Variability in Phytochemicals, A-Galactosides, Sucrose Composition and *in Vitro* Protein Digestibility of Common Bean (*Phaseolus vulgaris* L.) Varieties

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Abstract: The variability in the phytochemicals, α-galactosides, the sucrose composition and the *in-vitro* protein digestibility of common bean varieties released from research centres were investigated. Concentrations for α-galactosides (raffinose and stachyose) and phytochemicals (lectins, saponins, phytic acid, protease inhibitors and tannins) varied significantly ($P < 0.05$) amongst the common bean varieties. Mean values for raffinose, stachyose, total α-galactosides, sucrose, trypsin inhibitors, tannins, phytic acid, saponin and lectins were 3.14 mg g^{-1}, 14.86 mg g^{-1}, 17.99 mg g^{-1}, 24.22 mg g^{-1}, 20.68 TUIx10^3 g^{-1}, 17.44 mg catechin equivalent g^{-1}, 20.54 mg g^{-1}, 1.01 g 100 g^{-1} and 4.75 g kg^{-1} PHA based on dry weight basis respectively. *In vitro* protein digestibility varied significantly ($P < 0.05$) among the bean varieties and had a positive significant correlation with sucrose content and negative correlations with trypsin inhibitors, tannins, lectins, α-galactosides and saponins. The correlation matrix indicated that variability in α-galactosides, the protein digestibility, the phytochemical composition and the sucrose contents of beans existed. In addition, a protective role against diseases was correlated with the amount of phytochemicals quantified in the bean samples. Amongst the studied bean varieties, Roba has the potential to be used as a raw material in the food-processing industry owing to its higher *in vitro* protein digestibility, lower phytochemicals composition and other beneficial nutritional parameters.

Keywords: *Phaseolus vulgaris*; Protein Digestibility; A-Galactosides; Phytochemicals; Sucrose

1. Introduction

Common beans (*Phaseolus vulgaris* L) are considered to be one of the major sources of dietary proteins. They are most widely cultivated and consumed in Latin America, India and Africa as a whole seed (Salunkhe and Kadam, 1989). In Ethiopia, common beans are used as the least expensive protein source for (resource) poor people who cannot afford to buy expensive meat. Furthermore, beans are produced primarily by small-scale farmers and function as a cash-generating crop in the central rift valley of Ethiopia (Dawit and Demelash, 2003).

Protein quality in leguminous seeds does not, however, reach the same level as in animal products. This is due to various factors, among the most well-known are their unbalanced amino acid composition, the low true digestibility of protein and the presence of phytochemicals in the seeds (Bressani and Elias 1980; Norton *et al*., 1985). Common beans synthesize several undesirable chemical substances termed phytochemicals that are known to exert deleterious effects when ingested by humans or animals. The endogenous phytochemicals present in common beans are produced by the plant to protect itself against environmental stress. In general, phytochemicals are compounds that impair health by destroying nutrients/vitamins or by reducing the uptake of such essential elements by different mechanisms. They give an astringent taste, odour and flavour and which can cause adverse physiological responses or diminish the bioavailability of certain nutrients and hinder utilization of common beans for human nutrition and animal feed (Salunkhe, 1982). Phytochemicals inhibit protein and carbohydrate digestibility; interfere with mineral bioavailability, induce pathological changes in intestine and liver tissue thus affecting metabolism, inhibit a number of enzymes and bind nutrients making them unavailable (Bressani and Sosa 1990; Bressani 1993).

The main phytochemicals found in *Phaseolus vulgaris* are enzyme inhibitors, tannins, lectins (phytohaemagglutinins), phytic acid (phytate), flatulence-causing α-galactosides and saponins (Liener, 1989). Flatulence-causing α-galactosides are oligosaccharides of the raffinose-series family which include raffinose, stachyose and verbascose. α-galactosides contribute to flatulence production in humans and mono-gastric animals due to a lack of the necessary α-galactosidase enzyme which helps to break down raffinose-series oligosaccharides during the consumption of dry beans. Hence, they are considered as unwanted components as a consequence of the accumulation of gas in the intestinal tract is discomfort, abdominal rumblings, cramps, pain and diarrhea after bean ingestion. The α-galactosides are also associated with a low food intake in animal experiments (Frias *et al*., 2000). Phytic acid has long been recognized as a phytochemicals which affects the bioavailability of minerals (Ca^{2+}, Mg^{2+}) and trace elements such as Zn^{2+}, Fe^{2+}, Cu^{2+} and Mn^{2+} (Reddy *et al*., 1982). Tannins are a group of polyphenols which form insoluble complexes with protein and inhibit several enzymes (Bressani, 1993). Trypsin inhibitors (enzyme inhibitors) are capable of binding to the trypsin enzyme, thus inhibiting its activity, interfering with the digestion of proteins and resulting in an increased pancreatic secretion and hypertrophy of the pancreas (Birk, 1989). Saponins are bitter tasting, foam producing glycosides and detected by their hemolytic activity and surface-active properties (Duhan *et al.,* 2001). Lectins, the sugar-binding proteins that agglutinate animal red blood cells, are the main toxic components in *Phaseolus vulgaris* (Liener, 1983). A number of investigators

