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Content and Bioaccessibility of Phenolics and Flavonoids in Processed Germinated Brown Chickpea (Cicer Arietinum L.)

Short title: Digestible bioactive components in germinated chickpea

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Abstract

Germination is a traditional pre-process used for improving the cooking and sensory quality of legumes. The study explored the availability of bioactive components in chickpea fortified through germination in mineral fortified soak water. Whole chickpea grains were germinated in water enriched with either iron or zinc at two levels. A portion of grains was dehulled. Both whole and dehulled grains were cooked by two methods, namely, pressure cooking or microwave oven. The sample germinated in plain water served as control. All samples were analyzed for total and in vitro digestible bioactive components. The remaining soak water was also analyzed for presence of bioactive components. The results indicated that the process of dehulling improved the content and digestibility of total phenolics and tannins in fortified chickpea in comparison to control grain. For flavonoids, the results were opposite. In general, cooking reduced the content and digestibility of bioactive components; however, percent digestibility from cooked grains was better. Differences were found in digestibility of bioactive among iron and zinc fortified grains. In conclusion, the dehulling and cooking processes influenced the content and digestibility of bioactive components in mineral fortified germinated chickpea.

Keywords: Cicer Arietinum; Germination; Dehulling; Cooking; Phenolics; Tannins; Flavonoids

Introduction

Legumes are categorized as healthy grains on account of their nutritional composition and presence of several phytochemicals [1]. Evidence regarding their disease prevention role is emerging in many epidemiological, clinical and experimental studies [2, 3]. Many legume seeds have also been reported to exhibit alpha-amylase and alpha-glycosidase inhibition properties [3, 4]. Specifically, chickpea cultivars were associated with 58-62% of alpha amylase inhibitory activity indicative of anti-diabetic properties [5].

Generally dry legume seeds need some processing to convert them to edible form. Thermal treatments such as puffing, roasting, boiling, pressure cooking or frying develop flavors and improve the textural quality and palatability of legumes remarkably. Some legumes are also consumed as germinated grains with or without further processing. Germination improves the digestibility of grains by developing new enzymes, partially breaking down protein and starch and destroying anti-nutrients [6, 7, 8]. During pre-germination process, especially soaking, the bioactive components lixiviate in water and the water could affect the rate and kind of bioactive components which move out of the grain hulls. Germination has also been reported to influence the antioxidant components and activities of legumes [9, 10]. Legumes are also milled to remove the outer portion of grain which improves their cooking characteristics further. Outer layer of grains (husk) is a rich source of bioactive components; hence, dehulling can change its content. Cooking can cause considerable changes in the content of bioactive components and antioxidant activity [11].

Physiological availability of nutrients and bioactive components is a prime determinant of food and nutrition security and human

health. Hence, the inherent or externally added constituents of any food should be absorbed for the ultimate utilization by individuals. The bioavailability of any food component is influenced greatly by factors related to food or to the host. The food matrix, the processing conditions, and overall composition of food determines how much of an ingested component is absorbed by the body. For this reason, it is important to study the bioaccessibility of essential constituents from food [12].

Legumes serve as an important source of protein in SouthEast Asian countries where they are part of everyday diet. While as such they are good sources of vitamins and minerals, it is possible to fortify them to improve the micronutrient profile. Iron and zinc deficiencies are commonly seen across all age group, especially in developing countries and are recognized as major public health problems. Fortification of staple foods can be one of the strategies to overcome malnutrition [13]. Apart from industrial fortification of staples, it is also possible to incorporate fortificants at domestic level in traditional processes to improve malnutrition. In the present study, whole chickpea grains were germinated using iron or zinc fortified soak water and thereafter processed further. The objective was to study the effect of dehulling and cooking on total and digestible (bioaccessible) bioactive components in chickpea geminated in mineral fortified soak water.

Materials and methods

Materials

The material used for the study was whole chickpea (*Cicer arietinum*L.) of Desi variety with a brown color coat purchased from local market in one batch. All experiments were carried out in duplicate/triplicate with analytical grade chemicals and double distilled water. Chemicals were procured from (i) SD Fine Chemicals, India and (ii) HiMedia Company, India. The enzymes used for the digestibility experiments were pepsin (Batch No. 3-0060), pancreatin (Batch No. 0-0864), diastase (Batch No. 0695/195/270511) and papain (Batch No. 0993/493/130811). The dialysis tubing was procured from Sigma Aldrich Co. USA with a molecular mass cut off of 10 kDa.

Methods

Study Design

The study involved fortifying germinated *Desi*chickpea through enriching the soak water with minerals (iron or zinc at two levels), dehulling a part of germinated grains and cooking both whole and dehulled grains with two methods. The control sample was germinated in plain water. All samples were analyzed for total and digestible bioactive components. The soak water, in which grains were soaked prior to germination, was also analyzed for presence of bioactive components.

