Mung bean			Chickpea		
	TPC (mg GAE/g)	TFC (mg QE/g)	% RSA (DPPH)	TPC (mg GAE/g)	TFC (mg QE/g)	% RSA (DPPH)
Raw (Control)	4.12 ± 0.34^a	2.45 ± 0.06^a	40.62 ± 0.28^a	4.44 ± 0.26^b	2.51 ± 0.36^b	50.31 ± 0.46^a
Boiled	3.95 ± 0.60^a	1.55 ± 0.08^a	65.20 ± 0.39^b	3.73 ± 0.17^a	1.37 ± 0.26^a	63.20 ± 0.34^b
Sprouts	3.96 ± 0.18^a	5.12 ± 0.06^b	65.91 ± 0.56^b	5.16 ± 0.20^c	3.57 ± 0.18^c	71.47 ± 0.56^c
Microgreen	4.69 ± 0.32^a	8.62 ± 0.86^c	76.58 ± 0.98^c	5.89 ± 0.22^d	8.78 ± 0.45^d	75.53 ± 0.90^d

All the values are in triplicate ($n=3$) and represented by mean $\pm$ SE. Means with different letters are significantly different at $p < 0.05$ using Tukey's test. TPC total phenolic content, TFC total flavonoid content, RSA radical scavenging activity, n number of treatments, SE standard error

reduction in TPC after boiling is likely due to leaching into cooking water and polyphenol oxidase activity, which degrades polyphenols [42]. Additionally, polyphenols loss may also be influenced by their binding with macromolecules like proteins and carbohydrates [39, 43]. Moreover, germinated chickpea samples exhibited an increase in phenolic content, similar to sunflower seeds, where germination led to a 232% rise in TPC [44]. Similar trends were observed across the developmental stages of mung bean and chickpea, with microgreens exhibiting the highest phenolic content, followed by sprouts, while seeds showed the lowest levels [45]. Generally, sprouts and microgreens exhibit a higher bioactive profile than seeds due to the activation of metabolic pathways during germination. Additionally, microgreens, owing to their active photosynthetic stage, synthesize greater amounts of secondary metabolites [46, 47].

3.3 Total flavonoid content

In mung bean samples, TFC ranged from 1.55 ± 0.08 to 8.62 ± 0.86 mg QE/g. The boiled seeds showed only a slight, non-significant ($p < 0.05$) decrease in TFC, whereas, sprouting and microgreens led to significant increase of 109% and 252%, respectively, compared to raw seeds. The TFC content in chickpea samples varies from 1.37 ± 0.26 to 8.78 ± 0.45 mg QE/g. Boiled seeds showed a 45% reduction in TFC ($p < 0.05$) compared to raw chickpea seeds. Both sprouts and microgreens showed an increase of 42% and 250%, respectively in TFC, as compared to the raw sample (Table 2).

The decrease in TFC after boiling in both mung beans and chickpeas likely due to heat degradation and leaching into the water, as flavonoids are sensitive to thermal processing [47]. Similar findings were reported by Franke et al. [48], where the flavonoid content decreased in legumes after processing. In contrast, sprouting and microgreens development led to a significant increase in TFC in both legumes. This increase is attributed to enhanced phenylalanine ammonia-lyase (PAL) activity during germination, which stimulates flavonoid biosynthesis and converts existing compounds into more bioavailable forms [49]. Microgreens, harvested during vigorous metabolic stages, showed higher flavonoid levels, supporting the idea that they contain more phytonutrients than mature plants [19].

3.4 Antioxidant activity

The DPPH scavenging activity in mung bean samples varies from 40.62 ± 0.28 to $76.58 \pm 0.98\%$. The DPPH scavenging activity in boiled seeds ($65.20 \pm 0.39\%$) and sprouts ($65.91 \pm 0.56\%$) showed an increase of approximately 61% as compared to the raw mung bean ($40.62 \pm 0.28\%$) seeds. Both boiled seeds and sprouted samples showed comparable levels, with no significant ($p < 0.05$) difference. Microgreens ($76.58 \pm 0.98\%$) exhibited an 89% increase ($p < 0.05$) in scavenging activity. In chickpea samples, DPPH scavenging activity varies from 50.31 ± 0.46 to $75.53 \pm 0.90\%$. Boiled chickpeas ($63.20 \pm 0.34\%$) showed a 26% increase in scavenging activity compared to raw chickpea seeds ($50.31 \pm 0.46\%$). Sprouting ($71.47 \pm 0.56\%$) and microgreens ($75.53 \pm 0.90\%$) formation resulted in an increase of around 42% and 50%, respectively, in DPPH scavenging activity, as compared to the raw sample (Table 2).

