Preparation and evaluation of hemicellulose films and their blends

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ABSTRACT

Cell wall hemicelluloses are useful in various industries due to their ability to form edible and biodegradable films with plasticizers such as glycerol. The objective of this research was to extract hemicelluloses from *Caesalpinia pulcherrima* and *Tamarindus indica*, produce film blends from them, and evaluate their properties and their product development potential. The extraction process provided yields of 25% galactomannan (GalCp) from *C. pulcherrima* and 20% xyloglucan (XilTi) from *T. indica*. The apparent M_w obtained by GPC were 4.35×10^6 g/mol and 2.03×10^6 g/mol for GalCp and XilTi respectively. The ratio of mannose/galactose in GalCp determined by ^{13}C NMR was (2 : 1), while the ratio of glucose/xylose/galactose in XilTi obtained by ^{13}C NMR was (1.0: 1.2: 1.0). All filmogenic solutions from these hemicelluloses showed non-Newtonian rheological behavior at 25 °C. The IR spectra were consistent with the presence of GalCp and XilTi and were fully assigned. Galactomannan film exhibited a higher permeability to moisture than xyloglucan and the blends. All films showed less than 20% opacity; thus, they were relatively transparent. Thermogravimetric analysis indicated that the blend films had good thermal stability. The use of these blends permits a wider range of properties to be obtained than the hemicelluloses alone. Thus, these materials may serve as useful biodegradable and environmentally friendly materials for food packaging applications.

Keywords:

Cell wall polysaccharide

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Polymer blends

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Characterization

1. Introduction

Hemicelluloses are among the most abundant biopolymers found in nature. Different agri-based materials often contain about 20-30% hemicelluloses. Because they are edible, biodegradable, and environmentally friendly, there has been increasing interest in using hemicelluloses for a variety of applications (Hansen & Plackett, 2008; Christopher, 2012). Two of the biopolymers in the hemicellulose family are galactomannans and xyloglucans. Galactomannans are widespread in nature; their basic structure comprises a central core of β -(1 $\rightarrow$ 4)-linked D-mannopyranose (Manp) to which α -(1 $\rightarrow$ 6)-linked D-galactopyranosyl (Galp) units are attached (Fig. 1a). These polysaccharides are commonly extracted from the endosperm of numerous seed plants, particularly the *Leguminosae* family where they are involved with energy-reserve and hydration functions. Because of their specific physicochemical properties such as high molecular weight, water solubility, non-ionic character, and absence of toxicity (Gidley & Reid, 2006; Pollard et al, 2010; Albuquerque et al, 2014), they can be used in food, pharmaceutical, biomedical, cosmetic, textile and paper industries, especially as emulsion stabilizer, viscosifier, film, gelling agent, soluble dietary fiber, and hydraulic fracturing material (Albuquerque et al, 2014; Srivastava & Kapoor, 2005; Vendruscolo, et al., 2005; Cerqueira et al., 2009a, 2011; Mikkonen et al., 2007; Pinheiro et al., 2011).

Xyloglucans can be extracted from leguminous seeds, where they serve a storage function. The xyloglucan macromolecular structure contains a β -(1 $\rightarrow$ 4)-D-glucopyranose-linked backbone partly substituted by α -(1 $\rightarrow$ 6)-D-xylopyranosyl side chains, which in certain cases are further substituted by a β -(1 $\rightarrow$ 2)-D-galactopyranosyl residue (Fig. 1b) (Reid, 1985; Souza et al, 2014). Most of these polysaccharides form films that are brittle and tend to crack during handling and storage. For this reason, small non-volatile low-molecular-weight compounds are typically added as plasticizers to reduce brittleness and increase flexibility. However, the addition of most of the plasticizers may cause significant changes in the barrier properties of the films (Sothornvit & Krochta, 2005; Skurtys, et al., 2010; Antoniou, et al., 2014). Commercial applications most commonly use xyloglucan extracted from *Tamarindus indica* seeds, although other plant sources have been proposed in the literature. Important commercial applications of xyloglucan in the food, pharmaceutical and medical areas have been demonstrated (Antoniou, et al., 2014; Itoh et al., 2008; Souza, et al., 2014).

Commonly used plasticizers in polysaccharide-based films are polyols(e.g., glycerol,sorbitol and polyethylene glycol) because of their resemblance in structure to the polysaccharides and their hygroscopic nature that allow them to attract and retain water in the system. The efficiency of each plasticizer is related to its molecular size, shape,number of oxygen atoms, spacing of oxygen atoms and waterbinding ability (Antoniou, et al., 2014; Haq, Hasnain & Azam, 2014; Mali, Sakanaka & Grossmann, 2005; Mikkonen et al., 2007; Sothornvit & Krochta, 2001).

Polymer blending is known to be an excellent method to vary polymer properties and to generate new products (Paul & Bucknall, 2000). Because polyblends do not involve chemical reactions, they can more easily overcome regulatory/safety hurdles and represent a faster path towards commercial development. Reports of blends of two pure hemicelluloses are relatively rare in the literature. In previous studies hemicelluloses have been used to produce films and coatings. A study by Qi et al. (2015) showed that the increase in the ratio of quaternized hemicelluloses (QH)/carboxymethyl cellulose (CMC) made the blends structurally dense, resulting in a significant increase in tensile strength and decreased water vapor permeability (WVP). However, when excess QH was added, there was a decrease in the tensile strength of the films and increased WVP. The transparencies decreased with the QH/CMC ratio. Ruiz et al (2013) reported that the incorporation of hemicelluloses extracted from wheat straw under certain proportions in blends of κ -carrageenan/locust bean gum with glycerol as plasticizer slightly increased the water vapor barrier properties, opacity, and thermal properties of the blend.

