Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 103 (2007) 161–172
Food Chemistry
www.elsevier.com/locate/foodchem

Effect of processing on antinutrients and *in vitro* protein digestibility of kidney bean (*Phaseolus vulgaris* L.) varieties grown in East Africa

Emire Admassu Shimelis, Sudip Kumar Rakshit *

Food Engineering and Bioprocess Technology Program, Asian Institute of Technology, P.O. Box 4 Klong Luang, Pathumthani 12120, Bangkok, Thailand

Received 25 October 2005; received in revised form 3 August 2006; accepted 4 August 2006

Abstract

The effects of hydration, autoclaving, germination, cooking and their combinations, on the reduction/elimination of antinutrients, flatus-producing compounds and the improvement of *in vitro* protein digestibility of three selected *Phaseolus vulgaris* varieties were investigated. Reduction in the amount of total α-galactosides was attained by employing hydration process and was due to the differential solubility of the individual oligosaccharides and their diffusion rates. Due to their heat-sensitive nature, saponins, trypsin inhibitors and phytohaemagglutinins, diminished drastically to undetectable amounts when heating processes (cooking and autoclaving) were employed. Hydration and germination processes were less effective in reducing trypsin inhibitors, saponins and phytohaemagglutinins as compared with cooking/autoclaving processes. Germination process reduced stachyose, raffinose, phytic acid and tannins which was due to metabolic activity. The combination of germination followed by autoclaving processes yielded the most promising result in this study. The bean variety Roba exhibited better protein digestibility on processing and thus has high potential to be used as a raw material for the manufacturing of value-added products.

Keywords: Kidney bean; Processing methods; Antinutrtients; Protein digestibility; East Africa; *Phaseolus vulgaris* L.

1. Introduction

Kidney bean (*Phaseolus vulgaris* L.) is the most widely produced and consumed food legume in Africa, India, Latin America and Mexico (FAO, 1993). These beans have numerous health benefits, e.g. heart and renal disease risks (Anderson, Smith, & Washnock, 1999); lower glycemic index for persons with diabetes (Viswanathan et al., 1989); increased satiation (Leathwood & Pollet, 1988); and cancer prevention (Hangen & Bennink, 2002). Furthermore, kidney beans are regarded as an important source of protein and minerals for livestock feed production, as well as, potential raw materials for processing into human food (Salunkhe, 1982; Gupta, 1987). However, kidney beans have low protein digestibility, attributed to the presence of antinutrients, some of which also diminish the bioavailability of trace elements and proteins. The antinutrients (e.g. trypsin inhibitors, phytic acid, saponins, phytohaemagglutinins and tannins) and α-galactosides (e.g. raffinose, stachyose and verbascose) are some of the undesirable components in beans that could limit their protein and carbohydrate utilization.

Inactivation and/or removal of undesirable components are very essential in improving the nutritional quality and organoleptic acceptability of beans and in turn help to effectively utilize their potential as human food and animal feed. Different processing methods such as boiling (Jood, Mehta, Singh, & Bhat, 1985), hydration and germination (Matella et al., 2005) have been used to increase the utilization of kidney beans. Germination has often been proposed as a means by which the nutritional quality of bean seeds might be improved. This improvement is usually a result of break down of complex macromolecules such as starch

* Corresponding author. Tel.: +66 2 524 6215; fax: +66 2 524 6200.
E-mail address: rakshit@ait.ac.th (S.K. Rakshit).

0308-8146/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2006.08.005

and proteins into smaller and more digestible molecules, while at the same time lowering the amounts of antinutritional factors (Chang & Harrold, 1988; Labaneiah & Luh, 1981). Some studies revealed that during germination process, the amount of phytase, an enzyme responsible for the breakdown of phytic acid, is increased (Reddy, Sathe, & Salunkhe, 1982). Kataria, Chauhan, and Punia (1989) also found out that hydration followed by sprouting for up to 60 h reduced the levels of polyphenols in mung beans.

Improved kidney bean varieties are constantly given to farmers from agricultural research centres/stations of East African countries. However, information regarding the effect of processing on the levels of antinutritional substances is very limited. Modest work has been carried out on the interactive effect of various domestic processing methods in reducing/eliminating the antinutritional composition, flatulence-causing factors and improvement of *in vitro* protein digestibility of kidney beans. Germination, autoclaving, cooking and hydration are some of the processing methods employed in this study.

The purpose of this work was to investigate the effects of processing methods on the reduction/elimination of antinutritional and flatulence-producing factors in kidney beans. This would help determine simple and cost-effective processing options for developing countries in order to make use of all the nutritional values of the beans.

2. Materials and methods

2.1. Materials

The improved kidney bean (*P. vulgaris* L.) seeds used in this study were grown under similar field conditions and normal agronomic practices required for bean crops at Nazareth Agricultural Research Center, Ethiopia. The *P. vulgaris* kidney bean varieties used in the study were Roba, Awash and Beshbesh which were taken from agricultural research centres and were grown in the East and Great Lakes Regions of Africa. These beans were chosen amongst the eight varieties studied previously due to their respective low, medium and high antinutritional factors and *in vitro* protein digestibility (Shimelis & Rakshit, 2005b). The kidney bean seeds were sealed and placed in plastic bags, and stored at 4 °C before use. All chemicals and reagents used were either analytical or reagent grade.

2.2. Processing methods

The processing methods used for the reduction/elimination of antinutritional factors were soaking (hydration), germination, cooking, autoclaving and their combinations. After each step for every particular processing method, the samples were frozen, lyophilized, and ground to pass a 60 mesh sieve screen. For each treatment procedure, tannin analysis was conducted immediately after the sample was ground to avoid oxidation reaction. Antinutritional factors, raffinose family oligosaccharides and *in vitro* protein digestibility were also analyzed using standard procedures.

2.2.1. Hydration

Kidney bean seeds free from cracks, dust and other foreign materials were soaked at room temperature for 12 h (overnight) in plain water (pH 6.9) and sodium bicarbonate solution (0.05%, pH 8.2) . The seed-to-solution ratio was 1:3 (w/v). The unabsorbed liquid was drained off, and the seeds were rinsed twice in distilled water, blended and lyophilized (Freeze dry system, LABCONCO Corporation, Kansas City, USA). The lyophilized samples were milled into flour (60-mesh size) and stored in air tight dark brown polyethylene bottles at room temperature until further analyses.

2.2.2. Germination

Kidney bean seeds were accurately weighed into large plastic petri-dishes and soaked in distilled water (bean to water ratio of 1:5 w/v) for 12 h at 25 °C. The petri-dishes were covered with perforated aluminium foil and kept in the dark chamber at 25 °C to sprout for 4 days in filter paper (Whatman filter paper No. 541) lined petri dishes. Leaching losses were minimized by collecting all drip water at the bottom of the petri-dish. Sodium azide at a concentration of 0.01% (w/v) was added to distilled water and was used for hydration and germination processes to prevent microbial growth. During germination, distilled water (containing sodium azide) was sprinkled on the seeds (twice a day). Seeds that have sprouted for 1–4 days were immediately frozen (along with the excess water) and lyophilized. Unsoaked and non-germinated seeds served as control. Seeds were then ground in a Wiley mill fitted with a 40-mesh screen and stored at 4 °C until further analyses.

2.2.3. Cooking

After hydration for 12 h, the seeds were rinsed with distilled water and cooked (water = two times the weight of soaked seeds) according to the method of Rani, Jood, and Sehgal (1996). Cooking of pre-soaked seeds was performed at 97 °C which was previously determined for each bean variety (Shimelis & Rakshit, 2005a). Similarly, unsoaked samples were also cooked, using a seed-to-water ratio of 1:3 (w/v) at 97 °C for 35 min. The cooking water was drained off, and the seeds were rinsed twice in distilled water, crushed and lyophilized. Cooking process was carried out in duplicate samples of each variety. The processed (crushed and lyophilized), unprocessed, and soaked dried samples were then ground in a cyclone mill using a 0.5 mm sieve, and then stored in air tight plastic containers at 4 °C. These samples were used in *vitro* protein digestibility, antinutrients and raffinose family oligosaccharides analyses.

2.2.4. Autoclaving

The clean seed samples were autoclaved (Model KT-30LD, ALP Co., Ltd., Tokyo, Japan) at 10,547 kg/m^2 pres-

sure with a temperature of 121 °C in plain water (1:3 w/v or 1 g: 3 ml) for 30 min according to the method of Vijayakumari, Siddnuraju, and Janardhanan (1996). Similar procedures were used for seeds soaked overnight in plain water and sodium bicarbonate solution, and then autoclaved for 20 min. Subsequently, the seeds were rinsed with distilled water, lyophilized and powdered in a cyclone mill of 60-mesh size. After treatments of the samples, the protein digestibility, antinutrients and oligosaccharides remaining in the bean seeds were then analyzed.

