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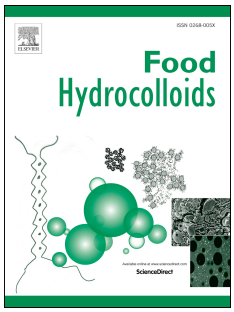

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Author statement

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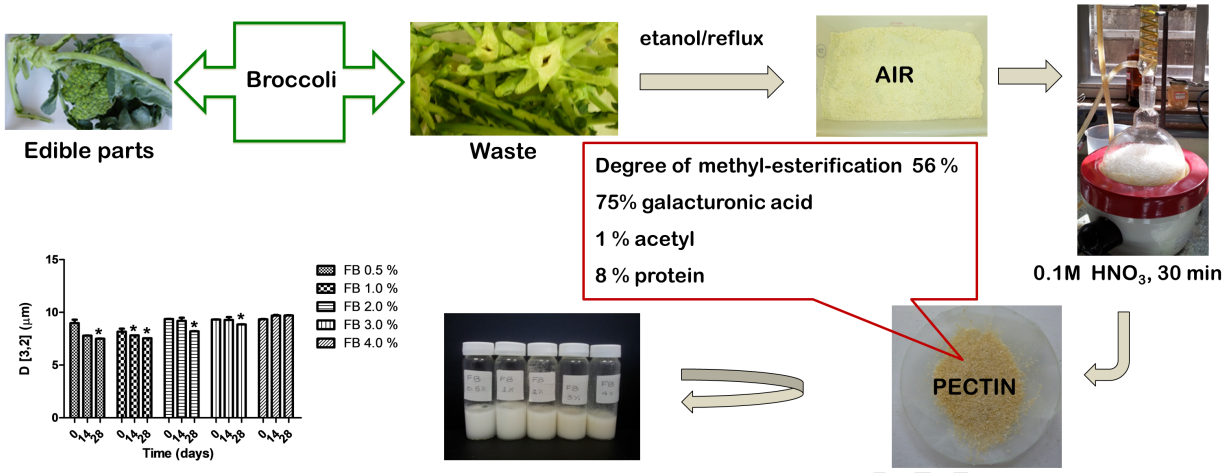

Pectins from food waste: Characterization and functional properties of a pectin extracted from broccoli stalk

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Abstract

Currently about 60-75% of world broccoli production is wasted during harvesting. The aim of the present study was to extract and characterise the pectin present in broccoli stalks and to evaluate its functional properties. The stalks were initially treated with boiling ethanol to remove compounds such as pigments and free sugars and then the pectin (FB) was extracted with 0.1 M nitric acid for 30min. The pectic fraction FB (18% yield) was found to contain 75% galacturonic acid with a degree of methyl-esterification of 56%, and an acetyl content of 1.1%. Rhamnose and galactose were the main neutral sugars present. NMR analyses showed that FB was composed of homogalacturonan and rhamnogalacturonan I substituted with β-1,4-D-galactan. The molar mass of FB was 72.2 x 10^3 g/mol and the viscosity of a 5% (w/w) solution in 0.1M NaCl at pH 4 showed shear thinning behaviour with a low shear Newtonian plateau of ~100Pa.s at 25°C. At the same concentration FB showed a weak gel like behaviour. FB (0.5 to 4%, w/w) was also able to stabilize medium chain triglyceride oil in water emulsions. The results suggest that broccoli stalk could be used as an alternative source of commercial pectins.

Keywords: Brassica oleracea L. var. italica; cell wall; chemical characterization; interfacial properties.

1. Introduction

The cell wall of vegetables consists of cellulose, hemicelluloses, pectin, glycoproteins, phenolic compounds and enzymes. Pectin is the anionic component of the cell wall made up mainly of galacturonic acid. It is used in a wide range of food products because of its ability to form gels (Endreß & Christensen, 2009; Voragen, Coenen,

Verhoef, & Schols, 2009). Pectin is considered as a safe food additive by different government food agencies and since 2017 has been given an acceptable daily intake of "not specified" by the Food and Agriculture Organization of the United Nations/World Health Organization Joint Expert Committee on Food Additives (IPPA, 2018). As dietary soluble fiber, pectin is associated with some health benefits, such as the decrease of circulating cholesterol levels, reduction of the response in post-prandial glucose and increase in satiety resulting in reduction in energy intake (Voragen et al., 2009; Vriesmann & Petkowicz, 2013). Besides that, it has been demonstrated that pectin, native or partially degraded ("modified pectins"), is able to promote modulation of the immune system and can prevent and reduce carcinogenesis (Maxwell, Belshaw, Waldron, & Morris, 2012; Zhang, Xu, & Zhang, 2015).

Although pectin is widely found in all land plants, it is commercially extracted from just a few sources, mainly citrus peel or apple pomace. Due to this limitation, the amount of pectin produced is also limited. In addition, poor citrus and/or apple crops can lower the supply of pectin. In 2016, there was a shortage of pectin and the average price exceeded $21/kg (Food Business News, 2016), justifying the search for new raw materials. In addition, the growing preference for the consumption of healthy products and the use in pharmaceutical and cosmeceutical sectors increases the demand for pectin. The global pectin market revenue was valued at USD 1 billion in 2019 and it is expected to reach USD 1.5 to 1.9 billion by 2025 (Grand View Research, 2019; Research and Markets, 2019). The consumption of any fruit or vegetable, fresh or processed, produces waste and can raise environmental concerns. Increasing efforts are currently being made to develop sustainable solutions for food waste management and some have been recently investigated as alternative sources of pectin, such as those from okra (Kpodo et al., 2017), artichoke (Sabater, Corzo, Olano, & Montilla, 2018), ponkan (Colodel, Vriesmann, Teófilo, & Petkowicz, 2018), eggplant (Kazemi, Khodaiyan, & Hosseini, 2019), black carrot (Sucheta, Misra, & Yadav, 2020), pea (Gutöhrlein, Drusch, & Schalow, 2020) and pomegranate (Shakhmatova, Toukach, & Makarova, 2020).

Broccoli (*Brassica oleracea* L. var. italica) is believed to be native to the eastern Mediterranean area and nowadays is cultivated worldwide with increasing production rate. According to data from the FAO (FAOSTAT, 2019), in the period of twelve years, from 1994 to 2016, the world production of cauliflower and broccoli increased 107%. In the same period, considering the increase in the world population, the consumption per capita increased by 57%. The cultivation of broccoli is associated with the production of a considerable amount of waste products, such as leaves, stalks and non-marketable vegetables that are discarded (Ares et al., 2013). The main edible part is broccoli florets

and it has been reported that the waste produced from broccoli by horticulture represents around 60-75% of the production (Gavilanes-Terán, Jara-Samaniego, Idrovo-Novillo, Bustamante, Moral, & Paredes, 2016; Hu et al., 2011). Considering the world production in 2017 (25,984,758 tonnes, combined reports with cauliflower) and assuming that 60% of production is wasted, more than 15 million tonnes of waste were generated in 2017.

