Extraction, purification and characterization of galactomannans from non-traditional sources

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ABSTRACT

This work presents a methodology for the extraction of galactomannans from seeds of four different species of Leguminosae (*Adenanthera pavonina*, *Caesalpinia pulcherrima*, *Gleditsia triacanthos* and *Sophora japonica*) to be used e.g. in the food and biomedical industries. The galactomannans were obtained by aqueous extraction followed by a precipitation with ethanol. This methodology is simpler and easier to perform than other existing extraction and purification methodologies, and because it avoids the use of organic solvents (other than ethanol), it is able to generate food grade substances and is environmentally friendlier. The yield of extraction in different stages of the process, monosaccharide composition, as well as physical and chemical parameters of the isolated galactomannans were determined and compared with previously published results. The mannose/galactose ratio of the extracted galactomannans ranged from 1.35 (*A. pavonina*) to 5.75 (*S. japonica*). The intrinsic viscosity ranged from 11.34 dL/g (*C. pulcherrima*) to 8.74 dL/g (*S. japonica*), while the viscosity average molecular mass ranged between 1.81×10^6 Da and 1.17×10^6 Da (*A. pavonina* > *C. pulcherrima* > *G. triacanthos* > *S. japonica*). The results confirm the suitability of the extraction and purification procedure to obtain galactomannans from non-traditional sources.

1. Introduction

In the last decade there has been a growing interest in the development of thermoplastic materials from biodegradable polymers, particularly those derived from renewable resources (Paes, Yakimets, & Mitchell, 2008; Petersen et al., 1999).

Biobased packaging is defined as packaging containing raw materials originating from agricultural sources, i.e. produced from renewable, biological raw materials such as starch and bioderived monomers. To date, biodegradable packaging has attracted great attention, and numerous projects are under way in this field. One important reason for this attention is the marketing of environmentally friendly packaging materials. Furthermore, the use of biodegradable packaging materials has the greatest potential in countries where landfill is the main waste management tool.

The biodegradable polymers have also interest for biomedical engineering, featuring two major advantages over non-biodegradable polymers: they are gradually absorbed by the human body, and some of them are able to regenerate tissues, through the interaction of their biodegradation with immunologic cells (Chu, 2003).

Galactomannans are present in the endosperm of numerous plants, particularly the Leguminosae, and they have several functions, including reserve of carbohydrates (Reid & Edwards, 1995). Galactomannans are polysaccharides built up of a β-(1–4)-D-mannan backbone with single D-galactose branches linked α-(1–6). Their mannose/galactose (M/G) ratios differ according to the species (Kök, Hill, & Mitchell, 1999). They are water soluble hydrocolloids which form highly viscous, stable aqueous solutions (Neukom, 1989). Galactomannans can often be used in different forms for human consumption. Featuring different physicochemical properties, galactomannans are a versatile material used for many applications: they are excellent stiffeners and stabilizers of emulsions, and the absence of toxicity allows their use in the textile, pharmaceutical, biomedical, cosmetics and food industries (Srivastava & Kapoor, 2005; Vieira, Mendes, Gallão, & de Brito, 2007). Most galactomannans used in pharmaceutical technology and cosmetics are usually unpurified gums (Üner & Altinkurt,

2004). In some occasions galactomannans have been used in binary mixtures with other polysaccharides such as: xanthan gum, agar and kappa-carrageenan, to form gels with new properties (Bresolin, Milas, Rinaudo, Reicher, & Ganter, 1999; Fernandes, Gonçalves, & Doublier, 1991; Vendruscolo, Andreazza, Ganter, Ferrero, & Bresolin, 2005).

The three major galactomannans of commercial importance in food and non-food industries are guar gum (GG, *Cyamopsis tetragonolobo*, M/G ratio: 2:1), tara gum (TG, *Caesalpinia spinosa*, M/G ratio: 3:1) and locust bean gum (LBG, *Ceratonia siliqua*, M/G ratio: 3.5:1 (Dakia, Blecker, Roberta, Watheleta, & Paquota, 2008).

