

# In Vitro Determination of the Indigestible Fraction in Foods: An Alternative to Dietary Fiber Analysis

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Dietary fiber (DF) intakes in Western countries only accounts for about one-third of the substrates required for colonic bacterial cell turnover. There is a general trend among nutritionists to extend the DF concept to include all food constituents reaching the colon. In this line, a method to quantify the major nondigestible components in plant foods, namely, the indigestible fraction (IF), is presented. Analytical conditions for IF determination are close to physiological. Samples, analyzed as eaten, were successively incubated with pepsin and  $\alpha$ -amylase; after centrifugation and dialysis, insoluble and soluble IFs were obtained. IF values include DF, resistant starch, resistant protein, and other associated compounds. IF contents determined in common foods (cereals, legumes, vegetables, and fruits) were higher than DF contents. Calculated IF intakes were close to the estimated amount of substrates reaching the colon. IF data could be more useful than DF data from a nutritional point of view; therefore, IF is proposed as an alternative to DF for food labeling and food composition tables.

**Keywords:** *Dietary fiber; resistant starch; indigestible fraction; vegetable foods, analysis*

## INTRODUCTION

The role of dietary fiber (DF) in nutrition and health is well established (Anderson et al., 1990; Kritchevsky and Bonfield, 1995; Ling, 1995). Knowledge of the beneficial effects of high dietary fiber diets toward the prevention of cardiovascular diseases and several types of cancer, as well as the inclusion of DF supplements in slimming diets, has led to the development of a large and yielding market for DF-rich products. Products offered to the consumer include traditional foods (meat, dairy products, breakfast cereals, biscuits, breads, etc.) enriched with different amounts of fiber from various sources, as well as dietary supplements including tablets, capsules, etc.

Determination of the DF content in foods and food products for labeling purposes requires a simple and accurate method. Among the many procedures for DF analysis developed in the last two decades, the most widely used at present is the official AOAC method of Prosky et al. (1988). Some errors associated with this analytical procedure have been described (Mañas and Saura-Calixto, 1993, 1995; Mañas et al., 1994).

The objective of the AOAC method for DF determination is to measure the content of the fraction traditionally defined as fiber (i.e., nonstarch polysaccharides and lignin). The main steps in the experimental protocol are the enzymatic treatments to remove protein and starch. However, there is recent evidence that a significant part of the starch content in foods, namely, resistant starch (RS) (Englyst et al., 1982; Asp, 1992),

escapes digestion and absorption in the human small intestine, along with other dietary substances not included in the DF definition such as protein, oligosaccharides, certain polyphenolic compounds, etc. (Cummings and MacFarlane, 1991; Bravo, 1998). These substances can be fermented by the colonic microflora, having physiological effects similar to those of the DF.

The general trend among nutritionists is to extend the concept of DF to include all major food constituents reaching the colon to be substrates for the fermentative microflora. (Saura-Calixto, 1988; Asp et al., 1996; Prosky, 1999). This new, extended DF concept demands a modification of the available analytical procedures so all these compounds could be quantified in a single analysis. DF in food composition tables, as well as in food labeling, would need to be changed accordingly.

In this line, the aim of this work was to propose a simple and reliable method to determine the indigestible fraction (IF) of foods, which would comprise most components of vegetable foods that escape digestion and absorption in the small intestine, reaching the colon where they are susceptible to bacterial fermentation. IF values would be more accurate from both nutritional and commercial points of view.

