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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

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Abstract

The content of nutrients (protein, starch, ash, calcium, iron, phosphorous and thiamin) and antinutritional components (dietary fiber fractions, phytic acid and tannin), and in vitro bioavailability of calcium and iron and in vitro digestibility of protein and starch were determined in control, germinated and dehulled green gram, cowpea, lentil and chickpea. Germination caused significant ($P<0.05$) increase in protein, thiamin, in vitro iron and calcium bioavailability and in vitro starch and protein digestibility contents of all the legume samples. Further increase in mentioned parameters was observed after dehulling the germinated legumes. Phytic acid and tannin were reduced by 18–21% and 20–38%, respectively, on germination and more reduction was observed in dehulled over germinated samples. There were negative correlations between nutrients bioavailability and digestibility with antinutritional factors.

Keywords: Germination; Dehulling; Antinutrients; In vitro mineral availability; In vitro starch and protein digestibility; Legumes

1. Introduction

Legumes play an important role in the agriculture and diet of many developing countries and are a major source of dietary nutrients for many people. However, their role appears to be limited because of several factors including low protein and starch digestibility (Kataria, Chauhan, & Punia, 1989; Negi, Boora, & Khetarpaul, 2001), poor mineral bioavailability (Kamchan, Puwastien, Sirichakwal, & Kongkachuichai, 2004; Rao & Prabhavathi, 1982) and high antinutritional factors (Das, Chaturvedi, & Nagar, 1999; Ramulu & Udayasekhara, 1997; Savelkoul, Vanderpoel, & Tamminga, 1992). It has been reported that protein and thiamin (Sattar, Durrani, Mahmood, Ahmad, & Khan, 1989), mineral bioavailability (Ghanem & Hussein, 1999; Rao & Prabhavathi, 1982) and protein and starch digestibility (Kataria, Chauhan, & Punia, 1992; Preet & Punia, 2000) increased, whereas phytic acid (Ayet et al., 1997; Kataria et al., 1989; Sattar et al., 1989) and tannin (Ayet et al., 1997; Savelkoul et al., 1992) decreased during germination of legumes. Most researchers have studied the effect of soaking and germination on nutritional quality of legumes, but information on effect of a combination of processes such as soaking, germination and dehulling on improvement of nutritional quality of legumes is scarce. Therefore, the aims of this work were (a) to study the effect of soaking and germination individually and in combination with dehulling on proximate composition; antinutrients such as dietary fiber fractions, phytic acid and tannin; minerals, viz. iron, calcium and phosphorous; in vitro iron and calcium bioavailability and in vitro starch and protein digestibility, and (b) to analyse data statistically to establish regression equations for predicting in vitro iron and calcium bioavailability as well as in vitro starch and protein digestibility by substituting antinutritional factors in equations.

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2. Materials and methods

2.1. Sample preparation

Green gram (*Phaseolus aureus*), cowpea (*Vigna catjang*), lentil (*Lens culinaris*) and chickpea (*Cicer arietinum*) were obtained from local market. Legume seeds were cleaned, washed and soaked in 4–5 volumes of water (22–25 °C) for 12 h under ambient laboratory conditions. At the end of the period, the water was drained and the seed samples were allowed to germinate under a wet muslin cloth for 24 h and then dried in a cabinet dryer (Magumps, Mumbai, India) at 50 ± 5 °C for 16–18 h. A portion of germinated samples was dehulled in a dehusker (Versatile dhal mill, designed and developed by Central Food Technological Research Institute, Mysore, India). Ungerminated seeds served as control. All the three samples, (1) control (ungerminated), (2) germinated and (3) dehulled (after germination) were milled to flour in a plate mill (Bhavani Industries, Bangalore, India). The processing of samples was done in one batch and processed samples were stored in airtight containers for further analysis.

