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Enzymatic determination of soluble and insoluble dietary fiber in rice and wheat bran

Alex Augusto Gonçalvez, Eliana Badiale-Furlong, Leonor Almeida de Souza-Soares, Simone Treibs Siervs

Fundação Universidade de Rio Grande-FURG, Rio Grande, Brazil

SUMMARY. The information about dietary fiber presents controversies in many research areas such as in nomenclature, related illnesses, recommended quantities and terminology, mainly because of lack of analytical data. Different needs and interests for the dietary fiber composition of foods and forages have led to a proliferation of methods for its analysis. This research, a further adaptation of the enzymatic method of Asp et al. (1983) for its application is proposed for rice and wheat bran, byproducts of agroindustries in the southern region of Rio Grande do Sul (Brazil). The inclusion of Amyloglucosidase in the proposed methodology contributed to the decrease in the content of residual starch at the end of the experiment, like Prosky et al (1992). To increase the efficiency of the enzyme system inthis type of samples, other changes were made with respect to incubation time and proteolytic enzyme concentration. In the final adaptation, a decrease of 51.33% of the starch content was observed in rice bran (RB) and of 52.93% in wheat bran (WB). This decrease was also verified in the model system (MS) (52.08%), which demonstrates the adequacy of the proposed adaptation. With respect to the residual protein, it was verified that the measures adopted provoked a reduction of 42.15% (RB), 52.19% (WB) and 42.11% (MS) as compared to the original method. Then the proposed conditions has been shown to be efficient in decreasing the level of interference (indigestible starch and protein) in the quantification of dietary fiber in rice and wheat bran.

Key words: Dietary fiber, enzymatic determination, rice bran, wheat bran.

RESUMEN. Determinación enzimática de la fibra dietética soluble e insoluble en salvados de arroz y trigo. Las informaciones sobre fibra dietética muestran controversias en diferentes áreas de investigación, referentes a la nomenclatura, enfermedades relacionadas, cantidades recomendadas y terminología, principalmente por la carencia de datos analíticos. Diferentes necesidades e intereses, con relación a la composición de la fibra dietética en los alimentos, han llevado a una proliferación de métodos para este tipo de análisis. En este trabajo fue propuesta una adaptación del método enzimático de Asp y col. (1983), especifico para salvados de arroz y trigo, ambos sub-productos de la agroindustria del sur de Rio Grande do Sul, Brasil. La inclusión de la enzima amiloglucosidasa dentro de la metodología propuesta favoreció la reducción del contenido de almidón residual al final de la experiencia (Prosky y col., 1992). Para aumentar la eficiencia del sistema enzimático para estos tipos de muestras, fueron realizados otros cambios, relacionados con los tiempos de incubación y concentración de las enzimas proteolíticas. Al final de la adaptación fue verificada una disminución de 51.33% en el contenido de almidón en el salvado de arroz (RB) y 52.93% en el salvado de trigo (WB). Esta caída también fue verificada en el sistema modelo (MS) (52.08%), lo que demostró la adecuación de la adaptación propuesta. Con respecto a la proteína residual, se verificó que los cálculos adaptados provocaron una reducción de 42.15% (RB), 52.19% (WB) y 42.11% (MS), cuando fueron comparados con el método original. Así, las condiciones propuestas se mostraron eficientes para disminuir los niveles de interferencia (ocasionada por almidón y proteína no digeribles) en el cálculo de la fibra dietética de salvado de arroz y trigo.

Palabras clave: Fibra dietética, determinación enzimática, salvado de arroz, salvado de trigo.

INTRODUCTION

A great concern of professionals in the area of Nutrition and Food Science nowadays is related to the adequate consumption of dietary fiber (1). Thus there is a necessity to obtain quantitative and qualitative data about its presence in food. The information about fiber presents controversies in many research areas such as in nomenclature, related illnesses, recommended quantities and terminology, mainly becasuse of lack of analytical data.

