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Effect of Simulated Digestion on Antioxidant Activity of Processed Germinated Brown Chickpea (Cicer Arietinum L.)

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Abstract

The objective of the present investigation was to determine the antioxidant activity of chickpea grains (Cicer arietinum L.) germinated in mineral fortified soak water and treated with digestive enzymes. Chickpea grains were germinated in water fortified with either iron or zinc at two levels, a part of grain was dehulled, and both whole and dehulled grains were cooked either under pressure or in microwave. Samples germinated in plain water served as control. Antioxidant activity of all samples was determined pre- and post-digestive enzyme treatment following a simulated digestion protocol using three assays. Results revealed that both processing variables and simulated digestion process affected the antioxidant activity of the grain significantly. Total antioxidant activity assay and free radical scavenging activity showed higher antioxidant activity in dehulled grains whereas reducing power assay showed whole grains to be having better activity. Cooking lowered the antioxidant activity of pre-digestion extracts to some extent and between pressure and microwave cooking, former retained higher activity. Post-digestion treatment significantly reduced the antioxidant activity of all aqueous extracts to varying extent as seen in all assays. In conclusion, the antioxidant activity of germinated chickpea was influenced both by processing treatments and process of digestion.

Keywords: Antioxidant Activity; Reducing Power; Free Radical Scavenging Activity; Dehulling; Cooking; Fortification

Introduction

Legumes are an essential part of human dietaries, contributing towards energy and protein requirements of more than 50% of world population. They complement the protein quality of cereals and are an affordable alternative to animal proteins. Apart from being good source of essential minerals, vitamins and dietary fiber. However, they also contain some anti-nutritional factors such as trypsin inhibitors, tannins and phytates which form complexes, reduce digestibility and lower the nutrient absorption in legumes [1]. They are also known for providing phytochemicals with proven health benefits. They contain several phenolic compounds and among these two important isoflavones are biochanin A and formononetin. They are associated with low glycemic index and lower risk of CVDs, are beneficial for cancer and good for weight loss and obesity [2].

While there are a huge variety of legumes grown globally, the most common ones are soybean, chickpea, red gram, mung bean, lentils, kidney bean and black gram. There are many cultivars of chickpea (*Cicer arietinum*) grown all over the world, and they can be differentiated with seed coat color varying from light cream to dark brown. Segev et al. [3] examined 17 chickpea lines with different coat color, and found that dark color seeds had significantly higher content of polyphenols, flavonoids and antioxidant capacity. Darker varieties also have much higher dietary fiber content [2].

Chickpea is used in either whole or dehulled form and processed in many ways – roasting, steaming, boiling, frying etc. Chickpea flour is the basis of many snacks. It has a pleasant flavour and good functional characteristics which improve product quality

[4, 5]. The grains are also used in germinated form, which improves it's nutritional quality further by reducing antioxidants, and increasing protein and starch digestibility. Germination has also been shown to increase the concentration of phenolic constituents thereby increasing the antioxidant activity [6]. Particularly Desi variety was linked to high anti-inflammatory activity of germinated grain [7]. An increase of almost 100-fold in isoflavones content in chickpea on germination was reported by Wu et al. [8].

In any food, bioactive constituents are largely responsible for high antioxidant activity. Antioxidant content and properties could be affected by many factors like cooking, refining and soaking. In addition to external factors, after ingestion antioxidant activity could alter due to many steps in digestion process. Since physiological digestion involves action of multiple enzymes in a constantly changing gut environment, the food components are broken down to simple moieties to facilitate absorption. This process may affect the antioxidant activity of food materials. On this premise, the present study was planned to determine the antioxidant activity of germinated chickpea post a simulated digestion process. The impact of dehulling and cooking of germinated grain on antioxidant activity was also investigated.

Materials and Methods

Materials

The brown variety of chickpea (*Cicer arietinum* L.) used in the present study, also commonly called as 'Desi variety' to differentiate from white chickpea, was sourced from local market. Chemicals were procured from SD Fine Chemicals, India and HI Media Company, India. The enzymes used for the simulated digestion 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 cutoff of 10 kDa.

Study Design

This paper includes part of the results of a larger study wherein whole chickpea grains were germinated in mineral fortified soak water and analyzed for nutritional quality, bioactive components and antioxidant activity. The first part of the paper reported on the nutritional quality [9], second part on the bioactive components [10], and the third part on the effect of simulated digestion on the antioxidant activity of same samples is reported here. For fortification of grains, two levels of iron or zinc were used, a part of grains dehulled, and whole and dehulled grains were cooked either by pressure or in microwave. The control sample was germinated in plain water. The samples were subjected to simulated digestion using human digestive enzymes and the aqueous extracts were analyzed for antioxidant activity using three assays. Antioxidant activity was also determined in pre-digestion extracts (without the enzyme treatment) for comparison.

Methods

Processing of Sample

Detailed procedure for the processing of samples is included in previously published paper [10] and can be referred. In brief, chickpea was germinated in mineral fortified soak water, and a part of grain dehulled. The levels of fortification of soak water were (ferrous sulfate equivalent to 100 or 200 mg of iron) or zinc (zinc sulfate equivalent to 50 or 100 mg of zinc). Both whole and dehulled grains were cooked by two methods, pressure cooking (PC) and microwave cooking (MW), and homogenized. Raw samples were also used as controls. All analysis was done on freshly prepared samples. Moisture content of all samples was determined using oven drying method [11]. The variations are referred to as A. control, B. germinated with 100 mg iron, C. germinated with 200 mg iron, D. germinated with 50 mg zinc and E. germinated with 100 mg zinc.

Simulated Digestion of Samples

The homogenized slurries were treated with enzymes sequentially with appropriate pH adjustments using dialysis membrane and the digesta was used for analysis of antioxidant activity (referred to as '***post-digestion extracts'***). A set of samples was also analyzed without the enzymes treatment and these are referred to as '***pre-digestion'*** extracts. The detailed procedure of simulated digestion following the procedure of Luten et al. [12] is included in the earlier paper [10].