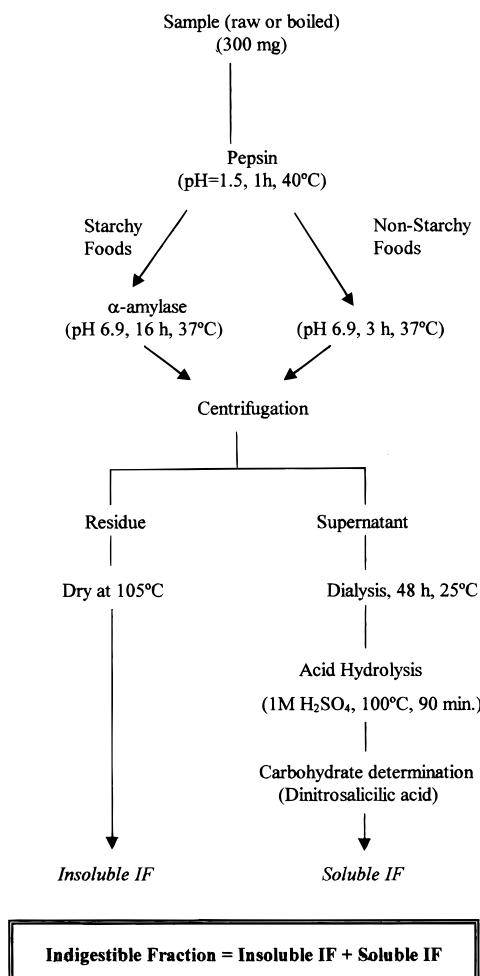
## MATERIALS AND METHODS

**Sample Preparation.** Common dietary foods, including cereals, legumes, vegetables, and fruits, were chosen as representative of both starchy and nonstarchy foods. All products were purchased in local supermarkets. The individual foods were (cereals) white bread (Bimbo S.A., Barcelona, Spain), spaghetti (Gallo S.A., Barcelona, Spain), rice (Nomen S.A., Valencia, Spain), Corn Flakes (Kellogg's, S.A., Barcelona, Spain), and biscuits (Maria Fontaneda S.A., Spain), (legumes) lentils, chickpeas, and white and pinto beans (Koifer S.A., León, Spain) and frozen peas (Pescanova S.A., Vigo, Spain),

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**Figure 1.** Scheme of the method for determination of the IF of foods. Enzymatic incubations of food samples as eaten (raw or cooked) are performed according to their starch content. After hydrolysis and centrifugation, insoluble IF residues are dried and quantified gravimetrically. Soluble IF is determined spectrophotometrically (dinitrosalicylic acid) after dialysis to remove products of enzymatic digestion, and acid hydrolysis of constituent polysaccharides.

(vegetables) potato crisps (Matutano S.A., Barcelona, Spain), potatoes, and tomatoes, and (fruits) ripe bananas, apples, and oranges.

All foods were analyzed as eaten. White bread, Corn Flakes, biscuits, crisp potatoes, tomatoes, bananas, apples, and oranges were analyzed raw, while rice, spaghetti, lentils, chickpeas, white beans, pinto beans, peas, and potatoes were boiled before the analysis. Those samples with a fat content higher than 5% of the dry matter (biscuits and crisps) were defatted with light petroleum prior to the analysis using a Soxhlet apparatus (Soxhlet System HT Tecator, Höganäs, Sweden).

Sample preparation and analysis were carried out in the same tube. Raw samples were homogenized in 10 mL of HCl-KCl buffer under controlled conditions (speed level 2, 1 min) using a Polytron homogenizer (Polytron PCU Kinematica GmbH, Switzerland). Those foods consumed cooked were boiled in tap water (5 mL) in capped tubes until edible (cooking time ranging from 15 to 90 min for pasta and beans, respectively). A 10 mL sample of HCl-KCl buffer was added after boiling, and samples were homogenized as described.

**Determination of the Indigestible Fraction.** The procedure to determine the indigestible fraction of foods is summarized in Figure 1. To 300 mg of raw or cooked food, prepared as described above, was added 0.2 mL of a pepsin (Cat. No. 7190, Merck, Darmstadt, Germany) solution containing 300 mg of pepsin/mL of HCl-KCl (0.05 M HCl and 0.03 M KCl, respectively) buffer, pH 1.5. Samples were incubated

for 1 h at 40 °C in a water bath with constant shaking. Then, 9 mL of Tris-maleate buffer (0.1 M, pH 6.9) was added and the pH checked. α-Amylase (1 mL of a 120 mg/mL solution in Tris-maleate buffer; Sigma, A-3176) was added, and the samples were incubated in a water bath at 37 °C for 16 h with constant shaking.

