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Antioxidant Potential and Type II Diabetes Related Enzyme Inhibition Properties of Raw and Processed Legumes in Indian Himalayas

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ABSTRACT

In vitro antioxidant potential and type II diabetes related enzyme inhibition capacity was analyzed in methanolic extract of raw and processed seeds of seven prominent legume genotypes, originated in Indian Himalayas. In raw seeds, total free phenolic content ranged from 2.18 ± 1.9 (small-seeded urd bean) to 13.11 ± 2.4 (bold-seeded grass pea) mg gallic acid/g extract dry weight basis (dwb), while total flavonoids varied between 1.89 ± 0.61(lima bean) and 0.41 ± 0.9 (small-seeded urd bean**)** mg catechin/ g of the extracts, dwb. Raw seed extracts exhibited scavenging capacity against DPPH (30.80 - 66.40 %), superoxides (43.78-71.22%) and hydrogen peroxide (11.19-53.78%) along with ferric reducing/antioxidant power (FRAP, 37.87-161.32 µmol/g extract dwb) and inhibition of ß-carotene degradation (23.45-49.11%). In type II diabetes related enzyme inhibition activity, the value varied from 8.11% (urd bean) to 21.34% (lima bean) for α-amylase and from 27.12% (urd bean) to 87.54% (grass pea) for α-glucosidase in raw seed extracts under *in vitro* bioassay. Among the processing methods, sprouting followed by direct cooking showed significant enhancement of antioxidant activity along with balanced levels of enzyme inhibition capacity, while soaking + cooking as well as roasting showed diminishing effects. Oil-frying exhibited mixed effects. Bold-seeded lima bean, grass pea and black-seeded common beans were superior to lentil, small-seeded urd bean and white-seeded beans. Phenolic content was correlated with antioxidant properties and enzyme inhibition activity, but this association was stronger in sprouting and direct cooking than raw seeds and other three methods.

INTRODUCTION

Demands for functional and therapeutic foods using plant natural products to combat chronic human diseases are increasing day by day (Dixon and Sumner, 2003). Legumes such as beans, peas and lentils are highly rich in bioactive compounds and different polyphenols have antioxidant potential, hindering the formation of free radicals (Dixon and Sumner, 2003; Hooda and Pal, 2012). These naturally occurring phenolic compounds are predominantly present in edible legumes and possess antimutagenic and antioxidant activities (Boateng *et al*., 2008).

Legumes constitute an important part in hill-based agro-biodiversity and traditional uses of plant natural products for food and medicinal purposes in different parts of the Himalayas (Chhetri *et al*., 2005; Kumari *et al*., 2011; Talukdar, 2013d).

However, scientific evaluations of Himalayan legumes as indigenous food and medicinal items have been started only very recently (Talukdar and Talukdar, 2012a, b; Talukdar, 2013d,f), and no reports are available regarding antioxidant potential of legumes originated in this region. The study revealed that various legume recipes/preparations are being used regularly in treating chronic diseases such as jaundice, gastrointestinal diseases and diabetes (Chhetri *et al*., 2005; Talukdar and Talukdar, 2012a; Talukdar, 2013f), in which type II diabetes (diabetes mellitus) emerges as the most threatening (Kelbe, 2005; Bhutia *et al*., 2011). Recently, stress markers of this disease have been identified in certain Indian Himalayan population (Bhutia *et al*., 2011). Growing evidences suggest that besides abnormal rise in blood glucose concentrations, reduction in plasma antioxidant level is another risk of type II diabetes (Kelbe, 2005). Although synthetic drugs are in use as enzyme inhibitors for clinical treatment of type II diabetes, these drugs are not without side effects (Kelble, 2005).

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Therefore, search for "hypoglycemic foods," showing manageable α-amylase and α-glucosidase inhibition activities as well as high antioxidant potential without any side effects are gradually gaining momentum.

Many people throughout the world use plants as safe and alternative medicine for their everyday health care needs (Chhetri *et al*., 2005; Kumari *et al*., 2011; Talukdar, 2013f). However, to exploit the potential of economically viable indigenous foods and medicine, it is important to focus on their antioxidant potential and diabetes-related enzyme inhibition properties. The objective of the present study was, therefore, framed to analyze the total free phenolic content, total flavonoids, antioxidant activity (free radical scavenging capacity, reducing power and inhibition of β-carotene oxidation) and type II diabetes-related enzyme inhibition properties in the methanolic extract of raw and indigenously processed seeds of seven predominant legume genotypes used extensively in Indian Himalayas.