The objective of this study was to develop a practical process to extract hemicelluloses from *C. pulcherrima* and *T. indica*, to evaluate them and their film blends for their physicochemical properties, and to assess their product development potential. *Caesalpinia pulcherrima* has good productivity and begins to produce seeds six months after planting. The seeds of *Tamarindus indica* are residues of the food industry from fruit pulp production in Brazil. Glycerol is a co-product from the biofuel industry. Thus, all the materials being utilized in this study are from renewable sources. The hemicellulose blends exhibit properties that qualify them for food packaging applications, and this may be an alternative use of these agricultural residues.

2. Materials and methods

The seeds of *Caesalpinia pulcherrima* were collected at the University of Fortaleza campus on March 2015. The *Tamarindus indica* seeds were obtained from Federal University of Ceará Campus from January to July 2015. Voucher specimens of the seeds of *C. pulcherrima* and *T. indica* had been deposited in the Herbarium Prisco Bezerra – EAC (UFC) under the numbers 563667 and 56965, respectively. The seeds were isolated from the pods, cleaned and stored in plastic containers at room temperature.

2.1 Hemicellulose Extraction

The extraction of galactomannan was adapted from the procedures described by Cerqueira et al. (2009b). Seeds were removed from the pods, cleaned and placed in a blender, and mechanically broken. In each seed, the endosperm was manually separated from the germ and the hull. The endosperm obtained was suspended in 96 % ethanol (w/w) in a proportion of 1:3 (seeds:ethanol) at 70 °C for 20 min to inactivate the enzymes and eliminate the low-molecular-weight compounds. Ethanol was removed and distilled water was added in a 1:10 (endosperm:water) proportion and the suspension was left overnight. The next day, the amount of water was refilled to an endosperm:water ratio of 1:10 and mixed in a blender for 5 min. Next, the viscous solution was filtered through a nylon net and precipitated by adding 96% ethanol (w/w) at a ratio of 1:2. The precipitate was washed with acetone, dried with hot air, and milled.

For xyloglucan extraction, the *T. indica* seeds were boiled in distilled water for 50 min, left in distilled water at 8°C for 12 h, and milled in distilled water at a ratio of 1:40 (w/v). The viscous extract obtained was filtered through nylon mesh and centrifuged at 10,000x g and 4 °C for 20 min. To the supernatant was added two volumes of 96% ethanol, the precipitate was resuspended in water (1:10 w/v), and the precipitation process repeated. The alcohol excess was discarded and the precipitate was immersed in acetone at a ratio of 1:5 (w/v) for 20 min, followed by drying under cold air flow (Freitas et al., 2003).

2.2. Hemicellulose Characterization

Nuclear magnetic resonance (NMR) spectra were recorded on a DRX 400 spectrometer from Bruker Corporation (Karlsruhe, Germany). The ¹³C NMR spectra were acquired at a repetition rate of 4 s with 90° pulses. The samples were placed

inD₂O in 5 mm tubes at a concentration of 7 mg/700 μL. Chemical shifts were referenced to tetramethylsilane at 0 ppm.

The weight-average molecular weights (M_w) of the galactomannan and xyloglucan samples were determined by gel permeation chromatography (GPC) with a Shimadzu instrument (Model LC-20AD, Kyoto, Japan) at room temperature using a PolySep linear column (7.8 x 300 mm, Waters, Milford, MA, USA), flow rate of 1.0 mL/min, polysaccharide concentration of 1 mg/mL, and 0.1 M NaNO₃ in water as the eluent. A Waters Model RID-10A (Milford, MA, USA) was used as the refractive index detector. The elution volume was corrected using ethylene glycol as an internal marker at 11.25 mL. The GPC was calibrated with pullulan samples (Shodex, Showa Denko, Kawasaki, Japan) as standards.

The intrinsic viscosity was performed at 25 ± 0.1 °C in an Ostwald Fenske number 200 capillary viscometer. A stock solution was first prepared, from which six dilutions were made covering a range of η_r (from 1.2 to 2.0), where η_r is the relative viscosity. The plots η_{sp}/C and $\ln \eta_r/C$ vs. C were used to estimate the intrinsic viscosity $[\eta]$ according to Huggins equation (Equation 1) and Kramer equation (Equation 2).

$$\eta_{sp}/C = [\eta] + k_H [\eta]^2 C \quad (1)$$

$$(\ln \eta_r)/C = [\eta] + k_K [\eta]^2 C \quad (2)$$

For rheology, the effect of shear rate on the viscosity of the galactomannan and xyloglucan aqueous solutions at concentrations of 0.2% and 1% (w/v) was evaluated using a rheometer from TA Instruments (Model AR-550, New Castle, DE, USA). Tests were performed at 25 °C.

The total nitrogen of galactomannan and xyloglucan was determined after digestion of the samples with sulfuric acid, according to the method of Baethgen & Alley (1989). Calculation was done relative to a standard curve obtained with ammonium sulfate, and the protein content was derived by multiplying the total nitrogen value by 6.25.