Germination and cooking of chickpea

For the control sample, a 100g portion of whole chickpea was washed and soaked in 300 ml of distilled water for 10 hours. For the fortified samples, the soak water was enriched with two levels of iron (ferrous sulfate equivalent to 100 or 200 mg of iron) or zinc (zinc sulfate equivalent to 50 or 100 mg of zinc). The soaked grains were transferred to a grain germinator, [wherein grains are kept moist to facilitate germination] and allowed to germinate for 48 hours at normal room temperature (28 ± 5°C) and lighting conditions. The germinated grains were divided into two portions, and one portion was manually dehulled. Manual dehulling was done by gently rubbing the grain between two fingers, since the husk portion was sufficiently loosened on account of soaking and germination, a gentle rub facilitated easy dehusking. A part of this was homogenized well in a blender and kept aside to be used as control and the rest was divided and cooked using two methods, namely pressure cooking (PC) and microwave (MW) cooking. For cooking, 50 ml water was added to 100 g germinated grain as cooking medium. Pressure cooking was done for 10 min on hot plate adjusted for maximum power in a standard pressure cooker with specifications of maximum 15 lbs pressure and 121°C temperature. The microwave (Model BPL-700T, 2,450 MHz, 1,200 watts, from BPL Sanyo Utilities and Appliances Ltd, Bangalore, India) was adjusted to quick set program and 100 g germinated grains were heated in oven continuously for 4 min. The cooking time was standardized by checking the softness of grain by pressing between two fingers. The cooked samples were homogenized with cooking water in a blender and used for further analysis. All analysis was done on freshly prepared samples. Moisture content of all samples was determined using oven drying method [14].

Analysis of bioactive components

To estimate total phenolics, flavonoids and tannin contents in chickpea, extracts were prepared by mixing known amount of sample slurry (raw and cooked) with glass double distilled water. The slurry was shaken for 3 hours and filtered through "Whatman" filter paper No.1 and used for all analysis.

Total polyphenol- A 0.2 ml of sample was mixed with 1.0 ml of Folin-Ciocalteau reagent (10 fold dilutions) and 0.8 ml of 2% Na_2CO_3. The volume was made up to 10 ml using water-methanol (4:6) as diluting fluid. This was allowed to stand for 30 min and absorbance read at 740 nm. Concentration was calculated using tannic acid as standard and the results expressed as mg tannic acid equivalents/100g sample [15].

Total flavonoids- This was estimated following the procedure explained by Arvouet-Grand et al. [16]. A 5.0 ml of 2% $AlCl_3$ in methanol was mixed with 5.0 ml of the extract solution and allowed to stand for 10 min. Absorption was read at 415 nm against a blank sample consisting of extract solution with 5.0 ml methanol

without $AlCl_3$. The total flavonoid content was determined using a standard curve with quercetin and expressed as mg quercetin equivalents/100g of sample.

Total tannins- Colorimetric estimation of tannins is based on the measurement of blue color formed by the reduction of phosphotungstomolybdic acid by tannin like compounds in alkaline solution [17]. A known amount of extract was mixed with 5.0 ml of Folin-Denis reagent and Na_2Co_3 solution and made up to 100 ml, mixed well and absorbance read at 760 nm after 30 min. Total tannin content was expressed as mg tannic acid equivalent/100 g of sample.

Digestible bioactive components

In vitro digestibility (also known as bioaccessibility) of total phenols, flavonoids and tannins was determined by simulated gastro-intestinal digestion using pepsin for the gastric stage followed by pancreatin and bile salts for the intestinal stage. The proportion of bioactive compound diffused through a semi-permeable membrane was considered as measure of the digestibility [18]. A known amount of sample was placed in a 250ml Erlenmeyer flask and 70 ml of water was added. The pH was adjusted to 2.0 with 6M hydrochloric acid. After 15 min the pH was checked and readjusted to 2.0, if necessary. A 3.0 ml of freshly prepared pepsin solution (16% pepsin in 0.1M hydrochloric acid) was added, volume made up to 100 ml with water and the samples incubated at 37^0C for 2 hours. Then the digests were frozen for 90 min. Titratable acidity was determined in an aliquot containing 5 ml of pancreatin bile extract mixture (0.4% pancreatin and 2.5% bile salt in 0.1M sodium bicarbonate) by titrating against 0.2M sodium hydroxide till the pH of 7.5 was obtained. A 20 ml of frozen digests (after thawing) were subjected to stimulated intestinal digestion by placing the dialysis tubing in Erlenmeyer flasks. The dialysis tubing contained 25 ml of sodium bicarbonate (equivalent to moles of sodium hydroxide determined by titratable acidity). The flasks were incubated in a shaker bath at 37^0C for 30 min (till the pH reached 5.0), 5.0 ml of pancreatin bile extract mixture was added and shaken for another 2 hours (till the pH reached 7.0). Then the dialysates were carefully transferred to graduated tubes and volume was measured. The collected dialysates were analyzed for total phenols, flavonoids and tannins as per the aforementioned procedures.

Statistical Analysis

Data were subjected to statistical analysis using statistical software SPSS 15.0 (SPSS Inc., Chicago, IL). Mean and standard deviation for all values were calculated. Data were analyzed using Student's *´T´* test to determine significant differences between (i) whole and dehulled samples, (ii) raw and cooked samples and (iii) PC and MW cooked samples. In addition, data were also subjected to analysis of variance to determine the significant differences between all variations of control germinated raw whole and dehulled grains (five variations each) and cooked whole and dehulled grains (ten variations each).