Epidemiological studies have shown that consumption of broccoli is associated with reduced risk of several types of cancer and cardiovascular disease mortality (Moreno, Carvajal, Lopez-Berenguer, & Garcia-Viguera, 2006; Zhang et al., 2011). These health-promoting properties, anti-carcinogenic in particular, have been mainly attributed to the high contents of glucosinolates found in broccoli (Ares, Nozal, & Bernal, 2013). Regarding the composition, apart from the high content of glucosinolates, they are also a rich source of flavonoids, vitamins, minerals and dietary fibre (Moreno et al. 2006). Houben, Jolie, Fraeye, Van Loey, & Hendrickx, (2011) performed a comparative study of the cell wall composition of broccoli, carrot, and tomato and found differences in the polysaccharide from the stem and florets of broccoli. However, most research on broccolis has been focused on the edible parts and scarce information is available concerning the by-products (Moreno et al., 2006). Christiaens, Van Buggenhout, Houben, Fraeye, Van Loey, & Hendrickx (2011a) and Christiaens, Van Buggenhout, Vandevenne, Jolie, Van Loey, & Hendrickx (2011b) have investigated the pectin structure–function relationship during processing in small broccoli stems collected just underneath the florets. Xu, Cao & Chen (2015) purified a water-soluble polysaccharide from fresh broccoli with no previous pretreatment of the plant material. The polysaccharide had arabinose, galactose and rhamnose in a molar ratio of 5.3:0.8:1.0. More recently, Urai, Kataoka, Nishida, & Sekimizu (2017) isolated a pectic fraction from an aqueous extract obtained from edible parts of broccoli. However, we are not aware of any studies concerning the acid extraction and characterization of pectins from broccoli stalk. Thus, the purpose of the present study was to use broccoli stalks to obtain pectin by acid extraction, characterise the polysaccharide and examine its emulsifying properties.

2. Material and Methods

2.1. Material

Broccoli stalks were obtained from a local greengrocer shop (Curitiba, Parana, Brazil), where they are routinely discarded. They were cut into small pieces, crushed in a blender with absolute ethanol and boiled under reflux for 20 min to give rise to the

alcohol insoluble residue (AIR). The AIR was isolated by filtration, washed with absolute ethanol, dried and used for pectin extraction.

2.2. Pectin extraction

Pectin was extracted from the AIR by heating in 0.1 M nitric acid under reflux for 30min, using a liquid to solid ratio of 25 (v/w). The extracts were isolated by centrifugation (15,400×g) for 20 min and treated with 2 volumes of ethanol. After being stored for 16 h at 4°C, the precipitated pectin was removed by centrifugation (15,400×g; 30 min), washed with ethanol dried under vacuum and named FB.

2.3. Chemical and macromolecular characterization of FB

2.3.1. Monosaccharide composition

The pectin was hydrolyzed with 2M TFA at 120 °C for 2h, reduced with $NaBH_4$ and then acetylated with pyridine–acetic anhydride as previously reported (Petkowicz, Vriesmann, & Williams, 2017). The alditol acetates were examined by gas chromatography-mass spectrometry (GC-MS) using a Varian gas chromatograph and mass spectrometer Saturn 2000 R, with helium as the carrier gas and a capillary column DB-225 (30m × 0.25mm i.d.), held at 50°C during injection for 1 min, then programmed to increase to 220°C at a rate of 40°C/min. The content of uronic acid was determined by the m-hydroxydiphenyl method (Blumenkrantz & Asboe-Hansen, 1973) using galacturonic acid as standard. The uronic acid was identified by high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) using the hydrolyzed pectin in a Dionex ICS-5000 instrument (Thermo Fisher Scientific, Sunnyvale, CA, USA), using a Carbopack PA-20 column (Thermo Fisher Scientific, Sunnyvale, CA, USA) as described by Nagel, Sirisakulwat, Carle, & Neidhart (2014). All the analyses were performed in triplicate.

2.3.2. Protein, acetyl and phenolic contents

Protein content was estimated by the Bradford method (1976) using BSA as standard. Phenolic content was determined using the Folin-Ciocalteau's reagent (Singleton & Rossi, 1965) and gallic acid as standard. Measurements were performed in triplicate.

2.3.3. Degree of methyl-esterification (DM) and acetyl content

The DM was determined by Fourier transform-infrared spectroscopy (FT-IR) from the areas of the absorption bands at 1749 cm^{-1} (methyl esterified carboxylic group) and 1630 cm^{-1} (carboxylic ion) as previously described (Vriesmann & Petkowicz, 2009). Spectra

were obtained from KBr-sample discs using a Perkin Elmer Spectrum RXI FT-IR Spectrometer (Perkin Elmer Instruments, Massachusetts, USA) in the range of 4000 to 400 cm^{-1} at a resolution of 4 cm^{-1} from 64 scans. The acetyl content was evaluated by the Hestrin method (1949) using penta-O-acetyl-β-D-galactopyranose as standard. Measurements were performed in triplicate.

2.3.3. Scanning electron microscopy with energy dispersive X-ray spectroscopy (SEM-EDS)

SEM-EDS was used for detection of mineral elements in FB. Previous to the analysis, the dried sample was submitted to metallization with gold (Balzers Union sputter-coater, model SCD 030). The sample was examined in a VEGA3 LMU microscope (Tescan, Czech Republic) equipped with a detector (SDD 80mm2) and AZ Tech Advanced software using a 15 kV accelerating voltage for 60 s.

2.3.4. Nuclear magnetic resonance spectroscopy (NMR)

^{13}C and ^{1}H-^{13}C HSQC spectra were obtained from samples in D_2O (10 mg/mL) at 70 °C using a Bruker Avance III 400 MHz spectrometer (Bruker, Germany). Chemical shifts were expressed as δ (ppm) relative to acetone δ (2.22/30.2). Data was processed and analyzed by a Bruker TopSpin software.

2.3.5. Molar mass distribution

The pectin was analyzed by high-performance size-exclusion chromatography (HPSEC) coupled to a Dawn DSP laser photometer (Wyatt Technology, Santa Barbara, CA, USA) and OPTILAB DSP interferometric refractometer (Wyatt Technology, Santa Barbara, CA, USA) detectors and Agilent 1100 series UV detector (Agilent Technologies, Santa Clara, CA, USA) set at 280 nm. Analyses were performed at room temperature using a Suprema column 3000 Å (PSS, Mainz, Germany) and 0.1M NaCl containing 0.005% sodium azide as eluent at a flow of 0.5mL/min. The sample (4.5 mg/mL) was solubilized in 0.1M NaCl and filtered (0.45 μm). The refractive index increment of the solvent–solute solution with respect to a change in solute concentration (dn/dc) was determined as 0.178 mL/g and the average molar mass (Mw) was calculated using a Wyatt Technology ASTRA software (Wyatt Technology, Santa Barbara, CA, USA).

2.4. *Investigation of interfacial properties of FB*

2.4.1. Foaming ability

The foaming ability of 0.5%, 1%, 1.5% and 4% (w/w) FB solutions was evaluated by the method proposed by Stiepel (1914). For this purpose, 40 mL of solutions were introduced to a stoppered 250 mL measuring cylinder and inverted 10 times. The total height and the height of the liquid were recorded every minute for four minutes, subtracting the liquid height from total height to have the total foam volume. Measurements were performed in triplicate. Reported results used the foam value at two minutes to allow the foam to settle and stabilize.