In boiled legumes, thermal processing may liberate bound antioxidant compounds and generate Maillard reaction products with radical-scavenging properties, thereby enhancing AOA [50]. Similarly, high temperature roasting has been shown to increase AOA in

almond kernels [51] and peanut kernel flour [52], likely due to Maillard reaction compounds induced by thermal processing. The increased AOA in germinated samples is likely attributed to the enhanced activity of endogenous antioxidant enzymes. Germination activates metabolic processes that enhance the synthesis and accumulation of bioactive compounds such as polyphenols, flavonoids, tocopherols, ascorbic acid and other antioxidant enzymes, which contribute to higher antioxidant capacity. Sprouts accumulate higher levels of polyphenols and organic acids while microgreens further promote the accumulation of light-induced antioxidants and other phytochemicals [45, 46]. This higher bioactive profile makes sprouts and microgreens valuable functional ingredients for fortifying staple foods with improved health benefits.

3.5 Anti-nutritional factors

Table 3 represents the impact of boiling and germination on anti-nutritional factors in mung bean and chickpea. A notable reduction ($p < 0.05$) in anti-nutritional components was observed in both legumes after boiling and germination. Polyphenols content in mung bean decreased by 28.1%, 24.4%, and 42.7% in boiled seeds, sprouts, and microgreens, respectively, compared to raw seeds. In chickpeas, polyphenol content decreased by 5%, 22.8%, and 51.4% in sprouts, boiled seeds, and microgreens, respectively. Tannins content in mung beans decreased by 25% and 45% in boiled seeds and microgreens, respectively, while in chickpeas, it decreased by 60.7% and 65.6%. Sprouts showed non-significant ($p < 0.05$) difference with raw seeds in both the legumes. Similarly, saponin content in legumes showed a non-significant change in boiled seeds and sprouts as compared to raw seeds, but a significant decrease of 6.8% and 20.3% in mung bean and chickpea microgreens, respectively. Phytates content in mung beans decreased by 41.2% and 51% in boiled seeds and microgreens, respectively, while sprouts showed no significant change. In chickpeas, phytates content decreased by 13.7%, 26.2%, and 71.4% in sprouts, boiled seeds, and microgreens, respectively. Microgreens consistently contained the least amount of anti-nutritional factors compared to other samples.

Anti-nutritional compounds are plant metabolites that interfere with nutrient absorption and negatively impact nutritious quality of foods [53]. However, their levels can be significantly reduced through traditional processing methods like boiling and germination [39] as observed in our findings. Zang et al. [54] reported a significant reduction in tannins in melon seeds after soaking and boiling. Germination further reduces

Table 3 Anti-nutritional factors ($\mu\text{g}/100\text{ g}$) in raw seeds, boiled seeds, sprouts and microgreens of mung bean and chickpea

Treatments	Polyphenols	Tannins	Phytic acid	Saponins
Mung bean				
Raw (control)	726.51 $\pm$ 37.11 ^c	462.16 $\pm$ 69.44 ^b	653.85 $\pm$ 38.46 ^b	632.57 $\pm$ 8.55 ^b
Boiled	522.46 $\pm$ 9.77 ^b	347.22 $\pm$ 46.29 ^{ab}	384.62 $\pm$ 38.46 ^a	626.78 $\pm$ 43.86 ^b
Sprouts	549.31 $\pm$ 3.84 ^b	439.81 $\pm$ 46.29 ^b	564.10 $\pm$ 96.79 ^b	615.09 $\pm$ 13.06 ^b
Microgreen	416.35 $\pm$ 13.84 ^a	254.63 $\pm$ 46.29 ^a	320.51 $\pm$ 58.75 ^a	589.74 $\pm$ 22.61 ^a
Chickpea				
Raw (control)	611.38 $\pm$ 12.58 ^c	439.81 $\pm$ 46.29 ^b	538.46 $\pm$ 38.46 ^c	686.61 $\pm$ 49.34 ^b
Boiled	471.71 $\pm$ 21.82 ^b	172.84 $\pm$ 11.65 ^a	397.44 $\pm$ 58.75 ^b	678.06 $\pm$ 72.69 ^{ab}
Sprouts	580.34 $\pm$ 18.33 ^c	404.32 $\pm$ 11.65 ^b	230.77 $\pm$ 38.46 ^a	664.67 $\pm$ 40.68 ^{ab}
Microgreen	297.24 $\pm$ 1.92 ^a	151.23 $\pm$ 11.65 ^a	153.85 $\pm$ 38.46 ^a	547.01 $\pm$ 39.16 ^a