Results and discussion

Effect of dehulling germinated chickpea on total and digestible bioactive components

The total, digestible and percent digestible total phenols (TP), tannins and flavonoids in raw, whole and dehulled chickpea are compiled in Table 1. The TP content of the control whole chickpea reduced significantly after dehulling from 170.2 mg/100g to 155.6 mg/100g, whereas in other variations (fortified grains) dehulling increased TP content in germinated grains. Fortification of grain with minerals influenced the TP content to a large extent and in most of the fortified grains; a higher TP content could be estimated. Digestible TP increased in all variation of chickpea following dehulling and was significant for control and sample with 200 mg Fe. When computed as percent digestibility, the control chickpea had highest value of 81.58 after dehulling, whereas all variations followed an opposite trend of showing higher percent digestibility for whole grains. Among variations, Zn fortified samples had least percent digestible TP. In a similar study with green gram (*Vigna radiata*) the results were slightly different. Dehulling did not affect the total and digestible phenolics in green gram significantly and among all variations, iron fortification reduced the bioactive components while zinc did not have any effect [19].

Data were further analyzed using ANOVA to examine if differences were significant within all variations of (i) whole and (ii) dehulled grains. The overall observations are as follows, mineral fortified grains had higher phenolic content in comparison to control with one exception of 200 mg iron fortified sample. This could possibly be explained on the basis that minerals bound phenolics, so they could not be leached out in soak water. However, this difference was reduced when bio accessibility of phenolics was examined. Grains with 100 mg iron fortification showed maximum bioaccessibility whereas zinc fortified dehulled grains had least. Similar trend was also seen in percent bioaccessible phenolic constituents. This indicates that zinc binding with phenolics was stronger and not easily broken down by digestive enzymes.

The process of soaking and germination itself can influence the phenolic constituents of legumes. Lopez-Amoros et al. [10] in their study on effect of germination on antioxidant components of beans, lentils and peas reported that germination modified the quantitative and qualitative phenolic composition. These were in turn influenced by the type of legume and the germination conditions. Vadivel et al. [3] also reported an increase in total phenolic constituents of wild legumes on germination. Fouad and Rehab [20] found a progressive increase in the phenolic content of lentils with an increase in sprouting time.

Table 1: Effect of dehulling of germinated chickpea on total and digestible bioactive components (mean±standard deviation per 100g)

Variations		Total	Digestible	
		(mg)	(mg)	(%)
Total phenols				
A. Distilled water (control)	Whole	170.2±0.00^{c}	108.6±0.57^{b}	63.79±0.33^{a}
	Dehulled	155.6***±0.00^{c}	126.9*±1.22^{ab}	81.58*±0.78^{a}
B. With 100 mg Fe	Whole	207.3±2.52^{a}	138.8±3.90^{a}	66.95±1.88^{a}
	Dehulled	234.8*±3.56^{a}	139.7±5.11^{a}	59.48*±2.17^{b}
C. With 200 mg Fe	Whole	144.0±0.00^{d}	104.8±0.00^{b}	72.80±0.00^{a}
	Dehulled	190.2*±2.82^{b}	121.4**±0.26^{b}	63.86**±0.14^{b}
D. With 50 mg Zn	Whole	184.4±0.00^{b}	91.3±0.97^{b}	49.51±0.53^{b}
	Dehulled	229.5*±1.86^{a}	103.9±2.09^{c}	45.26±0.91^{c}
E. With 100 mg Zn	Whole	186.7±1.70^{b}	92.4±8.41^{b}	49.40±4.60^{b}
	Dehulled	225.1*±8.59^{a}	103.0±7.37^{c}	45.74±3.27^{c}
Tannins				
A. Distilled water (control)	Whole	223.8±1.23^{c}	173.7±3.07^{b}	77.60±1.37^{a}
	Dehulled	286.5*±6.31^{bc}	190.8*±0.00^{a}	66.58*±0.00^{b}
B. With 100 mg Fe	Whole	247.4±1.94^{a}	192.8±3.53^{a}	77.93±1.43^{a}
	Dehulled	268.1*±0.00^{c}	190.9±3.31^{a}	71.20**±1.23^{a}
C. With 200 mg Fe	Whole	183.0±2.59^{e}	80.3±5.74^{d}	43.87±3.14^{c}
	Dehulled	328.0*±8.65^{a}	91.6*±0.34^{d}	27.94*±0.06^{e}
D. With 50 mg Zn	Whole	196.9±0.00^{d}	92.3±4.49^{d}	46.88±2.28^{c}
	Dehulled	317.0*±10.15^{ab}	110.9*±1.99^{c}	34.99*±0.62^{d}
E. With 100 mg Zn	Whole	235.8±2.04^{b}	152.4±3.77^{c}	64.60±1.60^{b}
	Dehulled	315.3*±10.65^{ab}	164.1±3.28^{b}	52.05*±1.04^{c}
Flavonoids				
A. Distilled water (control)	Whole	22.52±1.17^{a}	5.73±0.00^{a}	25.50±0.00^{a}
	Dehulled	18.40±1.97^{a}	3.44*±0.27^{a}	18.77*±1.43^{ab}
B. With 100 mg Fe	Whole	21.93±2.83^{a}	5.08±0.23^{a}	23.16±1.04^{a}
	Dehulled	15.41±0.00^{a}	2.61*±0.12^{a}	17.04*±0.76^{b}
C. With 200 mg Fe	Whole	19.72±0.00^{a}	4.83±0.13^{a}	24.52±0.68^{a}
	Dehulled	14.60*±0.67^{a}	2.36*±0.00^{a}	16.20*±0.00^{b}
D. With 50 mg Zn	Whole	22.56±0.32^{a}	5.61±0.21^{a}	26.21±0.99^{a}
	Dehulled	17.33±1.78^{a}	3.90±0.23^{a}	22.57±1.24^{a}
E. With 100 mg Zn	Whole	19.93±0.00^{a}	4.81±0.24^{a}	24.20±1.20^{a}
	Dehulled	17.40±0.53^{a}	3.10±0.00^{a}	19.23*±0.00^{ab}