2.3. Methods of analysis

2.3.1. Trypsin inhibitor activity

Trypsin inhibitor activity (TIA) was measured following the procedure by Kakade, Rackis, McGhee, and Pusk (1974).

2.3.2. Extraction and estimation of sugars by HPLC

The oligosaccharides were extracted from bean samples using AOAC official method 982.14 (AOAC, 2000). The analyses of sugars were carried out using high-performance liquid chromatography according to the analysis method by Doyon, Gaudreau, St-Gelais, Beaulieu, and Randall (1991). The liquid chromatograph used for this study was "Agilent 1100 series" with analytical column (APS-2 HYPERSIL, 5 μm, 250 × 4.6 mm, L × I.D, Thermo Electron Corporation, England) equipped with differential refractive index detector (Model, G1362A, Agilent technologies, Germany). The extracts of oligosaccharides were eluted with acetonitrile/water 73:27 (v/v) as a mobile phase at a pump rate of 1 ml/min.

2.3.3. Measurements of lectins, saponins, phytic acid and tannins

A competitive indirect ELISA assay for quantification of *P. vulgaris* lectins was conducted following the procedure of Burbano, Muzquiz, Ayet, Cuadrado, and Pedrosa (1999). The saponin contents of raw and treated bean samples were determined using a spectrophotometric method described by Hiai, Oura, and Nakajima (1976). Phytate content was evaluated using the method of Haug and Lantzsch (1983). Tannins were also determined following the method described by Makkar, Aderibige, and Becker (1998).

2.3.4. In vitro protein digestibility

Proteins from kidney beans were isolated for digestibility analysis, using the method of Satterlee, Bembers, and Kendrick (1975). *In vitro* protein digestibility of kidney bean samples was carried out using the multi-enzyme solution by Hsu, Vavak, Satterlee, and Miller (1977).

3. Results and discussions

The influence of hydration, germination, cooking and autoclaving, and their combinations on the levels of certain antinutrients (phytic acid, tannins, protease inhibitors, lectins and saponins), α-galactosides present in the seeds of *P. vulgaris* L. and enhancement in *in vitro* protein digestibility were studied.

3.1. Effect of hydration

The levels of oligosaccharides, sucrose, saponins, trypsin inhibitors, tannins and phytic acid in the samples have been affected by Hydration in distilled water and sodium bicarbonate solution as shown in Tables 1–3. However, lectins (phytohaemagglutinis) were not significantly ($P > 0.05$) reduced in all hydration media. Overnight hydration of seeds in either water or salt media have resulted significant reduction in the levels of oligosaccharides and sucrose concentrations for each case of three bean varieties. Amounts of total α-galactosides and trypsin inhibitors activity were reduced to 40–48% and 9–18%, respectively. This result confirms the earlier reports in legumes (Jood et al., 1985; Vijayakumari, Siddhuraju, & Janardhanan, 1997) and in cowpea (Wang, Lewis, Brennan, & Westby, 1997).

During hydration with distilled water and sodium bicarbonate solution, significant reduction had been observed in the levels of stachyose followed by raffinose contents for the three bean varieties being studied. Generally, these results indicated that the reduction of α-galactosides is higher in seeds soaked in sodium bicarbonate solution than in distilled water. Upadhyay and Garcia (1988) demonstrated that differential solubility of the individual oligosaccharides and their diffusion rates are two factors that could influence the sugar losses during hydration. Hydration in 0.05% sodium bicarbonate solution increased softening of the testa and cotyledons that resulted in the increase of sugar extraction. In addition, overnight hydration in salt solution along with subsequent cooking resulted in significantly lesser sugar content as compared to simple hydration in plain water with subsequent cooking of seeds.

Hydration process (12 h) had significantly reduced the phytic acid contents of all the three bean varieties. The percentage loss of phytic acid is higher with distilled water hydration compared to salt water hydration in all three varieties. This agrees with previous research conducted with wheat products (Khan, Zaman, & Elahi, 1986) and white variety of Bengal gram (*Cicer arietinum*) (Khan, Zaman, & Elahi, 1988). This demonstrated that in the presence of sodium bicarbonate solution, there was lesser loss of phytic acid. Hydration-induced reduction in phytate content in legumes may be attributed to the activity of phytase and diffusion of the products. An increase in the phytase activity with a decrease in the level of phytate as a result of hydration in faba bean had been shown in an earlier study by Eskin and Wiebe (1983). Unlike phytic acid, the percentage loss of tannins is higher with salt water hydration compared to distilled water hydration in all three bean varieties. Respectively, reduction in the concentration of saponins and trypsin inhibitors of beans soaked in salt solution was higher compared with samples soaked in

Table 1
Effect of processing methods on α-galactosides and sucrose contents of kidney bean of Roba variety

Treatment	Raffinose (g/100 g) (d.m.)	Reduction[a] (%)	Stachyose (g/100 g) (d.m.)	Reduction[a] (%)	Total α-galactosides (g/100 g) (d.m.)	Reduction[a] (%)	Sucrose (g/100 g) (d.m.)	Reduction[a] (%)
Unprocessed seeds (control)	0.34 ± 0.01^{a}		1.24 ± 0.06^{a}		1.58 ± 0.05^{a}		2.68 ± 0.01^{e}	
Water soaking (12 h in plain water)	0.22 ± 0.05^{b}	35	0.71 ± 0.01^{b}	43	0.93 ± 0.06^{b}	41	2.12 ± 0.07^{g}	21
Sodium bicarbonate soaking (12 h)	0.21 ± 0.02^{b}	38	0.66 ± 0.04^{b}	47	0.86 ± 0.01^{b}	46	2.00 ± 0.02^{gh}	25
Sprouting for 24 h	0.21 ± 0.01^{b}	38	0.53 ± 0.03^{cd}	57	0.73 ± 0.01^{c}	54	3.93 ± 0.04^{d}	47[b]
Sprouting for 48 h	0.18 ± 0.03^{c}	47	0.13 ± 0.04^{e}	90	0.31 ± 0.02^{f}	80	4.57 ± 0.02^{a}	71[b]
Sprouting for 72 h	ND	100	ND	100	ND	100	4.35 ± 0.01^{b}	62[b]
Sprouting for 96 h	ND	100	ND	100	ND	100	4.21 ± 0.01^{c}	57[b]
Cooking of un soaked seeds	0.19 ± 0.07^{c}	44	0.62 ± 0.02^{b}	50	$0.81 \pm 0.08\ b^{c}$	49	1.36 ± 0.03^{i}	49
Water soaking + cooking	0.18 ± 0.04^{c}	47	0.47 ± 0.03^{e}	62	0.64 ± 0.03^{d}	60	1.13 ± 0.06^{j}	58
Sodium bicarbonate soaking + cooking	0.16 ± 0.09^{d}	53	0.45 ± 0.01^{e}	64	0.61 ± 0.02^{d}	61	0.99 ± 0.01^{k}	63
Autoclaving un soaked seeds	0.14 ± 0.02^{d}	59	0.41 ± 0.07^{e}	67	0.55 ± 0.01^{de}	65	0.52 ± 0.02^{n}	81
Soaking (H_2O) + autoclaving	0.12 ± 0.08^{e}	65	0.30 ± 0.02^{d}	76	0.41 ± 0.04^{e}	74	0.86 ± 0.09^{l}	68
Soaking ($NaHCO_3$) + autoclaving	0.08 ± 0.05^{f}	77	0.29 ± 0.01^{d}	77	0.36 ± 0.09^{e}	77	0.68 ± 0.01^{m}	75
Sprouting for 24 h + autoclaving	0.15 ± 0.03^{d}	56	0.12 ± 0.05^{e}	90	0.27 ± 0.08^{f}	83	1.82 ± 0.04^{h}	32
Sprouting for 48 h + autoclaving	0.08 ± 0.04^{f}	76	0.06 ± 0.07^{f}	95	0.14 ± 0.09^{g}	91	2.39 ± 0.05^{f}	11
Sprouting for 72 h + autoclaving	ND	100	ND	100	ND	100	2.17 ± 0.02^{g}	19
Sprouting for 96 h + autoclaving	ND	100	ND	100	ND	100	2.06 ± 0.01^{g}	23

ND- not detectable.
[a–n] Means not sharing a common superscript letter with in a column are significantly different ($P < 0.05$).
[a] Reduction indicates % decrease over raw value.
[b] Increase in sucrose percentage.

