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Nutrient composition, protein quality and antinutritional factors of some varieties of dry beans (*Phaseolus vulgaris*) grown in Burundi

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Four varieties of dry beans (*Phaseolus vulgaris*) grown in four different areas of Burundi were analyzed for moisture, protein, fat, ash, mineral (potassium, calcium, magnesium, iron, copper, zinc, and phosphorus), essential amino acids (isoleucine, leucine, lysine, methionine, phenylalanine, threonine, valine) and antinutritional factors (raffinose oligosaccharide, trypsin inhibitor, hemagglutinin, tannin and phytic acid) contents. In-vitro digestibility of dry bean proteins was also evaluated. The concentrations observed for the proximate composition, protein quality, and antinutritional factors varied significantly ($P < 0.05$) among the 13 'variety–locality' combinations investigated in this study. Mean values for moisture, protein, fat, ash, and carbohydrate were 9·19%, 22·26%, 1·01%, 4·47%, and 72·33% respectively. They were 525, 55·2, 38·2. 7·63, 0·92, 7·33 and 456 mg/100 g for potassium, calcium, magnesium, iron, copper, zinc and phosphorus, respectively; and 7·35, 14·49, 13·49, 1·59, 9·85, 9·08 and 9·11 mg/g for isoleucine, leucine, lysine, methionine, phenylalanine, threonine and valine, respectively. Finally values for raffinose, stachyose, trypsin inhibitor, hemagglutinin, tannin and phytic acid were respectively 2·20 mg/g, 18·40 mg/g, 15·02 TUI $\times 10^{-3}$/g, 2·15 HU $\times 10^{-3}$/mg, 14·99 mg catechin equivalent/g and 16·50 mg/g. The levels obtained for different nutrients (except for some minerals) and antinutrients were in agreement with those found in dry beans in other areas of the world. Protein digestibility ranged from 67·47% (Calima variety from Kirimiro) to 71·99% (A410 variety from Imbo). These values were comparable to those obtained in other countries. Statistical analyses of data confirmed that nutrient content, protein digestibility and undesirable factors were influenced by both variety and locality. Relationships between some dry bean nutrients and between antinutritional factors and protein digestibility were also observed.

ABBREVIATIONS

A3B	A321 variety from Buragane
A3I	A321 variety from Imbo
A3K	A321 variety from Kirimiro
A3M	A321 variety from Moso
A4B	A410 variety from Buragane
A4I	A410 variety from Imbo
A4K	A410 variety from Kirimiro
A4M	A410 variety from Moso
CB	Calima variety from Buragane
CI	Calima variety from Imbo
CK	Calima variety from Kirimiro
CM	Calima variety from Moso
DB	Dore de Kirundo variety from Buragane
DI	Dore de Kirundo variety from Imbo
DK	Dore de Kirundo variety from Kirimiro
DM	Dore de Kirundo variety from Moso

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INTRODUCTION

Legume seeds are an important part of the human diet in many countries throughout the world, particularly in tropical and subtropical areas (Koehler *et al.*, 1987), constituting an important source of protein, vitamins (especially B-group vitamins), and mineral elements such as potassium, phosphorus, zinc, and magnesium (Meiners *et al.*, 1976). They are also a good source of carbohydrate and food fibres (Koehler *et al.*, 1987). Of the legumes grown in the world, dry beans are the legume most widely consumed in the world as whole seed (Doughty & Walker, 1982). Dry beans are especially cultivated in Latin America, India and Africa (Salunkhe & Kadam, 1989). In Burundi, dry beans are an essential component of human diets and the main source of protein. They are consumed as much by rural as by urban communities, and by rich and poor alike. According to CIAT data (1981), in Burundi, daily dry bean availability (*per capita*) (123 g) is the highest in the world. However, although dry beans are widely

consumed, they take a long time to cook, are poorly digested and cause flatulence.

The flatulence associated with dry beans is mainly caused by oligosaccharides, particularly raffinose, stachyose and verbascose (Vishalakshi *et al.*, 1980). These oligosaccharides are not digested by humans, who lack the necessary alpha-galactosidase, but are instead metabolized by intestinal bacteria, which produce carbon dioxide, hydrogen and methane, resulting in flatulence (Becker *et al.*, 1974).

The low digestibility of dry beans is often attributed to antinutritional factors such as trypsin inhibitor, hemagglutinin, tannin and phytic acid (Hernandez-Infante *et al.*, 1979; Antunes & Sgarbieri, 1980).

Because of their importance in human diets, dry beans are a legume of interest to many investigators. Most previous investigations have dealt with proximate composition and nutritive quality (Watt & Merrill, 1963; Milner, 1975; Besançon, 1978; Sotelo & Hernandez, 1980; Koehler *et al.*, 1987), antinutritional factors (Liener, 1962; Elias *et al.*, 1979; Pusztai *et al.*, 1979; Furuichi *et al.*, 1988; Elkowicz & Sosulski, 1982; Godon, 1985) and detoxification methods (Bressani *et al.*, 1963; Kakade & Evans, 1965; El Nahry *et al.*, 1977; Vishalakshi *et al.*, 1980; Sathe *et al.*, 1984*a,b*; Reddy *et al,* 1986). However, nutrient contents in dry beans vary with variety and locality where the legumes have grown (Quenzer *et al.*, 1978; Deshpande *et al.*, 1984*a*; Koehler *et al.*, 1987).