The inadequate use of techniques in the determination of the fiber content of a specific food may result in damage to the human organism. For example, in the analysis of beans, it was found that the fiber content obtained by acid and alkaline digestion is much smaller than that called "dietary fiber" or "physiological fiber" obtained after enzymatic digestion with evident implications in the calculation of the caloric value of that food (2).

It is known that part of the starch associated with the fiber fraction called "resistant starch" and which is difficult to analyze, may be metabolized by bacteria in the large intestine producing short chain fatty acids which take part in the energy

metabolism of the enterocyte and show constant action in the control of the intestinal pH and in the cellular proliferation of the mucosa (2,3).

The analytical evaluation of this fraction and the knowledgement of its distribution in vegetable food and its physiological action when present in diets are necessary for the elaboration of fiber rich products, since the consumption of fiber rich food has recently grown indiscriminately.

The enzymatic-gravimetric method, developed by Hellendoorn et al. (4) and later modified by Asp et al. (5), and more recently by Prosky et al. (6), determines the total content of the fiber fraction of the food. Although its execution is very fast when compared with others methods, and this method does not allow for the complete isolation of each component, although it can determine the soluble and insoluble fractions separately.

On a national level, few studies about the dietary fiber content of Brazilian food have been developed. The work of Filisetti-Cozzi & Lajolo (7) can be mentioned as a contribution to the elaboration of a system of reliable data about the total fiber content of Brazilian foods.

This work belongs to a research string that studies the exploitation of by-products of agroindustries (particularly rice and wheat bran) in the southern region of Rio Grande do Sul (Brazil) and was intended to evaluate the dietary fiber of the same ones. As there were no enzymes and no materials recommended for the official method (6), this research was a further modification of Asp et al. (5)'s enzymatic method proposed for application to rice and wheat bran. Such a method was adjusted better for our laboratory conditions, obtaining satisfactory results as well as that of diminishing the content of indigestible starch which is the main problem with this kind of matrix.

Deffated rice bran was used initially, because it shows a great potencial as a food ingredient when compared to wheat bran, which is already very much use, and also because it is abundant in the region.

Basically the modifications were made in the conditions used for enzymatic hydrolysis for in the gravimetric procedures, enzymatic and chemical stages were being used which extracting compounds which are not part of the fiber fraction (8).

MATERIAL AND METHODS

Reagents

The following reagents were used according to A.O.A.C. (9): phosphate buffer 0.1 M (pH 6.0), 95% ethanol, 78% ethanol, acetone p.a., HCl (1:1), NaOH (1:1), H_2SO_4 (0,0099 N) HCl, 15% potassium ferrocyanide, 30% zinc sulfate, Fehling A and B solutions, 1% fenolftalein, methylene blue solution and distilled water.

Enzymes

The following enzymes were used: Termamyl (α-amylase) 1:10 (120 L - NOVO - 120 KNU/g), Amyloglucosidase 1:10 (NOVO - 300 AGU/ml), Pepsin (Riedel de Haënag-Seelze-Hannover-10000 E/g) and Pancreatin (MERCK - 0.1 m Ansom - Protease E/mg; 7.5 FIP - Lypase U/mg; 10 Amylase U/mg).

Samples

The samples of deffated rice bran (RB) (*Oryza sativa*, L.) were obtained directly from a local rice processing plant, and the samples of wheat bran (WB) (*Triticum aestivum*, L.) were obtained from a wheat mill, in Rio Grande, Rio Grande do Sul (Brazil).

These samples were first sifted to remove chucks and dirt. After sifting, the samples were homogenized by crushing and sifted again. They were then packed and stored in freezers at -20°C.

To test the efficiency of the new method, a model system (MS) was made up from the chemical composition of the deffated rice bran, with the same granulometry of others samples and is described in Table 1.

TABLE 1
Proximate Composition (g/100 g) of Model System

Moisture	13.6	Endogenous humidity of each component
Protein	14.8	7.375 g of bovine albumin and 7.375 g of casein
Lipid	2.8	2.7 g of soya oil
Starch	36.4	36.4 g fo commercial corn starch
Insoluble fiber	26.1	26.1 g of grounded filter paper
Soluble fiber	4.5	4.5 g of pectin powder
Ash	10.9	1.39 g of P; 1.31 g of K; 0.035 g of Ca; 0.88 g of Mg; 0.0044 g of Na; 0.10253 g of Fe; 0.05445g of Mn; 0.0042 g of Cu; 0.04047 g of Zn

The quantities of each component of the ash were calculated according to Alencar & Alvarenga (11).