In nonstarchy foods or foodstuffs with negligible starch content, amylase treatment was unnecessary. Nonetheless, experimental conditions were maintained to reproduce conditions applied for starchy foods. Thus, Tris-maleate buffer was added after pepsin incubation, and samples were maintained at 37 °C, pH 6.9, to mimic the intestinal digestion step. Incubation times for nonstarchy foods were reduced to 3 h to facilitate analysis within a working day. Previous experiments with fruits (apples and oranges) showed no differences in the total IF values obtained after 3 vs 16 h (16.97% vs 16.66% IF for apples and 26.50% vs 26.23% IF for oranges after 3 and 16 h of incubation, respectively). This clearly shows that the presence or absence of amylolytic enzymes (amylase) and the length of incubation have no influence on the final IF values for nonstarchy foods. Nevertheless, for convenience authors can choose to run in parallel starchy and nonstarchy foods for longer incubation times bearing in mind it would not affect the final result.

At the end of the second incubation step (3 or 16 h), samples were centrifuged (15 min, 3000g) and supernatants removed. Residues were washed twice with 10 mL of distilled water and all supernatants combined. The residues were dried overnight at 105 °C and quantified gravimetrically as the insoluble indigestible fraction (iIF). Supernatants were transferred into dialysis tubes (12000–14000 MWCO; Dialysis Tubing Visking, Medicell International Ltd., London, U.K.), and dialyzed against water for 48 h at 25 °C (water flow 7 L/h). Dialysates were then hydrolyzed with 1 M sulfuric acid at 100 °C for 90 min, and the soluble indigestible fraction (sIF) was measured with dinitrosalicylic acid (Englyst and Cummings, 1988).

**Dietary Fiber Determination.** Dietary fiber in the selected foods was analyzed by the AOAC method (Prosky et al., 1988), modified in our laboratory (Mañas et al., 1994). Samples were treated with heat-stable α-amylase (Sigma, A-3306), protease (Sigma, P-3910), and amyloglucosidase (Sigma, A-9913), followed by centrifugation (15 min, 3000g) instead of filtration to separate the soluble and insoluble fractions. Dialysis against water, performed as described above, substituted ethanol precipitation of soluble dietary fiber (SDF). Both dialysates and residues from centrifugation were submitted to acid hydrolysis with sulfuric acid, and the constituent neutral sugars (NS) and uronic acids (UA) quantified. Klason lignin (KL) was quantified gravimetrically in the insoluble residues after acid hydrolysis.

UA were quantified spectrophotometrically by the Scott method (1979) using galacturonic acid as standard. NS were analyzed by GLC as alditol acetates (Englyst and Cummings, 1988). A Shimadzu GC-14 A chromatograph (Shimadzu Co., Kyoto Japan) fitted with a flame ionization detector and connected to a C-R4A Chomatopac computer system was used. A SP-2330 capillary column (30 m × 0.32 mm i.d., Cat. No. 2-4073, Supelco, PA) was used. Analytical conditions were as follow: column temperature 240 °C (isothermal), injector temperature 270 °C, detector temperature 270 °C, carrier gas nitrogen. IDF was calculated as UA + NS + KL, and SDF as UA + NS.

**Resistant Starch Determination.** RS was measured by the procedure of Goñi et al. (1996). Briefly, protein and digestible starch were removed after treatment with pepsin (Cat. No. 7190, Merck, Darmstadt, Germany; 40 °C, 1 h, pH 1.5) and α-amylase (Sigma, A-3176; 37 °C, 16 h, pH 6.9), respectively. After centrifugation, residues were dispersed with 2 M KOH to solubilize RS and incubated with amyloglucosidase (Cat. No. 102857, Boehringer Mannheim, Germany; 60 °C, 45 min, pH 4.75), and glucose was quantified spectrophotometrically using the GOD-PAP reagent (Cat. No. 676543, Boehringer Mannheim, Germany). RS was calculated as glucose × 0.9.