Table 2
Effect of processing methods on α-galactosides and sucrose content of kidney bean of Awash variety

Treatment	Raffinose (g/100 g) (d.m.)	Reduction[a] (%)	Stachyose (g/100 g) (d.m.)	Reduction[a] (%)	Total α-galactosides (g/100 g) (d.m.)	Reduction[a] (%)	Sucrose (g/100 g) (d.m.)	Reduction[a] (%)
Unprocessed seeds (control)	0.29 ± 0.07^{a}		1.84 ± 0.02^{a}		2.12 ± 0.02^{a}		1.73 ± 0.03^{e}	
Water soaking(12 h in plain water)	0.18 ± 0.01^{b}	38	0.98 ± 0.06^{b}	47	1.16 ± 0.04^{b}	45	1.36 ± 0.02^{g}	21
Sodium bicarbonate soaking(12 h)	0.17 ± 0.04^{b}	41	0.94 ± 0.01^{b}	49	1.11 ± 0.01^{b}	48	1.35 ± 0.00^{g}	22
Sprouting for 24 h	0.18 ± 0.06^{b}	38	0.87 ± 0.01^{c}	53	1.04 ± 0.04^{bc}	51	2.56 ± 0.07^{d}	48[b]
Sprouting for 48 h	0.11 ± 0.06^{c}	62	0.15 ± 0.01^{h}	92	0.26 ± 0.01^{f}	88	3.00 ± 0.02^{a}	73[b]
Sprouting for 72 h	ND	100	ND	100	ND	100	2.86 ± 0.01^{b}	65[b]
Sprouting for 96 h	ND	100	ND	100	ND	100	2.75 ± 0.01^{c}	59[b]
Cooking of un soaked seeds	0.16 ± 0.02^{b}	43	0.83 ± 0.02^{c}	55	0.99 ± 0.02^{bc}	53	0.89 ± 0.05^{i}	49
Water soaking + cooking	0.13 ± 0.03^{c}	55	0.59 ± 0.03^{e}	68	0.72 ± 0.05^{c}	66	0.76 ± 0.01^{j}	56
Sodium bicarbonate soaking + cooking	0.13 ± 0.01^{c}	55	0.57 ± 0.02^{e}	69	0.70 ± 0.01^{c}	67	0.67 ± 0.06^{k}	61
Autoclaving of un soaked seeds	0.12 ± 0.01^{c}	59	0.53 ± 0.01^{f}	72	0.65 ± 0.00^{cd}	69	0.38 ± 0.04^{l}	78
Soaking(H_2O) + autoclaving	0.08 ± 0.02^{cd}	72	0.41 ± 0.07^{f}	78	0.49 ± 0.01^{d}	77	0.64 ± 0.01^{j}	63
Soaking ($NaHCO_3$) + autoclaving	0.06 ± 0.05^{cd}	79	0.37 ± 0.05^{g}	80	0.43 ± 0.03^{e}	80	0.60 ± 0.02^{j}	65
Sprouting for 24 h + autoclaving	0.11 ± 0.03^{c}	62	0.66 ± 0.05^{d}	64	0.77 ± 0.05^{c}	64	1.21 ± 0.08^{h}	30
Sprouting for 48 h + autoclaving	0.06 ± 0.06^{cd}	79	0.08 ± 0.04^{i}	96	0.14 ± 0.02^{g}	93	1.54 ± 0.07^{f}	11
Sprouting for 72 h + autoclaving	ND	100	ND	100	ND	100	1.44 ± 0.09^{g}	17
Sprouting for 96 h + autoclaving	ND	100	ND	100	ND	100	1.37 ± 0.04^{g}	21

ND- not detectable.
[a–l] Means not sharing a common superscript letter with in a column are significantly different ($P < 0.05$).
[a] Reduction indicates % decrease over raw value.
[b] Increase in sucrose percentage.

water. From the nutritional point of view, hydration in plain water may be preferable than in sodium bicarbonate solution due to the susceptibility of some Vitamin B complex group to alkali. Thiamine and riboflavin, for example are known to be destroyed slowly in alkaline medium at room temperature (Swaminathan, 1974).

Our results indicated that the difference in the reduction of raffinose, stachyose, total α-galactosides and sucrose were not significantly different from hydration in plain water and sodium bicarbonate solution. Hence, water hydration would be sufficient if these sugars need to be reduced. Verbascose, fructose and maltose were not found

Table 3
Effect of processing methods on α-galactosides and sucrose content of kidney bean of Beshbesh variety

Treatment	Raffinose (g/100 g) (d.m.)	Reduction[a] (%)	Stachyose (g/100 g) (d.m.)	Reduction[a] (%)	Total α-galactosides (g/100 g) (d.m.)	Reduction[a] (%)	Sucrose (g/100 g) (d.m.)	Reduction[a] (%)
Unprocessed seeds (control)	0.24 ± 0.01^{a}		1.67 ± 0.01^{a}		1.90 ± 0.01^{a}		2.52 ± 0.05^{e}	
Water soaking (12 h in plain water)	0.15 ± 0.05^{b}	38	0.98 ± 0.02^{a}	41	1.15 ± 0.02^{b}	40	1.97 ± 0.01^{g}	22
Sodium bicarbonate soaking (12 h)	0.14 ± 0.03^{b}	42	0.93 ± 0.05^{b}	44	1.09 ± 0.01^{b}	43	1.94 ± 0.07^{g}	23
Sprouting for 24 h	0.16 ± 0.04^{b}	33	0.97 ± 0.01^{b}	42	1.13 ± 0.02^{b}	41	3.77 ± 0.03^{d}	50^{b}
Sprouting for 48 h	0.13 ± 0.01^{c}	46	0.15 ± 0.00^{f}	91	0.28 ± 0.03^{g}	85	4.34 ± 0.01^{a}	72^{b}
Sprouting for 72 h	ND	100	ND	100	ND	100	4.10 ± 0.07^{b}	63^{b}
Sprouting for 96 h	ND	100	ND	100	ND	100	3.95 ± 0.08^{c}	57^{b}
Cooking of unsoaked seeds	0.13 ± 0.01^{b}	46	0.82 ± 0.04^{c}	51	0.94 ± 0.05^{c}	51	1.82 ± 0.08^{h}	28
Water soaking + cooking	0.11 ± 0.09^{c}	54	0.57 ± 0.03^{d}	66	0.67 ± 0.03^{d}	65	1.74 ± 0.00^{hi}	31
Sodium bicarbonate soaking + cooking	0.10 ± 0.07^{cd}	58	0.53 ± 0.06^{d}	68	0.63 ± 0.02^{d}	67	1.71 ± 0.01^{hi}	32
Autoclaving of un soaked seeds	0.09 ± 0.06^{cd}	63	$0.48 \pm 0.01\ d^{e}$	71	0.56 ± 0.07^{e}	71	0.77 ± 0.02^{k}	69
Soaking(H_2O) + autoclaving	0.08 ± 0.03^{cd}	67	0.38 ± 0.08^{e}	77	0.46 ± 0.01^{f}	76	1.52 ± 0.04^{i}	40
Soaking($NaHCO_3$) + autoclaving	0.06 ± 0.00^{de}	75	0.35 ± 0.00^{e}	79	0.41 ± 0.03^{f}	78	1.41 ± 0.09^{j}	44
Sprouting for 24 h + autoclaving	0.12 ± 0.02^{c}	50	0.62 ± 0.03^{cd}	63	0.73 ± 0.03^{cd}	62	1.69 ± 0.07^{hi}	33
Sprouting for 48 h + autoclaving	0.07 ± 0.01^{de}	71	0.26 ± 0.04^{ef}	84	0.33 ± 0.01^{g}	83	2.19 ± 0.08^{f}	13
Sprouting for 72 h + autoclaving	ND	100	ND	ND	ND	100	1.99 ± 0.05^{g}	21
Sprouting for 96 h + autoclaving	ND	100	ND	ND	ND	100	1.79 ± 0.06^{h}	29

ND- not detectable.
[a–k]Means not sharing a common superscript letter with in a column are significantly different ($P < 0.05$).
[a] Reduction indicates % decrease over raw value.
[b] Increase in sucrose percentage.

to be within detectable levels in HPLC analysis in three varieties of beans that was studied.