MATERIALS AND METHODS

Seed materials

Fresh, healthy and dry seeds (10% moisture) of seven genotypes such as lima bean (*Phaseolus lunatus* L. bold seeded type), common beans (*Phaseolus vulgaris* L., black-seeded), common bean (white seed coat), urd bean (*Vigna mungo* L., small-seeded), lentil (*Lens culinaris* Medik.), pea (*Pisum sativum* L.) and grass pea (*Lathyrus sativus* L., bold-seeded) were collected in Eastern Indian Himalayas during an earlier survey (2009-2011) on traditional legumes grown and used by local ethnic tribes in The Himalayas (Talukdar and Talukdar, 2012a). Soon after collection, the immature and damaged seeds were removed and the mature seeds were sun-dried for 24 h and stored in plastic containers in glass desiccators, until further use.

Processing methods of seed samples

Seed samples from each of the seven genotypes were randomly divided into six batches with five replicates; the first batch was analyzed without any treatment and considered raw seeds, while the remaining five batches were processed. Whole seeds of the second batch (25 g in each replicate) were washed with distilled water in a 1:10 (w/v) ratio and directly cooked about 30 min with fresh distilled water at 85–90 °C (direct cooking).

The third batch of samples was soaked first in distilled water in a 1:10 (w/v) ratio for 8 h at 25 °C and then cooked as before (soaking + cooking). After germination in sterile Petri dishes, the sprouts of the fourth batch of samples were separated, thoroughly washed and ground in the flour mill, passed through a sieve of 0.5 mm and the obtained flour (sprouting) was stored in plastic bags, in darkness at 4 °C (Gharachorloo *et al*., 2012). The fifth batch of samples was ground in fine powder, sieved and fried in sunflower oil at 85–90 °C for 10 min (oil-frying). The sixth batch of seed materials was roasted in an iron pot for 30 min at 100–110 °C along with clean fine sand to prevent the burning of the seed coat and to ensure the uniform distribution of heat; the seeds were then separated by using a sieve and allowed to cool to room temperature. All the processed and raw samples were frozen at −80 °C, dried and finely powdered.

Preparation of methanolic extract

One gram of dried flour of raw and processed seeds/materials was treated with petroleum ether (1:10, w/v) overnight on a magnetic stirrer, centrifuged at 2,500 × *g* for 20 min; the supernatant was then discarded. The defatted residue was then air-dried and extracted exhaustively with 50 ml of chilled aqueous methanol (methanol: water, 80:20 v/v) at the ratio of 1:10 (w/v) for 2 h at room temperature. The samples were centrifuged at 3,000 × *g* for 20 min and the supernatant was removed. Extraction was repeated twice and supernatants were pooled and evaporated using a rotary vacuum evaporator at 40 °C and freeze-dried in a lyophilizer for 1 h. The phenolic concentrate was made to a final volume of 10 ml with distilled water and stored at -80 °C until analysis. All measurements were done in five replicates.

Total phenolic (TP) and flavonoid (TF) content

TP content of methanolic extracts was estimated following earlier method (Singleton *et al*., 1999) with Folin-Ciocalteu reagent at 750 nm. TP content was expressed as Gallic acid (GAE) equivalent/ g extract on a dry weight basis (dwb). TF was estimated spectrophotometrically using the earlier method (Zhishen *et al*., 1999) based on the formation of a flavonoid-aluminium complex and was expressed as equivalent to catechin (CAE) in mg/ g of the extracts dwb.

Radical scavenging activity

Free radical scavenging activity of the methanolic extract (100 µl, 1 mg/ml) was analyzed by DPPH scavenging assay with absorbance at 517 nm (Wettasinghe and Shahidi, 2000). For superoxide radical scavenging activity (SRSA) assay, light-induced reduction of nitro-blue tetrazolium (NBT) by superoxide radicals was used at 560 nm (Zhishen *et al*., 1999). For hydrogen peroxide (H_2O_2) scavenging activity, 1 ml of extract (250 µg/ml) was mixed with 2.4 ml of 0.1 M phosphate buffer (pH 7.4), and then 0.6 ml of a 43 mM solution of H_2O_2 in the same buffer were added (Wettasinghe and Shahidi, 2000). After 40 min, the absorbance of reaction mixture was taken at 230 nm against a blank solution (phosphate buffer without H_2O_2). Radical scavenging activity was calculated from absorbance values in each case and expressed as inhibition percentage.