Analysis of Antioxidant Activity

Total Antioxidant Activity by Phosphomolybdenum Method

This assay is based on the reduction of Mo (VI) to Mo(V) by the sample analyte and the subsequent formation of green phosphate/ Mo(V) complex at acidic pH [13]. Briefly, an aliquot of 0.1 mL sample was combined with 1.0 mL of reagent solution consisting of 0.6M sulphuric acid, 28mM sodium phosphate and 4mM ammonium molybdate, and incubated at 95ºC for 90 min. After cooling the samples, the absorbance was measured at 695 nm against blank and results calculated as µmoles total antioxidant activity (TAA) per g of sample.

Free Radical Scavenging Activity Using 2,2-Diphenyl-1-Picrylhydrazyl (DPPH)

A commercial oxidizing radical DPPH is reduced by antioxidants and its disappearance at a characteristic wavelength is monitored by decrease in optical density [14]. To the test extracts taken in different concentrations with equalized volume of 1.0 mL with methanol, 4.0 mL of 0.1mM methanolic solution of DPPH was added, mixed, allowed to stand for 20 min in dark at room

temperature and absorbance measured at 517 nm. The control was prepared as above without any sample. Changes in free radical scavenging activity (FRSA) was expressed as inhibition percentage.

Ferric Reducing Antioxidant Assay (Reducing Power)

In this assay, Fe^{3+}/ferricyanide complex is reduced to the ferrous form by antioxidants. The Fe^{2+} formed is monitored by measuring the formation of Perl's Prussian blue at 700 nm [15]. Different amounts of extracts in 1.0 mL of distilled water were mixed with 2.5 mL phosphate buffer (0.2M, pH 6.6) and 2.5 mL potassium ferricyanide (1%). The mixture was incubated at 50°C for 20 min. A 2.5 ml of 10% TCA was added and the mixture centrifuged at 3000 rpm for 10 min. Upper layer of the solution (2.5 mL) was mixed with distilled water (2.5 mL) and 0.5 mL $FeCl_3$ (0.1%), and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. All analysis was run in triplicate and averaged.

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 antioxidant activity of (i) whole and dehulled samples, each five variations (ii) raw and cooked samples, each five and ten variations and (iii) pressure cooked and microwave cooked samples, each ten variations. The effect of digestive enzyme treatment on antioxidant activity was also computed as percent retention of antioxidant activity.

Results and Discussion

The results of the study are compiled in Table 1-3 and figures 1-4.

Total Antioxidant Activity (TAA)

The antioxidant activity of a compound can either be a radical scavenging activity based on single electron transfer or be a hydrogen abstraction reaction. The TAA is a guide that describes the ability of antioxidants present in food to scavenge preformed free radicals. The TAA depends on the synergistic and redox interactions between the different compounds available in the food [16]. The results of TAA of aqueous extracts of germinated chickpea treated with digestive enzymes along with the control (pre-digestion and post-digestion) are presented in Table 1. Part I of table compiles data on whole and dehulled grains with statistical comparison. Pre-digestion TAA of dehulled control was lesser than the whole control, though in variations fortified with mineral salts, the results were opposite with whole grains having lesser activity. Variation B showed highest TAA in whole and dehulled grains. All changes that occurred following dehulling in controls and variations B, C, D were significant with exception of variation E. Post digestion TAA of chickpea was significantly higher among dehulled control and variations B, C, and D and lesser in variation E. Percent retention TAA post digestive enzymes treatment of chickpea ranged between 25.6-37.8% in dehulled grains and 15.0 to 37.4% in whole grains. Dehulled control and variations C and D were significantly higher than their whole counterpart, variation B was similar and E was lower. In post-digestion phase fortified samples had an overall higher retention of TAA indicating that presence of minerals did not interfere with antioxidant activity of chickpea.

When a comparison was made between PC and MW samples, the TAA data showed a definite trend for few sets of values. All aqueous extracts of PC samples- both pre-digestion as well as post-digestion showed higher TAA (exception being PC, pre-digestion extract of variation C, which was low, though not significantly different). Similar trend was seen for pre-digestion extracts of whole grains also, though in post-digestion data, there were some differences seen with three MW samples (control, variations C and D) having lower values. These results show that PC samples retained the TAA better in both dehulled and whole germinated chickpea grains.

On examining the data to see the differences between dehulled and whole grains, a similar trend was reflected with PC dehulled samples showing higher TAA than whole grains in pre-digestion activity and in post-digestion extracts, control and few fortified variations showing higher activity. The percent retention data did not reveal any particular trend with varying range of 18.8-38.9% for dehulled and 15.1-41.8% for whole grains.

To examine the effect of cooking as such on TAA of germinated chickpea, data was compared with the raw counterpart, and overall observations can be summarized as follows – for the dehulled grains, pre-digestion extracts of MW cooked samples were significantly lower than raw counterparts. PC samples had significantly higher or similar antioxidant activity. For post-digestion extracts, all values were lower than raw counterparts and some were significant. Percent retention of antioxidant activity was also lower for all samples with one exception of MW control. For the whole grains, the pre-digestion aqueous extracts of all cooked samples (both PC and MW) exhibited lower antioxidant activity, post-digestion extracts also followed similar trend with two exceptions (PC, variation B and MW cooked, variation D). Percent retention of TAA, showed mixed results of being higher for some and lower for others. It can be said that cooking reduced the TAA of germinated grains with or without the simulated digestion procedure.