The ash content of galactomannan and xyloglucan was obtained using moisture-free porcelain crucibles. Each crucible containing 2 g sample was heated in a muffle furnace (Model SP-1200DRP7, Thermo Scientific, MA, USA), at 50 °C per 15 min up to 550 °C and remained for 6 h until complete incineration of all organic material. The crucible with sample was then transferred to a desiccator containing silica gel and allowed to cool to room temperature. The crucible containing the incinerated sample was

then weighed and the ash content calculated as the percentage of the weight of the residue versus the initial weight of the sample (AOAC, 2000).

2.3 Preparation of Hemicellulose Films

The polysaccharides were dissolved in distilled water by using a TE-102 TURRATEC homogenizer (Marca Tecnal, Brazil) operating at 20,500 rpm for 15 min. For the formulation of films and blends the experimental design shown in Table 1 was followed. The final concentration of all the solutions was 1% w/v. Glycerol (0.5% w/v) was added as a plasticizer to each solution, and the mixture was stirred again for 5 min (Godbillot et al., 2006). The filmogenic solutions were then centrifuged (10,000 g, 20 min, 4 °C), subjected to vacuum deaeration to remove bubbles, and then distributed in Petri plates (Model PB-PP-90-15, 90 mm in diameter and 15 mm in height) with approximately 30 g in each plate. The samples were dried at 40 °C with air circulation for a period of approximately 24 h. After the formation of the films, they were stored at 25 °C with 50% relative humidity in a desiccator until use.

2.4. Evaluation of Hemicellulose Films

The galactomannan and xyloglucan film samples were ground to a fine powder and analysed as KBr pellets via Fourier transform infrared (FTIR) spectroscopy. The FTIR spectra of the samples were recorded with a spectrophotometer from ABB Bomem (Model FTLA 2000, Cary, NC, USA) in the range of 400 - 4000 cm^{-1} .

Thermogravimetric analysis (TGA) was conducted on the GalCp and XilTi film samples using an analyzer from TA Instruments (Model Q50, New Castle, DE, USA). Approximately 10 mg of each sample was placed in a platinum pan and heated over a temperature range of 25 - 900 °C at 10 °C/min under an oxidative atmosphere.

Water vapor permeability (WVP) was determined gravimetrically at 25 °C, based on the ASTM E96-00 method (ASTM, 2000). The films were cut (discs with a diameter of 50 mm), placed at the top of a permeation cell containing distilled water, and then the cells were placed in a desiccator containing dried silica at 24 °C and 47% RH. The cells were weighed eight times over a 24-h period in intervals of at least 1 h. The calculations were carried out according to the ASTM method.

The opacity of the films was determined with a Minolta colorimeter (Model Cr 410, Minolta, Japan). Opacity of a material is an indication of how much light that passes through it: the higher the opacity, the lower the amount of light that

passthrough the material. Generally, the opacity is calculated from reflectance measurements. The opacity of the samples was determined according to the Hunter lab method, as the relationship between the opacity of each sample on a black standard (Y_b) and the opacity of each sample on a white standard (Y_w) (Eq. (3)). The measurements were repeated three times for each film.

$$\text{Opacity} = Y_b/Y_w \cdot 100 \quad (3)$$

The film thickness was measured with a manual digital micrometer (No. 293–140, Mitutoyo, Japan) with a sensitivity of 0.001 mm. For each sample, eight measurements of thickness were made, and the mean and the standard deviation were reported.

All data were expressed as mean $\pm$ standard deviation ($n \geq 3$). Differences among mean values were tested for statistical significance at the $p < 0.05$ level using analysis of variance (STATISTICA 6.0, StatSoft Inc., Tulsa, USA) and the Tukey test.

3. Results and discussion

3.1. Hemicellulose Extraction

The extraction of galactomannan *C. pulcherrima* showed a yield of 25% (w/w) relative to seed weight, while a xyloglucan yield of 20% (w/w) was obtained from *T. indic* seeds (Table 2). The results obtained for *C. pulcherrima* galactomannan were similar to those reported by Cerqueira et al. (Cerqueira, et al., 2009b), but for xyloglucan the yield obtained was lower than that reported by Deters et al. (Deters, Schröder, Smiatek, & Hensel, 2005). Previously many plants have been studied and shown to be potential sources of galactomannan as given in a recent review (Mirhosseini & Amid, 2012), where a GalCp yield of 19–43% was reported for guar gum, 33% for *Cassia brewsteri*, and 24.9% for mesquite. Among the 88 species of legumes studied, only 11 species showed gum yields higher than 20%, 17 exhibited yields between 15 and 20%, and 31 had yields lower than 10% (Crisostomo, 2008).

The seed xyloglucan is mainly stored in the cotyledons, while the galactomannan is stored in cell walls of endosperm cells of the seed, wherein the amount of protein is significantly different. According to Srivastava & Kapoor (2005), the seed coat contains several impurities with high contents of protein and fiber. The presence of protein is not desirable and needs to be minimized during the process of extraction of the seed gums.

