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COMPARACIÓN DE VALORES DE FIBRA DIETÉTICA EN DOS VARIEDADES DE COWPEA (*Vigna Unguiculata L.* WALP) DE VENEZUELA, USANDO MÉTODOS QUÍMICOS Y ENZIMÁTICOS GRAVIMÉTRICOS

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ABSTRACT

Two varieties of uncooked beans Vigna unguiculata ("Unare"and "Tuy") analysed by proximate analysis showed values of proteins, fats, carbohydrates and ash very similar between the varieties of "Unare" and "Tuy". The chemical-gravimetric method to quantify the neutral detergent fiber (NDF) showed the highest value for the variety "Tuy" (35.3%) compared to "Unare" (27.1%). The enzymatic-gravimetric method Hellendoorn showed that the values of non-carbohydrate available for the varieties "Tuy" (15.5%) and "Unare" (21.7%) were lower than those from the NDF method. The enzymatic-gravimetric method of Prosky, showed that the variety "Tuy" had values of insoluble dietary fiber(IDF) (15.4%) and total dietary fiber (SDF + IDF) (16.1%) higher than the variety "Unare." In conclusion Prosky's method seems to be the most suitable for quantifying DF in the analysed samples. More research is needed to clarify the validity of the quantification of DF.
Key words: legumes, cowpea, dietary fiber, enzymatic-gravimetric and chemical-gravimetric methods.

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INTRODUCTION

Legumes are, together with cereals, fruits, fish, and olive oil, one of the principal components of the mediterranean diet. Legumes are an excellent source of protein, carbohydrates, fiber, minerals, and other nutrients (1). Legumes are also a nutritious and economical source for populations of developing countries and play an important role in their diets (2).

The legume *Vigna unguiculata L.* (Walp) (cowpea, caupi, blackeye pea, frijol, etc), is an annual plant; it has adapted to diverse soil types, rainfall levels, temperature and altitudes (3-5). It is consumed in many forms: young leaves, green pods and green seeds are used as vegetables, and dry seeds are widely used in various food preparations for human nutrition, especially in Africa.

The average world production of cowpea is 300 - 1000 Kg/ha and its world production reached 3,6 million metric tons in 2003 (6). Recent improvements in the genetics of cowpea have resulted in a greater production within traditional growing regions in the tropics and have stimulated adaptation allowing the cowpea to grow in previously unsuitable regions. The Venezuelan production was 581 kg of legume seeds/ha in 1990. In 1994, it was estimated an apparent consumption for the social strata III, IV and V of the Venezuelan population of 44,5 g/person/day of legume seeds (7,8). These facts demonstrate the importance of studying the nutritional value of cowpea beans as well as the dietary fiber content.

The definition of dietary fiber (DF) as the sum of lignin and the plant polysaccharides that are not digested by the endogenous secretions of the human digestive tract (9,10) is a physiological, not a chemical definition. It covers a wide variety of chemical substances with different physical properties. DF was classified according to its solubility, as soluble dietary fiber (SDF) and insoluble dietary fiber (IDF). As a consequence of such differences in its hydratation capabilities, each fiber type produces a different physiological response (10,11). The DF content and proximate analysis provided an evaluation of protein, carbohydrate, fats and mineral composition of legumes (12,13). Two general categories of methods have been proposed to analyze DF, based on the chemical principles employed. The first category uses selective extraction of nonfiber materials and gravimetric determination of the residue as fiber. Typical methods in this category are the crude fiber method (AOAC, 1975), the detergent fiber approach (14) and the enzymatic digestion approach (15 – 17). The second category of methods is an approach toward defining fiber in terms of its content (18) for which the method of Prosky and the method of Asp for FD determination are widely used in the world. However, the neutral detergent fibre (NDF) method of Southgate et al., 1978, is used for insoluble fiber determination in legumes and cereals (19).

However, Marlett et al (1989) showed that the proportion of the total dietary fiber extracted as soluble fiber is dependent on the method of analysis and could explain part of the differences among values that have been reported by different investigators, and also the effect of the method of analysis on the fractionation of fiber-derived sugars varied with the food (20). On the other hand it is reasonable to consider IDF as the most reliable value for the estimation of DF for the varieties of legume used in these experiments because of its higher proportion in comparison with SDF (21, 22).

