Food Hydrocolloids 73 (2017) 262–273

Contents lists available at ScienceDirect

Food Hydrocolloids

journal homepage: www.elsevier.com/locate/foodhyd

Insoluble soybean polysaccharides: Obtaining and evaluation of their O/W emulsifying properties

M.C. Porfiri [a, b], J. Vaccaro [a], C.A. Stortz [b, c], D.A. Navarro [b, c], J.R. Wagner [a, b], D.M. Cabezas [a, b, *]

[a] Laboratorio de Investigación en Funcionalidad y Tecnología de Alimentos (LIFTA), Departamento de Ciencia y Tecnología, Universidad Nacional de Quilmes, Roque Sáenz Peña 352, B1876BXD, Bernal, Provincia de Buenos Aires, Argentina
[b] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina
[c] Departamento de Química Orgánica (CIHIDECAR), Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria, Pabellón 2, C1428, Buenos Aires, Argentina

ARTICLE INFO

Article history:
Received 16 February 2017
Received in revised form
2 June 2017
Accepted 24 June 2017
Available online 26 June 2017

Keywords:
Soybean
Polysaccharide
O/W emulsion

ABSTRACT

The aims of this work were to obtain different samples of insoluble soybean polysaccharides (ISPS) from defatted soy flour and to study their potential application as O/W emulsifier. In this regard, the insoluble residue (okara) resulting from an aqueous extraction (60 °C, pH 9.0), was submitted to an acidic extraction (pH 3.5, 120 °C) without or with a pretreatment (high pressure homogenization or sonication). The insoluble residues of these extractions were dried (oven, 70 °C or vacuum post-treatment with 2-propanol, 40 °C) yielding different ISPS samples. Aqueous dispersions of ISPS samples (1–2% w/w, pH 3 and 7), were used to prepare coarse and fine O/W emulsions. Emulsion stability against creaming and coalescence processes, and the rheological behavior were analyzed. ISPS samples obtained by okara pretreatment and vacuum dried post-treatment with 2-propanol allow to produces emulsions with high values of flocculation degree, increasing the stability of the particle size, and allowing the formation of stronger gel-like emulsions. These pretreatments expose internal sites of the polysaccharide and protein structures, increasing their superficial hydrophobicity and, therefore, allow a strong absorption of the macromolecules at the oil-water interface and/or the formation of external layers, increasing the rigidity of the interfacial film and contributing to the formation of hydrated flocs. Also, these treatments could solubilize certain compounds in okara that would interfere negatively in the formation of the interfacial film. Particularly, sample obtained by high pressures homogenization of the okara presented the best emulsifying properties and it was not significantly affected by variations in the pH of the emulsion. The results of this research work demonstrate a high potential of application of the ISPS samples as O/W emulsifier, under acid and neutral conditions, increasing the added value of an important by-product of the soybean industry.

1. Introduction

Proteins and polysaccharides play a key role in the structure formation and stabilization of oil-in water (O/W) emulsions. Proteins are commonly used as emulsifiers due to its ability to prevent droplet aggregation and coalescence (Dickinson, 2009; Phillips & Williams, 2001). On the other hand, polysaccharides are often incorporated to stabilize the emulsion droplets against gravitational separation by increasing the viscosity of the aqueous phase or by the formation of a gel (Chuah, Kuroiwa, Kobayash, & Nakajima, 2009; Liu, Verespej, Alexander, & Corredig, 2007). Various studies report that polysaccharides may also interact with protein adsorbed at the interface enhancing the emulsion stability. In this way, only polysaccharides with sufficient hydrophobic character to adsorb strongly at the oil-water interface or those directly complexed with proteins are capable of making and stabilizing O/W emulsions (Akhtar, Dickinson, Mazoyer, & Langendorff, 2002; Evans, Ratcliffe, & Williams, 2013; Neirynck et al., 2007).

* Corresponding author. LIFTA, Departamento de Ciencia y Tecnología, Universidad Nacional de Quilmes, Roque Sáenz Peña 352, B1876BXD, Bernal, Provincia de Buenos Aires, Argentina.
E-mail address: dario.cabezas@unq.edu.ar (D.M. Cabezas).

http://dx.doi.org/10.1016/j.foodhyd.2017.06.034
0268-005X/

Soybean proteins are widely recognized for their low price, high nutritional quality and versatile functional properties (Wang, Johnson, & Wang, 2004). The first step in the production of protein isolate is an extraction under alkaline conditions (pH 7.5–9, up to 80 °C) (Berk, 1992; Pearson, 1983). The insoluble residue left after this alkaline extraction is called 'okara'. Most okara is treated as an industrial waste, because it contains about 80% moisture and spoils quickly (Maeda, 2000). However, this by-product has various components that can be isolated in fractionating steps to produce high-value products such as proteins and carbohydrates that can be used as food additives. In fact, the soluble fraction obtained by heating okara in weak acidic conditions (pH 3–3.5, 120 °C) allows the extraction of the soluble soybean polysaccharides (SSPS) (Nakamura, Fujii, Tobe, Adachi, & Hirotsuka, 2012). SSPS has various functions, such as dispersion, stabilization, emulsification, and adhesion (Maeda, 2000; Porfiri, Cabezas, & Wagner, 2016). Furthermore, this acid extraction generates an insoluble fraction containing a complex polysaccharide with high protein content. There is no specific literature about this fraction, named insoluble soybean polysaccharides (ISPS) in the present research.

In recent years, the concepts of applying modification by mechanical treatments in carbohydrates polymers research have gained much attention. For example, high pressure treatments are used to break binding forces (hydrogen bridges and hydrophobic forces) of the highly branched regions in pectin materials without the degradation of the main chain (covalent bonds) (Chen et al., 2012; Michel & Autio, 2001). Moreover, the exerted shear stresses of a sonication process are large enough to alter the molecular conformation of the polysaccharide and/or to disrupt entangled structures (Ogutu, Mu, Elahi, Zhang, & Sun, 2015). These physical treatments can not only alter the particle size and surface area of these polymers but also improve their functional properties (Chen, Gao, Yang, & Gao, 2013).

The aims of this research were to evaluate and compare the chemical composition and the O/W emulsifying properties of ISPS samples obtained under different extraction conditions.

2. Materials and methods

2.1. Material

Defatted solvent-free soy flour (DSF), prepared under controlled conditions (not thermally inactived to avoid protein denaturation), was provided by Terminal 6 S.A. (Santa Fe, Argentina). Refined sunflower oil (Molinos Cañuelas, Argentina) was purchased in a local supermarket. The chemical reagents used in this work were of analytical grade.

2.2. Obtaining samples of insoluble soybean polysaccharides

2.2.1. Preparation and treatment of the okara

Defatted soybean flour was ground to a particle size lesser than 150 μm. ISPS was prepared by adapting the extraction method of Porfiri et al. (2016). The defatted soy flour was extracted by adding 11 times the weight of distilled water, adjusting the dispersion to pH 9.0 with 5M NaOH, and gently stirring during 30 min at 60 °C. The residue obtained after centrifugation (7000 ×g, 4 °C, 15 min), called *okara* (Furuta, Takahashi, Tobe, Kiwata, & Maeda, 1998), was suspended in distilled water at a concentration of 25.0% w/w and adjusted at pH 3.5 with 38% HCl. As shown in Fig. 1, portions of okara dispersion were subjected separately to the following homogenization processes:

I) High pressure valve homogenization: dispersion was subjected to a treatment in a two-valve high pressure homogenizer in 3 cycles, at 1000 bar and 100 bar in the first and second valve, respectively (GEA Niro Soavi).
II) High intensity ultrasonic homogenization: dispersion was subjected to a treatment in a probe-type ultrasonic homogenizer (Sonics Vibra Cells- 7070 J, 75% power, 3 min 30″ on, 15″ off-).

2.2.1.1. Particle size determination of okara dispersions. De Brouckere (D [4,3]) mean diameters of particles of the okara dispersions, without and with previous homogenization treatment, were determined by laser diffraction with a particle size analyzer (Malvern Mastersizer 2000E, Malvern Instruments Ltd., Worcestershire, U.K.). Refractive indexes of 1.33 and 1.45 were used for water (dispersant) and okara particles, respectively (Preece et al., 2015). Samples were diluted in the water bath of the dispersion system (Hydro 2000MU).

2.2.2. Obtaining and drying procedure of acid insoluble fractions

Okara dispersions, without and with previous homogenization treatment, were heated in autoclave at 120 °C for 2 h. After that, they were centrifuged at 7000×g at 4 °C for 15 min and wet insoluble fractions were obtained. These fractions were dried by the following procedures:

a) Forced-air drying oven at 70 ± 2 °C to a constant weight, obtaining the samples: ISPS-U-O (untreated okara, oven dried), ISPS-V-O (valve homogenized okara, oven dried) and ISPS-S-O (sonicated okara, oven dried) (Fig. 1).
b) Vacuum drying post-treatment with 2-propanol (Kalapathy & Proctor, 2001). The high moisture content (>87%, w/w) of the wet insoluble fractions did not allow the use of a vacuum drying oven because of the high processing times required without a previous moisture reduction. Therefore, in order to reduce these moisture content, the insoluble fractions were dispersed in an equal volume of 2-propanol, the pH was adjusted to 3.5 and allowed to settle for 4 h s. The pellet was collected, centrifuged, dispersed in 70% 2-propanol, stirred for 30 min and centrifuged. The washing was repeated with 100% 2-propanol. The precipitate was dried in a vacuum drying oven at 40 °C (pressure less than 50 mm Hg). This process allows obtaining the samples: ISPS-U-P (untreated okara, treated with propanol), ISPS-V-P (valve homogenized okara, treated with propanol) and ISPS-S-P (sonicated okara, treated with propanol) (Fig. 1).

