

Pectin from *Cucurbita moschata* Pumpkin Mesocarp

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Abstract

Pumpkin has been mentioned as a great source of natural and low-cost pectin and has been regarded as a functional food for applications in food and pharmaceutical formulations. In this work, low-methoxyl pectin was extracted from pumpkin *Cucurbita moschata* species by acid hydrolysis and characterized by chemical techniques. Infrared spectroscopy characterized isolated pectin, ¹H, ¹³C Nuclear Magnetic Resonance, Gel Permeation Chromatography, elemental analysis, rheology and metal determination. ¹H NMR was used to quantify the DM which was 44%. The determined molecular mass was 9.5×10^5 g/mol. Rheological study of continuous shear of pectin solutions showed shear-thinning behaviour and gel formation in presence of calcium ions. The results suggest that this pumpkin pectin may be used both in food and pharmaceutical ionotropic gels formulations.

Keywords: Pectin; Pumpkin; NMR; Rheology; GPC

Introduction

Pumpkin is a basic food which has a social and economic role for the society with many functional elements such as vitamins and it is a low-cost source of pectin [1-5]. Some authors have also reported on pumpkin pectin extraction and characterization for industrial or pharmaceutical applications [1,6-10]. Pectin is a complex structure of molecules present in cell walls of all plants [11]. The polysaccharide comprises polymers containing galacturonic acid, rhamnose, arabinose and galactose and other different monosaccharides and it is recognized that the three major polymeric constituents are homogalacturonan, rhamnogalacturonan I and rhamnogalacturonan II [12,13]. The composition, structure, and physiological properties of pectin can be influenced by extraction conditions as well as source, location and many other environmental factors. The network of pectin must be broken to be extracted. This may involve extraction with calcium chelating agents, dilute bases, or dilute acids. Alternatively, fragments of polysaccharides can be released using enzymatic degradation. Pectin's are typically extracted from citrus fruits and apple pomace. It is traditionally used as a gelling agent for jellies and marmalades and it can

also act as a thickener, gelling agent, stabilizer, emulsifier, cation-binding agent, etc. [14]. It is listed among the ingredients of many food products.

Pectin is a biopolymer especially valuable for medicine, food production as well as for applications in bioactive encapsulation in spray-drying, ionotropic gelation, and other formulations [15-18]. Pectin's also offer health benefits to consumers, for example, they are being increasingly recognized as important precursors of substrates for gastrointestinal functions and structures. Foods rich in fibers, such as pectin's, guar gums and starch are usually recommended for diabetics, because they can reduce the glycemic response and thus reduce the need for insulin [19]. The polysaccharide is also effective on lowering the cholesterol level in blood, removing heavy metal ions from the body, stabilizing blood pressure, and restoring intestinal functions [20]. The search for new pectin-containing raw materials is an important task of science and industry [21], especially from abundant and low cost raw material. In this work, pectin was extracted from pumpkin *Cucurbita moschata* species and characterized by different techniques.

Experimental

Extraction of pectin

For extraction of pectin, mass of about 5 kg of pumpkin pulp was pressed in an expeller type press (INCOMAP, Brazil) with a force adjusted to 805 N. Pumpkin mesocarp was processed and used for pectin extraction (approx. 209 g). The processed mesocarp was dried in an oven at 60°C for 12 hours forming a paste that was used for the extraction procedure. For extraction by acid hydrolysis, 2 L of 0.1 mol/L HCl solution was stabilized at 65°C and after that, approx. 200 g pumpkin pulp was added to the solution and let extracting for 2 hours. After precipitation and washing with ethanol PA (1:10-solution: ethanol), filtration and freeze-drying, 4.7 g of pectin was obtained. After purification by dialysis membranes remained 2.5 g of pectin, which was used for further analysis (yield calculated from pumpkin dry fiber of approx. 6.9%).

Infrared spectroscopy (FTIR)

The Fourier transform IR spectrum (FT-IR) of pectin was recorded using a Shimadzu IR spectrophotometer (model 8300) in the range of 400 cm⁻¹ and 4000 cm⁻¹ as KBr pellet.

¹H and ¹³C Nuclear magnetic resonance (NMR)

NMR spectra of 0.1% (w/v) solutions in D₂O were recorded at 70°C on a Fourier Transform Bruker Avance DRX 500 spectrometer with an inverse multinuclear gradient probe-head equipped with z-shielded gradient coils, and with Silicon Graphics. Sodium 2, 2-dimethylsilapentane-5-sulphonate (DSS) was used as the internal standard (0.00 ppm for ¹H).