Currently the international trends demand the introduction of alternative sources of seed gums (Joshi & Kapoor, 2003) and it is therefore important to search for alternative renewable sources for e.g. the production of edible and biodegradable films and coating materials. In particular, Latin American sources of galactomannans are not well known, in spite of the rich biodiversity of the local flora and of the favorable climate for their production (Azero & Andrade, 2002).

Mikkonen et al. (2007) show the use of galactomannan sources as an alternative for film formation. The mechanical and thermal properties from different film formulations (containing GG and LBG with glycerol and sorbitol) show that galactomannans with different M/G ratio have different behaviours, pointing at a promising use of biobased-galactomannan films and coatings as edible packaging materials (Mikkonen et al., 2007).

In the present work, four non-traditional galactomannans were isolated from seeds of *Adenanthera pavonina*, *Caesalpinia pulcherrima*, *Gleditsia triacanthos* and *Sophora japonica* and a simple methodology using only ethanol and water as solvents was developed for their extraction in view of their use e.g. in the demanding area of food industry. All the galactomannans from those plants were obtained by aqueous extraction followed by precipitation with ethanol, but the extraction procedure of galactomannan from the seeds of *S. japonica* required an acidic pretreatment in order to effectively separate the hull from the endosperm. Other works used solvents as chloroform (Mirzaeva, Rakhmanberdyeva, Kristallovich, Rakhimov, & Shtonda, 1998) and petroleum ether (Amin, Ahmad, Yin, Yahya, & Ibrahim, 2007; Üner & Altınkurt, 2004) to extract galactomannans, which are not authorized in the food industry (List of Codex Specifications For Food Additives, 2008). The extraction yield of each of the galactomannans was determined, as well as their monosaccharide composition, M/G ratio, purity, intrinsic viscosity and viscosity average molecular mass, thus providing a convenient measure of the hydrodynamic volume of individual polymer coils.

2. Materials and methods

2.1. Plant material

The pods of *A. pavonina and C. pulcherima* were collected in Fortaleza, Federal University of Ceará (Ce-Br) during January 2006. The pods of *G. triacanthos* and *S. japonica* were collected in the Botanic Garden in Porto, Portugal, during April 2006. The seeds were manually separated and kept in a cool, dry place until further use.

Adenanthera pavonina is a plant from the family Leguminosae, native from tropical Asia. It is used in reforestation, as ornamental plant and constitutes an important source of wood. The baking of the seeds and of the wood allows its use in the treatment of pulmonary infections, and also in the treatment of chronic ophthalmia (Fonseca & Perez, 2003).

Caesalpinia pulcherrima is an ornamental plant found throughout India, but it can be found in other countries as well, especially

in Brazil. It also belongs to the family Leguminosae. Some of the constituents extracted from *C. pulcherrima* were found to possess anti-tumour (Che, McPherson, Cordell, & Fong, 1986; Patil et al., 1997) and antimicrobial properties (Ragasa, Ganzon, Hofilena, Tamboong, & Rideout, 2003).

Gleditsia triacanthos belongs to the family Leguminosae, grows in America, Middle Europe and Mediterranean area (Üner & Altınkurt, 2004). Galactomannans are the main polysaccharide constituents from the endosperm of the seed of *G. triacanthos* (Manzi, Mazzini, & Cerezo, 1984).

Sophora japonica, also a Leguminosae, is native from China where it is widely cultivated and used as a hemostatic agent in traditional Chinese medicine (Ishida, Umino, Tsuji, & Kosuge, 1989; Tang, Lou, Wang, & Zhuang, 2001). This tree is currently spread all over the world, including Portugal. Chemical constituents of the seeds of these plants such as triterpenes, phospholipids, alkaloids, amino acids, polysaccharides and fatty acids have been reported (Grishkovets & Gorbacheva, 1995; Mukhamedova & Glushenkova, 1997). According to previous studies (Smirnova, Mestechkina, & Sherbukhin, 2004), the galactomannan from *S. japonica* exhibits a high M/G ratio (greater than 5). Its unusually low content in galactose can be very interesting for possible commercial application of this gum because it has been recognised that galactomannans which are less substituted show a greater synergistic effect with e.g. xanthan gum (Fernandes, 1995; Scborsch, Gamier, & Doublier, 1997).

