

Determination of total, soluble and insoluble dietary fibre in two new varieties of *Phaseolus vulgaris* L. using chemical and enzymatic gravimetric methods

O. E. Garcia,^a R. B. Infante^{a*} & C. J. Rivera^b

^aLaboratorio de Investigaciones Bioanálisis-Nutrición, Escuela de Nutrición y Dietética, Facultad de Medicina, U.C.V., Apartado Postal No. 47176, Los Chaguaramos, Caracas, Venezuela

^bLaboratorio de Investigaciones Bioanálisis-Nutrición, Escuela de Bioanálisis, Facultad de Medicina, U.C.V., Apartado Postal No. 47176, Los Chaguaramos, Caracas, Venezuela

(Received 3 March 1995; accepted 30 October 1995)

Two new varieties of uncooked legumes were analysed by proximal analysis and biochemical methods. The 'Montalban' variety of black kidney beans showed values of protein, fat, carbohydrate and ash very similar to those of the 'Tacarigua' variety. The application of chemical-gravimetric methods to measure acid detergent fibre (ADF) and neutral detergent fibre (NDF) yielded higher values of NDF (32.1%) for 'Montalban' than for 'Tacarigua' (15.4%), while ADF values were higher for 'Tacarigua' (14.5%) than for 'Montalban' (6.9%). The application of the Hellendoorn enzymatic-gravimetric method showed similar values of unavailable carbohydrate for both varieties; however, these values (21.6%) were higher than those previously reported for food grain legumes in the literature using the same method. The determination of soluble dietary fibre (SDF) yielded insignificant values for the 'Tacarigua' variety; the insoluble dietary fibre (IDF) was higher for the 'Montalban' variety (16.8%) and the total dietary fibre was higher for the 'Montalban' variety (19.9%) using the Prosky method. These differences could possibly be due to the higher content of SDF and to the starch quality of the 'Montalban' variety. © 1997 Elsevier Science Ltd. All rights reserved

INTRODUCTION

The family of Leguminosae is distributed throughout most continents, occurring as 600 genera and approximately 13 000 species. In Latin America a significant number of species and varieties are consumed by much of the human population, as they represent an inexpensive source of protein (Cardenas, 1986; Jaffé, 1986). In Venezuela the legume seeds have a high level of consumption in the order: *Phaseolus vulgaris* > *Pisum sativum* > *Cajanus cajan*.

P. vulgaris is a herb, an annual plant, prostrate or erect, often climbing, with a well-developed central root, reaching an average of 90 cm deep and presenting spherical or irregular nodules. Stems are thin, twisted and angular; innervate leaves are alternates. Flowers are borne on axillary branches. Generally there are few flowers; colours are white, yellow, pink or violet.

Pods are thin (7.5–20 cm × 1.0 cm × 1.5 cm), straight or slightly curved; borders are rounded; colour may be

yellow, green or deep green, sometimes spotted with pink or purple; the number of seeds varies from 1 to 12.

P. vulgaris is a polymorphic species and it has not been possible to classify it into subspecies. Several cultivators have divided it into five races according to its seed size, and approximately 500 varieties are known. The plant is both wild and domesticated; it has adapted to diverse soil types, rainfall levels, temperature and altitudes.

The average world production of *P. vulgaris* is 500 kg ha⁻¹, although the variations from one country to another are often large: for the USA, the mean is 1200 kg ha⁻¹, for Brazil 600 kg ha⁻¹, the Dominican Republic 4000–5000 kg ha⁻¹ and for Mexico 450 kg ha⁻¹ (Kay, 1979).

The analytical methods for dietary fibre can be divided into two types: those that measure total dietary fibre and are usually gravimetric, and those that quantify the various components of dietary fibre usually by analytical means, such as colorimetry, gas-liquid

*To whom correspondence should be addressed.

chromatography, high-performance liquid chromatography (Lanza & Butrum, 1986).

