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Analytical Methods

Isolation and characterisation of cell wall polysaccharides from legume by-products: Okara (soymilk residue), pea pod and broad bean pod

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ABSTRACT

Cell wall material (CWM) from three legume by-products: okara (O), pea pod (PP) and broad bean pod (BBP) were isolated. CWM was sequentially extracted with a chelating agent to obtain S1, and with dilute alkali (S2), 1 M alkali (S3), 4 M alkali (S4) and $NaClO_2$/acetic acid (S5) to leave a cellulose-rich residue (RES). The S1 fraction was different in each by-product (O: 7.2%; PP: 5.5%; BBP: 29.4 %). S2 (O: 7.4%; PP: 3.2%; BBP: 8.1%) and S5 (O: 9.8%; PP: 5.6%; BBP: 10.8%) were low in pea pod and similar in okara and broad bean pod. S1, S2 and S5 were characterised as pectin-rich fractions, whereas S3 and S4 were hemicellulose-rich fractions. There was a high percentage of S3 in pea pod (O: 8.9%; PP: 11.5%; BBP: 6.8%) and S4 in okara (O: 17.8%; PP: 7.1% BBP: 5.7%). RES was the fraction with the highest percentage of cellulose, and the remainder was pectin and hemicellulose material (O: 48.9%; PP: 67.1%; BBP; 39.2%). In addition, the swelling and water retention capacity of these by-products indicates their potential application as a texturing additive.

1. Introduction

Most legumes are consumed after a simple industrial process, in which the pod is removed to prepare the seed as frozen, tinned or fresh food. Peas and broad beans are widely consumed, and are grown all over the world; world production of peas in 2007 was 8.3 million tons, with 4.9 million tons for broad beans (http://faostat.fao.org/site/567/default.aspx#ancor). Soybean is a legume which is well-known for its beneficial properties (Mateos-Aparicio, Redondo-Cuenca, Villanueva-Suarez, & Zapata-Revilla, 2008) and its annual production was 216.1 million tons (http://faostat.fao.org/site/567/default.aspx#ancor) in 2007. Soybeans are normally processed to obtain protein isolates or other end products such as soymilk and tofu. During this process, a fibre-rich residue called okara is removed. The large quantities of by-products generated during the processing of plant food involve an economic and environmental problem due to their high volumes and elimination costs. Today, they are considered a promising source of functional compounds (Carle et al., 2001). After isolation of the main constituent, there are abundant remains which represent an inexpensive material that has been undervalued until now, when it was only used as fuel or fertiliser.

Polysaccharides from dietary fibre are of importance among functional compounds due to the well-known role that dietary fibre plays in many physiological processes and in the prevention of different diseases (Rodríguez, Jiménez, Fernández-Bolaños, Guillén, & Heredia, 2006). Dietary fibre has a potential prebiotic character, in other words, that beneficially affects the growth and/or activity of intestinal flora, particularly bifidobacterium, which plays an important role in protecting against pathogens. This prebiotic effect is due to the fermentation process, which produces short-chain fatty acids, principally acetate, propionate and butyrate. Butyrate has been found to act as a protective agent against experimental tumorogenesis of these cells. Propionate could be related to hypocholesterolemic effects (Martínez-Villaluenga, Frías, & Vidal-Valverde, 2005). Furthermore, dietary fibre is a significant factor in the reduction of cholesterol levels in some hyperlipidemic individuals, and can also be used to improve glucose tolerance in diabetes (Gray, 2006). It increases faecal bulk and reduces gastrointestinal transit time. Dietary fibre also seems to have a positive effect on diarrhoea and constipation and as a treatment for irritable bowel (Bosaeus, 2004). It has anti-inflammatory and anti-carcinogenic effects on the digestive system (Gray, 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 fewest dietary changes to our diet in today's lifestyle. Dietary fibre has all the characteristics required to be considered an important ingredient, and this has led to the development of a large potential market for fibre-rich products and ingredients. In recent years, there has been a trend to find new sources of dietary fibre that can be used

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as ingredients in the food industry. Foods fortified with fibre are usually made with the addition of unrefined cereals; however, other sources such as vegetables and fruits are also used (Rodríguez et al., 2006). The by-products from okara, pea pod and broad bean pod all have dietary fibre as their most important fraction. Therefore, the by-products of these legumes could be added to different foods to provide these beneficial properties. Nevertheless, few studies have been found in the literature on the importance and characterisation of polysaccharides in dietary fibre in okara (Redondo-Cuenca, Villanueva-Suárez, & Mateos-Aparicio, 2008), although there are rather more for soybean seed (Maeda, 2000; Ouhida, Pérez, & Gasa, 2002; Redondo-Cuenca, Villanueva-Suárez, Rodríguez-Sevilla, & Mateos-Aparicio, 2007; van Laar, Tamminga, Williams, Verstegen, & Schols, 2000) and soybean meal (Huisman, Schols, & Voragen, 1998, 1999; Huisman, Weel, Schols, & Voragen, 2000). No information was found about the other two by-products.