3.2. Effect of germination

During the germination process, levels of raffinose oligosaccharides was significantly ($P < 0.05$) reduced (Tables 1–3). The highest reduction in stachyose and raffinose concentrations reaching its undetectable level was obtained at the end of the second day of germination. This reduction in raffinose oligosaccharides was comparable to its maximum reduction in black eye bean and pink bean samples during 4-day germination (Labaneiah & Luh, 1981; Silva & Luh, 1979). However, it was higher as compared with green gram and black gram varieties (Gupta & Wagle, 1980) and navy beans (Snauwaret & Markakis, 1976) at 4-day germination. These differences can be attributed to the origin and variety differences as well as the distinction in the levels of endogenous α-galactosidase activity in different beans. Furthermore, during the 48 h germination time, maximum reduction was observed in the levels of stachyose followed by raffinose levels. This may be attributed to germination process wherein α-galactosidase first attacks stachyose and then raffinose.

This study also indicated that in subsequent stages of germination, the increase in sucrose content was proportional to the stage of germination. After 48 h of germination, none of the oligosaccharides could be identified, indicating that the sucrose content may have increased at the expense of oligosaccharides of raffinose family. Germination influences the raffinose family oligosaccharides through mobilization of raffinose and through growth of stachyose. A number of studies have indicated that the content of raffinose family oligosaccharides in legumes decreases during germination due to the action of α-galactosidase, which selectively cleaves the galactose from raffinose, stachyose, and verbascose and leaving behind sucrose (Muzquiz, Rey, Cuadrado, & Fenwick, 1992; Siddhuraju, Becker, & Makkar, 2000; Vijayakumari et al., 1997).

It also appears that germination may be useful in removing/reducing certain unwanted heat-stable components like phytates and tannins. During the 4-day germination period, phytic acid contents of Roba, Awash and Beshbesh varieties progressively decreased from 23.51 to 1.88, 24.06 to 5.06 and 17.34 to 0.69 mg/g, respectively (Tables 4–6). Previous reports on the effects of germination on phytic acid in beans indicated that as a result of increased enzyme activity, 20–70% or more of the phytic acid is hydrolyzed during germination (Deshpande, 1985; Reddy et al., 1982) depending on the type of bean and the increase in phytase activity. Similarly, Noor, Bressani, and Elias (1980) also reported that a notable reduction (over 70%) in the phytic acid content of mung beans after 4-days of germination was obtained. The reduction (over 75%) of phytic acid in all three bean varieties used in this study (Tables 4–6) indicated that an increased in hydrolysis of phytates during germination led to the liberation of inorganic phosphates for plant growth from organic phosphorus containing compound (phytate). The breakdown of phytate during germination is attributed to the increased activity of the endogenous phytase (enzyme activity). Since phytic acid has been considered to be one of the factors responsible for reducing minerals bioavailability, its reduction during germination may have enhanced the nutritional

Table 4
Effect of processing methods on the levels of heat-stable antinutrients and *in-vitro* protein digestibility of Roba variety

Treatment	Tannins[a] (mg/g) (d.m.)	Reduction[b](%)	Phytic acid (mg/g) (d.m.)	Reduction[b](%)	Protein digestibility (%)	Increment[c](%)
Unprocessed seeds (control)	5.37 ± 0.01^{a}		23.51 ± 0.01^{a}		80.66 ± 0.02^{e}	
Water soaking (12 h in plain water)	4.03 ± 0.03^{b}	25	19.28 ± 0.07^{b}	18	84.69 ± 0.01^{d}	5
Sodium bicarbonate soaking (12 h)	3.92 ± 0.02^{b}	27	20.22 ± 0.05^{b}	14	85.51 ± 0.01^{d}	6
Sprouting for 24 h	3.66 ± 0.01^{c}	32	6.34 ± 0.02^{e}	73	88.74 ± 0.01^{c}	10
Sprouting for 48 h	1.38 ± 0.07^{de}	74	3.05 ± 0.02^{f}	87	87.11 ± 0.03^{cd}	8
Sprouting for 72 h	1.25 ± 0.03^{de}	77	2.59 ± 0.01^{f}	89	84.00 ± 0.04^{d}	4
Sprouting for 96 h	1.28 ± 0.01^{de}	76	1.88 ± 0.01^{g}	92	81.47 ± 0.01^{e}	1
Cooking of un soaked seeds	3.55 ± 0.01^{c}	34	17.64 ± 0.01^{c}	25	87.11 ± 0.03^{cd}	8
Water soaking + cooking	1.61 ± 0.00^{d}	70	8.23 ± 0.01^{d}	65	90.31 ± 0.02^{b}	12
Sodium bicarbonate soaking + cooking	1.71 ± 0.02^{d}	68	8.45 ± 0.02^{d}	64	90.34 ± 0.00^{b}	12
Autoclaving of un soaked seeds	1.51 ± 0.06^{d}	72	8.18 ± 0.01^{d}	65	86.31 ± 0.02^{cd}	7
Soaking(H_2O) + autoclaving	1.35 ± 0.01^{de}	75	8.01 ± 0.03^{d}	66	92.76 ± 0.01^{ab}	15
Soaking($NaHCO_3$) + autoclaving	1.34 ± 0.04^{de}	75	8.21 ± 0.01^{d}	65	92.84 ± 0.01^{ab}	15
Sprouting for 24 h + autoclaving	0.19 ± 0.04^{e}	97	0.57 ± 0.05^{h}	98	87.48 ± 0.03^{cd}	9
Sprouting for 48 h + autoclaving	ND	100	ND	100	94.12 ± 0.01^{a}	17
Sprouting for 72 h + autoclaving	ND	100	ND	100	94.22 ± 0.02^{a}	17
Sprouting for 96 h + autoclaving	ND	100	ND	100	93.79 ± 0.01^{a}	16

ND- not detectable.
[a–h] Means with the same superscript letters with in a column are not significantly different at $P < 0.05$ level.
[a] As D-Catechin equivalent (mg/g) on dry weight basis.
[b] Reduction indicate % decrease over raw values.
[c] Increment indicate % increase over raw values.

quality (with respect to mineral bioavailability) of beans. After 4-day germination, lectins, trypsin inhibitors, saponins, tannins and phytic acid were also significantly reduced to as much as 14%, 17%, 58%, 76% and 96%, respectively (Tables 4–9).

The observed reduction in tannin content in sprouted kidney bean seeds was attributed to the formation of hydrophobic association of tannins with seed proteins and enzymes (Sharma & Sehgal, 1992). Some loss of tannins during germination may be due to the leaching of tannins into the water. According to Goldstein and Swain (1965), tannins have been found to inhibit digestive enzymes and thereby lowering its digestibility in most nutrients, especially protein. Trypsin inhibitors, saponins, and lectins were affected significantly after 48 h of germination process as shown in Tables 7–9.

Table 5
Effect of processing methods on the levels of heat-stable antinutrients and *in-vitro* protein digestibility of Awash variety

Treatment	Tannins[a] (mg/g) (d.m.)	Reduction[b](%)	Phytic acid (mg/g) (d.m.)	Reduction[b](%)	Protein digestibility (%)	Increment[c](%)
Unprocessed seeds (control)	17.55 ± 0.01^{a}		24.06 ± 0.01^{a}		71.14 ± 0.01^{e}	
Water soaking (12 h in plain water)	13.51 ± 0.01^{b}	23	19.97 ± 0.07^{b}	17	74.01 ± 0.02^{d}	4
Sodium bicarbonate soaking (12 h)	13.16 ± 0.02^{b}	25	20.69 ± 0.03^{b}	14	74.00 ± 0.03^{d}	4
Sprouting for 24 h	11.41 ± 0.01^{c}	35	15.65 ± 0.04^{d}	35	75.42 ± 0.01^{d}	6
Sprouting for 48 h	4.04 ± 0.04^{e}	77	9.14 ± 0.03^{e}	62	77.55 ± 0.03^{c}	9
Sprouting for 72 h	4.38 ± 0.02^{e}	75	5.06 ± 0.08^{f}	79	74.71 ± 0.02^{d}	5
Sprouting for 96 h	4.34 ± 0.02^{e}	75	5.09 ± 0.06^{f}	79	72.57 ± 0.00^{e}	2
Cooking of un soaked seeds	11.40 ± 0.01^{c}	35	17.32 ± 0.01^{c}	28	78.28 ± 0.01^{c}	10
Water soaking + cooking	6.50 ± 0.01^{d}	63	8.67 ± 0.00^{e}	64	78.98 ± 0.00^{c}	11
Sodium bicarbonate soaking + cooking	6.85 ± 0.03^{d}	61	8.91 ± 0.01^{e}	63	78.88 ± 0.03^{c}	11
Autoclaving of un soaked seeds	6.88 ± 0.01^{d}	61	8.43 ± 0.05^{e}	65	76.12 ± 0.04^{cd}	7
Soaking(H_2O) + autoclaving	6.75 ± 0.03^{d}	62	8.42 ± 0.01^{e}	65	82.53 ± 0.06^{b}	16
Soaking($NaHCO_3$) + autoclaving	6.69 ± 0.01^{d}	62	8.90 ± 0.01^{e}	63	81.82 ± 0.02^{b}	15
Sprouting for 24 h + autoclaving	2.34 ± 0.05^{f}	87	1.28 ± 0.05^{g}	95	77.24 ± 0.00^{c}	9
Sprouting for 48 h + autoclaving	ND	100	ND	100	83.97 ± 0.02^{a}	18
Sprouting for 72 h + autoclaving	ND	100	ND	100	84.11 ± 0.02^{a}	18
Sprouting for 96 h + autoclaving	ND	100	ND	100	83.72 ± 0.01^{a}	18

ND: not detectable.
[a–g] Means with the same superscript letters with in a column are not significantly different at $P < 0.05$ level.
[a] As D-Catechin equivalent (mg/g) on dry weight basis.
[b] Reduction indicate % decrease over raw values.
[c] Increment indicate % increase over raw values.

