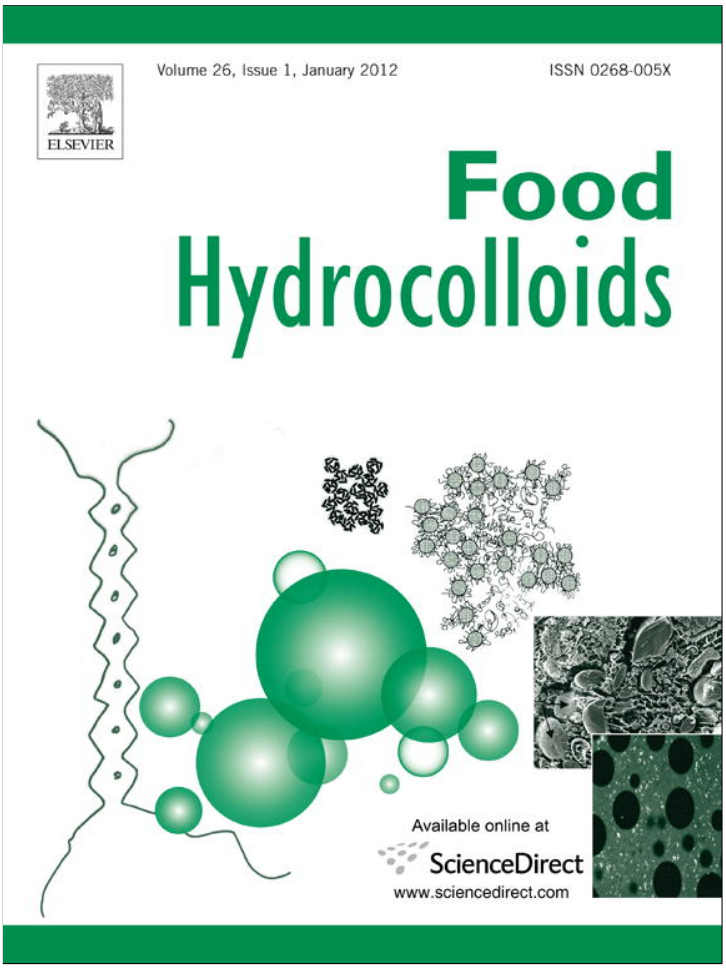

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Butternut and beetroot pectins: Characterization and functional properties

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ABSTRACT

The physicochemical characteristics and functional properties of butternut (*Cucumis moschata* Duch. ex Poiret) and beetroot (*Beta vulgaris* L. var. conditiva) pectins obtained by enzymatic extraction from by-products of vegetable processing have been evaluated. The molecular mass distribution was determined using Gel Permeation Chromatography using light scattering, refractive index and UV detectors and the samples were found to be highly heterogeneous and polydisperse. M_w values of 136,000 and 1,309,000 g/mol were determined for butternut and beetroot pectins respectively. Butternut pectin had a high degree of methyl esterification. In the presence of high concentrations of sugar and at low pH, this pectin did not form gels but instead produced viscous solutions. Solutions showed pseudoplastic flow behaviour with a shear thinning index of 0.68 as determined from the Power law model. Beetroot pectin had a low degree of methyl esterification and formed gels with addition of Ca^{2+} at concentrations of 10 mg/g pectin or higher. The maximum value of the storage modulus was obtained at a Ca^{2+}/GalA ratio of 0.25. The thermal stability of gels suggested that hydrogen bond interactions prevailed in the absence of Ca^{2+}, whereas electrostatic junction zones increasingly developed between pectin chains as the calcium concentration increased. Aqueous solutions of butternut and beetroot pectins significantly reduced surface tension and both samples were able to form stable oil-in-water emulsions. It was found that protein and/or polyphenol – rich fractions present in the pectins adsorbed at the oil–water interface and were responsible for the emulsification properties.

1. Introduction

Hydrocolloids are widely used in the food industry because of their ability to thicken and gel aqueous solutions and to stabilize emulsions and dispersions (Phillips & Williams, 2009). It is also now very well recognised that they are a source of dietary fibre and can provide a range of health benefits (Phillips & Williams, 2009). Pectin is an abundant and multifunctional component of the cell wall of all land plants and it is a high value functional food ingredient widely used as a gelling agent (Endress & Christensen, 2009; Willats, Knox, & Mikkelsen, 2006). It is believed to consist of a homogalacturonan (HG), rhamnogalacturonan-I (RG-I) and rhamnogalacturonan-II (RG-II). It is thought that these three polysaccharide domains are covalently linked to form a pectic network throughout the primary cell wall matrix and middle lamellae (Willats et al., 2001). The plant primary cell wall contains proteins and the presence of hydroxyproline rich protein has been reported in pectin (Kravtchenko, Arnould, Voragen, & Pilnik, 1992). Recently, Nuñez, Fishman, Fortis, Cooke, and Hotchkiss (2009) identified extensin as the protein that formed complexes with the pectin extracted from sugar beet as well as with commercial pectin of the same origin, complexes that were not due to ionic interaction. Pectins as defined for use in food are high molecular mass heteropolymers containing a majority of GalA units. These GalA residues of HG can be methyl-esterified at C-6 and carry acetyl groups on O-2 and O-3 (Vincken et al., 2003). However, pectins are derived from the breakdown of more complex protopectins which are present in the plant tissue, and also contain a range of neutral sugars, including rhamnose, galactose, arabinose and lesser amounts of other sugars (May, 2000). Pectins are highly heterogeneous with respect to their molecular mass and chemical structure. The average molecular mass of pectins from various vegetable sources is typically in the range of 10^4-10^5 Da (Izydorczyk, Cui, & Wang, 2005).

The pectin network must be disrupted to enable extraction from vegetable tissues. This may involve extraction with calcium-chelating agents, dilute alkali or dilute acid. Alternatively, pectic polysaccharide fragments can be released through the use of

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degrading enzymes. The plant cell wall is mainly composed of cellulose, hemicellulose, pectin, and proteins. Therefore, the use of enzymes such as cellulases which are able to degrade the plant cell wall facilitates the isolation of pectins. The extracted materials will be heteropolymolecular and polydisperse having a diversity of chemical structures and molecular sizes (MacDougall & Ring, 2004). The functionality of the pectin is dictated by the chemical fine structure (Willats et al., 2006). The major sources of commercial pectin are citrus peel and apple pomace and it is extracted from the raw material with hot aqueous mineral acid at pH ~ 2 (May, 2000; May, 1990). The composition, structure, and physiological properties of pectin might be influenced by conditions of extraction as well as source, location and many other environmental factors.

Extracted pectin is used as a functional food ingredient and it is listed among the ingredients in many food products (EU code, E440). Worldwide annual consumption is estimated at around 45 million Kg (Willats et al., 2006). Based on the degree of methyl-esterification (DM), which is the proportion of carboxyl groups present in the esterified form, pectins with DM lower than 50% are considered to be low-methoxyl (LM) pectins, while those with DM equal to or greater than 50% are considered to be high-methoxyl (HM) pectins. The formation of gels with HM pectins requires conditions of low pH (~3) and high sugar content (~65%). In contrast, LM pectins may form gels in the presence of calcium over a wide range of pH with or without sugar (Fu & Rao, 2001).

The emulsifying properties of sugar beet pectin have been established (Funami et al., 2007; Leroux, Langendorff, Schick, Vaishnav, & Mazoyer, 2003; Siew & Williams, 2008; Williams et al., 2005) and it is apparent that proteinaceous and ferulic acid moieties covalently attached to the pectin molecules have a significant role to play.

