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

Shimelis Admassu

Food Engineering Program, Chemical Engineering Department, Addis Ababa University, P O Box 33381, Ethiopia  E-mail: shimelisemire@yahoo.com

**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 TUx10⁻³ 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 Demelah, 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 (Lien, 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 (Ca²⁺, Mg²⁺) and trace elements such as Zn²⁺, Fe²⁺, Cu²⁺ and Mn²⁺ (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
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. α-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 °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\(_3\)CN/ H\(_2\)O 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 spectrophotometer (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), a-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 vitro 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-Galactosidases and Sucrose

The a-galactosides and sucrose contents of eight common bean varieties studied are presented in Table 1. Stachyose was the major a-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 awash). Stachyose concentrations from 12.38 mg g\(^{-1}\) (in Roba) to 18.41 mg g\(^{-1}\) (in Gobirasha). 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 a-Galactosides, sucrose and total oligosaccharide compositions of eight common bean varieties (mean ± SD, n=3).

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

\(\text{a}\)Means with different superscript letters within a column indicate statistically significant differences (\(P < 0.05\)).

\(\text{Total oligosaccharides (mg g}\(^{-1}\)) = \text{a-galactosides (mg g}\(^{-1}\)) + \text{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
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 \textit{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 \textit{et al.} (1982) and Aw and Swanson (1985). Tannins concentration levels 0.3-29.3 mg catechin equivalent g\(^{-1}\) were reported by Sathe \textit{et al.} (1983); Aw and Swanson (1985). Deshpande and Cheryan (1983); Reddy \textit{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 Coordinates
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 \textit{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 \textit{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 \textit{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 \textit{et al.} (1976), Rockland \textit{et al.} (1979) and Augustin \textit{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 \textit{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 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\(^{b}\) | 1.92 ± 0.01\(^{c}\) | 5.38 ± 0.01\(^{c}\) | 80.66 ± 0.03\(^{a}\) |
| Gobirasha | 22.94 ± 0.09\(^{c}\) | 0.75 ± 0.04\(^{c}\) | 27.25 ± 0.07\(^{b}\) | 6.43 ± 0.07\(^{c}\) | 23.55 ± 0.01\(^{b}\) | 68.87 ± 0.07\(^{c}\) |
| Beshbesh  | 17.34 ± 0.10\(^{c}\) | 1.32 ± 0.08\(^{a}\) | 29.27 ± 0.09\(^{a}\) | 9.98 ± 0.02\(^{a}\) | 28.79 ± 0.14\(^{b}\) | 65.64 ± 0.04\(^{b}\) |
| Gofta     | 20.09 ± 0.07\(^{c}\) | 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\(^{c}\) | 4.52 ± 0.03\(^{d}\) | 17.56 ± 0.08\(^{d}\) | 71.15 ± 0.02\(^{c}\) |
| Mexican   | 16.81 ± 0.02\(^{b}\) | 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\(^{c}\) | 17.97 ± 0.04\(^{e}\) | 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 (P vulgaris lectin)

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           | 0.64*               | -0.68** | -0.67**    | -1      |                |           |           |         |          |     |      |
| Sucrose               | -0.89**             | 0.95**  |            | -0.46*  | 1              |           |           |         |          |     |      |
| α- Galactosides       | 0.76**              | 0.81**  | 0.76**     | -0.44*  | 0.92**         | -         | 1         | 0.86**  | 1        |     |      |
| Raffinose             | -0.76**             | 0.81**  | 0.76**     | -0.44*  | 0.92**         | -         | 1         | 0.86**  | 1        |     |      |
| Stachyose             | -0.89**             | 0.75**  | 0.88**     | -0.76** | -              | -         | 0.57*     | 1       |          |     |      |
| Lectins               | -0.49*              | 0.76**  | 0.44*      | -0.74** | -              | -         | -         | -       |          |     |      |
| Saponins              | 0.97**              | -       | -0.49*     | -       | -              | -         | -         | -       | 0.41*    | 1   |      |
| Ash                   | 0.97**              | -       | -0.49*     | -       | -              | -         | -         | -       | 0.41*    | 1   |      |
| Zinc                  | 0.97**              | -       | -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 saponins 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 maculatus, a major storage pest of many legumes. Thus, the presence of lectins is of considerable importance in preventing C. maculatus 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) (Olipionia spenserellia) 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.