The protein yield (1%) found for the galactomannan from *C. pulcherrima* was below the values reported in the literature for galactomannan obtained from seeds of *Cassia grandis* and *Cassia spectabilis*. Values between 4% and 6% of proteins were

found for the galactomannan extracted from *Mimosa scabrella*. Although there are other unconventional sources of galactomannans, such as *Sophora japonica* and honey locust, where protein levels are very low (less than 1%), the protein content of galactomannan meets the range specified by the US Pharmacopeia, which allows a limit up to 10% (Joshi & Kapoor, 2003; Vendruscolo et al., 2009; Kapoor et al., 1998; Pinheiro et al., 2011). In addition, there are sources of galactomannans where protein content can range from 8.3% to 16.8% (Cunha et al., 2009).

The ash contents in the two hemicelluloses (0.38% for GalCp, 1.87% for XilTi) studied in this work were lower than those found in the aforementioned sources, which varied widely depending on the source. For example, in locust bean gum, the concentration varied between 1% and 4% (Panegassi, et al., 2000). Generally, commercial gums and even some galactomannans from unconventional sources contain lower than 1% ash (Bourbon et al, 2010; Pinheiro, et al. 2011). The ash content of *T. indicaxyloglucan* reported in this work was slightly lower than the 2.1% reported by Alves (2013).

3.2. Gel Permeation Chromatography (GPC)

The GPC chromatograms of the galactomannan from *C. pulcherrima* and xyloglucan from *T. indica* are shown in Fig. 2. The M_w for GalCp was found to be 4.35×10^6 g/mol and M_n 3.8×10^5 g/mol. These values bracketed the range of molecular weights reported by Pollard et al. (2010), who showed a range of molecular weights from 0.9 to 3.1×10^6 g/mol for locust bean gum galactomannan. The size of chain is associated with mannosyl transferase enzyme which participates in the regulation of the size or chain termination step during the biosynthesis of the less substituted galactomannans. Since GalCp has a different mannose/galactose ratio from locust bean gum, the slightly different molecular weight distribution is not unexpected.

For *T. indica* xyloglycan M_w was found to be 2.03×10^6 g/mol and M_n 1.63×10^5 g/mol. These values also bracketed the molecular weights found by Sierakowski (2007) and Sousa (2014), which were 1.1×10^6 g/mol and 1.2×10^6 g/mol, respectively. In view of the broad molecular weight distribution of the xyloglucan, these molecular weights were probably very similar values. The Mark-Houwink equation $\eta = 11.55 \times 10^{-6} [(1 - \alpha) \times M_v]^{0.98}$, where $\alpha = 1 / [(M / G) + 1]$ and $[\eta]$ is expressed in dL/g (Cerqueira et al., 2009b). The viscosity-average molecular weight (M_v) obtained in this study was 1.09×10^6 , lower than the value of 1.75×10^6 reported earlier (Cerqueira et al., 2009b). It

was also lower than the value found by GPC: $M_w = 4.35 \times 10^6$. This variance can be explained by the difference in M_w versus M_v and the higher sensitivity of the RI detector used in GPC.

3.3. Viscometry

The viscometric methods are based on a polymer solution whose viscosity increases with increasing concentration, and the data provide information regarding the size of polymer in solution and polymer-solvent interactions (Kwaambwa et al., 2007). The intrinsic viscosity characterizes the hydrodynamic properties of a macromolecule (Azero & Andrade, 2002). The Huggins' constant (K_H) reflects solute-solvent interactions and the state of aggregation of the polymer molecules; in suitable solvents or flexible macromolecules, $0.3 < K_H < 0.7$. However, it is possible to obtain values greater than 1.0 in the case of molecular aggregation (Morris et al., 1981).

The mean values obtained for intrinsic viscosity, Huggins' coefficient and Kramer's constant are shown in the lower part of Table 2. In this study, the high values obtained for the Huggins' constant reflect intermolecular aggregation in the samples. This can be attributed to the existence of non-specific physical entanglements in polysaccharide solutions. The associations in galactomannans are known to occur in polymer segments containing at least six mannose consecutive units not substituted by galactose (Dea et al., 1986).

The intrinsic viscosity values $[\eta]$ of the galactomannans of *C. pulcherrima* and the xyloglucan from *T. indica* were 8.33 dL/g and 2.39 dL/g, respectively. Arcanjo (2011) reported $[\eta]$ values of 10.21 dL/g for *C. pulcherrima* galactomannan and 9.28 dL/g for guar gum. In comparison, Sousa (2014) reported $[\eta]$ values of 9.64 dL/g and 3.51 dL/g for *C. pulcherrima* galactomannan and *T. indica* xyloglucan, respectively. These results may be related to the stage of maturation of the seeds, as observed by Silva (2005), who noted the increase in intrinsic viscosity during seed growth. The lower value for the intrinsic viscosity of xyloglucan may also be related to the higher amount of substituted glucose which reduces the interactions between the D-glucopyranose chains.

The critical concentration (C^*) corresponds to the limit of dilute polymer solution, where the steric and frictional interactions of neighboring polymer coils are negligible. The parameter $C^*[\eta]$ reflects the total volume occupied by polymer chains (Silva, 2014). It was 0.12 and 0.41 at 25 °C for galactomannan and xyloglucan, respectively.

