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Multifunctional antioxidant activity of polysaccharide fractions from the soybean byproduct okara

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ABSTRACT

An alcohol insoluble residue (AIR) from okara, a byproduct from soymilk, was mainly composed by indigestible carbohydrate (55.7%). After sequential extraction, three alkali-soluble fractions (12.7% yield) and an insoluble residue (RES, 58.7% yield) were obtained. Soluble polysaccharide fractions showed *in vitro* reduction power (11–26 μmol Trolox Equivalent (TE)/g dry weight (dw)) and free radical scavenging activity (63–78 μmol TE/g dw). The highest antioxidant activity was exhibited by 0.05 M NaOH-soluble fraction, rich in pectins. The 1 M KOH-soluble fraction had a mixture of hemicellulosic and pectic polysaccharides, and 4 M KOH-soluble fraction contained the bulk of xyloglucans, although some pectins could also be present. RES contained cellulose along with residual pectins. FT-IR spectra of okara and AIR exhibited an absorption band at 1740 cm^{-1} of carboxylic ester from pectins, which lacked in the fractions. Moreover, they showed absorption bands at 1650 and 1550 cm^{-1} of proteins, and at 900–890 cm^{-1} of β-glycosidic linkages. Potential antioxidant activity of okara cell-wall polysaccharides could be attributed to pectins, although the contribution of residual proteins cannot be ruled out.

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1. Introduction

Epidemiological studies have shown that consumption of fruits and vegetables is associated with reduced risk of chronic diseases. In consequence, an increased consumption of products from vegetable origin which contain high levels of dietary fibre and phytochemical constituents has been recommended (Kris-Etherton et al., 2002; Willett, 1994). As a consequence, various naturally occurring substances are receiving continuous attention as antioxidants. A promising group of antioxidative compounds is thought to be polysaccharides and their fractions, which carry polyhydroxyl groups (Dourado et al., 2004; Luo, Cai, Yan, Sun, & Corke, 2004).

Okara is a byproduct from the soymilk industry. Raw okara, also called soy pulp, is a white yellowish material consisting of the insoluble material from soybean seeds which remains in the filter sack when pureed soybeans are filtered for the production of soymilk (O'Toole, 1999). Okara is enriched in cell-wall polysaccharides. Characterization of this byproduct, including the protein, oil, dietary fibre, and mineral composition, along with un-specified monosaccharides, and oligosaccharides can be found in the literature (O'Toole, 1999; Préstamo, Rupérez, Espinosa-Martos, Villanueva, & Lasunción, 2007; Redondo-Cuenca, Villanueva-Suárez, & Mateos-Aparicio, 2008; Surel & Couplet, 2005; van der Riet, Wight, Cilliers, & Datel, 1989).

Several health effects of okara have been assessed. Recently, *in vitro* experiments have indicated that okara is a potential source of antioxidant components (Amin & Mukhrizah, 2006), showing that protease hydrolysate from okara yielded antioxidant activity (Yokomizo, Takenaka, & Takenaka, 2002). Our group has previously examined the effects of okara in rats, concluding that okara might be useful as a weight-loss dietary supplement (Préstamo et al., 2007) and might protect the gut environment in terms of antioxidant status and prebiotic effect (Jimeĭnez-Escrig, Tenorio, Espinosa-Martos, & Rupérez, 2008). Also we have suggested that under simulated physiological conditions okara may release potential bioactive peptides (Jiménez-Escrig, Alaiz, Vioque, & Rupérez, 2010). Specifically, a protein concentrate from okara is obtained by precipitation, followed by an enzymatic hydrolysis to release potential bioactive peptides. In the <1 kDa molecular weight

Abbreviations: ABTS, 2,2′-azinobis-(3-ethyl benzothiazoline-6-sulfonic acid); $ABTS^{\bullet+}$, ABTS radical cation; ANOVA, one-way analysis of variance; dw, dry weight; FRAP, ferric-reducing antioxidant power; GLC, gas–liquid chromatography; FT-IR, Fourier transformed infrared spectra; 0.05MSF, 0.05 M NaOH-soluble fraction; 1MSF, 1 M KOH-soluble fraction; 4MSF, 4 M KOH-soluble fraction; LC-ESI-MS/MS, Liquid chromatography electrospray ionization tandem mass spectrometry; RP, reduction power; RT, room temperature; TE, Trolox equivalent; NS, neutral sugar; TPTZ, 2,4,6-tri(2-pyridyl)-s-triazine; Trolox, 6-hydroxy-2,5,7,8-tetrametylchroman-2-carboxylic acid; UA, uronic acid.