| Varieties | L, a | Seed Color¹ |
|-----------|------|-------------|
| Roba      | 58.54 ± 0.10⁹ | 6.49 ± 0.08⁹ | 25.39 ± 0.13⁹ |
| Gobirasha | 30.30 ± 0.19⁸ | 12.57 ± 0.11⁹ | 5.87 ± 0.24⁹ |
| Beshbesh  | 61.67 ± 0.25⁹ | 7.51 ± 0.12⁹ | 17.77 ± 0.20⁹ |
| Gofta     | 55.73 ± 0.39⁸ | 8.19 ± 0.11⁹ | 22.50 ± 0.19⁹ |
| Awash     | 69.34 ± 0.55⁸ | 2.18 ± 0.06⁹ | 13.31 ± 0.19⁹ |
| Mexican   | 73.94 ± 0.20⁹ | 1.69 ± 0.06⁹ | 11.08 ± 0.35⁹ |
| Redwolaita| 28.82 ± 0.64⁹ | 14.39 ± 0.09⁹ | 5.71 ± 0.62⁹ |
| Tabor     | 57.48 ± 0.29⁸ | 8.65 ± 0.15⁹ | 18.41 ± 0.28⁹ |

¹L, (lightness), a (chroma) and b (hue)
²Means with different superscript letters within a column indicate statistically significant differences (P < 0.05)
All values are means of triplicates ± standard deviation
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 phytochemicals for human consumption through large-scale cultivation. Roba was found to be the best variety in terms of higher in-vitro 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, Gobirasha 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.

6. Acknowledgments
The author appreciates the unreserved assistance of Dr. Gulilate Haake while carrying out the experiments at the Bioprocess Technology laboratory of Asian Institute of Technology, Bangkok, Thailand. The author also wish to thank Dr. Aberra Deressa, Alemtsehay Assefa, Bussarins Kosin and Bernice B. Polohan for their valuable support and encouragement during the experimentation and manuscript preparation.

