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Scleroglucan compatibility with thickeners, alcohols and polyalcohols and downstream processing implications

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ABSTRACT

Thickening capacity and compatibility of scleroglucan with commercial thickeners (corn starch, gum arabic, carboxymethylcellulose, gelatin, xanthan and pectin), glycols (ethylene glycol and polyethylene glycol), alcohols (methanol, ethanol, 1-propanol and isopropanol) and polyalcohols (sorbitol, xylitol and mannitol) was explored. Exopolysaccharides (EPSs) from *Sclerotium rolfsii* ATCC 201126 and a commercial scleroglucan were compared. Compatibility and synergism were evaluated taking into account rheology, pH and sensory properties of different thickener/scleroglucan mixtures in comparison with pure solutions. *S. rolfsii* ATCC 201126 EPSs induced or increased pseudoplastic behaviour with a better performance than commercial scleroglucan, showing compatibility and synergy particularly with corn starch, xanthan, pectin and carboxymethylcellulose. Compatibility and a slight synergistic behaviour were also observed with 30% (w/v) ethylene glycol whereas mixtures with polyethylene glycol (PEG) precipitated. Scleroglucan was compatible with polyalcohols, whilst lower alcohols led to scleroglucan precipitation at 20% (v/v) and above. PEG-based scleroglucan downstream processing was compared to the usual alcohol precipitation. Downstream processed EPSi (with isopropanol) and EPS-p (with PEG) were evaluated on their yield, purity, rheological properties and visual aspect pointing to alcohol downstream processing as the best methodology, whilst PEG recovery would be unsuitable. The highest purified EPSi attained a recovery yield of ~23%, similar to ethanol purification, with a high degree of purity (88%, w/w *vs.* EPS-p, 8%, w/w) and exhibited optimal rheological properties, water solubility and appearance. With a narrower molecular weight distribution (M_w, 2.66×10^6 g/mol) and a radius of gyration (R_w, 245 nm) slightly lower than ethanol-purified EPSs, isopropanol downstream processing showed to be a proper methodology for obtaining a refined-grade scleroglucan.

1. Introduction

Scleroglucan from *Sclerotium* species showed to be a highly stable exopolysaccharide (EPS) over a wide pH range, in the presence of electrolytes, ethanol and glycols (Fosmer, Gibbons, & Heisel, 2010; Moresi, Lo Presti, & Mancini, 2001; Schmid, Meyer, & Sieber, 2011). By virtue of these and further rheological properties, scleroglucan has been proposed for different industrial applications such as in cosmetics, enhanced oil recovery or agroindustrial packaging (Moresi et al., 2001; Rau, 2004; Schmid et al., 2011).

Gelling, film forming and fat replacement β-glucan properties might be also applicable in the food industry, where synergic hydrocolloid combinations can be sought to gain additional advantages such as moisture retention, control of rheological properties or improved texture (Laroche & Michaud, 2007; Sadar, 2004; Sikora, Juszczak, Sady, & Krawontka, 2003; Sutherland, 2002).

Glucans are FDA-approved and 'Generally Recognized As Safe' (GRAS) and particularly for β-glucans, their biological activity has been related to cancer-preventing, immune-enhancing, tumour inhibition, hypocholesterolemic, hypoglucemic and wound repair effects (Jung, Park, Park, & Hong, 2008; Miranda et al., 2008; Rice et al., 2005; Zhang, Cui, Cheung, & Wang, 2007).

The relevance of studying an EPS from submerged fermentation, in comparison to yeast cell wall glucans, has been previously emphasized (Miranda et al., 2008). *Sclerotium rolfsii* ATCC 201126 secrete copious amounts of scleroglucan (Fariña, Siñeriz, Molina, & Perotti, 2001). Mainly due to the high molecular weight and the very stiff triple helical conformation, scleroglucan aqueous solutions exhibit well recognized rheological properties (Brigand, 1993; Yanaki, Kojima, & Norisuye, 1981).

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Previous work reported that scleroglucan would be compatible, without synergism, with thickeners such as guar gum, locust bean gum, alginates, gelatin, polyacrylamides, xanthan, carrageenans, and cellulose derivatives (Brigand, 1993). Synergism between corn starch and *S. rolfsii* scleroglucan was also described (Viñarta, Molina, Figueroa, & Fariña, 2006). This background led to a deeper investigation on scleroglucan as a compatible and synergistic candidate when combined with commercial thickeners. Biopolymer behaviour in the presence of glycols, alcohols and polyalcohols was also herein examined.

Compatibility and synergism of scleroglucans from *S. rolfsii* ATCC 201126 and a commercial scleroglucan were comparatively assessed in the light of rheological behaviour, pH and sensory properties of thickener/scleroglucan mixtures.

Additionally, downstream processing with polyethylene glycol of the scleroglucan produced at fermenter scale was compared to the usual alcohol purification protocol. Particular attention was paid to the incidence of downstream processing on yield, purity and rheological properties of the final biopolymer obtained.

2. Materials and methods

2.1. Materials for compatibility and synergism assays

Commercial grade corn starch (MAIZENA® Duryea, Refinerías de Maíz SAICF, Buenos Aires, Argentina, 99.4% purity), gum arabic (Anedra, San Fernando, Argentina), carboxymethylcellulose (Chemicon, Temecula, CA, USA), gelatin (Difco, Detroit, MI), commercially available xanthan and pectin from apple (Sigma Chemical Co., St. Louis, MO, USA) were used.

Glycols comprised ethylene glycol (EG) (Dorwil, Buenos Aires, Argentina), polyethylene glycol 4000 (PEG 4000) (BDH, UK) and polyethylene glycol 6000 (PEG 6000) (Merck, Darmstadt, Germany). Polyalcohols were xylitol, sorbitol (Sigma Chemical Co., St. Louis, MO, USA) and mannitol (Carlo Erba, Milan, Italy). Lower alcohols included methanol, 1-propanol (Sintorgan S.A., Buenos Aires, Argentina), ethanol and isopropanol (Cicarelli Reagents S.A., Santa Fe, Argentina).