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have demonstrated that the poor digestibility and biological utilization of beans is directly related to the phytochemicals content of beans (Pusztai *et al*., 1975; Rao and Belavady 1978; Maga, 1982; Singh and Krikorian 1982; Johnson *et al*., 1986; Laurena *et al*., 1994; Shimelis, 2005).

Most of the research on Ethiopian common beans has been related to varietal selection where the criteria for selection have always been adaptation, resistance to disease, rate of maturation, yields, seed size, color and specific agronomic traits, but never nutritive quality. Information on the composition of phytochemicals and protein digestibility of improved common beans released from research centers has not been available at national level in the Ethiopian context (EARO, 2002). This information would, therefore, be of great interest to Ethiopia because the knowledge provided would help to orient the work of breeders involved in varietal selection, give baseline information for exporters and processors on the levels of unwanted components and protein digestibility which, in turn, would help to develop suitable, simple and inexpensive processing techniques for the reduction or removal of those factors. Furthermore, it could boost the utilization of common bean varieties as value-added products at small-scale industry level in the developing countries by local food processors.

The present study aims to evaluate the variability in concentration of phytochemicals, α-galactosides, sucrose and find out the levels of protein digestibility of common bean varieties grown in Ethiopia. The study also compares the obtained results to those observed in beans grown in other areas of the world which can be influenced by genotype, environmental and varietals interaction. The outcome of this study could contribute to the intensive utilization of *Phaseolus vulgaris* in the form of processed commercial products at industry level through large-scale cultivation of selected varieties in developing countries, exclusively in the East and Great Lakes regions of Africa where common beans are utilized to a great extent.

2. Materials and Methods

2.1. Common Bean Varieties

The common beans used in this study were grown at Nazareth Agricultural Research Centre of Ethiopia under similar field conditions and normal agronomic practices required for bean crops. The eight varieties of *Phaseolus vulgaris* used for the study were Awash 1 (G-4445) and Mexican 142 (G-11239) (Export types), Beshbesh (XAN76 x BAT85), Gobirasha (ICA-15541), Gofta (G-2816), Redwolita (local collection), Roba 1 (A-176) and Tabor (A-788) (Food types) which were released from Haramaya, Awassa, Jimma and Nazareth Research Centres. The test samples were clean, uniform in size with natural colour, good appearance, and free from abnormal odors, broken seeds, dust and other foreign materials including living or dead insects. The bean samples were finely ground in analytical mill (Cole-Parmer, Cole-Parmer Instrument Company, Model 4301-02, U.S.A) and sieved through a 0.5 mm mesh screen. Samples were stored at 4°C prior to analyses. All chemicals and reagents used were either analytical or reagent grade.

2.2. A-Galactosides and Other Sugars Analyses by HPLC

The α-galactosides and other sugars were extracted from the bean samples using the AOAC official method (AOAC, 2000). The analyses of sugars were carried out using high-performance liquid chromatography (HPLC) according to Doyon *et al*., (1991). The liquid chromatograph used for this study was "Agilent 1100 series" with analytical column (APS-2 HYPERSIL, 5µm, 250 x 4.6 mm, L x I.D, Thermo Electron Corporation, England) equipped with differential refractive index (RI) detector (Model, G1362A, Agilent technologies, Germany). Individual sugar standards were allowed to dry for 12hrs at 60 ^{0}C under vacuum. Subsequently, standard solutions of raffinose-series oligosaccharides (raffinose, stachyose, verbascose; procured from Sigma Chemical, St. Louis, MO, USA) and other sugars (glucose, sucrose, fructose and maltose; purchased from Sigma Chemical, St. Louis, MO, USA) were prepared at a concentration of 1mg ml^{-1} (stachyose, raffinose, verbascose) and 5mg ml^{-1}(other sugars) each and used for calibrating a selected plot.