**Table 1. Indigestible Fraction and Total Dietary Fiber Content of Foods (Mean values  $\pm$  SD;  $n = 4$ ; Percent on Dry Basis)**

	indigestible fraction	AOAC dietary fiber <sup>a</sup>		
		IDF	SDF	TDF <sup>b</sup>
Corn Flakes	11.61 $\pm$ 0.11	2.47 $\pm$ 0.16	1.08 $\pm$ 0.09	3.55 $\pm$ 0.12
biscuits	10.28 $\pm$ 1.04	0.65 $\pm$ 0.16	2.75 $\pm$ 0.59	3.40 $\pm$ 0.63
rice	12.13 $\pm$ 0.83	3.22 $\pm$ 0.25	0.89 $\pm$ 0.06	4.11 $\pm$ 0.23
spaghetti	14.01 $\pm$ 0.82	3.33 $\pm$ 0.15	3.03 $\pm$ 0.07	6.36 $\pm$ 0.12
white bread	13.80 $\pm$ 0.45	0.81 $\pm$ 0.12	3.13 $\pm$ 0.08	3.84 $\pm$ 0.12
		Cereals		
white beans	35.59 $\pm$ 0.51	15.22 $\pm$ 0.24	7.76 $\pm$ 0.36	22.98 $\pm$ 0.59
pinto beans	43.67 $\pm$ 2.21	14.30 $\pm$ 0.50	11.77 $\pm$ 0.48	25.04 $\pm$ 0.81
lentils	29.15 $\pm$ 0.47	18.85 $\pm$ 0.12	2.70 $\pm$ 0.08	21.55 $\pm$ 0.22
chickpeas	28.03 $\pm$ 0.92	13.12 $\pm$ 1.05	2.15 $\pm$ 0.12	15.27 $\pm$ 1.01
peas	42.30 $\pm$ 3.58	17.83 $\pm$ 1.30	5.83 $\pm$ 0.22	23.36 $\pm$ 0.92
		Legumes		
potatoes	12.18 $\pm$ 0.89	3.82 $\pm$ 0.46	7.28 $\pm$ 0.24	11.10 $\pm$ 0.46
crisp potatoes	15.04 $\pm$ 0.77	10.48 $\pm$ 0.05	2.84 $\pm$ 0.15	13.32 $\pm$ 0.36
tomatoes	34.52 $\pm$ 1.54	26.48 $\pm$ 0.90	2.70 $\pm$ 0.42	29.18 $\pm$ 0.90
		Vegetables		
bananas	36.08 $\pm$ 3.21	9.43 $\pm$ 0.22	5.44 $\pm$ 0.18	14.87 $\pm$ 0.89
apples	16.97 $\pm$ 0.45	7.12 $\pm$ 0.57	5.05 $\pm$ 0.49	12.10 $\pm$ 0.56
oranges	26.50 $\pm$ 0.95	10.87 $\pm$ 0.23	4.44 $\pm$ 0.16	15.31 $\pm$ 0.12
		Fruits		

<sup>a</sup> Determined by the AOAC modified procedure (Mañas et al., 1994). SDF = soluble dietary fiber; IDF = insoluble dietary fiber; TDF = total dietary fiber. <sup>b</sup> TDF calculated as SDF + IDF.

**Other Determinations.** Resistant protein was quantified in the insoluble IF residues using an automated nitrogen analyzer (LEKO, FP-2000, LEKO Corp., Michigan). Protein was calculated as N  $\times$  6.25.

Ash content was determined gravimetrically after calcination in a muffle furnace at 550 °C for 16 h.

**Statistics.** Results are expressed as the mean  $\pm$  standard deviation. Comparison of IF and DF values was performed by linear regression using a Statgraphics Plus program, version 2.1 (Statistical Graphics Corp., Rockville, MD).