Ferric reducing antioxidant power (FRAP)

Freshly prepared FRAP reagent (3.0 ml) was mixed with 100 ml of diluted samples of methanolic extracts (Benzie and Strain, 1999). After 10 min incubation at 37 °C, the absorbance changes at 593 nm in the test mixture were compared to those obtained from standard mixture of ferrous sulphate ($FeSO_4.7H_20$). The increasing concentration of Fe^{3+} is expressed as µmol of Fe^{2+}/g dwb (Benzie and Strain, 1999).

β-Carotene-linoleic acid test

β carotene (type I, synthetic, Sigma-Aldrich) of 10 mg was dissolved in 10 ml of chloroform and 3 ml of it was added to 20 µl of linoleic acid and 200 µl Tween ® 40 (Sowndhararajan *et al*., 2010). After removing chloroform under reduced pressure, 40 µl of sample was mixed with 3 ml of emulsion and incubated for 1 hr at 50 °C. The absorbance was recorded at 470 nm against a blank (emulsion without β-carotene). Inhibition of ß-carotene oxidation was expressed on a percent basis.

α-Amylase inhibition assay

To 100 µl of 0.02 M sodium phosphate buffer (pH 6.9) and100 µl of porcine α-amylase (Sigma-Aldrich, Bangalore, India) solution (4.5 Units/ml/min) methanolic sample extract (100 µl, 1 mg/ml) was added and pre-incubated at 25 °C for 10 min (Worthington, 1993). After addition of 1% starch solution (100 µl), the reaction mixture was then incubated at 25 °C for 30 min. The reaction was stopped by the addition of 1.0 ml of dinitrosalicylic acid (HiMedia, Mumbai, India) color reagent (Worthington, 1993). The test tubes were then incubated in a boiling water bath for 5 min and then cooled to room temperature. The reaction mixture was then diluted (10-fold) with distilled water and the absorbance was measured at 540 nm with the control (buffer instead of extract), and the percent of enzyme inhibition was calculated.

α-Glucosidase inhibition assay

Methanolic sample extract (100 µl, 1 mg/ml) was mixed with 100 µl of 0.1 M phosphate buffer (pH 6.9) and 100 µl of yeast α-glucosidase (Sigma Aldrich, India) solution (1 Unit/ml/min) and pre-incubated at 25 °C for 5 min (Worthington, 1993). Then, 100 µl of *p*-nitrophenyl-α-D-glucopyranoside was added and the reaction mixture was incubated at 25 °C for 10 min. After the incubation period, the absorbance were taken at 405 nm and allegorized to a control (100 µl of buffer instead of the extract). The results were expressed on a percent basis. Synthetic anti-diabetic drug acarbose (1mg/ml, Sigma Aldrich, India) was used as positive control for both α-amylase and α-glucosidase activity inhibition bioassay.

Statistical analysis

All data were expressed as means ± standard errors of five replicates. Significant ($P < 0.05$) differences with raw seeds was performed with't-test'. One-way ANOVA with Duncan's Multiple Range Test was performed using the SPSS v.10 statistical software (SPS Inc., USA) for multiple comparison and separation of means. Correlation analysis was carried out using 'Microsoft data analysis tool pack 2007' at $P < 0.05$.

RESULTS AND DISCUSSION

TP and TF content in raw and processed seeds

The TP and TF content of the methanolic extract from the defatted raw seeds of seven genotypes was presented in table 1. TP content ranged from 2.18 ± 1.9 to 13.11 ± 2.4 mg GAE/g extract dry weight basis (dwb), while TF content varied between 1.89 ± 0.61 and 0.41 ± 0.9 mg CAE/ g of the extracts, dwb. The values are higher compared to the earlier report in different leguminous genotypes, differing in seed size, coat color, flower color and ploidy levels (Sreeramulu *et al*., 2009; Sowndhararajan *et al*., 2010; Talukdar, 2009a, b; 2011a,b, 2012d). The high TF level may be due to high anthocyanin and flavonol glycosides in beans and peas (Boateng *et al*., 2008; Talukdar, 2012a).

