Determination of insoluble dietary fiber compounds: cellulose, hemicellulose and lignin in legumes

Determinacion de los componentes insolubles de la fibra dietética en legumbres: celulosa, hemicelulosa y lignina.

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ABSTRACT

Insoluble dietary fiber was analyzed in raw chick peas, kidney beans and lentil samples by detergent fiber methods (NDF,ADF). NFD with enzyme modification (ENDF). The values were 24.9%, 21.6% and 17.4% by lentils, kidney beans and chick peas respectively . The ADF results obtained by manual (9.83%) and automatic (Dosi-fiber instruments) (9.13%) procedure showed statistical difference ($p<0.05$). Insoluble fiber amount obtained by modified detergent method (ENDF) was compared with insoluble dietary fiber (IDF) by AOAC method and statistical significant differences were obtained for lentils and chick peas ($p<0.001$) and non differences for kidney beans.

Key words: Insoluble fiber. Cellulose. Hemicellulose. Lignine. Legumes.

RESUMEN

Los métodos de fibra detergente (NDF, ADF) se han aplicado a la determinación de fibra dietética insoluble en garbanzos, lentejas y alubias crudas. El método NDF fue modificado con la adición de enzimas (ENDF). Este método permite la determinación individual de celulosa, hemicelulosa y lignina que presentan composición quimica asi como efectos fisiológicos diferentes. Los resultados de ADF obtenidos de forma manual (9.83%) y automática (dosi-fiber) (9.13%) mostraron diferencias estadísticas significativas ($p<0.05$). El contenido de fibra dietética neutra modificado enzimáticamente (ENDF) y realizado de forma automática se comparó con el contenido de fibra dietética insoluble (IDF) determinado por el método de la AOAC obteniendose diferencias estadísticamente significativas para las muestras de lentejas y garbanzos ($p<0.001$).

Palabras clave: Fibra insoluble. Celulosa. Hemicelulosa. Lignina. Legumbres.

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INTRODUCTION

Legumes are second to cereals as important sources of dietary fiber (DF), protein and starch. Recent studies have indicated that dietary fiber (DF) may protect against cardiovascular diseases, diabetes, obesity, colon cancer and other diverticular diseases (McPherson,1992).

The intake of dietary fiber in Spain is 22.4 g/person/day (Southgate's method), 35.9% of which comes from cereals, 28.3% vegetables, 21.9% fruits and 10.0% legumes (Saura-Calixto and Goñi, 1993). DF intake in the Spanish diet has decreased over the past decades. A higher contribution of fruits, vegetables and legumes is found in Spain rather than other European countries (Saura-Calixto and Goñi, 1993).

Gravimetric methods for the analysis of dietary fiber can be divided into two groups. The first (detergent acid and neutral methods, ADF and NDF) consists in the gravimetric determination of residue previously treated with acid and neutral detergent solutions. These methods determine the insoluble fraction and their individual components (Van Soest´s methods). The second group uses amylolytic and/or proteolytic enzymes determinating insoluble and soluble fraction.

The detergent methods sometimes leads to overestimate due to the imcomplete removal of starch, proteins and fats (Robertson and Horvath, 1992). Mongeau and Brassard (1982) incorporated amylase pancreatic in the NDF method to permit a correct digestion of starch.

The purpose of this research was: (1) to compare the traditional manual procedure for determination of ADF with automatic procedure realized by Dosi-Fiber instrument. (2) To determine cellulose, hemicellulose and lignine by ADF and ENDF in the three most important legumes consumed in Spain. (3) To compare insoluble dietary fiber by detergent method with enzymatic modification (ENDF) and AOAC´s method.

MATERIAL & METHODS

Sample Preparation

Chick peas (*Cicer arietinum, c. v.* "Blanco lechoso"), common beans (*Phaseolus vulgaris*), with a white seed coat (*c. v.* "riñon") and lentils (*Lens culinaris, c. v.* "castellana") were purchased from established commercial sources in

September 1992.

Approximately 100 g of each raw legume was ground with a Wiley mill to pass through a 40 mesh screen and stored in a screw cap bottle at -40°C.

Proximate Analyses

Ash was determined by ignition in a muffle furnace; moisture was analyzed by drying for 3 h at 130°C. Fat was determined by the Soxhlet procedure (AOAC, 1984). Protein was obtained using the Kjeldhal´s method.