2.2. Chemicals

The chemicals required for the study were obtained from SD Fine, Qualigen Laboratories Pvt. Ltd., Mumbai, India. The enzymes used were obtained from Himedia Company, Mumbai, India. Glucose oxidase peroxidase kit (Code No: B0112/Lot No: 5354) was procured from Span Diagnostics Ltd., Surat, India.

2.3. Chemical analysis

Moisture, fat, ash (minerals) and tannin contents were estimated by standard AOAC methods (AOAC, 1990). The nitrogen content was estimated by Kjeldhal method, based on the assumption that plant proteins contain 16 g/100 g nitrogen, protein content was calculated using the formula, protein = nitrogen × 6.25. Thiamin was analysed by oxidation to thiochrome, which fluoresces in UV light (Raghuramulu, Nair, & Kalyansundaram, 1983). Insoluble and soluble dietary fiber was analysed by separation of non-starch polysaccharides by enzymatic gravimetric method (Asp, Johansson, Hallmer, & Siljeström, 1983). Phytic acid was extracted and determined by estimation of phosphorous according to the precipitate analysis method of Thompson and Erdman (1982). The conversion factor 3.55 for phosphorous to phytic acid was used (Sen & Bhattacharyya, 2003). The samples were ashed in a muffle furnace and ash solution was prepared by dry ashing. Iron was estimated colorimetrically by α-α- dipyridyl method (AOAC, 1990). An in vitro method for the determination of bioavailability of nonheme iron from foods was investigated. Sample was extracted with pepsin–HCl at pH 1.35 and subsequently the pH was adjusted to pH 7.5 and filtered. Ionizable iron was determined in the filtrate by the α-α- dipyridyl method. Percent iron bioavailability is predicted using the following regression equation; $Y = 0.4827 + 0.4707X$, where Y is the percent available iron and X is the percent ionizable iron (Rao & Prabhavathi, 1978). Calcium was analysed by precipitation as calcium oxalate and subsequent titration by potassium permanganate (AOAC, 1990). In vitro bioavailability of calcium was determined by a simulated gastrointestinal digestion using pepsin for the gastric stage followed by pepsin and bile salts for the intestinal stage (Luten et al., 1996). The content of calcium diffused through a semi permeable membrane was determined by precipitation and titration method (AOAC, 1990). Phosphorous was estimated colorimetrically by Taussky and Shorr (1953) method. The enzymatic method of Batey and Ryde (1982) was used for total starch analysis. In vitro starch digestibility was determined by modification of method of Kumar and Venkataramann (1976) by using enzymatic glucose oxidase peroxidase kit instead of dinitrosalicylic reagent. Glucose was used as a standard and the degree of hydrolysis was expressed as mg of glucose liberated from the samples after correction for blank values and percent in vitro starch digestibility was calculated on the basis of total starch content using the following equation; glucose released (g) × 0.9 × 100/g of total starch. In vitro protein digestibility was estimated by enzymatic method of Akeson and Stahmann (1964).

2.4. Statistical analysis

The analysis was carried out in four replicates for all determinations. The mean and standard deviation of means were calculated. The data were analysed by one-way analysis of variance (ANOVA). A multiple comparison procedure of the treatment means was performed by Duncan's new multiple range test (Duncan, 1955). The correlation coefficients were computed and regression equations were made. Significance of the differences was defined as $P < 0.05$.

3. Results and discussion

The results in Table 1 showed that moisture in control samples ranged from 8.5 to 11.7 g/100 g. There was a reduction in moisture on germination in all samples and an increase of it after dehulling. Fat content of control seeds ranged from 0.89 g/100 g in lentil to 5.45 g/100 g in chickpea. On germination, there was a statistically significant ($P < 0.05$) decrease of fat content, which could be due to total solid loss during soaking prior to germination (Wang, Lewis, Brennan, & Westby, 1997) or use of fat as an energy source in sprouting process. The results are comparable with findings of Venderstoep (1981) for germinated green gram and lentil. Protein and thiamin levels of control samples were within previously reported ranges (Gopalan, Sastri, & Balasubramanian, 1989; Savage, 1988). Protein and thiamin contents after germination increased significantly ($P < 0.05$) by 6.1–9.7% and