Compared to raw seeds, processing had significant ($P < 0.05$) effect on phenolic content of seven genotypes (Table 1). Sprouting enhanced TP content 1.2 to 2.1-fold and TF content 1.4 to 2.5-folds. TP and TF content was also increased in direct cooking of all genotypes and in oil-fried beans, but it was reduced significantly ($P < 0.05$) in oil-fried lentil and peas. Soaking + cooking markedly reduced TP content in all genotypes but increased TF level in beans by about 1.3-1.5-fold (Table 1). Lowest amount of TP and TF was estimated in roasting. The study showed that legume sprouts are the excellent source of dietary phenolic antioxidants, and beans are nutritionally better than lentil and peas under processing. Beneficial effect of sprouting on seed phenolic content was also found in other legumes, also (Ramesh *et al*., 2011; Vadivel *et al*., 2011; Kim *et al*., 2012).

Antioxidant activity of the methanolic extracts of raw and processed seeds

The DPPH, SRSA and H_2O_2 scavenging activity, FRAP and inhibition capacity of ß-carotene oxidation was significantly ($P < 0.05$) higher in raw seeds of bold-seeded lima bean, grass pea and black-seeded common beans in comparison to white-seeded beans and small-seeded urd beans (Fig. 1A-C, 2A,B). Radical scavenging activity, FRAP value and inhibition of ß-carotene oxidation was either higher or comparable to different other legumes, millets and pseudo-cereals (Siddhuraju and Becker, 2007; Boateng *et al*., 2008; Nsimba *et al*., 2008; Sreeramulu *et al*., 2009; Ramesh *et al*., 2011; Vadivel *et al*., 2011). Since phenolic compounds present in the extract are good electron donors, they may accelerate the conversion of H_2O_2–H_2O. The H_2O_2 is a diffusible free radical within cell, and its dual roles as inducer of stresses and a signaling molecule to stresses have been strongly confirmed in different legumes (Talukdar, 2012c, 2013 c). In lentil, pea and beans, antioxidant defense in scavenging of H_2O_2 have been studied under diverse modes of stresses (Talukdar, 2012a-c,d, 2013a-c, e). Compared to raw seeds, antioxidant activity was significantly enhanced under sprouting and direct cooking, exhibiting increase of 1.5-2-fold for DPPH, 1.2-1.5-fold for superoxide, 2-2.5-fold for H_2O_2, 1.8-3.0-fold for FRAP and 2-3-fold for inhibition of β-carotene oxidation across the seven genotypes. After sprouting, direct cooking emerged as second most efficient processing methods. Higher magnitudes of activity were noticed in bold-seeded lima bean and grass pea, and were followed by black-seeded beans, lentil and peas (Fig. 1A-C, 2A, B). By contrast, antioxidant and free radical scavenging capacity in all genotypes was significantly ($P < 0.05$) reduced in soaking +

cooking and roasting, while low to moderate activity was noticed in oil-fried lentil and pea (fig. 1A-C). Increasing antioxidant potential in sprouted seeds might be due to photosynthetic activity in the sprouts leading to the synthesis of different phytochemicals, including polyphenolic compounds and their subsequent mobilization by the activation of enzymes like polyphenol oxidase (Kim *et al*., 2012). Beneficial effect of direct cooking might be due to release of bound polyphenols during disintegration of cell wall (Xu *et al*., 2008). Contrary to this, negative impact of soaking prior to cooking on polyphenols might be attributed to softening of cell wall tissues, accompanied by solubilization and subsequent leaching of bound polyphenols into the soak water and then degradation of phenolic compounds while being cooked at an elevated temperature (Boateng *et al*., 2008). Obviously, soaking + cooking process is not suitable for antioxidant potential of present legumes. Oxidative damage due to excess free radicals appears to be a feature of most human diseases including type II diabetes (Kelble,2005). Thus, dietary antioxidants from sprouted and directly cooked legumes may be supplemented against free radical damage of cellular DNA, lipids and proteins.

Table. 1: Total phenolics (TP, mg GAE/g extract dwb) and flavonoid (TF, mg CAE / g dwb) content in raw and differentially processed seed samples of seven edible legumes in Indian Himalayas