The definition of dietary fibre (DF) as the sum of lignin and the plant polysaccharides that are not digested by the endogenous secretions of the human digestive tract (Trowell, 1976) is a physiological, not a chemical, definition. It covers a wide variety of chemical substances with different physical properties as well as various physiological effects (Kritchevsky, 1988). Although the total dietary fibre (TDF) method approved by the Association of Official Analytical Chemists (AOAC) has been chosen by many laboratories (Lee *et al.*, 1992), there are reports of DF analysis using neutral detergent fibre (NDF) and acid detergent fibre (ADF) methods, which are often used for legume grains (Méndez *et al.*, 1993).

In the present study the amounts of insoluble (IDF), soluble (SDF) and total (TDF) dietary fibre were determined in two varieties of *P. vulgaris* by the method of Prosky *et al.* (1988). NDF and ADF were determined by the methods of Van Soest (1963), Van Soest and Wine (1967) and by the method of Hellendoorn *et al.* (1975). The results obtained were compared to establish their relative merits.

MATERIALS AND METHODS

The seeds of two varieties of *P. vulgaris* labelled 'Tacarigua' and 'Montalban' (black beans) were purchased from the National Center of Agropecuary Research (CENIAP), Maracay, Aragua State, Venezuela.

Both new varieties are erect plants that belong to the family Leguminosae. The average seed number per pod is six for the 'Tacarigua' variety. The seed colour is opaque black; they are short (10.9 mm×6.7 mm) and have an average weight of 0.24 g per grain. The period from sowing to harvest is 75–80 days; the average yield is 1683 kg ha⁻¹ (Ortega & Barrios, 1972).

The seeds of 'Montalban' variety are opaque black, packed into curved pods, short (10.2 mm×6.2 mm), with an average weight of 0.205 g per grain. It takes 78–80 days from sowing to harvest, with an average yield of 2150 kg ha⁻¹ (Ortega *et al.*, 1987).

The legume grains were ground in a Tecator mill (Multiciclon, Sweden) with a 0.5-mm screen, and then passed through a 60-mesh sieve. About 400 g of each variety were dried and stored in sealed flasks.

Milled samples were analysed for moisture, protein, fat, ash and crude fibre according to standard procedures (AOAC, 1990). The factor 6.25 was used throughout for conversion of nitrogen to crude protein.

Acid detergent method

One gram of each sample was boiled for 1 h with a solution of cetyltrimethylammonium bromide in 1 N H₂SO₄. After filtration the residue was washed with hot water, acetone and alcohol, then dried, weighed and

ashed for 4 h in a muffle furnace at 525°C. The residues were ashed to correct for inorganic matter (Van Soest 1963).

Neutral detergent method

One gram of sample was boiled for 1 h in a sodium lauryl sulphate solution at neutral pH. After filtration the residue was washed with hot water, acetone and ethanol, then dried, weighed and ashed for 4 h in a muffle furnace at 525°C. The residues were ashed to correct for inorganic matter (Van Soest & Wine, 1967).

Hellendoorn method

One gram of sample suspended in 100 ml of 0.1 N HCl was digested with 1 mg ml⁻¹ of pepsin (Sigma Chemical Co., St. Louis, MO, USA), then, after adjusting the pH to 6.8, it was digested with 1 mg ml⁻¹ pancreatin (Sigma) according to Hellendoorn *et al.* (1975), with the following modification: instead of lyophilising the undigested residue (IDF), it was washed with hot water through a preweighed sintered glass filter Gooch crucible under vacuum (the soluble fraction passes through) (Goering & Van Soest, 1970).

Prosky method

TDF was determined following sample digestion with thermostable α -amylase (Sigma) at pH 6.0 for 30 min at 100°C and allowing to cool. The pH was adjusted to 7.0 and the sample was incubated with protease VII (Sigma) for 30 min at 60°C. After cooling, the sample was adjusted to pH 4.5 and incubated with amylase glucosidase at 60°C for 30 min. All incubations were carried out in a boiling water bath with continuous shaking. A 250-ml volume of 95% ethanol preheated to 60°C was added and the sample was allowed to precipitate at room temperature for 60 min. Preweighed crucibles, containing celite previously washed with 78% ethanol, were used to filter the enzyme digest. The residue was washed with 78% ethanol, 95% ethanol and acetone. Determination of protein, ash and calculation of TDF was as described by Prosky *et al.* (1988) and the AOAC gravimetric method no. 955.29 (AOAC, 1990).