The purpose of this study was to determine the amounts of insoluble (IDF), soluble (SDF) and total (TDF) dietary fiber of two varieties of V. unguiculata, “Tuy” and “Unare”, using three methods:

1. Prosky et al. (15) to determine TDF;
2. FDN to measure the IDF was determined by the method of Van Soest (14), Van Soest and Wine (23);
3. The method of Hellendoorn et al. (16) was used to measure IDF.

The results were analyzed and compared between the two varieties of Unare and Tuy cowpea in order to compare the contents of IDF provided by the three methods above, and SDF by the method of Prosky et al.

MATERIALS AND METHODS

The seeds of two varieties of cowpea, V. unguiculata, ”Unare” and “Tuy” were purchased from the Centro Nacional de Investigaciones Agropecuarias (CENIAP), Maracay, Estado Aragua, Venezuela. The “Tuy” variety is a cream coloured seed and the “Unare” variety is a white coloured seed.

Sample preparations

Dried Unare and Tuy seed samples were ground (using Multiciclon, Tecator. Sweden) and passed through a 1 mm screen before analysis. The nitrogen content of each of the two varieties was determined according to the Kjeldahl method and the percentage of crude protein was calculated using the factor 6.25. The moisture, crude lipid, crude fiber, and ash contents of the milled samples of the two varieties of cowpea were determined in accordance with the standard methods of the AOAC (1990) (24). The carbohydrate content of each of the two cowpea varieties was obtained by difference.

Dietary fiber analisys

Method of Prosky (15). Total dietary fiber (TDF) was determined using the Prosky et al method. The samples, run by duplicate, were first digested with heat-stable α-amylase (Thermamyl) and then with amyloglucoxidase and protease VIII (Sigma Chemical Co., USA) ; residual fractions were left in the crucible, dried overnight at 105 ± 5 ºC in a forced-air oven, cooled in a desiccator and weighed. One duplicate was used as purified fiber fraction and the other was incinerated at 525 ºC to determine ash content. In brief, the procedure used to determine the IDF content using the Prosky method was as follows, preweighed crucible filters containing celite, previously washed with hot water were used to filter the enzyme digest, this was washed with two 10-ml portions of water. The filtrate and the water used for washing were saved for SDF determination. The residue was washed with 95% ethanol and acetone, then dried in an air oven, cooled in a dessicator and weighed for IDF determination. Determination of protein and ash were used to correct IDF. SDF was determined in the combined filtrate and washing solution from the IDF procedure as described above. This solution was adjusted to 100g with water and precipitated with 95% ethanol preheated to 60ºC. After filtration through a preweighed crucible containing celite, the crucible was dried overnight in a 105 ºC oven. Determination of protein, ash and calculations of SDF were as described by the Prosky et al method(15). All the above samples were tested qualitatively for the presence of contaminating starch using iodine.

Neutral detergent fiber (NDF) (14,23). The procedure used to determine the NDF was as follows, one gram of sample was boiled for 1 hr at neutral pH. After filtration the residue was washed with hot water, acetone and ethanol, then dried, weighed and ashed for 4 hr in a furnace at 525 ºC to correct for any inorganic matter (15). This method gives a good estimate of insoluble fiber (cellulose, hemicellulose and lignin) and its use continues on a smaller scale for assessing food for human consumption.

Hellendoorn (16). The procedure used was as follows, 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., USA), then after adjusting the pH to 6.8, it was digested with 1 mg ml-1 pancreatin for one hr and washed with hot water through a preweighed sintered glass filter Gooch crucible under vacuum (21).

Statistical analysis

The statistical significance of differences between means was assessed by Student's unpaired t test using the SPSS (11 versions, U.S.A).