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cut-off ultra-fraction the amino acid sequence, TIIPLPV, of a peptide from soybean lipoxygenase-1 with a calculated mass 751.48 Da is identified using LC–ESI-MS/MS techniques.

Thus as a part of a continuing study on the biological activities of okara, we have examined the fractionation of the polysaccharides from okara cell-wall material by alkaline extraction. The chemical composition and structural features from FT-IR spectra, along with the antioxidant activities of the polysaccharide fractions *in vitro* are reported. No studies dealing with the antioxidant activities of the polysaccharides of a byproduct from soybean processing were found.

2. Materials and methods

2.1. Materials and preparation of alcohol insoluble residue (AIR)

Okara was provided as a fresh byproduct from soybean [(*Glycine max* (L.) Merr.)] by *Toofu-Ya* S.L., a local food processing industry (Arganda del Rey, Madrid, Spain). Due to its high water content (~79%) okara was preserved at −18 °C until preparation. Afterwards, the byproduct was freeze-dried, ground (1.0 mm mesh), placed in sealed vacuum bags, and stored at −18 °C. Freeze-dried okara was defatted by Soxhlet extraction of the oil using diethyl ether (James, 1995). Then, protein was extracted (3 h × 3, room temperature (RT)) from the residue with 1.5% (w/v) sodium dodecyl sulfate (SDS) solution containing 10 mM 1,4-dithioerythritol; after centrifugation (3000 × *g*, 20 min), the residue was washed with distilled water (Huisman, Schols, & Voragen, 1998). Afterwards, soluble sugars were extracted using 85% ethanol (60 min, 50 °C) and subsequent centrifugation (3000 × *g*, 15 min) (Rupérez & Toledano, 2003). The residue was washed with acetone, centrifuged (3000 × *g*, 15 min), then dried in oven at 60 °C overnight and used as the starting material for cell-wall fractionation. This product was called alcohol insoluble residue (AIR) throughout the paper.

2.2. Sequential extraction of AIR

For the purpose of characterization and evaluation of their antioxidant activities, okara polysaccharides in the AIR (5 g) were sequentially extracted, based on the procedure described (Ruperez, Selvendran, & Stevens, 1985), with 0.05 M NaOH, 1 M KOH, and 4 M KOH (each extraction 500 mL, 1 h, RT). The mixtures in each step of the fractionation were filtered through a No. 3 sintered glass funnel under reduced pressure. Afterwards, the filtered soluble fractions were treated with 2 M acetic acid until neutral pH, and then dialyzed (12–14 kDa) against running tap water (7 L/h) for at least 48 h. After dialysis, the soluble fractions were concentrated to 100 mL in a rotatory evaporator at 50 °C. Aliquots of each soluble fraction were freeze-dried and stored until analysis. The final residue was also washed with distilled water until neutral pH, dialyzed and freeze-dried. As a result, a total of three soluble fractions (0.05 M NaOH-soluble, 0.05MSF; 1 M KOH-soluble, 1MSF; 4 M KOH-soluble, 4MSF), and an insoluble residue (RES) were obtained from the AIR of okara.

2.3. Carbohydrate analysis

Soluble fractions from the sequential extraction were hydrolyzed with 1 M H_2SO_4 (100 °C, 90 min), whereas the final residue was pre-treated with 12 M H_2SO_4 (30 °C, 1 h), followed by hydrolysis with 1 M H_2SO_4 (100 °C, 90 min) and the neutral sugars (NS) and uronic acids (UA) released in the soluble fractions and the RES were analyzed. NS composition was determined by gas–liquid chromatography (GLC) as alditol acetates with inositol as internal standard (Rupérez, Ahrazem, & Leal, 2002). A Shimadzu gas chromatograph model GC-14A was used. The column was a Supelco SP-2330 capillary fused silica, 30 m × 0.32 mm i.d., 0.2 μm film thickness. The oven, injector, and detector temperatures were 240 °C (isothermal), 270 °C, and 270 °C, respectively. UA were quantified spectrophotometrically with 3,5-dimethylphenol as the reagent and galacturonic acid as the standard by the Scott method (Scott, 1979).