In Burundi, most of the research on dry beans has been related to varietal selection. The criteria for selection have always been resistance to disease, yields, and rate of maturation, but never nutritive quality (ISABU, 1987). A study of the composition and nutritive quality of dry beans would therefore be of great interest to Burundi, because the knowledge provided would also help to orient the work of investigators involved in varietal selection.

The purpose of this study was to determine the nutrient and antinutrient composition and nutritive protein quality for some dry bean varieties grown in Burundi in order to compare them to those observed in dry beans grown in other areas of the world. At the same time, the results obtained should permit us to select the worst variety (rich in antinutrients and presenting the lowest digestibility) of all 'variety–locality' combinations studied, to be used in future research (consisting of reduction of flatulence and improvement of protein quality by lactic fermentation), and also to verify the influence of variety and locality on nutrient contents, and the existence of relationships either between some nutrients or between antinutritional factors and protein digestibility in dry beans from Burundi.

MATERIALS AND METHODS

Source and preparation of dry beans

Four varieties of dry beans, *Phaseolus vulgaris* (A321, A410, Calima and Dore de Kirundo), grown in four different areas of Burundi, were used in this study.

These varieties were grown by personnel of the Agronomic Science Institute of Burundi. Beans were sown in March 1990 and harvested in June of the same year. Samples were collected after harvesting and sun-drying of the beans. For each variety and locality, three 1-kg portions of dry bean seeds, representing the harvest of three parcels from the same site, were collected, treated with insecticide and put in plastic bags. In all, 39 lots (instead of 48) were constituted.† They were placed in an aluminium box and transported by air to the Food Science and Technology Laboratory of Laval University, Québec, Canada.

For each lot, two samples of 100 g of dry bean seeds were taken, rinsed three times in distilled water to eliminate insecticide, dried in a ventilated oven at 55°C for 24 h, and finely ground in a cyclotex (Tecator, Sweden). The bean powder obtained (80 mesh) was placed in plastic containers and stored at room temperature (20°C). Subsamples were then taken for subsequent analyses of each variety of dry bean. Concentrations of nutrients were always calculated on a dry weight basis.

Chemical analyses

Proximate composition

Moisture, fat, mineral ash and total nitrogen were determined according to procedures 14.003, 7.056, 14.006 and 47.021–47.023 (Kjeldahl method with Kjell–Foss automatic 16210, A/SN Foss Electric, Denmark), respectively, of the Association of Official Analytical Chemists (AOAC, 1980). Carbohydrate content was calculated by difference.

Minerals (except phosphorus) were determined by atomic absorption spectroscopy using a Model IL 751 AA/AE Spectrophotometer (Boston, MA), according to AOAC (1980) procedures: potassium (3000, 3008, 3009, 7094: unheated and without dilution with 3 N HCl), calcium, magnesium (2109, 2110, 2112, 2113, 7094: unheated and without dilution with 3 N HCl), iron, zinc, copper (7091, 7094: unheated and without dilution with 3 N HCl). Phosphorus was assayed by the colorimetric method of Fiske and Subbarow (1925). Certified commercial standards (Sigma Chemical Co., St Louis, MO) were run with every determination.

Amino acid levels of bean powder were determined after 24 h hydrolysis with 6 N HCl according to the method of Spackman *et al.* (1958) using an LKB 4400 amino acid analyzer (Biochrom Ltd, Cambridge, UK). Tryptophan is destroyed by acid hydrolysis and was not determined.

Raffinose oligosaccharides

Extraction (method of Agbo et al., *1985, slightly modified).*To about 1 g of sample in a 50-ml polyethylene centrifuge tube, 10 ml of 80% ethanol:water (v/v) was

† Lots for the A410 variety from Kirimiro and those for the Dore de Kirundo variety from Buragane and Imbo were not collected.

added for the first extraction. The sample was thoroughly mixed using a vortex mixer and then shaken in a water-bath at 80°C for 15 min. The mixture was then centrifuged for 5 min at 653 *g* using a Sorvall RC-5B centrifuge (Du Pont Company, Newtown, USA). The supernatant was transferred to another 50-ml polyethylene tube collector. To the sample residue in the first centrifuge tube, 5 ml of 80% ethanol:water (v/v) was added and the extraction performed as previously described. Finally, 10 ml of 80% ethanol:water (v/v) was added to the same sample residue and the extraction repeated as in the two first steps. The three supernatants were combined in a second polyethylene centrifuge tube, and 2 ml of 10% lead acetate:water (w/v) was added in order to deproteinize the solution. The mixture was shaken using a vortex mixer until homogeneous, and then centrifuged for 10 min at 653 *g*. The extract obtained was transferred to another centrifuge tube, and a solution of 0·5 ml of 10% oxalic acid:water (w/v) was added. This extract was centrifuged for 20 min at 653 *g* to remove the lead oxalate, and the clear extract was then quantitatively transferred to a 25-ml volumetric flask, and brought to volume with deionized water.

Estimation of HPLC (method of Doyon et al., 1991). The liquid chromatograph used was a 'Waters Millipore, Ville-St-Laurent, Qc, Canada' equipped with a UV detector (model 481; 210 nm) and refractometer (model 410). Before chromatography, the prepared extracts were purified by passage through a Sep-pak C18 (Waters Associates, Milford, MA). After this, a standard solution of raffinose oligosaccharides (raffinose, stachyose; Sigma Chemical Co., St Louis, MO) was prepared at a concentration of 1 mg/ml each. Fifteen microlitres of standard solution was injected into the column (separation column Ion-300, Mandel Sci., Rockwood, Ont., Canada) using a 100-μl microsyringe. Finally, the extracts were injected into the HPLC column after filtration through 0·45 μm SM111 cellulose acetate filters (Sartorius, Baxter, Mississauga, Ont., Canada). Samples were eluted with 0·0065 N sulfuric acid at a pump rate of 0·4 ml/min. Then, the unknown samples injected into the HPLC column were quantified by comparison with the standard sugars and their concentration in raffinose oligosaccharides was expressed as mg/g.