All the values are in triplicate ($n = 3$) and represented by mean $\pm$ SE. Means with different letters are significantly different at $p < 0.05$ using Tukey's test. n number of treatments, SE standard error

anti-nutrients by the activation of amylase, phytase, and protease enzymes which improve the absorption of proteins and minerals [55]. Phytic acid degradation is accelerated by the activities of phytase and acid phosphatase, which hydrolyze phytic acid into lower inositol phosphates and free phosphate, these enzymes are upregulated by polyamines that support sprout growth [56]. Additionally, sprouting activates several catabolic enzymes, including polyphenol oxidase, which hydrolyse tannins, along with tannin-protein complex formation and leaching during soaking and germination [57, 58]. Similarly, a significant decrease in saponin levels after 48 h of germination has been reported in quinoa [59]. The results are consistent with the study of Pal et al. [60], where germination significantly lowered tannins, oxalic acid, and phytic acid in horse gram. Therefore, germination remains a highly effective traditional approach for reducing antinutrients in legumes, as confirmed in this study.

3.6 Minerals content

Mineral content varied significantly ($p < 0.05$) in all samples (Table 4). Ca content was higher ($p < 0.05$) in sprouts and microgreen as compared to other samples. The lowest Ca level was found in boiled seeds of mung bean (11.67 ± 0.001 mg/100 g) and chickpea (19.35 ± 0.001 mg/100 g). Similarly, Mg and Na contents were also found higher ($p < 0.05$) in microgreens as relative to other samples. The Fe content in microgreens and sprouts was showed insignificant ($p < 0.05$) difference as compared to raw seeds in both legume crops. Similar to Ca content, other minerals also showed significantly ($p < 0.05$) lower levels in boiled mung bean and chickpea seeds.

The reduction in mineral levels after boiling can be linked to the rupture of cell walls, which increases mineral migration into the cooking medium. This phenomenon is well-documented in various studies, highlighting significant mineral loss during boiling due to the water solubility of these minerals. For instance, Owade et al. [61] reported Fe levels ranging from 66 to 75 mg/100 g, Zn from 5.33 to 12.91 mg/100 g, and Ca from 24.3 to 24.6 mg/100 g in boiled and dried cowpea leaves, indicating that both processing methods contribute to mineral depletion. Similarly, a decrease in K levels was reported in boiled melon seeds highlighting the impact of boiling on mineral loss [54]. On the other hand, germination enhances the bioavailability of minerals by breaking down anti-nutritional factors that bind to minerals, thereby improving their absorption [58]. Additionally higher mineral contents are observed in the germinated stage due to enhanced

Table 4 The minerals content in raw seeds, boiled seeds, sprouts and microgreens of mung bean and chickpea

Treatments	Ca (mg/100 g)	Mg (mg/100 g)	Na (mg/100 g)	Fe (mg/100 g)
Mung Bean				
Raw (control)	12.91 ± 0.002^b	15.48 ± 0.003^b	14.71 ± 0.003^b	13.30 ± 0.002^b
Boiled	11.67 ± 0.001^a	9.80 ± 0.001^a	13.56 ± 0.003^a	10.22 ± 0.001^a
Sprouts	14.95 ± 0.002^c	15.23 ± 0.002^b	15.55 ± 0.001^c	13.00 ± 0.001^b
Microgreen	16.38 ± 0.001^d	16.56 ± 0.004^c	16.30 ± 0.003^d	13.75 ± 0.001^b
Chickpea				
Raw (control)	20.47 ± 0.003^b	13.18 ± 0.001^b	15.34 ± 0.001^b	11.16 ± 0.001^b
Boiled	19.35 ± 0.001^a	10.80 ± 0.003^a	13.60 ± 0.001^a	10.68 ± 0.003^a
Sprouts	22.12 ± 0.003^c	14.44 ± 0.001^c	15.90 ± 0.002^b	11.46 ± 0.002^b
Microgreen	29.09 ± 0.002^d	15.36 ± 0.003^d	17.82 ± 0.001^c	11.37 ± 0.001^b

All the values are in triplicate and represented by mean $\pm$ SE. Means with different letters are significantly different at $p < 0.05$ using Tukey's test

metabolic activity, nutrient mobilization from seed reserves combined with mineral uptake from the growing medium [62, 63]. For example, sprouting lentils led to significant increases in calcium and potassium contents by 41–58% and 28–30%, followed by increase in zinc and manganese levels [64]. In microgreens, mineral accumulation is further influenced by the composition of the specific growing medium and light conditions [65]. The observed increase in mineral profile in germinated chickpea and mung bean has the potential to contribute to the alleviation of micronutrient deficiencies.