The prepared extracts (unknown samples) were eluted with CH_3CN/ H_2O 73:27 (v/v) as a mobile phase at a pump rate of 1ml min $^{-1}$ in to the HPLC column and were quantified by comparison with the standard sugars. The results obtained from HPLC analysis were expressed in mg g^{-1}.

2.3. Determination of Trypsin Inhibitor Activity

Trypsin inhibitor activity (TIA) in common bean flour was measured according to Smith *et al*., (1980).

2.4. Measurements of Lectins, Saponins, Phytic Acid and Tannins

A competitive indirect ELISA assay for quantification of *Phaseolus vulgaris* lectins was conducted following the procedure of Burbano *et al*., (1999). The saponin contents of bean samples were determined using the method described as Hiai *et al*., (1976). Phytic acid content was evaluated using the method of Haug and Lantzsch (1983). Tannins were also determined following the method described by Makkar *et al.,* (1998).

2.5. Zinc, Total Ash Composition and Colour Measurement

Zinc analysis was carried out using the method reported on Issac and Johnson (1975) atomic absorption spectrophotometer **(**Hitachi, Model Z-8230, Japan). Total ash composition of the seed flour was performed according to official methods (AOAC, 2000). Common beans were monitored for their colour by using colour flex spectrocolorimeter (Model no. 45/0, Hunter Lab Reston, VA, USA, 2002). The parameters recorded were **L**, **a** and **b** co-ordinates of the CIE scale.

2.6. *In Vitro* Protein Digestibility Analysis

Proteins from common beans were isolated for digestibility analysis, using the method of Satterlee *et al.*, (1975). In *vitro* protein digestibility analysis of common bean samples was carried out with a mixture of three enzymes: trypsin (porcine pancreatic trypsin type IX, with 15,500 BAEE units per mg protein), α-chymotrypsin (bovine pancreatic chymotrypsin, type II, 76 units per mg protein) and peptidase (porcine intestinal mucosa, grade III, 102 units per gm solid). All these enzymes were procured from Sigma Chemical Co., St Louis, MO, USA. The multi-enzyme solution was freshly prepared before each series of tests, and its activity was determined using casein (bovine milk, purchased from Sigma Chemical Co., St Louis, MO, USA) of known *in vivo* apparent digestibility with the method described by Hsu *et al.*, (1977).

3. Experimental Design and Statistical Analyses

The experiment was laid out using complete randomized design (CRD). Data were scrutinized using analysis of variance (ANOVA), followed by least significant difference (LSD) for multiple comparisons among treatment means at 5% level of significance. Statistical analyses were performed using SPSS/12 software for windows. All values were presented as means of triplicates ± standard deviation.

4. Results and Discussion

4.1. A-Galactosides and Sucrose

The α-galactosides and sucrose contents of eight common bean varieties studied are presented in Table 1. Stachyose was the major α-galactoside contained in all the samples analyzed, which also contained significant quantities of raffinose. Nevertheless, verbascose, fructose, glucose and maltose were not detected in HPLC analyses of all common bean samples. The sucrose content of common beans ranged from 17.27 mg g^{-1} (in Beshbesh) to 28.58 mg g^{-1} (in Mexican). Raffinose concentrations ranged from 2.35 mg g^{-1} (in Awash) to 4.34 mg g^{-1} (in Gobirasha) and stachyose concentrations from 12.38 mg g^{-1} (in Roba) to 18.41mg g^{-1} (in Beshbesh). Comparable concentrations of raffinose family oligosaccharides and sucrose have been reported for common beans grown in Canada (Sosulski *et al.*, 1982) and in Burundi (Barampama and Simard, 1993). However, some investigators, Agbo (1982); Sathe *et al.*, (1983); Reddy *et al.*, (1984); Salunkhe and Kadam (1989); Burbano *et al.*, (1990) who assayed common bean varieties grown in the USA for flatus factor, have observed raffinose (2-10 mg g^{-1}) and stachyose (2.0-56.2 mg g^{-1}) concentrations higher than those obtained for common bean varieties grown in Ethiopia and used in this study. Similarly, for common bean varieties grown in different Spanish areas, flatus factors were reported as 0.9-5.6 mg g^{-1} raffinose, 18.3-29.3 mg g^{-1} stachyose, 0.4-2.7 mg g^{-1} verbascose and 12.8-28.9 mg g^{-1} sucrose (Burbano *et al.*, 1990; Muzquiz, 1999). However, cultivars of *Phaseolus vulgaris* grown in Brazil (Trugo *et al.*, 1990) have observed raffinose (0.5-1.4 mg g^{-1}), stachyose (3.2-4.7 mg g^{-1}) and sucrose (3.0-3.7 mg g^{-1}). These values are lower compared to the beans analyzed in the present study. Jood *et al.*, (1985) also reported that the raffinose and sucrose content of Rajmah (red bean) from India (Hissar) were 0.89 % and 1.58 % respectively. From the comparisons, it can be concluded that the concentration of these oligosaccharides varies depending on the location of growth and the variety used.