EPS I and EPS II scleroglucans ($M_w \sim 5.2 \times 10^6$ Da) produced under batch culture mode by *S. rolfsii* ATCC 201126, recovered at 48 and 72 h, respectively, and ethanol-purified as previously described (Fariña et al., 2001) were used. Commercial scleroglucan, LSCL ($M_w = 4.5 \times 10^5$ Da) was acquired from CarboMer Inc. (USA).

2.2. Polymer solutions for compatibility and synergism assays

Polymer solutions (0.2%, w/v) were prepared as follows: EPS I, EPS II and LSCL were added to the appropriate volume of distilled water and hydrated overnight under magnetic stirring at 400 rpm and 25 °C. Magnetic stirring at 60 °C was continued until polymer became dissolved (~48–72 h for EPS I and EPS II; ~72–96 h for LSCL). Xanthan (XAN) was prepared by mixing the polysaccharide with distilled water at 400 rpm and 40 °C until dissolved. Pectin (PEC) and gum arabic (GA) were dispersed in distilled water at 80 °C and thoroughly homogenized for 3 h by magnetic stirring (400 rpm, 40 °C). Carboxymethylcellulose (CMC) was dispersed in distilled water and stirred (400 rpm) until complete dissolution. Gelatin (GEL) was prepared according to the manufacturer instructions. The appropriate amount of corn starch (CS) was first dispersed in cold distilled water and the resultant starch slurry was then cooked in a boiling water bath (10–15 min) with gentle manual stirring until the paste was ready.

Moisture contents were taken into account in order to compensate the desired final concentrations. If necessary, volume was corrected by addition of distilled water, and blend was left aside to reach 25 °C. Potassium sorbate (0.025%, w/v) (Merck, Darmstadt, Germany) was added as preservative.

2.3. Evaluation of scleroglucan compatibility and synergism with thickeners, glycols and polyalcohols

Sensory properties (colour, aspect and consistency) as well as apparent viscosity of both, pure aqueous solutions (EPSs, thickeners, glycols and polyalcohols) and the respective blends were evaluated. Two substances were considered compatible when the rheological and sensory properties experienced by the mixture were not adversely affected with respect to those of the components. Blends with superior rheological properties than those of each individual component were considered synergic.

Polymer controls were characterized according to their rheological behaviour, pH, colour, aspect and consistency. Apparent viscosity measurements were carried out at 25 °C with a rotational viscometer (Cannon® LV 2000, Cannon® Instrument Co., State College, PA, USA) (see Section 2.7). Rheological parameters were estimated by fitting viscosity data to the Ostwald–de-Waele model:

$$\eta = K\gamma^{(n-1)}$$

where η represents the apparent viscosity, γ the shear rate, K the consistency coefficient and n the flow behaviour index (Rau, Müller, Cordes, & Klein, 1990).

Thickener concentrations described in the literature were used to allow comparisons, namely (%, w/v): CMC, 0.5; CS, 2; GA, 12.5; GEL, 3; PEC, 2.2 and XAN, 0.6. Thickener/scleroglucan ratios (per weight) were as follows: CMC/EPS, 2.5/1; CS/EPS, 10/1, GA/EPS, 62.5/1; GEL/EPS, 15/1; PEC/EPS 11/1 and XAN/EPS, 3/1. Thickener/EPS blends were prepared by mixing equal volumes of twofold concentrated polymer solutions at room temperature. EPS final concentration in the mixture was always 0.2% (w/v). LSCL proportions were equal to EPSs.

Compatibility and synergism with glycols and polyalcohols were tested at 20, 30 and 40% (w/v), except for mannitol (20, 25 and 30%, w/v), and pH and η were measured (see Section 2.7).

Assays were run in triplicate from independent assays and the statistical significance of data was assessed according to one-way ANOVA and Tukey–Kramer multiple comparisons tests (GraphPad Instat Biostatistics package, version 3.0).

2.4. Compatibility with lower alcohols

Methanol, ethanol, 1-propanol and isopropanol at 20, 40 and 60% proportions were mixed with scleroglucan to evaluate its ability to remain in solution. Samples were incubated at 5 °C for 24 h and then centrifuged (8000 × *g*, 15 min, 5 °C). Precipitated EPS was dried at 105 °C until constant weight.

2.5. Studies on the influence of post-fermentation EPS downstream processing

Scleroglucan from *S. rolfsii* ATCC 201126 was produced in batch culture mode during 72 h under optimized conditions (Fariña et al., 2001) in a 4-L stirred-tank reactor (LH 210 Series) fitted with baffles and two six-flat bladed Rushton turbine impellers with a 2-L working volume. At the end of fermentation, isopropanol or PEG precipitation methods were applied, thus obtaining EPS-i and EPS-p, respectively. To evaluate the influence of purification steps in EPS-i properties, three polysaccharide samples (EPSi-A, EPSi-B and EPSi-C) were independently obtained according to the number of re-precipitation stages.

Downstream processing started with a 3-fold dilution of culture broth with distilled water, neutralization and homogenization (Fariña et al., 2001). After heating at 80 °C for 30 min, it was centrifuged (10,000 × *g*, 30 min, 20 °C). The EPS from clear supernatant was recovered and divided into two fractions. One fraction was precipitated by adding an equal volume of 40% (w/v) PEG 4000 followed by vigorous magnetic stirring at room temperature until white polymer threads were visible (2–5 min) (Johal, 1991). EPS was recovered with a fine sieve (Macotest A.S.T.M. N°60) and the precipitate was named EPS-p.

The second supernatant fraction was precipitated by adding an equal volume of isopropanol and vigorously mixed with a glass rod. This mixture was allowed to stand overnight at 5 °C to complete EPS precipitation (EPS-i) and finally, the whole precipitate was recovered with a fine sieve, as above. From this precipitate, a sample (EPSi-A) was kept for future analyses and the rest was re-dissolved in distilled water and re-precipitated with isopropanol as above. EPS was recovered with a fine sieve and a fraction of this precipitate (EPSi-B) was kept. A third re-dissolution/re-precipitation step was carried out in order to obtain the polysaccharide sample identified as EPSi-C.