2.4. Fourier Transform Infrared spectroscopy

AIR and polysaccharide fractions were incorporated into KBr and pressed into a 1 mm disk. Fourier Transform Infrared (FT-IR) spectra of samples were obtained at a phase resolution of 4 cm^{-1} and averaging 30 scans/min. Spectra were recorded in the transmittance mode from 4000 to 400 cm^{-1} using a FT-IR 4200 Jasco Fourier Transform spectrophotometer (Rupérez et al., 2002). Five replicate spectra were obtained for each sample.

2.5. In vitro antioxidant activity

2.5.1. Preparation of the extracts

An aliquot (0.3 g) of each polysaccharide soluble fraction was placed in a centrifuge tube; 40 mL of water were added, and the tube was thoroughly and constantly shaken at room temperature for 1 h. Then it was centrifuged (2500 × *g*, 10 min), and the supernatant was recovered. Extracts were produced in triplicate and used to measure the *in vitro* antioxidant activity (Rupérez et al., 2002).

2.5.2. Reduction power

The antioxidant activity, in terms of reduction power (RP) towards Fe(III), of the aqueous extract was evaluated (Benzie & Strain, 1996; Jiménez-Escrig, Jiménez-Jiménez, Pulido, & Saura-Calixto, 2001). Briefly, 900 μL of FRAP reagent [10 mmol/L TPTZ in 40 mmol/L HCl (2.5 mL), plus 20 mmol/L $FeCl_3 6H_2O$ (2.5 mL), plus 0.3 mol/L acetate buffer pH 3.6 (25 mL)] was mixed with 90 μL water and 30 μL of test sample or reagent blank. Readings at the absorption maximum (595 nm) were taken using a Beckman DU-640 spectrophotometer at 37 °C. The readings at 4 and 30 min were selected for calculation of RP values. The results were expressed as Trolox equivalents [TE/g dry weight (dw)].

2.5.3. Radical scavenging activity

The ability of polysaccharide fractions to act as a radical scavenging compound was tested. The analysis was performed using an ABTS decolourization assay (Re et al., 1999) with some modifications (Jiménez-Escrig, Dragsted, Daneshvar, Pulido, & Saura-Calixto, 2003). ABTS radical cation ($ABTS^{\bullet+}$) was produced by reacting ABTS (0.12 mmol) with a 2.45 mmol/L potassium persulfate (10 mL). The mixture was left in the dark at room temperature for 12–16 h before use. The $ABTS^{\bullet+}$ solution was diluted with water to an absorbance of 0.70 ± 0.02 at 658 nm on a Beckman DU-340 spectrophotometer. Radical scavenging activity (RSA) of the tested samples was measured by mixing 0.1 mL of the samples with 3.9 mL of diluted $ABTS^{\bullet+}$ solution, and the absorbance reading was taken for 10 min. The results were expressed as TE/g dw.

2.6. Statistical analysis

Results are expressed as mean values ± standard deviation. Comparison of means of at least three measurements, using a significance level of $P < 0.05$, was performed by one-way analysis of variance (ANOVA). Statgraphic version 5.1 was used.

Table 1
Yield (%)[a] and monosaccharide composition of polysaccharide fractions from okara cell-wall material expressed as g/100 g dry weight, as mol%[b] and as a percentage of that particular sugar in the AIR (in parentheses).