2.4.2. Dynamic surface tension

The dynamic surface tension of 4% (w/w) FB solution was measured by the maximum bubble pressure method using a PC-500LV SensaDyne tensiometer (SensaDyne Instrument Division, USA) after calibration with water and ethanol. Measurements were performed in triplicate at 25°C in the LV operating mode.

2.4.3. Emulsifying properties

2.4.3.1 Preparation of emulsions

FB was solubilized in water (0.5, 1, 2, 3, 4%, w/w) (in the presence 0.005% sodium azide) with stirring for 16h at 25°C. The emulsions were prepared by mixing 15 mL of FB solution to 1.5 mL of medium chain triglyceride oil (MCT) for 4 min at 24,000 rpm using an IKA T25 digital Ultra Turrax mixer (IKA Werke GmbH & Co. KG, Germany).

2.4.3.2. Emulsion droplet size

The droplet size of emulsions was measured by laser diffraction shortly after preparation and after 1, 3, 7 days and then weekly (up to 35 days) using a Malvern Mastersizer 2000 (Malvern Instruments, Worcestershire, UK). The refractive indices of MCT (1.45) and water (1.33) were used. The measurements were performed in triplicate and the average value reported.

2.5. Rheological analyses

For rheological analyses, 5%, w/w FB was solubilized in 0.1M NaCl at pH4 with stirring for 18 h at 25°C. The sample was analyzed after resting for at least 1h. The steady shear viscosity was determined at shear rates of 0.01-1000s^{-1} and the storage and loss moduli were determined by small deformation oscillation measurements at a frequency of 0.01-1 Hz which was within the linear viscoelastic region as determined by stress sweep tests (0.01-100 Pa) at a constant frequency of 1Hz. All the measurements were performed in triplicate using a Thermo Scientific HAAKE MARS II rheometer (Thermo

Fisher Scientific Inc., Karlsruhe Germany) coupled to circulator (thermostatic water bath HAAKE K15 and HAAKE DC5 temperature control module) and a UTMC (Peltier) unit. A P35TiL parallel plate measurement system was used and the experiments were performed at 25°C.

3. Results and discussion

3.1. Extraction and chemical composition of pectin

Broccoli stalks were treated with ethanol giving rise to the AIR, free of pigments and low molar mass compounds, which was used for pectin extraction. The yield of pectin (FB) was 18.2% ±0.6 based on AIR. A similar yield (19.3%) was described for pectins from fresh watermelon rind (Petkowicz et al., 2017). On the other hand, the yield was higher that found by Ponmurugan et al. (2017) for pectins extracted from sunflower head using ultrasound assisted extraction (8.9%) and lower than the pectin content reported for citrus peel (~23 %) which is the main source of commercial pectins (Ciriminna, Chavarría-Hernández, Hernández, & Pagliaro., 2015). Houben et al. (2011) prepared AIR from broccoli stem and found that 16% of AIR was galacturonic acid, which was considered to be due to pectin, a value lower than that found in the present study for the pectin isolated by acid extraction (FB). When the authors performed sequential extraction with water, chelator, and sodium carbonate to isolate pectins, they recover 80.8% of the estimated content.

It is well known that extraction conditions dramatically affect the yield, composition and physico-chemical properties of pectins (Vriesmann & Petkowicz, 2013). Experimental design can be used to find the optimal conditions to obtain the highest yield of pectin (Pasandide, Khodaiyan, Mousavi, & Hosseini, 2017) and new approaches such as ultra-high pressure- (Guo et al., 2012), microwave- (Fishman, Chau, Hoagland, & Hotchkiss, 2006) and ultrasound-assisted (Grassino, Brncic, Vikic-Topic, Roca, Dent, & Brncic, 2016) extractions, also enhance the yield and improve properties. It is important to point out that, in the present study the pectin was extracted by conventional acid extraction and the extraction conditions were set based in previous studies performed with other raw materials (Petkowicz et al., 2017; Vriesmann, Teófilo & Petkowicz, 2011). Thus, it is possible that higher yields of pectin from broccoli stalk could be achieved if an experimental design and/or new technologies were used.

Table 1 gives the chemical composition of FB. The pectin had 74.7% galacturonic acid, in agreement with the specification set by the Food and Agriculture Organization of the United Nations/World Health Organization Joint Expert Committee on Food Additives (not less than 65%) (May, 1990). Among the neutral monosaccharides, galactose was

the main component followed by rhamnose. These are typical from rhamnogalacturonan-I (RG-I) domains. Glucose (3%), probably arising from non-pectic polysaccharides was also found. Pectic fractions isolated by sequential extraction from broccoli stem had arabinose, not galactose, as the main neutral monosaccharide (Houben et al., 2011). A pectin with immunostimulatory properties purified from an aqueous extract of edible parts of broccoli also had galacturonic acid, arabinose, galactose, rhamnose and glucose in a molar ratio of 12: 7.3: 4.9: 1.2: 1.0 (Urai et al., 2017). The apparent discrepancy is probably due to the hydrolysis of the more labile linkages with arabinose in the acidic extraction conditions (Thibault, Renard, Axelos, Roger, & Crépeau, 1993). The presence of arabinans and arabinogalactans as neutral side chains of RG-I in broccoli stem was reported by Schäfer, Stanojlovic, Trierweiler, & Bunzel (2017). Besides that, according to these authors, storage for 7 days at 20 °C could result in a decrease of arabinose and galactose from RG-I side chains.

The relative amount of RG-I and homogalacturonan (HG) was calculated as described by M'sakni et al. 2006. FB was composed of 69.3% HG and 25.9% RG-I. The ratio (Ara+Gal)/Rha = 2.8 was lower than that found for pectins from watermelon rind which had (Ara+Gal)/Rha = 8.0 - 8.7 (Petkowicz et al., 2017), indicating that FB had shorter side chains attached to the RG-I region. When the same ratio was estimated for pectins extracted from broccoli stem by sequential extraction, the values ranged from 7.1 to 15.3 (Houben et al., 2011), much higher than that found in the present study. Again, the difference is probably due to hydrolysis of the side chain in the acidic extraction conditions. Besides that, it was observed that pectins with the longest side chains were extracted with water because they are loosely bound to the cell wall (Houben et al., 2011). The use of harsher conditions, such acid extraction, enables to extract more linear domains of pectin that are more tightly bound to the cell wall compared to more substituted regions.

FB displayed a low amount of phenolics and a high protein content (Table 1), comparable to sugar beet pectin (10.4%) (Thibault, De Dreu, Geraeds, & Rombouts, 1988). Ca, S and K were detected in FB by SEM-EDS analysis (Ca > S > K), S being from the protein moiety of FB. It has been demonstrated that the high level of protein in sugar beet pectin plays an important role in the ability of sugar beet pectin to stabilize oil-in-water emulsions (Chen, Qiu, Gan, Liu, Zhu & Yin, 2016; Funami, Nakauma, Ishihara, Tanaka, Inoue, & Phillips, 2011). Thus, the results suggest that FB could display good emulsifying properties. However, differently from sugar beet pectin, FB had a low acetyl content (1.1%) which corresponds to a degree of acetylation of 6.3%. The high acetyl level in sugar beet pectin (degree of acetylation 16-35%; Leroux, Langendorff, Schick,

Vaishnav, & Mazoyer 2003) is known to affect the capability of forming gels by the conventional methods of pectin gelation (Funami et al., 2011).