Precipitated polymers (with isopropanol: EPSi-A, EPSi-B, EPSi-C, and with PEG: EPS-p) were freeze-dried and milled to give different purity-grade scleroglucan powder preparations. Samples were analyzed for protein content by the Lowry method using BSA as standard. Reducing sugars were measured according to the Somogyi-Nelson method (Hodge & Hofreiter, 1962) with glucose as standard. Total carbohydrates were determined by the phenol–sulphuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956) with dextran as standard.

2.6. Preparation of downstream-processed scleroglucan samples

To obtain polymer solutions (0.2%, w/v), EPSi-A, EPSi-B, EPSi-C and EPS-p were added to the appropriate volume of distilled water and hydrated during 3 h under magnetic stirring (400 rpm, 25 °C). Thereafter, magnetic stirring at 60 °C was continued until polymer became completely dissolved (12, 6 and 3 h for EPSi-A, EPSi-B and EPSi-C, respectively; and 3 h for EPS-p). Moisture contents (%, w/w: EPSi-A, 23; EPSi-B, 27; EPSi-C, 3 and EPS-p, 32) were taken into account to obtain the desired final concentration. If necessary, volume was corrected by addition of distilled water, and blend was left aside to reach 25 °C.

2.7. Apparent viscosity measurements

Rheological properties were assessed with a rotational viscometer with narrow gap concentric cylinders or spindles (Cannon LV 2000) equipped with a Temperature Controlled Unit TCU 1000 and a Small Sample Adapter. Measurements (for 8-mL samples) were carried out with a TL-5 spindle, at 25 °C and at shear rates between 0.396 and 79.2 1/s. Readings were taken after 2 min of rotation and data presented are average of at least three measurements.

2.8. Molecular weight (M_w) determination by laser light scattering (LLS)

EPS M_w was determined by size exclusion chromatography coupled to laser light scattering (SEC-LLS). Scleroglucan solutions (2 g/L) were prepared in the mobile phase according to the protocols above (Section 2.2 for EPS I, EPS II and LSCL; Section 2.6 for EPSi-A, EPSi-B, EPSi-C and EPS-p).

Samples (200 μL) were injected in duplicate into a Waters-Breeze liquid chromatograph with a binary pump (model 1525) and an automatic sample injector (Waters 717 plus) coupled to a differential refractometer (DR) (Waters 2414, model 410, 30 °C) and a Light Scattering detector (LS) (Dawn DSP). SEC involved the use of an Ultrahydrogel 5-column set (Waters, 7.8 × 300 mm; pore sizes: 120, 250, 500, 1000 and 2000 Å) with exclusion limits of 5×10^3, 8×10^4, 4×10^5, 1×10^6 and 7×10^6 g/mol. The filtered (0.2 μm; GHP Acrodisc GF) mobile phase, 0.1 M $NaNO_3$ plus 0.02% (w/v) NaN_3, was delivered at a flow rate of 0.8 mL/min.

The system was calibrated using narrow-band pullulan standards (Shodex Standard, Showa Denko), dissolved in the mobile phase (1 mg/mL) and filtered (0.2 μm) prior to injection.

Table 1
Rheological parameters and pH of scleroglucans, commercial thickeners and scleroglucan/thickener blends.

	Ostwald–de-Waele parameters		pH
	Consistency coefficient, K (mPa s^n)	Flow behaviour index, n	
Polymer (%, w/v)			
CMC (0.5)	NA	NA	6.74
CS (2)	245.1	0.36	7.69
EPS I (0.2)	1098.0	0.21	8.38
EPS II (0.2)	1031.0	0.21	7.70
GA (12.5)	NA	NA	4.50
GEL (3)	NA	NA	6.85
LSCL (0.2)	274.0	0.27	6.68
PEC (2.2)	NA	NA	2.56
XAN (0.6)	43.4	0.63	6.26
Polymer blends[a]			
CMC + EPS I	1749.0	0.21	6.85
CMC + EPS II	1277.0	0.24	6.53
CMC + LSCL	297.3	0.44	6.68
CS + EPS I	3820.0	0.08	6.45
CS + EPS II	3820.0	0.08	6.45
CS + LSCL	2612.0	0.41	6.58
GA + EPS I	1075.0	0.24	4.71
GA + EPS II	746.4	0.26	4.73
GA + LSCL	334.3	0.35	4.66
GEL + EPS I	90.9	0.56	6.17
GEL + EPS II	76.2	0.55	6.23
GEL + LSCL	274.0	0.27	6.17
PEC + EPS I	1836.0	0.23	2.80
PEC + EPS II	1578.0	0.28	2.76
PEC + LSCL	581.8	0.38	2.75
XAN + EPS I	1509.0	0.24	6.64
XAN + EPS II	1441.0	0.22	6.59
XAN + LSCL	625.1	0.29	6.15

Viscosity measurements were carried out at 25 °C and at shear rates between 0.396 and 79.2 1/s. Data were fitted to the Ostwald–de-Waele model. Standard errors were all below 10%. For details, see Section 2. NA: not applicable model.

[a] Polymer concentrations in blends were the same as in the above column and were obtained by mixing equal volumes of the respective twofold concentrated solutions.

3. Results and discussion

3.1. Compatibility and synergism with thickeners

The occurrence of synergism between scleroglucan and CMC, CS, GA, GEL, PEC and XAN in aqueous solution was evaluated. Rheological data of thickeners, scleroglucans and polymer blends were fitted to the Ostwald–de-Waele model (Supplementary material 1). Larger pseudoplasticity was characterized by higher values of K and lower values of n (Table 1).

Supplementary material related to this article found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2012.10.065.

EPS I and EPS II performance was generally superior to that for LSCL. Better suspending and emulsifying properties were also previously associated to the outstanding rheological behaviour of EPS I and EPS II in comparison to the commercial glucan (Viñarta, François, Daraio, Figueroa, & Fariña, 2007).