METHODOLOGY

The centesimal composition of the samples were made in triplicate and determined according to the A.O.A.C. (9) procedures for moisture, ash, protein, lipid and raw fiber.

The method described by Asp et al. (5) was used as the standard method. This uses a rapid system of enzymatic assays in the quantification of soluble and insoluble dietary fiber, with some adaptations specific to our laboratory. Based on the standard method, sucessive changes were made with respect to the incubation time and the inclusion fo other amylolitic enzyme in the process (Table 2).

TABLE 2
Modifications to the enzymatic method of Asp et al. (5)

Methods	Changes
Standar	2 ml of Termamyl (15 min.) 400 mg of Pepsin (60 min.) 400 mg of Pancreatin (60 min.)
First Modification	The amount of enzymes were doubled 2 mil of Termamyl (15 min.) 800 mg of Pepsin (60 min.) 800 mg of Pancreatin (60 min.)
Second Modification	Amyloglucosidase was added to the system 2 ml of Termamyl (15 min.) 2 ml of Amyloglucosidase (30 min.) 800 mg of Pepsin (60 min.) 800 mg of Pancreatin (60 min.)
Third Modification	Termamyl action time was increased 2 ml of Termamyl (30 min.) 2 ml of Amyloglucosidase (30 min.) 800 mg of Pepsin (60 min.) 800 mg of Pancreatin (60 min.)
Fourth Modification	The enzymes were solubilized 2 ml of Termamyl (30 min.) 2 ml of Amyloglucosidase (30 min.) 5 ml of Pepsin (60 min.) 5 ml of Pancreatin (60 min.)
Fifth Modification	Amyloglucosidase action time were increased 2 ml of Termamyl (30 min.) 2 ml of Amyloglucosidase (60 min.) 5 ml of Pepsin (60 min.) 5 ml of Pancreatin (60 min.)

The differente combinations of time, temperature, pH and enzyme concentrations were chosen by a preliminary study of enzymatic activity for each enzyme according A.O.A.C. (9). The specific activity was: for Termamyl (21 μg product/min.μg protein) and for Pancreatin (2,4 μg product/min.μg protein). For Termamyl, the ideal incubation temperature/time is 95°C/15 min. Thus, the utilization of incubation temperature of 70°C was used to increase the incubation time (30 min.) and not to cause its denaturation.

The soluble and insoluble fiber fractions in the rice and wheat bran and model system samples were made in triplicate and quantified according to the following procedure: First, 1 g of sample was dissolved in 25 ml of phosphate buffer solution (0,1M- pH 6.0); 2 ml of Termamyl solution (1:10) was added, and the mixture was incubated in a water bath (70°C) for 30 minutes with occasional stirring; 2 ml of Amyloglucosidase (1:10) was then added and incubated in a water bath (60°C) for 30 minutes with constant stirring. When cool, the pH was adjusted to 1.5 with HCl 1:1 and 5 ml of pepsin (160 mg/ml) was added and incubating inwater bath at 40°C with stirring for 60 minutes. The pH was then adjusted to 6.8 with NaOH, 5 ml of pancreatin was added and the mixture incubated in water bath (40°C) with stirring for 60 minutes. The pH was adjusted to 4.5 with HCl 1:1, and the contents allowed to sediment for 1 hour, before being filtered in a previously weighed sintered glass crucible (ASTM porosity 4.4 - 5.0, 50 ml Pyrex, CORNING N° 32940 GOOCH). The precipitate was washed twice with 10 ml of distilled water, twice with 10 ml of 95% ethanol and twice with 10 ml of acetone. It was then dried at 105°C to constant weight and incinerated at 550°C for at least 5 hours, and weighed again after being cool in the desiccator, thus obtaining the value of insoluble fiber. The filtrate was collected in a beaker and completed to 100 ml with distilled water; 400 ml of 95% ethanol were then added and incubated in water bath at 60°C for one hour. The mixture was then filtered in a previously weighed crucible, washed twice with 10ml of 78% ethanol, twice with 10 ml of 95% ethanol and twice with 10 ml of acetone before drying to constant weight at 105°C and finally incinerated at 550°C for at least 5 hours and then cooled in the desiccator to obtain the value of soluble fiber.