2.2. Polysaccharide extraction

The polysaccharide extraction of *A. pavonina*, *C. pulcherrima* and *G. triacanthos* was performed with ethanol and distilled water. In this process, the seeds were removed from the pods, cleaned and placed in a blender, where they were mechanical broken. Following this operation, the endosperm was manually separated from the germ and the hull, suspended in ethanol (purity 99.8%, Riedel-de Haën, Germany) in a proportion 1:3 (seeds:ethanol) at 70 °C during 15 min to inactivate the enzymes and eliminate low-molecular-weight compounds (Egorov, Mestechkina, & Shcherbukhin, 2003; Egorov, Mestechkina, & Shcherbukhin, 2004). The ethanol was decanted and distilled water was added in a proportion of 1:5 (endosperm:water), the suspension was left to rest for approximately 24 h. Then water, in a proportion of 1:10, (suspension:water) was added and mixed in a blender for 5 min.

Only in the case of the *S. japonica* seeds this procedure was not efficient because the black hull remained attached to the endosperm and consequently the gum contained a high level of impurities and was brownish. In order to obtain a purer gum, two different de-hulling pre-treatments (procedures A and B) of the *S. japonica* seeds with acid were tested, as described below.

2.2.1 Procedure A
The seeds of *S. japonica* were peeled using sulfuric acid (purity 98%, Fluka, Germany) (1:1) in a water bath at 100 °C for 1.5 h. This treatment with acid at an elevated temperature carbonized the hull which was removed by successively rinsing firstly with water (3 × 200 mL) and further with ethanol (250 mL) (purity 99.8%, Riedel-de Haën, Germany) until the husks and acid were mainly removed. After this pre-treatment, the procedure was similar to the one performed for the other seeds, differing only in the temperature of the extraction process. In this case, after adding the water in a proportion of 1:5 (endosperm: water), the mixture was heated at 80 °C during 2 h and left to rest approximately 22 h at room temperature. Da Silva and Gonçalves (1990) and Smirnova et al. (2004) showed that the increase of temperature in extraction pro-

cedures of galactomannans with high M/G ratio originates higher extraction yields and leads to galactomannan with different intrinsic viscosities.

2.2.2. Procedure B

This method differs from procedure A only in the bath temperature and in its duration. Thus, procedure B consisted in placing the seeds in sulfuric acid (1:1) in an oil bath at 120 °C for 10 min. After this pre-treatment, the procedure was similar to procedure A.

2.3. Polysaccharide purification

The endosperm mixed in the blender was filtered through a nylon net followed by a centrifugation step at 3800g (Sigma 4K, B. Braun, Germany) during 20 min at 20 °C. The precipitation of the galactomannan was achieved by adding the supernatant to ethanol (purity 99.8%, Riedel-de Haën, Germany) at a ratio of 1:2. The ethanol was decanted and the precipitated galactomannan was lyophilized and kept in a dry place until further use.

Fig. 1 shows the flow chart representative of the extraction and purification processes for the galactomannans of the four seeds considered in this work.