EPS I, EPS II and LSCL were able to induce (CMC, GA, GEL and PEC) or increase (CS and XAN) pseudoplastic behaviour in all mixtures (Table 1). Positive synergism was observed between scleroglucans (EPS I, EPS II and LSCL) and CMC, CS, PEC and XAN, as witnessed by the substantial increase in pseudoplasticity of these mixtures with respect to both individual polymers (Table 1). In the case of GA, positive synergism was only observed with LSCL (Table 1) and no synergism was observed between scleroglucans and GEL.

Compatibility also implied no precipitation and no variations in colour or transparency of the mixtures. Blends were not deleteriously affected with time and aroma of the mixtures was kept invariable, while texture was slightly improved. No significant variations were imparted to the pH of the mixtures, in comparison to the individual thickener (Table 1).

Amending preliminary reports on the non-synergic compatibility of scleroglucan with gelatin, xanthan and cellulose derivatives (Brigand, 1993), scleroglucans from *S. rolfsii* ATCC 201126 showed synergism with XAN and CMC. The synergism previously noted between CS and EPS II (Viñarta et al., 2006) was also herein evidenced for EPS I and LSCL (Table 1). The best synergistic combinations were obtained between CS and scleroglucans. EPS I and EPS II showed synergism in most of the cases, whilst LSCL showed more pronounced effects when associated to CS.

The stiffness of scleroglucan triple helix provides architectural stability and confers exceptional viscosity to aqueous solutions (Bluhm, Deslandes, Marchessault, Pérez, & Rinaudo, 1982; Pérez & Vergelati, 1985). Additionally, protruding β-1,6-linked residues with extreme conformational flexibility encourage lateral glucose disorder and prevent intermolecular association beyond the triple helical stage, thus avoiding lateral packing and precipitation (Bluhm et al., 1982).

Particular conformations adopted by individual polysaccharide chains make possible the association with water, with themselves or other polymers, thus affecting network development and rheological behaviour (Morris, 1986). Helical or ribbon-like regular shapes can line up with one another and form interchain physical bonds giving rise to network cavities where water molecules are trapped. Conversely, areas of irregular conformation cannot be associated. These polysaccharide interactions may explain the synergistic behaviour observed in the present work. Depending on the nature of the junction zones, they will be more or less easily disrupted by shear forces leading to the release of water and viscosity fall (Morris, 1986).

The EPS concentrations required to observe a synergic rheological behaviour (2 g/L) were lower than those recommended in the literature for other polysaccharides (*e.g.* 5 g/L jamilano + 4.5 g/L carrageenan). Additionally, sensory properties were not affected by scleroglucans, conversely to the effects described after the addition of similar amounts of xanthan (García-Ribera, Monteoliva-Sánchez, & Ramos-Cormenzana, 2002). This would be particularly interesting in foodstuffs manufacture, where the use of additives with ability to modify rheological parameters is frequently applied to improve the quality of the final product (Gómez-Díaz & Navaza, 2002; Sikora et al., 2003).

3.2. Compatibility and synergism with glycols and polyalcohols

Scleroglucan/EG blends exhibited compatibility and kept the typical scleroglucan pseudoplastic behaviour at different EG concentrations (20–40%, w/v) and only LSCL mixtures showed a moderate synergism as EG concentration increased (Table 2 and Supplementary material 2).

Supplementary material related to this article found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2012.10.065.

EPS I, EPS II and LSCL precipitated when combined with PEG 4000 and PEG 6000 at all assessed concentrations, indicating their incompatibility. Consequently, they were considered as potential precipitants for scleroglucan recovery and purification (Johal, 1991).

Table 2
Rheological parameters and pH of scleroglucans, ethylene glycol and scleroglucan/ethylene glycol blends.

		Ostwald–de-Waele parameters		pH
		Consistency coefficient, K (mPa s^n)	Flow behaviour index, n	
Scleroglucan/EG blends				
EPS I		690.1	0.24	8.36
EPS II	+20% (w/v) EG	858.0	0.22	8.71
LSCL		325.0	0.23	6.52
EPS I		1341.0	0.18	7.65
EPS II	+30% (w/v) EG	1020.0	0.19	7.81
LSCL		395.6	0.31	8.21
EPS I		942.9	0.29	7.12
EPS II	+40% (w/v) EG	973.8	0.31	7.53
LSCL		409.4	0.44	7.52

Viscosity measurements were carried out at 25 °C and at shear rates between 0.396 and 79.2 1/s. Data were fitted to the Ostwald–de-Waele model. Consistency coefficient reference values (mPa s^n) for scleroglucans (0.2%, w/v) were: EPS I, 1098.0, EPS II, 1031.0 and LSCL, 274.0. Reference pH values for EG were: 8.17, 8.07 and 7.80 for 20, 30 and 40% (w/v) EG aqueous solutions, respectively. Standard errors were all below 10%.

No synergism was detected between scleroglucan and xylitol, sorbitol and mannitol. Nevertheless, polyol solution viscosity was slightly increased in the presence of scleroglucan (data no shown), demonstrating their compatibility. These findings would be consistent with previous reports on the scleroglucan solubility in mixtures containing up to 50% polyols (Brigand, 1993).

3.3. Compatibility with lower alcohols

In agreement with earlier reports (Brigand, 1993), EPS I, EPS II and LSCL precipitated with all the alcohols assayed (methanol, ethanol, 1-propanol and isopropanol) at all the proportions evaluated (20, 40 and 60%, v/v). However, differences could be noted in the precipitated polysaccharide appearance. At 20% (v/v) alcohol a more hydrated precipitate was obtained. As the alcohol proportion increased (40–60%, v/v), precipitated polysaccharide became more compact. This might be relevant for downstream processing design, since denser precipitates can be more easily recovered.

With methanol and 1-propanol, scleroglucan recovery yield was 100% at all proportions (20, 40 and 60%, v/v). Ethanol and isopropanol at the highest proportions (40–60%, v/v) led to 100%-precipitation, while at the lower proportion (20%, v/v), yields of 95 and 99.25%, for ethanol and isopropanol respectively, were found.