7. References
Agbo, N.G. 1982. Genetic, physico-chemical and structural parameters affecting texture of dry edible beans. Ph.D Thesis. Michigan State University, USA.
AOAC. (Association of Official Analytical Chemists). 2000. Official methods of analysis of the association of official analytical chemists: Food composition; additives; natural contaminants. William, H. (eds.). Volume II, 17th edition. Washington, D.C. Official method 982.14.
Augustin, J. Beck, C.B. Kalbfleish, G. Kagel, L.C. and Matthews, R.H. 1981. Variation in the vitamin and mineral content of raw and cooked commercial Phaseolus vulgaris classes. Journal of Food Science 46:1701-1706.
Aw, T.L. and Swanson, B.G. 1985. Influence of tannins on Phaseolus vulgaris protein digestibility and quality. Journal of Food Science 50: 67-71.
Barampama, Z. and Simard, E.R. 1993. Nutrient composition, protein quality and anti nutritional factors of some varieties of haricot beans (Phaseolus vulgaris L.) grown in Burundi. Food Chemistry 47: 159-167.
Birk, Y. 1989. Protein protease inhibitors of plant origin and their significance in nutrition. In: Huisman, J., Van der Poel, TFB, Liener, I.E. (eds). Recent advances of research in anti nutritional factors in legume seeds. Proceedings of the first international workshop of antinutritional factors (ANF’s) in legume seeds. Pudoc, Wageningen. pp.83-94.
Bressani, R. and Elias L.G. 1980. Nutritional value of legume crops for humans and animals. In: Summerfield, R.J., Bunting, A.H.(eds). Advances in legume science. Kew, Richmond, London: Royal Botanical Gardens. pp. 135-155.
Bressani, R. and Sosa, J.L. 1990. Effect of processing on the nutritive value of canavalina Jack bean (Canavalia ensiformis, L.). Plant Food and Human Nutrition 40:207-214.
Bressani, R. 1993. Grain quality of common beans. Food Reviews International 9: 217-297.
Burbano, C., Muzquiz, M., Ayet, G., Cuadrado, C. and Pedrosa, M. 1999. Evaluation of antinutritional factors of selected varieties of Phaseolus vulgaris. Journal of the Science of Food and Agriculture 79: 1468-1472.
Dawit, A. and Demelash, S. 2003. Haricot bean marketing and export performance, constraints and opportunities. Research report no.54. Ethiopian Agricultural Research organization.pp.18.
Deshpande, S.S., Sathe, S.K. and Salunkhe, D.K.1984. Haricot beans of Phaseolus: A review. Part 3. Critical Reviews in Food Science and Nutrition 21:137-195.
Deshpande, S.S. and Cheryan, M. 1983. Changes in phytic acid, tannins and trypsin inhibitory activity on soaking of haricot bean (Phaseolus vulgaris). Nutrition Report International 27: 371-377.
Doyon, G., Gaudreau, G., St-Gelais, D., Beaulieu, Y. and Randall, J.C.1991. Simultaneous HPLC Determination of Organic Acids, Sugars and Alcohols. Canadian Institute of Science and Technology Journal 24: 87-94.
Duhan, A., Khetarpaul, N. and Bishnoi, S. 2001. Saponin content and trypsin inhibitor activity in processed and cooked pigeon pea cultivars. International Journal of Food Sciences and Nutrition 52: 53-59.
EARO (Ethiopian Agricultural Research Organization). 2001. Crop protection research program strategy. Addis Ababa, Ethiopia.
EARO (Ethiopian Agricultural Research Organization). 2002. Food science and post harvest technology research program strategy. Addis Ababa, Ethiopia.
Flanagan, P.R. 1984. A model to produce pure zinc deficiency in rats and its use to demonstrate that dietary phytate increases the excretion of endogenous zinc. Journal of Nutrition 114: 493-502.
Frias, J., Vidal-Valverde, C., Sotomayor, C., Diaz-Pollan, C. and Urbana, G. 2000. Influence of processing on available carbohydrate content and anti nutritional factors of chickpeas. European Food Research Technology 210: 340-345.
Furuichi, Y., Sawada, M. and Takahashi, T. 1988. Anti nutritional factors in tora-mame seeds on the Japanese cultivars of Phaseolus vulgaris. Nutrition Report International 37:713-722.
Gatehouse, A.M.R. and Boulter, D.1983. Assessment of the anti metabolic effects of trypsin inhibitors from cowpea (Vigna unguiculata) and other legumes on the development of the bruchid beetle. Callosobruchus maculates. Journal of the Science of Food and Agriculture 34: 345-350.
Gatehouse, A.M.R., Dewey, F.M. and Dove, J. 1984. Effects of seed lectins from Phaseolus vulgaris on the
development of larvae of Callosobruchus maculatus; Mechanism of toxicity. *Journal of the Science of Food and Agriculture* 35: 373-380.

Gatehouse, A.M.R., Hilder, V. and Boultier, D. 1992. Potential of plant derived genes in the genetic manipulation of crops for insect resistance. *In: Plant genetic manipulation for crop protection, Gatehouse, AMR, Hilder, VA. and Boultier, D. (eds.). CAB International, Wallingford, UK.* pp.155-181.

Haug, W. and Lantzsch, H.J. 1983. A sensitive method for the rapid determination of phytate in cereals and cereal products. *Journal of the Science of Food and Agriculture* 34: 1423-1426.

Hernández-Infante, M., Sousa, V., Montalvo, I., and Tena, E. 1998. Impact of microwave heating on hemagglutinins, trypsin inhibitors and protein quality of selected legume seeds. *Plant Foods for Human Nutrition* 52:199-208.

Hiai, S., Oura, H. and Nakajima, T. 1976. Color reaction of some sapogenins and saponins with vanillin sulfuric acid. *Planta Medica* 29: 116-122.

Hsu, H.W., Vavak, D.L., Satterlee, L.D. and Miller, G.A. 1977. A multienzyme technique for estimating protein digestibility. *Journal of Food Science* 42:1269-1273.

Issac, R.A. and Johnson, W.C. 1975. Collaborative study of wet and dry ashing techniques for elemental analysis of plant tissue by atomic absorption spectrophotometer. *Journal of Association of Analytical Chemists* 58: 436-440.

Janzen, D.H., Justler, H.B. and Liener, I.E. 1976. Insecticidal action of the phytohaemagglutinin in black beans on a bruchid beetle. *Science* 192: 795-796.

Johnson, I.T., Gee, J.M., Price, K.R., Curl, C.L. and Fenwick, G.R. 1986. Influence of saponins on gut permeability and active nutrient transport *in vivo.* *Journal of Nutrition* 116:2270-2277.