RESULTS

The proximate composition for the "Unare" and "Tuy" seeds are presented in Table 1. Moisture was a little higher (9.05%) than that reported by Granito et al (25) for vigna sinensis (7.74%), but below of the reported by Herrera et al (22). The protein values were very similar for both varieties and are comparable to results reported elsewhere (19,25,26,27). The fat contents were below 1%, and the median fat content of Unare was significantly higher than that of Tuy variety, but both are under the 2% reported in the literature for vigna sinensis (25,27). Values of crude fiber, did not differ significantly between Tuy and Unare and are comparable to values reported in the literature (19,22,27,28). Carbohydrates obtained by difference were higher for the "Tuy" variety than that of the "Unare" variety. The major component of the seed is carbohydrate followed by crude protein and crude fiber.

Table 2 summarises the determination of DF, using chemical and enzymatic gravimetric methods. TDF was significantly higher for Unare than for the Tuy variety. Meanwhile, SDF did not show significative differences between the two varieties; however, those values were below the reported in the literature for vigna sinensis (1.2%) (22). IDF resulted with higher values for Tuy than for the Unare variety with significative difference, although the higher value was below the value reported in the literature (23.7%), for the vigna sinensis (22,29).

The results of applying the method of NDF for the "Unare" and "Tuy" varieties are shown in table 2, the NDF method showed significative differences between both varieties, the higher value corresponded to the Tuy variety. These values may be considered as IDF with its principal components as cellulose, lignin and hemicellulose, however each variety practically doubled the value of IDF when using the Prosky method.

Table 2 shows the results obtained by the Hellendoorn method. The IDF contents showed significative differences between both varieties, The "Unare" Hellendoorn value was higher (1.4 times) than that of "Tuy" variety. Interestingly the Prosky IDF value of the "Tuy" variety resulted much closer to the value shown by the Hellendoorn method if it is assumed to be IDF.

DISCUSSION

It is evident from the proximate composition of these two varieties of cowpea, Unare and Tuy, that they each posses a significant amount of protein and carbohydrate. However, it is known that vegetable protein in particular

TABLE 1

Proximal analysis of legume (*Vigna unguiculata*) grains[a].

Legume variety	Humidity	Protein	Fat	Carbohydrate	Crude fiber	Ash
Unare	11.03± 0.47*	26.27± 0.08	0.87± 0.04*	50.63± 0.27*	6.41± 0.03	4.62±0.02*
Tuy	9.05± 0.24	25.45± 0.04	0.70± 0.02	52.82± 0.04	6.57± 0.03	5.72±0.02

[a] Values are averages of five determinations for each variety, expressed as percentage ± standard deviation for each 100 g of wet sample. Unpaired t test * $p< 0.05$.

has low digestibility in vivo; the protein in beans, for example, can have as low a digestibility as 65% whether this undigestible protein should be regarded as a part of the fiber complex or not is a matter of discussion (30). Crude fiber is still used in human foods in particular in developing countries despite having a value 2-5 times lower than that of FD (31).

The values for SDF for both the Unare and Tuy varieties of cowpea (vigna unguiculata) were below 1% and from the value reported by Herrera et al 98 (1.2%) (22) for a similar variety "frijol rojo"(Vigna sinensis L), using the method of Prosky et al. (15). However, keeping in mind the criticism of Marlett et al. (20) and Wolters et al. (32) about the low efficiency of the Prosky method to measure SDF, we decided to use IDF as the most reliable value for the estimation of DF in both varieties of Unare and Tuy cowpea (24). Differences between TDF and IDF values using the Prosky method were found for "Unare" and "Tuy" varieties (table 2). However, the magnitude of the differences in the content of DF should not be a limiting factor to restrict any of these varieties for human consumption, even the protein content which is more important due to its nutrient properties, did not show any significant differences between both varieties.

Assuming that NDF is a method for IDF determination a clear difference between this method and the method of Prosky, for the "Unare" sample, was found. A higher IDF estimation obtained with the NDF method (2.66 times the Prosky value) suggests the presence of hemicellulose or resistant starch (29). The differences between these two methods were also described for two varieties of Phaseolus vulgaris with a similar observation of higher values of IDF from the NDF method than the IDF of the Prosky method (15). Although the NDF method is useful for measuring IDF, it suffers from an incomplete removal of starch; therefore the modification including an enzymatic step necessarily be incorporated in upcoming experiments (33). The fraction of the neutral detergent fiber (NDF) for both varieties of bean was compared. The comparative values for NDF content between these cowpea varieties showed the "Tuy" variety 1.29 times NDF higher than the "Unare" variety. When the IDF from the Prosky method, was compared using the same exercise, the "Tuy" variety was 1.5 times higher than the "Unare variety". The same procedure was applied for comparing IDF-Hellendoorn contents between both varieties and "Unare" was 1.4 times higher than the "Tuy" variety.