No statistically significant differences were observed in the precipitation yields between the different alcohols evaluated. Nevertheless, more compact and defined precipitates were obtained with isopropanol, a fact that could facilitate and improve scleroglucan recovery and quantification.

Ethanol precipitation has been recognized as a purification step able to remove impurities from polysaccharides (Zhang et al., 2007). In food or pharmaceutical industries, the use of ethanol (considered as GRAS by FDA) is usually preferred than isopropanol. Similar observations were made on xanthan gum, whose chemical properties and physiological effects were equivalent when purified with either ethanol or isopropanol (U.S. FDA, 2003, 2004).

3.4. Influence of downstream processing

3.4.1. Purified scleroglucans

At the end of fermentation, EPSi-A, EPSi-B and EPSi-C were obtained at different steps of isopropanol precipitation, while

EPS-p by PEG precipitation (see Section 2), with recovery yields of 48.9, 29.5, 22.9 and 117.2%, respectively. Lower yield values for EPSi-B and EPSi-C would be likely due to the larger number of purification steps, while the apparently high yield in EPS-p might be an artefact (EPS-PEG co-precipitation).

Care must be taken with the interpretation of yields because crude products often contain cells, organic materials and salts which can be co-precipitated from fermentation broth along with the polymer (Margaritis, 1985). This rationale may explain the high yields in EPS-p and EPSi-A, obtained after less purification steps. EPSi-C yield was similar to values reported for EPS I and II (~20–25%), produced under identical conditions and downstream processed with ethanol (Fariña et al., 2001).

Reducing sugars gradually decreased with the progress of isopropanol purification (steps A–C), whilst for EPS-p the value was relatively low (Table 3). Total sugars for EPSs precipitated with isopropanol indicated high polysaccharide content in comparison to EPS-p (Table 3). Total sugars content higher than 100% for less purified isopropanol-EPSs (EPSi-A and EPSi-B, Table 3), could be due to the overestimation of sugars dragged from culture broth.

The total sugars content in EPSi-C was about 90% (w/w), reason why it could be considered a refined grade scleroglucan and a good candidate for biotechnological applications requiring high purity grade (Schmid et al., 2011; Survase, Saudagar, Bajaj, & Singhal, 2007; Wang & McNeil, 1996).

In contrast, the lower total sugars content in EPS-p (~8%, w/w) which account for real polysaccharide, might reflect the inefficient EPS precipitation in addition to the co-precipitation of PEG, as shown by LLS (see below). Accordingly, this previously suggested methodology (Johal, 1991) would be inappropriate for downstream processing. Although not systematically evaluated in this work, time (2–5 min) or temperature (room temperature) during this procedure, different from the alcohol precipitation protocol (overnight, 5 °C), may be not discarded as factors that could influence EPS PEG-recovery.

The low protein content for EPSi-C was similar to values previously found for EPS I and EPS II (1.9 and 1.6%, w/w, respectively) (Fariña et al., 2001). EPS-p protein content (~9%, w/w) indicated that PEG recovery would be also inappropriate according to this parameter.

3.4.2. Rheological properties

Apparent viscosity data of purified scleroglucan solutions were fitted to the Ostwald–de-Waele model and exhibited the usual pseudoplastic behaviour (Fig. 1).

In agreement with a refined purity grade biopolymer, the best performance corresponded to EPSi-C (Fig. 1 and Table 3) exhibiting the highest *K* value. Conversely, lower *K* values experienced by less purified polymers (EPSi-A and EPS-p, Table 3) were coherent with the low viscosity produced by crude polymers (Bluhm et al., 1982).

As often reported for many other β-glucans, scleroglucan properties can be influenced by recovery methods (Sletmoen & Stokke, 2008) and solution properties vary differently depending on the purity grade (Rodgers & Goffett, 1976; Survase et al., 2007). Heterogeneities can be caused by unsuitable extraction processes such as careless alkalinization or thermal post-biosynthetic treatments or even, excessive aeration or shearing, which may denature or degrade the polysaccharide giving rise to differences between commercial batches (Laroche & Michaud, 2007; Sletmoen & Stokke, 2008; Zentz, Verchère, & Muller, 1992).

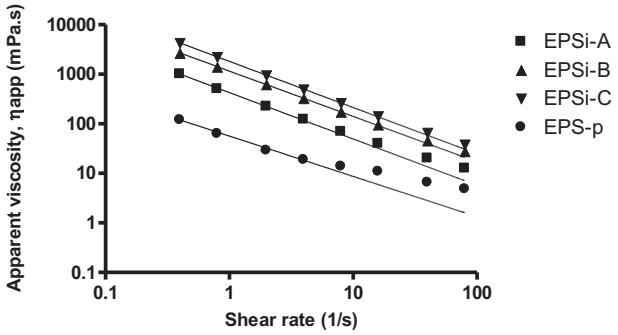

Fig. 1. Rheological behaviour in distilled water (η = 0.8904 mPa s) at 25 °C for scleroglucans (0.2%, w/v) from *S. rolfsii* ATCC 201126 after different downstream processing (for nomenclature, see Section 2). Data were fitted to the Ostwald–de-Waele model. For the sake of clarity, standard errors were not plotted.

Lyophilization and milling processes may also be a reason for reduced viscosity and/or poor solubility (Johal, 2006; Rau, 2004; Rau et al., 1990). Water removal steps can significantly affect the microstructure of polysaccharide particles and, interparticle H-bond formation during this process may acquire a significant influence (Hromádková et al., 2003).

Accordingly, it has been already suggested to state the sample history when referring results on β-glucan behaviour and biological properties (Sletmoen & Stokke, 2008). A high purity grade is essential for the understanding of conformation and conformational transitions associated to a certain biological activity. The use of impure β-glucan preparations has become problematic at the time of identifying the component or structural motif inducing a biological effect (Sletmoen & Stokke, 2008).

3.4.3. Characteristics of purified EPSs

Aspect, colour, moisture content and other associated characteristics exhibited variations for each purified EPS (Table 3 and Fig. 2).