IDF was determined as described by Prosky *et al.* (1988). Briefly, preweighed crucibles containing celite previously washed with water, were used to filter the enzyme digest; this was washed with two 10-ml portions of water. The filtrate and the water washings were saved for SDF determination. The residue was washed with 95% ethanol and acetone, then dried in an oven cooled in a desiccator and weighed for IDF determination. Determination of protein, ash and calculation of SDF were as described by Prosky *et al.* (1988).

SDF was determined in the combined filtrate and washings solution from the IDF procedure as described above. This solution was adjusted to 100 g with water and precipitated with 95% ethanol preheated to 60°C. After filtration through a preweighed crucible containin

Table 1. Proximal analysis of legume (*Phaseolus vulgaris*) grains

Variety	Humidity	Protein	Fat	Carbohydrate	Crude fibre	Ash
'Tacarigua'	6.35 ± 0.28	24.5 ± 0.03	1.69 ± 0.04	59.4 ± 0.7	4.03 ± 0.01	4.21 ± 0.02
'Montalban'	8.45 ± 0.35	24.8 ± 0.6	1.45 ± 0.07	54.2 ± 0.1	7.43 ± 0.2	4.01 ± 0.01

Values are averages of five determinations (each determination was done in triplicate) for each variety, and are expressed as percentage (\pm standard deviation) for each 100 g of dry sample.

celite, the residue was washed successively with 78% ethanol, 95% ethanol and acetone. The crucible was dried overnight in a 105°C air oven. Determination of protein, ash and calculation of SDF were as described by Prosky *et al.* (1988).

All the DF samples obtained by the chemical and enzymatic gravimetric methods were tested qualitatively for the presence of contaminating starch using iodine.

Statistics

Means were compared by Student's *t*-test, using the GraphPad Instat program (GPIS, 1989).

RESULTS AND DISCUSSION

Table 1 shows values of the proximal analyses for the 'Tacarigua' and 'Montalban' black beans. The protein values were very similar for both varieties and are comparable to results reported elsewhere (Joslyn, 1970).

The fat contents also were similar to values reported in the literature (Marques *et al.*, 1990), both varieties being below 2%. Similarly, the values for carbohydrates and ash were in the range of the results reported by Joslyn (1970) and Leung (1970). Table 2 summarises the determination of DF, using the method of Prosky *et al.* (1988).

The addition of SDF to IDF did not reach the TDF values for the 'Tacarigua' variety. Possibly the real SDF content is underestimated, as Marlett *et al.* (1989) demonstrated that 25% of SDF was lost during the Prosky method. Similar results were found for TDF, SDF and IDF in four food vegetable products (non-legumes); i.e. the SDF + IDF value was different from the TDF value (Wolters *et al.*, 1992).

Comparison between 'Tacarigua' and 'Montalban' varieties using the Prosky method showed differences for TDF and IDF values. However, considering the criticism of Marlett *et al.* (1989) and Wolters *et al.* (1992), it is reasonable to consider IDF as the most reliable value for the estimation of DF, in particular for the grain varieties discussed here. 'Montalban' has a higher DF content than 'Tacarigua'. However, the magnitude of this difference should not be of relevance for human consumption of these varieties.

Table 2 also shows the results after applying the methods of NDF and ADF. Those values are generally considered to represent IDF, where the principal components are cellulose and lignin for the ADF method, while cellulose, lignin and hemicellulose are determined by the NDF method.

Table 2. Dietary fibre determination in two varieties of legume grains

Dietary fibre method	'Tacarigua'	'Montalban'
TDF	17.74 ± 0.42 ^a	19.95 ± 1.37 ^b
IDF	12.36 ± 0.1 ^a	16.85 ± 0.55 ^b
SDF	0.31 ± 0.36 ^a	3.66 ± 0.68 ^b
NDF	15.48 ± 1.94 ^a	32.12 ± 1.66 ^b
ADF	14.5 ± 1.66 ^a	6.93 ± 1.66 ^b
Hellendoorn	20.4 ± 1.76 ^a	21.61 ± 1.5 ^b

Values are averages of five determinations for each variety (each determination was done in triplicate) and are expressed as percentage (\pm standard deviation) for each 100 g of dry sample.