## RESULTS AND DISCUSSION

**Indigestible Fraction in Foods.** The IF is defined as the part of vegetable foods that is not digested nor absorbed in the small intestine, reaching the colon where it is a substrate for the fermentative microflora. As such, it 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 method for IF determination (Figure 1) combines in a single procedure the methodologies for DF (Mañas et al., 1994) and resistant starch (Goñi et al., 1996) analysis. It is worth noting that, in this method, (i) samples are analyzed as eaten (fresh, boiled, or fried) and (ii) analytical conditions (pH, temperatures, incubation times) are close to physiological ones.

The content of IF and DF in the analyzed food samples is shown in Table 1. As expected, IF values were always higher than DF values due to the presence of other constituents different from nonstarch polysaccharides (NSP) and lignin. Table 2 shows the IF composition. As can be seen, substantial amounts of resistant starch and resistant protein were present in the insoluble residue, as well as minor amounts of minerals.

RS, present in raw and processed foods, is defined as the sum of starch and the products of starch degradation not absorbed in the small intestine of healthy individuals (Asp, 1992). In the large intestine, RS is fermented almost quantitatively, being one of the main substrates for the colonic microflora (Asp et al., 1996). Although

there are specific methods for RS determination, we consider that a joint determination of DF and RS in foods would be of value due to the similar physiological behavior and effects of both indigestible fractions.

On the other hand, resistant protein (RP) is also included as a component of the IF (Table 2). Estimates suggest that between 6 and 18 g of nitrogen-containing compounds enters the colon on a daily basis (Cummings and MacFarlane, 1991; Andersson et al., 1996). Most of this protein is of endogenous origin (MacFarlane and MacFarlane, 1995), although dietary protein also contributes to the nitrogen entering the colon. It has been estimated that between 3 and 9 g of RP reaches the large intestine daily, depending on the physical form of the food and the protein in the diet (Chacko and Cummings, 1988). These physiological grounds support the inclusion of RP in the definition of IF.

Minerals remaining in the iIF residues (Table 2), unreleased after enzymatic treatments, would probably be strongly attached to the plant cell wall. Therefore, they would not be absorbed within the small intestine, reaching the colon where absorption could take place after fermentation of cell wall polysaccharides. Nonetheless, they constitute a minor fraction of lesser analytical importance, since their content lies within the standard deviations of IF values.

Gravimetric values of the iIF residues after subtraction of RS, RP, and ash would correspond mainly to NSP and lignin, i.e., to the insoluble dietary fiber (IDF) fraction according to its traditional definition. On the other hand, the composition of the sIF would be similar to that of the soluble DF (soluble nondigestible polysaccharides) because both fractions correspond to the retentate after dialysis of supernatants from the enzymatic hydrolysis. In fact, sIF and SDF values showed a good linear correlation ( $r = 0.90$ ,  $p \leq 0.01$ ); small differences between both values are due to the different experimental conditions in IF and DF analysis (sample preparation, incubation times, pH, and temperatures).