Significant differences between whole and dehulled grains on application of 'T' test; * P<0.05, **: P<0.01, ***: P<0.001, values without notation are not significantly different.Differing superscripted small letters in a column indicate significant difference among all variations of (i) whole grains and (ii) dehulled grains.

The tannin content of chickpea increased in all variations significantly on dehulling. The highest tannin in whole (247.4 mg/100g) and dehulled grain, (328.0 mg/100g) was found in variation B and C respectively. Mineral fortification either increased or decreased tannin content of chickpea, and no specific trend was seen. These alterations could be due to loss or retention of tannins in soak water mediated by presence of external minerals. Digestible tannin increased in chickpea due to dehulling in four samples and increases in variation A, C and D was significant. Variation C (with 200 Fe) showed least tannin digestibility in whole and dehulled grain (80.3 and 91.6 mg/100g respectively). Percent digestible tannin reduced significantly in all variations of chickpea in dehulled

grain. In a similar study with green gram dehulling did not affect the content and digestibility of tannins, though the fortification with iron had an effect. The iron fortified samples had lesser digestibility compared to control and zinc fortified samples [19]. Application of ANOVA revealed significant differences among tannin content of both whole and dehulled grain variations. The bioaccessibility was in the lower range for mineral fortified samples with exception of samples with 100 mg iron indicating that presence of minerals affected tannin bioaccessibility.

Total, digestible and percent digestibility of flavonoids reduced in chickpea due to dehulling which shows that the husk portion could be good source of flavonoids. The flavonoid content ranged from 14.60-22.56 mg/100g in chickpea which was markedly lesser than TPs and tannins. Reduction of total flavonoids in variation C was significant (by 5.12 mg/100g). Digestible flavonoids reduced significantly in control chickpea and in variations with iron whereas changes in variation with zinc were not significant. Highest percent flavonoids digestibility was seen in variation D (26.21%). The overall percent digestible flavonoids was in lower range in comparison to TP and tannins. Application of ANOVA did not reveal any significant differences among all variations of either whole or dehulled grains for content and bioaccessibility of flavonoids, though differences were observed in percent bioaccessibility.

Sharma et al. [5] analysed the bioactive components of five cultivars of Indian brown chickpea and reported range of values for total phenolics, tannins and flavonoids as 203-255 mg, 170-210 mg, and 31-36 mg/100g respectively. The range of values for all constituents were similar to the present study. A high content of phenolics and flavonoids in the whole grains can be attributed to their high concentration in the hull portion as observed by Kanatt et al. [21], who found that the hull extract of chickpea was a rich source of phenolics and flavonoids.

Effect of cooking on total and digestible bioactive components in germinated chickpea

Table 2 presents the content and digestibility of phenolics, tannins and flavonoids in cooked whole chickpea. The content of TP as well as its digestibility were significantly higher in PC than MW cooked samples in all variations of whole chickpea. Highest TP content (129.7 mg/100g) and digestibility (128.5 mg/100g) was found in PC control sample. Fe fortification at higher level (200 mg) and Zn fortification at both levels resulted in decreased content and digestibility of TP than control. However, both control and Fe fortified variations had better percent digestibility of TP (>90%), whereas Zn fortification reduced it to some extent. This shows that zinc bound phenolic constituents to a larger extent as was seen in dehulled raw sample (Table 1). Application of ANOVA revealed significant differences among all variations considered together, both for content and digestibility of phenolics. However, for percent bioaccessibility, there were no differences among control and iron fortified samples whereas zinc fortified samples were significantly lower.

When effect of cooking was analyzed as comparison between raw and cooked samples, cooking lowered the TP content significantly in all variations, both control and fortified. Digestibility was also lesser, though it was significant only for few variations. Percent digestibility was much higher in comparison to raw for all variations and was marginally significant. To summarize the overall effect, cooking decreased the TP content but improved the availability.