Butternut (*Cucurbita moschata* Duch ex Poiret) and beetroot (*Beta vulgaris* L. var conditiva) are non conventional sources that could be exploited for pectin production (Fissore et al., 2011; Fissore, Ponce, Stortz, Rojas, & Gerschenson, 2007).

The object of this research work was to study the potential of butternut and beetroot pectins for use as additives in the food industry. The extraction process of the pectins involved the use of non-pectolytic enzymes such as cellulases, an environmentally friendly alternative to the industrial pectin extraction through hot mineral acids (Endress, Mattes, & Norz, 2005).

2. Materials and methods

2.1. Sample preparation

With the aim of testing the technological potential of pectins extracted from unconventional sources through novel extractive procedures, two samples of pectin were used in the present research work. Sample A was obtained from butternut pumpkin mesocarp with the aid of cellulase enzyme (Fissore et al., 2007). Sample B was obtained from beetroot tissue with the aid of cellulase after an alkaline pre-treatment (Fissore et al., 2011). The extraction procedures are summarized as follows:

Sample A: 10 g butternut (*C. moschata* Duch ex Poiret) cell wall material (CWM) were digested under stirring (100 rpm) in 1000 mL 0.05 M-sodium citrate buffer (pH 5.2) with 0.01% (w/w) sodium azide (final concentration) and 0.05 g cellulase (C9422; cellulase from *Trichoderma viride*, Sigma, USA). This treatment was performed for 20 h, at 30 °C.

Sample B: In order to saponify the esterified ferulic dimers present in beetroot CWM, alkaline hydrolysis (30 min; 25 °C) was performed prior to buffer/enzyme treatment (Fissore et al., 2011). 10 g beetroot (*B. vulgaris* L. var. *conditiva*) CWM were treated with 2 M - NaOH solution (500 mL) at 25 °C for 30 min under constant agitation and then filtered under vacuum and washed once with deionized (Milli-Q™, USA) water, using a glass fibre filter (Schleicher & Schuell, Dassel, Germany). The residue obtained was poured into a beaker containing 1000 mL of 0.05 M - sodium citrate buffer solution with 0.01 g/100 g of sodium azide (final concentration). The pH was adjusted to 5.2 with citric acid and the residue was then submitted to digestion with stirring (100 rpm) in 1000 mL 0.05 M - sodium citrate buffer (pH 5.2) with 0.01% (w/w) sodium azide (final concentration) and 0.05 g cellulase (C9422; cellulase from *T. viride*, Sigma, USA). The procedure was performed for 20 h, at 30 °C.

Insolubles obtained after both enzymatic digestions were separated by filtration through glass fibre filter paper (Schleicher & Schuell, Germany) under vacuum and cell wall polysaccharides (CWP) were finally precipitated from each supernatant through ethanol addition (2 volumes). These CWPs were collected through filtration under vacuum, using glass fibre filter paper and they were, finally, freeze-dried.

In previous research work, Fissore et al. (2011; 2007) have characterised the chemical composition of samples through chemical and enzymatic spectrophotometric methods, as well as through derivatization of the neutral sugars (NS) to the alditol acetate derivatives followed by separation and quantification by gas–liquid chromatography (GLC) coupled to flame ionization or to electron impact-mass spectrometric detection in order to identify unusual NS of the RG-II. The type of uronic acids present was confirmed and identified as GalA and glucuronic acid by monosaccharide reduction with carbodiimide before the GLC method described above. Sample A consisted of 55% (w/w) GalA, 7.5% (w/w) protein, DM = 80% (mol/mol) and degree of acetylation, DA = 16% (mol/mol) (Fissore et al., 2007).

Sample B contained 54% (w/w) GalA, 6% (w/w) protein with DM = 2% (mol/mol) and DA = 2% (mol/mol) (Fissore et al., 2011).

2.2. Ferulic acid content

The UV absorption spectrum of 0.05% (w/w) solutions of the fractions was determined from 190 to 600 nm using a Perkin Elmer Lamda 25 UV/VIS Spectrophotometer (USA). The ferulic acid (FA, *trans*-4-hydroxy-3-methoxycinnamic acid) content in the samples was determined by measuring the UV absorbance at a wavelength of 310 nm. A calibration curve was constructed using ferulic acid obtained from Aldrich. The FA content of samples was calculated from the measured intensity.

2.3. FTIR analysis

Fourier transform infrared spectroscopy (FTIR) spectra were recorded using a Perkin Elmer Spectrum RX I FT-IR Spectrometer (USA) in the range 4000 to 500 cm^{-1} at a resolution of 8 cm^{-1}.

2.4. Molecular mass distribution

Pectin samples (0.25%, w/w) were dissolved in 0.1 M NaCl solution with addition of 0.1 M ethylenediaminetetraacetic acid (EDTA) to complex any calcium ions present. The solutions were left overnight on a roller-mixer (SRT2, Staurt, Scientific, UK) at room temperature.

The molecular mass distribution of the pectin samples was determined using gel permeation chromatography (GPC), coupled to multiangle laser light scattering (MALLS; DAWN DSP Laser Photometer, Wyatt Technology Corp., USA), refractive index (RI;

Optilab DSP Interferometric Refractometer, Wyatt Technology Corp., USA), and ultraviolet (310 nm; UV Agilent 1100 series, USA) detectors. A 200 μL amount of 0.1% (w/w) polymer solution was passed through two Suprema columns (3000 Å and 30,000 Å) (PSS, D-55120 Mainz, Germany) packed with 10 μm beads of polyhydroxymethacrylate copolymer network in series to a multiangle laser light scattering and a refractive index detector (MALLS/RI) (Optilab DSP, Wyatt Technology Corporation, Santa Barbara Ca93103) at a flow rate of 0.5 mL/min with 0.1 M NaCl as the eluent. The eluent was passed through a degasser (Gastorr 153, Japan) before being pumped using a Knauer HPLC Pump K-501 into the GPC system. All samples were filtered through a 0.45 μm nylon filter prior to injection to the GPC system. A dn/dc value of 0.131 mL/g for pectin was used (Williams et al., 2005), and the data were analysed using the Berry equation.

2.5. Rheological characterization

Due to its high DM, the gelling capacity of the butternut pectin was assessed in the presence of sugar at low pH. Samples were prepared by dissolving pectin (1% w/w) in water and heating to 70 °C; 65% (w/w) sucrose was added with continuous stirring and when the sugar was dissolved, the pH was adjusted to 3.5 with tartaric acid.

Due to its low DM, the gelling capacity of the beetroot pectin was assessed in the presence of calcium ions. The 1% (w/w) pectin systems were prepared by dissolving the pectin in water at 70 °C with continuous stirring and adding $CaCl_2$ to give Ca^{2+} concentrations of 5, 10, 20, 30, 40, 50 mg Ca^{2+}/g pectin.

All samples were rheologically characterized after setting for 30 min. Steady shear viscosity and small deformation oscillation experiments were performed using a controlled stress rheometer (AR 2000; TA Instruments, USA) fitted with cone and plate geometry (50 mm, 2° steel cone, 43 μm gap) and a solvent trap. Sample aliquots were placed onto the rheometer plate and rheological measurements were performed at 25 °C after 20 min of equilibration. The flow properties (viscosity and stress) were obtained at 25 °C at shear rates of 10^{-1}–10^{3} s^{-1}. The Power Law and Herschel–Bulkley equations were considered to fit the stress data obtained. In the small deformation oscillation experiments, stress sweeps were performed on each pectin solution to locate the linear viscoelastic region. Frequency sweeps were performed at 25 °C in the region of ω = 0.1–500 rad/s at a strain amplitude within the linear viscoelastic region. Temperature sweeps were performed by cooling at 2 °C/min from 70 to 20 °C, at a constant frequency of 1 Hz. All measurements were performed in duplicate.