3.4. ^{13}C NMR Spectroscopy

The ^{13}C NMR spectrum of the *T. indica* xyloglucan is given in Fig.3a. The signals at about 105, 102 and 99 ppm corresponded to the anomeric carbons of β -D-galactopyranose, β -D-glucopyranose, and α -D-xylopyranose, respectively (York et al, 1996, Lima et al, 1995, Busato, Vargas-Rechia & Reicher, 2001, Sumathi & Ray 2002). From the integrations of these signals, the approximate ratios of monosaccharides Glu:Xyl:Gal were determined to be 1.0 : 1.2 : 1.0. At the 67-61 ppm region, the signals at ca. 66.4 and 66.8 ppm could be assigned to the C-6 unit of substituted β -D-glucopyranoses, and 60.3 ppm for C-6 units of free β -D-galactopyranoses. From the literature data, the signal at 61.6 ppm could be assigned to C-5 of α -D-xylopyranose (York et al., 1996; Lima et al, 1995; Busato, Vargas-Rechia & Reicher 2001).

Fig.3b shows the ^{13}C NMR spectrum of *C. pulcherrima* galactomannan. The peaks at 99 ppm and 100 ppm corresponded to galactose C-1 and mannose C-1, respectively (Buriti et al., 2014). The mannose/galactose (M/G) ratio was obtained by integrating the signals at 99 ppm and 100 ppm. The *C. pulcherrima* galactomannan found in this work had a M/G ratio of 2:1, which was similar to that of guar gum (Painter et al., 1979), but lower than the M/G ratios of 2.83:1, 3.6:1, and 3.65:1 reported for other galactomannans (Cerqueira et al., 2009b; Braga et al., 2011; Buriti et al., 2014).

3.5. Rheological Measurements

The viscosity of 1% GalCp solution decreased with increasing shear rate. Partial replacement of GalCp with XilTi decreased the viscosity of the solution, since XilTi had a lower viscosity than GalCp at equal concentration (Fig.4a). The high viscosity of *C. pulcherrima* galactomannan in this study is related to its chemical structure, as the mannose/galactose ratio influences its intrinsic viscosity as well as its higher molecular weight. It is likely that intra- and inter-molecular hydrogen bonding may also cause some aggregation, which may further enhance viscosity (Pollard et al 2010). In contrast, the xyloglucan has a lower molecular weight. It also has larger substituent groups (α -D-xylose and β -D-galactose substituted α -D-xylose), which hinder the formation of intermolecular hydrogen bonds and aggregation. Another possible factor is that the amount of intramolecular hydrogen bonds reduces the hydrodynamic radius that is related to the intrinsic viscosity of this polymer (Freitas et al 2005). In any case, a set of shear-thinning curves were observed for the solutions of hemicellulose blends.

The rheological behavior of the fluids can be divided into Newtonian and non-Newtonian, depending on the relationship between shear stress and the applied shear rate (Rao, 1999; Steffe, 1996). The shear stress is usually expressed as a function of shear rate in the flow curves as shown in Fig.4B. All samples that show a nonlinear relationship between the shear stress and the strain rate at 25 °C are characterized as non-Newtonian fluid. Dea & Morrison (1975) noticed that a guar gum solution at 1% w/v showed pseudoplastic behavior, but when the concentration was less than 0.3% pseudoplasticity decreased and approached the Newtonian behavior. The decrease in apparent viscosity with increasing shear rate could be attributed to structural change of the polymer due to the hydrodynamic forces generated and enhanced alignment of molecules in the direction of the applied force (Ferreira, Guimaraes & Maia, 2008).

The pseudoplastic behavior was clearly observed for all solutions in Figure 4B. The degree of shear thinning also decreased from F0 to F6. The pseudoplastic behavior was frequently reported in formulations containing natural and synthetic gums or polymers (Yasar, Togrul & Arslan, 2007). At low shear rates, the polymer chains that make up the solution are disoriented, interlaced or unfolded while maintaining an irregular internal order to generate a higher viscosity (Toneli et al., 2005). At higher shear rates, chain entanglements, electrostatic and hydrophobic interactions decrease, thereby resulting in a drop in the apparent viscosity of the samples (Horne, 1998).

3.6. Fourier transform infrared spectroscopy

Infrared spectra of GalCp and XilTi films were obtained and given in the Supplemental Information Fig. S.1. Stretching vibrations of primary and secondary alcohols C-O bond were found at 1260-1350 cm^{-1} (Coates, 2000). It was suggested that this stretching vibration can be related to the interactions between the two polymers and with glycerol that was used as plasticizer; it is known that glycerol changes the thermal properties of blends (Pinheiro et al 2010). In general, the plasticizers decrease the intermolecular forces between the polymer chains, allowing a greater absorption of water molecules (Carneiro-da-Cunha et al., 2009). All the spectra contained a 1600 – 1700 cm^{-1} band due to the residual water present in the samples. This band was often found in polysaccharide films (e.g., Wu et al, 2012; Kurt & Kahyaoglu, 2014). In addition, the GalCp spectra showed an intense absorption band in the region 3000-3500 cm^{-1} , which could be assigned to the O-H stretching vibration. A band between 2926 and 2899 cm^{-1} could be attributed to the symmetric and asymmetric C-H vibration

(Tavares et al, 2012; Yuen et al., 2009; Cerqueira et al., 2011). An absorption in the region 1350-1450 cm^{-1} could be assigned to symmetric stretching of the CH_2 and C-OH groups (Wang & Somasundaran, 2006). The band at 1160 cm^{-1} associated with the vibrational angle δ module tension (C-O) was assigned to the stretching vibration in the pyranose ring (Figueiró et al., 2004).