Table 1. The α-Galactosides, sucrose and total oligosaccharide compositions of eight common bean varieties (mean ± SD, n=3).

Varieties	Raffinose (mg g^{-1})	Stachyose (mg g^{-1})	α-Galactosides (mg g^{-1})	Sucrose (mg g^{-1})	Total Oligosaccharides (mg g^{-1})[X]
Roba	3.36 ± 0.07[b]	12.38 ± 0.01[e]	15.74 ± 0.04[e]	26.84 ± 0.01[b]	42.58 ± 0.03[c]
Gobirasha	4.43 ± 0.05[a]	14.16 ± 0.04[c]	18.59 ± 0.05[c]	23.44 ± 0.02[e]	42.03 ± 0.04[c]
Beshbesh	2.89 ± 0.01[c]	18.41 ± 0.07[a]	21.30 ± 0.04[a]	17.27 ± 0.01[g]	38.57 ± 0.03[d]
Gofta	4.34 ± 0.00[a]	14.19 ± 0.01[c]	18.53 ± 0.01[c]	24.05 ± 0.03[d]	42.58 ± 0.02[c]
Awash	2.35 ± 0.03[d]	16.67 ± 0.06[b]	19.02 ± 0.05[b]	25.24 ± 0.01[c]	44.26 ± 0.03[b]
Mexican	2.82± 0.03[c]	16.31 ± 0.02[b]	19.13 ± 0.03[b]	28.58 ± 0.07[a]	47.71 ± 0.05[a]
Redwolaita	2.48 ± 0.08[d]	13.03 ± 0.01[d]	15.51± 0.05[d]	28.18 ± 0.01[a]	43.69 ± 0.03[b]
Tabor	2.40 ± 0.00[d]	13.69 ± 0.05[d]	16.09 ± 0.03[d]	20.16 ± 0.08[f]	36.25 ± 0.06[e]

[a-g] *Means with different superscript letters within a column indicate statistically significant differences (P < 0.05).*

[X] *Total oligosaccharides (mg g^{-1}) = a-galactosides (mg g^{-1}) + sucrose (mg g^{-1})*

4.2. Trypsin Inhibitor Activity

There was a significant difference (P < 0.05) in trypsin inhibitor activities between varieties (Table 2). Gobirasha and Beshbesh varieties had higher mean values 27.25 and 29.27 TUI mg^{-1} respectively, while Roba had the lowest (4.59) TUI mg^{-1}. The variations in the activity of trypsin inhibitors ranged from 4.59 to 29.27 TUI mg^{-1} on dry matter basis of *Phaseolus vulgaris.* These are in agreement with the findings of Sosulski *et al.*, (1982) and Barampama and Simard (1993) for common bean varieties grown in different countries. Higher concentrations have been reported for trypsin inhibitors of different varieties and species of legumes (Thorn *et al.*, 1983; Khokhar and Chauhan, 1986; Kantha *et al.*, 1986). A significant (P < 0.01) negative correlation between trypsin inhibitor and protein digestibility is in agreement with the findings of

Hernández-Infante *et al.* (1979) and Furuichi *et al.*, (1988). The negative relation which exists between the trypsin inhibitor and protein digestibility may be implicated in the reduction of nutritive protein quality in common beans.

4.3. Tannins

Tannins concentration ranged from 5.38 (in Roba) to 28.79 mg catechin equivalent g^{-1} (in Beshbesh) and there were significant ($P < 0.05$) differences among the varieties tested. The results revealed that a negative correlation was observed between tannins and the *in-vitro* protein digestibility of common beans (Table 3). Similarly, a highly significant ($P < 0.01$) negative correlation between tannins concentration and protein digestibility has also been reported by Sosulski *et al.* (1982) and Aw and Swanson (1985). Tannins concentration levels 0.3-29.3 mg catechin equivalent g^{-1} were reported by Sathe *et al.* (1983); Aw and Swanson (1985). Deshpande and Cheryan (1983); Reddy *et al.* (1985) have observed lower tannins concentrations (0.34 to 26.50 mg catechin equivalent g^{-1}) for common bean varieties grown in the USA. Barampama and Simard (1993) have also reported on common beans grown in Burundi with a wider range of tannins concentration (0.11 to 28.78 mg g^{-1}). Thus, tannins might contribute to the reduction of nutritional quality of protein in common beans confirmed by the results of this study.