FB can be classed as a high methoxyl pectin (HM, DM > 50%) which is in agreement with a previous report from Houben et al. (2011), who found that the pectins from AIR of broccoli stem were HM (DM 63.1%).

3.2. NMR and molar mass analysis

^{13}C and $^{1}H/^{13}C$ HSQC NMR analyses (Figure 1) confirmed the presence of a partially methyl-esterified homogalacturonan in FB. Signals of C1/H1 from 1,4-linked esterified and unesterified α-D-Gal*p*A residues were observed at 101.1/4.97 and 99.4/5.10 ppm, respectively. Signals at 68.2/4.02; 68.7/3.93 and 78.2/4.47 ppm were assigned to C2/H2, C3/H3 and C4/H4, respectively. C5/H5 from unesterified GalA was found at 70.3/5.00 and that from esterified residues appeared at 70.8/5.09 and 70.5/5.05 ppm. The C-6 from esterified and unesterified GalA was identified at 170.6 and 172.2 ppm, respectively in the ^{13}C-NMR spectrum (data not shown). The signal at 52.8/3.83 ppm was assigned to the methyl group from esterified GalA units. The $^{1}H/^{13}C$ HSQC spectrum also detected the presence of acetyl groups at 20.0/2.07.

Characteristic peaks from RG-I were also observed. Signal of α-L-Rha*p* (C1/H1 at 99.1/5.22 ppm) was seen in the resonance region of the anomeric atoms. The signals at 75.6/4.12 and 77.6/4.16 ppm were assigned to 1,2- and 1,2,4-linked α-L-Rha*p* residues, respectively. C3/H3 was observed at 72.9/3.78 ppm and C5/H5 at 68.0/3.76 ppm. The C4/H4 of 1,2- and 1,2,4-linked α-L-Rha*p* units appeared at 73.5/3.70 and 78.6/4.44, respectively. The upfield region had C6/H6 signals of 1,2-linked α-L-Rha*p* at 16.4/1.25 ppm and 1,2,4-linked α-L-Rha*p* at 16.9/1.32. The $^{1}H/^{13}C$ spectra showed that in FB, the rhamnose units were mainly substituted with β-1,4-D-galactans, C1/H1, C2/H2, C3/H3, C4/H4, C5/H5 and C6/H6 at 104.3, 73.4/3.92, 73.7/3.95, 77.6/4.16, 73.6/3.95 and 60.8/3.81 ppm, respectively. All the assignments were based on the literature (Colodel et al., 2018; Ovodova, Bushneva, Shashkov, Chizhov, & Ovodov, 2005).

The LS, RI and UV elution profiles obtained from HPSEC-MALLS are depicted in Figure 2A. FB showed a main peak eluting between 7 and 10 mL detected by RI and LS The fact that the RI and LS peaks do not superimpose is an indication of the polydispersity of the sample since the RI signal is sensitive to the concentration of eluting species while the LS signal is also sensitive to their molar mass. This is confirmed in Figure 2B which shows the molar mass of the eluting species decreasing with elution volume. Furthermore, the eluting species do not give a UV signal indicating

that they do not contain proteinaceous components. The elution profiles in Figure 2A also show a minor peak eluting at 11.5 mL which is detected by both RI and UV (280ηm), consistent with the presence of 8.1% protein. This peak does not give a LS signal indicating it has a low molar mass. FB had an average molar mass of 72.2 x 10^3 g/mol and the weight-average radius of gyration was 27.5 ηm. The Mw was higher than those described for pectins extracted from watermelon rind (34.5 – 40.4 x 10^3 g/mol; Petkowicz et al., 2017), but much lower than those described for pectins isolated by sequential extraction from raw broccoli (158 kDa to > 788 kDa; Christiaens et al., 2011a) and for pectins obtained by aqueous extraction from the pods of six okra genotypes (791 -1,693 x 10^3 g/mol; Kpodo et al., 2017).

It has been pointed out that chemically or enzymatically modified pectins display anti-cancer and anti-metastatic properties and one of the modified polysaccharides was patented as a mammalian anti-cancer agent. The aim of the modification is to reduce the molar mass and Ara and increase β-Gal, thus increasing the accessibility to β-galactan chains and the ability of binding to galectin-3 (Gal3), a β-galactose-binding lectin, implicated in cancer and metastase (Maxwell et al., 2012). The chemical features of FB, such as low molar mass and high levels of β-1,4-galactans suggest that this pectin might also possess pharmacological properties. This possibility is currently under investigation.

3.3. Rheological properties of 5% (w/w) pectin solution

The rheological properties of a 5% (w/w) solution of FB in 0.1M NaCl at pH 4 were investigated and the steady shear viscosity is presented as a function of shear rate in Figure 3A. The viscosity of FB was strongly shear rate dependent. Similar shear thinning behaviour, with high shear rate dependence, was observed for a low methoxyl pectin (DM ~21%) isolated from okra (*Abelmoschus esculentus* L.) genotype Agbagoma at 4% (w/v) (Kpodo et al., 2017). The viscosity of FB at low shear was ~100 Pa s, higher than the values found for pectins from okra genotype Asontem (~10 Pa s) and watermelon rind (~30 Pa s) at the same concentration, but close to that measured for the pectin from okra genotype Agbagoma at 4% (w/v) (Kpodo et al. 2017; Petkowicz et al., 2017). Due the marked shear thinning behaviour of FB, when the shear rate was reduced to 1 s^{-1} the viscosity was lowered to 6 Pa s. Despite the decrease, the viscosity was higher than that observed for a highly branched pectin from gabiroba (*Campomanesia xanthocarpa* Berg) fruit at 5% (w/v), which had a viscosity of ~ 0.2 Pa s at shear rate 1 s^{-1} (Barbieri**,** Amaral, Ruthes, Petkowicz, Kerkhoven, & Silveira, 2019). As observed by Kpodo et al. (2017), the viscosity of pectins seems not to be a simple function of molar mass. Dranca, Vargas, & Oroian (2020) found a positive correlation

between dynamic viscosity and GalA content which could explain the higher viscosity of FB since okra and gabiroba pectins had lower GalA (33.5-54.2%) than FB (~75%).

Small deformation oscillation measurements were performed in the linear viscoelastic region to evaluate the viscoelastic properties of FB at 5% (w/w) (Fig. 3B). The values of the storage modulus (G') were higher than the loss modulus (G'') in the range from 0.01 to 1 Hz and exhibited frequency dependence, indicating a weak gel behaviour. At the same concentration and frequency range, pectins from gabiroba (Barbieri et al., 2019) and watermelon rind (Petkowicz et al., 2017) also displayed G' > G''. On the other hand, the values of the moduli were higher for FB. Positive correlation between Mw as well as GalA content and the values of G' and G'' was reported for pectins from apple pomace (Dranca et al., 2020). However, comparing the viscoelastic properties, GalA and Mw of FB and pectins from gabiroba and watermelon rind, no correlation was found.