2.4. Determination of polysaccharide yield

The yield is one of the most economically important aspects of polysaccharide extraction and purification, and it was determined in three stages of the process ($Y1$, $Y2$ and $Y3$), for an initial mass of 50 g seeds of each species. $Y1$ was calculated dividing the mass of recovered dry endosperm (m_r) by the initial mass of seeds (m_i) thus determine the yield in the stage where the hull and the germ are removed manually, it represents the yield of the pre-treatment process. $Y2$ was calculated dividing the result of the difference between the mass of recovered endosperm (m_r) and the mass obtained from the filtration and centrifugation (after drying in a oven until constant weight at 105 °C) (m_f) by the mass of recovered endosperm (m_r), it represents the yield of the purification process. $Y3$ represents the total yield of the extraction and purification
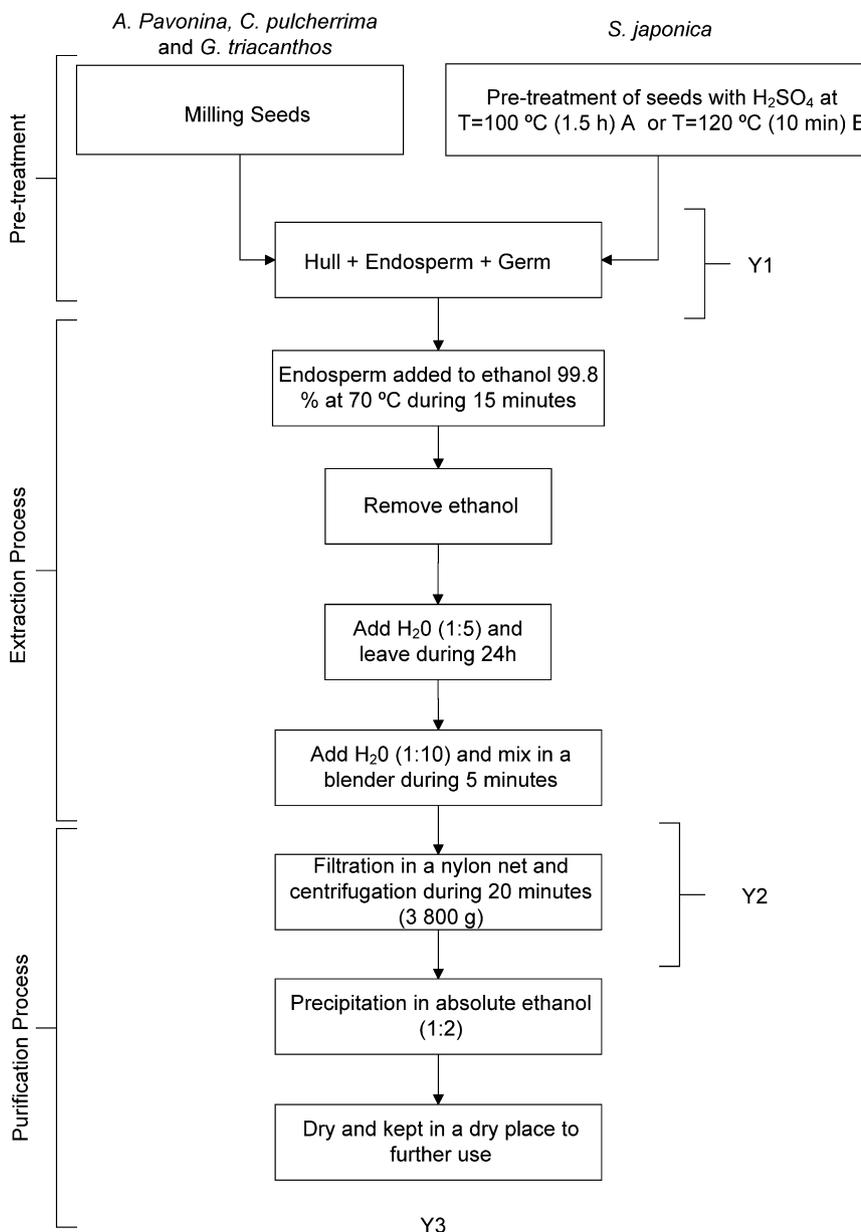

Fig. 1. Flow chart representative of the extraction and purification processes of the galactomannans. $Y1$, $Y2$ and $Y3$ are the points of the procedure where the yield was calculated.

processes and was calculated dividing the mass of lyophilized galactomannan (m_l) by the initial mass of the seeds (m_i).

2.5. Polysaccharide analyses

Polysaccharide analyses were performed as described in Ferreira, Mafra, Soares, Evtuguin, and Coimbra (2006). Neutral sugars (2 mg) were released through an acid treatment using 0.2 mL 11 M H_2SO_4 for 3 h at 20 °C followed by 2.5 h in 1 M H_2SO_4 at 100 °C, reduced with sodium borohydride, acetylated with acetic anhydride using methylimidazole as catalyst, and the alditol acetates formed were analyzed by gas chromatography (Carlo Erba 6000, Carlo Erba, Milan, Italy) with a split injector (split ratio 1:60) and a flame ionization detector. The column was a DB-225 (J & W, USA) with 30 m × 0.25 mm and film thickness of 0.25 μm; the oven temperature program was: 220 °C during 5 min, being then the temperature raised at a rate of 20 °C min^{-1} to 230 °C and maintained at this temperature for further 6 min. The flow rate of the carrier gas (H_2) was set at 1 mL/min at 220 °C. The injector temperature was 220 °C and the flame ionization detector temperature was 230 °C. The hydrolysis of all samples was performed in duplicate and each one was injected twice.