4.3.1. Relation between Tannins and Colour Co-ordinates

Colour measurement was done to correlate the tannins content of bean varieties with their colour value. **L** (whiteness) values obtained for the samples studied ranged from 28.82 to 73.94 among the different common bean varieties (Table 4). The export-type beans such as Mexican and Awash had the highest **L** value 73.94 and 69.34 respectively, and the Redwolaita variety had the lowest **L** value. The colour scale value of **a** (red) ranged from 1.69 to 14.39 in different common bean varieties. The highest **a** value was obtained (14.39) in Redwolaita and lowest (1.69) in Mexican varieties. The highest **b** (yellow) value of 25.39 was obtained in Roba and the lowest of 5.71 in Redwolaita.

There were differences in the surface colour of the eight varieties of beans. The tannins concentration seemed to be influenced by the colour of the common bean seeds. Coloured bean seeds (Table 4), Beshbesh and Gobirasha varieties indeed presented higher tannins concentrations than the other bean seeds studied (Table 2). This observation is in agreement with the findings given by Sotelo and Hernández (1980) for tannins in common bean varieties grown in the USA. Mexican and Awash are exporting type varieties from Ethiopia due to their high white colour quality and reasonable protein digestibility. Roba and Redwolaita are the most popular varieties in farming society in Ethiopia, especially in the central rift valley and southern areas, due to their colour preference, acceptability and food-making qualities. Though white and light creamy beans would be preferred from a protein digestibility point of view, it may not be the only basis for the purchase of such products from and in Ethiopia.

4.4. Phytic Acid

The phytic acid composition of common bean varieties studied is presented in Table 2. The level of phytic acid varied among the eight varieties of common bean from 16.81 to 24.07 mg g^{-1}. The highest phytic acid content is found in Awash and the lowest in Mexican. The ANOVA indicated that phytic acid mean difference was significant at 0.05 levels. Deshpande and Cheryan (1983) have reported that, for common bean varieties grown in the USA there is a concentration of phytic acid ranging from 18.1-27.5 mg g^{-1}. Barampama and Simard (1993) reported that phytic acid varied from 12.37 to 23.60 mg g^{-1}. However, Muzquiz *et al.*, (1999) reported a low value of phytate (3.10-5.01 mg g^{-1}) for common bean varieties grown in different areas of Spain. Phytate reduces the bioavailability of minerals and the solubility, functionality and digestibility of protein and carbohydrate in common beans (Reddy *et al.*, 1982). Therefore, special attention must be given to eliminating or reducing the levels of phytic acid during the preparation of weaning food formulations to assure its high protein quality and mineral bioavailability.

4.4.1. Effect of Phytic Acid on Zn

The results of this study show that bean varieties had a range of zinc content varying from 15.39 mg kg^{-1} to 28.03 mg kg^{-1} (Table 5). The significant ($P < 0.01$) positive correlation for total ash and phytic acid; and negative correlation with ash and zinc is presented in Table 3. The results of this study confirm that phytic acid and zinc have a significant negative correlation. The amount of phytic acid, the type and amount of protein and the total Zn content have a major impact on the amount of Zn absorbed from foods (Lopez *et al.*, 2002). Phytic acid strongly binds Zn in the gastrointestinal tract and reduces its availability for absorption and re-absorption (Flanagan 1984).

Zinc concentrations in the eight varieties varied from 15.39 mg kg^{-1} to 28.22 mg kg^{-1} (Table 5). These values are comparable to concentrations reported by Meiners *et al.* (1976), Rockland *et al.* (1979) and Augustin *et al.* (1981). Zinc is an essential trace micronutrient involved in the immune function, in the activation of many enzymes, normal healthy growth and reproduction Umeta *et al.* (2000). Therefore, zinco-protein supplementation of formulated bean-based foods can reduce protein-energy malnutrition (PEM) disease which is common in countries like Ethiopia.

Table 2. Phytochemical composition and *in vitro* protein digestibility of the bean varieties (mean ± SD, n=3).