The tannin content was in the range of 127.3-190.7 mg/100g for PC samples and 116.9-198.5 mg/100g for MW cooked samples. MW cooking increased tannin content and digestibility for iron fortified samples but lowered it for Zn fortified samples and differences were marginally significant. The percent digestibility did not follow any specific trend but was higher for control and in lower ranges for mineral fortified samples. When cooked samples were compared with raw, the tannin content was lower, though digestibility was similar indicating that cooking did not influence the availability of tannin in germinated chickpea. Application of ANOVA revealed significant differences in tannin content among all variations. Bioaccessibility was higher in variations A and B followed by E, and then by C and D. Similar trend was seen for percent bioaccessibility of tannins too.

The flavonoid content of whole cooked chickpea was in the lower range in comparison to raw in all variations, though differences were not significant. However, cooking improved the digestibility of flavonoids in control and Zn fortified samples. Percent digestibility was also higher than raw samples. The method of cooking (under pressure or in microwave) did not influence the flavonoids content or availability.A further analysis using ANOVA did not reveal significant differences among all variations put together except for PC sample with 100 mg iron, which was marginally lower for flavonoid content. However, for digestibility, significant differences were seen among all. The differences were lesser in percent digestibility with seven variations showing similar values and the rest in lower ranges.

The data on content and digestibility of bioactive components of dehulled germinated chickpea grains are compiled in Table 3. The phenolic compounds of PC and MW cooked variations were in the range of 118.0-155.5 and 85.3-130.1 mg/100g respectively. MW cooked samples had significantly lesser TP content and digestibility than PC samples. To analyze the effect of cooking, when cooked samples were compared with raw dehulled grains, both TP content and digestibility were significantly lesser, though in terms of percent digestible components, cooked grains were better. Similar observations could be made for tannin content and digestibility, wherein they were much lesser than raw samples but percent digestibility was higher. No specific trend was seen between methods of cooking. The flavonoids followed a pattern similar to

Table 2: Effect of cooking on total and digestible bioactive components in whole chickpea (mean±standard deviation per 100g)

Variations		Total		Digestible			
		(mg)	N	(mg)	N	(%)	N
Total phenols							
A. Distilled water (control)	PC	129.7±2.62^{a}	*	128.5±2.10^{a}	*	99.12±1.61^{a}	*
	MC	107.7*±0.00^{c}	**	99.63*±5.32^{cd}	-	92.49*±4.94^{a}	*
B. With 100 mg Fe	PC	119.9±1.47^{b}	**	115.7±0.00^{b}	-	96.49±0.00^{a}	*
	MC	110.4**±0.71^{c}	**	108.9*±1.11^{c}	-	98.681.00^{a}	*
C. With 200 mg Fe	PC	122.0±0.00^{ab}	*	106.1±1.66^{c}	-	86.97±1.37^{a}	*
	MC	94.57***±0.00^{d}	**	88.57*±0.68^{d}	*	93.66*±0.71^{a}	*
D. With 50 mg Zn	PC	105.2±0.00^{c}	**	78.13±1.39^{e}	-	74.11±1.51^{b}	*
	MC	90.44***±3.47	***	67.87*±2.06^{f}	*	75.03±2.28^{b}	*
E. With 100 mg Zn	PC	97.17±2.88	*	78.49±2.52^{e}	-	80.78±8.77^{b}	*
	MC	82.71*±1.43	*	59.57**±1.04^{f}	*	72.06*±1.25^{b}	*
Tannins							
A. Distilled water (control)	PC	190.7±0.00^{ab}	*	179.50±0.99^{a}	-	93.59±0.00^{a}	-
	MC	163.2*±0.63^{d}	**	155.73*±2.61^{b}	-	95.42±1.60^{a}	-
B. With 100 mg Fe	PC	176.1±2.85^{bc}	*	159.20±8.37^{ab}	-	90.40±4.75^{a}	-
	MC	198.5*±4.65^{a}	*	149.85*±6.93^{b}	*	75.50±3.49^{b}	-
C. With 200 mg Fe	PC	170.7±4.62^{cd}	-	44.75±0.00^{d}	-	26.21±0.00^{c}	-
	MC	171.2*±1.56^{c}	-	56.81*±8.96^{d}	-	33.17*±5.23^{c}	-
D. With 50 mg Zn	PC	127.3±6.62^{e}	*	56.94±0.00^{d}	-	44.73±0.00^{c}	-
	MC	116.9*±1.14^{e}	**	52.72*±2.55^{d}	-	45.09±2.17^{c}	-
E. With 100 mg Zn	PC	179.5±3.74^{bc}	*	128.62±1.78^{c}	-	72.04±1.37^{b}	-
	MC	168.3*±1.39^{cd}	*	119.29*±3.63^{c}	-	70.89±2.17^{b}	-
Flavonoids							
A. Distilled water (control)	PC	16.80±2.24^{a}	-	6.32±0.08^{ab}	-	37.74±0.45^{a}	-
	MC	18.40±0.00^{a}	-	6.64±0.00^{a}	*	36.20±0.00^{a}	-
B. With 100 mg Fe	PC	13.20±1.13^{b}	-	4.12±0.22^{def}	-	31.28±1.74^{a}	-
	MC	15.89±0.51^{a}	-	4.58±0.00^{de}	-	28.83±0.00^{bc}	-
C. With 200 mg Fe	PC	14.68±1.63^{a}	-	4.04±0.08^{f}	-	26.92±1.28^{bc}	-
	MC	14.77±0.90^{a}	-	3.79±0.24^{g}	*	25.71±1.59^{c}	-
D. With 50 mg Zn	PC	17.42±1.48^{a}	-	6.23±0.21^{ab}	-	35.86±1.14^{a}	-
	MC	16.90±0.00^{a}	-	5.86±0.06^{bc}	-	34.79±0.31^{a}	-
E. With 100 mg Zn	PC	15.62±0.78^{a}	-	5.16±0.17^{cd}	-	33.07±1.15^{a}	*
	MC	17.35±1.23^{a}	-	5.35±0.00^{c}	-	30.83*±0.00^{ab}	-