Beside changes in the content of bioactive compounds, some studies showed that various heat treatments can increase the total antioxidant capacity (TAC) of different food groups. While studying the antioxidant properties of predictive food models, Oghbaei and

Table 1: Effect of dehulling and cooking on total antioxidant activity (μ mol/g sample, vitamin C equivalent) in pre- and post-digestion aqueous extracts of germinated chickpea

Variations		Dehulled			Whole		
		Pre-digestion	Post-digestion	Percent Retention	Pre-digestion	Post-digestion	Percent Retention
Part I. Effect of dehulling							
A. Control		22957 ±734	6692 ±38	29.2 ±0.2	25222* ±117	3787** ±106	15.0*** ±0.4
B. Fe (100mg)		30212 ±238	11406 ±128	37.8 ±0.4	26594** ±0	9957** ±47	37.4 ±0.2
C. Fe (200 mg)		25950 ±66	6643 ±119	25.6 ±0.5	24142* ±720	4535* ±171	18.8** ±0.7
D. Zn (50 mg)		18930 ±598	6720 ±121	35.5 ±0.8	17562* ±147	4601** ±261	26.2** ±1.5
E. Zn (100mg)		23972 ±111	7221 ±272	30.1 ±1.1	23130 ±412	8222* ±5	35.5* ±0.0
Part II. Effect of cooking							
A. Control	PC	24220 ±311	6173 ±9	25.5 ±0.0	19807 ±333	3401 ±54	17.2 ±0.3
	MW	19202** ±390	6155 ±498	32.0* ±2.6	19280 ±65	3616* ±133	18.7* ±0.7
B. Fe (100mg)	PC	27101 ±1164	10549 ±953	38.9 ±3.5	25858 ±0	10804 ±414	41.8 ±1.6
	MW	22752* ±1126	8298* ±32	36.5 ±0.1	23193* ±0	9640 ±522	41.5* ±2.2
C. Fe (200 mg)	PC	23123 ±419	4353 ±482	18.8 ±2.1	19420 ±306	2095 ±12	10.8 ±0.1
	MW	17930** ±490	3603* ±278	20.1* ±1.5	18257* ±166	2758** ±23	15.1** ±0.1
D. Zn (50 mg)	PC	18150 ±78	5098 ±165	28.8 ±0.8	13337 ±136	3198 ±24	24.0 ±0.2
	MW	15325** ±409	3308** ±158	21.6* ±1.0	12790 ±235	3764* ±195	29.4* ±1.5
E. Zn (100mg)	PC	22389 ±85	6774 ±110	30.3 ±0.5	20167 ±346	5830 ±122	28.9 ±0.6
	MW	22566 ±803	6210 ±410	27.5 ±1.8	18470* ±89	5580 ±517	30.2 ±2.8
Part III. Statistical analysis for effect of cooking – Comparison between raw and cooked grains							
A. Control	PC	ns	**	***	**	*	*
	MW	*	ns	*	***	ns	*
B. Fe (100mg)	PC	ns	ns	ns	***	ns	ns
	MW	*	***	ns	***	**	*
C. Fe (200 mg)	PC	ns	ns	*	*	**	**
	MW	**	ns	*	**	**	*
D. Zn (50 mg)	PC	**	ns	**	***	*	**
	MW	ns	**	**	**	*	**
E. Zn (100mg)	PC	**	ns	ns	*	***	**
	MW	ns	ns	ns	**	*	ns

PC: Pressure cooked, MW: Microwave cooked. Values equalized for 50% moisture, comparison between dehulled and whole (part I); PC and MW (part II) and raw and cooked grains (part III) on application of T test; *: P<0.05, **: P<0.01, ***: P<0.001, ns: not significant. For the values without notation differences are not significant.

Prakash [17], reported a retention of 54.8% of post-digestion TAA in green gram + greens curry mix, which was retained well on refrigerated storage for 3 months to 53.3%. However, frozen storage and dehydration combined with ambient storage brought it down to 33.5 and 35.0% respectively. In a similar model, when green gram was replaced with chickpea, the post digestion retention was 56.0% initially, which on refrigerated storage reduced to 50.9%, on frozen storage to 45.7% and on ambient storage with dehydration to 44.1%. [18].

The profiles and quantities of polyphenols and tannins in foods are affected by processing due to their highly reactive nature, which in turn can impact their anti-oxidant activity and the nutritional value of foods [19]. As per one study, germinated soybean, lentil and vicia were studied for their antioxidant activity by several methods and it was observed that germination modified the antioxidant activity indicating differences which exist among the types of legumes as well as in germination conditions, which are not always related to the content of some of the antioxidant compounds [20].

Free Radical Scavenging Activity (FRSA)

The ability of antioxidant components to battle free radicals and make them inactive could be estimated by percent inhibition of DPPH which is a source of free radicals. Fig. 1 and 2 depict results of pre- and post-digestion activity for dehulled and whole chickpea with all variations. The results of statistical analysis for these data and post-digestion percent retention of activity are compiled in Table 2 and Table 3 respectively. To facilitate discussion and statistical analysis, the 4th concentration (highest concentration; pre- and post-digestion in chickpea was 120 and 150 mg respectively) is used as the basis in all variations. The range of percent inhibition among variations for dehulled and whole chickpea for pre-digestion were 46.4-88.5 and 36.8-86.7% respectively, whereas for post-digestion, values were much lower with the range 11.5-25.5 and 11.1-23.4% respectively. The minimum percent activity in chickpea was in variations with added iron and the control, whereas variations with zinc exhibited higher percent activity in comparative range for both pre- and post-digestion extracts.