Table 1
Effect of germination and dehulling on moisture, fat, protein, thiamin, and ash contents of legume flours (on dry weight basis/100 g)[a]

Sample	Moisture (g)	Fat (g)	Protein (g)	Thiamin (mg)	Ash (g)
Green gram					
Raw	10.9 ± 0.1^{a}	1.29 ± 0.02^{b}	27.7 ± 0.3^{c}	0.56 ± 0.02^{c}	4.03 ± 0.09^{a}
Germinated	8.0 ± 0.2^{c}	1.21 ± 0.01^{c}	29.1 ± 0.1^{b}	0.71 ± 0.03^{b}	3.88 ± 0.04^{b}
Dehulled	10.0 ± 0.1^{b}	1.7 ± 0.01^{a}	29.9 ± 0.6^{a}	0.85 ± 0.04^{a}	3.79 ± 0.01^{b}
SE (df = 9)	0.084	0.007	0.208	0.016	0.028
Cowpea					
Raw	8.5 ± 0.4^{a}	1.16 ± 0.04^{b}	25.7 ± 0.1^{c}	0.64 ± 0.02^{c}	3.62 ± 0.01^{a}
Germinated	6.1 ± 0.1^{c}	1.07 ± 0.02^{c}	27.2 ± 0.2^{b}	0.69 ± 0.03^{b}	3.53 ± 0.01^{b}
Dehulled	7.6 ± 0.3^{b}	1.48 ± 0.01^{a}	28.4 ± 0.3^{a}	0.85 ± 0.02^{a}	3.47 ± 0.06^{b}
SE (df = 9)	0.174	0.012	0.146	0.011	0.018
Lentil					
Raw	11.7 ± 0.1^{a}	0.89 ± 0.06^{b}	26.5 ± 0.5^{c}	0.51 ± 0.03^{c}	2.44 ± 0.02^{a}
Germinated	10.0 ± 0.2^{c}	0.78 ± 0.02^{c}	28.5 ± 0.2^{b}	0.68 ± 0.04^{b}	2.28 ± 0.14^{b}
Dehulled	11.0 ± 0.3^{b}	1.2 ± 0.09^{a}	29.6 ± 0.2^{a}	0.81 ± 0.03^{a}	2.13 ± 0.13^{b}
SE (df = 9)	0.025	0.031	0.187	0.016	0.055
Chickpea					
Raw	9.9 ± 0.3^{a}	5.45 ± 0.02^{b}	22.1 ± 0.5^{c}	0.34 ± 0.009^{c}	3.14 ± 0.17^{a}
Germinated	7.1 ± 0.2^{b}	5.18 ± 0.02^{c}	24.2 ± 0.2^{b}	0.42 ± 0.01^{b}	2.94 ± 0.01^{b}
Dehulled	6.5 ± 0.3^{b}	5.7 ± 0.04^{a}	27.2 ± 0.3^{a}	0.51 ± 0.009^{a}	2.87 ± 0.02^{b}
SE (df = 9)	0.154	0.013	0.195	0.005	0.048

SE = standard error of means.
df = degree of freedom.
All mean scores bearing different superscripts in columns in each sample are significantly different on application of Duncan's new multiple range test ($P<0.05$).
[a]Values are expressed as mean ± standard deviation ($n = 4$).