PC: pressure cooked; MW: microwave cooked. Significant differences between PC and MW cooked grains on application of 'T' test; * P<0.05, **: P<0.01, ***: P<0.001, values without notation are not significantly different. Column N shows significant differences between raw (Table 1) and cooked grains. Differing superscripted small letters in a column indicate significant difference among all variations together.

whole grains with the content being lesser and percent digestibility being higher than raw grains.

The data were also analyzed using ANOVA for all variations put together and the results showed significant differences among variations for phenolic content. Again, there was no specific trend but zinc fortified samples had lower content and digestibility. Similar trend was seen for tannins also. The differences were lesser in flavonoid content and digestibility. These differences can possibly be explained by the presence of varying amount of mineral uptake by the grains during germination (the process being further influenced by dehulling), which in turn influences the extent of

Table 3: Effect of cooking on total and digestible bioactive components in dehulled chickpea (mean±standard deviation per 100g)

Variations		Total		Digestible			
		(mg)	N	(mg)	N	(%)	N
Total phenols							
A. Distilled water (control)	PC	118.0±0.00^{c}	*	122.1±1.59^{a}	-	103.1±0.92^{a}	**
	MC	109.1***±3.79^{c}	*	94.35*±1.89^{c}	*	86.49**±1.73^{b}	-
B. With 100 mg Fe	PC	155.5±2.26^{a}	*	118.1±2.84^{ab}	*	75.96±1.83^{c}	*
	MC	130.1*±2.16^{b}	**	109.6*±0.88^{b}	*	84.24**±0.68^{bc}	*
C. With 200 mg Fe	PC	125.4±2.07^{bc}	*	89.53±2.33^{d}	*	71.39±1.87^{d}	-
	MC	106.1*±1.20^{c}	**	83.71*±1.74^{d}	*	78.93*±1.64^{bc}	*
D. With 50 mg Zn	PC	119.6±1.58^{c}	**	102.3±2.12^{c}	-	85.54±1.77^{bc}	*
	MC	105.5*±6.72^{c}	**	77.64**±2.15^{e}	-	73.57*±2.03^{d}	*
E. With 100 mg Zn	PC	117.9±5.44^{c}	**	86.71±0.00^{d}	-	73.52±0.00^{d}	*
	MC	85.28**±1.37^{d}	**	69.87*±1.21^{f}	-	81.96*±1.40^{bc}	*
Tannins							
A. Distilled water (control)	PC	198.4±2.28^{b}	*	178.9±1.00^{a}	-	90.16±0.50^{a}	*
	MC	171.8*±0.56^{d}	*	144.1*±2.64^{c}	*	83.86**±1.54^{a}	*
B. With 100 mg Fe	PC	202.9±4.39^{ab}	*	158.6±0.92^{b}	*	78.14±0.45^{bc}	-
	MC	214.5*±0.00^{a}	*	153.8*±6.26^{bc}	*	71.73*±2.92^{c}	-
C. With 200 mg Fe	PC	210.9±3.91^{ab}	*	118.5±3.82^{de}	*	56.20±1.23^{d}	*
	MC	202.1*±0.00^{ab}	*	88.92*±2.47^{g}	-	44.00*±1.22^{e}	*
D. With 50 mg Zn	PC	202.0±3.46^{ab}	*	100.9±1.54^{f}	-	70.24±1.51^{c}	*
	MC	185.7*±1.03^{c}	*	107.2±2.19^{ef}	-	57.74*±1.25^{d}	*
E. With 100 mg Zn	PC	177.4±2.18^{d}	*	126.5±3.45^{d}	*	71.33±1.95^{c}	*
	MC	169.8*±1.32^{d}	*	147.5*±5.74^{bc}	-	86.87*±3.38^{ab}	*
Flavonoids							
A. Distilled water (control)	PC	15.30±1.32^{ab}	-	5.39±0.15^{a}	-	35.22±0.99^{ab}	**
	MC	16.41±0.00^{a}	-	6.01±0.25^{a}	-	36.66±1.54^{a}	**
B. With 100 mg Fe	PC	10.41±0.72^{bc}	*	4.03±0.15^{bc}	*	38.80±1.40^{a}	**
	MC	12.40*±0.00^{ab}	-	4.31±0.20^{b}	*	34.82*±1.58^{ab}	**
C. With 200 mg Fe	PC	8.74±1.72^{d}	-	3.20±0.06^{c}	*	36.60±0.69^{a}	**
	MC	11.23*±1.15^{b}	-	3.72±0.05^{c}	*	32.19*±1.52^{b}	**
D. With 50 mg Zn	PC	12.82±0.00^{ab}	-	4.59±0.15^{b}	*	35.92±1.30^{ab}	**
	MC	12.88±0.72^{ab}	-	4.29±0.10^{b}	*	33.37±0.76^{b}	**
E. With 100 mg Zn	PC	10.40±0.00^{cd}	*	3.23±0.09^{c}	-	31.29±0.8^{b}	**
	MC	12.83±0.74^{ab}	-	4.18±0.11^{b}	*	32.74±0.91^{b}	**