3.7 Functional groups analysis

The FTIR spectra (Figure 1) reveal key absorption bands indicative of biochemical constituents in mung bean and chickpea, respectively. In both spectra, characteristic peaks were observed at specific wavenumbers, corresponding to fundamental molecular vibrations. The broad peak at $\sim 2919\text{ cm}^{-1}$ in sprout and $\sim 2924\text{ cm}^{-1}$ in boiled samples corresponds to C-H stretching vibrations of lipids and carbohydrates. The characteristic absorption around $1641\text{--}1654\text{ cm}^{-1}$ is attributed to the stretching vibrations of amide I (C = O) in proteins, which is prominent across all samples, suggesting the retention of protein structure despite processing. The region around $996\text{--}1001\text{ cm}^{-1}$ signifies the presence of polysaccharides or carbohydrate-related functional groups. The microgreen sample showed a peak at 1027 cm^{-1} , which was not clearly seen in other treatments, suggesting possible alterations in polysaccharide or glycosidic linkages due to the microgreen growth stage. The changes in functional groups due to germination were also observed in FTIR studies conducted in mung bean [66] and black chickpea [67]. Furthermore, minor shifts in peak positions between raw, sprout, and processed samples indicate modifications in biochemical composition during different growth and treatment stages.

The variations observed in FTIR spectra among different treatments suggest structural changes in macromolecules, particularly proteins, carbohydrates, and lipids. These changes may be attributed to enzymatic activity, metabolic transformations, and thermal processing effects. The FTIR results provide insights into the biochemical transformations occurring at different growth and processing stages, reinforcing the role of microgreens as a nutritionally dynamic food source.

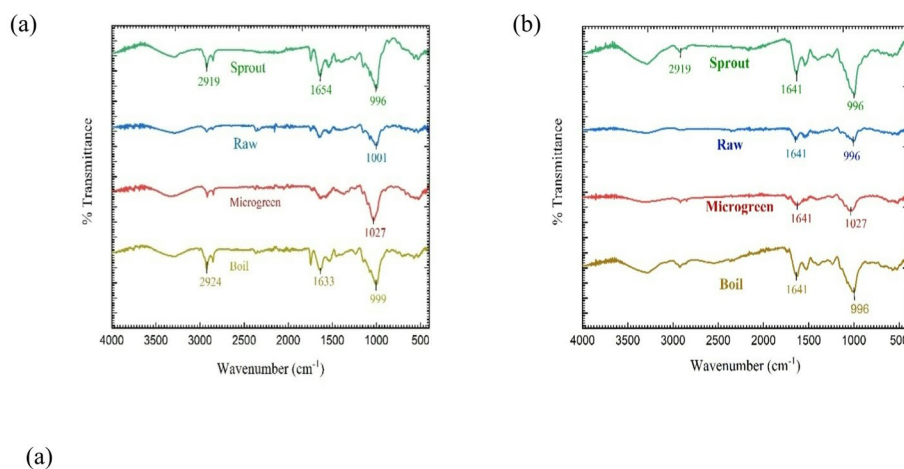


Fig. 1 Comparative FTIR spectrum of raw seeds, boiled seeds, sprouts, and microgreens of (a) mung bean and (b) chickpea

3.8 Chemometric techniques

To interpret the variation among samples, principal component analysis (PCA) was conducted for the eight samples including raw, boiled, spouted seeds and their microgreens of mung beans and chickpea to identify potential clustering patterns. PCA generated 16 orthogonal principal components, which were further refined through varimax rotation to enhance interpretability.

The first PC1 exhibited highest eigenvalue (8.74), explaining 54.67% of the total variance. Eigenvalues for the second, third, and fourth components were 3.44, 1.68 and 1.20, respectively and accounted for 21.54, 10.52, and 7.55% of total variance in dataset. The remaining twelve subsequent PCs (PC5 to PC16) had low eigenvalues, explaining less than 6% of the total variance. Hence, based on Kaiser's rule, only the first four PCs, accounting for 94.28% of the total variance were considered for further analysis. Table 5 represents the key varimax-rotated principal components from the 16 variables along with their statistical loadings.