Table 2
Effect of germination and dehulling on antinutritional factors in legume flours (g/100 g on dry weight basis)[a]

Sample	Soluble dietary fiber	Insoluble dietary fiber	Total dietary fiber	Phytic acid	Tannin
Green gram					
Raw	3.42 ± 0.06^{b}	16.58 ± 0.07^{a}	20.0 ± 0.09^{b}	0.61 ± 0.02^{a}	0.66 ± 0.02^{a}
Germinated	4.71 ± 0.07^{a}	15.8 ± 0.02^{b}	20.51 ± 0.07^{a}	0.5 ± 0.03^{b}	0.59 ± 0.01^{b}
Dehulled	2.57 ± 0.06^{c}	12.6 ± 0.06^{c}	15.16 ± 0.02^{c}	0.29 ± 0.03^{c}	0.36 ± 0.01^{c}
SE (df = 9)	0.027	0.026	0.041	0.014	0.004
Cowpea					
Raw	2.19 ± 0.08^{b}	25.18 ± 0.34^{b}	27.38 ± 0.26^{b}	0.6 ± 0.01^{a}	0.47 ± 0.01^{a}
Germinated	2.3 ± 0.02^{a}	25.8 ± 0.11^{a}	28.1 ± 0.13^{a}	0.48 ± 0.02^{b}	0.34 ± 0.01^{b}
Dehulled	1.34 ± 0.01^{c}	21.48 ± 0.06^{c}	22.82 ± 0.07^{c}	0.29 ± 0.01^{c}	0.25 ± 0.01^{c}
SE (df = 9)	0.024	0.161	0.186	0.007	0.006
Lentil					
Raw	0.85 ± 0.01^{b}	15.62 ± 0.03^{a}	16.47 ± 0.02^{b}	0.19 ± 0.01^{a}	0.75 ± 0.01^{a}
Germinated	1.48 ± 0.06^{a}	15.52 ± 0.08^{b}	17.0 ± 0.02^{a}	0.15 ± 0.02^{b}	0.61 ± 0.01^{b}
Dehulled	0.34 ± 0.01^{c}	11.39 ± 0.08^{c}	11.73 ± 0.06^{c}	0.1 ± 0.01^{c}	0.36 ± 0.01^{c}
SE (df = 9)	0.018	0.033	0.019	0.004	0.005
Chickpea					
Raw	1.27 ± 0.02^{b}	25.91 ± 0.04^{a}	27.18 ± 0.06^{b}	0.48 ± 0.02^{a}	0.53 ± 0.01^{a}
Germinated	2.41 ± 0.08^{a}	25.56 ± 0.08^{b}	27.98 ± 0.03^{a}	0.38 ± 0.02^{b}	0.44 ± 0.01^{b}
Dehulled	0.54 ± 0.01^{c}	19.69 ± 0.02^{c}	20.23 ± 0.02^{c}	0.24 ± 0.01^{c}	0.3 ± 0.01^{c}
SE (df = 9)	0.022	0.027	0.019	0.008	0.006

SE = standard error of means.
df = degree of freedom.
All mean scores bearing different superscripts in columns in each sample are significantly different on application of Duncan's new multiple range test ($P<0.05$).
[a]Values are expressed as mean ± standard deviation ($n = 4$).

Table 3
Effect of germination and dehulling on mineral content and % bioavailability of iron and calcium of legume flours (on dry weight basis/100 g)[a]