2.2.3. Okara and ISPS samples yields

Yields (w/w %) were calculated as the weight of dried okara or ISPS sample obtained with 100 g of DSF (Equation (1)). The okara dispersion without previous homogenization was oven-dried at 70 °C to obtain the dried okara sample.

$$\text{Yield}(\%) = (\text{Okara or ISPS sample produced}(g) / \text{Initial weight of DSF}(g)) \times 100 \quad (1)$$

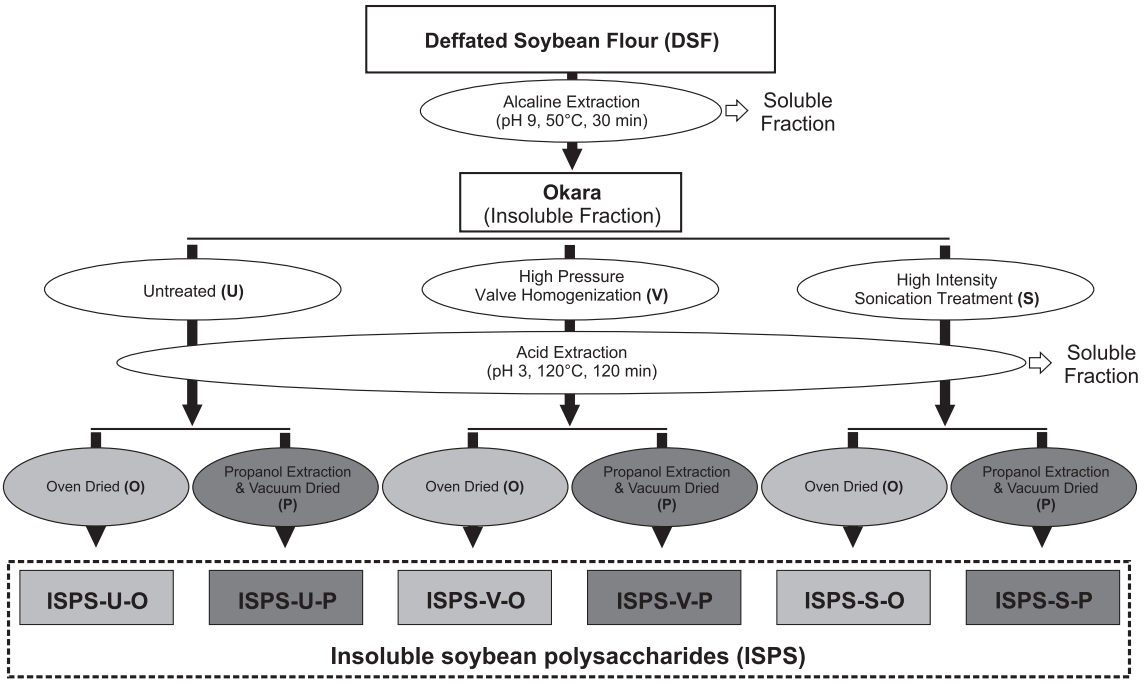

Fig. 1. Flow diagram of the process used for producing different insoluble soybean polysaccharide fractions.

2.3. Chemical composition

The proportions of neutral monosaccharides were determined after hydrolysis with pure trifluoroacetic acid (90 min, 120 °C), in order to detect cellulosic and fiber materials (Morrison, 1988). Hydrolyzates were derivatized to the alditol acetates (Albersheim, Nevins, English, & Karr, 1967) and analyzed by gas-liquid chromatography (GLC) using a capillary column (30 m × 0.25 mm) coated with SP-2330 (0.20 mm) on a HP-5890 Gas Chromatograph equipped with a flame ionization detector (FID). Nitrogen was used as the carrier gas, with a flow rate of 1 ml/min and a split ratio of 100:1. Chromatography runs were programmed starting at 200 °C, 2 °C/min to 230 °C (hold for 20 min), while the injector and detector were set at 240 °C. The percentage of the different monosaccharides was calculated by considering that the FID responses are proportional to the molecular weight of the alditol acetates. Every hydrolysis step was carried out in duplicate.

Uronic acid contents of the sample were determined by a colorimetric method using *m*-hydroxydiphenyl, and expressed as galacturonic acid (GalA) (Blumenkrantz & Asboe-Hansen, 1973). Degree of esterification was expressed as percent methoxy groups as determined by a titration method (Lira-Ortiz et al., 2014).

The protein content of samples was determined by Micro-Kjeldahl (N × 6.25) using a colorimetric procedure for determining the nitrogen content calibrated with ammonium sulphate (Nkonge & Balance, 1982). Total carbohydrates were determined by difference. The lipid amount was negligible given the process for obtaining the soybean flour used as starting material.

2.4. FTIR analysis

Infrared spectra of the ISPS samples were registered in the range of 850–2150 cm^{-1} on a Fourier-Transform Infrared Analyzer (FTIR) Shimadzu IR-Affinity (Shimadzu Co., Japan) equipped with an attenuated total reflectance diamond module (GladiATR, Pike Technologies, USA). IR spectra were measured as an average of 45 scans, a resolution of 4.0 cm^{-1}.

2.5. Preparation of aqueous dispersions of ISPS samples

Aqueous dispersions were prepared by dissolving the ISPS samples (1.0 and 2.0 w/w) in 8 mM sodium citrate buffer (pH 3.0 and 7.0), with the addition of 0.02% w/v sodium azide to retard the microbial growth. To promote dispersion, samples were allowed to stand overnight and subsequently were stirred (25 °C, 50 rpm, 30 min) using a magnetic stirrer.

2.6. Surface and interfacial tension

ISPS samples (0.1 g protein/ml) were dispersed in 8 mM sodium citrate buffer (pure water, pH 3.0 and 7.0). These dispersions were left overnight at room temperature to fully hydrate, and then mixed by using an Ultraturrax T-25 homogenizer using a 25 N-10 G dispersing tool (10,000 rpm, 2 min, IKA Labortechnik GmbH & Co, Germany). The du Noüy ring (platinum ring) method was used to determine the surface (air – aqueous phase) and interfacial (sunflower oil - aqueous phase) tensions of each dispersion at 25 °C using a LAUDA Tensiometer model TD3 (Lauda Dr. R. Wobser GmbH & Co. KG, Lauda-Königshofen, Germany). As a result of adsorption of the surface active compounds, the surface (or interfacial) tension at equilibrium decreased from the value of the clean interface γ^0 to a value γ. Therefore, the interfacial and surface pressures at equilibrium (π^i_e and π^s_e, respectively, where i stands for interfacial and s represents surface) were calculated as:

$$\pi^i_e = \gamma^{i_0} - \gamma^i (mN/m) \quad (2)$$

$$\pi^s_e = \gamma^{s_0} - \gamma^s (mN/m) \quad (3)$$

where the symbols 0 and e represent the initial and equilibrium stages, respectively.

2.7. Interfacial rheology

Rheological properties of the oil-water interface generated by

Table 1
Extraction yield and chemical analysis of the insoluble soybean polysaccharide samples.

Sample	Sample/DSF	Total Carbohydrates	Sugar Composition (Mol %)								Protein	Ash
	Yield (W/W %)	(DWB%)	Rha	Fuc	Ara	Xyl	Man	Gal	Glc	Gal A	(DWB%)	(DWB%)
Okara	21.88^{d}	62.85^{e}	3.27^{d}	2.02^{b}	16.25^{d}	4.53^{a}	1.63^{a}	29.06^{d}	22.93^{a}	20.30^{ab}	33.57^{a}	3.58^{b}
ISPS-U-O	13.32^{c}	54.11^{c}	3.12^{d}	2.14^{c}	6.44^{c}	6.70^{bc}	3.67^{c}	16.73^{c}	40.11^{b}	21.10^{b}	42.77^{d}	3.12^{a}
ISPS-V-O	12.56^{b}	59.17^{d}	2.71^{bc}	1.94^{b}	5.46^{b}	7.51^{d}	3.82^{c}	13.89^{a}	44.49^{de}	20.17^{a}	37.16^{b}	3.67^{b}
ISPS-S-O	12.52^{b}	58.13^{d}	2.80^{bc}	2.02^{b}	6.57^{c}	7.17^{cd}	3.66^{c}	14.69^{b}	42.01^{c}	21.08^{b}	38.10^{b}	3.77^{b}
ISPS-U-P	11.53^{a}	50.83^{a}	2.98^{cd}	2.19^{c}	5.01^{a}	7.43^{d}	3.60^{c}	14.98^{b}	43.66^{cd}	20.15^{a}	46.08^{f}	3.09^{a}
ISPS-V-P	11.45^{a}	55.93^{c}	2.07^{a}	1.68^{a}	5.08^{a}	6.72^{bc}	3.85^{c}	14.44^{b}	46.01^{e}	20.15^{a}	40.86^{c}	3.21^{a}
ISPS-S-P	11.21^{a}	52.20^{b}	2.52^{b}	2.00^{b}	5.17^{ab}	6.27^{b}	3.16^{b}	15.65^{bc}	45.01^{de}	20.23^{ab}	44.24^{e}	3.56^{b}

Mean values of three determinations; ISPS: insoluble soybean polysaccharide; DSF: Defatted solvent-free soy flour; Rha: rhamnose; Fuc: fucose; Ara: arabinose; Xyl: xylose; Man: mannose; Gal: galactose; Glc: glucose; Gal A: galacturonic acid. Values with different letters in each column are significantly different ($P < 0.05$).

the ISPS samples were studied by using an AR-G2 rheometer (TA Instruments, New Castle, DE) equipped with a du Noüy ring (platinum ring) geometry. For this, 30 ml of aqueous dispersion of 0.5% of ISPS (8 mM sodium citrate buffer, pH 3.0 and 7.0) were placed in a beaker (6.7 cm in diameter) and the ring was lowered to make contact with the surface. In order to increase the repeatability the gap was zeroed and kept constant at the position of 10,900 μm. The same volume of sunflower oil was carefully placed above the aqueous phase, generating an oil-water interface. Oscillatory shear measurements were conducted at constant frequency of 0.1 Hz and strain of 5%, which was measured to be within the linear viscoelastic regime. The temperature was set to 25 °C and the experimental data were obtained by recording G′ and G″ as a function of time immediately after interface generation (Cabezas, Pereira Ortiz, Wagner, & Porfiri, 2017).