2.6. Interfacial properties

2.6.1. Surface tension

The surface tension of 1% (w/w) pectin aqueous solution was measured using a Profile Analysis Tensiometer PAT-1 (SINTERFACE Technologies, Germany). The surface tension was monitored as a function of surface age until a plateau value was reached. Prior to each measurement, calibration of the instrument was carried out using deionized water. Determinations were performed in triplicate.

2.6.2. Preparation of emulsions

Pectin solutions were prepared by stirring pectin in water with gentle heating at 65 °C to ensure complete dissolution. 16 g of pectin solution at various concentrations (0.5–5.0%, w/w) were added to 4 g of middle-chain triglyceride (MCT) oil. The mixtures were subjected to shaking for 30 s immediately prior to homogenization for 1–5 min using the Ultra-Turrax mixer (24,000 rpm) and the optimum emulsification time was determined.

2.6.3. Droplet size

Droplet size was measured using the Malvern Mastersizer 2000 (Malvern, UK) in triplicate. A few drops of the emulsion were added to water in the dispersion unit of the instrument until the obscuration was ~15%.

2.6.4. Emulsion stability

Measurements were carried out to determine the effect of pectin concentration on droplet size distribution and stability. The emulsions were stored at room temperature and the droplet size was measured over a period of weeks. Gum Arabic emulsions were also prepared in order to compare their performance with those of pectin emulsions. Additionally, the emulsion stability was followed

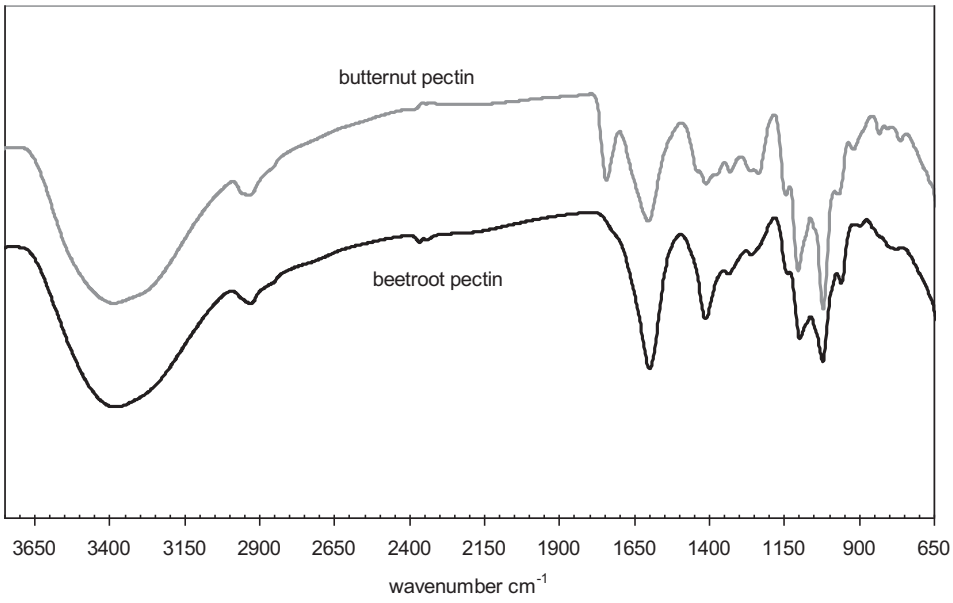

Fig. 1. FTIR spectra recorded for butternut and beetroot pectins.

in time by Image Analysis using a Flow Particle Image Analyzer Sysmex FPIA 3000 (Sysmex Corporation, Japan). Experiments were carried out in triplicate.

2.6.5. Surface active components of pectin

A 0.1% (w/w) pectin solution was prepared by gentle heating in water with stirring. A 20% (w/w) MCT oil emulsion was prepared with each sample by mixing with an Ultra-Turrax mixer (24,000 rpm) for 3 min. The emulsion was then centrifuged at 1000 × g for at least 10 h until complete separation of the aqueous and oil phases was achieved.

The molecular mass distribution of the pectin solution prior to emulsification and of the aqueous supernatant layer obtained after centrifuging the emulsion, were determined by GPC. The amount of pectin adsorbed onto the oil droplets was calculated from the change in the intensity of the refractive index elution profile before and after emulsification. Experiments were performed in duplicate.

2.7. Statistical analyses

Non-linear fittings were performed through Prism 5 Statistical Software for Windows (GraphPad, USA), as well as through the Solver function of the Excel program for Windows XP (Microsoft, USA). Statistical analyses was carried out through ANOVA (level of significance, $\alpha = 0.05$) followed by pairwise multiple comparisons using Tukey's significant difference test (Sokal & Rohlf, 1995).

3. Results and discussion

3.1. Ferulic acid content

The amount of ferulic acid (FA) present in the samples was found to be ~0.1 g/100 g and ~0.03 g/100 g for butternut and beetroot, respectively. It must be stated that there are no reports of the presence of ferulic acid in pumpkin mesocarp although other