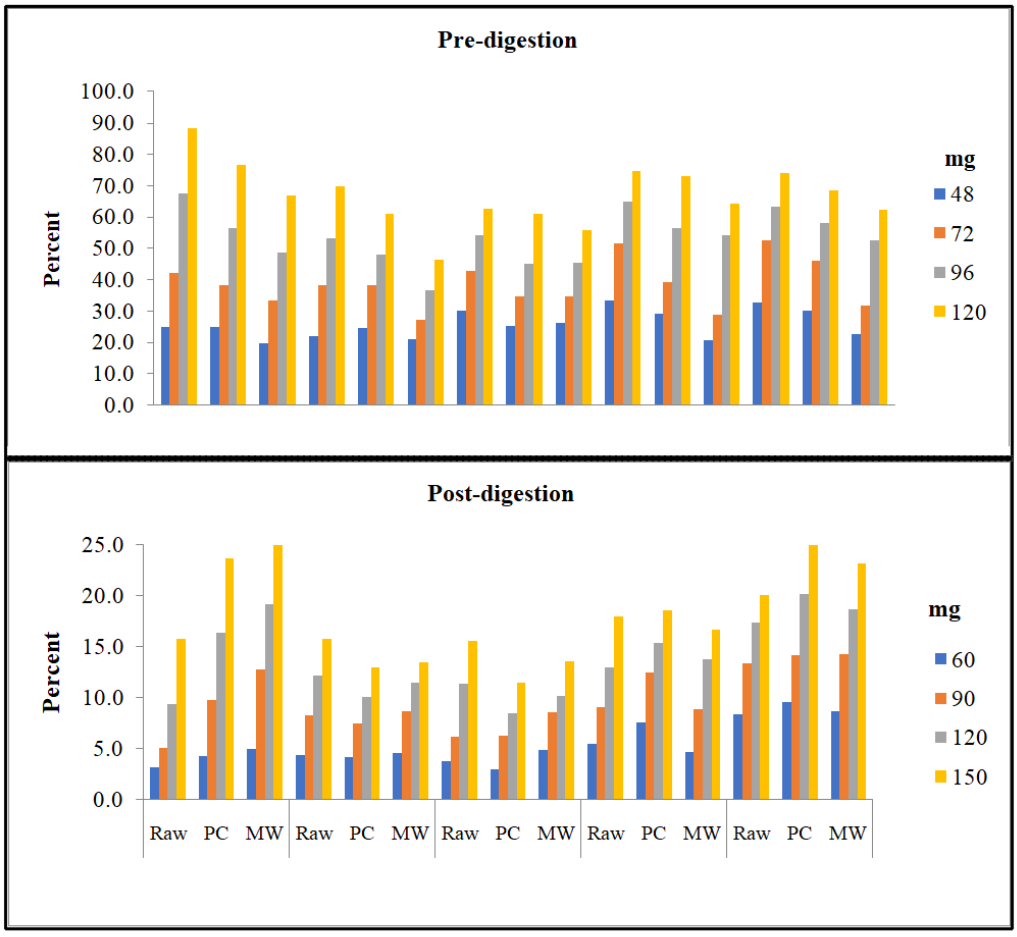

Figure 1: Effect of cooking on pre- and post-digestion free radical scavenging activity of dehulled chickpea

The comparison of dehulled and whole raw grains revealed that in most of variations the former showed significantly higher percentage (range of increase 1.74-14.92%). In one of our previous study done on similar lines, green gram (*Vigna radiata*) showed opposite trend, with whole grains in most of variation showing significantly higher activity with the range of differences between 7.8-37.4%. This shows that the antioxidant activity was individual and specific to legumes [21].

Percent inhibition of sample cooked by PC and MW was compared and it was observed that among both dehulled and whole grains, PC sample exhibited significantly higher values (exception, variation E of whole chickpea). The range of maximum difference between PC and MW in dehulled and whole chickpea variations was 14.6 and 12.4% respectively. The antioxidant activity of raw was compared against PC and MW cooked grains to bring out the effect of cooking on DPPH inhibition properties. Both cooking methods resulted in lesser activity than control in dehulled and whole grains with MW samples showing greater effect. Cooking affected dehulled grains more than whole grains and maximum difference was 23.3% in dehulled MW cooked variation B.

The post-digestion FRSA of chickpea was much lesser than pre-digestion, (higher concentration used but lesser percentage of inhibition resulted). The trend of changes among variations in post-digestion results were same as pre-digestion except control raw sample exhibiting lower activity than cooked counterparts. It was observed that antioxidant activity of mineral fortified samples, especially with iron, reduced significantly after heat treatments. The high temperature of cooking could increase the binding of phenolic compounds with iron or minerals and act as catalysts for converting bioactive components to other isomers which have less activity.