Determination of indigestible protein and starch

The bran samples were separated at the end of each filtration, and dried in an oven (105°C) for an hour before determining protein and starch.

The total nitrogen content was dosed according tothe microkjeldahl method, methodology described by A.O.A.C. (9), using a factor of 5.95 (for cereals) for the protein calculation. Starch was determined according to the methodology described by A.O.A.C. (9).

RESULTS AND DISCUSSION

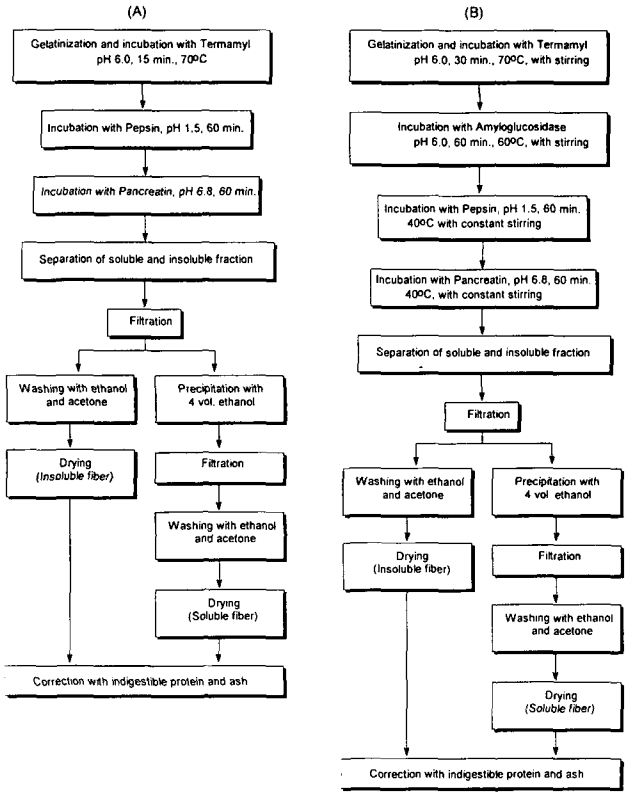
The centesimal composition (moist base) of the rice and wheat bran are in agreement with the literature as shown in Table 3.

TABLE 3
Proximate composition (g/100 g) of rice bran (RB) and wheat bran (WB)

Components	Rice Bran	Wheat Bran
Moisture	13.55 ± 1.43	13.96 ± 0.51
Ash	10.92 ± 0.71	4.92 ± 0.47
Protein	14.75 ± 0.80	13.73 ± 0.75
Lipid	2.68 ±0.18	6.84 ± 0.34
Starch	36.40 ±0.82	40.97 ±0.32
Crude Fiber	7.10 ± 0.17	7.08 ± 0.15

To get a better view of the proposed procedure and to have a comparison to the original method, the main stages are described in the following flowsheet (Fig. 1).

FIGURE 1
Simplified flowsheet of (A) original method of Asp et al. (4) and (B) proposed method (fifth modification)



The results for the contents of soluble and insoluble fiber, residual starch and protein of the rice bran, wheat bran and model system are presented in Table 4 and 5.

It was verified that the first modificationof the method did not affect the results for the total contents of dietary fiber of the bran samples, which changed from 39.6 g/100 g to 38.9 g/100 g (RB) and from 47.4 g/100 g to 45.1 g/100 g (WB). However, when a new enzyme system (Amyloglucosidase) was added (second modification), a decrease inthe content of total dietary fiber was observed giving values of 34.8 g/100 g and 43.7 g/100 g, results already obtained by Prosky et al. (6).