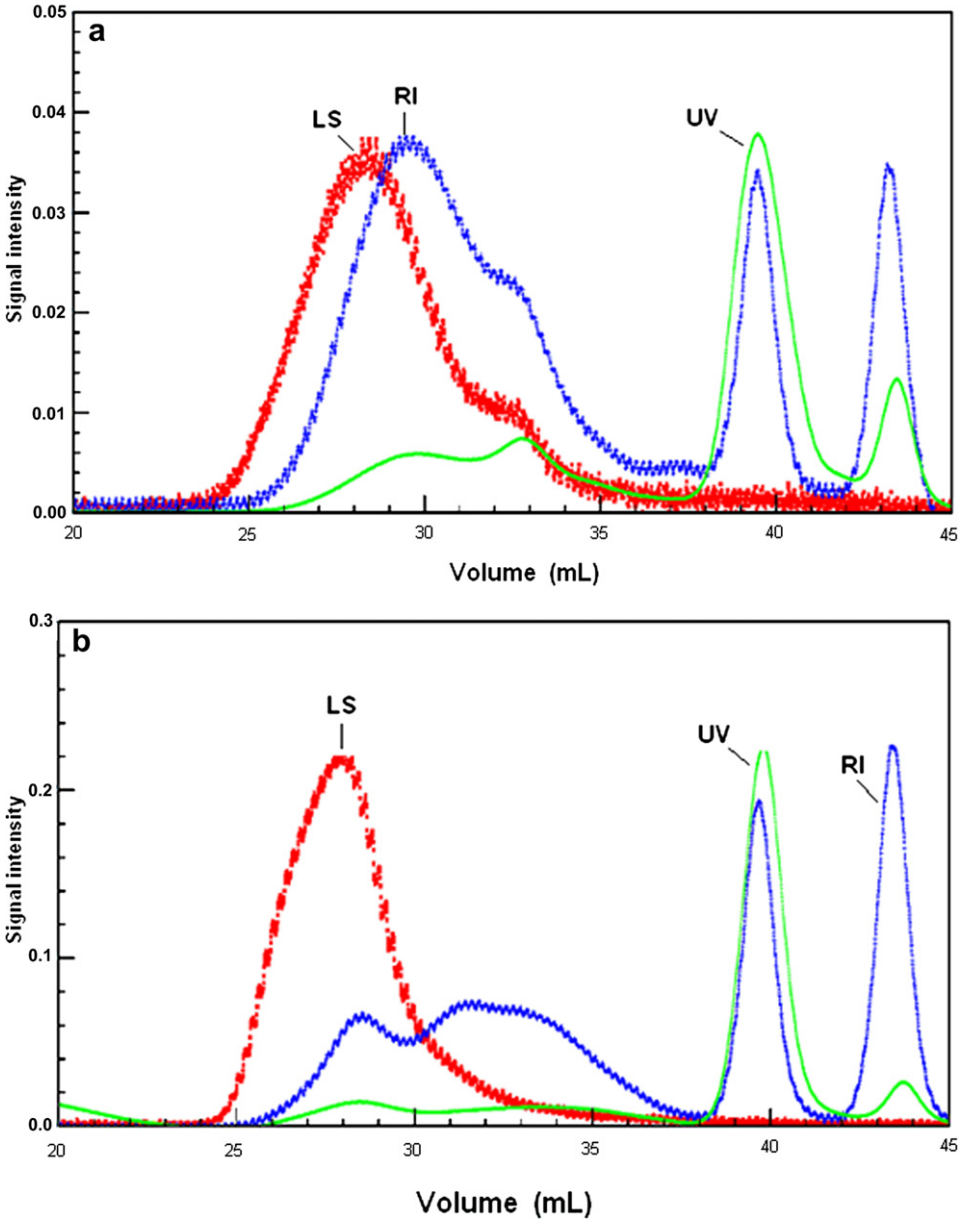

Fig. 2. GPC profiles for butternut (a) and beetroot (b) pectins.

phenolic compounds have been found (Dragovic-Uzelac, Delonga, Levaj, Djakovic, & Pospisil, 2005; Peričin, Krimer, Trivič, & Radulovič, 2009). The *B. vulgaris* family has been found to contain ferulic acid (Ralet et al., 2005) but given the alkaline pretreatment performed to extract the beetroot pectin, a very low FA content was expected. Oosterveld, Beldman, Schols, and Voragen (1996) reported the existence of FA in sugar beet pectin fractions isolated by different techniques, some of which involved saponification. The FA content in those samples was in the 0.03–0.18% range. Buchholt, Christensen, Fallesen, Ralet, and Thibault (2004) also isolated sugar beet pectin fractions by saponification and obtained fractions with FA contents of 0.69–0.84%.

3.2. FTIR analysis

The FTIR spectra can be observed in Fig. 1. The wavelength range of 950 and 1200 cm^{-1} is considered as the 'finger print' region for carbohydrates as it allows the identification of major chemical groups (Cerná, Barros, Nunes, Rocha, Delgadillo, Copikova & Coimbra, 2003). In this spectral region, the characteristic peaks corresponding to the typical profile of polygalacturonic acid (964, 1020, 1095 and 1130 cm^{-1}) are present in both butternut and beetroot (Fig. 1) pectin spectra (Kačuráková, Capek, Sasinková, Wellner, & Ebringerová, 2000; Lee, Warner, & Inglett, 2005). Three other peaks observed at 1242 (attributable to –C–O–C–), 1322 and 1402 cm^{-1} by Lee et al. (2005) for polygalacturonic acid are also well distinguished for beetroot pectin, but are masked for butternut pectin (Fig. 1). Other noticeable differences between the isolated pectins is the absence of the typical band at 1750 cm^{-1} in the beetroot spectrum, which corresponds to the carbonyl stretching of the methyl esterified carboxylate group. The phenolic ester peak at 1517 cm^{-1} corresponding to the feruloyl ester is absent which is consistent with the low FA content previously reported.

A broad band at 2935 cm^{-1} that corresponds to the OH-stretching in the carboxylic group, and one at ≈ 3363 cm^{-1} typical of the –OH stretch in the carbohydrate backbone can be observed in the spectrum of butternut and beetroot pectins (Fig. 1). Both are characteristic of polysaccharides from cell walls (Coimbra, Barros, Barros, Rutledge, & Delgadillo, 1999).

3.3. Molecular mass distribution

The molecular mass distribution of both pectins (Fig. 2) was determined using gel permeation chromatography (GPC) coupled to multiangle laser light scattering (MALLS), refractive index (RI) and ultraviolet (UV, 214 nm) detectors. RI is a sensitive measurement of concentration, whereas UV absorbance is sensitive also to the chemical nature of the eluting species, particularly the aromatic components. LS is sensitive to concentration and molecular mass (Mahendran, Williams, Phillips, Al-Assaf, & Baldwin, 2008). From the RI elution profiles we can observe that the major portion of both samples elutes between 25 and 38 mL and that these fractions contain a small amount of protein and/or phenolic compounds since there are also two peaks observed by UV. There are also RI and UV peaks at ~40 mL and 43 mL corresponding to lower mass components. The component at 40 mL has a large UV peak indicating it contains protein and/or phenolics.

The molecular mass values calculated from MALLS are in Table 1 and indicate a weight average molecular mass (M_w) of 136,000 g/mol for butternut pectin and 1,309,000 g/mol for beetroot pectin. The polydispersity index of natural polysaccharides is usually in the range of 1.5–2.0. In our case, the butternut fraction presented a polydispersity index of ~2.0 and beetroot of ~5.0.

Table 1
Average molecular mass for butternut and beetroot pectins determined through gel permeation chromatography (GPC).

Average molecular mass (kDa)	Butternut pectin	Beetroot pectin
M_n	67.2	253.4
M_w	136.0	1309.0
M_z	256.2	3037.0

Number average molecular mass (M_n), weight average molecular mass (M_w), z-average molecular mass (M_z).

3.4. Rheological characterization

3.4.1. Flow behaviour of butternut pectin

As noted earlier the butternut pectin has a high DM value and hence the gelling capacity was studied at low pH and high sugar concentration. The system consisted of 1% (w/w) butternut fraction in water with 65% (w/w) of sucrose and a pH of 3.5. A 65% (w/w) sucrose solution was evaluated as the control system. However, it was found that the viscosity of the system increased but that gelation did not occur. This is likely to be due to the fact that this sample has a low GalA content and a high DA and in addition has a relatively low M_w.

The viscosity – shear rate flow curves for the sample and control are shown in Fig. 3. The control system showed a Newtonian behaviour with a constant viscosity of 0.134 Pa s. The butternut pectin system showed a Newtonian plateau of 0.725 Pa s at low shear rates and pseudoplastic behaviour at higher shear rates. At shear rates above 34 s^{-1}, the Power Law adequately fitted ($R^2 = 0.999$) to the experimental data of stress (Pa) *vs* shear rate (s^{-1}) and the consistency index (K) and the flow behaviour index (n) were found to be 2.11 ± 0.40 Pa.s^n and 0.68 ± 0.03, respectively.