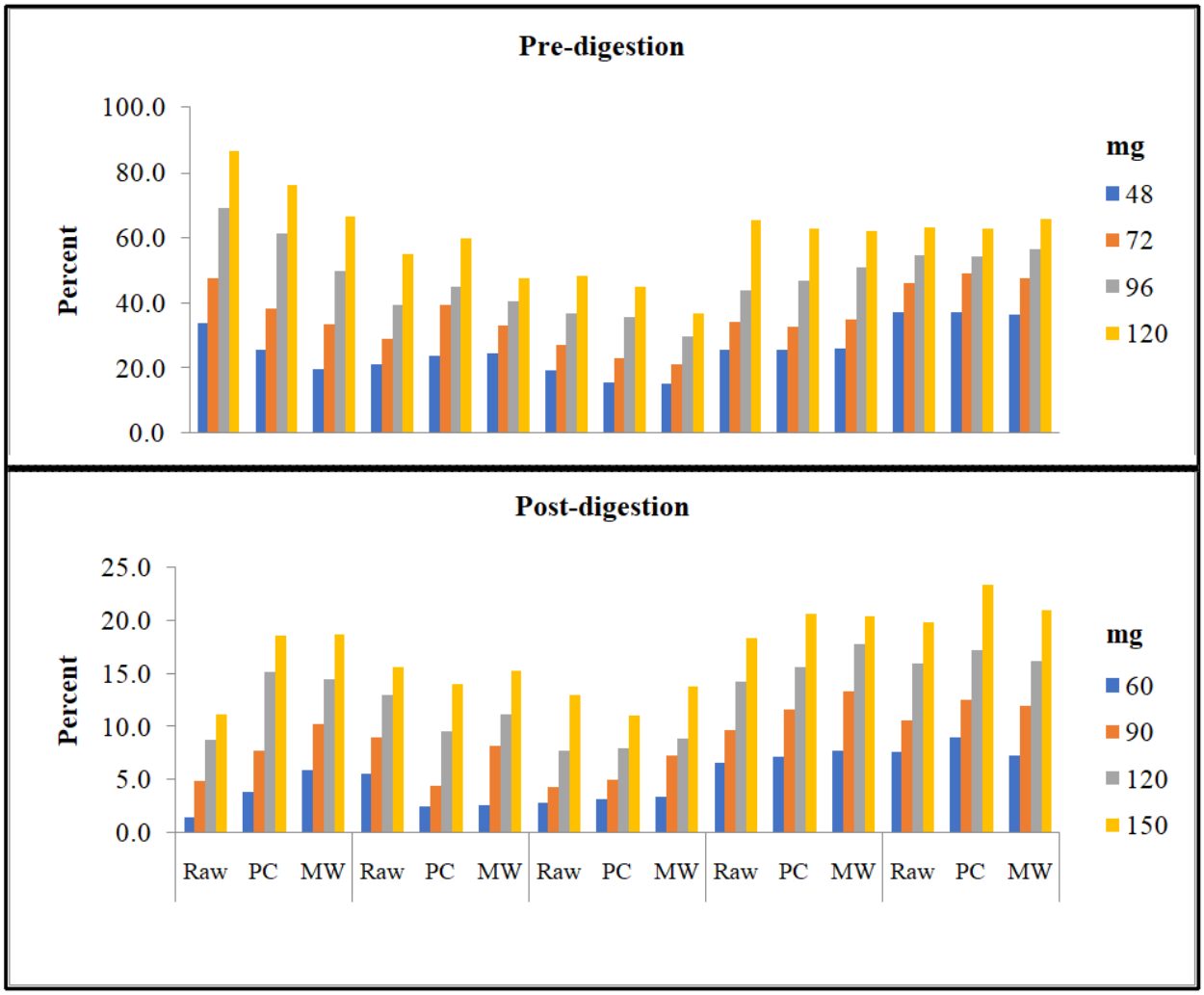

Figure 2: Effect of cooking on pre- and post-digestion free radical scavenging activity of whole chickpea

The statistical differences between whole and dehulled did not follow same trend. For many samples the differences were non-significant. For few, where differences existed, they were more in pre-digestion extracts than post-digestion samples (Table 2). The significant changes in post-digestion comparisons including between raw dehulled Vs raw whole, or between differently cooked samples or between raw and cooked samples were less than what was seen for pre-digestion extracts. When the retention of post-digestion FRSA was computed as percent of pre-digestion activity (Table 3), the overall observations showed that the raw samples had lesser retention in comparison to cooked samples except for few PC variations and MW samples showed higher retention than PC ones.

Many researchers have reported that some compounds like flavonols which are not available either in raw or soaked grains are seen in germinated grains and their presence depends upon different factors like light, soaking time etc. [6]. The soak water fortification could affect the compounds involved in antioxidant activity. Gupta et al. [1] analysed the antioxidant capacity of 40 genotypes of chickpea grown in India and reported a range of 4.7-35.4 mg/100g of total phenolics and 32.6-58.9% FRSA in the grain. A considerable variation depending on the genotype was found. Whereas Kaur et al. [22] reported an average of 56.21 + 2.50 and 9.77+0,45 mg/100 g of phenols and flavonols respectively in ten Desi variety of chickpea with mean FRSA of 49.4%. The antioxidant activities of polyphenols are said to alter on account of heat applications, enzymatic or chemical oxidations. These processes change the proportion of cis- and trans-isomers, cause intra molecular trans esterification of polyphenols and their polymerization. This altered nature of polyphenols could show different antioxidant activity. Partially oxidized polyphenols may exhibit higher antioxidant activity than the corresponding non-oxidized forms [23].

Reducing Power

Reduction of ferric to ferrous by donating hydrogen atom could be used for expressing antioxidant properties of foods. Fig. 3 and 4 depicts the antioxidant activity measured as reducing power in dehulled and whole chickpea respectively. Fourth concentration for pre- and post-digestion of whole and dehulled chickpea (120 and 150 mg respectively) is used for comparison and discussion. The statistical differences and percent retention of post digestion activity for these data are also included in Table 2 and 3 respectively. The changes in all variations from A to E were very similar to FRSA assay and samples fortified with iron exhibited least optical density (OD) in pre- and post-digestion estimations. The maximum reducing power activity of dehulled and whole chickpea was found to be 0.425 (variation D, PC) and 0.47 (variation A, raw) respectively. The comparison of raw dehulled and whole grains showed that except for control, no other variations were significantly different.

The reducing power activity of whole grain was higher than dehulled counterparts (except variation C). A comparison of PC and MW cooked samples revealed that the former showed higher activity and range of difference was 0.01-0.05 OD. The differences in whole grains were larger than dehulled ones (whole, variation A, C and dehulled variation A, B showed significant differences). Lastly, comparison between raw and cooked (PC/MW) chickpea samples showed that the raw grains had higher activity. Dehulled control and whole variation B exhibited largest difference among PC and MW cooked grains, respectively. Dehulled and whole control and variation with zinc exhibited significantly lesser activity than raw counterpart. Post- digestion estimation of reducing power followed similar trend to pre-digestion and even when the concentration used for assay was higher than pre-digestion, activity was less. The antioxidant activity could be reduced during digestion procedure on account of exposure to strong acidic and alkaline solutions and enzymes.

The differences in highest activity of pre- and post-digestion for dehulled and whole set of germinated chickpeas was 0.13 and 0.09 OD respectively which indicates that irrespective of higher concentration used for post-digestion test, antioxidant activity decreased by 32.1 and 20.8% in dehulled and whole set of grains, respectively. The comparison between whole and dehulled sets of chickpeas showed that all iron fortified whole grains showed lesser activity while the rest demonstrated higher activity. The accumulation of external iron in hull portion could block antioxidant components and their activity. The differences in reducing power properties of PC and MW cooked dehulled grains were not significant but in whole grains, control and PC variation with iron were significantly higher. After cooking (PC/MW) variations A, B and C of dehulled set showed lesser reducing power activity than raw sample but rest of variation in this set and entire variations of whole chickpea set exhibited more activity after cooking (PC/MW). The changes were statistically insignificant except between dehulled raw versus PC (C, D and E) samples and whole versus PC samples (A, B and C).

Phenolic compounds in whole grains exist in either bound or free form and the process of germination enhances the inherent enzymic activities in the grain [24, 25, 26]. Studies in literature report an increase in antioxidant activity of legumes on germination and cooking [27, 28]. It has also been observed that enzymic digestion of phenolic compounds as may happen during the process of digestion in human gastrointestinal tract can increase the antioxidant potential of grains by releasing the phenolics from the bound matrix [29, 30].