Uronic acids were determined by the 3-phenylphenol colorimetric method as described in Ferreira et al. (2006). Samples were prepared in duplicate by hydrolysis in 0.2 mL 11 M H_2SO_4 for 3 h at 20 °C followed by 1 h in 1 M H_2SO_4 at 100 °C. The uronic acids determined were quantitatively accounted as galacturonic acid.

The purity of the polysaccharides was evaluated both by the total amount of monosaccharides obtained in the monosaccharide composition and by the amount of manose + galactose present per mg of sample.

2.6. Macromolecular characterization

Viscosities of dilute solutions were measured at 25 ± 0.1 °C with a Cannon Fenske capillary viscometer (ASTM-D2515, Series 100), using exactly 10 mL of solution sample.

Solutions were prepared to have relative viscosities, η_{rel}, from about 1.2–2.0, to assure good accuracy and linearity of extrapolation to zero concentration. The intrinsic viscosity, $[\eta]$, was determined from Huggins' (Eq. (1)) and Kramer's (Eq. (2)) equations, where k_H and k_K are the Huggins' and Kramer's coefficients, respectively, η_{sp} is the specific viscosity and C is the solution concentration.

$$\frac{\eta_{sp}}{C} = [\eta] + k'[\eta]^2 C \qquad (1)$$

$$\frac{\ln_{\eta rel}}{C} = [\eta] + k''[\eta]^2 C \qquad (2)$$

Viscosity average molecular masses, M_v, were calculated using the Mark–Houwink relationship given by Doublier and Launay (1981) for guar gum as modified by Gaisford, Harding, Mitchell, and Bradley (1986) to take into account the different values of M/G of the galactomannans.

$$\eta = 11.55 \times 10^{-6}[(1 - \alpha) \times \overline{M_v}]^{0.98} \qquad (3)$$

Where $\alpha = 1/[(M/G) + 1]$ and $[\eta]$ is expressed in dL/g.

3. Results and discussion

3.1. Pre-treatment, extraction, purification and global yield

The extraction yield was measured for the processes of polysaccharide pre-treatment (Y1), extraction and purification (Y2) and for the global process (Y3). Table 1 shows the results of the yields Y1, Y2 and Y3.

Y1 is the yield after pre-treatment, where the hull and germ are removed from the endosperm. This yield is a measure of the ease with which hull and germ can be separated from the endosperm. It is also a measure of the relative amount of endosperm in the seed. The highest value of Y1 (67.13%) was obtained for G. triacanthos, while A. pavonina and C. pulcherrima had similar values (43.73% and 45.27%, respectively). These results show that the pre-treatment was more effective in removing the hull and germ from the seeds of A. pavonina and C.pulcherrima than for the seeds of G. triacanthos.

The yield Y2 was measured after the filtration and centrifugation processes; here most of the hull that is still attached to the endosperm after the extraction process is removed. In this stage, C. pulcherrima seeds showed the highest value of yield (66.19%), while G. triacanthos presented the lowest value (42.40%). This is a direct consequence of the previous pre-treatment step: G. triacanthos endosperm was carrying much more attached material than A. pavonina and C. pulcherrima, therefore such material was now removed in more significant amounts, thus decreasing the yield value obtained for the first species. The opposite has happened with C. pulcherrima.

The best global yield was obtained for G. triacanthos and C. pulcherrima, which have values close to 25% (24.73% and 25.70%, respectively).