3.4.2. Gelling capacity of beetroot pectin

Given its low DM, the gelling properties of the beetroot fraction were evaluated in the presence of different concentrations of calcium ions which are known to specifically bind to sites along the (1,4) linked α-D-galacturonic acid chains (Fry, 1986). The results are presented in Fig. 4. As can be observed, systems with Ca^{2+} concentration lower than 10 mg/g pectin gave a predominantly viscous response. In the absence of Ca^{2+} (0 mg/g pectin), G'' was higher than G' and considerably dependent on frequency (Fig. 4a) which is characteristic of the mechanical spectra of polymer solutions at concentrations below the coil overlap concentration (Ross-

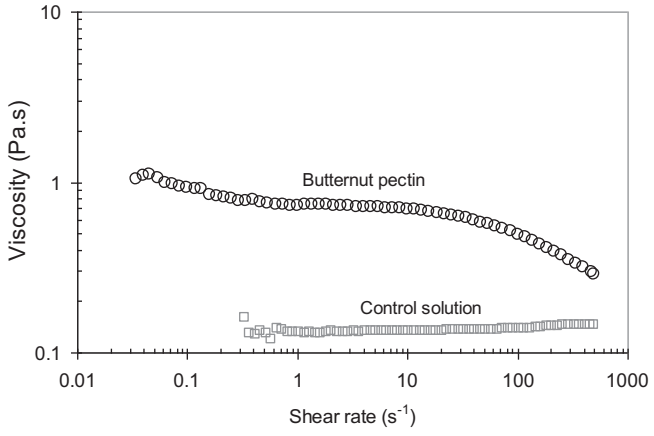

Fig. 3. Flow behaviour (25 °C) of an aqueous system containing butternut pectin (1% w/w) in the presence of sugar (65% w/w) at pH = 3.50. A control solution of 65% (w/w) sucrose was run for comparison.

Murphy, 1994). A similar mechanical spectrum was showed by the system with the lowest calcium concentration (5 mg Ca^{2+}/g pectin). Strong gels were obtained at Ca^{2+} concentrations of 10 mg Ca^{2+}/g pectin and above. Networks constituted from 20 mg Ca^{2+}/g pectin shown a G' value of ~100 Pa, with only a slight dependence on frequency, which is characteristic of the mechanical spectra of strong physical gels (Doublier, Launay, & Cuvelier, 1992; Lapasin & Pricl, 1995; Ross-Murphy, 1994). The viscous modulus (G'') showed a characteristic minimum herein between 3 and 8 rad/s (Lapasin & Pricl, 1995). This minimum of $G''(\omega)$ can often be observed within the frequency window, giving an indication of the presence of a low-frequency loss peak (Lefebvre & Doublier, 2005). This phenomenon may correspond to a period of oscillation which is long compared with the relaxation times of the network junction zones in water solvent but short compared with their lifetime (Grassi, Lapasin, & Pricl, 1996). In physical gels, the junction zones have finite energy and lifetime; besides, their number, size, and position fluctuate with time and temperature.

Systems containing 30, 40 or 50 mg Ca^{2+}/g pectin showed mechanical spectra with some similarities between them but different from that of 20 mg Ca^{2+}/g pectin-system. Their G' values were close to 1000 Pa, increasing slightly with the frequency. The G'' profile of 30 mg Ca^{2+}/g pectin system showed similar frequency dependence between 0.1 and 500 rad/s, showing an almost imperceptible minimum close to 0.1 rad/s. It can be thought that this profile corresponds to a strong and well constituted visco-elastic gel in the entire frequency window assayed. On the other hand, beetroot systems containing 40 or 50 mg Ca^{2+}/g pectin showed mechanical spectra with G' values slightly more frequency dependent than that of 30 mg Ca^{2+}/g pectin-system. At the same time, their G'' profiles were considerably more dependent on frequency with a minimum observed near 0.1 rad/s. This suggests some instability of the gel network developed from Ca^{2+} addition at these two highest concentrations. It is known that the elastic modulus (G') is proportional to the number of cross links from which a network is developed (Doublier et al., 1992).

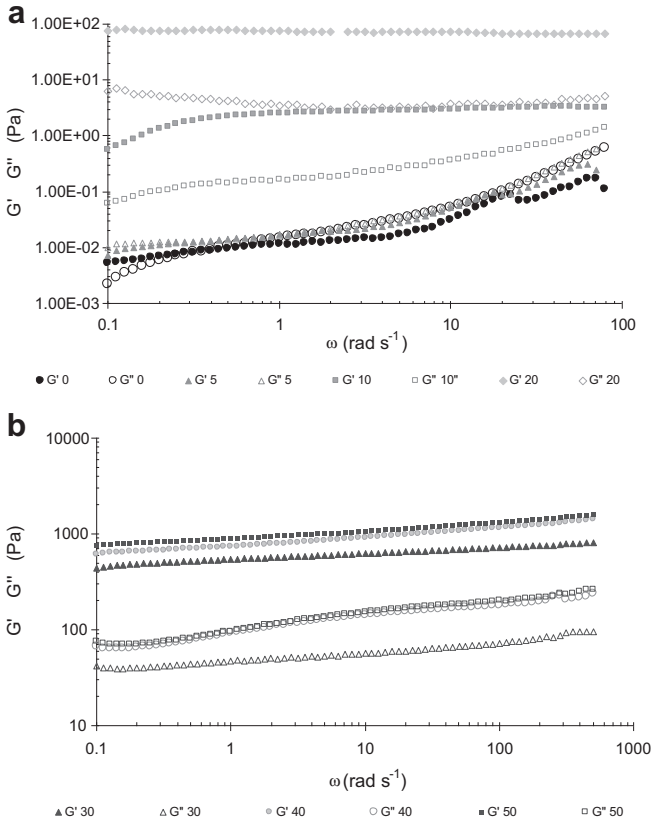

Fig. 4. Mechanical spectra (G' and G'' against angular frequency, ω) recorded at 25 °C from aqueous systems constituted by beetroot pectin (1% w/w) and one of the following calcium concentrations: 0, 5, 10 or 20 mg Ca^{2+}/g pectin (a) and 30, 40 or 50 mg Ca^{2+}/g pectin (b).

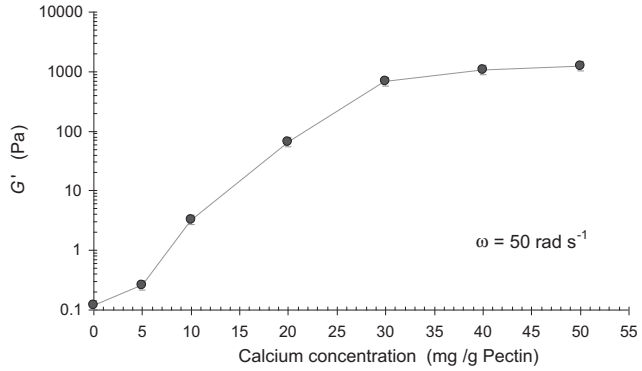

Fig. 5. Influence of calcium concentration on the storage moduli (G') of beetroot pectin gels, recorded at constant angular frequency (ω) and temperature (25 °C). Bars correspond to the standard deviation ($n = 3$).

The influence of Ca^{2+} concentration on the G' modulus is shown in Fig. 5, considering the values recorded at $\omega = 50$ rad/s from the corresponding spectrum. G' values increased continuously up to ~30 mg Ca^{2+}/g pectin, showing no further increase in the density of calcium cross links (junction zones) at higher amounts of Ca^{2+}. This concentration of Ca^{2+} corresponds to a Ca^{2+}/GalA ratio of 0.25, i.e. 1 Ca^{2+} bound for every 4 carboxylate groups. This is less than expected from stoichiometric considerations (0.5) but is in agreement with the values reported by Siew, Williams, and Young (2005) and Fang et al. (2008) for low methoxyl pectin and high methoxyl pectin. It is also consistent with the egg-box model (Grant, Morris, Rees, Smith, & Thom, 1973) in which one calcium ion per four carboxylate groups is the expected ratio for calcium-mediated association of two polygalacturonate strands (Morris, Powell, Gidley, & Rees, 1982).