Moreover, the bands at 1027 cm^{-1} could be attributed to the CH_2 torsional vibrational band and 1076 cm^{-1} to primary alcohol $-\text{CH}_2\text{-OH}$ stretching (Wang & Somasundaran, 2006). The bands at 815 and 872 cm^{-1} referred to the stretching in the anomeric conformations α -D-galactopyranose and β -D-mannopyranose, respectively (Cerqueira et al., 2011, Figueiró, et al., 2004). A band in the region 890-920 cm^{-1} could be assigned to the C1-OH deflection and C-OH axial strain of C-4, respectively (Wang & Somasundaran, 2006).

In addition, the FTIR spectra for xyloglucan (Fig. S.1) showed bands at 820 cm^{-1} and 870 cm^{-1} , ascribed to the stretching of α -D-xylose linkages and β -D-galactose in xyloglucan. The wavenumbers between 800 and 1200 cm^{-1} constituted the "fingerprint" region for xyloglucan (Cui et al., 2007).

In blends of galactomannan and xyloglucan the presence of the same FTIR bands could also be seen; however, there was a decrease in the intensity of O-H bands and the band corresponding to C-H symmetric and asymmetric vibrations. According to Jamroz et al. (2007), the band at 981-1132 cm^{-1} was the result of vibrational stretching (C-O) in alcohol (C-O-H). These vibrations were reported by Cerqueira et al. (2011) and Antoniou et al. (2014) to be from galactomannan and showed little change with the addition of a plasticizer, particularly glycerol. The same bands were reported by Cerqueira et al. (2012) for galactomannan films with increasing glycerol concentration. Haq et al. (2014) showed that with the addition of PEG 400 these absorption bands increased significantly in intensity, indicating greater interaction between the hydroxyl groups of the polymer with the plasticizer; at high concentrations they could induce conformational changes in the polymer.

3.7. Thermal Analysis

Thermogravimetric analysis (TGA) was used to characterize thermal decomposition and evaluate thermal stability of films in air with plasticizers present. A summary of the TGA data is shown in Table 3, and the actual TGA curves are given in the Supplementary Information (Fig. S.2). The data indicated that the changes in weight

of samples without plasticizer occurred near 110 °C with weight loss of 11.8% and 12.1% for GalCp and XilTi, respectively. This might be associated with water loss (Cerqueira et al, 2012, Leceta et al, 2013). Another weight loss event was observed at ca. 300 °C(68.4% and 67.6%), probably associated with the disintegration of the macromolecular chains of galactomannan and xyloglucan, respectively, followed by degradation of the hexoses(Cerqueira et al, 2011,2012; Giancone et al, 2011; Martins et al., 2012; Mu et al., 2012; Liyanage et al., 2015).

According to Cunha et al. (2007), the presence of impurities lowered the degradation temperature, thereby influencing the thermal stability of galactomannan. By removing the impurities in samples with a higher mannose/galactose ratio, there was an increase in thermal stability. Studies by Cerqueira et al. (2011) confirmed this fact and also noted that the galactomannan with a higher amount of mannose had higher thermal stability. As reported by Dressler et al. (2003), a galactomannan with less galactose gave higher crystallinity, resulting in stronger interactions between polymeric structures and degradation at higher temperatures, compared to a galactomannan with a larger number of galactose side branches. Furthermore, the change in molecular weight due to purification could lead to variations in thermal stability (Cerqueira et al., 2011).

According to Table 3 and Fig. S.2C, the F0 sample incurred a weight loss of 9.9% around 110 °C and a loss of 29.0% at about 200 °C, which was related to the evaporation of glycerol (Cerqueira et al., 2012; Martins et al., 2012; Mu et al., 2012). The loss of 44.8% at 340 °C was associated with the disintegration of the polymeric chains, and the loss of 15.9% at 500 °C with the formation of degradation products. (When the analysis was performed in synthetic air atmosphere, only inorganic solid was observed at these temperatures). In F1 blend (Fig. S.2D), there was a loss of 27.1% near 100 °C and a loss of 56.4% at 260 °C, followed by a weight decrease of 16.1% at 480 °C. The F2 formulation (Fig. S.2E) showed a loss of 20.8% at 100 °C, another weight decrease of 27.0% at about 200 °C related to the glycerol evaporation (Schlemmer & Sales, 2010), and weight loss of 35.8% and 15.6% at 300 °C and 500 °C respectively, associated with the degradation of galactomannan and xyloglucan. The F3 formulation (Fig. S.2F) showed a weight loss of 10.6% near 100 °C, 28.5% loss at 200 °C, and polysaccharide degradation at 300 °C and 550 °C, with weight losses of 41.1% and 18.4% respectively, and no more observed weight loss beyond 550 °C, where only inorganic matter was present.