Trypsin inhibitor

Trypsin inhibitor activity was assayed according to the AACC 71–10 (AACC, 1980) method. One trypsin unit (TU) is defined as the elevation of 0·01 absorbance unit at 410 nm for 10 ml of reaction mixture. Trypsin inhibitor activity is expressed in trypsin units inhibited (TUI).

Hemagglutinin

Hemagglutinins were evaluated according to the method of Valdebouze *et al.* (1980). One hemagglutinin unit (HU) is defined as the smallest quantity of sample necessary to cause agglutination under experimental conditions. Hemagglutinin activity is expressed in hemagglutinin units per mg of sample.

Tannins

Tannins were assayed according to the modified vanillin–HCl method of Price *et al.* (1978). Analyses were done on samples previously dried in a ventilated oven at 55°C for 24 h. Catechin (Sigma Chemical Co., St Louis, MO) was used as the reference standard, and tannin concentration was expressed in mg of catechin equivalent.

In order to correct for interference from natural pigments in dry beans, a sample blank was prepared by subjecting the original extract to the conditions of the reaction, but without vanillin reagent.

Phytic acid

Phytic acid was evaluated by the method of Eskin *et al.* (1980). Phytic acid was first concentrated on anion exchanged resin. After inorganic phosphate elution with 0.05 M NaCl, phytate was eluted with 0·7 M NaCl. Phytate concentration in eluate was then determined by reaction with the Wade reagent (0·03% $FeCl_3.6H_6O$ and 0·3% sulfosalicylic acid in distilled water). Final color development was measured at 500 nm with a spectrophotometer.

Protein digestibility

Protein digestibility was evaluated according to the method of Hsu *et al.* (1977). This method involves the measurement of the drop in pH which occurs following the digestion of the protein with a mixture of three enzymes: trypsin (porcine pancreatic trypsin type IX, with 14 190 BAEE units per mg powder), chymotrypsin (bovine pancreatic chymotrypsin type II, 60 units per g powder) and peptidase (porcine intestinal peptidase grade III, 40 units per g powder). All these enzymes were purchased from Sigma Chemical Co., St Louis, MO). Percent protein digestibility (Y) was calculated from the equation $Y = 210{\cdot}464 - 18{\cdot}10X$, where X is the pH change after 10 min.

Statistical analysis

Data were subjected to analysis of variance, Duncan–Waller Multiple Range Test and calculation of correlation coefficient, where applicable (Steel & Torrie, 1980).

RESULTS AND DISCUSSION

Proximate composition

Concentrations of moisture, protein, fat, ash and carbohydrate for the different 'variety–locality' combinations studied are presented in Table 1. For all the combinations, significant differences ($P < 0{\cdot}05$) were observed in the nutrient contents.

Proximate composition concentrations varied from

Table 1. Proximate nutrient contents of dry beans from Burundi
(means of three replicate analyses, expressed as % (DWB))

'Variety–locality' combination	Moisture	Protein	Fat	Ash	Carbohydrate
A3B	9·35^{abc}	17·75^{g}	1·13^{bcd}	4·79^{bc}	76·33^{ab}
A3I	8·85^{c}	24·31^{b}	0·88^{gh}	4·82^{bc}	70·07^{f}
A3K	9·16^{abc}	23·99^{bc}	1·02^{def}	4·25^{def}	70·73^{ef}
A3M	8·87^{c}	22·18de	1·07^{cde}	4·34^{cdef}	72·47^{cd}
A4B	8·71^{c}	23·06^{cd}	0·91^{fgh}	4·36^{cdef}	71·71de
A4I	8·87^{c}	27·54^{a}	0·66^{i}	5·44^{a}	66·39^{g}
A4M	9·11^{bc}	22·32de	1·03^{def}	4·57^{bcd}	72·11^{cd}
CB	9·36^{abc}	22·23de	0·96^{efg}	4·43^{cde}	72·40^{cd}
CI	9·21^{abc}	22·67de	0·78^{hi}	5·04^{ab}	71·55de
CK	10·08^{a}	19·25^{f}	1·23^{ab}	4·24^{def}	75·35^{b}
CM	9·11^{bc}	23·89^{bc}	0·96^{efg}	4·01^{ef}	71·49de
DK	9·94^{ab}	18·13^{g}	1·27^{a}	3·88^{f}	76·79^{a}
DM	8·96^{c}	22·03^{e}	1·19^{abc}	3·91^{f}	72·93^{c}
Mean	9·19	22·26	1·01	4·47	72·33
Coeff. Var.	5·06	2·80	8·82	6·73	0·90

[a-i] Means in the same column with different superscripts are significantly different ($P < 0.05$).