Jood, S., Mehta, U., Singh, R. and Bhat, C.M. 1985. Effect of processing on flatus-producing factors in legumes. *Journal of Agricultural and Food Chemistry* 33: 268-271.

Kantha, S.S., Hettiarachchy, N.S. and Erdman, J.W. 1986. Nutrient, anti nutrient contents, and solubility profiles of nitrogen, phytic acid, and selected minerals in winged bean flour. *Cereal Chemistry* 63:9-13.

Khokhar, S. and Chauhan, B.M. 1986. Anti nutritional factors in moth bean (*Vigna aconitifolia*); varietal differences and effects of methods of domestic processing and cooking. *Journal of Food Science* 51:591-594.

Laurena, A.C., Revilleza, M.J.R. and Mendoza, E.M.T. 1994. Polyphenols, phytate, cyanogenic glycosides, and trypsin inhibitor activity of several Philippine indigenous food legumes. *Journal of Food Composition and Analysis* 7:194-202.

Liener, I.E. 1983. Toxic constituents in legumes. *In: Chemistry and Biochemistry of Legumes.* Arora, S.K. (eds.). Oxford and IBH, New Delhi. pp.217-257.

Liener, I.E. 1989. Anti nutritional factors. *In: legumes. Chemistry, technology and human nutrition.* Matthews, R.H. (eds). Marcel Dekker, NewYork, pp.339-382.

Lopez, H.W., Leenhardt, F., Coudray, C. and Remesy, C. 2002. Mineral and phytic acid interaction: is it a real problem for human nutrition? *International Journal of Food Science and Technology* 37: 727-729.

Maga, J. 1982. A review. Phytate: Its chemistry, occurrence, food interactions, nutritional significance, and methods of analysis. *Journal of Agricultural and Food Chemistry* 30: 1-9.

Makkar, H.F.S., Aderibige, A.O. and Becker, K. 1998. Comparative evaluation of nontoxic and toxic varieties of *Jatropha curcas* for chemical composition, digestibility, protein degradability and toxic factors. *Food Chemistry* 62:207-215.

Meiners, C.R., Derise, N.L., Lau, H.C., Crews, M.G. and Ritchey, S.J. 1976. The contents of nine mineral elements in raw and cooked mature dry legumes. *Journal of Agricultural and Food Chemistry* 24: 1126-1130.

Mirelman, D., Galune, S N. and Lotan, R. 1975. Inhibition of fungal growth by wheat germ agglutinin. *Nature (Land)* 256:414-416.

Muzquiz, M., Burbano, C., Ayet, G. and Pedrosa, M.M. 1999. The investigation of anti nutritional factors in *Phasolus vulgaris.* Environmental and varietal differences. *Biotechnology of Agronomy and Social Environments* 4: 210-216.

Norton, G., Bliss, F.A. and Bressani, R. 1985. Biochemical and nutritional attributes of grain legumes. *In: Grain legume crops. Summerfield, R.J., Roberts, E.H. (eds). London: Collins.* pp.73-114.

Oluwatosin, O.B. 1999. Genotype x environment influence on cowpea (*Vigna unguiculata* (L) Walp) antinutritional factors: 1-Trypsin inhibitors, tannins, phytic acid and haemagglutinin. *Journal of the Science of Food and Agriculture* 79: 265-272.

Pusztai, A., Grant, G. and Palmer, R. 1975. Nutritional evaluation of kidney beans (*Phasolus vulgaris*): the isolation and partial characterization of toxic constituents. *Journal of the Science of Food and Agriculture* 26: 149-156.

Pusztai, A., Clarke, E.M.W., King, T.P. and Stewart, J.C. 1979. Nutritional evaluation of kidney beans (*Phasolus vulgaris*): Chemical composition, lectin content and nutritional value of selected cultivars. *Journal of the Science of Food and Agriculture* 30:843-848.

Rao, P.U. and Belavady, B. 1978. Oligosaccharides in pulses: varietal differences and effects of cooking and germination. *Journal of Agricultural and Food Chemistry* 26: 316-322.

Reddy, N.R., Pierson, M.D., Sathe, S.K. and Salunkhe, D.K. 1984. Chemical, nutritional and physiological aspects of haricot bean carbohydrates. A review. *Food Chemistry* 13: 25-68.