Fraction	Yield[a]	Neutral sugar							UA	Total sugar
		Rha	Fuc	Ara	Xyl	Man	Gal	Glc		(%)
Okara		0.3 ± 0.1 0.8[b]	0.5 ± 0.1 1.3[b]	5.7 ± 0.1 16.5[b]	2.7 ± 0.1 7.8[b]	1.5 ± 0.3 3.6[b]	10.4 ± 0.2 25.0[b]	11.9 ± 0.4 28.6[b]	7.3 ± 0.5 16.3[b]	40.3
AIR	100	0.4 ± 0.0 0.8[b]	0.5 ± 0.1 0.9[b]	8.6 ± 0.2 17.9[b]	4.4 ± 0.1 9.2[b]	1.4 ± 0.3 2.4[b]	13.1 ± 0.1 22.7[b]	16.1 ± 0.2 27.9[b]	11.2 ± 0.7 18.1[b]	55.7
0.05MSF	3.5	0.4 ± 0.0 1.3[b](6.2)	0.7 ± 0.1 2.3[b](8.6)	4.9 ± 0.2 18.0[b](3.5)	1.5 ± 0.1 5.5[b](2.1)	0.5 ± 0.0 1.5[b](2.2)	8.6 ± 0.3 26.3[b](4)	1.1 ± 0.1 3.4[b](0.4)	14.7 ± 0.4 41.7[b](8.1)	32.4
1MSF	2.8	0.0 ± 0.0 0[b](0)	0.5 ± 0.0 1.5[b](4.6)	5.0 ± 0.3 16.9[b](2.6)	8.4 ± 0.9 28.5[b](8.7)	5.3 ± 0.8 15.0[b](17.2)	5.8 ± 0.1 16.4[b](2.0)	3.6 ± 0.1 10.2[b](1)	4.4 ± 0.4 11.5[b](1.8)	33.0
4MSF	6.4	0.0 ± 0.0 0[b](0)	0.9 ± 0.1 2.2[b](1.5)	5.1 ± 0.3 13.9[b](5)	13.5 ± 0.5 36.7[b](25.6)	2.2 ± 0.0 5.0[b](13.1)	8.2 ± 0.2 18.6[b](5.2)	5.7 ± 0.1 12.9[b](3)	5.1 ± 0.1 10.7[b](3.8)	40.7
RES	58.7	0.6 ± 0.0 0.8[b](60.4)	1.1 ± 0.1 1.4[b](88.7)	11.5 ± 0.3 16.5[b](53.9)	5.8 ± 0.2 8.3[b](53.1)	2.1 ± 0.1 2.5[b](60.4)	17.0 ± 0.2 20.3[b](52.3)	31.2 ± 0.6 37.2[b](78.1)	11.8 ± 0.3 13.1[b](42.5)	81.1
Recovery	71.4	67	117	65	90	93	64	82	56	

Mean value of three determinations ± SD; Yield (%) by gravimetry; AIR = alcohol insoluble residue; 0.05MSF = 0.05 M NaOH-soluble fraction; 1MSF = 1 M KOH-soluble fraction; 4MSF = 4 M KOH-soluble fraction; RES = Insoluble residue; Rha = rhamnose; Fuc = fucose; Ara = arabinose; Xyl = xylose; Man = mannose; Gal = galactose; Glc = glucose; UA = uronic acid.

3. Results and discussion

3.1. Chemical extraction of okara polysaccharides

Freeze-dried okara, the main byproduct from soymilk and tofu processing, contained 40.3% total sugar (on a dw basis) from non-starch polysaccharides (NSP) and its main monosaccharides were Glc, Gal, uronic acid and Ara (Table 1). Other main components of raw okara were protein (28.5–33.4%), fat (9.8–19.8%) and ash (3.5%) (Préstamo et al., 2007; Redondo-Cuenca et al., 2008). Okara consists of the insoluble parts from soybean seed, while soybean meal is the industrial residue from oil extraction. On a dw basis, soybean meal is predominantly composed of protein (57.3%), with minor amounts of NSP (15.4%) and low molecular weight carbohydrates (11.7%) (Huisman et al., 1998).

After the extraction of fat, protein and soluble sugars, an AIR was obtained which accounted for 63.6% of the initial okara. In a similar way (Mateos-Aparicio et al., 2010), it is reported an AIR prepared from okara that accounts for 77.2%, meanwhile the water unextractable fraction obtained by other authors from raw soybean meal accounted only for 15.7% (Huisman et al., 1998).

AIR contained higher carbohydrate content (55.7%) from NSP than starting okara (Table 1). The most important neutral sugars in the AIR were Glc, Gal, Ara, and Xyl; UA accounted for an 11.2%. Gal is reported to be the major constituent sugar in soybean meal, followed by Glc, Ara, and UA (Huisman et al., 1998). These discrepancies could be due to the different soybean byproduct studied. Man content in okara and AIR (Table 1), was probably coming from galactomannans in soybean hull (Huisman et al., 1998). The kind of UA in AIR was not specifically determined, but it is reported that the UA present in soybean cotyledon meal is primarily galacturonic acid (Huisman et al., 1998).