2.8. Preparation of the O/W emulsions

2.8.1. Coarse O/W emulsions

Refined sunflower oil (oil mass fraction, $\Phi_m = 0.3$) and the different ISPS aqueous dispersions in a range of 1.0–2.0% w/w were used to prepare emulsions. Coarse emulsions were prepared at room temperature in an Ultraturrax T-25 homogenizer using a 25 N-10 G dispersing tool (25,000 rpm, 2 min, IKA Labortechnik GmbH & Co, Germany) (Cabezas, Madoery, Diehl, & Tomas, 2012). The behavior of these emulsions as a function of the storage time was analyzed for 90 min.

2.8.2. Fine O/W emulsions

Coarse emulsions previously obtained with 2% of different ISPS samples (ISPS–U–P, ISPS-V-P and ISPS-S-P) were homogenized in a two-valve high pressure homogenizer (400 bar, 1 cycle, GEA Niro Soavi). The behavior of these fine emulsions as a function of the storage time was analyzed for 28 days.

2.9. Evaluation of the emulsifying properties of the ISPS samples

2.9.1. Particle size determination of emulsions

De Brouckere (D [4,3]) mean diameters of particles were determined by laser diffraction with a particle size analyzer (Malvern Mastersizer 2000E, Malvern Instruments Ltd., Worcestershire, U.K.). D [4,3] are more sensitive to an increment of droplet size than Sauter mean diameters (D [3,2]) (Cortés-Muñoz, Chevalier-Lucia, & Dumay, 2009). The refractive indexes for water and sunflower oil were 1.33 and 1.47, respectively. Samples were diluted in the water bath of the sample dispersion unit (Hydro 2000MU).

Furthermore, D [4,3] were measured after 2 min of sonication at 40 W with the ultrasonic probe of the dispersion system. The purpose of this treatment was break down the flocs that would be present in the emulsions (Porfiri et al., 2016). Subsequently, flocculation degree (FD) and coalescence index (CI) were analyzed. The FD was calculated by Equation (2):

$$FD = \frac{D[4,3] - D[4,3]_{sonic}}{D[4,3]_{sonic}} \quad (4)$$

Where D [4,3] and D $[4,3]_{sonic}$ are the volume-weighted diameters, measured before and after the sonication treatment, respectively.

The CI of coarse and fine emulsions were calculated by Equations (3) and (4), respectively:

$$CI = \frac{D[4,3] - D[4,3]_{initial}}{D[4,3]_{initial}} \times 100 \quad (5)$$

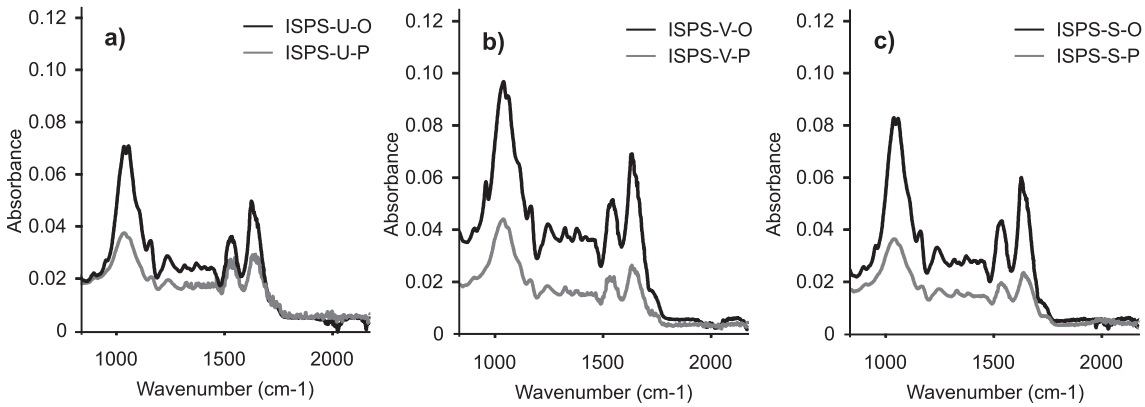

Fig. 2. Fourier transform infrared spectra of the 850-2150 cm^{-1} region of a) ISPS-U, b) ISPS-V and c) ISPS-S samples. Abbreviations are described in Fig. 1. Mean values (n = 4).

Where D $[4,3]_{initial}$ and D [4,3] are the volume-weighted diameters measured in initial and stored (90 min) emulsions, respectively.

$$CI = \frac{D[4,3]_{sonic} - D[4,3]_{sonic,\ initial}}{D[4,3]_{sonic,\ initial}} \times 100 \quad (6)$$

Where D $[4,3]_{sonic,\ initial}$ and D $[4,3]_{sonic}$ are the volume-weighted diameters measured in initial and stored (28 days) emulsions after the sonication treatment, respectively.

2.9.2. Creaming kinetics of the coarse O/W emulsions

Coarse O/W emulsions tend to separate a "serum" lower layer and a "creamed" upper layer. The kinetics of creaming of these emulsions was determined by manually measuring the change in the height of the serum layer over time using 10 ml graduated and transparent cylinder (Mc Clements, 1999). The percentage of serum layer is then given by Equation (5):

$$\%\,Serum \quad layer = \frac{V_{Sl} - V_E}{V_E} \times 100 \quad (7)$$

where V_E is the volume of the emulsion in the graduated cylinder and V_{Sl} is the volume of the serum layer.

2.9.3. Optical characterization of fine O/W emulsions

The destabilization process of the fine emulsions was analyzed from the backscattering (%BS) profiles of each emulsion obtained

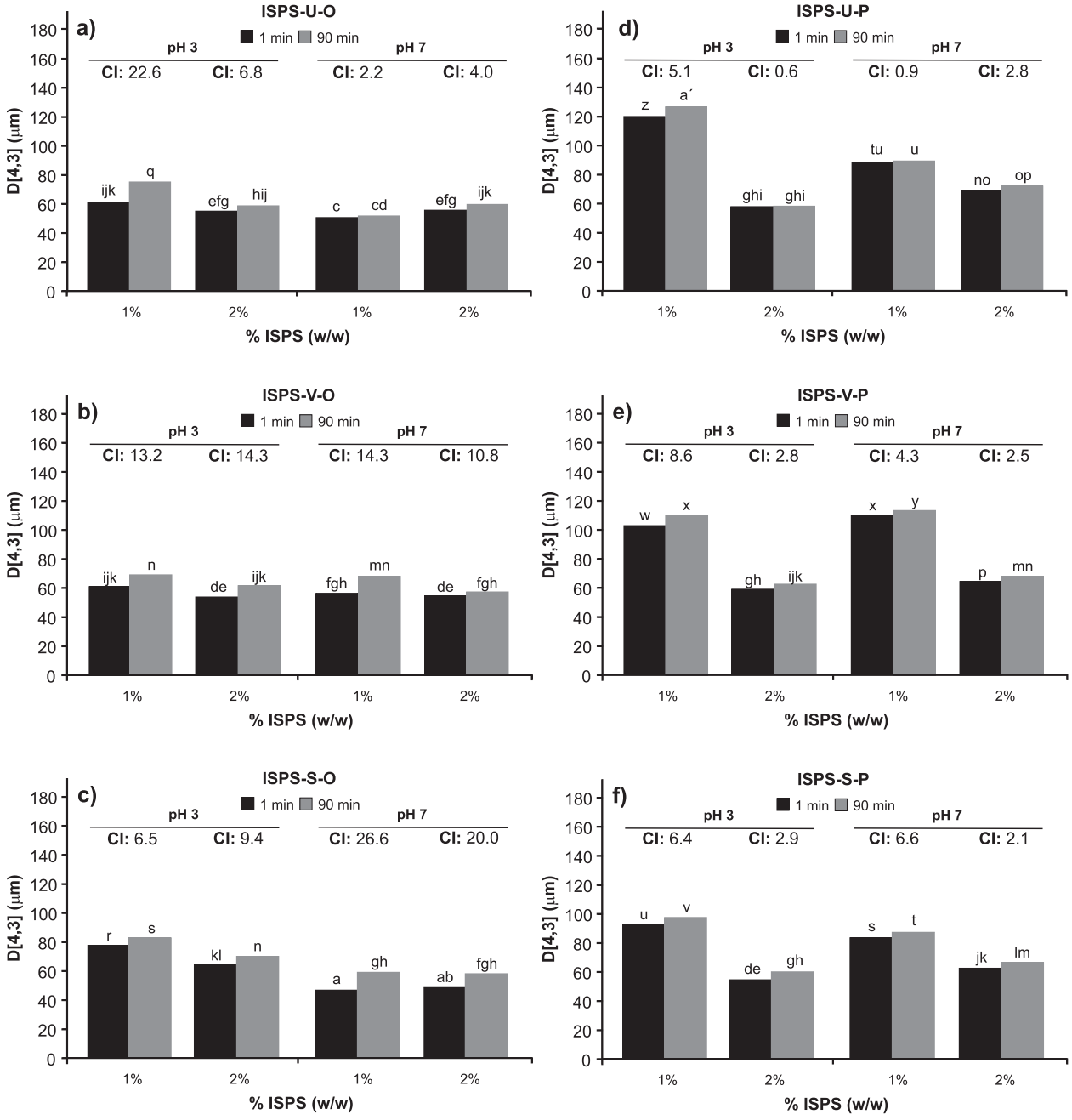

Fig. 3. De Brouckere mean diameters (D [4,3]) of O/W emulsions ($\Phi_m = 0.3$) obtained in Ultra-Turrax homogenizer with the addition of different concentrations (1–2% w/w) of insoluble soybean polysaccharide samples at pH 3 and 7. Abbreviations are described in Fig. 1. Mean values (n = 3). Values with different letters are significantly different ($P < 0.05$).