López -Barrios et al. [31] measured the antioxidant activity of protein hydrolysates from germinated black beans (*Phaseolus vulgaris* L.) subjected to simulated gastrointestinal digestion and reported a lower activity in comparison to controls (undigested samples). This is similar to our observations. The retention of post-

Table 2: Statistical analysis for antioxidant activity (FRSA and Reducing power) of germinated chickpea – Inter sample differences

Description	Comparison		A. Control	B. Fe (100 mg)	C. Fe (200 mg)	D. Zn (50 mg)	E. Zn (100 mg)
Pre-digestion (FRSA)	Dehulled Vs whole		ns	ns	*	ns	*
	Dehulled	PC Vs MW	ns	*	**	ns	*
		Raw Vs PC	*	*	ns	ns	*
		Raw Vs MW	**	**	*	ns	**
	Whole	PC Vs MW	*	*	*	ns	ns
		Raw Vs PC	*	ns	**	ns	ns
		Raw Vs MW	**	*	*	*	*
Post-digestion (FRSA)	Dehulled Vs whole		ns	ns	ns	ns	ns
	Dehulled	PC Vs MW	ns	ns	ns	ns	*
		Raw Vs PC	*	*	*	ns	ns
		Raw Vs MW	**	ns	*	ns	ns
	Whole	PC Vs MW	ns	ns	ns	ns	ns
		Raw Vs PC	ns	ns	ns	ns	ns
		Raw Vs MW	*	ns	ns	*	ns
Pre-digestion (reducing power)	Dehulled Vs whole		*	ns	ns	ns	ns
	Dehulled	PC Vs MW	*	ns	**	ns	ns
		Raw Vs PC	**	ns	ns	ns	*
		Raw Vs MW	*	ns	ns	*	ns
	Whole	PC Vs MW	*	**	ns	ns	*
		Raw Vs PC	ns	*	*	ns	ns
		Raw Vs MW	ns	ns	*	ns	ns
Post-digestion (reducing power)	Dehulled Vs whole		*	ns	**	**	ns
	Dehulled	PC Vs MW	ns	ns	ns	ns	ns
		Raw Vs PC	ns	ns	*	*	***
		Raw Vs MW	ns	ns	ns	ns	ns
	Whole	PC Vs MW	*	*	*	ns	ns
		Raw Vs PC	*	*	*	ns	ns
		Raw Vs MW	ns	**	ns	ns	ns

FRSA: Free radical scavenging activity, PC: Pressure cooked, MW: Microwave cooked. Level of significance; * :P<0.05,**: P<0.01,***: P<0.001, ns: not significant.

Table 3: Percent retention of FRSA and reducing power on post-digestion among all samples of germinated chickpea

Antioxidant activity	Samples		A. Control	B. Fe (100 mg)	C. Fe (200 mg)	D. Zn (50 mg)	E. Zn (100 mg)
Free radical scavenging activity	Dehulled	Raw	10.4	17.5	18.2	17.4	23.5
		PC	21.4	16.6	13.9	21.0	29.4
		MW	28.7	24.8	18.2	21.4	30.0
	Whole	Raw	10.1	23.7	15.9	21.8	25.6
		PC	19.9	16.0	17.8	25.0	27.3
		MW	21.8	23.4	24.2	28.7	24.6
Reducing Power	Dehulled	Raw	57.1	64.6	59.2	43.5	53.3
		PC	68.2	65.1	49.4	39.5	57.0
		MW	63.0	59.4	47.1	39.8	59.5
	Whole	Raw	52.2	61.2	48.5	61.4	54.8
		PC	59.2	66.8	63.3	63.6	55.1
		MW	65.5	61.8	59.8	78.7	61.6

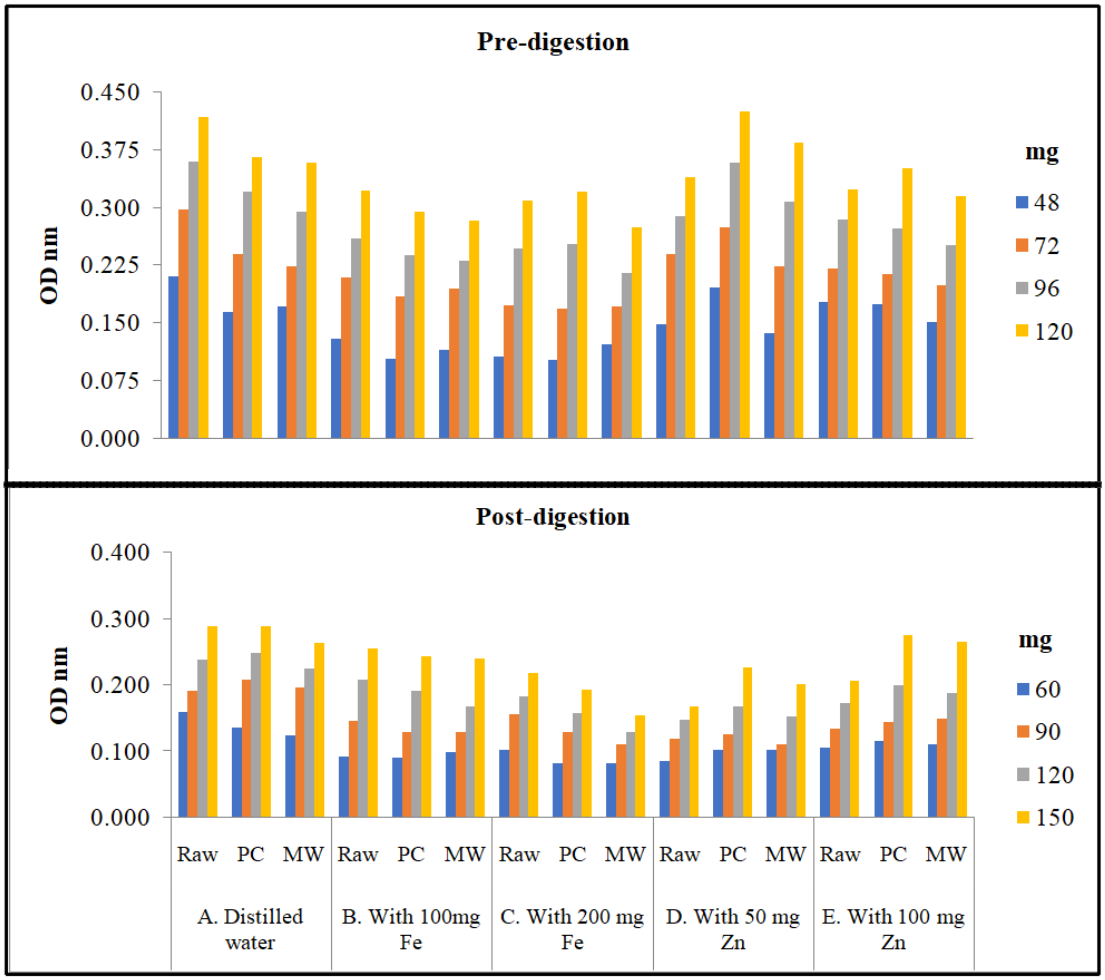

Figure 3: Effect of cooking method on pre- and post-digestion reducing power of dehulled chickpea