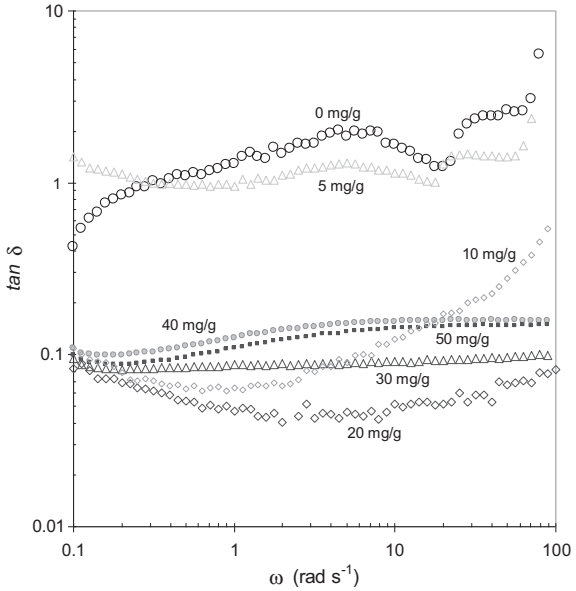

Fig. 6. Loss factor ($tan\ \delta$) plotted against frequency (25 °C) for beetroot systems containing different calcium concentrations (mg Ca^{2+}/g pectin).

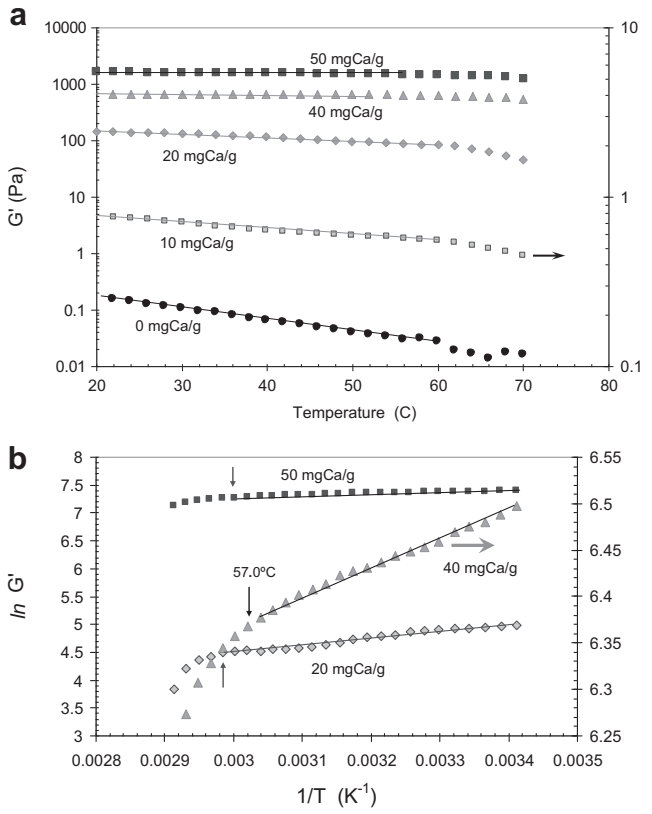

Fig. 7. (a) Storage (G') modulus profiles recorded during a cooling ramp performed at 2 °C/min from 70 to 20 °C (1 Hz constant frequency) for beetroot pectin systems with different calcium concentrations (mg Ca^{2+}/g pectin). (b) Experimental data from some systems are plotted in the Arrhenius form. Arrows pointing right indicate the secondary axis on which the G' profile is plotted. Each vertical arrow (b) indicates the point at which the slope changes.

Siew et al. (2005) showed that the maximum amount of Ca^{2+} ions bound to pectin and other polyelectrolytes (alginate and polyacrylate) occurred when the effective linear charge density had a value of ~1. Siew et al. (2005) noted that the distance between the carboxylate groups along the pectin chain was greater than the hydrodynamic radii of the Ca^{2+} ions. They, therefore, argued that it was not possible for a Ca^{2+} ion to interact simultaneously with two carboxylate groups and hence monocomplexes were formed which had a net positive charge, i.e. charge reversal. These positively charged groups were then able to interact with carboxylate groups on adjacent chains to form junction zones.

Differences observed between the mechanical spectra obtained from the studied concentrations of Ca^{2+} in the beetroot pectin systems can be better seen from plotting *tan δ* (G''/G' or dissipated energy/stored energy) against frequency (Fig. 6). Hookean or ideal solids show *tan δ* values of 0 whereas Newtonian (ideal) liquids are characterized by *tan δ* values > 1 or tending to infinite (Steffe, 1996, pp. 316–317). Between these extremes, viscoelastic materials such as polysaccharide solutions and gels are found and the differences above analysed from the mechanical spectra are more evident in the *tan δ* profiles. Fig. 6 shows that 0 and 5 mg Ca^{2+}/g systems were characterized by *tan δ* values of ~1. The system without Ca^{2+} showed a greater dependence on frequency, with *tan δ* values in general higher than 1. A minimal amount of Ca^{2+} (5 mg Ca^{2+}/g) led to lower frequency dependence but the system was fluid (*tan δ* ~ 1). Above 5 mg Ca^{2+}/g of pectin, systems showed *tan δ* < 1 which indicates that the networks are more elastic. The system with 30 mg Ca^{2+}/g pectin showed a *tan δ* ~0.1 and was frequency

Table 2
Activation energy (E_a)[a,b] calculated from the elastic modulus (G') scans against temperature, which were recorded during a cooling ramp performed at 2 °C/min on the beetroot systems.

Calcium concentration (mg/g pectin)	E_a (kJ/mol)
0	38.1 ± 0.2^{A}
10	7.0 ± 0.3^{B}
20	11.0 ± 0.6^{C}
30	0.6 ± 0.2^{D}
40	2.5 ± 0.3^{E}
50	1.8 ± 0.3^{E}

[a] Mean and standard deviation ($n > 4$) are shown.
[b] The same capital letter into a column means non significant differences ($p < 0.05$).

Table 3
Surface tension (ST) of aqueous systems of butternut and beetroot pectins compared to commercial pectins, gum Arabic, sodium dodecyl sulphate (SDS) and water.

Sample	ST (mN/m)
Water	71.1
Butternut pectin	*60.4*
Beetroot pectin	*54.4*
Sugar beet pectin	54.5
High DM citrus pectin	62.7
Low DM apple pectin	69.2
Gum Arabic	57.1
SDS	37.9

Names in italics correspond to the pectins analised in the present work.

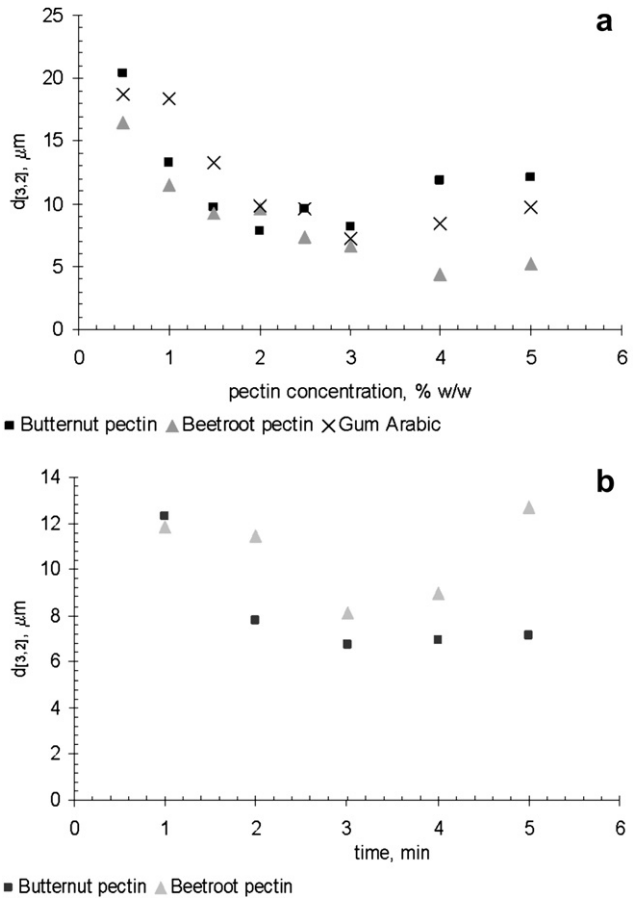

Fig. 8. Effect of beetroot or butternut pectin concentration (a) and time of stirring (b) on the emulsion droplet size. Arabic gum behaviour is also reported for comparison (a).