Table 6
Effect of processing methods on the levels of heat-stable antinutrients and *in-vitro* protein digestibility of Beshbesh variety

Treatment	Tannins [a] (mg/g) (d.m.)	Reduction[b](%)	Phytic acid (mg/g) (d.m.)	Reduction[b](%)	Protein digestibility (%)	Increment[c](%)
Unprocessed seeds (control)	28.79 ± 0.01^{a}		17.34 ± 0.03^{a}		65.63 ± 0.01^{d}	
Water soaking (12 h in plain water)	21.88 ± 0.01^{b}	24	14.05 ± 0.04^{b}	19	68.91 ± 0.02^{cd}	5
Sodium bicarbonate soaking (12 h)	21.31 ± 0.01^{b}	26	14.74 ± 0.03^{b}	15	69.58 ± 0.01^{cd}	6
Sprouting for 24 h	19.29 ± 0.03^{c}	33	12.49 ± 0.01^{c}	28	72.20 ± 0.06^{c}	10
Sprouting for 48 h	5.77 ± 0.03^{g}	80	2.25 ± 0.00^{e}	87	76.14 ± 0.02^{b}	16
Sprouting for 72 h	7.21 ± 0.01^{f}	75	1.39 ± 0.02^{f}	92	70.88 ± 0.01^{e}	8
Sprouting for 96 h	7.21 ± 0.02^{f}	75	0.69 ± 0.01^{g}	96	66.95 ± 0.01^{d}	2
Cooking of un soaked seeds	21.01 ± 0.03^{b}	27	12.83 ± 0.05^{c}	26	71.55 ± 0.02^{c}	9
Water soaking + cooking	12.09 ± 0.00^{e}	58	6.69 ± 0.01^{d}	61	73.52 ± 0.01^{bc}	12
Sodium bicarbonate soaking + cooking	12.96 ± 0.04^{e}	55	6.76 ± 0.02^{d}	61	72.85 ± 0.03^{c}	11
Autoclaving of un soaked seeds	14.37 ± 0.08^{d}	50	6.94 ± 0.04^{d}	60	69.58 ± 0.04^{cd}	6
Soaking (H_2O) + autoclaving	12.80 ± 0.02^{e}	56	6.59 ± 0.01^{d}	62	74.77 ± 0.01^{bc}	14
Soaking ($NaHCO_3$) + autoclaving	12.91 ± 0.01^{e}	55	6.59 ± 0.00^{d}	62	74.83 ± 0.01^{bc}	14
Sprouting for 24 h + autoclaving	1.25 ± 0.04^{h}	96	0.37 ± 0.05^{g}	98	75.89 ± 0.01^{b}	16
Sprouting for 48 h + autoclaving	ND	100	ND	100	79.91 ± 0.02^{a}	22
Sprouting for 72 h + autoclaving	ND	100	ND	100	80.14 ± 0.02^{a}	22
Sprouting for 96 h + autoclaving	ND	100	ND	100	80.02 ± 0.02^{a}	22

ND: not detectable.
[a–h] Means with the same superscript letters with in a column are not significantly different at $P < 0.05$ level.
[a] As D-Catechin equivalent (mg/g) on dry weight basis.
[b] Reduction indicate % decrease over raw values.
[c] Increment indicate % increase over raw values.

Conflicting data also existed on the protease inhibitory and lectin activities from various researchers. El-Hag, Haard, and Morse (1978) reported about 50% reduction in trypsin inhibitor activity in *P. vulgaris* during 10-day germination. However, Kakade and Evans (1965) found only a 5.48% reduction in trypsin inhibitor activity of navy beans during 4-day germination. Sathe, Deshpande, and Salunkhe (1984) also observed that germination of the Great Northern beans caused a reduction in trypsin and chymotrypsin inhibitor factors. However, King and Puwastien (1987) reported that trypsin inhibitor activity was unaffected by germination as shown by a change of 74.7–73.7 TUI/mg sample for 0–120 h incubation time, respectively. Furthermore, Noor et al. (1980) did not find any significant change in trypsin inhibitor activity when mung beans were germinated for 4 days. In this study it

Table 7
Effect of processing methods on heat-sensitive antinutrients of Roba variety

Treatment	Lectin[b] (g/kg PHA)	Reduction[a] (%)	TIA (TIU[c]/mg) (d.m.)	Reduction[a](%)	Saponins (g/100 g) (d.m.)	Reduction[a](%)
Unprocessed seeds (control)	1.92 ± 0.01^{a}		4.59 ± 0.00^{a}		0.94 ± 0.02^{a}	
Water soaking (12 h in plain water)	1.90 ± 0.00^{a}	1	4.18 ± 0.01^{b}	9	0.84 ± 0.00^{b}	11
Sodium bicarbonate soaking (12 h)	1.90 ± 0.00^{a}	1	4.10 ± 0.01^{b}	11	0.72 ± 0.01^{c}	23
Sprouting for 24 h	1.82 ± 0.01^{b}	5	4.13 ± 0.01^{b}	10	0.76 ± 0.01^{c}	19
Sprouting for 48 h	1.71 ± 0.02^{c}	11	3.91 ± 0.01^{c}	15	0.54 ± 0.01^{d}	43
Sprouting for 72 h	1.65 ± 0.02^{c}	14	3.94 ± 0.02^{c}	14	0.47 ± 0.01^{e}	50
Sprouting for 96 h	1.65 ± 0.01^{c}	14	3.89 ± 0.01^{c}	15	0.47 ± 0.00^{e}	50
Cooking of un soaked seeds	0.24 ± 0.01^{d}	88	2.98 ± 0.01^{d}	35	0.45 ± 0.00^{e}	52
Water soaking + cooking	ND	100	2.52 ± 0.02^{e}	45	0.17 ± 0.01^{e}	82
Sodium bicarbonate soaking + cooking	ND	100	2.39 ± 0.03^{e}	48	ND	100
Autoclaving of un soaked seeds	ND	100	ND	100	ND	100
Soaking (H_2O) + autoclaving	ND	100	ND	100	ND	100
Soaking ($NaHCO_3$) + autoclaving	ND	100	ND	100	ND	100
Sprouting for 24 h + autoclaving	ND	100	ND	100	ND	100
Sprouting for 48 h + autoclaving	ND	100	ND	100	ND	100
Sprouting for 72 h + autoclaving	ND	100	ND	100	ND	100
Sprouting for 96 h + autoclaving	ND	100	ND	100	ND	100

ND: not detectable.
[a–e] Means with the same superscript letters with in a column are not significantly different at $P < 0.05$ level.
[a] Reduction indicate % decrease over raw values.
[b] Lectin as PHA (*P. vulgaris* lectin).
[c] TUI: trypsin units inhibited per mg of dried sample.