However, according to Garcia et al (21), NDF is considered to represent the level of IDF irrespective of which method is used. The following relationship was established: NDF/IDF, which should express the rate of fiber, using the neutral detergent method and the Prosky method for each variety, resulted in 2.66 for the "Unare" variety and 2.29 for the "Tuy" variety. These value rates suggest that, there is no significant difference between both varieties, but it is evident that there is a higher proportion of NDF to IDF for both varieties. Some researchers have modified the NDF sample by applying amylase to the sample for NDF and then by using the standard determination method of NDF (23,35). Enzymes were not applied in this study for NDF determinations, suggesting that the above difference might be due to the retained starch quality not being fully removed in the high-starch for both varieties and more notably the "Tuy" variety. However the iodine test did not reveal presence of starch. Perhaps the contamination of IDF with proteins which are non-removable by NDF method is responsible for such high values.

The difference between the NDF, Hellendoorn and

TABLE 2

Dietary fiber determination in two varieties of legume grains[a].

Dietary fiber methods	Unare	Tuy
TDF (IDF + SDF)[b]	11.09 ± 0.9*	16.17 ± 0.35
IDF	10.2 ± 0.56*	15.5 ± 1.0
SDF	0.89 ± 0.37	0.77 ± 0.03
NDF[b]	27.2 ± 1.9*	35.3 ± 1.7
Hellendoorn	21.7 ± 1.4*	15.4 ± 0.32

[a] Values are average of five determinations for each variety, expressed as percentage ± standard deviation for each 100 g of wet sample. Unpaired t test P< 0.05*.
[b] TDF, total dietary fibre; IDF, insoluble dietary fibre; SDF; soluble dietary fibre; NDF; neutral detergent fibre.

Prosky methods for IDF values between both Unare and Tuy varieties apparently resides in the properties of each method, in particular the values of IDF between the Prosky and Hellendoorn methods are closer and both methods use enzyme digestion. Also attention has to be paid to the proposition made by Saura-Calixto et al (34) about Indigestible Fraction (IF) in foods. The IF is defined as the part of vegetable foods that is not digested nor absorbed by the small intestine, and reaches the colon where it is a substrate for the fermentative microflora. As such,if comprises not only DF, but also other compounds of proven resistance to the action of digestive enzymes such as a fraction of dietary starch, protein, certain polyphenols, and other associated compounds. The indigestible fraction proposed here could be a suitable alternative to DF in food analysis.

The above results require more research and to apply the modification suggested by other researchers (32, 34) to be carried out to establish their validity for quantification of DF.

RESUMEN

Dos variedades de fríjol crudo Vigna unguiculata (“Unare” y “Tuy”) analizadas mediante un análisis proximal mostró valores de proteínas, grasas, hidratos de carbono y cenizas muy similares entre las variedades “Unare” y “Tuy”. El método químico-gravimétrico para cuantificar la fibra detergente neutro (FDN) produjo el mayor valor para la variedad “Tuy” (35,3%) en relación a “Unare” (27,1%). El método enzimático-gravimétrico Hellendoorn mostró que los valores de hidratos de carbono no-disponibles de las variedades “Tuy” (15,5%) y “Unare”, (21,7%) eran menores a los obtenidos con el método FDN. El método enzimático-gravimétrico Prosky arrojó valores de fibra dietética insoluble (FDI) (15,4%) y fibra dietética total (16,1%) de la variedad “Tuy” superiores a la variedad “Unare”. En conclusión el método de Prosky parece ser el más indicado en la cuantificación de DF de estas muestras. Mayor información es requerida para poder precisar la validez de la cuantificación de DF.

Palabras clave: leguminosas, fríjol, fibra dietética, métodos enzimático-gravimétrico y químico-gravimétrico.

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