After sequential extraction of the AIR, three alkali-soluble fractions and an insoluble residue (RES) were obtained. The yield of soluble fractions was 12.7%, while that of RES was the highest and amounted to 58.7% (Table 1).

The insoluble residue from the extraction of the water unextractable solids in soybean meal is more than three times lower (18%) (Huisman et al., 1998). Differences found in yields and composition between okara and soybean meal polysaccharides could be due to the different composition of the starting soybean byproduct used and to differences in the sequential extraction, comprising CDTA, alkali and $NaClO_2$/acetic acid treatment. On applying this extraction method to okara, it is reported five soluble fractions with a yield of 72.9% and a residue (21.9%) (Mateos-Aparicio et al., 2010).

Regarding monosaccharide composition of polysaccharide fractions from okara cell-wall material, 0.05MSF main sugar was UA, followed by Gal and Ara (Table 1). Molar ratio of Gal to Ara in this fraction was 1.5:1, and that of UA to Rha was 32:1. The material solubilised in the 0.05MSF extracts contained pectins. The sugar composition and the previous molar ratio for neutral sugar in 0.05MSF was quite similar to that of the diluted alkali-soluble fraction from soybean meal (Huisman et al., 1998) and to the CDTA-, diluted alkali-, and $NaClO_2$/acetic acid-soluble fractions from okara (Mateos-Aparicio et al., 2010), which contain soluble pectic substances.

Hemicelluloses are non-cellulosic polysaccharides other than pectins, which can be extracted from the cell-wall material with alkaline solutions, typically 1–4 M alkali (Huisman, Weel, Schols, & Voragen, 2000; Ruperez et al., 1985). In the case of 1MSF and 4MSF the major sugar was Xyl (8.4 and 13.5%, respectively) (Table 1). 1MSF gave similar values of Ara, Gal, and Man (5.0–5.8%), whereas in 4MSF Gal (8.2%) was the second major polysaccharide sugar. Uronic acids were present in relatively medium amount in both fractions (4.4 and 5.1%, respectively). It is described two similar soluble fractions but the monomeric composition in 1MSF varied because Gal was the major monomer followed by Ara, UA and Xyl (Mateos-Aparicio et al., 2010).

It has been described the presence of neutral and acidic sugars in similar extracts (relative strong alkaline) obtained from okara (Mateos-Aparicio et al., 2010), and soybean meal (Huisman et al., 2000) and from almond seeds (Dourado et al., 2004). Thus, the material solubilised from the 1MSF extracts probably contained a mixture of pectic and hemicellulosic polysaccharides, as it was evidenced by the relatively higher amounts of UA together with Xyl, Glc, Ara and Gal. In the same way, the presence of Ara, Gal or arabinogalactan side chains and xylogalacturonan regions is described in soybean meal (Huisman, Schols, & Voragen, 1999) and okara (Mateos-Aparicio et al., 2010). Sugar analysis of the 4MSF extract revealed a relative composition of Glc, Xyl, Gal and Fuc (6:18:10:1), characteristic of xyloglucans (Coimbra, Barros, Barros, Rutledge, & Delgadillo, 1998), also described in soybean meal (Huisman et al., 2000) and similar to the composition (6:14:11:1) described in okara (Mateos-Aparicio et al., 2010). However, some pectins

could also be present, as may be inferred from the occurrence of UA and Ara, as is also proposed (Mateos-Aparicio et al., 2010). In sum, the sugar composition of 1MSF and 4MSF may indicate the presence of xylans and xyloglucans in both fractions, along with residual contents of arabinans, galactans and arabinogalactans (Jarvis, 1982). RES showed Glc (31.2%) as the major polysaccharide sugar from cellulose, still leaving Gal (17%), Ara (11.5%) and UA (11.8%) from residual pectins. This composition is in agreement with a recently published work (Mateos-Aparicio et al., 2010).

The presence of these pectins might be attributed to the complex architecture of the cell wall, probably due to a high ramification degree that implies insolubility and association with other polymers, mostly α-cellulose (Ng, Parr, Ingham, Rigby, & Waldron, 1998; Selvendran, 1985).