Sample	Iron (mg)	Bioavailable iron (%)	Calcium (mg)	Bioavailable calcium (%)	Phosphorous (mg)
Green gram					
Raw	11.14±0.36[a]	10.9±0.1[c]	136±4.2[a]	15.7±0.7[c]	417±7.0[a]
Germinated	10.15±0.22[b]	18.3±0.2[b]	114±3.7[b]	24.7±0.6[b]	384±7.7[c]
Dehulled	9.05±0.36[c]	35.7±0.3[a]	71±3.6[c]	40.5±0.8[a]	401±1.1[b]
SE (df = 9)	0.159	0.122	1.944	0.397	3.028
Cowpea					
Raw	6.5±0.24[a]	11.2±0.3[c]	87±1.8[a]	22.6±0.5[c]	399±4.5[a]
Germinated	5.87±0.15[b]	19.7±0.2[b]	75±4.1[b]	38.2±0.3[b]	341±4.3[c]
Dehulled	5.52±0.12[c]	37.6±0.1[a]	54±2.1[c]	54.8±0.4[a]	378±2.0[b]
SE (df = 9)	0.089	0.102	1.448	0.243	1.912
Lentil					
Raw	8.52±0.24[a]	10.2±0.1[c]	77±2.4[a]	29.3±0.4[c]	467±12.4[a]
Germinated	6.73±0.18[b]	18.5±0.2[b]	63±3.7[b]	46.5±0.4[b]	420±5.7[c]
Dehulled	5.55±0.18[c]	40.4±0.2[a]	50±3.2[c]	59.6±0.5[a]	448±7.6[b]
SE (df = 9)	0.104	0.078	1.581	0.256	4.54
Chickpea					
Raw	4.68±0.24[a]	11.3±0.2[c]	222±3.7[a]	19.3±0.7[c]	366±5.5[a]
Germinated	3.77±0.11[b]	18.6±0.2[b]	176±3.5[b]	32.9±0.5[b]	325±5.5[c]
Dehulled	2.94±0.22[c]	38.6±0.3[a]	63±5.6[c]	48.1±0.7[a]	344±5.5[b]
SE (df = 9)	0.098	0.117	2.208	0.365	2.772

SE = standard error of means.
df = degree of freedom.
All mean scores bearing different superscripts in columns in each sample are significantly different on application of Duncan's new multiple range test ($P < 0.05$).
[a]Values are expressed as mean + standard deviation ($n = 4$).

Table 4
Effect of germination and dehulling on in vitro starch and protein digestibility in legume flours (on dry weight basis)[a]

Sample	In vitro starch digestibility			In vitro protein digestibility (%)
	Total starch (g%)	Glucose released (g%)	In vitro starch digestibility (%)	
Green gram				
Raw	46.7±0.8[a]	9.8±0.3[c]	18.9±0.6[c]	61.0±1.0[c]
Germinated	42.3±0.9[c]	16.2±0.2[b]	34.4±0.6[b]	72.7±0.8[b]
Dehulled	44.8±0.7[b]	27.5±1.5[a]	55.2±3.3[a]	77.7±0.8[a]
SE (df = 9)	0.433	0.469	0.994	0.469
Cowpea				
Raw	38.1±1.0[a]	9.1±0.3[c]	21.4±0.7[c]	63.8±0.6[c]
Germinated	35.2±0.5[c]	13.9±0.2[b]	35.6±0.7[b]	72.9±1.0[b]
Dehulled	37.0±0.9[b]	24.3±0.5[a]	59.1±1.4[a]	77.2±1.0[a]
SE (df = 9)	0.425	0.201	0.518	0.450
Lentil				
Raw	38.2±0.9[a]	10.8±0.4[c]	25.5±1.1[c]	65.6±1.1[c]
Germinated	34.3±0.5[c]	14.9±0.2[b]	39.1±0.7[b]	75.1±1.4[b]
Dehulled	37.3±0.5[b]	26.8±0.3[a]	64.7±0.7[a]	78.8±0.8[a]
SE (df = 9)	0.333	0.174	0.445	0.582
Chickpea				
Raw	42.4±0.4[a]	10.3±0.2[c]	21.8±0.5[c]	64.2±1.8[c]
Germinated	38.5±0.6[c]	15.6±0.4[b]	36.5±0.9[b]	73.4±0.7[b]
Dehulled	41.3±0.4[b]	27.2±0.2[a]	59.3±0.5[a]	77.6±1.0[a]
SE (df = 9)	0.253	0.162	0.364	0.649

SE = standard error of means.
df = degree of freedom.
All mean scores bearing different superscripts in columns in each sample are significantly different on application of Duncan's new multiple range test ($P < 0.05$).
[a]Values are expressed as mean ± standard deviation ($n = 4$).