The aim of this study is to characterise the polysaccharides from the cell wall of okara, pea pod and broad bean pod through sequential extraction with different extractive solutions. Knowledge of the cell wall structure of these by-products may allow the use of their polysaccharides as a functional ingredient in fortified food. In addition, swelling capacity (SC), water retention capacity (WRC) and oil retention capacity (ORC) of these by-products are evaluated.

2. Materials and methods

2.1. Materials

Okara is a by-product of soy milk production. Soybean (*Glycine max* L.), north-east variety (China) and organic crop were extracted with heat application according to the guidelines for the Japanese method. Okara was obtained from Vegetalia, S.L. (Castellón, Spain). Pea and broad bean pods were provided by Bonduelle, S.L. (Navarra, Spain). In the case of peas, the pods are separated from the seeds by shelling machines in the field; however, broad bean seeds are removed from the pods at the plant, as the first step in processing. By-product samples were freeze-dried (Telstar freeze-dryer, mod. Cryodos) and ground to a fine powder (particle size <1 mm).

2.2. Isolation and sequential extraction of polysaccharides from the by-products

The three by-products were defatted in a Soxhlet system by extraction with diethyl ether solvent. Defatted by-products were treated with a solution containing sodium dodecylsulphate and 1,4-dithioerythreitol (2×), leaving a residue without most of the proteins. They were subsequently extracted with 85% ethanol in constant mix at 60–70 °C during 30 min (2×) to deliver low molecular weight carbohydrate and to obtain cell wall material (CWM). Sequential extraction was based on the method described by Huisman et al. (1998) and Weightman, Renard, and Thibault (1994) with some modifications. CWM was sequentially extracted with 0.05 M CDTA + NH_4 oxalate in 0.05 M sodium acetate buffer (pH 4.8–5.2) stirred at 70 °C during 1 h to separate the S1 fraction. The remnant was then extracted with 0.05 M NaOH, 2 °C, 1 h (S2 fraction), 1 M KOH, room temperature, 2 h (S3 fraction), 4 M KOH, room temperature, 2 h (S4 fraction) and $NaClO_2$/acetic acid (pH 4.2–4.7), 75 °C, 2 h (S5 fraction). A cellulose-rich residue (RES) remained. Each extraction was done twice. Each extract and the residue was neutralised with glacial acetic acid, dialysed with 12–14 KDa membranes (Medicell Internacional Ltd.) against distilled water at 4 °C during four days, changing the water every 24 h. Each fraction was then freeze-dried.

2.3. Neutral sugar composition and uronic acid analysis

All the fractions (S1, S2, S3, S4, S5 and RES) were studied for their neutral sugars by gas chromatography according to Englyst, Quigley, and Hudson (1994), using β-D-allose (Fluka) as internal standard. The samples were treated with H_2SO_4 12 M at 35 °C during 30 min, followed by H_2SO_4 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 ionisation 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. 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. Uronic acid content was determined in the acid hydrolysates according to the colorimetric method of 3,5-dimethylphenol (Rodríguez, Redondo, & Villanueva, 1992), with a Pharmacia mod. LKB Ultrospec Plus Spectrophotometer, using galacturonic acid (Merck) as standard.

2.4. Methanol and calcium determination

These analyses were carried out on all the fractions. The analysis of methanol by GLC (Ng, Parr, Ingham, Rigby, & Waldron, 1998) was done by sonicating ~15 mg of samples plus 2 mL of distilled water during 10 min. Later, de-esterification was done by adding 0.8 mL of 2 M NaOH and placing in a water bath at 20 °C during 1 h with constant agitation. To neutralise, 0.8 mL of 2 M HCl was added, and it was placed in a water bath at 25 °C during 15 min. The neutralised liquid was filtered through a 0.45 μm Millex filter. The analysis was performed in a Perkin–Elmer Autosystem Chromatograph with SP-2330 column. Nitrogen was used as carrier gas and injector temperature was 250 °C, detector was 200 °C and oven temperature was 140 °C. Calcium was analysed in the ash content, after incineration of samples at 550 °C in a muffle furnace, by atomic absorption spectroscopy (AAS) in a Perkin–Elmer Analyst 200. Ash content was dissolved in 50% HCl plus 50% HNO_3 and filtered before analysis by AAS (Sagardoy Muniesa, 2002).

2.5. Physicochemical properties of the by-products

Three properties of defatted okara, pea and broad bean pods were studied: swelling capacity, water retention capacity and oil retention capacity based on the methods described by Robertson et al. (2000).

2.5.1. Swelling capacity (SC)

The sample (100 mg) was hydrated in a measuring cylinder with 10 ml distilled water at room temperature. After 18 h, the volume (ml) occupied was recorded, and SC was expressed as volume (ml)/g of original sample.

2.5.2. Water retention capacity (WRC)

The sample (500 mg) was hydrated in 30 ml distilled water in a centrifuge tube at room temperature. After 18 h, samples were centrifuged (3000*g*; 20 min). The supernatant was decanted and residue fresh weight was recorded. WRC was calculated as the amount of water retained by the pellet (g water/g sample dry weight).

2.5.3. Oil retention capacity (ORC)

The same protocol as above was followed, substituting extra virgin olive oil (acidity 0.7°) for distilled water. ORC was expressed as g oil/g sample dry weight.