Factor loading refers to the correlation between the original variable and the factor or rotated component obtained from PCA. The values indicate the significant contribution of proximate composition, minerals, antinutritional compounds, bioactive compounds and antioxidant activity to the total variance. According to the PCA theory, factor loading values of 0.7 or higher were used to determine the key variables and attributes in each PC [68]. PC1 was strongly influenced by protein, ash, flavonoids, magnesium, sodium, and iron; negatively contributed by carbohydrate. PC2 showed a positive contribution from phytic acid; and a negative from DPPH. PC3 was influenced by calcium and fat, while PC4 by crude fiber.

The score plots of proximate composition, minerals, anti-nutritional compounds, bioactive compounds, and antioxidant activity based on first two PCs is illustrated (Figure 2). The PC1-PC2 score plot highlight three distinct sample groups. Cluster I includes mung bean microgreens and chickpea microgreens owing to higher protein, minerals,

Table 5 Varimax rotated factor loadings of the significant principal components (PCs)

Variables	PC1	PC2	PC3
Protein	0.830	-0.480	
Fat	-0.442	0.139	0.832
Ash	0.909	-0.167	
Crude fiber	-0.118	0.156	
Carbohydrate	-0.841	0.483	
Phenolics	0.570	-0.501	
Flavonoids	0.870	-0.290	
DPPH	0.261	-0.801	
Polyphenols	-0.251	0.460	
Tannins		0.251	
Phytic acid	-0.131	0.816	
Saponins	-0.695	0.227	
Calcium	0.254	-0.295	0.843
Magnesium	0.963		
Sodium	0.835	-0.286	
Iron	0.802	0.304	
Eigen value	8.74	3.44	
Variance %	54.67	21.54	
Cumulative %	54.67	76.21	

The significant loadings are highlighted bold

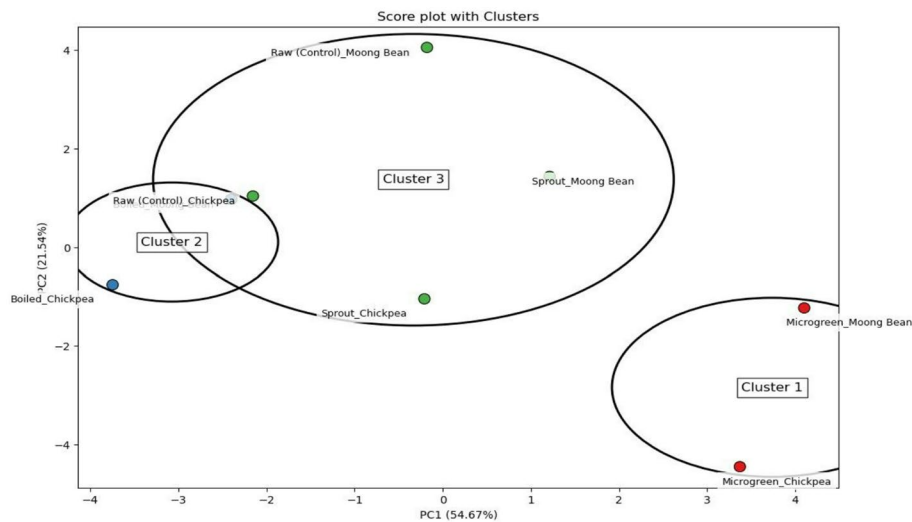


Fig. 2 Score plot of different samples in mung bean and chickpea

flavonoids, antioxidant activity and relatively lower content of anti-nutritional compounds. Cluster II consists of boiled chickpea and boiled mung bean owing to lower level of protein, minerals, flavonoids, phenolics, antioxidant activity, and moderate content of anti-nutritional compounds. Cluster III was related to raw and sprouted mung bean and chickpea due to their moderate content of antioxidants such as phenolics and flavonoids, higher content of antinutritional compounds, moderate to higher content of minerals, and lower to moderate content of protein.

Agglomerative hierarchical clustering (AHC) was applied to eight samples, including raw, boiled, and spouted seeds and their microgreens of mung beans and chickpeas. The samples were clustered based on their similarities and represented in a heatmap (Figure 3). AHC showed distinct clustering of microgreens, boiled, and raw and sprouted beans. However, raw and boiled beans were more closely related to each other than to their respective microgreens. First cluster consists of microgreens from mung beans and chickpea because of higher level of minerals, protein, phenolics, flavonoids and antioxidant activity as seen in PCA. The second cluster includes boiled chickpea and boiled mung bean owing to lower levels of protein, minerals, flavonoids, phenolics, antioxidant activity and moderate content of anti-nutritional compounds. The third cluster consists of all raw and sprouted mung beans and chickpea having higher level of anti-nutritional compounds, moderate to higher content of minerals and moderate content of antioxidants.