8·77 (A4B) to 10·08% (CK), from 17·75 (A3B) to 27·54% (A4I), from 0·66 (A4I) to 1·27% (DK), from 3·88 (DK) to 5·44% (A4I) and from 66·39 (A4I) to 76·79% (A3B), respectively, for moisture, protein, fat, ash and carbohydrate. These values are similar to those observed for the dry bean varieties grown in the USA (Watt & Merrill, 1963; Naivikul & d'Appolonia, 1978; Koehler *et al.*, 1987) for protein (17·5–28·7%), ash (3·2–5·0%) and moisture (8·3–11·3%); in Egypt (El Nahry *et al.*, 1977) and in Lebanon (Kuzayili *et al.*, 1966) for protein (21·7%); and in Brazil (De Moraes & Angelucci, 1971; Antunes & Sgarbieri, 1980) for protein (26·3%) and fat (2·0%).

Proximate composition concentrations were, however, lower than those published for some varieties grown in the USA (Koehler & Burke, 1981; Salunkhe & Kadam, 1989) for protein (21·1–39·4%), fat (1·2–2%) and moisture (11·5–11·9%); in Egypt (El Nahry *et al.*, 1977) for fat (2·57%) and moisture (13·26%), and also in Brazil (Antunes & Sgarbieri, 1980) and in Lebanon (Kusayili *et al.*, 1966) with regard to fat (2%) and moisture (10·4–11·7%). Concentrations observed were higher than those obtained for varieties from Lebanon (Kuzayili *et al.*, 1966) for moisture (6·7%) and carbohydrates (53·97–64·42%) and for varieties grown in the USA (Naivikul & d'Appolonia, 1978), in Egypt (El Nahry *et al.*, 1977) and in Brazil (Antunes & Sgarbieri, 1980) with regard to ash (USA: 3·45%; Egypt: 3·77%; Brazil: 3·2%). For protein, the lowest concentration was observed the A321 variety from Buragane (A3B). The protein concentration of the Dore de Kirundo variety was slightly higher than that of A3B, but lower than that of the Calima variety also grown in Kirimiro (CK). Protein concentration of DK (18·13%) was not significantly different from that of A3B. On the other hand, it was significantly lower than the protein concentration of CK.

Table 2. Relations between some nutrients and between protein digestibility and antinutritional factors in dry beans

Relation	Correlation coefficient	Probability	Significance of correlation[a]
Protein–Carbohydrate	−0·99	0·000 1	+++
Protein–Fat	−0·83	0·000 5	+++
Protein–Ash	0·46	0·115 5	—
Protein–Methionine	0·42	0·148 2	—
Ca–Mg	0·45	0·125 3	—
Fe–Cu	0·32	0·289 9	—
Fe–Zn	0·33	0·272 4	—
Zn–Cu	−0·14	0·657 5	—
Digestibility–Trypsin Inhibitor	−0·90	0·0001	+++
Digestibility–Hemagglutinin	0·19	0·526 2	—
Digestibility–Tannin	0·16	0·604 1	—
Digestibility–Phytic acid	−0·45	0·121 4	—
Protein–Trypsin Inhibitor	−0·31	0·294 2	—
Protein–Hemagglutinin	0·06	0·853 8	—
Protein–Tannin	0·20	0·507 7	—
Protein–Phytic acid	0·38	0·199 4	—
Ash–Phytic acid	0·76	0·002 5	++
Carbohydrate–Hemagglutinin	0·03	0·910 6	—

[a] +++ Very highly significant ($P < 0.001$); ++ highly significant ($P < 0.01$);–not significant ($P > 0.05$).

In this study, proximate composition concentrations of dry beans were significantly influenced ($P < 0.05$) by variety and locality, and also by the variety–locality interaction ($P < 0.01$), except for fat. This influence has also been reported by other investigators, e.g. Hosfield and Uebersax (1980), Deshpande *et al.* (1984*a*) and Koehler *et al.* (1987). Moreover, significant negative correlations were observed between protein concentration and fat ($r = -0.83$), and between protein and carbohydrate ($r = -0.99$), (Table 2). These correlations have also been reported by Deshpande *et al.* (1984*a*). No relationship was, however, observed between protein and ash, or between protein and methionine. The latter correlation has nevertheless been reported by Summerfield and Bunting (1978).

Mineral elements

Mineral element concentrations observed for dry bean varieties analyzed are presented in Table 3. There were significant differences ($P < 0.05$) among them. Values (mg/100 g) ranged from 442 (DK) to 631 (A4I) for potassium, from 24·8 (DK) to 72·6 (CM) for calcium, from 28·1 (DK) to 43·8 (A3B) for magnesium, from 6·02 (A4I) to 9·49 (CK) for iron, from 0·70 (A4M) to 1·28 (CK) for copper, from 6·35 (DM) to 8·79 (A3I) for zinc, and from 360 (CK) to 665 (A4I) for phosphorus.

Similar concentrations have been published for calcium (26·8–78·1 mg/100 g) in dry bean varieties grown in Brazil (De Moraes & Angelucci, 1971) and in

Table 3. Mineral element contents of dry beans
(means of three replicate analyses, expressed as mg/100 g (DWB))