3.4. Interfacial properties

The dynamic surface tension of 4% (w/w) FB solution measured by the maximum bubble pressure method was 46.72 mN/m, close to the value reported for gum arabic at the same concentration (47.91 mN/m) (Petkowicz et al., 2017). The ability of FB to lower surface tension is likely to be due to the proteinaceous components which have amphiphilic characteristics and are able to adsorb at the air – liquid interface.

The foaming ability of FB was measured and the foam volumes for aqueous solutions of FB at concentrations 0.5-2% (w/w) are shown in figure 4. The foam volume increased with FB concentration. However, at the highest concentration the amount of foam decreased. Similar results were obtained for pectins from watermelon rind and the unexpected behaviour was attributed to the high viscosity of the polymers (Petkowicz et al., 2017).

At the same concentrations, the foam volume produced by FB solutions was lower than those observed for pectins from watermelon rind, but it was almost three times higher than that reported for gum arabic at concentrations 0.5-1.5% (Petkowicz et al., 2017). The foam life time of 1% FB was 175 h, being notably more stable than the foam produced with watermelon rind pectins (foam life ~62h) (Petkowicz et al., 2017). The foaming ability of FB is believed to be due to the presence of protein, while the stability is provided by the carbohydrate component (Foegeding, Luck, & Davis, 2006; Perez, Sanchez, Patino, Rubiolo, & Santiago, 2012). The higher viscosity of FB compared to watermelon rind pectins (Petkowicz et al., 2017) is consistent with this hypothesis.

Figure 5A shows the values of surface weighted mean diameter D [3,2] for oil in water emulsions prepared using MCT and FB (0.5-4%, w/w) as a function of time. The ability of FB to form 10% v/v MCT oil-in-water emulsions was investigated using varying concentrations of FB (0.5-4%). The droplet size of the emulsions was found to be smallest at concentrations of 0.5% and 1% FB and then the droplet size increased slightly as the FB concentration increased further. Similar pattern was observed for volume weighted mean diameter D [4,3] as shown in figure 5B. The results indicate that 0.5% FB was sufficient to fully coat the surface of the droplets and prevent aggregation and coalescence. The fact that the droplet size was slightly higher as the FB concentration increased above 1% is likely to be due to the fact that the viscosity of the continuous phase increased thus inhibiting droplet formation on shearing using the Ultra-Turrax mixer. Similar behavior was observed for emulsions prepared with gum arabic using the same conditions when the concentration increased from 0.5% to 4% (Petkowicz et al., 2017). The droplet size for all of the emulsions remained almost constant over the 28day period. The droplet size distribution of the emulsion prepared with 2% FB shortly after preparation and after 35 days is depicted in figure 6. A main peak centered at about 10μm was observed with almost no changes over the storage period. The ability of sugar beet pectin to stabilise oil-in-water emulsions has been noted previously (Siew & Williams, 2008) and is believed to be due to the presence of both proteinaceous components and ferulic acid. The mechanism is the same as for gum arabic (Randall, Phillips, & Williams, 1988) with the proteinaceous components adsorbing onto the surface of the oil droplets and the carbohydrate component protruding out into the aqueous phase preventing aggregation through both electrostatic and steric repulsions.

4. Conclusion

Broccoli stalk can be used to obtain pectin with GalA content in the range set by FAO and EU for food application. This waste could be recovered for pectin extraction directly from farming during the harvesting period with no need of previous drying. The results indicated that the pectin could be used as a thickening and emulsifying agent in the food industry.

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Figure captions

Figure 1. $^{13}C/^{1}H$ heteronuclear single quantum correlation spectroscopy (HSQC) spectrum of FB in D_2O at 70 °C

Figure 2. HPSEC elution profile of FB using multi-angle laser light scattering (MALLS; 90° is shown), refractive index (RI) and ultraviolet (UV at 280 nm) detectors (A); molar mass curves superimposed on the RI chromatogram (B).

Figure 3. Viscosity dependence on shear rate of pectin FB at 5% (w/w) in 0.1M NaCl at pH 4 (A); frequency sweep of pectin FB at 5% (w/w) in 0.1M NaCl at pH 4 (B).

Figure 4. Volume of foam produced from 0.5 to 2% (w/w) solutions of pectin FB.

Figure 5. Dependence of surface weighted mean diameter D [3,2] (A) and volume weighted mean diameter D [4,3] (B) on time for emulsions prepared with different concentrations (0.5-4%, w/w) of pectin FB.

Figure 6. Droplet size distribution of the emulsion prepared with 2% FB shortly after preparation and stored over 35 days period.

Table 1. Chemical composition and molecular features of pectin FB extracted from broccoli stalk.

Monosaccharide composition (%)	
GalA	74.7 ±0.80
Rha	5.4 ±0.11
Fuc	0.2 ±0,02
Ara	1.5 ±0.08
Xyl	0.9 ±0.04
Man	0.6 ±0.11
Gal	13.6 ±0.34
Glc	3.2 ±0.21
HG[b]	69.3
RG-I[b]	25.9
(Ara+Gal)/Rha	2.8
DM[c]	56.2 ±0.09
Acetyl[d] (%)	1.1 ±0.04
Protein[d] (%)	8.1 ±0.07
Phenolics[d] (%)	0.4 ±0.03
Mw (g/mol)	7.218×10^4
Mw/Mn	5.72 ±0.07
Rg (ηm)	27.5

[a]Neutral monosaccharide determined by GC-MS; GalA quantified by spectrophotometry and identified by HPAEC-PAD
[b]HG= GalA – Rha and RG-I= 2(Rha) + Ara + Gal
[c]Determined by FT-IR
[d]Determined by colorimetric method