Gel permeation chromatography (GPC)

The peak molar mass (M_{pk}) of pectin was determined by gel permeation chromatography (GPC) using a Shimadzu instrument (Ultra hydrogel linear column, 7.8×300 mm), at room temperature, flow rate of 0.5 mL/min, polysaccharide concentration of 0.1% (w/v) and 0.1 mol/L NaNO_3 as the solvent. A differential refractometer was used as detector. The elution volume was corrected using the internal marker ethylene glycol at 11.25 mL. Pullulan samples (Shodex Denko) of molar mass 5.9×10^3 , 1.18×10^4 , 4.73×10^4 , 2.12×10^5 , and 7.88×10^5 g/mol were used as standards.

Elemental analysis: Protein content

The elemental analysis of carbon, hydrogen and nitrogen of pumpkin pectin was performed using a microanalyzer Carlo ERBA EA 1108.

Rheological measurements (REO)

All REO were performed using an Advanced Rheometer AR550 (DP Union). Geometry cone-and-plate (40 mm diameter and angle of $0^\circ 59' 1''$) was used for measurements of continuous flow. The viscosity of continuous flow was determined at 25°C in the shear range of $1\text{-}100 \text{ s}^{-1}$. Aqueous pectic solution at concentration of 1% (w/v) was prepared dissolving the polysaccharide overnight before measurements. Pectin/calcium solution was prepared dissolving 1% calcium (w/v), one hour before the rheological experiments. After complete calcium dissolution, the system was let to equilibrium for app. 20-30 minutes.

Metals content determination by inductively coupled plasma atomic emission spectrometry (ICP-OES)

Metals content was quantified by inductively coupled plasma atomic emission spectrometry (ICP), consisting of three steps: sample opening, construction of a standard curve and metals analysis. A Perkin-Elmer spectrophotometer model OPTIMA 4300 DV was used for the measurements.

Results and Discussion

Infrared spectroscopy

An overview of the IR spectrum of pectin is shown in FIG. 1. The "fingerprint" region of the spectrum (up to approx. 2000 cm^{-1}) includes the region of 1200 cm^{-1} - 1800 cm^{-1} as shown [22]. The band at 1743 cm^{-1} is indicative of the stretching group $\text{C}=\text{O}$ of non-ionized carboxylic acid (methylated or protonated). Its ionization (formation of salt) leads to their disappearance, and the appearance of stretch modes of COO^- in approx. $1600\text{-}1650 \text{ cm}^{-1}$ and $1400\text{-}1450 \text{ cm}^{-1}$, respectively [22]. The degree of methylation (DM) is defined as the amount of ester groups compared to the total amount of acid groups and carboxylic

ester. FTIR spectra can be useful to characterize the state of carboxylic acid groups but Nuclear Magnetic Resonance can give more precise results in DM calculations.

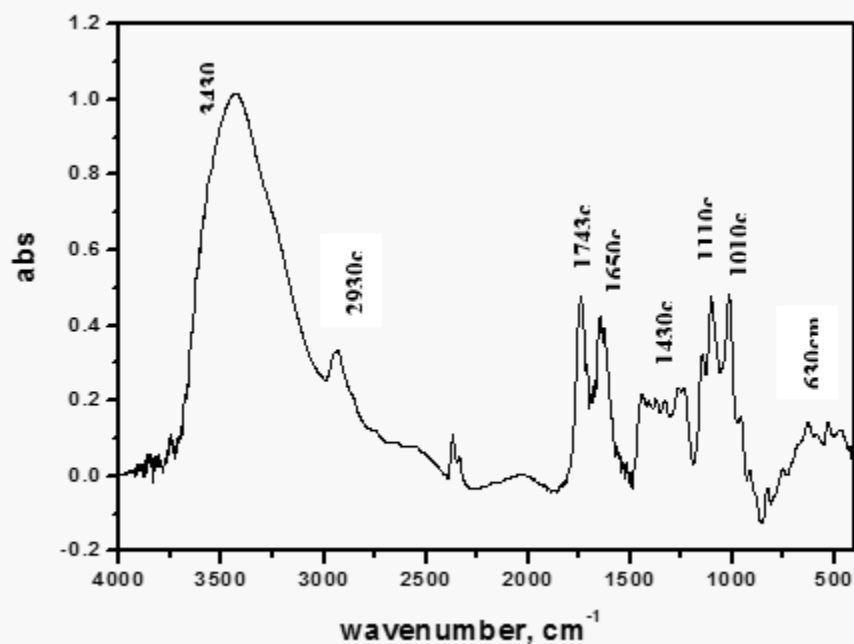


FIG. 1. FTIR main assignments of pumpkin pectin spectrum.