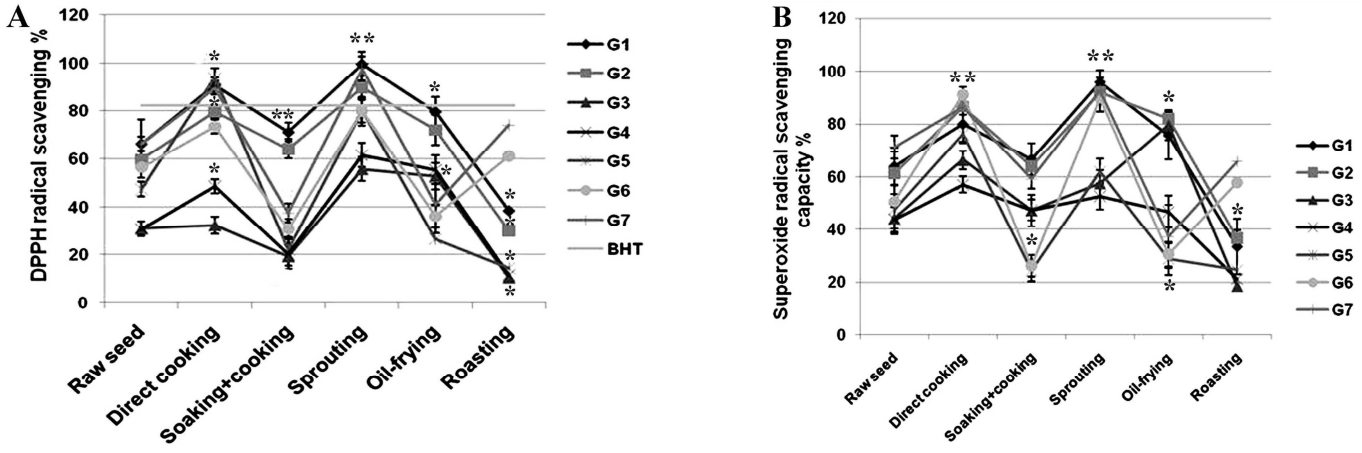
Genotype	Desig-nation		Raw seed	Cooking	Soaking + cooking	Sprouting	Roasting	Oil-frying
Phaseolus lunatus L. bold seeded	G1	TP	12.89 ± 0.1^{b}	13.35 ± 1.0^{b} (+ 1.04)	7.72 ± 1.3^{c} (- 1.7)	27.07 ± 1.0^{a} (+ 2.1)	3.87 ± 0.7^{d} (- 3.3)	16.54 ± 1.0^{b} (+ 1.3)
		TF	1.89 ± 0.61^{c}	1.89 ± 0.8^{c} (0)	2.84 ± 1.1^{b} (+ 1.5)	4.73 ± 1.1^{a} (+ 2.5)	0.95 ± 1.7^{d} (- 2.0)	1.91 ± 0.9^{c} (+ 1.01)
P. vulgaris L-black seeded	G2	TP	10.56 ± 0.2^{b}	17.95 ± 1.9^{a} (+ 1.7)	5.81 ± 1.2^{c} (- 1.8)	16.89 ± 1.2^{a} (+ 1.6)	3.21 ± 0.9^{c} (- 3.3)	13.51 ± 2.0^{a} (+ 1.3)
		TF	1.59 ± 1.4^{c}	1.63 ± 0.3^{c} (+ 1.03)	2.38 ± 0.8^{b} (+ 1.5)	3.49 ± 0.9^{a} (+ 2.2)	0.88 ± 0.3^{d} (-1.8)	1.55 ± 1.2^{c} (-1.03)
P. vulgaris L-white-seeded	G3	TP	3.88 ± 0.9^{b}	5.04 ± 1.0^{a} (+ 1.3)	2.91 ± 1.0^{c} (- 1.3)	4.67 ± 1.1^{a} (+ 1.2)	0.57 ± 0.1^{d} (- 6.8)	4.11 ± 1.5^{a} (+ 1.1)
		TF	0.78 ± 0.8^{b}	0.83 ± 1.0^{b} (+ 1.06)	1.01 ± 1.1^{a} (+ 1.3)	1.09 ± 1.8^{a} (+ 1.4)	0.39 ± 0.9^{b} (- 2.0)	1.01 ± 1.5^{a} (+ 1.3)
Vigna mungo, small-seeded	G4	TP	2.18 ± 1.9^{a}	2.60 ± 1.3^{a}(+ 1.2)	1.49 ± 1.1^{b} (- 1.5)	2.59 ± 2.0^{a} (+ 1.2)	0.49 ± 0.8^{c} (- 4.5)	2.40 ± 1.2^{a} (+ 1.1)
		TF	0.41 ± 0.9^{b}	0.39 ± 1.1^{b} (-1.04)	0.53 ± 1.0^{a} (+ 1.3)	0.58 ± 0.7^{a} (+ 1.4)	0.27 ± 1.3^{c} (- 1.5)	0.55 ± 1.0^{a} (+ 1.3)
Lens culinaris Medik	G5	TP	6.26 ± 0.5^{b}	11.89 ± 0.2^{a} (+ 1.9)	3.12 ± 1.5^{c} (- 2.0)	10.64 ± 0.9^{a} (+ 1.7)	1.14 ± 1.2^{d} (- 5.5)	4.19 ± 1.3^{c}(- 1.5)
		TF	1.12 ± 1.2^{c}	1.68 ± 1.0^{b} (+ 1.5)	0.62 ± 1.3^{d} (- 1.8)	2.35 ± 1.2^{a} (+ 2.1)	0.49 ± 1.1^{d} (- 2.3)	0.98 ± 1.6^{d} (- 1.1)
Pisum sativum L.	G6	TP	4.67 ± 2.2^{b}	8.41 ± 1.6^{a} (+ 1.8)	2.32 ± 1.2^{c} (- 2.0)	7.94 ± 1.3^{a} (+ 1.7)	1.21 ± 1.3^{d} (- 3.9)	3.89 ± 0.9^{b} (- 1.2)
		TF	1.22 ± 1.8^{c}	1.83 ± 1.7^{b} (+ 1.5)	1.83 ± 1.7^{b} (- 1.5)	2.20 ± 1.4^{a} (+ 1.8)	2.21 ± 1.5^{a} (+ 1.8)	1.22 ± 1.2^{c} (0)
Lathyrus sativus L.-bold-seeded	G7	TP	13.11 ± 1.4^{b}	18.35 ± 1.1^{a} (+ 1.4)	6.53 ± 1.8^{d} (- 2.0)	26.23 ± 2.2^{a} (+ 2.0)	3.76 ± 1.8^{e} (- 3.5)	8.19 ± 1.3^{c} (- 1.6)
		TF	1.74 ± 0.73^{b}	3.31 ± 0.8^{b} (+ 1.9)	1.16 ± 1.3^{b} (- 1.5)	4.35 ± 1.0^{a} (+ 2.5)	3.48 ± 1.3^{b} (+ 2.0)	1.69 ± 1.2^{b} (- 1.03)