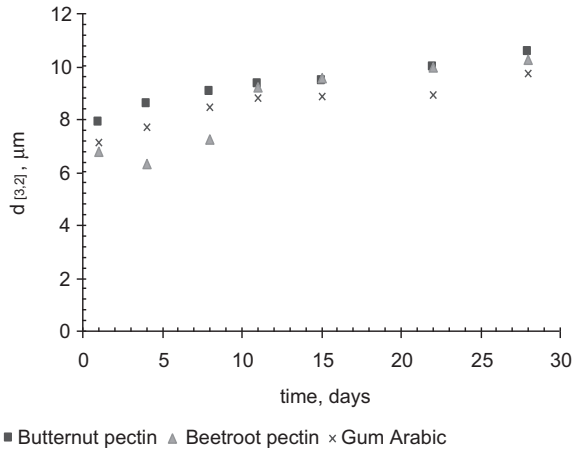

Fig. 9. Effect of pectin on emulsion stability. Droplet size (d) is plotted against the storage time of emulsion at room temperature. Gum Arabic behaviour is also reported for comparison.

independent in the entire frequency window assayed (Fig. 6), reflecting that observed in its mechanical spectrum (Fig. 4b). From these results, it can be suggested that this system was the most elastic gel.

Samples of the Ca^{2+}-pectin systems were studied on cooling at 2 °C/min and the results are shown in Fig. 7a. It is seen that the G' values increased slightly on cooling and it is likely that this is due to enhanced association due to, for example, hydrogen bonding, dipolar and van der Waals attractions (Kastner, Einhorn-Stoll, & Senge, 2012).

The temperature dependence of G' was evaluated through the Arrhenius relationship (Fig. 7b). The activation energies were then calculated and the results are shown in Table 2. It is noted that the activation energy for gel formation is significantly less in the presence of Ca^{2+} ions as expected since the Ca^{2+} ions promote junction zone formation. The lowest activation energy was observed in the presence of 30 mg Ca^{2+}/g pectin followed by 40 and 50 mg Ca^{2+}/g pectin (Table 2). The systems containing 10 and 20 mg Ca^{2+}/g were more dependent on temperature, probably due to reduced crosslinking by the Ca ions and consequently weaker junction zones. Once again 30 mg Ca^{2+}/g pectin was observed as the optimum Ca concentration for gelling of beetroot pectins.

3.5. Interfacial properties

3.5.1. Surface tension

The surface tension values (ST) of 1% (w/w) aqueous solutions of the butternut and beetroot pectins are shown in Table 3 together with those of sodium dodecyl sulphate (SDS), gum arabic and other pectins for comparison (Siew & Williams, 2008). It can be observed that both pectins are surface active and reduce the surface tension. The butternut fraction gave an ST value very similar to that of commercial citrus pectins whereas beetroot pectin gave a lower ST value than both sugar beet pectin and gum Arabic. The reduction in the surface tension is likely to be due to the relatively high level of protein present (Leroux et al., 2003; Williams et al., 2005).

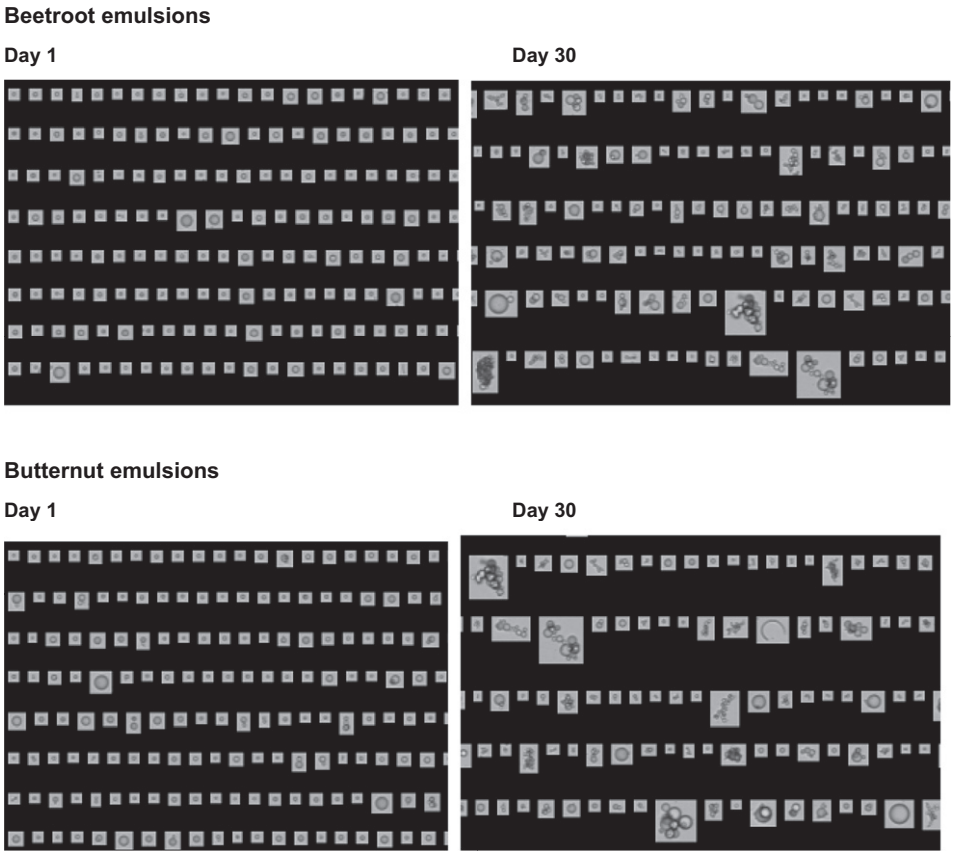

Fig. 10. Evaluation of the emulsion particle size by the Flow particle image analyzer (FPIA) in systems constituted by 10% w/w MCT oil and beetroot (3% w/w) or butternut pectin (4% w/w).

3.5.2. Effect of pectin concentration on droplet size

The droplet size of 10% (w/w) MCT oil in water emulsions prepared using different pectin and gum arabic concentrations (0.5–5%, w/w) was measured 1 h after preparation and the results are reported in Fig. 8. The droplet size decreased with increasing polysaccharide concentration up to an optimum value indicating full coverage of the oil droplets by the polymer molecules (Siew & Williams, 2008). The minimum droplet size ($d_{[3,2]}$) was obtained at a pectin concentration of 2% (w/w) for butternut pectin, 4% (w/w) for beetroot pectin and 3% (w/w) for gum arabic. These were the concentrations chosen to perform the subsequent experiments.