In the extraction of S. japonica galactomannan, the acid pre-treatment caused a progressive hydrolysis from external to internal components of the seed. The extraction yield obtained with pre-treatment B (3.33%) was significantly lower than that obtained with pre-treatment A (9.22%). During the pre-treatments it was observed that procedure B caused the carbonization of hull and also

Table 1
Pre-treatment, extraction and purification and global yields

Species	Y1 (%)	Y2 (%)	Y3 (%)
G. triacanthos	67.13 ± 0.64	42.40 ± 3.51	24.73 ± 2.08
A. pavonina	43.73 ± 1.75	44.67 ± 6.54	17.11 ± 4.15
C. pulcherrima	45.27 ± 0.50	66.19 ± 5.89	25.70 ± 3.20
S. japonica (A)	–	–	9.22 ± 0.53
S. japonica (B)	–	–	3.33 ± 0.21

Table 2
Physicochemical composition of the studied polysaccharides in other works

Species	M/G	η (dL/g)	$M_v \times 10^6$ (Da)	Yield (%)	References
G. triacanthos	1.5–2.6	–	–	15.4	Manzi et al. (1984)
	4.6	–	–	18	Mirzaeva et al. (1998)
	1.48–3.12	–	–	11.90–34.16	Sciarini et al. (2008)
	3.2–3.5	–	–	15–20	Leschziner & Cerezo (1970)
A. pavonina	1.8	–	–	–	Tavares (1999)
C. pulcherrima	2.8	13.75	2.1	25	Andrade et al. (1999)
S. japonica	4.8–5.3	10.29–12.11	1.19–1.40	2.5–10.38	Smirnova et al. (2004)
	5.26	–	–	4.3	Kooiman (1971)

damaged the endosperm, decreasing the extraction yield. This clearly shows that the extraction global yield is very much dependent on the pre-treatment step. These yield values are in close agreement with those reported in other works (see Table 2).

The extraction yields obtained for the galactomannan of *S. japonica* are much lower than those obtained for the other galactomannans, and this may be related with the temperature of the extraction process. Although the extraction of the galactomannan from *S. japonica* included 2 h in hot water, the other steps were performed at room temperature. This may explain the lower extraction yields once the high M/G ratio of the galactomannan from *S. japonica* lowers its solubility in cold water (Smirnova et al., 2004).

Finally, the variability of the yield results is a direct consequence of the use of different species (Fernandes, 1995).

The final milled galactomannan of *S. japonica* was a white mucilage containing low remains of the husk and germ fractions. The other galactomannans presented a light yellow colour; in fact, even when performing the precipitation in ethanol, some parts of the germ and pigments from the hull pass to the polysaccharide. This observation has been described by other authors, who reported the passage of pigment and tannins from the hull or from the germ to the endosperm (Avallone, Plessi, Baraldi, & Monzani, 1997; Dakia et al., 2008). The final stage of the global process was the drying step that can determine the color of the end product. In the present work the polysaccharides were lyophilized, thus minimizing browning and moisture absorption during long-term storage. In other works, as those of Dakia et al. (2008) and Sciarini, Maldonado, Ribotta, Pérez, and Léo (2008), the final product was dried in a oven at 100 °C and 35 °C, respectively; the combination of a relatively low water activity and high temperature can enhance the Maillard reaction which provokes browning of the galactomannan, thus changing the galactomannan's chemical properties which in turn may potentially have negative health effects such as those reported to occur in coffee and bread crust, related with acrylamide formation (Frank & Hofmann, 2000).

3.2. Polysaccharide composition

Table 3 shows the results of polysaccharide analyses which confirmed that mannose (Man) and galactose (Gal) are the major monosaccharides present in the polysaccharide material extracted from *G. triacanthos* (66.9% and 23.7%, respectively), *A. pavonina* (52.8% and 39.2%), *C. pulcherrima* (69.1% and 24.0%) and *S. japonica* (81.5% and 14.2%; 81.9% and 14.5%, for gums obtained by procedure A and B, respectively). All the extracted galactomannans contain minor amounts of other monosaccharides such as rhamnose (Rha), fucose (Fuc), arabinose (Ara), xylose (Xyl) and glucose (Glc). In the polysaccharides of *G. triacanthos*, *A. pavonina* and *C. pulcherrima* there are significant values of Ara monosaccharides (3.0–4.5%). This presence was also showed by Navarro, Cerezo, and Stortz (2002) in the galactomannan of *G. triacanthos* and by Nunes, Domingues, and Coim-

bra (2005) in galactomannans from green and roasted coffee. The presence of these minor components could be attributed to a more complex polysaccharide composition, as single Ara side chains such as those occurring in coffee (Nunes et al., 2005), and/or to contaminants proceeding from the seed coat (Da Silva & Gonçalves, 1990; Dakia et al., 2008).