TABLE 4
Soluble and insoluble fiber (g/100 g). Original and proposed methods

Methods	Rice Bran	Wheat Bran	Model System
ASP et al (5)	IF: 35.43±0.36 SF: 4.16±0.07 TF: 39.59±0.42 (c.v. =1.06)	IF: 40.01±0.05 SF:7.35±0.02 TF: 47.36±0.05 (c.v. = 0.13)	IF: 36.41±0.35 SF:5.48±0.58 TF: 41.89±0.85 (c.v. = 2.03)
First modification	IF: 31.03±0.66 SF: 7.91±0.16 TF: 38.94±0.62 (c.v. = 1.59)	IF: 38.01±0.48 SF: 7.11±0.21 TF: 45.13±0.34 (c.v. = 0.75)	-
Second modification	IF: 26.62±0.08 SF: 8.24±0.38 TF: 34.86±0.45 (c.v. + 1.29)	IF: 36.68±0.08 SF: 7.07±0.21 TF: 43.75±0.29 (c.v. = 0.66)	-
Third modification	IF: 26.54±0.30 SF: 6.26±0.22 TF: 32.80±0.33 (c.v. = 1.01)	IF: 36.13±0.30 SF: 5.31±0.12 TF:41.44±0.42 (c.v. = 1.01)	-
Fourth modification	IF: 26.15±0.05 SF: 5.57±0.34 TF: 31.71±0.29 (c.v. = 0.91)	IF: 30.26±0.05 SF: 5.64±0.17 TF: 35.90±0.67 (c.v. = 1.87)	-
Fifth modification (proposed)	IF: 26.14±0.09 SF: 4.52±0.04 TF: 30.66±0.05 (c.v. = 0.16)	IF: 29.84±0.41 SF: 5.13±0.17 TF: 34.97±0.05 (c.v. = 1.43)	IF: 27.07±0.30 SF: 5.52±0.28 TF: 32.59±0.56 (c.v. = 1.72)

IF= Insoluble fiber; SF = Soluble fiber; TF = Total dietary fiber; c.v. = Coefficient of variation

TABLE 5
Indigestible protein* and starch* before and after last modification

Methods	Rice Bran		Wheat Bran		Model System	
	Protein	Starch	Protein	Starch	Protein	Starch
Asp et al. (1983)	10.25	15.00	9.58	17.23	9.00	12.50
Fifth modification	5.93	7.30	4.58	8.11	5.21	5.99

* g/100 g of indigestible residue

Due to the fact that these samples are rich in starch, 36.4 g/100 g (RB) and 41 g/100 g (WB), the inclusion of this enzyme in the proposed methodology contributed to the decrease in the content of indigestible starch at the end of the experiment, as mentioned in the literature.

To increase the efficency of the enzyme system, other changes were made with respect to incubation time and

enzyme concentration since proteins also contribute grealty to the determined high values of fiber.

In the fifth modification a decrease of 51.33% of the starch content was noted in RB and of 52.93% in WB. This decrease was also verified in the model system (52.08%), which demonstrates the adequacy of the proposed methodology. Under these conditions, the interferences by the residual content became more acceptable for the gravimetric determinations.

With respect to the indigestible protein, it was verified that the measures adopted caused a reduction of 42.15% (RB), 52.19% (WB) and 42.11% (SM) as compared to the original method.

It can be considered that the remaining mass, after correction for compounds not totally removed, such as digested protein and starch, corresponds to the dietary fiber, as stated by Filisetti-Cozzi (8); since it is considered that in the digestive process allstarch, and protein are barely digested in highly rich fiber matrices.

CONCLUSIONS

The inclusion of a new enzyme and the increase in incubation time and concentration of each enzyme, have been shown to be efficent in decreasing the level of interference (*in vitro* indigestible starch and protein) in the quantificationof dietary fiber.

The level of confidence of the proposed methodology was confirmed by the use of the model system.

The values obtained for dietary fiber in the bran were 17.43 g/100 g (RB) and 22.28 g/100 g (WB).

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