All data are means ± SE of five replicates; means followed by same lower-case superscript letters are not significantly different at $P < 0.05$ by Duncan's Multiple Range Test; values within bracket denote changes in relation to raw seeds; + increase; - decrease; 0 no change.

Table. 2: Correlation study between total phenolics, flavonoids and antioxidant as well as type II diabetes-related enzyme inhibition properties in raw and processed seeds using Pearson's coefficient (r), $n = 10$.

Processing	Compounds [a]	DPPH	SRSA	H_2O_2	FRAP	BCB	AI	GI
Raw seeds	TP	0.703*	0.663*	0.611*	0.633*	0.658*	0.589	0.512
	TF	0.629*	0.534	0.522	0.593	0.576	0.564	0.532
Direct cooking	TP	0.666*	0.859*	0.834*	0.712*	0.706*	0.645*	-0.765*
	TF	0.706*	0.757*	0.810*	0.634*	0.669*	0.613*	-0.738*
Soaking + cooking	TP	0.634*	0.655*	0.643*	0.689*	0.596	0.434	0.567
	TF	0.661*	0.534	0.598	0.605*	0.469	0.532	0.573
Sprouting	TP	0.898*	0.953*	0.931*	0.878*	0.665*	0.665[a]	-0.711[a]
	TF	0.903*	0.888*	0.934*	0.816*	0.732*	0.638[a]	-0.675[a]
Roasting	TP	0.613*	0.608*	0.717*	0.653*	0.615*	0.516	0.578
	TF	0.632*	0.611*	0.643*	0.545	0.498	0.464	0.475
Oil-frying	TP	0.656*	0.627*	0.566	0.545	0.578	0.554	0.596
	TF	0.711*	0.605*	0.528	0.537	0.505	0.487	0.532

*Correlation is significant at $P < 0.05$, TP total phenolics; TF Total flavonoids; DPPH DPPH free radical scavenging activity; SRSA Superoxide radical scavenging activity; H_2O_2 Hydrogen peroxide scavenging activity; FRAP Ferric reducing/antioxidant potential; BCB β-Carotene bleaching assay; AI α-Amylase inhibition activity; GI α-glucosidase inhibition activity [a]Average values of seven genotypes were used.