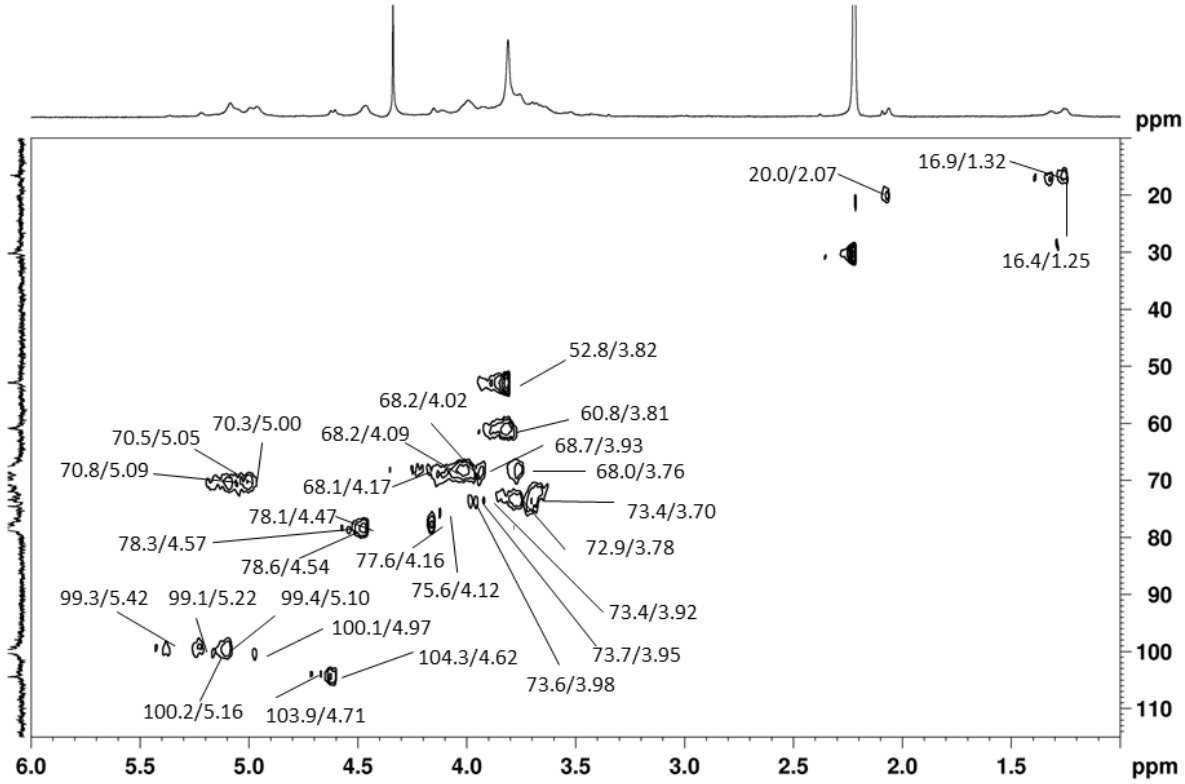

Figure 1

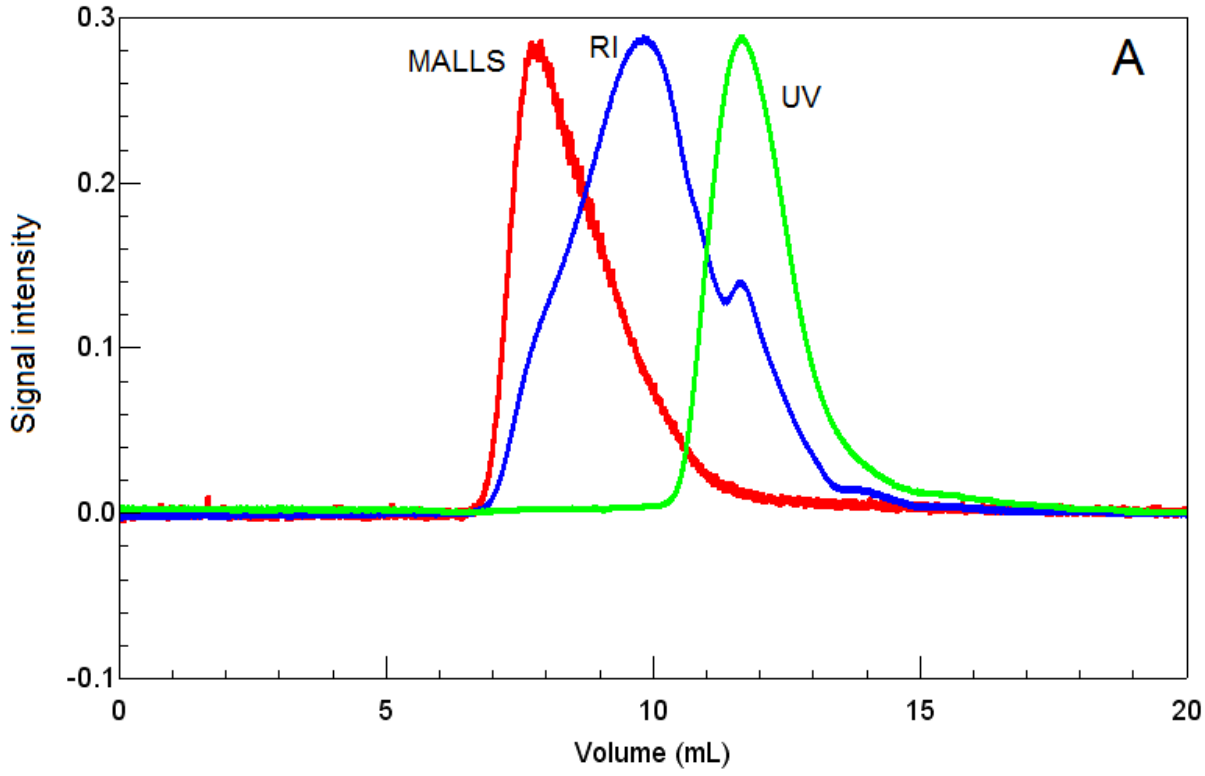

Figure 2A

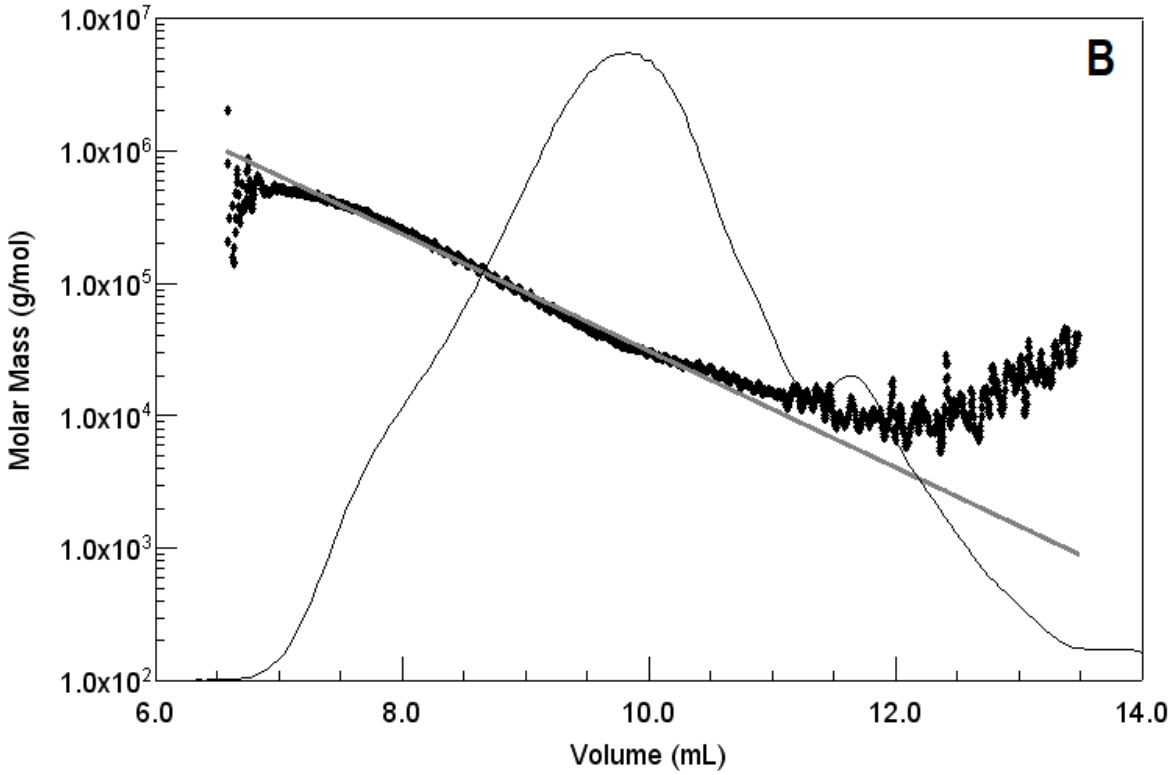

Figure 2B

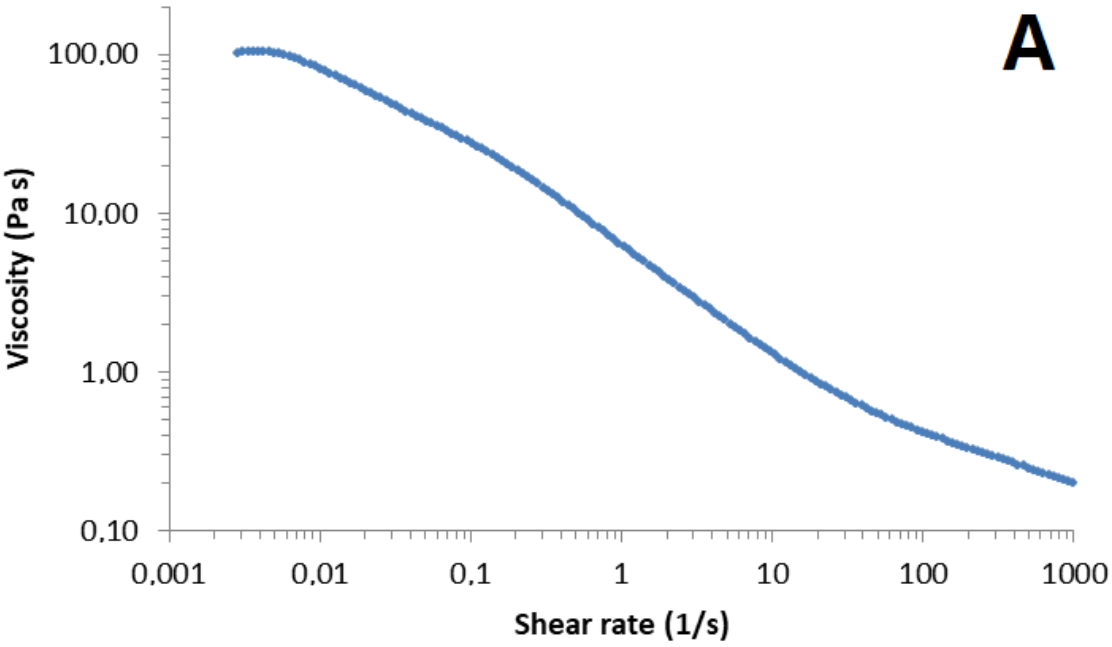

Figure 3A

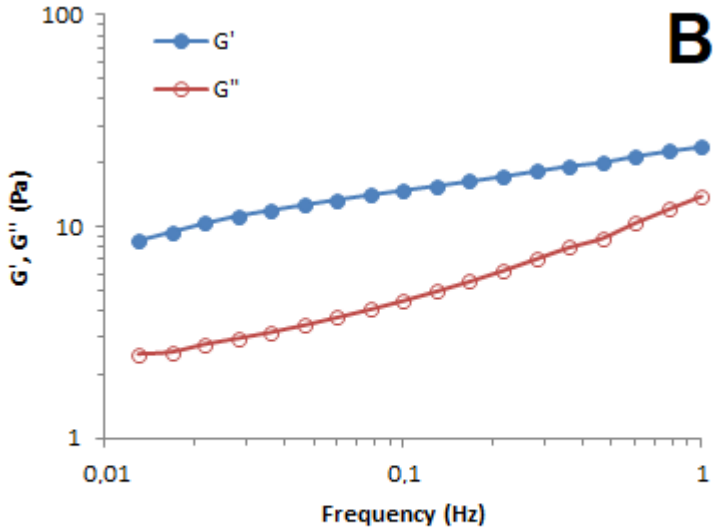

Figure 3B

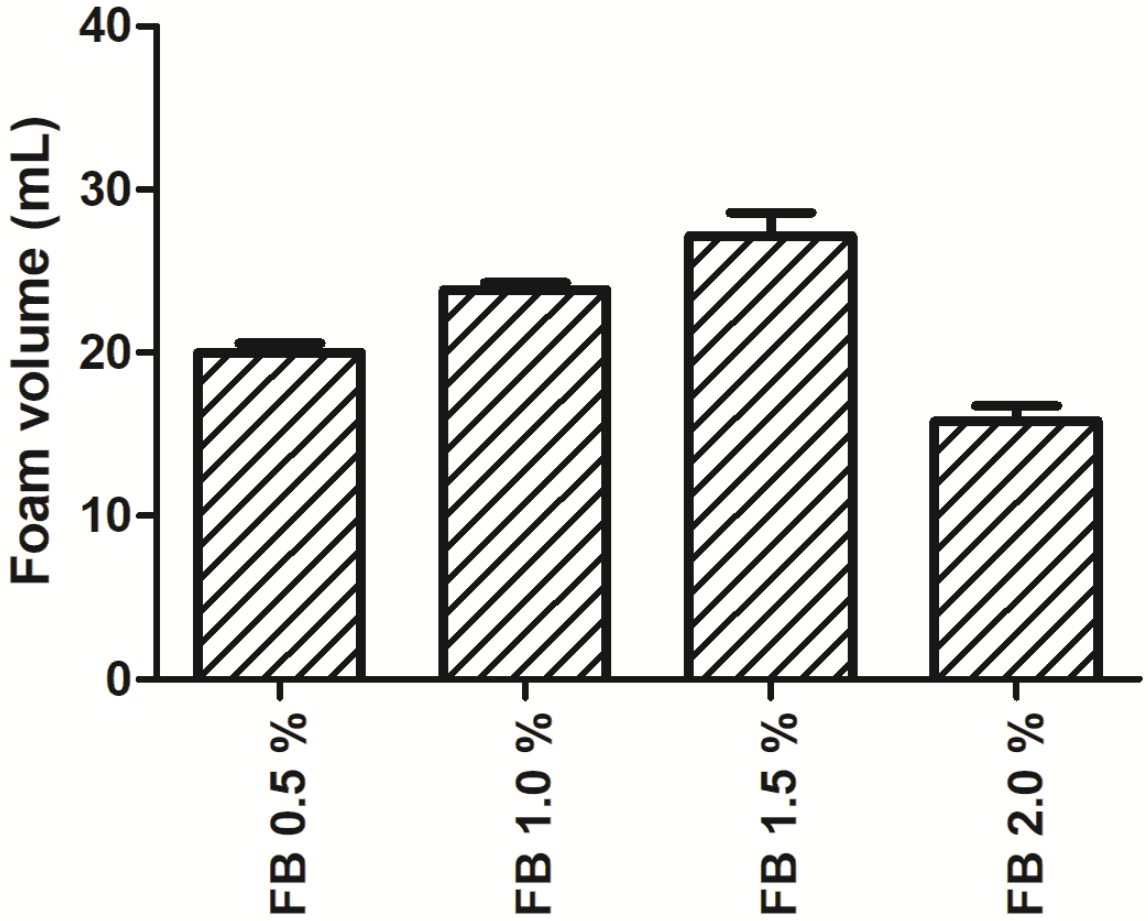