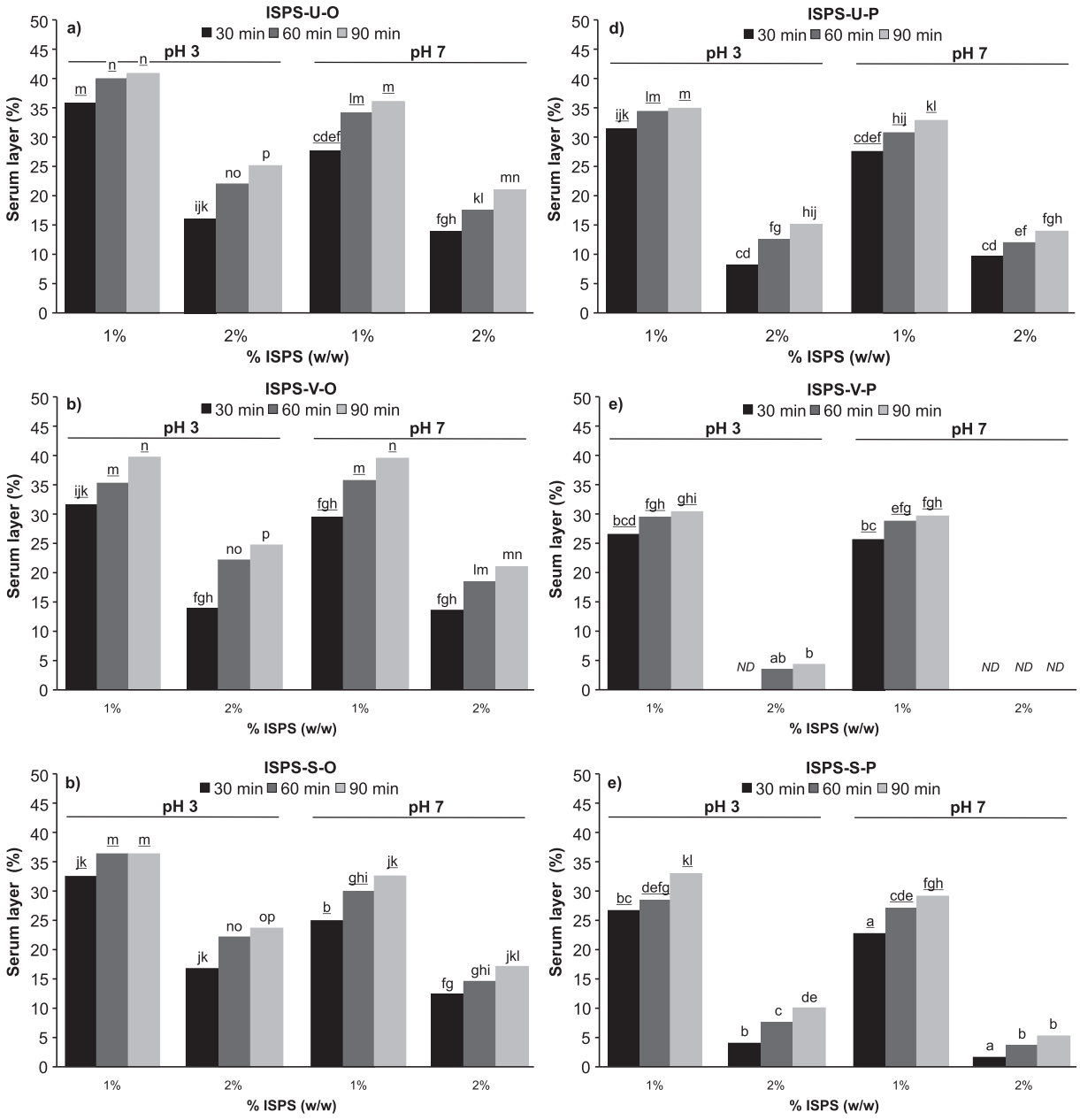

Fig. 4. Serum layer (%) of O/W emulsions (Φ_m = 0.3) obtained in Ultra-Turrax homogenizer with the addition of different concentrations (1–2% w/w) of insoluble soybean polysaccharide samples at pH 3 and 7. Abbreviations are described in Fig. 1. ND: not detectable. Mean values (n = 3). Values with different letters at each concentration (1%: underlined letters and 2%: not underlined letters) are significantly different ($P < 0.05$).

during 28 days at 24 °C (Cabezas et al., 2012). The %BS was measured using a vertical scan analyzer (QuickScan, Beckman-Coulter, USA).

Table 2
Surface (π^s_e) and interfacial (π^i_e) pressure at equilibrium of 8 mM sodium citrate buffer dispersion with 0.1% of different ISPS-P samples at pH 3.0 and 7.0. Mean values (n = 4).

	π^s_e (mN/m)			π^i_e (mN/m)		
pH	ISPS-U-P	ISPS-V-P	ISPS-S-P	ISPS-U-P	ISPS-V-P	ISPS-S-P
3.0	19.07^{a}	19.61^{ab}	20.17^{bc}	7.56^{a}	8.64^{c}	7.82^{ab}
7.0	20.57^{c}	20.32^{c}	20.69^{c}	8.04^{b}	9.04^{d}	8.58^{c}

Values with different letters in each parameter are significantly different ($P < 0.05$).

2.9.4. Light microscopy

Micrographs of the emulsions were obtained after being diluted 10 times with an optical microscope operating at 400 × magnification and fitted with a digital camera (Canon A570 IS; Malaysia) at 4 × optical zoom.

2.9.5. Rheology

Oscillatory rheology of the fine emulsions was studied using an AR-G2 rheometer (TA Instruments; New Castle, DE, USA) with parallel-plate geometry (gap 1000 μm, diameter 40 mm). Temperature (24 °C) was controlled with a water bath (Julabo ACW100, Julabo Labortechnik, Germany) associated with the rheometer. Experimental data were obtained by recording the storage or elastic modulus (G′) and the loss or viscous modulus (G″) as a function of

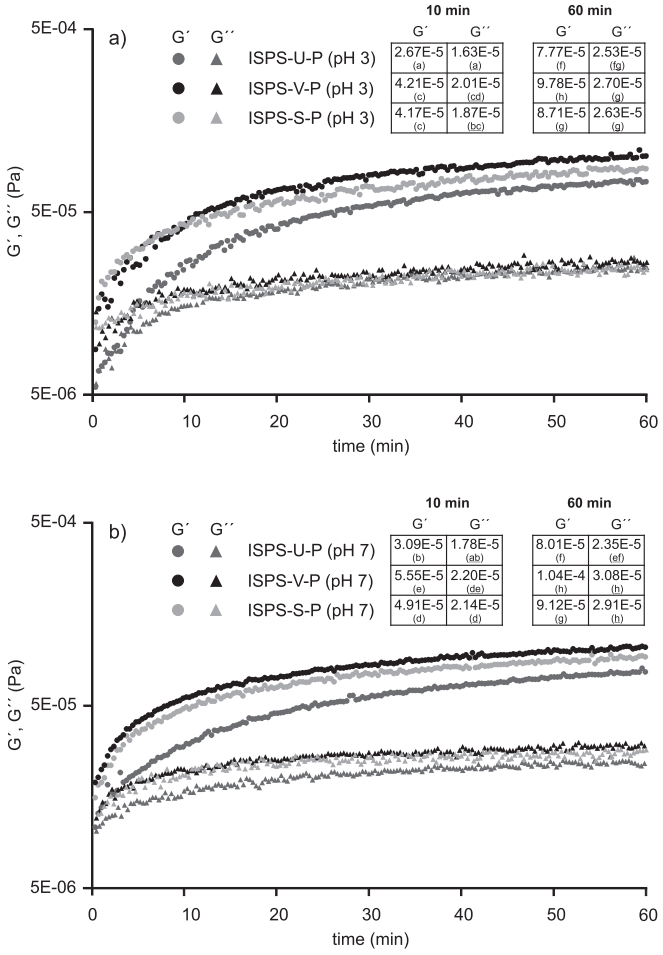

Fig. 5. Storage (G′) and viscous modulus (G″) at the oil-water interface as a function of time (0–60 min, 0.1 Hz, strain 5%) using 8 mM sodium citrate buffer with 0.5% of different ISPS-P samples at a) pH 3.0 and b) 7.0. Abbreviations are described in Fig. 1. Mean values (n = 3). Values with different letters in each viscoelastic moduli (G': not underlined letters and G": underlined letters) are significantly different ($P < 0.05$).

oscillation frequency (0.1–100 Hz range) within the linear viscoelasticity range previously determined by stress-sweeps (strain 1%).

2.9.6. Statistical analysis

Data were evaluated using analysis of variance (ANOVA) with the Statgraphics Centurion XV software (StatPoint Inc. 2005; USA). Assays were conducted at least in triplicate. Least significance difference (LSD) values were used to differentiate mean values, and significance as defined at $P < 0.05$.