Monosaccharide recovery ranged from 56 to 117%. The relatively low recovery for Gal and Ara (64–65%) could indicate the presence of a furanose ring form, which is more labile to acid hydrolysis. The recovery of UA (56%) was low, most probably because of insolubility and incomplete hydrolysis of pectic polysaccharides. Recoveries for Glc, Xyl and Man were high and varied from 82 to 93%.

The high total sugar recovered from soluble and insoluble polysaccharide fractions (93.8%), indicated that the loss of polysaccharidic material from AIR was less than 6.2%. In terms of polysaccharide content, approximately 8.4% of the AIR polysaccharides were solubilised during the alkaline extraction. Among these soluble fractions, the 4MSF led the major yield (55.8% of total soluble polysaccharides). The fact that around 85% of the AIR polysaccharides remained insoluble in the final residue suggested that they might be either cellulose-like polysaccharides or polysaccharides covalently linked to proteins (Cheung & Lee, 2000; Ooi & Liu, 2000).

We have to point out that the total carbohydrate content in the antioxidant extracts tested from okara was in the same/above range as those obtained by several authors when the antioxidant activity of polysaccharides is examined. I.e. when the fruit of *Lycium barbarum* is assayed the extraction of polysaccharides by different solvents leads to a crude extract with a total polysaccharide content around 58.0%, this percentage was lower in the case of the subsequent neutral and acidic fractions of polysaccharides (2.56 and 0.90%, respectively) (Wang, Chang, Inbaraj, & Chen, 2010). In the case of the antioxidant activity evaluation in the alga *Turbinaria conoides*, when a similar extraction procedure of polysaccharides is followed, a range of 35–58% of polysaccharides in the different polar extracts is quantified (Chattopadhyay et al., 2010). Another example is the optimization of extraction of polysaccharides from *Parkia speciosa* pod to measure its antioxidant activity; in this work when the best condition of extraction is used a value <10% of UA is given (Gan, Hj, Manaf, & Latiff, 2010). In fruits of *Physalis alkekengi* around 1.39–3.2% of total polysaccharide is quantified in different substrates obtained by solvent extraction (Ge, Duan, Fang, Zhang, & Wang, 2009), and when the subsequent polysaccharide fractionation is made no quantification of carbohydrates is done. In other recent works dealing with the antioxidant activity of crude polysaccharides from vegetables the concentration of total or individual carbohydrates has not been determined (Costa et al., 2010; Shi et al., 2010; Sun, Zhang, Zhang, & Niu, 2010). Thus, in our work, as far as purity of polysaccharides is concerned, the results reached in the soluble extracts tested are comparable with those found in the literature.

3.2. Fourier Transform Infrared spectroscopy

To have a more precise characterization of the polysaccharides present in okara and its cell-wall fractions the acquisition of FT-IR spectra in the region 3750–400 cm^{-1} was performed (Fig. 1). The possibility of using mid-infrared FT-IR spectroscopy for monitoring

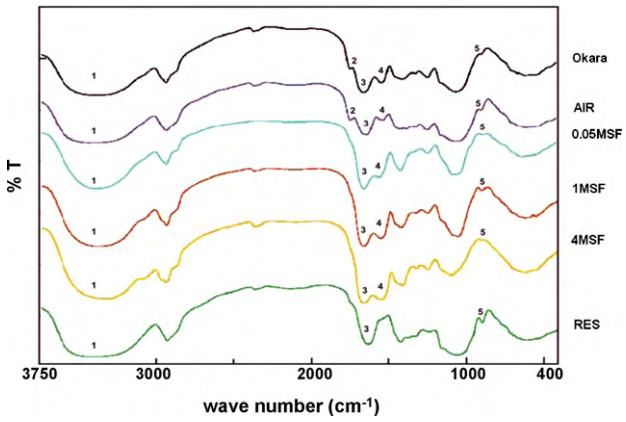

Fig. 1. FT-IR spectra of polysaccharide fractions from okara cell-wall material. %T = percentage of transmission. Okara = freeze-dried raw okara; AIR = alcohol insoluble residue; 0.05MSF = 0.05 M NaOH-soluble fraction; 1MSF = 1 M KOH-soluble fraction; 4MSF = 4 M KOH-soluble fraction; RES = insoluble residue. 1, O–H bond at 3400–3300 cm^{-1}; 2, ester bond at 1740 cm^{-1}; 3, amide I bond at 1650 cm^{-1}; 4, amide II bond at 1550 cm^{-1}; 5, β-glycosidic linkage at 900–890 cm^{-1}.

the main functional groups and glycosidic linkages present in the different polysaccharide fractions obtained from plant cell-walls (Kácurákova & Wilson, 2001), represents an important improvement in comparison with the traditional chemical–instrumental methods (Coimbra et al., 1998).

