

Analytical Methods

Soybean seeds and its by-product okara as sources of dietary fibre. Measurement by AOAC and Englyst methods

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Abstract

The composition of soybean seeds and its by-product okara has been studied in this work. Dietary fibre was analysed by Englyst et al. method, by enzymatic–gravimetric methods of AOAC and by the quantification of the monomers obtained from the AOAC residues after acid hydrolysis (AOAC plus hydrolysis). Total dietary fibre by the enzymatic–gravimetric methods of AOAC in okara (55.48 g/100 g dry matter) is more than twice that of soybean seeds (24.37 g/100 g dry matter). The proportion IF/SF is 11 in okara and 6 in soybean seeds. Dietary fibre results from enzymatic–gravimetric AOAC methods are higher in okara and soybean seed samples than those from the Englyst method (okara: 41.14 g/100 g dry matter; soybean seeds: 15.05 g/100 g dry matter), and AOAC plus hydrolysis (okara: 44.91 g/100 g dry matter; soybean seeds: 16.38 g/100 g dry matter). In the case of the insoluble fibre from both samples, AOAC plus hydrolysis gives significantly ($p < 0.001$) higher values than the Englyst method, whilst for soluble fibre the opposite occurs ($p < 0.001$). The main monomers in soybean seeds and okara fibre are glucose, galactose, uronic acids, arabinose and xylose. The proportion of each monomer is similar in soybean seeds and okara, so the healthy properties of soybean seeds fibre are also claimed for okara.

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1. Introduction

It is well known that dietary fibre plays an important role in many physiological processes and in the prevention of diseases of different origin (Rodríguez, Jiménez, Fernández-Bolaños, Guillén, & Heredia, 2006). Increased dietary fibre intake can be achieved by changing dietary habits, increasing the consumption of high fibre foods and consuming fibre-fortified foods and fibre supplements. Fibre fortification of common foods has the advantage of requiring the least dietary changes for our fast food/convenience food consuming population (Lo, 1989).

Dietary fibre has all the characteristics required to be considered as an important ingredient in the formulation

of functional foods (Heredia, Jiménez, Fernández-Bolaños, Guillén, & Rodríguez, 2002). The importance of food fibre has led to the development of a large potential market for fibre-rich products and ingredients and, in recent years, there is a trend to find new sources of dietary fibre that can be used as ingredients in the food industry (Chau & Huang, 2003). The most common fibre sources are bran from wheat, barley, corn, rice and oats; citrus fruits, grape, apple and sugar-beet fibre; soybean, peanut, pea and sunflower hull. Many of these sources of dietary fibre have been used in bread making and other cereal-based products (Anil, 2007).

On the other hand, while a few years ago the by-products generated during the processing of plant food constituted an economic and environmental problem, today they are considered a promising source of functional compounds (Carle et al., 2001). The components that remain

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after isolating the main constituent of the total by-products are abundant and represent an inexpensive material that has been undervalued until now, being only used as combustible or fertilizer (Grigelmo-Miguel & Martín-Belloso, 1999).

The most notable attributes of soybean seeds are their oil and protein contents, however, they are also a good source of dietary fibre and isoflavones. The production of soymilk and tofu results in a by-product called okara. In any country, discarding okara as waste is potentially an environmental problem because okara is highly susceptible to putrefaction. Okara also has a high moisture content ($\approx 80\%$), making it difficult to handle and too expensive to dry by conventional means. In light of its content in dietary fibre, okara is a suitable candidate for nutritional enrichment of cereal-based products. Finding convenient ways to incorporate okara into food could eliminate a possible source of pollution and add economic value to this currently valueless product (Rinaldi, Ng, & Bennink, 2000).

Soybean seeds fibre is supposed to have good nutritional and functional properties. The neutral taste and the absence of colour of such fibres makes them suitable for incorporation into food products without any change in their quality, unlike those from wheat fibres (Rinaldi et al., 2000; Sural & Couplet, 2005).