Varieties	Phytic acid (mg g^{-1})	Saponins (10^{-2}g g^{-1})	Trypsin inhibitors (TUI[1] mg^{-1})	Lectins (g kg^{-1} PHA)[2]	Tannins (mg g^{-1})	*In vitro* protein digestibility (%)
Roba	23.51 ± 0.12^{b}	0.96 ± 0.02^{d}	4.59 ± 0.02^{h}	1.92 ± 0.01^{e}	5.38 ± 0.01^{g}	80.66 ± 0.03^{a}
Gobirasha	22.94 ± 0.09^{c}	0.75 ± 0.04^{e}	27.25 ± 0.07^{b}	6.43 ± 0.07^{c}	23.55 ± 0.01^{b}	68.87 ± 0.07^{g}
Beshbesh	17.34 ± 0.10^{g}	1.32 ± 0.08^{a}	29.27 ± 0.09^{a}	9.98 ± 0.02^{a}	28.79 ± 0.14^{a}	65.64 ± 0.04^{h}
Gofta	20.09 ± 0.07^{e}	1.05 ± 0.03^{c}	24.09 ± 0.06^{c}	7.77 ± 0.04^{b}	19.69 ± 0.01^{c}	69.36 ± 0.01^{f}
Awash	24.07 ± 0.09^{a}	1.18 ± 0.01^{b}	20.89 ± 0.05^{e}	4.52 ± 0.03^{d}	17.56 ± 0.08^{d}	71.15 ± 0.02^{e}
Mexican	16.81 ± 0.02^{h}	1.16 ± 0.00^{b}	21.44 ± 0.08^{d}	4.49 ± 0.06^{d}	17.69 ± 0.06^{d}	72.33 ± 0.05^{d}
Redwolaita	18.27 ± 0.05^{f}	0.72 ± 0.03^{e}	17.97 ± 0.04^{g}	1.02 ± 0.09^{f}	11.15 ± 0.01^{f}	77.44 ± 0.01^{b}
Tabor	21.27 ± 0.01^{d}	0.94 ± 0.04^{d}	19.94 ± 0.01^{f}	1.90 ± 0.08^{e}	15.68 ± 0.09^{e}	73.57 ± 0.02^{c}

All values are means of three replicate analyses and expressed in dry weight basis
[1]Trypsin units inhibited; [2]Lectin as PHA (Pvulgaris lectin)
[a-b]Means with different superscript letters within a column indicate statistically significant differences (P < 0.05)

Table 3. Correlation coefficients among the bean varieties presented in matrix form.

	Protein digestibility	Trypsin inhibitors	Tannins	Phytic acid	Sucrose	α- Galactosides	Raffinose	Stachyose	Lectins	Saponins	Ash	Zinc
Protein digestibility	1											
Trypsin inhibitors	-0.94**	1										
Tannins	-0.98**	0.95**	1									
Phytic acid	-	-	-	1								
Sucrose	0.64*	-0.68**	-0.67**	-	1							
α- Galactosides	-0.89**	0.95**	-	-	-0.46*	1						
Raffinose	-	-	-	-	-	-	1					
Stachyose	-0.76**	0.81**	0.76**	-	-0.44*	0.92**	-	1				
Lectins	-0.89**	0.75**	0.88**	-	-0.76**	-	-	0.86**	1			
Saponins	-0.49*	0.76**	0.44*	-	-0.74**	-	-	-	0.57*	1		
Ash	-	-	-	0.97**	-	-	-	-	-	-	1	
Zinc	-	-	-	-0.49*	-	-	-	-	-	-	0.41*	1

***Highly significant (0.01< P < 0.001) and *Significant (0.01 < P < 0.05)*

4.5. Saponins and Lectins Concentrations

Concentrations of saponins and lectins of released varieties of common beans studied are presented in Table 2. For all varieties, significant differences ($P < 0.05$) existed in the saponins and lectin contents. Concentrations varied from 0.72 (in Redwolaita) to 1.32 g 100 g^{-1} (in Beshbesh), from 1.02 (in Redwolaita) to 9.98 g kg^{-1} (in Beshbesh) for saponins and lectins respectively. Concentrations of saponins were reported to be lower (0.44 to 2.05 g kg^{-1}) for common beans grown in Spain (Burbano *et al*., 1990) compared to this study. According to the results obtained, the lectins content of common bean varieties were significantly ($P < 0.05$) different. The content of lectin ranged from 1.02 g kg^{-1} (Redwolaita) to 9.98 g kg^{-1} (Beshbesh). These values are in agreement with the report of Burbano *et al*., (1990) for common beans varieties grown in Spain, and Barampama and Simard (1993) for common beans grown in Burundi. Concentrations of saponins varied from 0.72 to 1.32 g $100g^{-1}$. The Redwolaita variety had the lowest concentrations of saponins while Beshbesh had the highest concentration of saponins and lectins. Protein digestibility was affected by the composition of saponnins and lectins of common beans. It was pointed out earlier that digestibility was negatively influenced by lectins and saponins. This study indicated that saponins and lectins had significant positive correlations (Table 3).