Although in all cases the same previous hydration process was used (400 rpm, 3 h, room temperature), the time required for complete dissolution under identical conditions, was different for each EPS. For EPS-i, dissolution time decreased with the number of purification steps (A: 12 h, B: 6 h, C: 3 h), which would be advantageous since ethanol-purified EPSs usually required longer hydration and dissolution time (48–72 h). On the other hand, in agreement with its quite low scleroglucan content and despite the fast solution preparation (6 h), EPS-p led to low viscosity (Table 3).

Table 3
Purity grade parameters and solution properties of scleroglucans recovered from *S. rolfsii* ATCC 201126 culture broth, according to different downstream processes.

Scleroglucan[a]	Moisture content (%)[b]	Proteins (%)[b]	Total sugars (%)[b]	Reducing sugars (%)[b]	Consistency coefficient, *K* (mPa s^n)[c]	Flow behaviour index, *n*[c]	pH[c]
EPSi-A	23.43	4.37	111.51	31.10	426.3	0.06	7.33
EPSi-B	27.01	4.57	112.66	12.66	1159.0	0.08	8.33
EPSi-C	2.96	1.45	87.87	1.77	1813.0	0.07	8.30
EPS-p	32.47	8.61	8.31	7.65	56.3	0.18	7.10

[a] For details on nomenclature, see Section 2.
[b] (w/w), referred on a dry basis.
[c] EPS aqueous solution (0.2%, w/v) properties. *K* and *n* represent rheological parameters according to the Ostwald–de-Waele model. Viscosity measurements were carried out at 25 °C and at shear rates between 0.396 and 79.2 1/s. Standard errors were all below 10%.

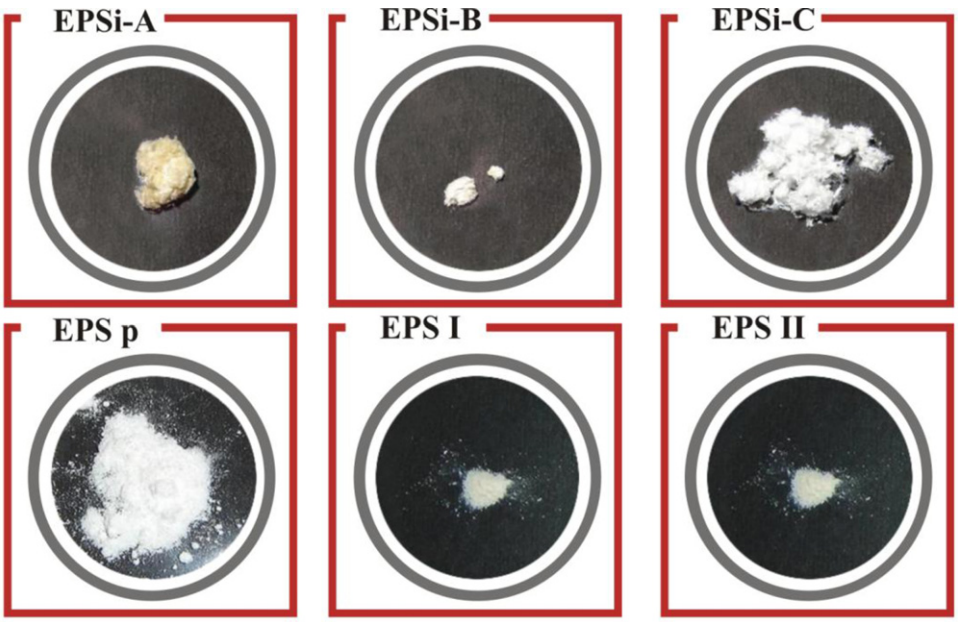

Fig. 2. Macroscopic appearance of scleroglucans from *S. rolfsii* ATCC 201126 after different downstream processing protocols. For nomenclature and technical details, see Section 2.

Differences in solubilization, particularly between EPS-i samples, might be mainly related to the purity grade but also, to conformational or microstructural features eventually modified during polysaccharide processing. Comparing EPS-i *vs*. ethanol-purified EPSs, differences could be related to the precipitating agent, which could induce conformational changes underlying a different solubility or microstructure.

Isopropanol precipitation led to a lower EPS moisture content (EPSi-C ~3%, w/w *vs*. EPS I-II, ~12–13%, w/w), which may be favourable to avoid sample contamination or deterioration. A better visual appearance (whiter and flaky, Fig. 2) also characterized EPSi-C, as referred for fine-grade polysaccharides (Schmid et al., 2011).

Not only yield is the main goal during downstream processing, but also the preservation of critical physico-chemical and biological properties, which may be influenced by the drying process or the precipitating agent. Differences on water uptake have been already reported for other β-glucans for which spray-drying was recommended (Hromádková et al., 2003). Accordingly, future investigation may involve the combination of isopropanol-purification along with spray-drying.

3.5. Molecular weight determination by laser light scattering (LLS)

EPSi-C exhibited a single peak at low retention times (Fig. 3), corresponding to a high molar mass compound. Compared to ethanol-purified EPSs (Fig. 3), the peak for EPSi-C was narrower, indicating a dominant proportion of molecules with a uniform molar mass. This fact may be linked to the better EPSi-C rheological properties (Table 1 for EPS I and II and Table 3 for EPSi-C).

Chromatograms for EPS-p and EPSi-A clearly showed two main peaks (Fig. 4). EPSi-A exhibited a quite low concentration (absent DR signal) of a compound with a very wide distribution (40–65 min, LS signal) of intermediate molar masses, and a low concentration of a high M_w compound (DR and LS signals at 30–35 min). Meanwhile, EPS-p profile showed a high concentration of a low molar mass compound (DR peak at 50–60 min), probably related to PEG co-precipitation which would be completely undesirable from the purification point of view, and a low concentration of a compound of high M_w (DR and LS signals at 30–35 min). Both for EPS-p and EPSi-A, peaks at 30–35 min would be related to the EPS at very low concentrations, while peaks at 65–70 min might be likely due to solvent interferences.