Samples Analysis

Six to eight raw samples of legumes were analyzed for acid and neutral detergent fiber by the method of Van Soest (Van Soest and Wine's, 1967, 1968) and insoluble fiber by the modified enzymatic-gravimetric method described by Prosky et al. (1988).(AOAC, 1995).

ADF analysis

The method of Van Soest and Wine (1968) was used to determine ADF, cellulose and lignin, in chick pea, kidney bean and lentil samples by using Dosi-fiber extractor (Selecta, Spain) (similar to Fibertec, Tecator) instead of hot plates and condensers. P^2 glass filtering crucibles with a porosity of 40-60 μm were used. This method has been previously applied in kidney bean samples by two procedures: with dosi-fiber extractor and without dosi-fiber extractor (manual method) in order to compare them. Students's test was applied to mean values thus obtained.

Dosi-fiber extraction includes 60 min of digestion with acid detergent solution, filtration with vacuum, three times washing with hot distilled water and twice with acetone. After being washed with acetone the crucibles were dried overnight at 100°C and weighed. The other procedure followed the same steps but without Dosi-fiber extractor (manual procedure).

ENDF analysis

Neutral detergent fiber was determined by the method of Van Soest and Wine (1967) with enzyme addition. The enzymatic-modification was as follows: 50 mL phosphate buffer (pH 6.0) within 0.1 mL heat-stable alpha-amylase (Termamyl, Sigma) was added to 1.0000 g of sample. It was heating 35 min at 100°C in Dosi-fiber extractor, then suction was applied to filter, and they were washed with hot distillated water to remove completely phosphate buffer. The crucibles were filled again with neutral detergent solution to continue with the method of Van Soest. The nitrogen content in ENDF residue was determided by Kjeldhal's method.

Insoluble dietary fiber analysis

The raw legume samples were analyzed by Prosky method (1988), (AOAC, 1995).

RESULTS AND DISCUSSION

Legume varieties selected for this study are the main ones consumed in our country. The composition percentage is showed in Table I.

Table I.- Composition percentage of raw legumes analyzed

	Moisture	Carbohydr.[a]	IDF[b]	Fat	Protein[c]	Ash
Chick peas	9.4	50.7	11.6	7.4	17.8	3.1
Kidney beans	10.7	42.6	20.4	1.6	20.9	3.8
Lentils	11.7	44.0	17.3	1.6	22.9	2.5

[a] Values obtained by differences
[b] AOAC (1995)
[c] N x 6.25

The ADF procedure was performed using a Dosi-Fiber apparatus with P^2 crucibles as well as the manual procedure.

The precision of the ADF was tested in eight samples of the same food. The determination was repeated in different days to include possible day-to-day variations. In raw common bean samples the values were 9.13% (Dosi-Fiber) and 9.83% (manual) (Table II). The coefficient of variation (C.V.) was 6.46% (Dosi-Fiber) and 5.59% (manual). The ADF values with both types of procedure was slightly higher ($p<0.05$) with the manual procedure. Minor time and manipulation as well as probably better digestion were obtained with the Dosi-Fiber apparatus. The last procedure was chosen to posterior studies.

Table II.- Comparison of two procedure for ADF determination in kidney beans samples (% dry matter).

Dosi-Fiber instrument	Manual procedure
8.92	10.08
9.19	9.07
9.15	9.54
9.78	9.47
9.70	10.21
8.21	9.30
9.69	10.36
8.42	10.59
X=9.13	X=9.83
SD=0.59	SD=0.55
CV(%)=6.46	CV(%)=5.59

Significantly different $p<0.05$

Chick pea, lentil and kidney bean samples were analyzed with the detergent method for the determination of ENDF, ADF, cellulose, hemicellulose and lignin using dosi-fiber instrument (Table III).

NDF method used alpha amylase heat-stable (ENDF) before the digestion with detergent solution to prevent the overestimation of insoluble fiber. The nitrogen content was determined in the ENDF residue to comprove the correct protein's remove. The digestion with alpha-amylase was achieved during one hour at 100°C and one hour (100°C) followed overnight at room temperature (data no shown here). We did not obtain different results.