4 Conclusion

This study demonstrated that processing methods and growth stages exert a significant influence on the nutritional, bioactive, and anti-nutritional composition of chickpea and mung bean. Germination and microgreen development enhanced protein levels, mineral availability, phenolic and flavonoid content, and antioxidant activity, while reducing anti-nutritional compounds. In contrast, boiling led to marked reductions in proteins, minerals, and phenolics.

Overall, sprouts and microgreens emerged as nutritionally superior to both raw and boiled seeds. Their enriched nutrient and bioactive profiles, combined with reduced

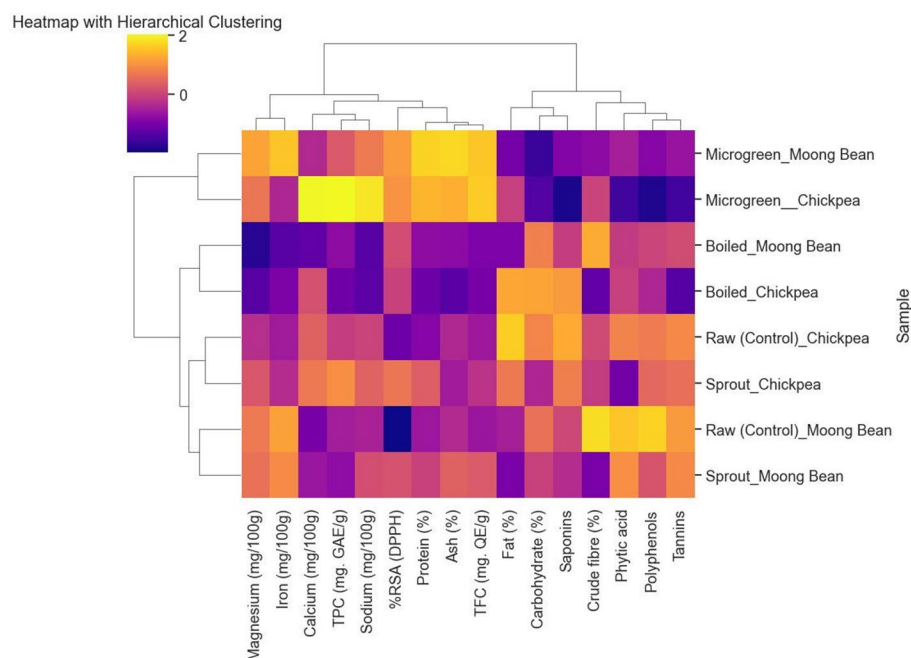


Fig. 3 Agglomerative hierarchical cluster analysis (AHC) and heatmap of bioactive compounds, antioxidant activity, minerals, and anti-nutritional compounds from raw, boiled, sprouts of chick pea and mung beans and their microgreens. The comparative variable levels are represented by color temperature, with the scheme indicating relative levels from minimum (blue) to maximum (yellow) for each variable

anti-nutritional factors, highlight their potential as functional foods. Promoting their consumption could address malnutrition, enhance dietary quality, and reduce the risk of lifestyle-related diseases, aligning with global nutrition and health initiatives.

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Author contributions

Conception and design of study: Rekha Kumari Meena; Acquisition of data: Lohit, Anusree Sobhanan; Analysis and interpretation of data: Lohit, Rekha Kumari Meena, Anusree Sobhanan, Himadri Shekhar Roy; Drafting the manuscript: Rekha Meena; Review and editing: Rekha Kumari Meena, Sunil Pareek. The final version of the manuscript, as published, has received the approval of all authors.

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Data availability

The complete set of original contributions is provided in the article. For additional information, please contact the corresponding authors.

Declarations

Ethics approval and consent to participate

The plant materials used in this study (mung bean *Vigna radiata* L. and chickpea *Cicer arietinum* L.) were procured as certified seeds from ICAR-Indian Agricultural Research Institute (IARI), New Delhi, India, an authorized national institute. All experimental plant materials were cultivated under controlled laboratory conditions, and no wild plant collection was involved. Therefore, no specific permits or licenses were required. The study complied with all relevant institutional, national, and international guidelines for the use of plant materials.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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