In agreement with the purification process, EPSi-B chromatogram (Fig. 4) showed a more similar profile to EPSi-C (Fig. 3), with a higher concentration of the high M_w compound, which evidenced the progress in the EPS purity grade. In terms of purity, the order of scleroglucans was EPSi-A < EPSi-B < EPSi-C, and minimal in the case of EPS-p.

The estimated M_w values for EPSi (A–C) and EPS-p were quite similar and in the order of 10^6 g/mol (Table 4). These values were slightly lower than those previously obtained in terms of intrinsic viscosity data for EPS I and EPS II (~5.2×10^6 Da; Fariña et al., 2001). Since M_w values represent an average, it is not surprising that macromolecules of greater size tended to precipitate at the beginning of isopropanol downstream processing, making this average value to be higher for the former EPS sample obtained (EPSi-A). Along subsequent precipitation steps, M_w tended to stabilize at slightly lower values (Table 4) and all EPSi polysaccharides showed a low polydispersity, denoting a basically uniform molar mass. The radius of gyration (R_w) also gave an idea of less expanded macromolecules as isopropanol purification proceeded (Table 4), indicating possible slight conformational changes as a result of post-fermentation processing.

The values for scleroglucan M_w and R_w were similar to those previously reported in the open literature (Lecacheux, Mustiere, Panaras, & Brigand, 1986; Yanaki et al., 1981; Yanaki & Norisuye, 1983). The viscometric radii of gyration (R_G) for EPS I and EPS II were herein estimated based on previous M_w and intrinsic viscosity data (Fariña et al., 2001) and according to the following equation (Aeberhardt, de Saint Laumer, Bouquerand, & Normand, 2005):

$$R_G = \sqrt[3]{\frac{\bar{M}_n[\eta]}{6^{3/2}\phi_0}}$$

where M_n is the average molecular weight in number, $[\eta]$ is the intrinsic viscosity and φ_0 is the Flory universal constant (2.1×10^{23}). Values of 253 and 252 nm for EPS I and EPS II, respectively, were

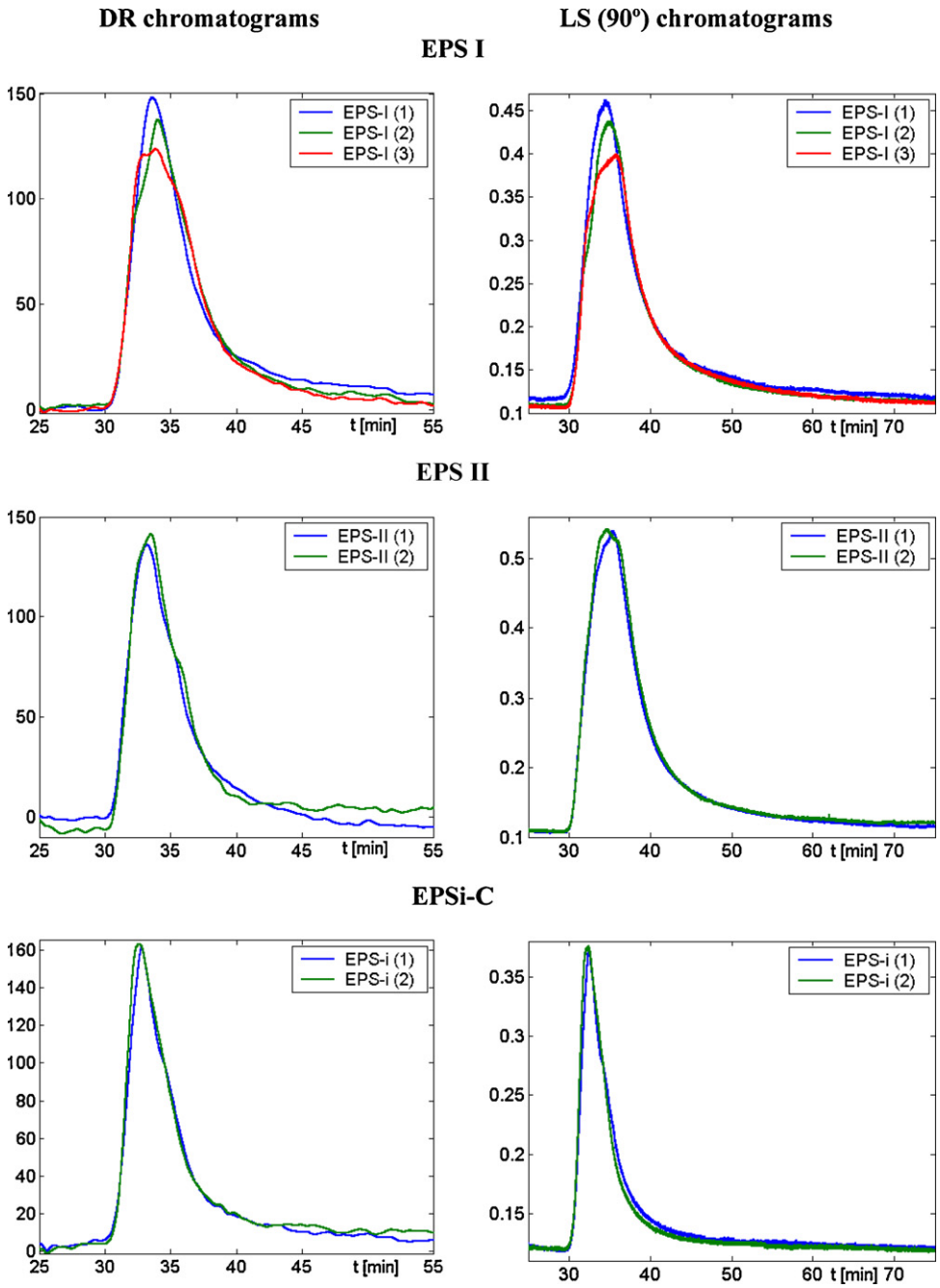

Fig. 3. SEC/RALLS chromatograms for ethanol- (EPS I and EPS II) and isopropanol-purified (EPSi-C) scleroglucans. Numbers in parenthesis and next to EPS indicate different runs. RALLS: Right Angle Laser Light Scattering.