**Dietary Fiber, Indigestible Fraction and Carbohydrate Gap.** From a physiological point of view,

**Table 2. Major Components of the iIF and sIF of Foods (Mean Values  $\pm$  SD;  $n = 4$ ; Percent on Dry Basis)**

	iIF	RS <sup>a</sup>	major iIF components			sIF
			RP <sup>b</sup>	minerals	NSP + KL <sup>c</sup>	
Corn Flakes	10.44 $\pm$ 0.12	3.02 $\pm$ 0.05	4.36 $\pm$ 0.84	0.45 $\pm$ 0.01	2.61	1.17 $\pm$ 0.23
biscuits	7.63 $\pm$ 1.08	1.59 $\pm$ 0.18	3.43 $\pm$ 0.16	0.50 $\pm$ 0.00	2.11	2.65 $\pm$ 0.41
rice	9.94 $\pm$ 0.86	2.53 $\pm$ 0.68	3.62 $\pm$ 0.25	0.51 $\pm$ 0.02	3.28	2.19 $\pm$ 0.31
spaghetti	11.91 $\pm$ 0.79	2.92 $\pm$ 0.64	4.49 $\pm$ 0.87	0.45 $\pm$ 0.00	4.05	2.10 $\pm$ 0.42
white bread	11.19 $\pm$ 0.40	2.49 $\pm$ 0.57	2.55 $\pm$ 0.43	0.56 $\pm$ 0.03	5.56	2.61 $\pm$ 0.24
Legumes						
white beans	28.34 $\pm$ 0.51	4.96 $\pm$ 0.90	4.30 $\pm$ 0.58	1.38 $\pm$ 0.01	17.70	7.25 $\pm$ 0.77
pinto beans	33.93 $\pm$ 1.56	5.48 $\pm$ 0.62	6.36 $\pm$ 0.26	1.39 $\pm$ 0.00	20.7	9.74 $\pm$ 0.85
lentils	27.30 $\pm$ 0.99	7.56 $\pm$ 0.60	6.01 $\pm$ 0.34	0.96 $\pm$ 0.03	12.77	1.85 $\pm$ 0.26
chickpeas	26.01 $\pm$ 0.98	4.35 $\pm$ 0.40	5.32 $\pm$ 0.75	0.76 $\pm$ 0.02	15.58	2.02 $\pm$ 0.59
peas	35.79 $\pm$ 2.65	10.03 $\pm$ 0.40	4.40 $\pm$ 0.31	1.40 $\pm$ 0.00	19.96	6.51 $\pm$ 1.17
Vegetables						
potatoes	6.68 $\pm$ 0.10	1.18 $\pm$ 0.14	0.15 $\pm$ 0.02	0.16 $\pm$ 0.00	5.19	5.50 $\pm$ 0.70
crisp potatoes	13.07 $\pm$ 0.72	3.27 $\pm$ 0.79	0.87 $\pm$ 0.11	0.65 $\pm$ 0.00	8.28	1.97 $\pm$ 0.40
tomatoes	31.21 $\pm$ 2.42		5.15 $\pm$ 0.25	2.57 $\pm$ 0.22	23.49	2.73 $\pm$ 0.38
Fruits						
bananas	34.63 $\pm$ 2.99	19.44 $\pm$ 1.49	4.34 $\pm$ 0.01	1.70 $\pm$ 0.02	9.15	1.45 $\pm$ 0.89
apples	13.90 $\pm$ 0.48		1.12 $\pm$ 0.09	0.43 $\pm$ 0.03	12.35	3.07 $\pm$ 0.46
oranges	23.52 $\pm$ 1.04		3.15 $\pm$ 0.01	0.39 $\pm$ 0.05	19.98	2.98 $\pm$ 0.54

<sup>a</sup> Resistant starch. <sup>b</sup> Resistant protein. <sup>c</sup> Nonstarch polysaccharides and Klason lignin determined by the difference iIF – RS – RP – minerals.

**Table 3. Differential Features of the Indigestible Fraction and Dietary Fiber<sup>a</sup>**

	indigestible fraction	dietary fiber
definition	major nondigestible food components	restricted to NSP + lignin
analytical conditions	physiological	not physiological
sample preparation	as eaten	milled and boiled
components of gravimetric residues	those included in the definition	NSP and lignin plus others (RS, RP, CT, etc.)
starch digestibility	not altered	artificially modified

<sup>a</sup> NSP = nonstarch polysaccharides; RS = resistant starch; RP = resistant protein; CT = condensed tannins.

IF values would be more representative than DF values. On the basis of bacterial growth, it has been estimated that up to 60 g of carbohydrates reaching the colon would be necessary to maintain the daily bacterial cell turnover (Cummings and MacFarlane, 1991; Stephen, 1991). However, DF intake in European countries only accounts for about 20 g of carbohydrate/day (Cummings and Frølich, 1993), which leaves what was called a "carbohydrate gap" of 40 g/day (Stephen, 1991).