8.0–33.0%, respectively, which could be due to biosynthesis during germination (Sattar et al., 1989; Venderstoep, 1981). Fat, protein and thiamin levels improved significantly after dehulling due to removal of hull portion and concentration of endosperm. The highest ash content was recorded in green gram (4.03 g/100 g) and the lowest in lentil (2.44 g/100 g). These results were in agreement with those reported earlier by several workers (Gopalan et al., 1989; Savage, 1988; Venderstoep, 1981). Leaching out of solid matter during pre germination soaking process could be the reason for significant reduction of mineral matter on germination. The further insignificant decrease of ash after dehulling could be contributed to removal of hull portion, which may have some amounts of minerals.

Table 2 presents the antinutritional factors of legume flours. Out of four legumes analysed, control lentil samples had the lowest percent of fiber fractions and cowpea had the highest. On germination, soluble and total dietary fiber fractions increased and insoluble dietary fiber fraction reduced significantly ($P<0.05$). There were marked reductions of all fiber fractions on dehulling of all legume samples studied. These data agree with the findings of Ramulu and Udayasekhara (1997) for dehulled green gram, pigeonpea, lentil and chickpea. Control samples contained considerable amounts of phytic acid (0.19–0.61 g/100 g). The 18–21% significant ($P<0.05$) decrease in phytic acid in germinated samples were comparable to the results reported for other germinated legumes including African oil bean (Enujiugha, Badejo, Iyiola, & Oluwamukomi, 2003), black gram and pea (Das et al., 1999) and pearl millet (Kumar & Chauhan, 1993). Decrease in phytic acid content during germination could be due to increase in phytase activity as reported by several authors in faba bean (Eskin & Wiebe, 1983), broad bean, chickpea and lentil (Egli, Davidsson, Juillerat, Barclay, & Hurrell, 2002) and several other legumes (Kyriakidis, Panayotou, Stavropoulou, & Athanasopoulos, 1998). Tannin levels in control samples ranged from 0.47 g/100 g in cowpea to 0.75 g/100 g in lentil. Germination significantly ($P<0.05$) reduced the tannin contents of all studied legumes as previously observed by Savelkoul et al. (1992) in pigoenpea, chickpea, black gram and green gram. After dehulling, there was little phytic acid and tannin detectable in cotyledons, indicating that most of the phytic acid and tannin are present in seed coat. Rao and Prabhavathi (1982) also reported similar results for some decorticated legumes.

Table 5
Correlation coefficients and the regression equations for the association between % of iron and calcium bioavailability and antinutritional factors in legume flours

Y	X	Regression equation	Correlation coefficient[a]
Green gram			
Bioavailable iron	Phytic acid	$Y = 58.131-78.209X$	−0.999
Bioavailable iron	Tannin	$Y = 65.06-80.92X$	−0.997
Bioavailable iron	Total dietary fiber	$Y = 95.9-4X$	−0.928
Bioavailable calcium	Phytic acid	$Y = 63.075-77.92X$	−0.999
Bioavailable calcium	Tannin	$Y = 69.611-79.452X$	−0.990
Bioavailable calcium	Total dietary fiber	$Y = 98.117-3.834X$	−0.898
Cowpea			
Bioavailable iron	Phytic acid	$Y = 62.098-85.982X$	−0.997
Bioavailable iron	Tannin	$Y = 63.866-116.131X$	−0.953
Bioavailable iron	Total dietary fiber	$Y = 133.608-4.244X$	−0.902
Bioavailable calcium	Phytic acid	$Y = 85.269-102.35X$	−0.993
Bioavailable calcium	Tannin	$Y = 89.594-144.523X$	−0.993
Bioavailable calcium	Total dietary fiber	$Y = 156.824-4.532X$	−0.806
Lentil			
Bioavailable iron	Phytic acid	$Y = 72.864-339.754X$	−0.982
Bioavailable iron	Tannin	$Y = 68.114-78.629X$	−0.996
Bioavailable iron	Total dietary fiber	$Y = 98.83-5.031X$	−0.936
Bioavailable calcium	Phytic acid	$Y = 93.922-332.675X$	−0.990
Bioavailable calcium	Tannin	$Y = 87.859-74.527X$	−0.972
Bioavailable calcium	Total dietary fiber	$Y = 105.734-4.022X$	−0.770
Chickpea			
Bioavailable iron	Phytic acid	$Y = 65.227-115.619X$	−0.986
Bioavailable iron	Tannin	$Y = 73.969-120.794X$	−0.990
Bioavailable iron	Total dietary fiber	$Y = 100.962-3.109X$	−0.937
Bioavailable calcium	Phytic acid	$Y = 77.252-119.508X$	−0.998
Bioavailable calcium	Tannin	$Y = 85.933-124.018X$	−0.995
Bioavailable calcium	Total dietary fiber	$Y = 104.314-2.82X$	−0.833