Table 4
Molecular weight (M_w) and radius of gyration (R_w) of scleroglucan EPSs according to SEC/LLS (DR + LS) determinations.

Scleroglucan[a]	Triple helix parameters					
	M_n (g/mol)	M_w (g/mol)	M_w/M_n	R_n (nm)	R_w (nm)	R_w/R_n
EPSi-A	3.50×10^6	3.50×10^6	1.00	296	297	1.00
EPSi-B	2.78×10^6	2.85×10^6	1.02	245	248	1.05
EPSi-C	2.62×10^6	2.66×10^6	1.02	245	247	1.03
EPS-p	2.65×10^6	2.81×10^6	1.06	253	260	1.11

M_n, number average molecular weight; M_w, weight average molecular weight; R_n, number average radius of gyration; R_w, weight average radius of gyration.
[a] For details on nomenclature, see Section 2.

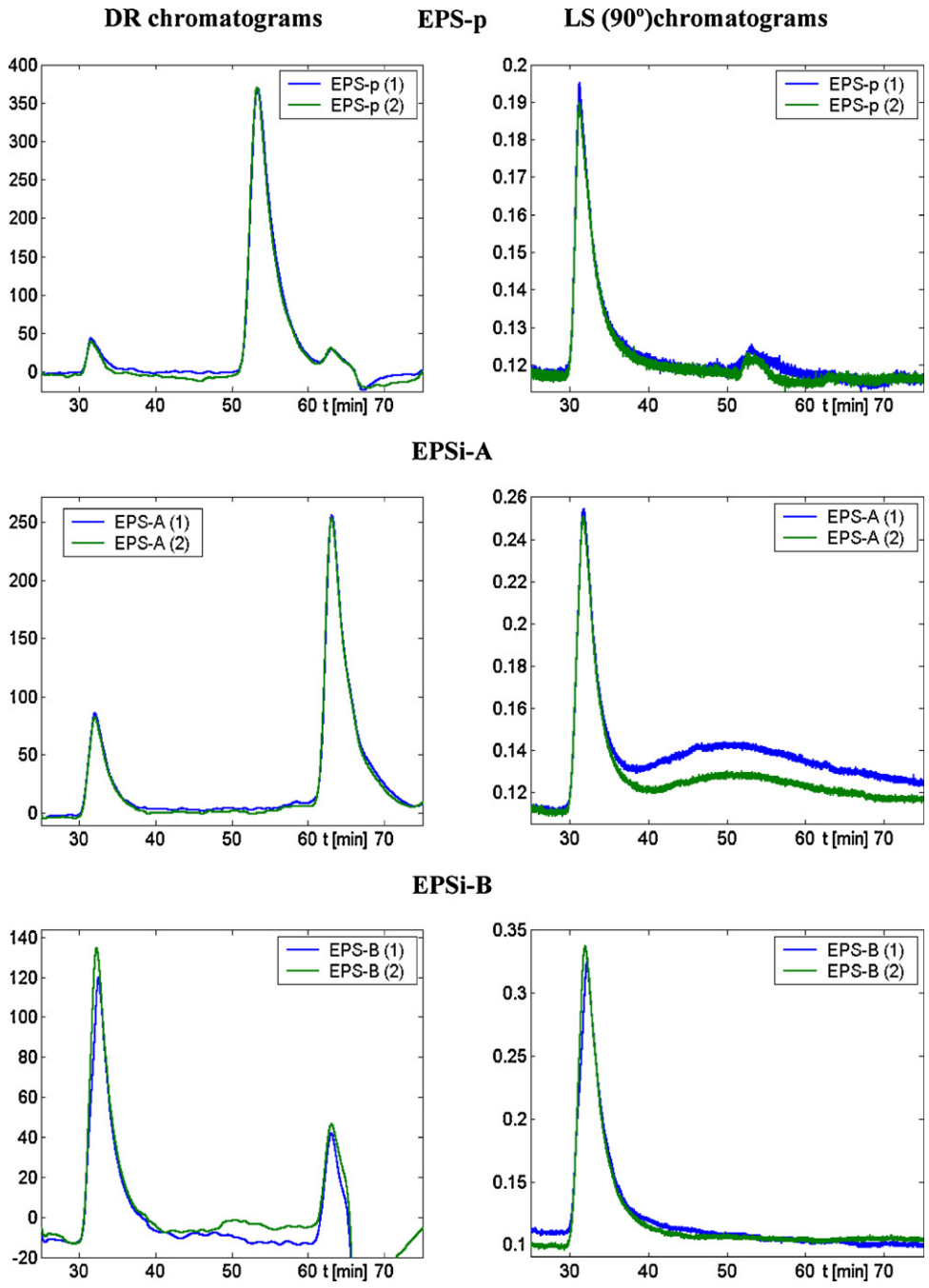

Fig. 4. SEC/RALLS chromatograms for PEG 4000- (EPS-p) and isopropanol-partially purified (EPSi-A and EPSi-B) scleroglucans. Numbers in parenthesis and next to EPS indicate different runs. RALLS: Right Angle Laser Light Scattering.

in the order of those obtained by LS (Table 4). Minor changes in R_G according to the EPS downstream processing were expectable (Hromádková et al., 2003).

4. Conclusions

Scleroglucan behaviour is a multifactorial response where EPS concentration, macromolecular characteristics, structure, charge, and solvent composition all interlace mutually. Compatibility and synergism of scleroglucan with commercial thickeners, particularly for lab fermenter-scale produced EPSs from *S. rolfsii* ATCC 201126 in comparison to commercial scleroglucan, can be significantly valuable at industrial level. In the food industry it may offer added value and innovation to the foodstuff, and new frontiers for the utilization of these EPSs in different industries may be opened.

The influence of downstream processing on the purity, appearance, solubility and rheological properties of obtained scleroglucans was clearly demonstrated, emphasizing the relevance of the number of purification steps in order to obtain high-purity grade polymers. Findings would be valuable since poorly processed glucan, although inexpensive, may be deprived of some biological properties when compared to a justifiable more expensive premium β-glucan purified to higher standards.

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