Figure 4

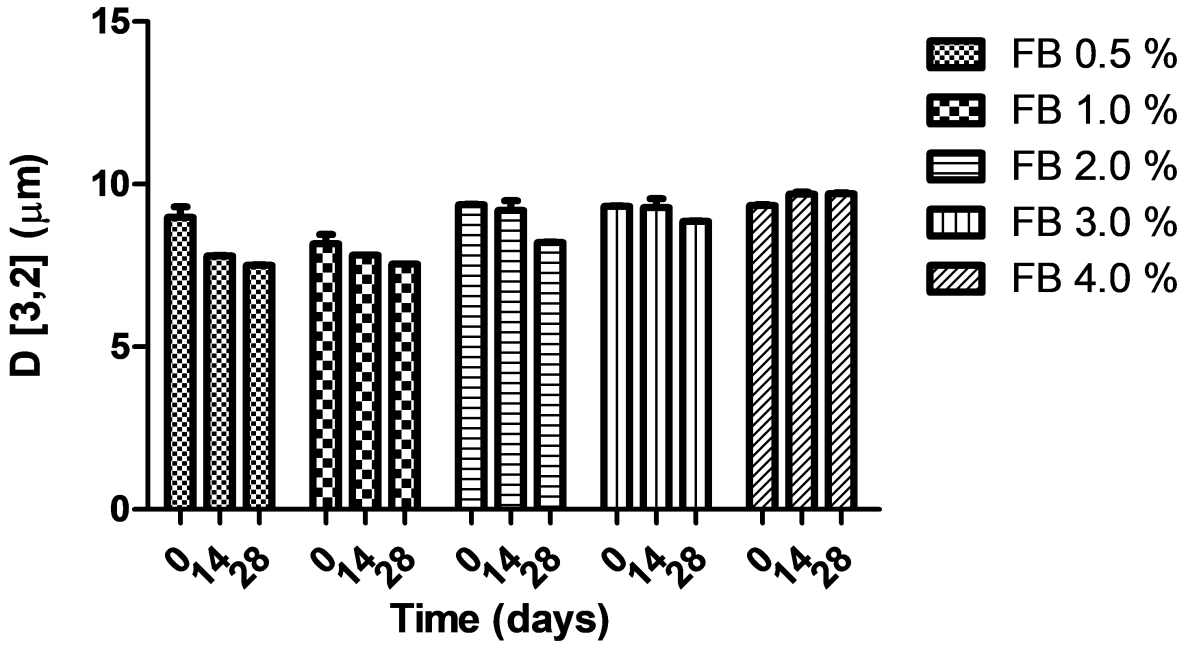

Figure 5A

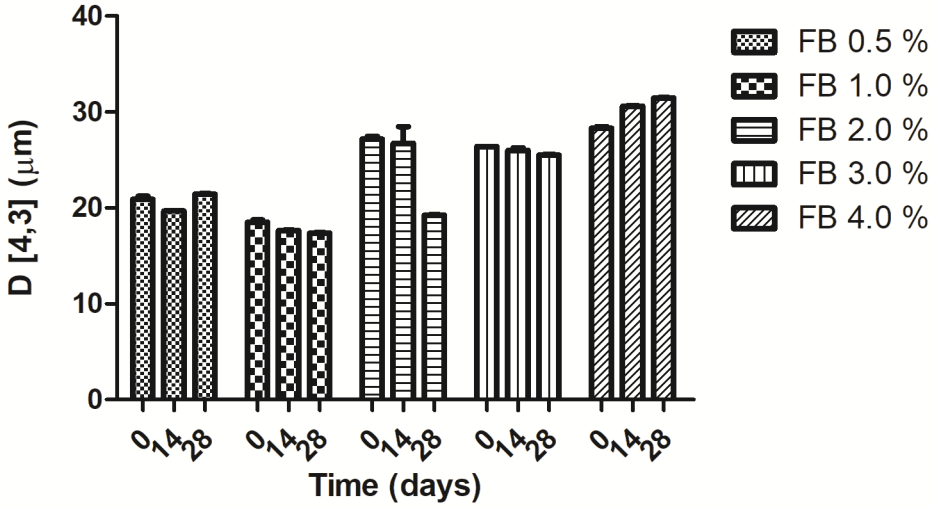

Figure 5B

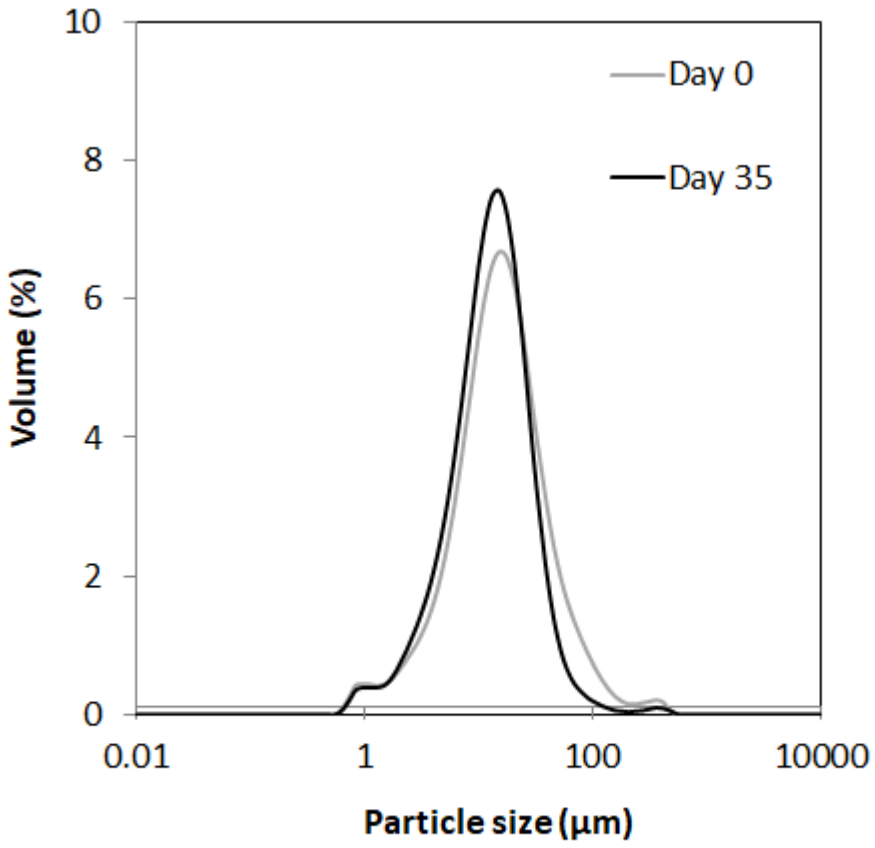

Figure 6

Highlights

- Acid extraction was used to obtain pectin from waste broccoli stalk.
- The pectin had a high degree of methyl-esterification and low acetyl content.
- Galacturonic acid content was in the range set by FAO and EU for food application.
- The protein content was comparable to sugar beet pectin.
- The pectin has application as a thickener and emulsifier.

Conflict of interest

The authors declare that they have no conflict of interest.