PC: pressure cooked; MW: microwave cooked. Significant differences between PC and MW cooked grains on application of 'T' test; * P<0.05, **: P<0.01, ***: P<0.001, values without notation are not significantly different. Column N shows significant differences between raw (Table 1) and cooked grains. Differing superscripted small letters in a column indicate significant difference among all variations together.

binding of bioactive compounds. Since the presence of minerals was more by adsorption, a larger variability in the content and digestibility of bioactive constituents was seen in dehulled grains in comparison to whole grains. The data on mineral content and bioaccessibility of these samples is available in a recently published paper from same study [22].

Xu and Chang [11] analyzed the total phenolic contents of soaked and cooked chickpea and reported that soaking and pressure boiling reduced the total phenols by 3.0 and 30.5% respectively, while losses were nil on steaming. Vadivel et al. [3] also analyzed the total free phenolics in ten types of soaked and cooked wild legumes and reported a mean reduction of 38% of total phenolics

on cooking. A reduction in phenolic constituents on soaking and cooking of *Cassia hirsute* L., an Indian underutilized legume was also reported by Vadivel et al. [4]. The results confirm our findings, wherein a considerable loss of phenolics on cooking was observed. The observations were similar to green gram wherein cooking reduced content of total bioactive constituents, however the availability was better from cooked grains [23].

The overall observations indicate that both the content and digestibility of bioactive components reduced significantly among all variations of chickpea on cooking. Losses were greater in dehulled grains and in Zn fortified samples, however, percent digestibility of bioactive components improved on cooking. According to Parada and Aguilera [24], thermal processing increases the digestibility through softening cell wall and release of compounds from food matrix. In our study, we found that though cooking reduced the content of bioactives, of the available constituents a higher percentage could be absorbed.

Bioactive components in soak water

Since, some of the bioactive components are water soluble and there is a possibility that these could also be dissolved in the soak water, we also analyzed the remaining soak water for total phenolics, tannins and flavonoids, and interestingly a considerable amount of these could also be measured in left over water (Table 4). Total phenols were in the range of 20.51-23.54 mg/100ml of water for control and Zn fortified samples. For iron fortified samples, there were variations with one sample having lesser and the other having more total phenols than the control. These variations can be explained on the binding ability of iron, though it did not follow any specific trend. For tannins, most of them were similar with one iron fortified sample showing lower value. Flavonoids did not show any difference and the amount was low. These results confirm the findings that process of soaking the grains for germination or added minerals can influence the bioactive components through leaching out or physical binding process.

Table 4: Bioactive components (mg per 100 ml) in soak water (plain and fortified) originated from leaching during soaking period

Variations	Total phenols	Tannin	Flavonoids
A. Distilled water (control)	23.54±0.94	31.95±0.35	3.82±0.69
B. With 100 mg Fe	13.34*±1.12	18.71*±0.31	5.43 ^{ns}±0.78
C. With 200 mg Fe	28.26*±1.43	30.82 ^{ns}±0.86	4.41 ^{ns}±0.92
D. With 50 mg Zn	20.75 ^{ns}±0.65	25.15 ^{ns}±2.15	1.98 ^{ns}±0.06
E. With 100 mg Zn	20.51 ^{ns}±0.58	29.69 ^{ns}±0.81	2.68 ^{ns}±0.00

Significant differences between control and fortified soak water on application of 'T' test; * P<0.05, ns: not significant.

Conclusion

The overall results show that chickpea grains were rich sources of phenolic compounds with a high digestibility in whole and dehulled grains. Mineral fortification increased the total phenolics and tannin content as well as digestibility on dehulling, though flavonoids were much lower. The content and digestibility of bioactive components was lesser in cooked chickpea; however, percent digestibility was better in cooked grains. Iron fortification during germination did not affect the digestibility of bioactives significantly, though Zn fortified samples showed lesser digestibility. The soak water remaining after the removal of grains for germination showed the presence of bioactive components indicating that a part of these were leached out in the soaking medium.

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Conflict of interest statement

Authors declare that they have no conflict of interest with anyone with respect to research reported in this paper.

References

1. Prakash J, Bacon G, Dube L. Pulses as nature's gift for precision food and precision nutrition. The Pulse Pod. Global Pulse Federation Magazine. 2017;12-14.