Among the different samples tested, major difference in IR spectra was due to the ester and carbohydrate bands. All the fractions tested showed a broadly stretched intense peak at around 3400–3300 cm^{-1} (sample concentration broads this absorption band and moves it to 3300 cm^{-1}), and a weak band at around 2930 cm^{-1}, which is the characteristic absorption of hydroxyl groups, and the C–H bond, respectively in the polysaccharide molecule (Coimbra et al., 1998; Xu et al., 2009). The relatively strong absorption peak at around 1740 cm^{-1} (Kácurákova & Wilson, 2001), which corresponds to a carboxylic ester band, was only present in okara and AIR, but absent in the extracts from pectic and hemicellulosic polymers, as they were in the salt form because of alkaline extraction. In addition some weak bands from 1400 to 1200 cm^{-1} were also characteristic FT-IR absorptions of a polysaccharide (Coimbra et al., 1998). Also, the fractions tested showed specific bands in the 1200–1000 cm^{-1} region. In polysaccharides, this region is dominated by ring vibrations overlapped with stretching vibrations of (C–OH) side groups, and the C–O–C glycosidic bond vibrations (Coimbra et al., 1998). The amide I band at 1650 cm^{-1}, is proposed for identification of proteins by IR spectroscopy (Kácurákova & Wilson, 2001). The amide II band at 1550 cm^{-1} indicates the presence of secondary amides, which are also related to the presence of proteins (Femenia, García-Conesa, Simal, & Rosselló, 1998). In our work, the absorption bands at 1650 and 1550 cm^{-1} due to proteins were found in all the fractions, although with different relative intensity: the higher intensity corresponded to 0.05MSF and 1MSF, while the lower intensity was found in AIR and RES. The presence of FT-IR absorption bands at 2930–2925, 1380–1375 and 898–890 cm^{-1} suggested the prevalence of the β-glycosidic linkage in the fractions tested (Kácurákova & Wilson, 2001). Accordingly, in the case of the insoluble residue – which contained the cellulose – these bands showed a relatively high intensity in comparison to the soluble fractions.

Okara is a byproduct containing a high amount of protein, showing values around 32.28% dw (Jiménez-Escrig et al., 2008). In the current work we followed a procedure to eliminate the protein in okara, and it was partly reduced (final protein content around 13% dw). It is described in the literature that when polysaccharides are isolated by solvent extraction on the purpose of antioxidant mea-

Table 2
Antioxidant activity of soluble polysaccharide fractions from okara cell-wall material.

Fraction	Reduction power	Free radical scavenging activity
0.05MSF	25.72 ± 2.51[a]	77.96 ± 5.29[a]
1MSF	11.38 ± 0.24[b]	68.67 ± 6.68[ab]
4MSF	10.71 ± 0.18[c]	63.27 ± 4.35[b]

Trolox equivalents per gram on dry weight basis; mean value of three determinations ± SD; values within the same column followed by different superscript symbols are significantly different ($P \leq 0.05$); 0.05MSF = 0.05 M NaOH-soluble fraction; 1MSF = 1 M KOH-soluble fraction; 4MSF = 4 M KOH-soluble fraction.

surements relatively high protein content is present in the tested extracts. This feature indicates the difficulty to eliminate the protein, which is bound in many cases to the polysaccharides present in the plant cell-wall. To support this statement the following examples are given: It is evaluated the bioactivity of a crude polysaccharide from the alga *Turbinaria ornata* in terms of antioxidant and anti-inflamatory activities and the tested extract containing the crude polysaccharide shows a value of 10% of protein (Ananthi et al., 2010). Also it is described, among the gel-chromatographic fractions obtained from a crude polysaccharide extract, a fraction which is a conjugate with portions of polysaccharide and protein (Ge et al., 2009). This feature seems to be reflected in the higher intensity at 1650 cm^{-1} *versus* 1000 cm^{-1} in the IR spectrum of this specific fraction. In contrast, it is recently investigated a group of sulfated heteropolysaccharides from *Bryopsis plumose* and their antioxidant activities (Song, Zhang, Zhang, & Wang, 2010). The content of protein with regard to total polysaccharide is relatively important in two fractions (8% *vs* 41%, and 9.5% *vs* 31%, respectively). However, in both fractions a higher band at 1650 cm^{-1} than at 1000 cm^{-1} is shown. Thus, the relative concentration of protein to carbohydrate could no be deduced from the corresponding intensities of these specific absorption bands in the IR spectra.