The protein amount in the ENDF residue was 0.44%

Table III.- Percentage of ADF and ENDF values in legumes (% dry matter)

	Moisture	ENDF	ADF	Cellulose	Hemicell.	Lignin
Chick peas						
X±S.D.	8.30	17.40±1.55	6.59±0.37	5.86±0.34	11.54	0.73±0.10
C.V.(%)		8.91	5.61	5.80		13.70
Kidney beans						
X±S.D.	10.66	21.57±1.50	9.13±0.59	8.02±0.41	12.44	1.11±0.18
C.V.(%)		6.97	6.46	4.66		16.03
Lentils						
X±S.D.	11.67	24.85±2.18	8.55±0.28	7.09±0.20	16.30	1.45±0.10
C.V.(%)		8.78	3.23	2.83		6.54

for chick peas, 2.16% for kidney beans and 0.40% for lentils (Table IV). These values suppose a slight overestimation between 0.08-0.47% in the percent value of ENDF.

Table IV.- Protein content (%) in the ENDF and IDF residues

	Protein in ENDF Residue	Protein in IDF Residue
Chick peas	0.44	8.99
Kidney beans	2.16	13.80
Lentils	0.40	8.53

ENDF content in raw legumes ranged between (17-25%) of which lentils presented the highest value. Hemicellulose was estimated by the difference between ENDF and ADF. It was the major contribution among the ENDF components (11-16%); chick peas and kidney beans had a similar amount (11-12%), and lentils presented the richest source (16%) (Table III). As in almost all sources of plant food fiber (Southgate, 1992) including cereal fiber (Mongeau and Brassard, 1982) the main component of legumes (chick peas, kidney beans and lentils) was hemicellulose. Cellulose showed inferior values (6-8%) and kidney beans presented the richest source of it. Lignin represented the smallest fraction (0.7-1.5%). Vidal-Valverde and Frias (1991), determined NDF in similar legume varieties. Our results were superior for chick peas and kidney beans. However, the hemicellulose proportion in NDF fraction was in the same range. ADF results were similars. The precision of this method was among (6.97-8.91 %CV) for ENDF and (3.23-6.46 %CV) for ADF values, which corresponds with the precison for this type of methods.

The insoluble dietary fiber (IDF) was determined by AOAC method in the same legume samples. The IDF content was 20.4% for kidney beans, 11,6% for chick peas and 17,3% for lentils (Table I).

Li and Cardozo, (1993) in cooked legumes reported superior IDF values by AOAC method. When they used dimethylsulfoxide (DMSO) for the same kind of samples they obtained similar results if it's compared with us. Prosky et al., (1992) and Hughes and Swason, (1989) published inferior values.

ENDF and IDF values were compared (Figure 1). Differences between both methods are ranged among 1.17-7.55%. Beans showed the smallest difference and lentils the highest. Mongeau and Brassard (1986) compared these methods in 16 different foods and obtained similar results.

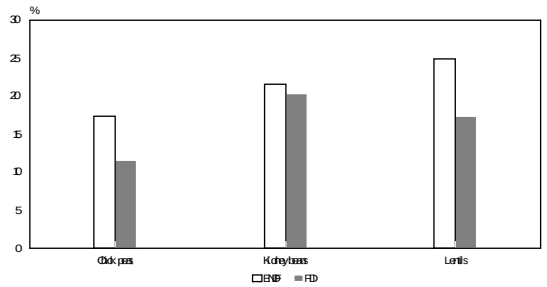

Figure 1.- Comparison between ENDF and IDF values

The present study only obtained these results in kidney beans. The statistical comparison between both methods showed a significant difference for lentils and chick peas ($p<0.001$) and a non-significant difference for kidney beans. The determination of nitrogen in ENDF and IDF residues gave a high amount of it in the latter. The proportion of the remaining nitrogen was between (8.5-13,8%) expresed in percentage of protein. The nitrogen content in ENDF residue was very small (Table IV). Detergent solution removed almost completely the nitrogen of the sample. Therefore, may be eliminate this step (nitrogen residue determination) using ENDF method but it isn't posible in IDF method.

The increase of ENDF respect to IDF could be due to insufficient starch hydrolysis. Vidal-Valverde et al, (1992) employed bacterial alpha-amylase during overnight previously NDF determination. When we used bacterial alpha-amylase (Termamyl) during a long time (18 h) didn´t produce a drop of the ENDF value. Alpha-amylase pancreatic was reported by Mongeau and Brassard (1995) for starch hydrolysis and they suggest to use of this enzyme in AOAC method.

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