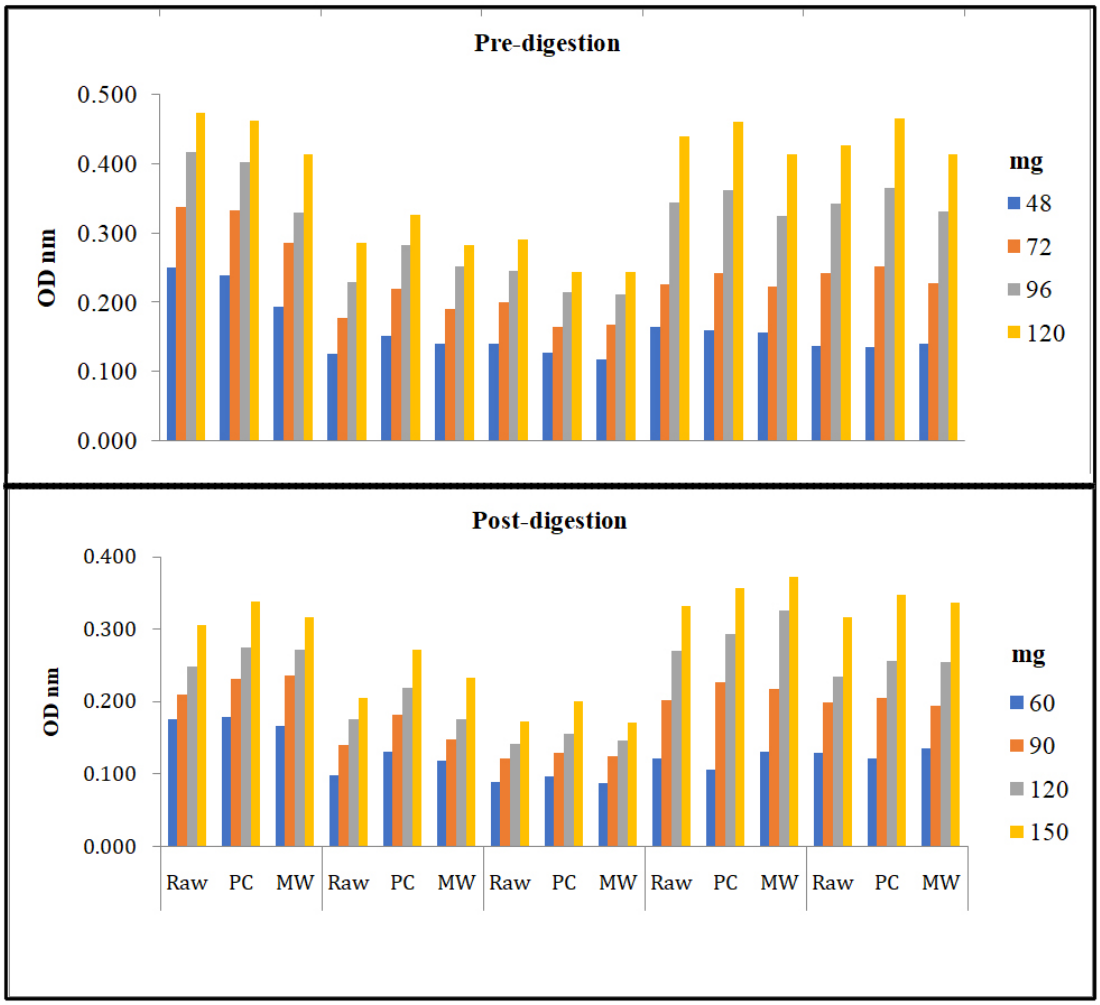

Figure 4: Effect of cooking method on pre- and post-digestion antioxidant activity reducing power of whole chickpea

digestion reducing power computed as percent of pre-digestion activity was much higher in comparison to FRSA retention as can be seen in Table 3. Though any specific trend could not be observed, the raw samples exhibited higher retention for many variations. And between cooking methods, differences were lesser. A higher level of antioxidant activity in post-digestion sample could be recorded using this method of assay.

Conclusion

The study reports the antioxidant activity of chickpea germinated in mineral fortified soak water with processing variables of dehulling, pressure cooking or microwave cooking along with respective controls. The aqueous extracts of samples, pre- and post-digestive enzyme treatment were analyzed for antioxidant activity using three assays and the major observations are summarized. Between whole and dehulled grains, the latter exhibited higher antioxidant activity when assayed using TAA and FRSA. Thermal treatments lowered antioxidant activity in pre-digestion extracts in comparison to raw control to varying extent. However, results with RP assay were different with whole grains showing better activity. Iron fortification reduced antioxidant activity to some extent in all samples. Post-digestion, all extracts showed significantly lesser activity which can be attributed to the process of digestion itself, exposure to enzyme action, change of pH and breaking down of macromolecules to smaller fractions which have different antioxidant potential. In conclusion, the digestion process can significantly affect the antioxidant activity of chickpea apart from the processing variables of germination, fortification, dehulling and cooking.

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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. Gupta N, Shrivastava N, Bhagyawant SS. Multivariate analysis based on nutritional value, antinutritional profile and antioxidant capacity of forty chickpea genotypes grown in India. *Journal of Nutrition and Food Science*. 2017;7(3):600

2. Jukanti AK, Gaur PM, Gowda CLL, Chibbar RN. Nutritional quality and health benefits of chickpea (*Cicer arietinum* L.): A review. *British Journal of Nutrition.* 2012;108:S11–S26

3. Segev A, Badani H, Kapulnik Y, Shomer I, Oren-Shamir M, Galili S. Determination of polyphenols, flavonoids, and antioxidant capacity in colored chickpea (*Cicer arietinum* L.) *Journal of Food Science*. 2010;75(2):S115-S119.