3. Results and discussion

3.1. Yields and chemical composition of okara and ISPS samples

The extraction yields and the chemical analysis of the okara and the six ISPS samples were determined (Table 1). Okara has a high content of carbohydrates and proteins, which are insoluble in alkaline conditions. Subsequently, this product was subjected to an acid extraction (2 h, pH 3, 120 °C) allowing obtaining six samples of insoluble soybean polysaccharides (ISPS) under different conditions (Fig. 1). According to previous works on soybean meal, this intense heating process (120 °C, 2 h) would dissociate the quaternary structures of the protein and denatures their subunits (Zhang et al., 2013). ISPS samples have higher protein content than okara due to the solubilization of the soybean soluble polysaccharides under acid condition at high temperature and pressure (Furuta et al., 1998). ISPS yield was lower in samples drying post-treatment with 2-propanol with respect to the oven dried ones. This may be due to the differential solubility of different components in the alcoholic and aqueous media, allowing to eliminate low molecular weight polysaccharide or peptides fragments. Furthermore, it was noted that fractions without a pretreatment of okara (ISPS-U) had a higher extraction yield, mainly those that were dried in an oven (Table 1). The lower yield of the ISPS-V and ISPS-S samples indicates that both pretreatments (valve homogenization and sonication) increased the percentage of soluble compounds in acid media during the autoclaving treatment, mainly proteinaceous material. This observation would be related to the weakening or rupture of non-covalent bonds (Van der Waals forces, hydrogen bridges and/or hydrophobic forces) of polysaccharide/protein aggregates by the high pressure (Mozhaev, Heremans, Frank, Masson, & Balny, 1996) or ultrasound waves treatments to the okara (Wang, Cheung, Leung, & Wu, 2010). The downsizing of the structures was evident in the smaller De Brouckere (D [4,3]) mean diameters of the valve homogenizated (29.1 ± 0.3 μm) or sonicated (34.4 ± 1.1 μm) okara-dispersions in relation to the untreated okara (64.8 ± 0.8 μm).

FTIR spectra presented the characteristic bands of the protein region, particularly amide I (AI, C–O stretching, ~1636 cm^{-1}) and amide II (AII, N–H deformation, ~1537 cm^{-1}) and the bands corresponding to carbohydrate region (900-1200 cm^{-1}). FTIR spectra show that the intensity of these spectral bands was substantially modified as a consequence of the homogenization processes (Fig. 2). In this sense, it was observed an increase of the band intensities of the ISPS samples obtained with a previous homogenization treatment (ISPS-V and ISPS-S), respect to that obtained without pretreatment (ISPS-U). In contrast to the previously mentioned, these untreated samples presented higher protein content than the homogenized samples under both drying methods. This behavior would confirm the rupture of non-covalent bonds of the polysaccharide/protein aggregates by the high pressure and ultrasound waves. These processes allow to expose internal sites of the polysaccharide and protein structures, modifying characteristics of the ISPS samples as their superficial hydrophobicity and, therefore, their functional characteristics. On the other hand, the intensity of the different bands of the oven-dried samples (ISPS-O) were significantly lower than those vacuum-dried samples post-treatment with 2-propanol (ISPS-P). The oven-dried treatment considerably reduces the unfolding effect on the molecular structures of the different homogenization processes. According to previous works, a decrease of the absorbance over the entire spectrum would be associated with an aggregation process (Ingrassia, Palazolo, Risso, & Wagner, 2016). The sugar compositions of the okara and the six ISPS samples are detailed in Table 1. A lower content of galactose and arabinose, and a higher content of mannose and glucose are observed in the ISPS samples than those observed in okara. This observation is due to the acid extraction of the soluble soybean polysaccharide (SSPS) which mainly consists of galactose and arabinose (Nakamura, Furuta, Kato, Maeda, & Nagamatsu, 2003). Furthermore, although significant differences were observed to the sugar composition a common polysaccharide matrix in the different ISPS samples would be identified. These fractions were composed mainly of glucose (>42%), galacturonic acid (>20%) and galactose (>13%). The high glucose content can be related to residues of cellulose present in the starting material, and different hemicelluloses described in the literature as part of the cotyledon (arabinogalactan, glucomannan and xyloglucan) (O'Toole, 2004; Redondo-Cuenca, Villanueva-Suárez, & Mateos-Aparicio, 2008). The insolubility of the samples in alkaline and

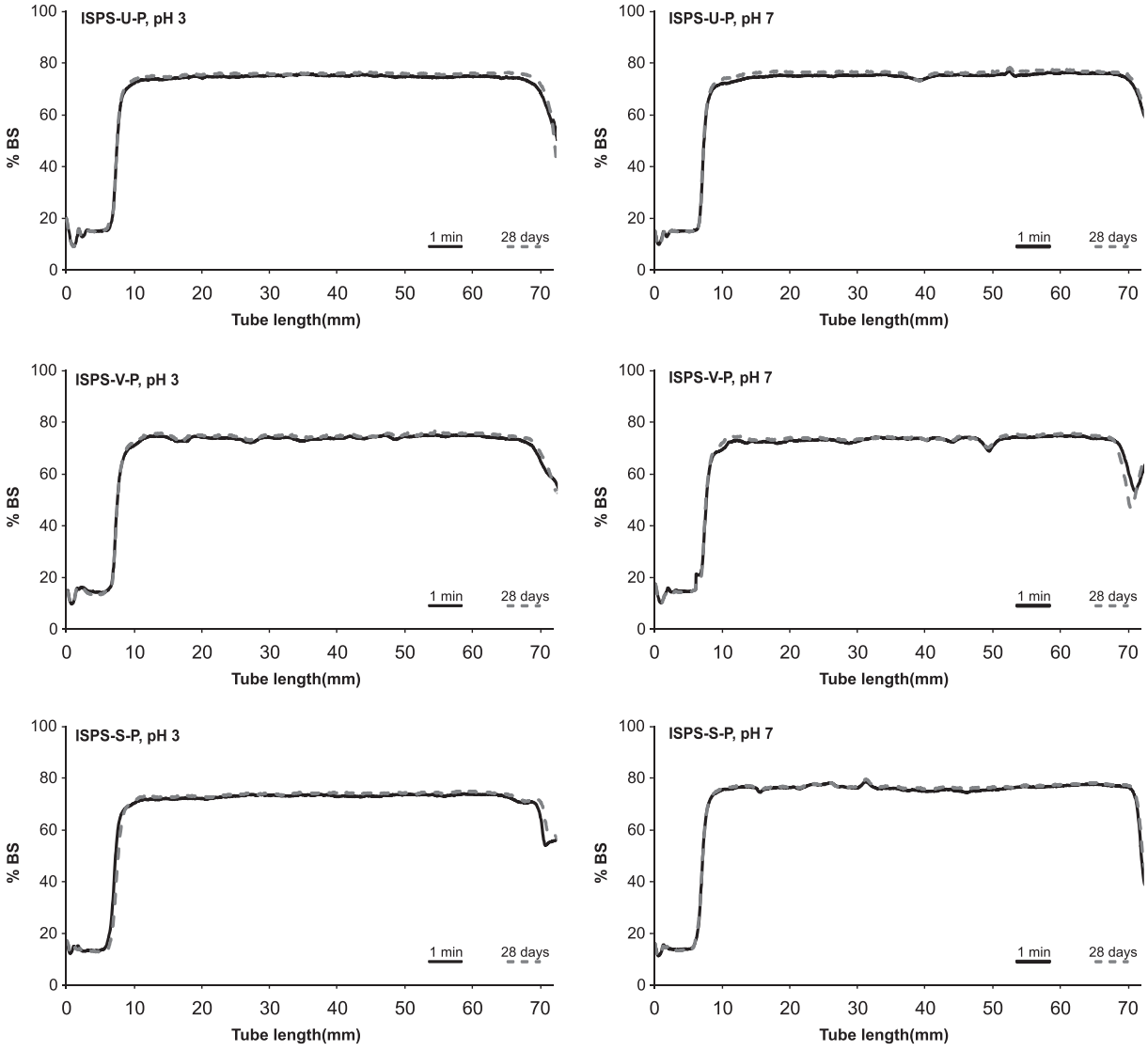

Fig. 6. Back scattering (%BS) profiles as a function of the tube length with storage time (28 day), for O/W emulsions ($\Phi_m = 0.3$) obtained in ultrasound homogenizer with the addition of 2% of different insoluble soybean polysaccharide samples at pH 3 and 7. Abbreviations are described in Fig. 1. Mean values (n = 3).

acid conditions could infer the presence of carbohydrates covalently attached to proteins in a glycoprotein matrix, for example forming arabinogalactan-proteins and glucomannan-proteins (Aspinall & Whyte, 1964; Fincher, Stone, & Clarke, 1983). This matrix, as observed in different gums and pectins, can lead surface-active compound potentially applicable in the formulation of emulsions (Dickinson, 2009).

3.2. Evaluation of the emulsifying properties of the ISPS samples

3.2.1. Particle size determination of O/W coarse emulsions

The particle size analysis (D [4,3]) allowed to evaluate the destabilization by coalescence of the different emulsions (Fig. 3). Oven-dried samples (ISPS-O, Fig. 3a, b, c) generally presented smaller particle sizes than those dried post-treatment with 2-propanol (ISPS-P, Fig. 3d, e, f), however, this difference was considerably reduced by increasing the concentration of the different emulsifiers to 2%. It should be noted that at this concentration ISPS-P samples were in all cases more stable against coalescence than ISPS-O samples with values of CI < 3, and a similar behavior at the different pH values.