Means in a row not followed by the same letter are significantly different ($P \leq 0.01$).

TDF, total dietary fibre; IDF, insoluble dietary fibre; SDF, soluble dietary fibre; NDF, neutral detergent fibre; ADF, acid detergent fibre.

The clear difference between the two methods, NDF and ADF, for the same sample, suggest the presence of hemicellulose in NDF; however, these values were much higher than those reported by Méndez *et al.* (1993).

Each of these methods was designed to measure DF depending on the amount of protein and starch present in each sample (Rodriguez *et al.*, 1992). Thus NDF and ADF methods are useful for measuring IDF, but both suffer from the loss of an indefinite amount of SDF and incomplete removal of starch from starch-rich samples.

Table 2 shows the results obtained by the Hellendoorn method. The high contamination of IDF with starch, and perhaps proteins, is evident. Hellendoorn considered that the protein and starch in the indigestible residues should be considered indigestible *in vivo*.

The comparison between both varieties using NDF showed a higher content of IDF for the 'Montalban' variety, which contrasts significantly with the results found for the two varieties using the Prosky method, as described above. Interestingly, a similar comparison could be done for the 'Tacarigua' variety, which presented a NDF value (assuming it is IDF) 3.1% higher than the IDF value from the Prosky method. This difference between the IDF values is significantly below the 19.5% difference found for the 'Montalban' variety using a similar comparison. The explanation for this quantitative difference is not clear. Some investigators have modified the NDF by digesting previously with amylase and then applying the standard determination of NDF (Rivera *et al.*, 1993; Wolters *et al.*, 1992). Enzymes were not applied for NDF determinations, which suggests that this difference might be due to the

starch quality of the 'Montalban' variety. This hypothesis seems to be supported by the ADF results, which yielded a difference of 7.57% when the two varieties were compared, favouring the 'Tacarigua' variety, but this assumption is not supported by the difference between ADF and IDF values by the Prosky method for the 'Montalban' variety which is 10.7% higher for IDF. No conclusion on the low ADF value for 'Montalban' can be drawn at present, since it is not known if there is physically inaccessible starch (for enzyme digestion) which could be hydrolysed by sulphuric acid. Some other studies on legume seeds have demonstrated that starch in instant flakes prepared from white beans is slowly digested *in vivo* (Tappy *et al.*, 1986). Further studies are being carried out to measure DF after boiling these beans, to measure pectin and probably other non-starch polysaccharides as part of SDF.

ACKNOWLEDGEMENTS

Acknowledgements are due to Dr. Neil R. Lynch and to Dr. Marian Ulrich (Instituto de Biomedicina, U.C.V.) for reading this manuscript, and financial support by C.D.C.H. from U.C.V. (Nos. 09-13-3259-94, 09-13-3129-94, 10-00-2939-92).