Calculation of the daily fiber intake from the foods analyzed in the present work, using consumption data from the Spanish Household Survey (MAPA, 1999), gave an estimated DF consumption of 21 g/day. However, when the values of IF included in Table 1 were used, the intake of nondigestible compounds was 41 g of IF/day. This approximation is closer to the estimated 60 g/day mentioned above, the values of IF thus representing more closely the amount of nondigested substances reaching the colon. Endogenous compounds, mainly nitrogen-containing products, would make up the final amount of substrate necessary to maintain the bacterial cell turnover. In a study with ileostomie patients consuming diets high and low in DF, Andersson et al. (1996) found that nondigested compounds recovered in the ileostomy effluents averaged 76 and 44 g/day, respectively, which does approximate the 41 g/day intake of the indigestible fraction calculated in the present work.

**Indigestible Fraction as an Alternative to Dietary Fiber.** Over the last century, food analysts have used the concept of crude fiber to represent the indigestible part of plant foods, which was determined after strong acid and alkali treatments. In the early 1970s

the concept of dietary fiber, with a more physiologic base, was introduced. Values of crude fiber in foods were markedly lower than those of DF and less representative from a physiological point of view, and eventually DF replaced crude fiber for food labeling and in food composition tables. At this time, however, there is sufficient scientific evidence of the fact that DF only represents part of the substrates escaping the action of digestive enzymes in the small intestine. The indigestible fraction proposed here could be a suitable alternative to DF in food analysis. The main facts availing both the concept of IF and the proposed method for its determination are summarized in Table 3. Considerations on the adequacy of the IF concept have already been discussed, yet there are additional aspects related to the analytical methodology followed by the AOAC procedure that demand further reflection.

First, enzymatic treatments in the analysis of DF are performed at temperatures (60–100 °C) different from those in the human gastrointestinal tract. Besides, pH conditions (4.5–7.5) do not reproduce the acid gastric pH (1–1.5). On the contrary, temperature, pH, and incubation times in the IF analysis mimic physiological digestive conditions.

On the other hand, all DF values correspond to foods dried, milled (sample preparation), and boiled (heat-stable  $\alpha$ -amylase treatment), irrespectively of whether they are consumed raw or processed. Therefore, DF values in food composition tables are not representative of the food as eaten in most cases. DF values of raw (e.g., breakfast cereals, bread, biscuits, fruits) or processed foods (e.g., snacks, french fries, etc.) actually correspond to the boiled product (boiled bread, boiled

oranges, boiled crisps, etc.). In the IF procedure, foods are analyzed as eaten to avoid artificial modifications during analysis.

There is a discrepancy between the definition of DF as NSP plus lignin, and its measurement with the present analytical method. It is always assumed that DF values calculated after ash and protein correction of gravimetric DF residues correspond exclusively to the substances aimed at in the AOAC procedure. However, significant amounts of RS, polyphenols, and other substances such as Maillard reaction products in processed foods are often found in gravimetric residues, involving both qualitative and quantitative errors (Siljeström and Asp, 1985; Saura-Calixto, 1987, 1988; Goñi et al., 1989; Saura-Calixto et al., 1993; Bravo and Saura-Calixto, 1998).