Reddy, N.R., Pierson, M.D., Sathe, S.K. and Salunkhe, D.K. 1985. Haricot bean tannins: a review of nutritional implications. *Journal of the American Oil Chemists' Society* 62:541-549.
Shimelis, A.

Reddy, N.R., Sathe, S.K. and Salunkhe, D.K. 1982. Phytate in legumes and cereals. *Advances in Food Research* 28: 1-92.

Rockland, L.B., Wolf, W.R., Hahn, D.M. and Young, R. 1979. Estimation of zinc and copper in raw and cooked legumes: An inter-laboratory study of atomic absorption and X-ray fluorescence spectroscopy. *Journal of Food Science* 44:1711-1713, 1719.

Salunkhe, D.K. 1982. Legumes in human nutrition. *Current Science* 51:387-394.

Salunkhe, D.K. and Kadam, S.S. 1989. CRC hand book of world food legumes: Nutritional chemistry, processing technology, and utilization. In: Legumes in human nutrition: future prospects. Salunkhe, D.K.(eds.). Volume III. CRC press, Inc. Boca Raton, Florida. pp.311-314.

Sathe, S.K., Deshpande, S.S., Reddy, N.R., Goll, D.E. and Salunkhe, D.K. 1983. Effects of germination on proteins, raffinose oligosaccharides and anti nutritional factors in the great northern beans (*Phaseolus vulgaris* L.). *Journal of Food Science* 48:1796-1800.

Sathe, S.K., Deshpande, S.S. and Salunkhe, D.K. 1984. Haricot beans of *Phaseolus*: A review. Part 1. Chemical composition: Proteins. *Critical Reviews in Food Science and Nutrition* 20: 1- 40.

Satterlee, L.D., Bembers, M. and Kendrick, J.G. 1975. Functional properties of the great northern bean (*Phaseolus vulgaris*) protein isolate. *Journal of Food Science* 40: 81-87.

Sequeira, L. 1978. Lectins and their role in host-pathogen specificity. *Annual Review of Phytopathology* 16: 453-481.

Shimelis, A.E. and Rakshit, S.K. 2005. Proximate composition and physico-chemical properties of improved dry bean (*Phaseolus vulgaris* L.) varieties grown in Ethiopia. *Journal of Food Science and Technology (LWT)* 38:331-338.

Shimelis, A.E. 2005. Influence of processing on anti nutrients, raffinose family oligosaccharides and in vitro protein digestibility of improved dry bean (*Phaseolus vulgaris* L.) varieties grown in Ethiopia. A Dissertation submitted in partial fulfilment of the requirements for the degree of Doctor of Engineering. Asian Institute of Technology. Bangkok, Thailand.

Singh, M. and Krikorian, A.P. 1982. Inhibition of trypsin activity in vitro by phytate. *Journal of Agricultural and Food Chemistry* 30:799-802.

Smith, C., Megen, W.V., Twalfhoven, L. and Hitchcock, C. 1980. The determination of trypsin inhibitor levels in foodstuffs. *Journal of the Science of Food and Agriculture* 31:341-350.

Sosulski, F.W., Elkowitz, L. and Reichert, R.D. 1982. Oligosaccharides in eleven legumes and their air-classified protein and starch fractions. *Journal of Food Science* 47:498-502.

Sotelo, A. and Hernandez, M. 1980. Nutritional evaluation of three varieties of beans (*Phaseolus vulgaris*) using chemical and biological methods. *Nutrition Report International* 22: 607-616.

Thorn, K.A., Tinsley, A.M., Weber, C.W. and Berry, J.W. 1983. Anti nutritional factors in legumes of the sonoran desert. *Ecological Food and Nutrition* 13: 251-256.

Trugo, L.C., Ramos, L.A., Trugo, N.M.F. and Souza, M.P. 1990. Oligosaccharide composition and trypsin inhibitor activity of *P. vulgaris* and the effect of germination on the α-galactoside composition and fermentation in the human colon. *Food Chemistry* 36:53-61.

Umeta, M., West, C.E., and Haudar, J. 2000. Zinc supplementation and stunted infants in Ethiopia a randomized controlled trial, *Lancet* 355:2021-2026.

Vadivel, V. and Janardhanan, K. 2000. Nutritional and anti-nutritional composition of velvet bean: an under-utilized food legume in South India. *International Journal of Food Sciences and Nutrition* 51: 279-287.