3.5.3. Optimum emulsification time

Once the optimum polymer concentration was determined, 10% (w/w) middle-chain triglyceride (MCT) oil emulsions were prepared with different emulsification times (1–5 min) using an Ultra-Turrax at 24,000 rpm and the droplet size was determined in order to find the optimum emulsification time. These results were confirmed by Flow Particle Image Analysis (FPIA). It was found that 3 min of emulsification yielded the lowest droplet size (Fig. 8b).

3.5.4. Emulsion stability

10% (w/w) MCT oil emulsions prepared with the optimum pectin and gum arabic concentrations were monitored as a function of time over a 30-day period of storage at 25 °C. The results are shown in Fig. 9.

Over the first 10 days, beetroot pectin had a smaller droplet size when compared with butternut pectin and gum arabic. All of the emulsions showed a slight increase in droplet size over the 30 days of storage, with no difference in the final droplet size between the pectins and gum arabic ($p < 0.05$; $n = 3$).

Images obtained using FPIA for emulsions prepared after day 1 and day 30 are shown in Fig. 10. It is noted that the increase in droplet size (Fig. 9) is mainly due to flocculation of the droplets although there is some evidence of droplet coalescence.

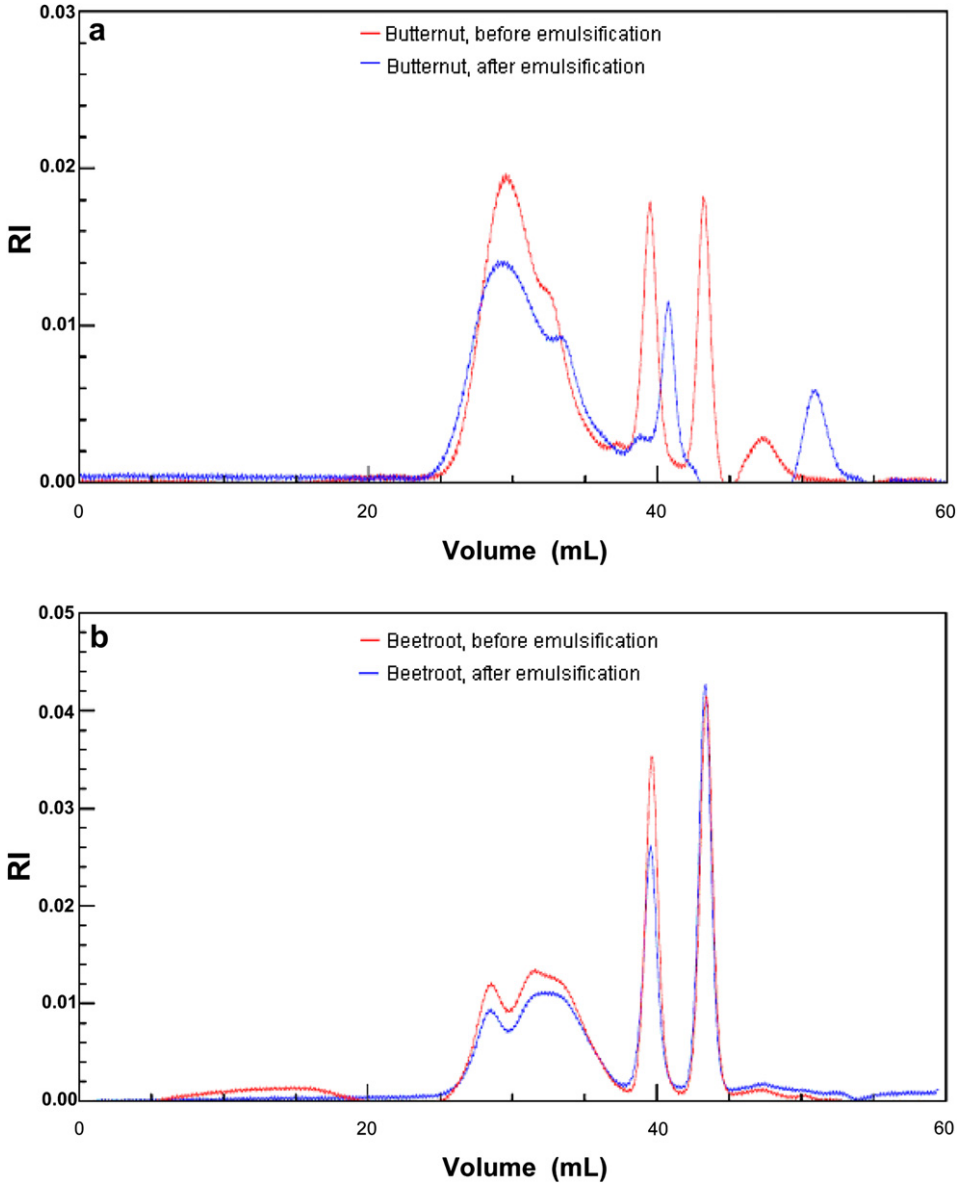

Fig. 11. Preferential adsorption of butternut (a) and beetroot (b) pectin during emulsification evaluated through GPC with RI detection.

3.5.5. Adsorption of pectin fractions during emulsification

The adsorption of the butternut and beetroot pectins onto oil droplets was investigated by measuring the molecular mass profile of the pectin before and after the emulsification process. Briefly, emulsion samples of 20% (w/w) MCT oil were prepared with beetroot and butternut pectins at a concentration of 0.1% (w/w). The emulsion samples were centrifuged for ~24 h until a clear aqueous phase was obtained at the bottom of the centrifuge tube. The aqueous layer was carefully taken out with a syringe connected to a needle and analysed with GPC-MALLS-RI. The GPC-RI chromatograms are presented in Fig. 11 (a and b) which show the RI signal profiles for butternut and beetroot pectin samples, respectively, before and after emulsification with 20% MCT oil. The RI signal is proportional to the concentration of solute (polymer) present, and hence from the change in intensity of the RI signals the amount of pectin adsorbed can be calculated. For the butternut pectin some high molecular mass material eluting between 25 and 35 mL is adsorbed but also a significant amount of lower molecular mass material which elutes at 40 mL and 43 mL. As discussed above, this material is known to be rich in protein and/or polyphenols. For the beetroot pectin there appears to be a smaller amount of material adsorbed which includes the higher molecular mass fractions eluting between 5 and 20 mL and 25 and 35 mL and some lower mass components eluting at 40 mL. All of these fractions give rise to a UV signal and hence are expected to contain protein and/or polyphenols. These findings are in keeping with results reported for the emulsification properties of sugar beet pectin in which fractions rich in protein and ferulic acid were found to adsorb at the oil–water interface (Siew & Williams, 2008).

4. Conclusions

The rheological and interfacial properties of butternut (*Cucumis moschata* Duch. ex Poiret) and beetroot (*B. vulgaris* L. var. conditiva) pectins previously extracted through enzymatic treatment were evaluated.

The butternut pectin had a high degree of methyl esterification but formed a viscous solution rather than a gel even in the presence of sugar and at low pH. Solutions showed pseudoplastic behaviour without yield stress (τ_0). On the other hand beetroot pectin had a low degree of methyl esterification and formed gels with addition of Ca^{2+} at concentrations of 10 mg/g pectin or higher. The maximum gel strength was obtained at a Ca^{2+}/GalA ratio of 0.25.

The surface tension of butternut pectin was similar to that reported for citrus pectin while the surface tension for beetroot pectin was lower and similar to that of sugar beet pectin. Both pectins were able to stabilize oil in water emulsions over a 30 day period and it is apparent that the fractions adsorbed at the oil–water interface are rich in protein and/or polyphenols.

Pectins recovered as by-products of vegetable processing, therefore, seem to be promising materials as food additives.

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