The value of M/G ratio obtained for *G. triacanthos* (M/G = 2.82) is in agreement with the values reported in the literature: 3.2 (Leschziner & Cerezo, 1970) and 1.48–3.12 (Sciarini et al., 2008). For *A. pavonina* the value of M/G reported by Tavares (1999) was high (1.8) when compared with the one obtained in this work (1.35); factors such as the degree of maturation of the seeds, the place of cultivation and differences in the extraction and purification procedures are known to play a determinant role in the M/G ratio and may justify the differences found in diverse literature sources. This means that comparisons such as the one made here are useful but should be made with these restrictions in mind. The value of M/G obtained for *C. pulcherrima* (2.8) is close to the value obtained by Andrade, Azero, Luciano, and Gonçalves (1999), where the extraction process was quite different, with the use of solvents as toluene, acetone and diethyl ether, and with a drying temperature of 35 °C. The M/G values obtained for the galactomannans of *S. japonica* extracted by procedure A and procedure B (M/G = 5.75 and M/G = 5.66, respectively) are statistically equal ($p < .05$) and are in good agreement with those reported by Smirnova et al. (2004) (M/G = 5.30) and by Kooiman (1971) (M/G = 5.28). These values are however very much lower than the M/G = 8 reported for green coffee (Nunes et al., 2005) and up to M/G = 20 and 45 in light and dark roasted coffee infusions (Nunes, Reis, Domingues, & Coimbra, 2006).

Anyway, the galactomannan of *S. japonica* exhibits a high M/G compared with the other galactomannans, thus rendering this galactomannan especially suitable for possible synergistic interactions. In general, galactomannans with higher relative values of Gal monosaccharides are readily soluble in H_2O but have less ability to form gels, while galactomannans with higher relative Man content have the tendency to interact with gelling polysaccharides. The galactomannan from *S. japonica* presents the lower value of Gal and the highest value of Man (see Table 3); this observation allows the establishment of the hypothesis that such galactomannan consists of long blocks of unsubstituted Man units, and is the most interesting galactomannan in terms of the possibility of interaction with other polysaccharides (e.g. covalent interactions and chemical bonds) (Srivastava & Kapoor, 2005).

Gleditsia triacanthos and *C. pulcherrima* have values of M/G very similar to the commercial tara gum (3.0) (Dakia et al., 2008), that is widely used as a thickening agent and stabilizer for food applications.

The results also show (see Table 3) that the extraction and purification process presented here allows purity values between 80.8% and 98.4%, as evaluated from the total monosaccharide content of the sample.

Table 3
Polysaccharide composition of the galactomannans from the four studied species

Species	Monosaccharide composition (% mol)							Total Man + Gal	Total (µg/mg)	M/G
	Man	Gal	Rha	Fuc	Ara	Xyl	Glc			
G. triacanthos	66.9 ± 0.9	23.7 ± 0.8	0.0 ± 0.0	1.3 ± 0.6	4.5 ± 0.5	1.4 ± 0.2	1.4 ± 0.2	741.6	808	2.82 ± 0.05
A. pavonina	52.8 ± 0.4	39.2 ± 1.1	1.0 ± 0.3	0.9 ± 0.2	4.2 ± 1.0	1.2 ± 0.2	0.8 ± 0.1	754.7	811	1.35 ± 0.03
C. pulcherrima	69.1 ± 1.5	24.0 ± 0.1	0.8 ± 0.0	0.8 ± 0.1	3.0 ± 1.4	1.2 ± 0.0	1.2 ± 0.2	841.7	897	2.88 ± 0.07
S. japonica (A)	81.5 ± 0.1	14.2 ± 0.4	0.9 ± 0.0	0.7 ± 0.2	0.7 ± 0.4	1.2 ± 0.1	0.9 ± 0.0	946.5	984	5.75 ± 0.13
S. japonica (B)	81.9 ± 0.8	14.5 ± 0.1	0.5 ± 0.0	0.8 ± 0.3	0.8 ± 0.5	0.5 ± 0.2	1.1 ± 0.1	882.6	913	5.66 ± 0.08