[a]Significant ($P<0.05$).

A significant ($P<0.05$) decrease found in iron, calcium and phosphorous contents on germination in present study (Table 3) is well documented by other authors (Das et al., 1999; Enujiugha et al., 2003; Giri, Pravatham, & Santhini, 1981). This is easily observable in the lower ash contents obtained in the germinated samples (Table 1). This reduction could be due to leaching of solid matter in soaking water. Further decline in iron and calcium levels after dehulling was observed, which may be contributed to presence of these minerals in hull portion. But there was a significant ($P<0.05$) improvement in phosphorous contents in dehulled samples compared to germinated samples. On germination, the percent bioavailable iron increased significantly ($P<0.05$) by 64.6%, 67.8%, 75.8% and 81.3% in chickpea, green gram, cowpea and lentil, respectively, over the control samples (Table 3). These data are in agreement with previous reports for germinated green gram, horse gram and red gram (Giri et al., 1981). The presence of tannin and phytic acid in seed coat as inhibitors was demonstrated to reduce iron absorption (Davies & Nightingale, 1975; Rao & Prabhavathi, 1982) by chelating the iron ion (McDonald, Mila, & Scalbert, 1996). The statistical analysis confirmed that, the percent biovailable iron correlated significantly ($P<0.05$) and negatively to phytic acid, tannin and total dietary fiber contents (Table 5). The process of germination and dehulling are associated with a significant ($P<0.05$) enhancement in the bioavailability of calcium (Table 3). Ghanem and Hussein (1999) also found an appreciable improvement in calcium bioavailability of germinated faba bean. Increase of calcium bioavailability after germination and dehulling of legume samples could be contributed to simultaneous reduction of phytic acid, tannin and dietary fiber. Several reports show the negative correlation of phytic acid and dietary fiber contents of foods with percent of calcium bioavailability (Allen, 1982; Cheryan, 1980; Ghanem & Hussein, 1999; Kamchan et al., 2004). While there is no doubt that unabsorbable complexes of calcium with uronic acid in hemicellulose fraction of dietary fiber and with phytic acid reduce the bioavailability of calcium (Allen, 1982; James, Branch, & Southgate, 1978). High negative correlation coefficient values of bioavailable calcium vs. phytic acid, tannin and total dietary fiber in all samples presented in Table 5 support the findings in this regard.

Increase in α-amylase activity during germination (Nnanna & Phillips, 1988; Sumathi, Malleshi, & Rao, 1995; Uriyo, 2001) could be a possible explanation of the total starch loss and appreciable improvement in percent

Table 6
Correlation coefficients and the regression equations for the association between % in vitro starch and protein digestibility and antinutritional factors in legume flours