'Variety–locality' combination	K	Ca	Mg	Fe	Cu	Zn	P
A3B	554^{b}	$66{\cdot}6^{b}$	$43{\cdot}8^{a}$	$6{\cdot}88^{de}$	$1{\cdot}01^{b}$	$7{\cdot}51^{bcd}$	496^{d}
A3I	562^{b}	$50{\cdot}5^{hi}$	$42{\cdot}5^{ab}$	$8{\cdot}80^{ab}$	$0{\cdot}98^{bc}$	$8{\cdot}79^{a}$	584^{c}
A3K	515^{cd}	$51{\cdot}9^{gh}$	$40{\cdot}8^{abc}$	$7{\cdot}94^{bc}$	$0{\cdot}86^{cde}$	$8{\cdot}17^{ab}$	363^{j}
A3M	519^{c}	$59{\cdot}1^{ed}$	$40{\cdot}9^{abc}$	$8{\cdot}62^{ab}$	$1{\cdot}02^{b}$	$7{\cdot}09^{cdef}$	400^{ghi}
A4B	572^{b}	$49{\cdot}3^{hi}$	$41{\cdot}2^{ab}$	$7{\cdot}09^{cd}$	$0{\cdot}79^{ef}$	$7{\cdot}81^{bc}$	418^{fgh}
A4I	631^{a}	$54{\cdot}4^{fg}$	$42{\cdot}2^{ab}$	$6{\cdot}02^{e}$	$1{\cdot}04^{b}$	$6{\cdot}37^{f}$	655^{a}
A4M	524^{c}	$62{\cdot}4^{c}$	$39{\cdot}3^{bcd}$	$7{\cdot}43^{cd}$	$0{\cdot}70^{f}$	$8{\cdot}02^{ab}$	431^{efg}
CB	486^{de}	$59{\cdot}8^{cde}$	$35{\cdot}5^{de}$	$7{\cdot}07^{cde}$	$0{\cdot}84^{de}$	$6{\cdot}47^{ef}$	449^{ef}
CI	628^{a}	$61{\cdot}10^{cd}$	$37{\cdot}1^{cde}$	$6{\cdot}83^{de}$	$0{\cdot}87^{cde}$	$6{\cdot}59^{ef}$	584^{b}
CK	475^{ef}	$56{\cdot}7^{ef}$	$36{\cdot}5^{de}$	$9{\cdot}49^{a}$	$1{\cdot}28^{a}$	$6{\cdot}87^{def}$	360^{j}
CM	447^{fg}	$72{\cdot}6^{a}$	$34{\cdot}1^{e}$	$8{\cdot}83^{ab}$	$0{\cdot}75^{ef}$	$7{\cdot}33^{bcde}$	382^{ij}
DK	442^{g}	$24{\cdot}8^{j}$	$28{\cdot}1^{f}$	$7{\cdot}12^{cd}$	$0{\cdot}96^{bcd}$	$7{\cdot}99^{ab}$	384^{hij}
DM	472^{efg}	$48{\cdot}2^{i}$	$34{\cdot}2^{e}$	$7{\cdot}09^{cd}$	$0{\cdot}79^{ef}$	$6{\cdot}35^{f}$	455^{e}
Mean	525	55·2	38·2	7·63	0·92	7·33	456
Coeff. Var.	3·69	3·82	6·74	8·48	8·98	7·48	5·04

$^{a-i}$ Means in the same column with different superscripts are significantly different ($P < 0{\cdot}05$).

Lebanon (Kuzayili *et al.*, 1966), and also for iron (7·8–9 mg/100 g) in dry beans grown in the USA (Koehler & Burke, 1981) and in Egypt (El Nahry *et al.*, 1971). Concentrations obtained were generally similar to those observed in other countries for phosphorus, iron and copper, but higher for zinc and lower for potassium, calcium and magnesium.

According to the results obtained in this study, mineral element levels were significantly influenced ($P < 0{\cdot}05$) by variety and locality. Interaction between variety and locality was also an important source of variation ($P < 0{\cdot}05$) for mineral element contents except magnesium. The influence of variety on mineral element concentrations in dry beans has also been observed by Quenzer *et al.* (1978) and Koehler *et al.* (1987), particularly for calcium and magnesium. Influences exerted by locality have been reported by Salunkhe and Kadam (1989) and also by Quenzer *et al.* (1978).

No correlation was observed between calcium and magnesium. However, such a correlation has been reported by other investigators, such as Quenzer *et al.* (1978).

Essential amino acids

Essential amino acid contents for the dry bean varieties analyzed are presented in Table 4. For all the amino acids studied, significant differences ($P < 0{\cdot}05$) among them were observed. Amino acid concentrations (mg/g) ranged from 5·95 (A3B) to 9·01 (A4I) for isoleucine, from 10·98 (A3B) to 17·57 (A4I) for leucine, from 10·47 (A3B) to 15·83 (A4I) for lysine, from 0·64 (DK) to 2·31 (A4M) for methionine, from 6·90 (A3B) to 11·98 (A4I) for phenylalanine, from 7·38 (A3B) to 10·95 (A4I) for threonine, and from 8·08 (A3B) to 10·02 (DK) for valine. The A410 variety from Imbo had the highest concentration of essential amino acids, while the A321 variety from Buragane showed the lowest. The same table shows high levels of lysine and low levels of methionine for the varieties analyzed. A comparison of Dore de Kirundo and Calima varieties revealed that Dore de Kirundo from Kirimiro (DK) had the lowest methionine concentration and that methionine content in the Calima variety from Kirimiro (CK) was also low but significantly higher than those of DK.

Some investigators, such as Koehler *et al.* (1987), have observed similar values, except for valine (9–14 mg/g), for dry bean varieties grown in the USA. Concentrations published by Sathe (1982) are also in agreement with those we obtained, except for methionine (0·5 mg/g) and leucine (5·3 mg/g). For Summerfield and Bunting (1978), essential amino acid levels in dry beans, except for leucine (1·94 mg/g), are similar to those observed in dry bean varieties of Burundi.