3.3. In vitro antioxidant activities

In the past decades, it has been found that the polysaccharides in plants are not only energy resources but they play key biological roles in many life processes as well. The bioactivities of polysaccharides and their conjugates can be affected by many factors including chemical components, molecular mass, structure, conformation, even the extraction and isolation methods (Xu et al., 2009). Ethanolic extracts from *Cassava* root exhibited hydroxyl radical and superoxide scavenging activities in a dose-dependent manner. These activities are attributed to a mucopolysaccharide mainly constituted by arabinogalactan (Charles & Huang, 2009). In an edible red seaweed (*Porphyra yezoensis*) the antioxidant activity (RSA, reduction power, and chelating activity) of different polysaccharide fractions correlates directly to the increasing sulfate group content or to the decreasing molecular weight of the polysaccharides (Zhou, Hu, Wu, Pan, & Sun, 2008). Similar evidence is found for the RP in different edible seaweeds (Rupérez et al., 2002). On this basis, the antioxidant potential of the aqueous extracts from AIR was estimated by their ability to reduce TPTZ–Fe(III) complex to TPTZ–Fe(II) complex. The FRAP (ferric-reducing antioxidant power) assay treats the antioxidants contained in the samples as reductants in a redox linked colorimetric reaction, and the value reflects the reducing power of the antioxidants (Xu et al., 2009), thus this activity was called throughout the manuscript reduction power (RP). The antioxidant capacities in terms of RSA towards ABTS radical of the soluble polysaccharide fractions were also evaluated (Table 2). It should be borne in mind that *in vitro* activities can only be considered potentially relevant in biological systems and that *in vivo* activities depend also on bioavailability and biotransformation. The extracts tested presented a similar change in the trend of antioxidant activity. That is, 0.05MSF had a significantly stronger RP and RSA among samples, followed by 1MSF and 4MSF. Accordingly, it is described the same tendency—that is the higher alkaline concentration the lower antioxidant activity- among different multifunctional antioxidant assays (lipid peroxidation, RP or RSA) among the different polysaccharide alkaline-extracts obtained from a sporous plant (Xu et al., 2009) and from rice bran (Zha, Luo, Zhang, & Hao, 2009). It is shown a positive correlation between uronic acids and antioxidant activities in brown alga (Zhou et al., 2008), tea (Chen, Zhang, & Xie, 2004), and mung bean sprout hypocotyl (Hayami, Motomura, & Nishizawa, 2007) polysaccharides. Similarly, 0.05MSF was the fraction with the highest content in UA among all tested fractions. Some authors link the antioxidant activity protection of polysaccharides to its bound-protein (Yang, Zhao, Lv, Yang, & Ruan, 2007). On the other hand, it is described that β-D-glucans from fungi and algae microorganisms themselves seem to have a low antioxidant activity in contrast to other cell-wall fractions with proteins (Jaehrig et al., 2008). A recent research (Gan et al., 2010), focusing on the RSA of alcohol insoluble polysaccharides from *P. speciosa* pod, concludes that the optimization of purity in polysaccharides leads to extracts containing <10% in terms of UA content. In this work a correlation between UA content and RSA is given.

4. Conclusions

On the basis of monosaccharide composition and FT-IR spectra of the different polysaccharide fractions obtained from the sequential extraction of okara cell-wall material, a strong link between structural proteins, cellulose-xyloglucans and pectin networks in this soybean byproduct was suggested. The antioxidant activities of the soluble fractions from okara would confirm the strong role of pectins in the reduction power and free radical scavenging activity of the cell-wall polysaccharides from edible plants found by other authors although the contribution of residual proteins cannot be ruled out.

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