Y	X	Regression equation	Correlation coefficient[a]
Green gram			
In vitro starch digestibility	Phytic acid	$Y = 88.215-111.532X$	−0.995
In vitro starch digestibility	Tannin	$Y = 97.019-113.39X$	−0.977
In vitro starch digestibility	Total dietary fiber	$Y = 135.167-5.335X$	−0.864
In vitro protein digestibility	Phytic acid	$Y = 93.067-48.43X$	−0.918
In vitro protein digestibility	Tannin	$Y = 95.807-47.219X$	−0.864
In vitro protein digestibility	Total dietary fiber	$Y = 106.513-1.942X$	−0.669
Cowpea			
In vitro starch digestibility	Phytic acid	$Y = 94.322-121.801X$	−0.999
In vitro starch digestibility	Tannin	$Y = 97.693-166.962X$	−0.969
In vitro starch digestibility	Total dietary fiber	$Y = 190.326-5.809X$	−0.873
In vitro protein digestibility	Phytic acid	$Y = 90.186-41.357X$	−0.944
In vitro protein digestibility	Tannin	$Y = 93.048-61.553X$	−0.995
In vitro protein digestibility	Total dietary fiber	$Y = 112.291-1.57X$	−0.657
Lentil			
In vitro starch digestibility	Phytic acid	$Y = 107.441-438.689X$	−0.993
In vitro starch digestibility	Tannin	$Y = 100.854-100.734X$	−0.999
In vitro starch digestibility	Total dietary fiber	$Y = 136.596-6.205X$	−0.904
In vitro protein digestibility	Phytic acid	$Y = 94.24-143.689X$	−0.951
In vitro protein digestibility	Tannin	$Y = 91.289-31.609X$	−0.917
In vitro protein digestibility	Total dietary fiber	$Y = 96.139-1.524X$	−0.649
Chickpea			
In vitro starch digestibility	Phytic acid	$Y = 96.647-156.674X$	−0.999
In vitro starch digestibility	Tannin	$Y = 108.214-163.027X$	−1.0
In vitro starch digestibility	Total dietary fiber	$Y = 137.309-3.904X$	−0.880
In vitro protein digestibility	Phytic acid	$Y = 91.597-54.173X$	−0.952
In vitro protein digestibility	Tannin	$Y = 95.358-55.806X$	−0.943
In vitro protein digestibility	Total dietary fiber	$Y = 99.01-1.085X$	−0.675

[a]Significant ($P<0.05$).

glucose released (Table 4). Total starch and glucose contents enhanced significantly ($P<0.05$) after dehulling. Germination significantly ($P<0.05$) increased the in vitro starch and protein digestibility of all the samples as compared to control samples by 53–82% and 14–18%, respectively (Table 4), which is supported by the findings of Kataria et al. (1989), Negi et al. (2001) and Archana, Sehga, and Kawatra (2001). Dehulling brought about further enhancement in starch and protein digestibility as reported also by Preet and Punia (2000) for dehulled cowpea. Dehulled samples had the highest levels of in vitro starch and protein digestibility that may be attributed to lowered levels of antinutrients. Findings about interaction of starch with fiber, phytic acid and tannin (Flores, Castanon, & McNab, 1994; Reddy, Sathe, & Salunkhe, 1982; Thorne, Thompson, & Jenkins, 1983), suppression of pepsin activity by dietary fiber and consequent reduction of in vitro protein digestibility (Horie, Sugase, & Horie, 1995; Mongeau, Sarwar, Peace, & Brassard, 1989), negative correlation between phytic acid and tannin with in vitro digestibility of protein support this study observations (Agarwal & Chitnis, 1995; Kumar & Chauhan, 1993). Significant negative correlations ($P<0.05$) were observed between in vitro starch and protein digestibility and phytic acid, tannin and total dietary fiber, with least correlation in case of fiber (Tables 5 and 6).

4. Conclusion

Germination improved protein, thiamin, in vitro iron and calcium bioavailability and in vitro starch and protein digestibility contents of all the legumes samples in this study, significantly ($P<0.05$). On dehulling the germinated legumes, further enhancement in mentioned parameters was observed. Phytic acid and tannin reduced by 47–52% and 43–52%, respectively, in dehulled samples over control. Significant ($P<0.05$) negative correlations were found between antinutritional factors and nutrients bioavailability and digestibility. Germination combined with dehulling process improved quality of legumes by enhancing the bioavailability and digestibility of nutrients and reducing the antinutrients.

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