The data for sample F4 (Fig.S.2G) and sample F5 (Fig.S.2H) showed a decrease in mass of 19.3% and 13.1%, close to 100 °C relating to the loss of water, weight losses of 29.9% and 29.5% at 200 °C due to the loss of glycerol according to Martins et al. (2012), and weight losses of 33.6% and 16.3% at 300 °C, and 19.6% and 36.4% at 550°C, respectively. For sample F6 (Fig.S.2I), it was possible to observe four events with weight losses of 17.5, 29.2, 32.8 and 18.9% at temperatures 100, 200, 300 and 550 °C respectively. As in the other samples, the higher temperature events indicated the gradual degradation of carbohydrates (Gomez et al., 2007; Fernandez et al., 2012). For comparison, the intensity of weight loss in biomass at 500 °C was related to the degradation of more complex aromatic structures with higher molecular weights, such as lignin and cellulose residues (El Ouaquoudi et al., 2014; Teh et al., 2014). Complexation and condensation of these lignocellulose structures at high temperatures could lead to high degradation temperatures (El Ouaquoudi et al., 2015).

If the TGA curves of F0 and GalCp were compared (the former with plasticizer and the latter without), one difference was the evaporation of the plasticizer at about 200°C; another difference was the degradation of the polysaccharide which was slightly elevated with the presence of the plasticizer (from 300°C to 340°C). If F6 and XilTi (with and without plasticizer) were compared, again the major change in TGA was the plasticizer evaporation at around 200°C; the degradation of the polysaccharide both occurred at around 300°C. Thus, there was a slight enhancement in thermal stability with glycerol plasticizer for GalCp but not for XilTi.

3.8. Water vapor permeability (WVP)

As for WVP, the galactomannan film (F0) showed the highest value among all the samples (Table 4, Column 3); however, there was no significant difference among the other six film samples (F1 – F6). Thus, galactomannan was more permeable to moisture than xyloglucan. The glycerol used as plasticizer in the films has hydrophilic character and may have been a factor that contributes to the increase in WVP values. Glycerol has low molecular weight and reduces polymer-polymer interactions, so it is easily inserted between the chains of polysaccharides, increases mobility of the molecules and causes more diffusion of water through the film matrix (Sobral et al., 2001; Vanin et al., 2005; Antoniou, et al., 2014). The blends formulated from galactomannan and xyloglucan are biocompatible, biodegradable and non-toxic and may be attractive in food packaging as an alternative to replace the traditional petroleum-based packaging materials. The films

blends contribute to a significant decrease in water vapor permeability and do not require the incorporation of hydrophobic agents, such as lipids and waxes(Wihodo & Moraru, 2013). This may be a useful characteristic for food packaging application, especially for fruits.

However, if there is an application that requires increased water vapor permeability, then it is possible to add an additional component such as collagen. Thus, the use of other additives, such as collagen and lipid, can permit further tuning (increase or decrease) of the water vapor permeation of our hemicellulose films, if needed.

3.9. Opacity

The opacity data of the same films are shown in Table 4 (Column 4). The opacity measurement scale ranges from 0% (fully transparent) to 100% (fully opaque); in this study the values of opacity for all films were below 20%, indicating that the films were relatively transparent(Fig.5).Although there were some deviations in the trend, film sample F0 (galactomannan) showed the lowest opacity (at about 16%) and film sample F6 (xyloglucan) among the highest in opacity (at about 19%).The galactomannan/xyloglucan blend films were mostly in between these values within the statistical error of the measurements.

In an earlier opacity work (Lima et al., 2010), a coating with 0.5% *A. pavanina* galactomannan, 1.5% collagen and 1.5% glycerol was reported to be less opaque than the coating with 0.5% *C. pulcherrima* galactomannan, 1.5% collagen, and no glycerol. Thus, collagen can promote transparency and can be an additional, optional ingredient in the hemicellulose film if needed.

3.10. Film thickness

The thicknesses of the films (Table 4, Column 2) ranged from 0.061 mm to 0.077 mm, with no significant difference ($p < 0.05$ %) among the samples, showing the uniformity of the films produced in this study. The thickness control is important to assess the uniformity, precision, and accuracy of the film-making process. According to Oliveira et al. (1996), film thickness has an impact on the film's mechanical properties and its barrier properties with respect to water vapor permeation. Studies reported by Park and Chinnam (1995) showed a linear relationship between water vapor permeability and thickness of the film for zein, gluten and cellulose derivatives.

4. Conclusions

Since hemicellulose is among the most common polysaccharides found in nature, it would be desirable to use it as a green and sustainable raw material for food-related and packaging applications. The extraction method developed in this work was shown to be feasible for obtaining the polysaccharides from seeds with yields between 20 and 30%, indicating that these sources can be exploited to obtain the hemicelluloses on a large scale. The hemicelluloses obtained had low levels of protein and ash, as compared with other sources of commercialized gum. Through the use of NMR, the ratio of monosaccharides in *C. pulcherrima* galactomannan and *T. indica* xyloglucan in the film formulations was determined.

The rheology data indicated that the solutions of films and blends formulated in this study had non-Newtonian behavior. Galactomannan film showed a higher permeability to moisture than xyloglucan and the blends. All films showed less than 20% opacity; thus they are relatively transparent. Thermogravimetric analysis of galactomannan and xyloglucan suggested that both had good thermal stability, and the films and blends formulated from these two hemicelluloses in the presence of glycerol as a plasticizer also demonstrated good thermal stability.

Thus, the characterization and test results suggested that both of these hemicelluloses and their blends have satisfactory properties that are compatible with food-related and packaging applications. These materials are available, edible, sustainable, biodegradable, non-toxic and environmentally friendly. The use of the blends permits a wider range of properties to be accessible than the hemicelluloses alone. Furthermore, no chemical reaction is needed to produce the blends, and the resulting blends should be cheap and safe to use. The results are relevant and signify the feasibility of manufacturing and producing food packaging films from hemicellulose derived from *C. pulcherrima* and *T. indica* seeds.