Particles higher than 80 μm in emulsions with 1% of fractions dried post-treatment with 2-propanol could be generated by the bridging flocculation mechanism. This mechanism loses effect by increase the fraction concentration, however, it may be causing the difference in particle size between the ISPS-O and ISPS-P emulsions. The size of individual droplets and the flocculation degree could not be determined applying low energy sonication with the ultrasonic probe of the dispersion system. This methodology was not possible to use in coarse emulsions given that the energy applied caused emulsion destabilization, mainly due to the tendency to coalesce of the higher droplets (Porfiri et al., 2016). However, the presence of flocs was probed through the microscope (data not shown).

3.2.2. Creaming stability of O/W coarse emulsions

Stability against this process increased for ISPS samples obtained from pretreated okara (ISPS-V, ISPS-S), particularly those dried post alcohol precipitation. In this sense, the oven dried samples produces emulsions with a final serum layer higher than

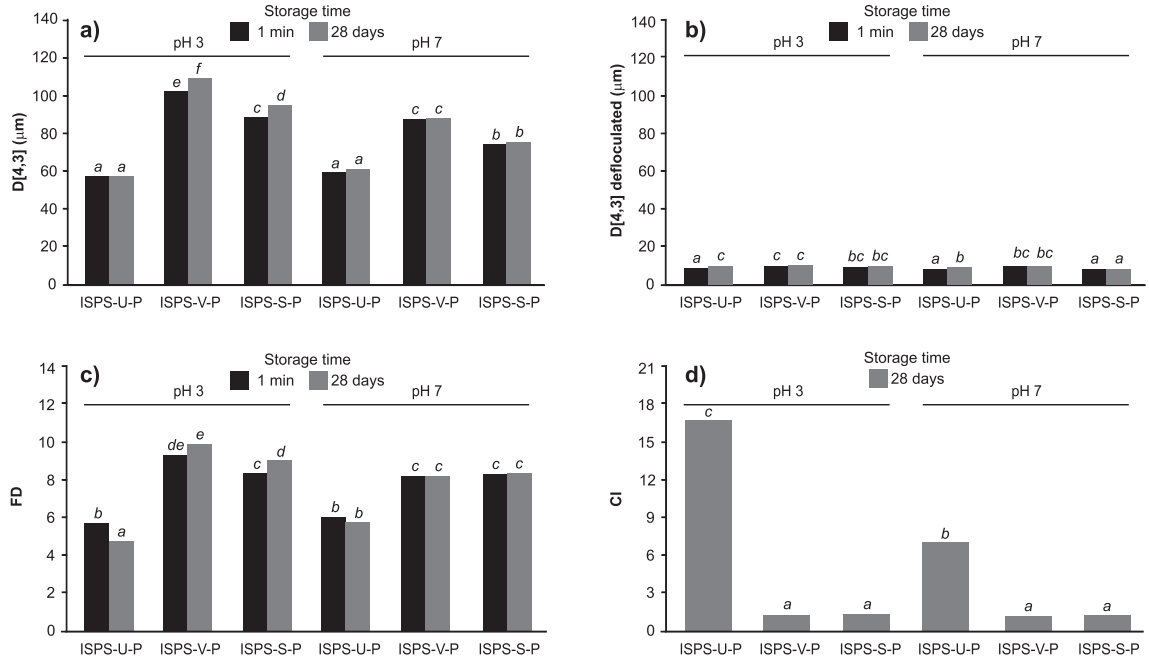

Fig. 7. De Brouker mean diameters without (D [4,3]) and with a subsequent deflocculating process by low energy sonication (D [4,3] deflocculated), Flocculation degree (FD) and coalescence index (CI) for O/W emulsions ($\Phi_m = 0.3$) obtained in ultrasound homogenizer with the addition of 2% of different insoluble soybean polysaccharide samples at pH 3 and 7. Abbreviations are described in Fig. 1. Mean values (n = 3). Values with different letters in each parameter are significantly different ($P < 0.05$).

32% and 17% at concentrations of 1% and 2%, respectively (Fig. 4a, b, c). In contrast, samples dried post-treatment with 2-propanol (ISPS-P) showed less than 15% of final serum layer at addition of 2% (Fig. 4d, e, f). Particularly, emulsions prepared with ISPS-V-P provided a serum layer of 4.2% at pH 3 and was not detectable at pH 7 (Fig. 4e). As it was previously suggested according the compositional characterization of the ISPS, both homogenization processes and the alcoholic treatment expose internal sites of the polysaccharide and protein structures, increasing their superficial hydrophobicity and, therefore, allow a strong absorption of the macromolecules at the oil-water interface. In addition, the homogenization treatments could solubilize certain compounds in okara as low molecular weight polysaccharide or peptides fragments. These compounds would generate a competitive absorption with ISPS in the interface during the emulsification process.

On the other hand, emulsions formulated at acidic and neutral conditions showed no significant differences using the ISPS-P samples at 2% as emulsifying agents, demonstrating versatility at different pH environments.

3.2.3. Selection of dispersions

The analysis of the coarse emulsions allowed selecting the ISPS dispersions with potential application as emulsifying agents. Conditions that generated coarse emulsions with greater stability against the coalescence and creaming processes were selected. In this regard, fine emulsions were prepared with the ISPS-P samples in a concentration of 2%.

3.2.4. Functional characterization of the selected ISPS dispersions

The effects of the ISPS samples on surface (π^s_e) and interfacial (π^i_e) pressure at equilibrium are shown in Table 2. All of the dispersions examined showed an increase in these values. In these sense, although significant differences were observed in the measurements, the nominal values of these parameters demonstrate a similar activity of the three ISPS samples in the analyzed conditions. This behavior shows that ISPS has emulsification potential and should be able to form a protective film around the oil droplets during the homogenization process and to stabilize an oil-in-water emulsion.

Interfacial G′ and G″ versus time are shown in Fig. 5. In all cases, the adsorption of the sample to the interface started immediately after the interface generation and G′ values were significantly higher than G″. This indicates the formation of gel-like interfacial films. At the different pH, ISPS-V-P and ISPS-S-P presented a faster arrange of macromolecules at the water/oil interface, promoting a stronger gel-like structure of the interfacial film (higher G′) in comparison with the ISPS-U-P sample.

The similar π^s_e and π^i_e values could be related to the formation of a predominantly proteic interfacial film. On the other hand, the increased exposure of active sites showed by the FTIR analysis (Fig. 2), particularly in ISPS-V and ISPS-S samples, would also allow the contribution of the polysaccharides either at the interface or in the formation of external layers, significantly modifying the rigidity of the interfacial film (for example, by increasing the values of G′). This better activity of the polysaccharides would be in direct relation with the increase of hydrophobic zones generated during the homogenization treatments (Ngouémazong, Christiaens, Shpigelman, VanLoey, & Hendrickx, 2015).

3.2.5. Optical characterization of fine O/W emulsions

The variation over time of the backscattering (%BS) values enable to discriminate between the particle migration (sedimentation, creaming) and particle size variation (flocculation, coalescence) processes (Cabezas et al., 2012). The %BS profiles were obtained for the fine O/W emulsions during 28 days at 24 °C (Fig. 6). These emulsions showed constant values of %BS along the length of the tube during 28 days of storage, indicating a high stability against the destabilization processes of creaming and/or coalescence.

3.2.6. Particle size determination of O/W fine emulsions

D [4,3] values before (Figura 5. a) and after (Figura 5. b)

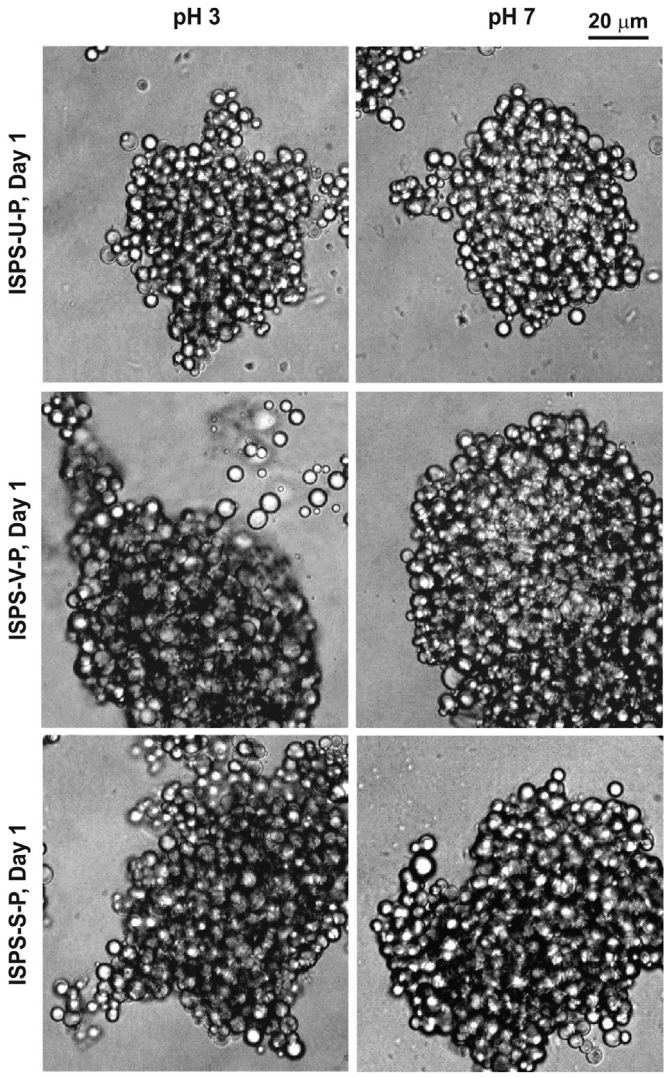

Fig. 8. Optical microscopy images of fine emulsions stabilized with 2% of different insoluble soybean polysaccharide samples after 1 day of storage at pH 3 and 7. Abbreviations are described in Fig. 1. Bar = 20 μm.

deflocculation by low energy sonication, allowed to analyze the effect of the destabilization process by coalescence and/or flocculation. ISPS samples produce emulsions with initial D [4,3] values in a range between 8.0 and 10.1 μm. High FD values (>5.7) were obtained immediately after preparing these six emulsions (Fig. 7c), and the presence of flocs was confirmed by optical microscopy (Fig. 8). Particularly, emulsions with pretreated okara samples (ISPS-V-P, ISPS-S-P) generated the largest flocs (FD > 8.1).