Table 4. Essential amino acid contents (mg/g (DWB)) and protein digestibility (%) for dry beans
(means of three replicate analyses; tryptophan not determined)

'Variety locality'–combination	Ile	Leu	Lys	Met	Phe	Thr	Val	In-vitro protein digestibility (%)
A3B	$5{\cdot}95^{c}$	$10{\cdot}98^{c}$	$10{\cdot}41^{h}$	$0{\cdot}93^{de}$	$6{\cdot}90^{g}$	$7{\cdot}38^{f}$	$8{\cdot}08^{d}$	$68{\cdot}68^{f}$
A3I	$7{\cdot}02^{bc}$	$15{\cdot}28^{b}$	$14{\cdot}51^{bc}$	$2{\cdot}00^{ab}$	$10{\cdot}35^{cd}$	$9{\cdot}81^{b}$	$8{\cdot}92^{bcd}$	$68{\cdot}38^{f}$
A3K	$8{\cdot}61^{a}$	$16{\cdot}86^{a}$	$15{\cdot}36^{ab}$	$1{\cdot}46^{bcd}$	$11{\cdot}23^{ab}$	$9{\cdot}85^{b}$	$9{\cdot}99^{a}$	$70{\cdot}79^{c}$
A3M	$7{\cdot}25^{b}$	$14{\cdot}56^{bc}$	$13{\cdot}28^{def}$	$1{\cdot}48^{bcd}$	$9{\cdot}72^{de}$	$9{\cdot}24^{bcd}$	$9{\cdot}17^{abc}$	$71{\cdot}99^{a}$
A4B	$7{\cdot}36^{b}$	$14{\cdot}15^{cd}$	$13{\cdot}13^{ef}$	$1{\cdot}03^{cde}$	$9{\cdot}51^{de}$	$8{\cdot}75^{cde}$	$8{\cdot}62^{cde}$	$70{\cdot}19^{d}$
A4I	$9{\cdot}01^{a}$	$17{\cdot}57^{a}$	$15{\cdot}83^{a}$	$1{\cdot}42^{bcde}$	$11{\cdot}98^{a}$	$10{\cdot}95^{a}$	$9{\cdot}66^{a}$	$69{\cdot}58^{e}$
A4M	$7{\cdot}38^{b}$	$14{\cdot}87^{bc}$	$13{\cdot}64^{cde}$	$2{\cdot}31^{a}$	$10{\cdot}03^{d}$	$9{\cdot}51^{bc}$	$9{\cdot}35^{abc}$	$70{\cdot}79^{c}$
CB	$7{\cdot}26^{b}$	$14{\cdot}74^{bc}$	$13{\cdot}52^{de}$	$1{\cdot}94^{ab}$	$10{\cdot}03^{d}$	$9{\cdot}17^{bcd}$	$9{\cdot}28^{abc}$	$68{\cdot}68^{f}$
CI	$6{\cdot}77^{bc}$	$14{\cdot}67^{bc}$	$14{\cdot}16^{cd}$	$2{\cdot}01^{ab}$	$10{\cdot}31^{cd}$	$8{\cdot}56^{de}$	$8{\cdot}13^{d}$	$68{\cdot}68^{f}$
CK	$6{\cdot}71^{bc}$	$12{\cdot}01^{e}$	$11{\cdot}81^{g}$	$1{\cdot}69^{abcd}$	$8{\cdot}33^{f}$	$7{\cdot}92^{ef}$	$8{\cdot}51^{cd}$	$67{\cdot}47^{g}$
CM	$7{\cdot}89^{ab}$	$15{\cdot}52^{b}$	$14{\cdot}11^{cd}$	$1{\cdot}97^{ab}$	$10{\cdot}91^{bc}$	$9{\cdot}20^{bcd}$	$9{\cdot}86^{ab}$	$71{\cdot}39^{b}$
DK	$7{\cdot}06^{bc}$	$13{\cdot}19^{d}$	$12{\cdot}45^{fg}$	$0{\cdot}64^{e}$	$9{\cdot}09^{ef}$	$8{\cdot}72^{cde}$	$10{\cdot}02^{a}$	$67{\cdot}67^{g}$
DM	$7{\cdot}35^{b}$	$14{\cdot}10^{cd}$	$13{\cdot}18^{def}$	$1{\cdot}79^{abc}$	$9{\cdot}68^{de}$	$8{\cdot}92^{c}$	$8{\cdot}52^{cd}$	$69{\cdot}28^{e}$
Mean	7·35	14·49	13·49	1·59	9·85	9·08	9·11	69·49
Coeff. Var.	9·32	4·89	4·67	28·35	5·56	6·04	6·42	0·55

$^{a\ h}$ Means in the same column with different superscripts are significantly different ($P < 0{\cdot}05$).