4. Acevedo Martinez KA, Yang MM, Gonzalez de Mejia E. Technological properties of chickpea (*Cicer arietinum*): Production of snacks and health benefits related to type-2 diabetes. *Comprehensive Reviews in Food Science and Food Safety.* 2021.

5. Hirdyani H. Nutritional composition of chickpea (*Cicer arietinum* L.) and value added products- A review. *Indian Journal of Community Health*. 2014;26(2):102-106.

6. Lopez-Amoros M, Hernande 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.

7. Domínguez-Arispuro DM, Cuevas-Rodríguez EO, Milán-Carrillo J, León-López L, Gutiérrez-Dorado R, Reyes-Moreno C. Optimal germination condition impacts on the antioxidant activity and phenolic acids profile in pigmented desi chickpea (*Cicer arietinum* L.) seeds. *Journal of Food Science and Technology.* 2018;55:638–647.

8. Wu Z, Song L, Feng S, Liu Y, He G, Yioe Y, Liu SQ, Huang D. Germination dramatically increases isoflavonoid content and diversity in chickpea (*Cicer arietinum* L.) seeds. *Journal of Agricultural Food Chemistry*. 2012;60(35):8606−8615.

9. 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, December issue. 103619.

10. Oghbaei M, Prakash J. Content and bio accesibility of phenolics and flavonoids in processed germinated brown chickpea (*Cicer arietinum* L.). *Journal of Nutritional Science and Healthy Diets.* 2021;2(1):12-20.

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

12. 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.

13. Oyaizu M. Studies on product of browning reaction produced from glucose amine. *Japan Journal of Nutrition*. 1986;44(6):307-315.

14. Shimada K, Fujikaw K, Yahar K, Nakamura T. Antioxidative properties of xanthine on autoxidation of soybean oil in cyclodextrin emulsion. *Journal of Agricultural Food Chemistry*. 1992;40(6):945-948.

15. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Annals of Biochemistry.* 1999;269(2):337–341.

16. Pellegrini N, Serafini M, Colombi B, Del Rio D, Salvatore S, Bianchi M, Brighenti F. Total antioxidant capacity of plant foods, beverages and oils consumed in Italy assessed by three different in vitro assays. *The Journal of Nutrition.* 2003;133(9):2812-2819.

17. Oghbaei M, Prakash J. Effects of processing and digestive enzymes on retention, bioaccessibility and antioxidant activity of bioactive components in food mixes based on legumes and green leaves. *Food Bioscience.* 2013;4:21-30.

18. Oghbaei M, Prakash, J. Effect of simulated *in vitro* digestion on antioxidant activity of processed *Cicer Arietinum* and *Amaranthus Caudatus* greens. *Nutrition and Food Toxicology*. 2017;1(2):53-62.

19. Dlamini NR, Dykes L, Rooney LW, Waniska RD, and Taylor JRN. Condensed tannins in traditional wet-cooked and modern extrusion-cooked sorghum porridges. *Cereal Chemistry*. 2009;86(2):191-196.

20. Zielinski H. Peroxyl radical trapping capacity of germinated legume seeds. *Food/Nahrung.* 2002;46(2):100-104.

21. Oghbaei M, Prakash J. Effect of digestive enzymes treatment on antioxidant properties of germinated green gram fortified with minerals. *Cereal Chemistry*. 2017;94(2):284-290.

22. Kaur K, Grewal SK, Gill PS, Singh S. Comparison of

cultivated and wild chickpea genotypes for nutritional quality and antioxidant potential. *Journal of Food Science and Technology*. 2019;56(4):1864–1876.

23. Nicoli MC, Calligaris S, Manzocco L. Effect of enzymatic and chemical oxidation on the antioxidant capacity of catechin model systems and apple derivatives. *Journal of Agricultural Food Chemistry*. 2000;48(10):4576-4580.

24. Han H, Baik B. Antioxidant activity and phenolic content of lentils (*Lens culinaris*), chickpeas (*Cicer arietinum* L.) peas (*Pisum sativum* L.) and soybeans (*Glycine max*), and their quantitative changes during processing. *International Journal of Food Science and Technology*. 2008;43(11):1971-1978.

25. Bhavya SN, Prakash J. Nutritional properties of iron fortified flatbreads enriched with greens and legumes. *Journal of Food Processing and Preservation*. 2021;45(5): e15495

26. 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.

27. Gharchorloo M, Tarzi BG, Baharinia M. The effect of germination on phenolic compounds and antioxidant activity of pulses. *Journal of the American Oil Chemists Society.* 2012;90(3):407-411.

28. Sánchez-Velázquez OA, Ribéreau S, Mondor M, Cuevas-Rodríguez EO, Arcand Y, Hernández-Álvarez AJ. Impact of processing on the in vitro protein quality, bioactive compounds, and antioxidant potential of 10 selected pulses. *Food Legumes*. [Early view.] 29th March, 2021.

29. Stanisavljević N, Jovanović Z, Čupić T, Lukić J, Đukić JM, Radović S, Mikić A. Extractability of antioxidants from legume seed flour after cooking and *in vitro* gastrointestinal digestion in comparison with methanolic extraction of the unprocessed flour. *International Journal of Food Science and Technology*. 2013;48(10):2096-2104.

30. Siddiqui A, Prakash J. Antioxidant properties of digestive enzyme treated fiber rich fractions from wheat, finger millet, pearl millet and sorghum: A comparative evaluation. *Cogent: Food and Agriculture.* 2015;1:1073815.1-15.

31. López-Barrios L, Antunes-Ricardo M, Gutiérrez-Uribe JA. Changes in antioxidant and anti-inflammatory activity of black bean (Phaseolus vulgaris L.) protein isolates due to germination and enzymatic digestion. *Food Chemistry*. 2016;203:417-424.