Most emulsions showed D [4,3] values almost constant along time, which indicates that the initially flocs do not disintegrate during storage. In contrast, the microstructure of such flocs was modified during storage depending on the type of emulsifier. This can be seen by analyzing the corresponding coalescence index (Fig. 7d): emulsions with pretreated okara (ISPS-V-P and ISPS-S-P, CI < 1.3%) showed an increased resistance to coalescence in relation to those with not-pretreated okara (ISPS-U-P, CI > 7.0%). The presence of hydrated flocs doing a gel-like structure, especially at higher FD values, would avoid the coalescence of the droplets due to the lower mobility of the system. In different protein samples, a positive relationship between the flocculation-creaming rates with surface hydrophobicity was detected (Wang, Li, Jiang, Qi, & Zhou, 2014). The unfolding structures of the ISPS-V-P and ISPS-S-P samples would increase the interfacial activity of these protein/polysaccharide systems (possibly forming a glycoprotein matrix) at the interfacial level and/or by formation of external layers, increasing the rigidity of the interfacial film (Fig. 5) and contributing to the formation of hydrated flocs (Figs. 7 and 8c).

3.2.7. *Rheological properties of O/W fine emulsions*

Storage (G′) and viscous modulus (G″) were analyzed as a function of oscillation frequency (0.1–100 Hz, strain 1%). The evolution of these rheological parameters during storage (28 days), particularly at 1 Hz, was used to compare the fine emulsions (Fig. 9). Storage modulus were higher than loss modulus (G′ ≫ G") maintaining this behavior in a wide range of frequencies. This observation indicates the formation of a strong gel-like material, which is directly related to the presence of the mentioned hydrated flocs (Mc Clements, 1999). Samples with pretreated okara (Fig. 9. c–f), showed higher G′ and G″ values and a better rheological stability during the storage time than the sample without pretreatment (Fig. 9. a, b). Both homogenization processes and the alcoholic treatment generates emulsions with high values of flocculation degree ($FD_{ISPS\text{-}V \text{ or } ISPS\text{-}S} > FD_{ISPS\text{-}U}$, Fig. 7c), increasing the stability of the particle size ($CI_{ISPS\text{-}V \text{ or } ISPS\text{-}S} < CI_{ISPS\text{-}U}$, Fig. 7d), and allowing the formation of stronger gel-like emulsions (Fig. 9). Particularly, ISPS-V-P gives emulsions with greater physical (Figs. 6 and 7) and rheology (Fig. 9) stability over storage time and it was not significantly affected by variations in the pH of the emulsion.

4. Conclusion

The present research analyzed different insoluble fractions in both alkaline (pH 9, 60 °C, 30 min) and acidic conditions (pH 3, 120 °C, 90 min) of defatted solvent-free soy flour. These fractions, called insoluble soybean polysaccharides (ISPS), have a high content of polysaccharides and protein. The compositional analyses of these samples allow inferring the presence of carbohydrates covalently attached to proteins in a glycoprotein matrix (arabinogalactan-proteins and glucomannan-proteins). High pressure homogenization and ultrasonic sonication pretreatments on the alkaline-insoluble residue (okara) and an alcoholic treatment expose internal sites of the polysaccharide and protein structures, increasing their superficial hydrophobicity and, therefore, allow a strong absorption of the macromolecules at the oil-water interface and/or the formation of external layers, increasing the rigidity of the interfacial film and contributing to the formation of hydrated flocs. Also, these treatments could solubilize certain compounds in okara (low molecular weight polysaccharide or peptides fragments) that would interfere negatively in the formation of the interface during the emulsification process. These behaviors were evidenced by the formation of stronger gel-like emulsions with higher stability of the particle size, which is associated directly with the formation of hydrated flocs. It should be noted that variations in pH (acid and neutral conditions) did not cause significant differences in the activity of the samples with an okara pretreatment (ISPS-V or ISPS-S). Particularly, the sample obtained by high pressures homogenization of the okara (ISPS-V-P) presented the best emulsifying properties. The results of this research work demonstrate a high potential of application of the ISPS samples in the production of O/W emulsions, under acid and neutral conditions, increasing the added value of an important by-product of the

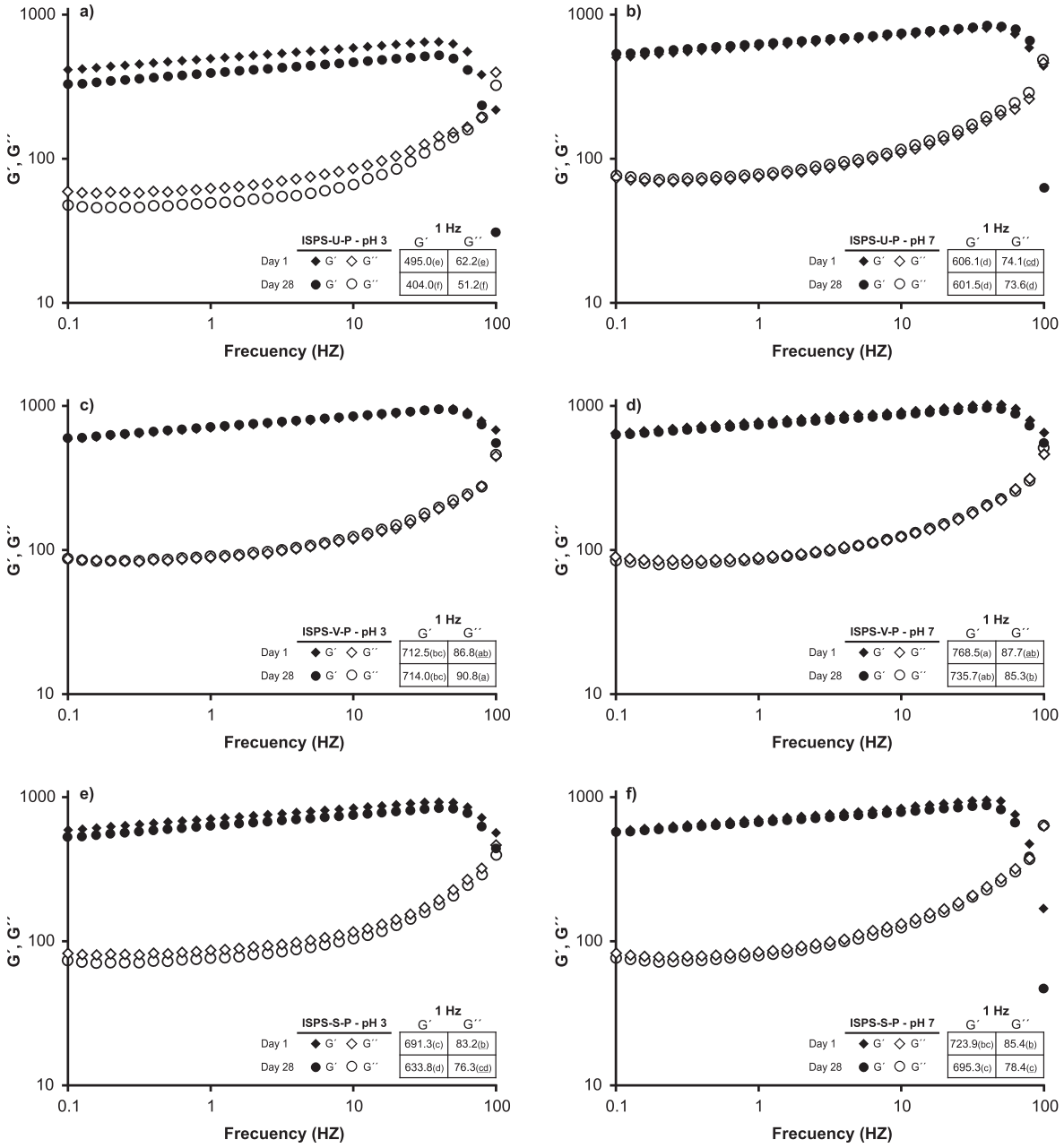

Fig. 9. Storage (G′) and viscous modulus (G″) as a function of oscillation frequency (0.1–100 Hz, strain 1%) for O/W emulsions ($\Phi_m = 0.3$) obtained in ultrasound homogenizer with the addition of 2% of different insoluble soybean polysaccharide samples after 1 and 28 days of storage at pH 3 and 7. Abbreviations are described in Fig. 1. Mean values (n = 3). Values with different letters in each viscoelastic moduli (G': underlined letters and G": not underlined letters) are significantly different ($P < 0.05$).

soybean protein isolates or soluble soybean polysaccharides (SSPS) industries.

Acknowledgements

The authors of this article gratefully acknowledge the financial support of the Universidad Nacional de Quilmes (I + D Grant PUNQ 53/1007). Agencia Nacional de Promoción Científica y Tecnológica (PICT-2015-0084, PICT-2014-1267). Authors wish to thank Terminal 6 S. A. (Santa Fe, Argentina) and Dra. Mabel Tomás (CIDCA).