REFERENCES

- AOAC (1990). *Official Methods of Analysis* (15th edn). The Association of Official Analysis Chemists, Arlington, VA, USA.
- Cardenas, L. (1986). Caracterización botánica de las leguminosas en la Alimentación animal. *Revista Alcance*, No. 35 (Marzo), Facultad de Agronomía, U.C.V., pp. 13–15.
- Goering, H. K. & Van Soest, P. J. (1970). *Forage fiber analysis (apparatus, reagents, procedures, and some applications)*. Agriculture Handbook No. 379, Agricultural Research Service, United States Department of Agriculture.
- GPIS (1989). Graphpad Instat Program. Version 1.0. San Diego, USA.
- Hellendoorn, E. W., Noordhoff, M. G. & Slagman, J. (1975). Enzymatic determination of the indigestible residue (dietary fiber) contents of human food. *J. Sci. Food. Agric.*, **26**, 1461–1468.
- Jaffé, W. G. (1986). Las leguminosas para consumo humano. Ventajas y desventajas. En: *Las leguminosas en la alimentación animal*. Revista Alcance, No. 35 (Marzo), Facultad de Agronomía, U.C.V., pp. 87–95.
- Joslyn, M. (1970). *Methods in Food Analysis*, 2nd edn. Academic Press, New York.
- Kay, D. E. (1979). *Food Legumes*. The Tropical Products Institute, London.
- Kritchevsky, D. (1988). Dietary fibre. *Annu. Rev. Nutr.*, **8**, 301.
- Lanza, E. & Butrum, R. R. (1986). A critical review of food fiber analysis and data. *J. Am. Diet. Assoc.*, **86**, 732–740.
- Lee, S. C., Prosky, L. & De Vries, J. W. (1992). Determination of total, soluble and insoluble dietary fiber in food enzymatic, gravimetric method, MES-TRIS buffer: collaborative study. *J. Assoc. Off. Anal. Chem.*, **75**, 395–416.
- Leung, W. T. W. (1970). *Tablas de Composición de Alimentos para Uso en America Latina*, 2nd edn. Editorial Interamericana, Mexico, 60 pp.
- Marlett, J. A., Chesters, J. G., Longacre, M. J. & Bogdanske, J. J. (1989). Recovery of soluble dietary fiber is dependent on the method of analysis. *Am. J. Clin. Nutr.*, **50**, 479–485.
- Marques, M. H., Casanova, S., Resender, M. C. & Figueiredo Da Silva, M. (1990). Estudio comparativo de metodos analiticos de determinacao de fraca 'fibra da dieta' insoluble, em alimentos ricos em amidos. *Arch. Lat. Nutr.*, **40**, 107–115.
- Méndez, M. H. A., Derivi, S. C. N., Fernandes, M. L. & Gomes de Oliveira, A. M. (1993). Insoluble dietary fiber of grain food legumes and protein digestibility. *Arch. Lat. Nutr.*, **43**, 66–72.
- Ortega, S. & Barrios, A. G. (1972). 'Tacarigua': nueva variedad de caraota negra (*Phaseolus vulgaris* L.). *Rev. Agron. Trop.*, **22**(4), 435–438.
- Ortega, S., Barrios, A. G. & Tešara, J. (1987). 'Montalban': nueva variedad de caraota negra (*Phaseolus vulgaris* L.). *Rev. Agron. Trop.*, **37**(1-3), 117–120.
- Prosky, L., Asp, N. G., Schwizer, T. F., De Vries, J. W. & Furda, I. (1988). Determination of insoluble, soluble and total dietary fiber in foods and food products: interlaboratory study. *J. Assoc. Off. Anal. Chem.*, **71**, 1017–1023.
- Rivera, C. J., Gerardi, A. E., Infante, R. B., Carrasco, H. J. & Rodriguez, O. (1993). Dietary fiber analysis of cassava using gravimetric methods. *Arch. Lat. Nutr.*, **43**, 73–74.
- Rodriguez, Ma. D., Redondo, A. & Villanueva, Ma. J. (1992). Study of dietary fibre content in cucumber by gravimetric and spectrophotometric methods. *Food Chem.*, **43**, 295–298.
- Tappy, L., Würsch, P., Randin, J. P., Felber, J. P. & Jéquier, E. (1986). Metabolic effects of pre-cooked instant preparations of bean and potato in normal and diabetic subjects. *Am. J. Clin. Nutr.*, **43**, 30–36.
- Trowell, H. (1976). Definition of dietary fiber and hypothesis that it is a protective factor in certain diseases. *Am. J. Clin. Nutr.*, **29**, 417.
- Van Soest, P. J. (1963). Use of detergents in the fibrous feeds. I. Preparation of fiber residues of low nitrogen. *J. Assoc. Off. Agric. Chem.*, **46**, 825–829.
- Van Soest, P. J. & Wine, R. H. (1967). Use of detergents in the analysis of fibrous foods. IV. Determination of plant cell-wall constituents. *J. Assoc. Off. Anal. Chem.*, **50**, 50–55.
- Wolters, M. G. E., Verbeek, C., van Westerop, J. J. M., Hermus, R. J. J. & Vorgen, A. G. J. (1992). Comparison of different methods for determination of dietary fiber. *J. Assoc. Off. Anal. Chem.*, **75**, 626–634.