Table 8
Effect of processing methods on heat-sensitive antinutrients of Awash variety

Treatment	Lectin[b] (g/kg PHA)	Reduction[a](%)	TIA (TIU[c]/mg) (d.m.)	Reduction[a](%)	Saponins (g/100 g) (d.m.)	Reduction[a] (%)
Unprocessed seeds (control)	4.52 ± 0.01^{a}		20.89 ± 0.04^{a}		1.18 ± 0.01^{a}	
Water soaking (12 h in plain water)	4.49 ± 0.03^{a}	1	17.75 ± 0.01^{bc}	15	1.05 ± 0.07^{b}	11
Sodium bicarbonate soaking (12 h)	4.45 ± 0.01^{a}	1	17.13 ± 0.02^{bc}	18	1.01 ± 0.01^{b}	14
Sprouting for 24 h	4.42 ± 0.01^{b}	2	18.38 ± 0.01^{b}	12	0.88 ± 0.01^{c}	25
Sprouting for 48 h	4.21 ± 0.06^{c}	7	17.34 ± 0.04^{bc}	17	0.61 ± 0.03^{d}	48
Sprouting for 72 h	4.20 ± 0.03^{c}	7	17.75 ± 0.05^{bc}	15	0.42 ± 0.06^{e}	64
Sprouting for 96 h	4.22 ± 0.00^{c}	7	17.35 ± 0.02^{bc}	17	0.48 ± 0.01^{e}	59
Cooking of un soaked seeds	0.49 ± 0.02^{d}	89	15.67 ± 0.01^{c}	25	0.38 ± 0.02^{e}	68
Water soaking + cooking	ND	100	8.36 ± 0.02^{d}	60	0.12 ± 0.01^{e}	90
Sodium bicarbonate soaking + cooking	ND	100	7.32 ± 0.03^{e}	65	ND	100
Autoclaving of un soaked seeds	ND	100	ND	100	ND	100
Soaking (H_2O) + autoclaving	ND	100	ND	100	ND	100
Soaking ($NaHCO_3$) + autoclaving	ND	100	ND	100	ND	100
Sprouting for 24 h + autoclaving	ND	100	ND	100	ND	100
Sprouting for 48 h + autoclaving	ND	100	ND	100	ND	100
Sprouting for 72 h + autoclaving	ND	100	ND	100	ND	100
Sprouting for 96 h + autoclaving	ND	100	ND	100	ND	100

ND: not detectable.
[a–e] Means with the same superscript letters with in a column are not significantly different at $P < 0.05$ level.
[a] Reduction indicate % decrease over raw values.
[b] Lectin as PHA (*P. vulgaris* lectin).
[c] TUI-Trypsin Units Inhibited per mg of dried sample.

was observed that on germination of kidney beans for 96 h, a reduction in trypsin inhibitory factors for Roba, Beshbesh and Awash varieties were 15%, 16% and 17 %, respectively (Tables 7–9). After 24 h of germination, lectin concentration for Awash variety remained unchanged. However, reduction of lectin content in Roba and Beshbesh varieties were 14% and 18%, respectively (Tables 7 and 9). Chen and Pan (1977) observed a reduction of 84–100% in the haemagglutinating activity of eight varieties of peas and beans seeds during 4-day germination. However, Palmer, Mcintosh, and Puszati (1973) demonstrated that germination for 8-days did not remove the toxicity. As shown in Tables 7–9, over 50% of the saponins in all three bean samples were reduced considerably through germination and this is in agreement with the report for food legumes (Jood et al., 1985). In brief, the raffinose family oli-

Table 9
Effect of processing methods on heat-sensitive antinutrients of Beshbesh variety

Treatment	Lectin[b] (g/kg PHA)	Reduction[a] (%)	TIA (TIU[c]/mg) (d.m.)	Reduction[a] (%)	Saponins (g/100 g) (d.m.)	Reduction[a] (%)
Unprocessed seeds (control)	9.98 ± 0.02^{a}		29.27 ± 0.08^{a}		1.32 ± 0.08^{a}	
Water soaking (12 h in plain water)	9.90 ± 0.00^{a}	1	27.51 ± 0.02^{b}	6	1.17 ± 0.01^{b}	11
Sodium bicarbonate soaking (12 h)	9.80 ± 0.01^{a}	2	26.64 ± 0.06^{b}	9	1.12 ± 0.02^{b}	15
Sprouting for 24 h	9.29 ± 0.01^{b}	7	26.05 ± 0.08^{bc}	11	1.05 ± 0.01^{c}	21
Sprouting for 48 h	8.49 ± 0.02^{c}	15	24.59 ± 0.04^{c}	16	0.73 ± 0.00^{d}	45
Sprouting for 72 h	8.19 ± 0.01^{d}	18	24.88 ± 0.07^{c}	15	0.55 ± 0.01^{e}	58
Sprouting for 96 h	8.18 ± 0.01^{d}	18	24.59 ± 0.03^{c}	16	0.55 ± 0.02^{e}	58
Cooking of un soaked seeds	0.82 ± 0.03^{e}	92	22.54 ± 0.03^{d}	23	0.42 ± 0.06^{f}	68
Water soaking + cooking	ND	100	12.59 ± 0.05^{e}	57	0.13 ± 0.01^{g}	90
Sodium bicarbonate soaking + cooking	ND	100	11.71 ± 0.07^{e}	60	ND	100
Autoclaving of un soaked seeds	ND	100	ND	100	ND	100
Soaking (H_2O) + autoclaving	ND	100	ND	100	ND	100
Soaking ($NaHCO_3$) + autoclaving	ND	100	ND	100	ND	100
Sprouting for 24 h + autoclaving	ND	100	ND	100	ND	100
Sprouting for 48 h + autoclaving	ND	100	ND	100	ND	100
Sprouting for 72 h + autoclaving	ND	100	ND	100	ND	100
Sprouting for 96 h + autoclaving	ND	100	ND	100	ND	100

ND: not detectable.
[a–e] Means with the same superscript letters with in a column are not significantly different at $P < 0.05$ level.
[a] Reduction indicate % decrease over raw values.
[b] Lectin as PHA (*P. vulgaris* lectin).
[c] TUI: trypsin units inhibited per mg of dried sample.

gosaccharides, tannins and phytic acid were mostly affected by germination process. However, lectins, saponins and trypsin inhibitors were not reduced significantly. Moreover, the increase in sucrose concentration was proportional to the stage of germination.

3.3. Effect of cooking and autoclaving

Amount of raffinose oligosaccharides were significantly ($P < 0.05$) reduced during heat processing (cooking, autoclaving) and their combinations (Tables 1–3). Higher losses of raffinose and stachyose were found when soaked seeds were autoclaved. After the seeds were autoclaved, the losses of sucrose and oligosaccharides content were significantly higher than other treatments. Autoclaving caused a decrease of both raffinose and stachyose on all kidney bean samples. This was due to the decomposition of fructose which is a part of both sugars that decomposes at 103–105 °C (Stecher, 1968). Buckle and Samboudi (1990) and Van der Poel et al. (1990) also reported a loss of amino acids and soluble sugars after hydration and/or cooking of some legume seeds. Cooking and autoclaving inactivate/destroy proteinaceous antinutrients (protease inhibitors, lectins) and saponins down to undetectable levels. Certain reports suggested that inactivation of heat-sensitive factors may not always be complete. Padhye and Salunkhe (1980) reported unusual stability of the trypsin inhibitor of black gram towards heat. Ghorpade, Kadam, and Salunkhe (1986) and Kadam and Smithard (1987) reported that trypsin inhibitory activity was appreciably destroyed by heat as obtained in this study.

Trypsin inhibitors, lectins and saponins, due to their heat-sensitive nature were significantly reduced to undetectable amounts by the heating processes (cooking and autoclaving). Hydration and cooking reduced and eliminated the antinutritional factors in legumes and improved its digestibility (Khokar & Chanhan, 1986). Dry heat treatment was also shown to inhibit the activity of trypsin inhibitors (Marquez, Fernandez, & Alonso, 1998). Optimal cooking enhances the protein quality of legumes. Kakade and Evans (1966) observed a significant improvement in the *in vitro* protein digestibility of navy beans after a mild heat treatment. The length of cooking is an important factor that affects the protein quality of cooked legumes. Cooking for more than 30 min at 121 °C, without a previous hydration decreased the nutritive value of legume proteins (Elias, Conde, Munoz, & Bressani, 1973). Autoclaving improved the *in vitro* protein digestibility of legumes and may be attributed not only to the removal of antinutrients but also to the structural disintegration of the native protein, including enzyme inhibitors (Moneam, 1990). The initial increase in the protein digestibility during heat treatment is partly due to inactivation of trypsin inhibitor, lectins and increased protein denaturation. The subsequent reduction in protein quality with further heating is usually due to the loss of certain essential amino acids (Elias, Bressani, & Braham, 1982).

Furthermore, cooking significantly ($P < 0.05$) lowered the phytic acid contents in kidney beans. Water hydration followed by cooking of kidney beans resulted in the reduction of phytic acid contents due to hydrolysis (Tables 4–6). It was noted that over 99% of the total phytic acid were in a water-soluble form which could serve as a means of removing or lowering phytic acid levels in *P. vulgaris* (Lolas & Markakis, 1975). Cooking and autoclaving processes significantly reduced tannins and phytic acid concentration on pre soaked samples, but their percentage reduction remained similar for these processes. The results of this study were consistent with those mentioned by previous investigators that cooking and autoclaving processes reduced tannins by 27–35% and 50–72%, respectively. Saponins, trypsin inhibitors and haemagglutin activities were also completely eliminated by heat treatment process while tannins, phytic acid, α-galactosides contents were only partly affected by the processing methods (hydration, cooking, autoclaving and their combinations) due to their heat-stable nature.