4.5.1. Role of Saponins and Lectins Concentrations Towards Disease Resistance

The results of this study reveal that, among the varieties studied, large quantities of phytochemical composition especially lectins and saponins were obtained in the Beshbesh variety. Many roles have been attributed to lectins, and it has been suggested that they play a protective role against insect, fungal and pathogenic bacterial attacks in the field and under storage conditions ((Janzen *et al*., 1976; Mirelman *et al*., 1975; Sequeira, 1978, Shimelis, 2005). Gatehouse *et al*., (1984) reported that lectins purified from *Phaseolus vulgaris* seeds were shown to be toxic to the development larvae of the bruchid beetle *Callosobruchus maculates,* a major storage pest of many legumes. Thus, the presence of lectins is of considerable importance in preventing *C. maculates* from attacking the seeds. Beshbesh was released by the crop protection program of Nazareth Agricultural Research Centre for its resistance to disease (EARO, 2001). Beshbesh is highly resistant to bean stem maggots (BSM) (*Ophiomyia spencerella*) which represent a principal insect pest of beans in southern Ethiopia and other East African countries like Uganda, Kenya, Tanzania and Zimbabwe (EARO, 2001).

Analogous suggestions were further corroborated by the reports of Gatehouse and Boulter (1983) and Gatehouse *et al*.,(1992), which confirmed that resistance to pests can lead to an increment in phytochemicals composition in the seed of common beans. A report by Oluwatosin (1999) also supports that increase in resistance to pests demonstrated in an enlargement in phytochemical concentration for cowpea varieties. Consequently, the presence of a high concentration of phytochemicals (lectins and saponins) in the Beshbesh variety verifies a correlation with its resistance to disease. On the other hand, Redwolaita has lower concentrations of lectins (1.02 g kg^{-1} PHA), saponins (0.72 g 100 g^{-1}) and immense quantities of sucrose concentration (28.18 mg g^{-1}). Accordingly, this shows that Redwolaita might be susceptible to many field and storage pest diseases.

Table 4. Colour analysis of common bean seeds.

Varieties	Seed Color[1]		
	L	a	b
Roba	58.54 ± 0.10^{d}	6.49 ± 0.08^{e}	$25.39 \pm 0.13^{a*}$
Gobirasha	30.30 ± 0.19^{g}	12.57 ± 0.11^{b}	5.87 ± 0.24^{f}
Beshbesh	61.67 ± 0.25^{c}	7.51 ± 0.12^{d}	17.77 ± 0.20^{c}
Gofta	55.73 ± 0.39^{e}	8.19 ± 0.11^{c}	22.50 ± 0.19^{b}
Awash	69.34 ± 0.55^{b}	2.18 ± 0.06f	13.31 ± 0.19^{d}
Mexican	$73.94 \pm 0.20^{a*}$	1.69 ± 0.06g	11.08 ± 0.35^{e}
Redwolaita	28.82 ± 0.64^{f}	$14.39 \pm 0.09^{a*}$	5.71 ± 0.62^{f}
Tabor	57.48 ± 0.29^{d}	8.65 ± 0.15^{c}	18.41 ± 0.28^{c}

[1]*L (lightness), a (chroma) and b (hue)*

[a*-g] *Means with different superscript letters within a column indicate statistically significant differences ($P < 0.05$)*

All values are means of triplicates ± standard deviation

Table 5. Zinc composition and ash contents of common bean varieties expressed according to dry weight basis.

Varieties	Zn (mg kg^{-1})	Ash ($10^{-2}g\ g^{-1}$)
Roba	15.99 ± 0.07^{e}	3.93 ± 0.01^{b}
Gobirasha	23.91 ± 0.16^{c}	3.84 ± 0.02^{b}
Beshbesh	28.03 ± 0.22^{a}	3.12 ± 0.01^{e}
Gofta	27.60 ± 0.13^{b}	3.36 ± 0.00^{d}
Awash	17.21 ± 0.23^{d}	4.26 ± 0.16^{a}
Mexican	17.91 ± 0.16^{d}	2.86 ± 0.04^{f}
Redwolaita	28.22 ± 0.00^{a}	3.27 ± 0.09^{d}
Tabor	15.39 ± 0.01^{e}	3.59 ± 0.03^{c}