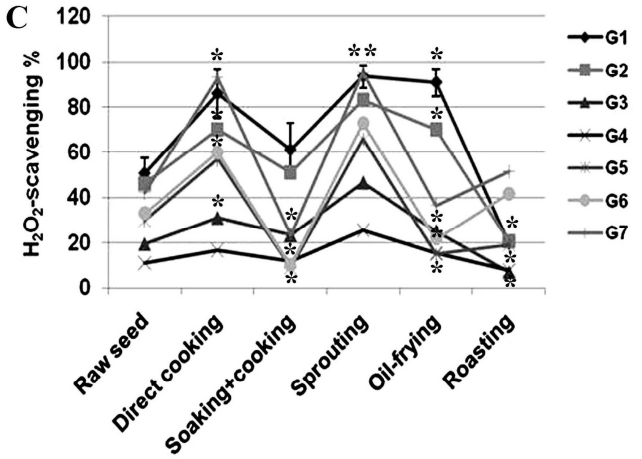


Fig. 1: DPPH (A), Superoxide radical (SRSA) (B) and hydrogen peroxide (H_2O_2) scavenging activity (C) in methanolic extract of raw and processed seeds of seven legume genotypes (designated as G1- G7 following table 1). Values are mean ± standard error of five independent determinations (n = 5). Single asterisk (*) and double asterisks (**) denote the significant ($P < 0.05$) differences with raw seeds (control) in some genotypes and in all genotypes, respectively. BHT was standard for DPPH %.

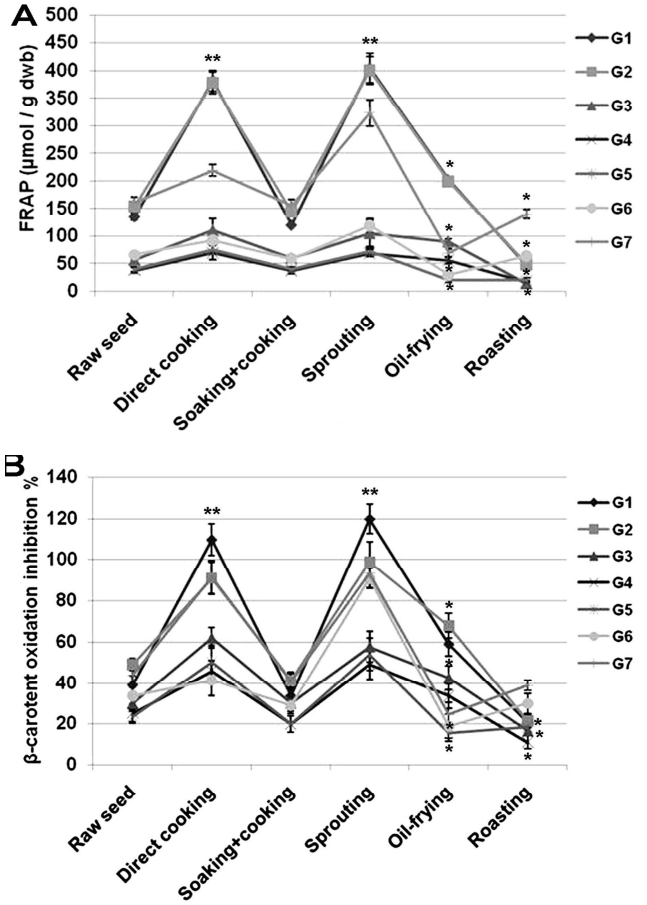


Fig. 2; Ferric reducing antioxidant potential (FRAP) (A) and β-Carotene oxidation inhibition (B) in methanolic extract of raw and processed seeds of seven legume genotypes (designated as G1- G7 following table 1). Values are mean ± standard error of five independent determinations (n = 5). Single asterisk (*) and double asterisks (**) denote the significant ($P < 0.05$) differences with raw seeds (control) in some genotypes and in all genotypes, respectively.