References

Akhtar, M., Dickinson, E., Mazoyer, J., & Langendorff, V. (2002). Emulsion stabilizing properties of depolymerized pectin. *Food Hydrocolloids, 16*, 249–256.

Albersheim, P., Nevins, D. J., English, P. D., & Karr, A. (1967). A method for the analysis of sugars in plant cell-wall polysaccharides by gas-liquid chromatography. *Carbohydrate Research, 5*, 340–345.

Aspinall, G. O., & Whyte, J. N. C. (1964). Polysaccharides of soy-beans. Part I. Galactomannans from the hulls. *Journal of the Chemical Society, 0*, 5058–5063. http://pubs.rsc.org/en/Content/ArticleLanding/1964/JR/JR9640005058#!divAbstract.

Berk, Z. (1992). *Technology of Production of edible flours and protein products from soybeans* (pp. 73–96). Rome: Food and Agriculture Organization of the United Nations (FAO).

Blumenkrantz, N., & Asboe-Hansen, G. (1973). New method for quantitative determination of uronic acids. *Analytical Biochemistry, 54*, 484–489.

Cabezas, D. M., Madoery, R., Diehl, B. W. K., & Tomas, M. C. (2012). Emulsifying properties of different modified sunflower lecithins. *Journal of the American Oil Chemists' Society, 89*, 355–361.

Cabezas, D. M., Pereira Ortiz, M., Wagner, J. R., & Porfiri, M. C. (2017). Effect of salt content and type on emulsifying properties of hull soy soluble polysaccharides

at acidic pH. *Food Research International, 97*, 62–70.
Chen, J., Gao, D., Yang, L., & Gao, Y. (2013). Effect of microfluidization process on the functional properties of insoluble fiber dietary. *Food Research International, 54*, 1821–1827.
Chen, J., Liang, R.-H., Liu, W., Liu, C.-M., Li, T., Tu, Z.-C., et al. (2012). Degradation of high-methoxyl pectin by dynamic high pressure microfluidization and its mechanism. *Food Hydrocolloids, 28*, 121–129.
Chuah, A. M., Kuroiwa, T., Kobayash, I., & Nakajima, M. (2009). Effect of chitosan on the stability and properties of modified lecithin stabilized oil-in-water monodisperse emulsion prepared by microchannel emulsification. *Food Hydrocolloids, 23*, 600–610.
Cortés-Muñoz, M., Chevalier-Lucia, D., & Dumay, E. (2009). Characteristics of submicron emulsions prepared by ultra-high-pressure homogenization: Effect of chilled or frozen storage. *Food Hydrocolloids, 23*, 640–654.
Dickinson, E. (2009). Hydrocolloids as emulsifiers and emulsion stabilisers. *Food Hydrocolloids, 23*, 1473–1482.
Evans, M., Ratcliffe, I., & Williams, P. A. (2013). Emulsion stabilisation using polysaccharide–protein complexes. *Current Opinion in Colloid & Interface Science, 18*, 272–282.
Fincher, G. B., Stone, B. A., & Clarke, A. E. (1983). Arabinogalactan-Proteins: Structure, biosynthesis, and function. *Annual Review of Plant Physiology, 34*, 47–70.
Furuta, H., Takahashi, T., Tobe, J., Kiwata, R., & Maeda, H. (1998). Extraction of water-soluble soybean polysaccharides under acidic conditions. *Bioscience, Biotechnology and Biochemistry, 62*, 2300–2305.
Ingrassia, R., Palazolo, G. G., Risso, P. H., & Wagner, J. H. (2016). Glycosylation, denaturation and aggregation of soy proteins in defatted soy flakes flour: Influence of thermal and homogenization treatments. *International Journal of Food Properties*. http://dx.doi.org/10.1080/10942912.2016.1238388.
Kalapathy, U., & Proctor, A. (2001). Effect of acid extraction and alcohol precipitation conditions on the yield and purity of soy hull pectin. *Food Chemistry, 73*, 393–396.
Lira-Ortiz, A. L., Reséndiz-Vega, F., Ríos-Leal, E., Contreras-Esquivel, J. C., Chavarría-Hernández, N., Vargas-Torres, A., et al. (2014). Pectins from waste of prickly pear fruits (opuntia albicarpa scheinvar 'reyna'): Chemical and rheological properties. *Food Hydrocolloids, 37*, 93–99.
Liu, J., Verespej, E., Alexander, M., & Corredig, M. (2007). Comparison on the effect of high-methoxyl pectin or soybean-soluble polysaccharide on the stability of sodium caseinate-stabilized oil/water emulsions. *Journal of Agricultural and Food Chemistry, 55*, 6270–6278.
Maeda, H. (2000). Soluble soybean polysaccharide. In G. O. Phillips, & P. A. y Williams (Eds.), *Handbook of hydrocolloids (Chapter 17)*. Boca Ratón: CRC Press.
Mc Clements, D. J. (1999). In F. M. Clydesdale (Ed.), *Food Emulsions: Principles, practice and techniques*. New York: CRC Press.
Michel, M., & Autio, K. (2001). Effects of high pressure on protein-and-polysaccharide-based structures. In M. E. G. Hendrickx, D. Knorr, L. Ludikhuyze, A. V. Loey, & V. Heinz (Eds.), *Ultra high pressure treatments of foods* (pp. 189–214). New York: Springer Publishing Company.
Morrison, I. M. (1988). Hydrolysis of plant cell walls with trifluoroacetic acid. *Phytochemistry, 27*, 1097–1100.
Mozhaev, V. V., Heremans, K., Frank, J., Masson, P., & Balny, C. (1996). High pressure effects on protein structure and function. *Proteins, 24*, 81–91.
Nakamura, A., Fujii, N., Tobe, J., Adachi, N., & Hirotsuka, M. (2012). Characterization and functional properties of soybean high-molecular-mass polysaccharide complex. *Food Hydrocolloids, 29*, 75–84.
Nakamura, A., Furuta, H., Kato, M., Maeda, H., & Nagamatsu, Y. (2003). Effect of soybean soluble polysaccharides on the stability of milk protein under acidic conditions. *Food Hydrocolloids, 17*, 333–343.
Neirynck, N., Van der Meeren, P., Lukaszewicz-Lausecker, M., Cocquyt, J., Verbeken, D., & Dewettinck, K. (2007). Influence of pH and biopolymer ratio on whey protein–pectin interactions in aqueous solutions and in O/W emulsions. *Colloids and Surfaces a, 298*, 99–107.
Ngouémazong, E. D., Christiaens, S., Shpigelman, A., VanLoey, A., & Hendrickx, M. (2015). The emulsifying and emulsion-stabilizing properties of pectin: A review. *Comprehensive Reviews in Food Science and Food Safety, 14*, 705–718.
Nkonge, C., & Balance, G. M. (1982). A sensitive colorimetric procedure for nitrogen determination in Micro-Kjeldahl digests. *Journal of Agricultural and Food Chemistry, 30*, 416–420.
Ogutu, F. O., Mu, T. H., Elahi, R., Zhang, M., & Sun, H. N. (2015). Ultrasonic modification of selected polysaccharides-review. *Journal of Food Processing & Technology, 6*, 446–453.
O'Toole, D. K. (2004). Soybean: Soymilk, Tofu, and okara. In C. Wrigley (Ed.), *Encyclopedia of grain science* (pp. 185–195). Cambridge: Elsevier Ltd.
Pearson, A. M. (1983). Soy proteins. In B. J. F. Hudson (Ed.), *Developments in food protein* (pp. 67–108). Londres: Applied Science Pub.
Phillips, G. O., & Williams, P. A. (2001). Introduction to food proteins. In G. O. Phillips, & P. A. Williams (Eds.), *Handbook of food proteins* (pp. 1–12). Cambridge: Woodhead Publishing Ltd.
Porfiri, M. C., Cabezas, D. M., & Wagner, J. R. (2016). Comparative study of emulsifying properties in acidic condition of soluble polysaccharides fractions obtained from soy hull and defatted soy flour. *Journal of Food Science and Technology, 53*, 956–967.
Preece, K. E., Drost, E., Hooshyar, N., Krijgsman, A., Cox, P. W., & Zuidam, N. J. (2015). Confocal imaging to reveal the microstructure of soybean processing materials. *Journal of Food Engineering, 147*, 8–13.
Redondo-Cuenca, A., Villanueva-Suárez, M. J., & Mateos-Aparicio, I. (2008). Soybean seeds and its by-product okara as sources of dietary fibre. Measurement by AOAC and Englyst methods. *Food Chemistry, 108*, 1099–1105.
Wang, Z. M., Cheung, Y. C., Leung, P. H., & Wu, J. Y. (2010). Ultrasonic treatment for improved solution properties of a high-molecular weight exopolysaccharide produced by a medicinal fungus. *Bioresource Technology, 101*, 5517–5522.
Wang, H., Johnson, L. A., & Wang, T. (2004). Preparation of soy protein concentrate and isolate from extruded-expelled soybean meals. *Journal of the American Oil Chemists' Society, 81*, 713–717.
Wang, Z., Li, Y., Jiang, L., Qi, B., & Zhou, L. (2014). Relationship between secondary structure and surface hydrophobicity of soybean protein isolate subjected to heat treatment. *Journal of Chemistry*, 10, 475389. http://dx.doi.org/10.1155/2014/475389.
Zhang, Y., Zhao, W., Yang, R., Ahmed, M. A., Hua, X., Zhang, W., et al. (2013). Preparation and functional properties of protein from heat-denatured soybean meal assisted by steam flash-explosion with dilute acid soaking. *Journal of Food Engineering, 119*, 56–64.