Table 4
Physical–chemical parameters of galactomannans extracted from the species considered in the present work (M_v – viscosity average molecular mass)

Species	Intrinsic viscosity Huggins̕ extrapolation (dL/g)	Intrinsic viscosity Kraemer̕s extrapolation (dL/g)	Intrinsic viscosity (average values) (dL/g)	Huggins̕ coefficient, k_H	M_v ($\times10^{-6}$)
G. triacanthos	10.06	10.78	10.42	1.13	1.62
A. pavonina	8.85	9.37	9.11	1.10	1.81
C. pulcherrima	10.91	11.77	11.34	1.27	1.75
S. japonica (A)	9.58	10.43	10.01	1.33	1.34
S. japonica (B)	8.93	8.55	8.74	1.25	1.17

3.3. Macromolecular characterization

The determination of intrinsic viscosity provides a measurement of the hydrodynamic volume occupied by the isolated polymer chains in a given solvent and depends primarily on the molecular structure and molecular weight of the polysaccharides as well as on the solvent quality.

The values for k_H depend on solute-solvent interactions and on the state of aggregation of the macromolecules; in a good solvent and for flexible macromolecules, $k_H = 0.3$; however it can be higher than one in case of aggregation (Sittikijyothin, Torres, & Gonçalves 2005).

Table 4 shows the values of physical–chemical composition of the studied galactomannans; k_H values of 1.10–1.33 probably reflect some intermolecular aggregation in the samples.

In a previous work, Andrade et al. (1999) showed that the galactomannan of C. pulcherrima has an intrinsic viscosity of 13.75 dL/g and a viscosity average molecular mass of 2.10×10^6 Da (Andrade et al., 1999); however, lower values were found in this work. These differences can be mainly explained by the extraction and purification processes, which are known to influence the intrinsic viscosity and, therefore, the viscosity average molecular mass. This is notorious, e.g., in the results shown for S. japonica extracted by pretreatments A and B; in this case, the processing at a higher temperature has eventually lead to a more extensive degradation of the polymer chains, which has in turn lowered the intrinsic viscosity and, together with it, the viscosity average molecular mass.

The galactomannans of G. triacanthos and C. pulcherrima have similar M/G ratios (2.82 and 2.88, respectively) but exhibit different intrinsic viscosities (10.42 and 11.34, respectively). For a certain M/G ratio, the galactomannans can differ in the distribution of galactose units along the mannan backbone. This distribution, though not fully understood yet, is believed to be important for the functional properties of these polysaccharides (Dakia et al. 2008).

4. Conclusions

Galactomannans are used by the industry in commercial form as, e.g., Locust Bean Gum, Guar Gum and Tara Gum, and new sources are important as an alternative to these traditional galactomannan sources. In this work galactomannans were extracted and purified from four species of seeds of plants from the family Leguminosae through an improved extraction and purification procedure which uses only ethanol and water. It is simpler and easier to perform than most of the published procedures and, as it avoids the use of non-food grade solvents it is food grade itself and environmentally friendlier.

This procedure allows a galactomannan yield of 24.73% – 25.70 starting from the seeds of G. triacanthos and C. pulcherrima, 17.11% for A. pavonina and 3.33–9.22% for S. japonica. The polysaccharide composition features a high content of Man and Gal, being the M/G ratio between 1.35 and 5.75 (A. pavonina < G. triacanthos < C. pulcherrima < S. japonica (B) < S. japonica (A)). G. triacanthos and C. pulcherrima present values of the M/G ratio very close to the com-

mercial Tara Gum, and S. japonica, with a high Man monosaccharide content (leading to M/G over 5) can be interesting for possible synergistic interactions with other polysaccharides.

The fact that this extraction and purification methodology has been applied with success to four galactomannans with very different M/G ratios gives a clear indication that it may be used with other galactomannans as well. The results have also shown that the extracted galactomannans show adequate characteristics to be used in the food industry.

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