The content of nutrients in the kidney bean seeds and interaction of nutrients under various processing conditions have a great influence on nutrient bioavailability (Reddy et al., 1982; Sathe et al., 1984). In order to maintain the nutritional value of food subjected to heat treatment, it is necessary that the heating temperature and the length of processing will reach, but will not exceed the optimum temperature required to eliminate the effect of inhibitors without altering basic nutrients. Overall, it was observed that cooking and autoclaving removed most of the heat sensitive antinutritional factors while the heat stable and soluble components were removed at a limited extent.

3.4. Combination of hydration, germination and heat processing

It was observed that hydration and germination leads to a reduction in raffinose, stachyose, phytates and tannins. The heat processing methods on the other hand led to a reduction in heat sensitive antinutrients like lectins, trypsin inhibitors and saponins. Hence, a combination of these processes was attempted by heating the beans just after they have germinated.

The combinations of presoaked germinated and autoclaved seeds were able to eliminate/reduce heat-stable and heat-sensitive antinutrients. Phytic acid located on cotyledon of the bean seeds which was retained during germination processes was attacked by autoclaving process. Subsequently, the combined processes (germination with autoclaving) improved the *in vitro* protein digestibility for the Roba, Awash and Beshbesh kidney bean varieties. The combined processes remarkably eliminated the antinutrients (lectins, saponins, trypsin inhibitors, tannins and phytic acid), flatus-producing compounds (α-galactosides). It also increased the *in vitro* protein digestibility of all bean samples (Tables 4–6). The only problem with the combined process was the extended time required for the process of

germination. As in the case of sprouts sold in Thailand, the possibility of the consumer purchasing pre-germinated beans before use is also possible.

3.5. Protein digestibility of beans

The *in vitro* protein digestibility of raw and processed seeds of *P. vulgaris* L. is given in Tables 4–6. All three bean varieties showed significant increase in digestibility after treatments (hydration, cooking, autoclaving and germination) and thus improved its protein quality. The improvement of *in vitro* protein digestibility of kidney beans in all process treatments may be attributed not only to the removal/reduction of antinutrients but also to the structural disintegration of the native protein, including enzyme inhibitors and lectins, differential solubility of the individual oligosaccharides and their diffusion rates, phytase activity to break down phytic acid in the seeds and the development of endogenous α-galactosidase activity to diminish oligosaccharides. The bean proteins were broken down into amino acids without any change on its content during the germination period but the protein digestibility of these beans had greatly improved. Raw seeds of Roba, Awash and Beshbesh varieties exhibited *in vitro* protein digestibility of 80.7%, 71.1% and 65.6%, respectively. Furthermore protein digestibility of Roba, Awash and Beshbesh varieties after processing reached 94.2%, 84.1% and 80.1%, respectively. Autoclaving of sprouts had significant effects on protein digestibility of seed samples compared to that of the seeds subjected to single processes like hydration, cooking, autoclaving and germination. From the obtained experimental result of this study it can be concluded that only one processing method cannot produce desired removal of all antinutrients and required improvement in the *in vitro* protein digestibility of the beans. Germination of pre-soaked seeds and autoclaving can be used for obtaining enhanced protein quality of bean flour which could be used to assist in the preparation of bean-based value-added food products such as weaning foods to prevent protein-energy malnutrition.

4. Conclusions

Processing methods for kidney beans is very important primarily due to the high content of antinutrients and the difficulty in their digestion. Effective utilization of quality protein in beans as a source of food/feed, especially in the East and Great Lakes Regions of Africa is also important. For large populations, a simple and inexpensive processing technique which changes the seed composition of kidney beans and improves its acceptability is necessary.

The results obtained showed that germination significantly reduced certain unwanted and heat-stable antinutrient components such as phytates, tannins, and the flatulence-causing factors, whereas it did not have significant effects on saponins, protease inhibitory and lectin activities. On the other hand, cooking of pre-soaked beans in either water or saline solution or autoclaving appeared as an adequate method for reducing heat-sensitive antinutrients i.e. saponins, protease inhibitors and lectins. Based on the results of this study it can be concluded that, no single method can remove or eliminate most of the antinutrients and toxic factors. A combination of germination and autoclaving brought about all the desirable changes in kidney beans. Among all the varieties that were studied, Roba bean samples had better *in vitro* protein digestibility (protein quality) and can be used as an ingredient for manufacturing bean-based value-added products.

Acknowledgements

The author Shimelis would like to express heartfelt gratitude to the Ethiopian government and Asian Institute of Technology, Bangkok for providing financial support for this research work. He also wishes to thank Dr. Prapasri Puwastien of Institute of Nutrition, Mahidol University (Thailand) for her valuable assistance while carrying out the experiments. Special thanks are due to Miss Mary Rosary Caspillo, Mr. Dominic Ortega Trani and Miss Bernice Buot Polohan (SERD/AIT) for their valuable support during manuscript preparation.

References

Anderson, J. W., Smith, B. M., & Washnock, C. S. (1999). Cardiovascular and renal benefits of dry bean and soy bean intake. *American Journal of Clinical Nutrition, 70*, 464–474.

AOAC, (2000). Association of Official Analytical Chemists (AOAC). Official methods of analysis of the association of official analytical chemists: Food composition; additives; natural contaminants. In William, H. (Ed.), *Official methods of analysis of the association of official analytical chemists: Food composition; additives; natural contaminants*. Vol. II, 17th edition, Washington, D.C. Official method 982.14.

Buckle, K. A., & Samboudi, H. (1990). Effect of soaking and boiling treatments on the quality of winged bean seeds. *Journal of the Science of Food and Agriculture, 53*, 379–388.

Burbano, C., Muzquiz, M., Ayet, G., Cuadrado, C., & Pedrosa, M. M. (1999). Evaluation of antinutritional factors of selected varieties of *Phaseolus vulgaris*. *Journal of the Science Food and Agriculture, 79*, 1468–1472.

Chang, K. C., & Harrold, R. L. (1988). Changes in selected biochemical components, in vitro protein digestibility and amino acids in two bean cultivars during germination. *Journal of Food Science, 53*, 783–787.

Chen, L. H., & Pan, S. H. (1977). Decrease of phytates and hemagglutinating activity during germination of pea and bean seeds. *Journal of Food Science, 42*, 1966.

Deshpande, S.S., (1985). Investigations on dry beans (*Phaseolus vulgaris* L.): Microstructure, processing, and antinutrients. Ph.D. thesis, University of Illinois, Urbana-Champaign.

Doyon, G., Gaudreau, G., St-Gelais, D., Beaulieu, Y., & Randall, J. C. (1991). Simultaneous HPLC determination of organic acids, sugars and alcohols. *Canadian Institute of Science and Technology Journal, 24*, 87–94.

El-Hag, N., Haard, N. F., & Morse, R. E. (1978). Influence of sprouting on the digestibility coefficient, trypsin inhibitor and globulin proteins of red kidney beans. *Journal of Food Science, 43*, 1874–1875.

Elias, L. G., Bressani, R., & Braham, J. E. (1982). Reduction of digestibility of legume proteins by tannins. *Plant Foods for Human Nutrition, 4*, 43–55.

Elias, L. G., Conde, A., Munoz, A., & Bressani, R. (1973). Effect of germination and maturation on nutritive value of common bean (*Phaseolus vulgaris*). In W. G. Jaffe (Ed.), *Nutritional aspects of common beans and other legume seeds as animal and human food* (pp. 139–149). Ribeiráo Preto, Sáo Paulo, Brazil.

Eskin, N. A. M., & Wiebe, S. (1983). Changes in phytase activity and phytate during germination of two faba bean cultivars. *Journal of Food Science, 48*, 270–271.

FAO (1993). Production Yearbook, 1992. Vol. 26. Food and Agriculture Organization of the United Nations, Rome.

Ghorpade, V. M., Kadam, S. S., & Salunkhe, D. K. (1986). Thermal stability and changes in trypsin inhibitor during germination and cooking. *Journal of Food Science and Technology, 23*, 164.

Goldstein, J. L., & Swain, T. (1965). The inhibition of enzymes by tannins. *Phytochemistry, 4*, 184–192.

Gupta, Y. P. (1987). Antinutritional and toxic factors in food legumes: A review. *Plant Foods for Human Nutrition, 37*, 201–228.