All values are means of three replicates ± standard deviation
[a-f] Means with different superscript letters within a column indicate statistically significant differences (P < 0.05)

4.6. *In-vitro* Protein Digestibility

In vitro-protein digestibility of eight common bean varieties studied is presented in Table 2. An analysis of protein digestibility in the present study revealed a significant difference among the eight bean varieties at the 0.05 level. Significant ($P < 0.01$) negative correlation between phytochemicals (lectins, tannins and trypsin inhibitors) and α-galactosides along with *in-vitro* protein digestibility was investigated. Additionally, significant ($P < 0.05$) negative correlation between saponins and protein digestibility were examined. Protein digestibility had a significant ($P < 0.05$) positive correlation with the sucrose content of common beans (Table 3). The range of *in-vitro* protein digestibility of common beans varied from 65.64% (in Beshbesh) to 80.66% (in Roba). The protein digestibility values obtained are comparable to those reported (66.9 - 70.9%) by Deshpande *et al*., (1984) for common bean varieties grown in the USA; Barampama and Simard (1993) for common bean varieties grown in Burundi; Vadivel and Janardhanan (2000) for velvet bean varieties grown in South India. Pusztai *et al*., (1979) and Sathe *et al*., (1984). Lower values for protein digestibility (36.3-56.0%) have been also reported by Salunkhe and Kadam (1989) for different common bean varieties. The protein digestibility of common bean varieties studied by different investigators concluded that it is influenced by genotype environmental factors and varietals interaction.

Factors influencing the nutritional quality of common bean proteins include the amino acid pattern and degree of digestibility, as well as the quantity and quality of the other food proteins consumed along with the common bean proteins (Bressani and Elias 1980). The higher protein digestibility of Roba (80.66%) and the lowest TUI mg^{-1} concentrations among the varieties studied supports the popularity and acceptability of this variety by the consumers among the dozen released varieties of common bean from research centres in Ethiopia (Shimelis and Rakshit, 2005). Beshbesh had the highest TIA and the lowest protein digestibility and thus makes it less acceptable to the consumers in the central rift valley of Ethiopia. It is interesting to note that there is a market for the varieties with a higher trypsin inhibitor in a research-focussed role in cancer treatment. This has been the case in Ethiopia where the variety Beshbesh with its higher trypsin inhibitor can be imported by many buyers for this purpose. However, from a nutritional point of view a lower trypsin inhibitor level which increases protein digestibility is desirable.

The release of more varieties with higher digestibility and protein content, which require less cooking time and have other physico-chemical properties, is vital as a means of contributing to the reduction of malnutrition-related problems in the country as a whole. The design and development of bean-based food products from the varieties that contain higher protein quality can be carried out to increase new types of value-added products that are affordable for most of the consumers/farmers.

5. Conclusions

The variability in the concentrations of α-galactosides, phytochemicals, sucrose and *in-vitro* protein digestibility of eight common bean varieties is evident. Genetic variability was the predominant factor in the observed variability. In the study, correlation was observed among *in-vitro* protein digestibility, phytochemicals, α-galactosides, sucrose, zinc and ash compositions for the common bean varieties analyzed. An increase in phytochemicals composition, especially in saponins and lectins concentrations, can lead to an increase in disease resistance. Hence, analyses of phytochemicals and the sucrose composition of common beans can be used as a identifying factor of the pest resistance of the cultivars.

This study could help breeders to select common bean varieties with reduced levels of phytochemiclas for human consumption through large-scale cultivation. Roba was found to be the best variety in terms of higher *in-vitr*o protein digestibility, lower level of flatulence-causing factors, tannins, lectins, saponins and trypsin inhibitors. Thus, the Roba bean variety could be used as a raw material for the manufacturing of bean-based value added products at industry level through large scale cultivation which in turn could help the bean farmers of Ethiopia to increase earning in the market. Bean varieties such as Gofta, Goberasha and Tabor could also be used as a raw material in the food/feed processing industries after the reduction of unwanted components through the use of appropriate processing technology. Similarly, in

developing countries like Africa which utilize common beans to an immense extent, the release of potential varieties which have low phytochemical composition from research centres must be encouraged to support the nutritional requirements through the design and development of bean-based value added food products processed in agro-processing industries. Further investigations on product design and development, end-users preference and product diversification is required. Finally, attention must also be paid to the selection of genotypes (new cultivars) that meet consumer criteria in terms of dense nutrient content, preferred colour, required grain size, higher digestibility with fewer flatulence factors and low phytochemicals composition.

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