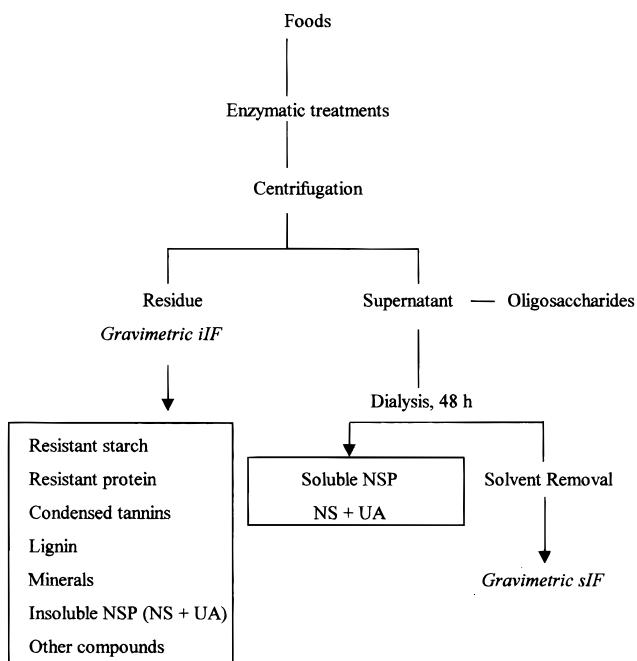
Another important consideration is the effect of analytical conditions on the digestibility of the starchy fraction in foods. Starch digestibility is altered artificially in the DF analysis because gelatinization of starch during the heat-stable amylase treatment would remove native resistant starch granules present in foods consumed raw, while milling of samples prior to analysis disrupts physically inaccessible resistant starch and renders it digestible. IF analysis follows experimental conditions previously tested to remove most digestible starch, avoiding alterations of naturally occurring RS.

Methodologically, filtration to separate IDF and SDF fractions is a difficult and time-consuming step in samples rich in soluble, viscous polysaccharides. Additionally, ethanol precipitation of filtrates is an important source of error in the quantification of SDF due to incomplete precipitation of SDF components on one side, and to coprecipitation of nonfiber components on the other (Mañas et al., 1994; Mañas and Saura-Calixto, 1995). The method for IF determination intends to minimize or avoid when possible the limitations associated with the AOAC method for DF: centrifugation and dialysis are performed instead of filtration and ethanol precipitation, and gravimetric corrections are avoided.

**Applications.** The IF method proposed here is characterized for its simplicity and versatility. The protocol, as described in the present work (Figure 1), is of application in food labeling and quality control. However, slight variations in the final steps of the method could expand its applications as schematized in Figure 2. Gravimetric quantification of the soluble IF residue can be performed after dialysis and solvent removal (i.e., by freeze-drying, vacuum-drying, etc.). Chemical analysis in both soluble and insoluble fractions can be performed to quantify NSP as neutral sugars (Englyst and Cummings, 1988) plus uronic acids (Scott, 1979). Also, other components such as resistant starch (Goñi et al., 1996), resistant protein (by the Kjeldahl method or any alternative procedure), condensed tannins (Reed et al., 1982), lignin (Edwards, 1973), or minerals (after calcination) in the insoluble fraction can be determined.

On the other hand, recent research has shown the nutritional importance of certain nondigestible oligosaccharides (NDO) (Van Loo et al., 1999), and their inclusion as DF components has been proposed (Cummings et al., 1997; Prosky, 1999). In such a case, oligosaccharides could also be quantified in the soluble IF prior to dialysis.

In summary, the proposed method is an attempt to quantify in a single analysis the major nondigestible components in plant foods. This method is based on a



**Figure 2.** Extended method for quantification and characterization of the IF. Enzymatic treatments of foods are as described in Figure 1. Individual IF constituents can be analyzed through available methodologies in both insoluble and soluble fractions (for a description see the text). iIF = insoluble indigestible fraction; sIF = soluble indigestible fraction; NSP = nonstarch polysaccharides; NS = neutral sugars; UA = uronic acids.

concept of the indigestible fraction that includes the main food constituents unavailable in the small intestine with nutritional relevance. IF values could be more useful than DF values from a nutritional point of view and could be a valid alternative to DF for food labeling and food composition tables.

#### ABBREVIATIONS USED

DF, dietary fiber; SDF, soluble dietary fiber; IDF, insoluble dietary fiber; IF, indigestible fraction; iIF, insoluble indigestible fraction; sIF, soluble indigestible fraction; NSP, nonstarch polysaccharides; NS, neutral sugars; UA, uronic acids; KL, Klason lignin; RS, resistant starch; RP, resistant protein; CT, condensed tannins; GLC, gas-liquid chromatography; NDO, nondigestible oligosaccharides.

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