Conflict of interest

The authors declare that they have no conflict of interest regarding this manuscript.

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Figure Captions

Figure 1. General structures of a) galactomannan with β - (1 $\rightarrow$ 4) connected D-mannopyranose units and α - (1 $\rightarrow$ 6) connected D-galactopyranosyl units; b) xyloglucan with glucose backbone and xylose and galactose side chains

Figure 2. Molecular weight distribution of galactomannan and xyloglucan via gel permeation chromatography

Figure 3. ^{13}C NMR spectra of (a) *T. indica* xyloglucan(lower plot) and (b) *C. pulcherrima* galactomannan (upper plot), both in D_2O

Figure 4. Rheological measurements: **A.** Apparent viscosity of 1% aqueous solutions of hemicelluloses as a function of shear rate; **B.** Shear stress versus shear rate of the same solutions

Figure 5. Pictures of galactomannan and xyloglucan films. A) Film F0 – GalCp only; B) Film F3- GalCp+XilTi blend; C) Film F6 – XilTi only

*Caesalpinia
pulcherrima* seeds

Galactomannan

Tamarindus indica
seeds

Xyloglucan

20.500 rpm
for 15 min

Dried, 40 ° C for 24 h

30 g
Solution

F0 film
GalCp

F3 blend
GalCp+XilTi

F6 film
XilTi

Figure 1. General structures of a) galactomannan with β - (1 $\rightarrow$ 4) connected D-mannopyranose units and α - (1 $\rightarrow$ 6) connected D-galactopyranosyl units; b) xyloglucan with glucose backbone and xylose and galactose side chains

Where Manp = mannopyranose, Galp = galactopyranose, Glcp = glucopyranose, and Xylp = xylopyranose.

875 **Figure 2.** Molecular weight distribution of galactomannan and xyloglucan via gel
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879 **Figure 4.** Rheological measurements: **A.** Apparent viscosity of 1% solutions of
880 hemicelluloses as a function of shear rate; **B.** Shear stress versus shear rate of the same
881 solutions.

882
883 **Figure 5.** Pictures of galactomannan and xyloglucan films. A) Film F0 – GalCp only;
884 B) Film F3 - GalCp+XilTi blend; C) Film F6 – XilTi only

Table 1. Experimental design of formulations of the films and the blends of galactomannan and xyloglucan; all films made from 1% w/v solutions of hemicellulose with 0.5% glycerol.

Sample	Galactomannan (%)	Xyloglucan (%)	Glycerol (%)
F0	67	0	33
F1	54	13	33
F2	40	27	33
F3	33	33	33
F4	27	40	33
F5	13	54	33
F6	0	67	33
GalCp	100	0	0
XilTi	0	100	0

Table 2. Composition (mean $\pm$ standard deviation) and viscometric parameters (intrinsic viscosity $[\eta]$, Huggins coefficient K_H , Kramer constant K_K , critical concentration C^*) of *C. pulcherrima* galactomannan and *T. indicax* xyloglucan

Hemicelluloses	GalCp	XilTi
Yield (μ g)	25.0 ± 0.5	20.0 ± 0.4
Protein (%)	1.00 ± 0.03	0.60 ± 0.01
Ash (%)	0.38 ± 0.03	1.87 ± 0.01
$[\eta]$ (dL/g)	8.33	2.39
K_H	1.85	4.06
K_K	6.74	5.13
$C^* [\eta]$	0.12	0.41

Table 3. Summary of TGA data for hemicellulose samples, all values in percent weight loss.

Sample	Degradation temperature			
	~100°C	200°C	300°C	500°C
GalCp	11.8	-	68.4	19.8
XilTi	12.1	-	67.6	18.7
F0	9.9	29.0	44.8	15.9
F1	-	27.1	56.4	16.1
F2	20.8	27.0	35.8	15.6
F3	10.7	28.5	41.2	18.4
F4	19.3	29.9	33.7	16.3
F5	13.1	29.5	36.4	19.6
F6	17.5	29.2	32.9	18.9

Table 4. Thickness,water vapour permeability (WVP)and opacity for hemicellulose films*

Sample	Thickness (mm)	WVP (g.mm/h.kPa.m ²)	Opacity (%)
F0	0.065 ± 0.032 ^a	2.475 ± 0.691 ^a	16.251 ± 0.273 ^a
F1	0.061 ± 0.006 ^a	1.605 ± 0.183 ^b	17.603 ± 0.040 ^b
F2	0.068 ± 0.020 ^a	1.800 ± 0.538 ^{ab}	17.378 ± 0.090 ^{ab}
F3	0.068 ± 0.007 ^a	1.789 ± 0.188 ^{ab}	19.698 ± 0.385 ^c
F4	0.073 ± 0.003 ^a	1.915 ± 0.087 ^{ab}	19.860 ± 0.953 ^c
F5	0.078 ± 0.011 ^a	2.036 ± 0.300 ^{ab}	17.504 ± 0.372 ^b
F6	0.073 ± 0.015 ^a	1.928 ± 0.411 ^{ab}	18.828 ± 0.030 ^c

*Values are given as mean ± standard deviation. Different letters in the same column indicate a statistically significant difference (P < 0.05).