2. Kris-Etherton PM, Hecker KD, Bonanome A, Coval SM, Binkoski AE, Hilpert KF. Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. American Journal of Medicine. 2002;113(9):71-88.

3. Vadivel V, Stuetz W, Scherbaum V, Biesalski HK. Total free phenolic content and health relevant functionality of Indian wild legume grains: Effect of indigenous processing methods. Journal of Food Composition and Analysis. 2011;24(7):935-943.

4. Vadivel V, Nandety A, Biesalski HK. Antioxidant potential and health relevant functionality of traditionally processed *Cassia hirsuta* L. seeds: an Indian underutilized food legume. Plant Foods for Human Nutrition. 2011;66:245-253.

5. Sharma S, Yadav N, Singh A, Kumar R. Antioxidant activity, nutraceutical profile and health relevant functionality of nine newly developed chickpea cultivars (Cicer arietinum L.). International Journal of Natural Products Research. 2013;3(2):44-53.

6. Ghavidel RA, Prakash J. The impact of germination and dehulling on nutrients, antinutrients, *in vitro* iron and calcium bioavailability and *in vitro* starch and protein digestibility of some legume seeds. *LWT Food Science and Technology*. 2007;40(7):1292-1299.

7. Luo Y, Xie W. Effect of soaking and sprouting on iron and zinc availability in green and white faba bean. *Journal of Food Science and Technology*. 2014;51(12), 3970–3976.

8. Oghbaei M, Prakash J. Effect of primary processing of cereals and legumes on its nutritional quality: a comprehensive review. *Cogent: Food and Agriculture*. 2016;2(1):1-29.

9. Fernandez-Orozco R, Frias J, Zielinski H, Piskula MK, Kozlowska H, Vidal-Valverde C. Kinetic study of the antioxidant compounds and antioxidant capacity during germination of *Vigna radiata cv. emmerald, Glycine max cv. Jutro, and Glycine max cv. Merit*. *Food Chemistry*. 2008;111(3):622–630.

10. Lopez-Amoros M, Hernandez T, Estrella I. Effect of germination on legume phenolic compounds and their antioxidant activity. *Journal of Food Composition and Analysis*. 2006;19(4):277–283.

11. Xu BB, Chang SKC. Effect of soaking, boiling and steaming on total phenolic content and antioxidant activities of cool season food legumes. *Food Chemistry*. 2008;110(1):1-13.

12. Prakash J. Enhancing the bioavailability of natural and synthetic fortificants from foods through alteration in compositional matrices. *Nutrition Foundation of India Bulletin*. 2014;35:5-8.

13. Neufeld LM, Aaron GJ, Garrett GS, Baker SK, Dary O, Van Ameringen M. Food fortification for impact: a data-driven approach. *Bulletin of World Health Organization.* 2016;94:631-632.

14. AOAC. Official Methods of Analysis. 17th ed. Association of Official Analytical Chemists, Arlington, USA. 2000.

15. Matthaus B. Antioxidant activity of extracts obtained from residues of different oilseeds. *Journal of Agricultural Food Chemistry.* 2002;50(12):3444-3452.

16. Arvouet-Grand A, Vennat B, Pourrat A, Legret P. Standardisation d'un extrait de propolis et identification des principaux constituants. *Journal Pharmacie Belgigue*. 1994;49(6):462-468.

17. Ranganna S. *Handbook of analysis and quality control for fruit and vegetable products*. 2nd Edn. Tata McGraw-Hill, New Delhi, India. 1986.

18. Luten J, Crews H, Flynn A,Van DP, Kastenmayer P, Hurrel R, Deelstra H, Shen L, Fairweather-Tait S, Hickson K, Farre R, Schlemmer U, Frohlich W. Interlaboratory trial on the determination of *in vitro* iron dialysability from food. *Journal of Science of Food and Agriculture*. 1996;72(4):415-424.

19. Oghbaei M, Prakash J. Nutritional properties of green gram germinated in mineral fortified soak water: I. Effect of dehulling on total and bioaccessible nutrients and bioactive components. *Journal of Food Science and Technology*. 2016;54(4):871-879.

20. Fouad AA, Rehab FMA. Effect of germination time on proximate analysis, bioactive compounds and antioxidant activity of lentil (*Lens culinaris* Medik.) sprouts. *Acta Sci. Pol. Technol. Aliment*. 2015;14(3):233–246.

21. Kanatt SR, Arjun K, Sharma A. Antioxidant and antimicrobial activity of legume hulls. *Food Research International*. 2011;44(10):3182–3187.

22. Oghbaei M, Prakash J. Effect of dehulling and cooking on nutritional quality of chickpea (Cicer arietinum L.) germinated in mineral fortified soak water. *Journal of Food Composition and Analysis*. 2020;94.

23. Oghbaei M, Prakash J. Nutritional properties of green gram germinated in mineral fortified soak water: II. Effect of cooking on total and bioaccessible nutrients and bioactive components. *Journal of Food Science and Technology*. 2016;54(4):880-889.

24. Parada J, Aguilera J. Food microstructure affects the bioavailability of several nutrients. *Journal of Food Science*. 2007;72(2):21-32.