Gupta, K. S., & Wagle, D. S. (1980). *Journal of Food Science, 45*, 394–395, p. 397.

Hangen, L., & Bennink, M. R. (2002). Consumption of black beans and navy beans (*Phaseolus vulgaris*) reduced azoxymethane-induced colon cancer in rats. *Journal of Nutrition and Cancer, 44*, 60–65.

Haug, W., & 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.

Hiai, S., Oura, H., & 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., & Miller, G. A. (1977). A multienzyme technique for estimating protein digestibility. *Journal of Food Science, 42*, 1269–1273.

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

Kadam, S. S., & Smithard, R. R. (1987). Effect of heat treatments on trypsin inhibitor and hemagglutinating activities in winged bean. *Plant Foods for Human Nutrition, 37*, 151.

Kakade, M. L., & Evans, R. J. (1965). Nutritive value of navy beans (Phaseolus vulgaris). *British Journal of Nutrition, 19*, 269–276.

Kakade, M. L., & Evans, R. J. (1966). Growth inhibition of rats fed raw navy beans (Phaseolus vulgaris L.). *Journal of Nutrition, 90*, 191–198.

Kakade, M. L., Rackis, J. J., McGhee, J. E., & Pusk, G. (1974). Determination of trypsin inhibitor activity of soy products: A collaborative analysis of an improved procedure. *Cereal Chemistry, 51*, 376–382.

Kataria, A., Chauhan, B. M., & Punia, D. (1989). Antinutrients and protein digestibility (*in vitro*) of mung bean as affected by domestic processing and cooking. *Food Chemistry, 52*, 9–17.

Khan, N., Zaman, R., & Elahi, M. (1986). Effect of processing on the phytic acid content of wheat products. *Journal of Agricultural and Food Chemistry, 34*, 1010–1012.

Khan, N., Zaman, R., & Elahi, M. (1988). Effect of processing on the phytic acid content of Bengal grams (*Cicer arietinum*) products. *Journal of Agricultural and Food Chemistry, 36*, 1274–1276.

Khokar, S., & Chanhan, B. M. (1986). Effect of domestic processing and cooking on *in vitro* protein digestibility of the north bean. *Journal of Food Science, 51*, 1083–1085.

King, R. D., & Puwastien, P. (1987). Effect of germination on the proximate composition and nutritional quality of winged bean (*Psophocarpus tetragonolobus*) seeds. *Journal of Food Science, 52*, 106–108.

Labaneiah, M. E. O., & Luh, B. S. (1981). Changes of starch, crude fiber, and oligosaccharides in germinating dry beans. *Cereal Chemistry, 58*, 135–138.

Leathwood, P., & Pollet, R. (1988). Effects of slow release carbohydrates in the form of bean flakes on the evaluation of hunger and satiety in man. *Appetite, 10*, 1–11.

Lolas, G. M., & Markakis, P. (1975). Phytic acid and other phosphorus compounds of beans (*Phaseolus vulgaris* L.). *Journal of Agricultural and Food Chemistry, 23*, 13.

Makkar, H. P. S., Aderibige, A. O., & 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.

Marquez, M. C., Fernandez, V., & Alonso, R. (1998). Effect of dry heat on the *in vitro* digestibility and trypsin inhibitor activity of chickpea flour. *International Journal of Food Science and Technology, 33*, 527–532.

Matella, N. J., Dolan, K. D., Stoeckle, A. W., Bennink, M. R., Lee, Y. S., & Uebersax, M. A. (2005). Use of hydration, germination, and α-galactosidase treatments to reduce oligosaccharides in dry beans. *Journal of Food Science, 70*, C203–C207.

Moneam, N. M. A. (1990). Effect of presoaking on faba bean enzyme inhibitors and polyphenols after cooking. *Journal of Agricultural and Food Chemistry, 38*, 1479–1482.

Muzquiz, M., Rey, C., Cuadrado, C., & Fenwick, G. R. (1992). Effect of germination on the oligosaccharide content of lupin species. *Journal of Chromatography, 607*, 349–352.

Noor, M. I., Bressani, R., & Elias, L. G. (1980). Changes in chemical and selected biochemical components, protein quality and digestibility of mung bean (*Vigna radiata*) during germination and cooking. *Plant Foods for Human Nutrition, 30*, 135–144.

Padhye, V. W., & Salunkhe, D. K. (1980). Studies on black gram trypsin inhibitor. *Journal of Food Biochemistry, 4*, 119.

Palmer, R., Mcintosh, A., & Puszati, A. (1973). The nutritional evaluation of kidney beans (*Phaseolus vulgaris*): the effect on nutritional value of seed germination and changes in trypsin inhibitor content. *Journal of the Science of Food and Agriculture, 24*, 937.

Rani, S., Jood, S., & Sehgal, S. (1996). Cultivar differences and effect of pigeon pea seeds boiling on trypsin inhibitor activity and *in vitro* digestibility of protein and starch. *Die Nahrung, 40*, 145–146.

Reddy, N. R., Sathe, S. K., & Salunkhe, D. K. (1982). Phytates in legumes and cereals. *Advanced Food Research, 28*, 1–92.

Salunkhe, D. K. (1982). Legumes in human nutrition. Current status and future research needs. *Current Science, 51*, 387–394.

Sathe, S. K., Deshpande, S. S., & Salunkhe, D. K. (1984). Dry beans of *Phaseolus*: A review, part 1. Chemical composition: proteins. *CRC Critical Reviews in Food Science and Nutrition, 20*, 1–46.

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

Sharma, A., & Sehgal, S. (1992). Effect of domestic processing, cooking and germination on the trypsin inhibitor activity and tannin content of faba bean (*Vicia faba*). *Plant Foods for Human Nutrition, 42*, 127–133.

Shimelis, A. E., & Rakshit, S. K. (2005a). 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., & Rakshit, S. K. (2005b). Antinutritional factors and *in vitro* protein digestibility of improved haricot bean (*Phaseolus vulgaris* L.) varieties grown in Ethiopia. *International Journal of Food Sciences & Nutrition, 56*, 377–387.

Siddhuraju, P., Becker, K., & Makkar, H. P. (2000). Studies on the nutritional composition and antinutritional factors of three different germplasm seed materials of an under-utilized tropical legume, *Mucuna pruriens* Var. *Utilis* (velvet bean). *Journal of Agricultural and Food Chemistry, 40*, 6048–6060.

Silva, H. C., & Luh, B. S. (1979). Changes in oligosaccharides and starch granules in germinating beans. *Canadian Institute of Food Science and Technology, 12*, 103.

Snauwaret, F., & Markakis, P. (1976). Effect of germination and gamma irradiation on the oligosaccharides of navy beans (*Phaseolus vulgaris*). *Lebensmittel-Wissenschaft und-Technologie /Journal of Food Science and Technology (LWT), 9*, 93.

Stecher, P. A. (1968). *The Merck index*. Rahway, NJ: Merck and Company, Inc., pp. 471.

Swaminathan, M. (1974). *Essentials of food and nutrition* (vol. 1). Madras: Ganesh and Co, 158–276.

Upadhyay, J. K., & Garcia, V. V. (1988). Effect of soaking and cooking on reduction of oligosaccharides of cowpea (*Vigna unguiculata* (L.) Walp.). *Philippines Journal of Food Science and Technology, 12*, 21–28.

Van der Poel, T. F. B., Blonk, J., van Zuilichem, D. J., & van Oort, M. G. (1990). Thermal inactivation of lectins and trypsin inhibitor activity during steam processing of dry beans (*Phaseolus vulgaris* L.) and effects on protein quality. *Journal of the Science of Food and Agriculture, 53*, 215–228.

Vijayakumari, K., Siddnuraju, K., & Janardhanan, K. (1996). Effect of soaking, cooking and autoclaving on phytic acid and oligosaccharide contents of the tribal pulse, Mucuna monosperma, Dc.ex.Wight. *Food Chemistry, 55*, 173–177.

Vijayakumari, K., Siddhuraju, P., & Janardhanan, K. (1997). Effect of domestic processing on the levels of certain antinutrients in *Prosopis chilensis* (Molina) Stunz seeds. *Food Chemistry, 59*, 367–371.

Viswanathan, M., Ramachandran, A., Indira, P., Snehalatha, C., Mohan, V., & Kymal, P. K. (1989). Responses to legumes in NIDDM subjects-lower plasma-glucose and higher insulin levels. *Nutrition Reports International, 40*, 803–812.

Wang, N., Lewis, M. J., Brennan, J. G., & Westby, A. (1997). Effect of processing methods on nutrients and antinutritional factors in cowpea. *Food Chemistry, 58*, 59–68.