Table 5. Raffinose oligosaccharides (Means of three replicate analyses, expressed as mg/g (DWB) and antinutritional factor (Means of three replicate analyses expressed as mg/g (DWB) for tannin and phytic acid, TUI/mg (DWB) for trypsin inhibitor; and HU/μg (DWB) for hemagglutinin) content of dry bean varieties

'Variety–locality' combination	Stachyose	Raffinose	Tannin	Phytic acid	Trypsin inhibitor	Hemagglutinin
A3B	$18{\cdot}85^{b}$	$2{\cdot}76^{ab}$	$8{\cdot}02^{f}$	$16{\cdot}75^{c}$	$12{\cdot}80^{gh}$	$0{\cdot}86^{e}$
A3I	$18{\cdot}66^{b}$	$2{\cdot}77^{ab}$	$0{\cdot}11^{h}$	$19{\cdot}89^{b}$	$17{\cdot}37^{cd}$	$0{\cdot}86^{e}$
A3K	$17{\cdot}99^{b}$	$2{\cdot}92^{a}$	$10{\cdot}06^{e}$	$13{\cdot}99^{d}$	$12{\cdot}14^{h}$	$1{\cdot}72^{d}$
A3M	$15{\cdot}95^{b}$	$2{\cdot}58^{b}$	$5{\cdot}35^{g}$	$13{\cdot}09^{d}$	$4{\cdot}77^{k}$	$1{\cdot}73^{d}$
A4B	$18{\cdot}90^{b}$	$2{\cdot}79^{ab}$	$28{\cdot}78^{a}$	$12{\cdot}87^{d}$	$14{\cdot}94^{ef}$	$0{\cdot}43^{f}$
A4I	$15{\cdot}58^{b}$	$2{\cdot}67^{b}$	$21{\cdot}99^{b}$	$23{\cdot}60^{a}$	$16{\cdot}49^{de}$	$0{\cdot}86^{e}$
A4M	$17{\cdot}79^{b}$	$2{\cdot}94^{a}$	$28{\cdot}14^{a}$	$14{\cdot}08^{d}$	$7{\cdot}17^{i}$	$1{\cdot}71^{d}$
CB	$17{\cdot}55^{b}$	$1{\cdot}27^{e}$	$15{\cdot}03^{d}$	$19{\cdot}55^{b}$	$19{\cdot}42^{bc}$	$3{\cdot}46^{c}$
CI	$15{\cdot}77^{b}$	$1{\cdot}99^{c}$	$19{\cdot}28^{c}$	$21{\cdot}11^{b}$	$21{\cdot}04^{b}$	$1{\cdot}71^{d}$
CK	$18{\cdot}51^{b}$	$1{\cdot}27^{e}$	$19{\cdot}12^{c}$	$16{\cdot}75^{c}$	$21{\cdot}59^{b}$	$0{\cdot}87^{e}$
CM	$18{\cdot}18^{b}$	$1{\cdot}33^{e}$	$18{\cdot}76^{c}$	$12{\cdot}37^{d}$	$5{\cdot}68^{j}$	$6{\cdot}83^{b}$
DK	$22{\cdot}60^{a}$	$1{\cdot}69^{d}$	$10{\cdot}85^{e}$	$13{\cdot}04^{d}$	$27{\cdot}89^{a}$	$1{\cdot}72^{d}$
DM	$22{\cdot}86^{a}$	$1{\cdot}65^{d}$	$9{\cdot}33^{ef}$	$17{\cdot}44^{c}$	$13{\cdot}96^{fg}$	$8{\cdot}89^{a}$
Mean	18·40	2·20	14·99	16·50	15·02	2·15
Coeff. Var.	10·61	7·12	8·88	7·56	9·18	37·94

$^{a-h}$ Means in the same column with different superscripts are significantly different ($P < 0{\cdot}05$).

Similar observations have been reported by Hernandez-Infante *et al.* (1979). Higher concentrations than those obtained in our study have been observed by Koehler and Burke (1981) and by Sathe (1982) for dry bean varieties grown in the USA. De Moraes and Angelucci (1971) have observed, in dry beans varieties from Brazil, lysine and methionine concentrations higher than those we obtained.

Essential amino acid concentrations, in dry bean varieties studied, were significantly influenced ($P < 0{\cdot}05$) by variety and locality but for valine and isoleucine. considerable variation ($P < 0{\cdot}05$) due to variety–locality interaction was also observed in amino acid contents in dry beans. However, correlation between protein and methionine levels, which has been reported by other investigators (Summerfield and Bunting, 1978) was not observed in this study.

Protein digestibility

Data on in-vitro digestibility of proteins of dry bean varieties studied are presented in Table 4. Significant differences ($P < 0{\cdot}05$) among them were observed. They ranged from 67·47% (CK) to 71·99% (A3M). The Calima variety from Kirimiro was the least digestible, followed by the Dore de Kirundo variety grown in the same area. However, the levels of digestibility for the two varieties were not significantly different.

Similar values (66·9–70·9%) to those obtained have been published by Deshpande *et al.* (1984*b*) for dry bean varieties grown in the USA. Pusztai *et al.* (1979) and Sathe *et al.* (1984*a*) have also observed levels of protein digestibility similar to ours, but for some dry bean varieties their values (43·5–74%) were slightly higher than those observed in this study. Lower values for protein digestibility (36·3–56·0%) have been reported by Salunkhe and Kadam (1989).

Protein digestibility for dry bean varieties studied was highly influenced ($P < 0{\cdot}01$) by variety and locality and by the variety–locality interaction. Deshpande *et al.* (1984*a*) have observed similar relations for protein digestibility in dry bean varieties grown in the USA.

Raffinose oligosaccharides

Flatus factor (raffinose, stachyose) concentrations of dry bean varieties studied are presented in Table 5. There were significant differences ($P < 0{\cdot}05$) among them. They ranged from 15·58 (A4I) to 22·86 mg/g (DM) for stachyose, and from 1·65 (DM) to 2·94 mg/g (A4M) for raffinose. On comparing the Dore de Kirundo variety (DK) to the Calima variety (CK), both grown in Kirimiro and considered the least digestible dry bean varieties of this study, it was possible to see that flatus factor concentrations were significantly higher for DK than for CK.