In this study, we have focused on the determination of dietary fibre from soybean seeds and its by-product okara. Fibre has been analysed by the Englyst method (Englyst, Quigley, & Hudson, 1994) and enzymatic–gravimetric AOAC methods (1995). Furthermore the residues, insoluble and soluble, obtained by the AOAC methods have been subjected to the same hydrolysis and monomers quantification (GLC and spectrophotometry) that are utilized in the Englyst method. In addition, the proximate composition of both samples (soybean seeds and okara) has been considered.

2. Materials and methods

2.1. Materials

Soybean seed (*Glycine max* (L.) Merrill) and okara samples were supplied by Toofu-Ya, S.L. (Arganda del Rey, Madrid, Spain). Soybean seed samples were blended and homogenised by grinding to a fine powder to pass through 0.4 mm sieve and stored at 4 °C prior to analysis. Okara samples were freeze-dried (Freeze-dryer Telstar, mod. Cryodos) and prepared in the same way as soybean seed samples.

2.2. Proximate composition

Moisture was determined by oven-drying method at 105 ± 1 °C. Fat was measured in a Soxhlet system by extraction with diethyl ether solvent. Total nitrogen content was analysed by the Kjeldahl procedure. The conver-

sion factor used to transform nitrogen into protein was 5.71. Ash content was determined by incineration of samples at 550 °C in a muffle furnace (James, 1995).

2.3. Dietary fibre

Enzymatic–gravimetric AOAC methods (AOAC., 1995): Isolation of dietary fibre (DF) was carried out with heat-stable alpha-amylase (termamyl) (pH 6, 100 °C, 30 min), protease (pH 7.5, 60 °C, 30 min) and finally amyloglucosidase (pH 4.5, 60 °C, 30 min). The obtained residues were filtered separating the liquid filtrate to soluble fibre (SF) analysis and the solid residue to insoluble fibre (IF) analysis. In both residues, SF and IF, the contents of protein and ash were calculated, and also dietary fibre content. *Englyst et al. method* (1994): Isolation of DF was carried out with termamyl (pH 5.2, 100 °C, 10 min) followed by treatment with a mixture of pancreatin and pullulanase (pH 7.0, 50 °C, 30 min). Four residues of DF were obtained for each sample. Two were destined to total dietary fibre (TF) analysis, adding HCl 5 M and acidified ethanol (30 min, in ice bath), and the other two to IF analysis, adding phosphate buffer (30 min, 100 °C). The insoluble residues obtained were hydrolysed with H₂SO₄ 12 M at 35 °C during 30 min followed by H₂SO₄ 2 M at 100 °C during 1 h. The released neutral sugars were transformed into alditol acetates with acetic anhydride in the presence of 1-methylimidazol. Quantification was performed in a Perkin–Elmer Autosystem chromatograph equipped with a hydrogen flame ionization detector. The column used was a SP-2330 (30 m long, 0.25 mm i.d., and 0.25 µm film thickness) and nitrogen served as carrier gas (22 psi). Temperatures of injector and detector were 275 °C and oven temperature was 235 °C. Retention times and peak areas were registered in a PE Nelson computer mod. 1020 and β -D-allose (Fluka) was used as internal standard. Soluble fibre was calculated as the difference between TF and IF. *Uronic acids* content was determined in the acid hydrolysates according to the colorimetric method of 3,5-dimethylphenol as modified by Rodriguez, Redondo, and Villanueva (1992), with a Pharmacia mod. LKB Ultrospec Plus Spectrophotometer, using galacturonic acid (Merck) as standard. *AOAC plus hydrolysis*: SF and IF residues, from enzymatic–gravimetric methods (AOAC., 1995), were submitted to the same hydrolysis, derivatization to transform neutral sugars into alditol acetates derivatives and GLC quantification, following the Englyst protocol (Englyst et al., 1994). Uronic acids were measured by the same colorimetric method (Rodriguez et al., 1992).

2.4. Statistical analysis

Results were calculated from the mean of six replicates and expressed in g/100 g dry matter. The significant differences between results from AOAC plus hydrolysis and the Englyst method were determined by analysis of variance

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