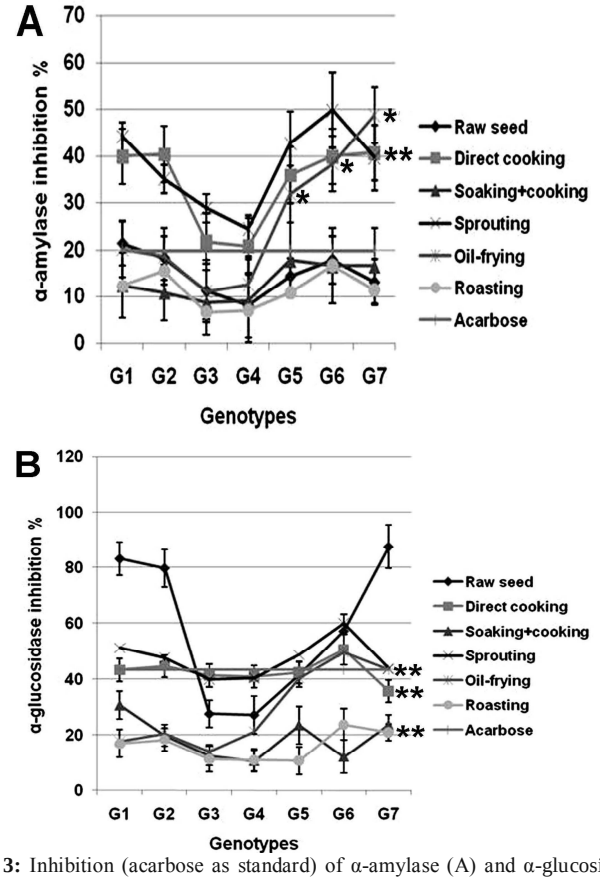


Fig. 3: Inhibition (acarbose as standard) of α-amylase (A) and α-glucosidase (B) enzyme activity in methanolic extract of raw and processed seeds of seven legume genotypes (designated as G1- G7 following table 1). Values are mean ± standard error of five independent determinations (n = 5). Single asterisk (*) and double asterisks (**) denote the significant ($P < 0.05$) differences with raw seeds (control) in some genotypes and in all genotypes, respectively.

Type II diabetes related enzyme inhibition in raw and processed seeds

In the methanolic extracts of raw seed, inhibition of α-amylase activity ranged from 8.11% (urd beans) to 21.34% (lima bean), while inhibition of α-glucosidase varied from 87.54% (grass pea) to 27.12% (urd bean) (Fig. 3A, B), indicating inhibition percentage was far lower for α-amylase but markedly higher for α-glucosidase in comparison to acarbose and earlier reports on cereals and millets (Randhir *et al*., 2008; Talukdar, 2013f). In sprouts and direct cooked seeds, inhibition of α-amylase was increased significantly ($P < 0.05$) over raw seeds in all genotypes (Fig. 3A). For α-glucosidase, inhibition capacity was decreased substantially in lima beans and grass pea but increased in white-seeded beans and small-seeded urd beans, and was unchanged in lentil and pea (Fig. 3B). Obviously, the α-amylase and α-glucosidase inhibition levels were adjusted during sprouting and direct cooking to those of the synthetic anti-diabetic agent acarbose. As high enzyme inhibition may be unsuitable for dietary intake due to anomalous carbohydrate digestion in human colon (Kelble, 2005), moderate level of inhibition as observed during sprouting and direct cooking has the potential to integrate into

dietary items of type II diabetic patients as natural substitute of synthetic drug. By contrast, soaking + cooking as well as roasting and oil-frying reduced enzyme inhibition levels even below acarbose levels, and thus are not recommended methods for dietary intake of legumes during type II diabetes.

TP and TF content (average of seven genotypes) was significantly ($P < 0.05$) correlated with enzyme inhibition in direct cooking and sprouting; this association became significantly positive for α-amylase but was strongly negative for α-glucosidase (Table 2). Rise in TP and TF content and concomitant decline in α-glucosidase inhibition percentage (to the acarbose level) in majority of the genotypes led to negative associations between them. Therefore, sprouting as well as direct cooking could be considered as mild and favorable therapeutic treatment for type II diabetes due to balanced change of enzyme inhibition during processing and the positive role of phenolics in enzyme inhibition capacity.

CONCLUSION

Methanolic extract of raw seed materials of seven widely used legume genotypes in Indian Himalayas contained very low to high antioxidant potential and undesirable type II diabetes-related enzyme inhibition properties. However, both the sprouting and direct cooking were the desirable processing methods to enhance antioxidant potential as well as managing of type II diabetes-related enzyme inhibition properties favorable to dietary integration. Phenolic content was significantly correlated with antioxidant properties and enzyme inhibition activity, and this association was stronger in sprouting and direct cooking than raw seeds and other three methods.

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