Similar concentrations to those obtained (raffinose: 2·6 mg/g; stachyose: 21·6 mg/g) have been reported by Sosulski *et al.* (1982) for dry bean varieties grown in Canada (raffinose: 3·7 mg/g; stachyose: 23·6 mg/g). However, some investigators (Naivikul & d'Appolonia, 1978; Agbo, 1982; Sathe *et al.*, 1983; Reddy *et al.*, 1984; Salunkhe & Kadam, 1989), who assayed dry bean varieties grown in the USA for flatus factor, have observed raffinose (2–10 mg/g) and stachyose (2–56·2 mg/g) concentrations higher than those obtained for dry bean varieties grown in Burundi.

According to the results obtained, highly significant differences ($P < 0{\cdot}01$) in seed raffinose content among localities or varieties were noted. However, significant differences in stachyose content due to variety were not observed. Finally, it has been observed that raffinose and stachyose contents of seed beans were significantly ($P < 0{\cdot}01$) influenced by the variety–locality interaction. These results are in agreement with those of Kosson and Bakowski (1986).

Antinutritional factors

Antinutritional factor (trypsin inhibitor, hemagglutinin, tannin, and phytic acid) concentrations for the dry bean varieties studied are presented in Table 5. Significant differences ($P < 0.05$) among them were observed. Antinutritional factor contents ranged from 4·77 (A3M) to 27·98 (DK) TUI $\times 10^{-3}$/g for trypsin inhibitor; from 0·43 (A4B) to 6·98 (DM) HU $\times 10^{-3}$/mg for hemagglutinin; from 0·11 (A3I) to 28·78 (A4B) mg catechin equivalent/g for tannin; and from 12·37 (CM) to 23·60 (A4I) mg/g for phytic acid.

Similar concentrations have been published for dry beans varieties grown in other countries for trypsin inhibitor (12–20 TUI $\times 10^{-3}$/g; Besançon, 1978; Elkowicz & Sosulski, 1982), hemagglutinin (3–13 HU $\times 10^{-3}$/mg; Besançon, 1978; Summerfield & Bunting, 1978), tannin (0·3–29·3 mg catechin equivalent/g; (Sathe *et al.*, 1983; Aw & Swanson, 1985), and phytic acid (18·1–27·5 mg/g; Deshpande & Cheryan, 1983; Sathe *et al.*, 1983). However, Deshpande and Cheryan (1983) and Reddy *et al.* (1985) have observed, for dry bean varieties grown in the USA, lower tannin concentrations (0·34–26·5 mg catechin equivalent/g) than those obtained in this study. The same investigators have also reported a phytic acid (21·6–27 mg/g) content higher than that observed in dry bean varieties grown in Burundi.

Results of variance analysis indicated that antinutritional factor concentrations were highly influenced ($P < 0.01$) by locality and combination of variety and locality. Significant differences in seed antinutritional factor contents among varieties were also observed for hemagglutinin ($P < 0.05$), trypsin inhibitor and tannins ($P < 0.01$), but not for phytic acid. These influences have also been reported by Pusztai *et al.* (1979) and Sosulski *et al.* (1982) for dry bean varieties grown in the USA. In addition to this double influence on tannin content, tannin concentration seemed to be influenced by the color of dry bean seeds. Colored bean seeds indeed presented higher tannin concentrations than those obtained in white bean seeds. This observation has also been made by Sotelo and Hernandez (1980) for tannin in dry bean varieties grown in the USA.

Some relationships were verified in this study between antinutritional factors and nutrients in beans. Results show a significant positive correlation ($r = 0.76$) between ash and phytic acid concentrations (Table 2). However, no correlation was observed between antinutritional factor and protein concentrations. Neither did any relationship exist between hemagglutinin, phytic acid or tannin content and in-vitro digestibility of protein, although a significant negative correlation between tannin concentration and protein digestibility has been reported elsewhere, e.g. by Sosulski *et al.* (1982) and Aw and Swanson (1985). In this study we have, however, observed a negative correlation ($r = -0.90$) between trypsin inhibitor and protein digestibility for the dry bean varieties analyzed (Table 2). This relationship has also been reported by Kakade and Evans (1965), Hernandez-Infante *et al.* (1979), Pusztai *et al.* (1979) and Furuichi *et al.* (1988). According to these investigators the negative relation which exists between trypsin inhibitor and protein digestibility may be implicated in the reduction of nutritive protein quality in dry beans.

CONCLUSION

In this study, composition, protein quality and antinutritional factor concentrations were determined in 13 'variety–locality' combinations of dry beans grown in Burundi. Two varieties presented the lowest digestibility, Calima (CK) and Dore de Kirundo (DK), both grown in Kirimiro. A comparison of the two varieties revealed that DK presented lower protein and methionine and higher raffinose, stachyose, hemagglutinin and trypsin inhibitor concentrations than CK. DK was thus considered the worst of all 'variety–locality' combinations studied.

Moreover, results obtained in this study permitted us to observe:

—the influence of variety and locality on composition, protein quality, and antinutritional factor concentrations in dry beans;
—relationships between some dry bean nutrients or between antinutritional factors and protein digestibility.

Generalization of the study to all dry bean varieties grown in Burundi could lead to results which would help investigators to select dry bean varieties with high nutritive value. Meanwhile, we are currently attempting to use a lactic acid fermentation to reduce flatulence and improve the protein quality of the DK variety, selected as being the worst of all varieties in the present study.

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