Determination of DM by ¹H nuclear magnetic resonance (NMR)

For DM determination, we used Grasdalen method of DM determination by ¹H NMR. By using this method [23,24], it is possible to characterize pectins having a specific DM, and specific gelling properties that are dependent on DM [23-25]. For the determination of DM, the integrals of H-5 adjacent to ester (I_{COOMe}) are compared with the sum of integrals of H-1 adjacent to the ester (I_{COOMe}) and H-5 adjacent to the carboxylate (I_{COO}). Due to proximity (or overlap) of the signals for H-1 and H-5COOMe, it is only possible to determine the full combined to H-1 and H-5COOMe ($I_{\text{H1}}+I_{\text{COOMe}}$). The value of DM was calculated as 44% (FIG. 2) and the pectin was classified as low-methoxyl. Low-methoxyl pectins have been studied for bioactive encapsulation applications using ionotropic gelation methods [15,17,26,]. We can also observe in the spectrum of the polysaccharide, a very large signal at 3.81 ppm related methyl groups binding to carboxyl groups of galacturonic acid. Signals around 2.1 ppm are related to acetyl groups and were not observed for the pectin. There are other signals related to D-galacturonic acid: H-1, 5.09 ppm; H-2, 3.76 ppm; H-3, 3.97 ppm; H-4, 4.41 ppm; H-5, 4.68 ppm [27].

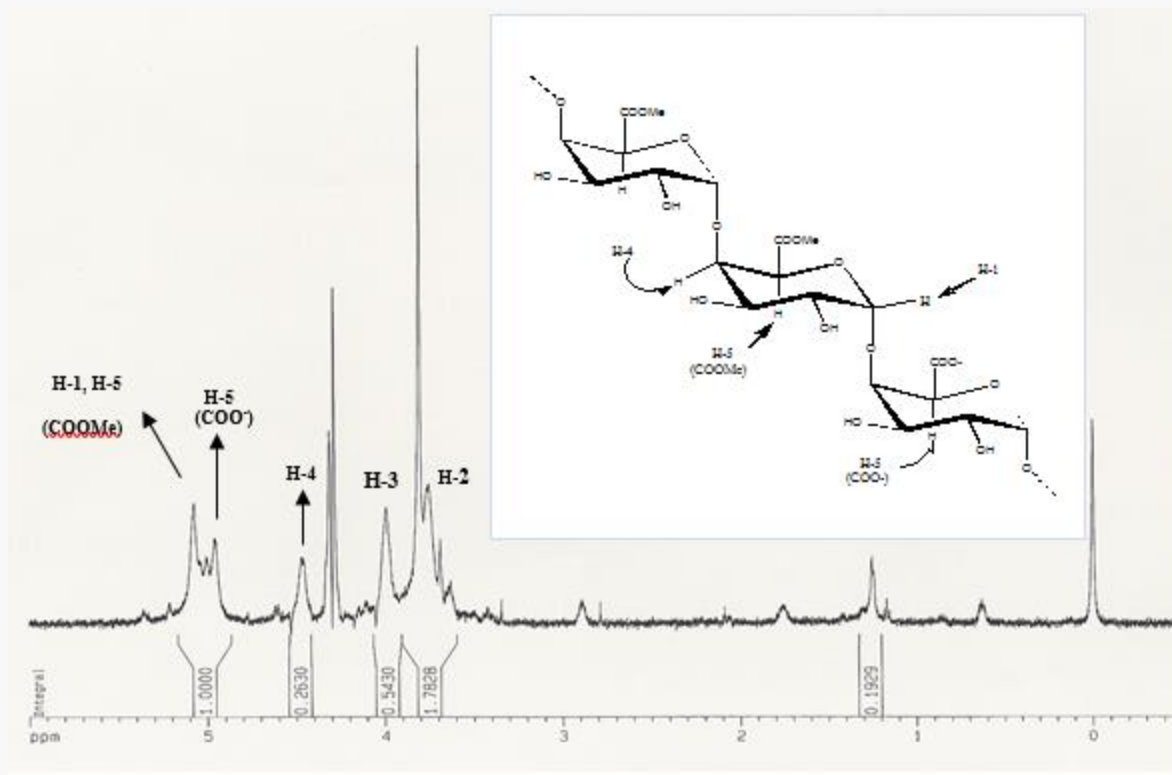


FIG. 2. ^1H NMR spectrum for pectin. Grasdalen method was used for DM calculation.

Characterization of pectin by ^{13}C NMR

The spectrum of ^{13}C nuclear magnetic resonance for the sample of pectin is shown in FIG. 3. In the spectrum of the polysaccharide, a signal at about 53.5 ppm was assigned to methyl groups attached to carboxylic groups of galacturonic acid [28], and a signal at 173 ppm was attributed to carboxylic groups linked to methyl groups [29]. Major and smaller signals can be observed between the region of 60 ppm and 110 ppm. Galacturonic acid (GaA) assignments are highlighted in FIG. 3. The major signs are assigned to D-galacturonic acid while the smaller signs are assigned to D-galactose, as shown in (TABLE 1) [27,30]. These chemical shifts are in good agreement with those related to the pattern of pectin studied by Tamaki et al. There is also less intense signal related to arabinan, galactan and rhamnose [30] but those signals are not intense compared to galacturonic acid ones.

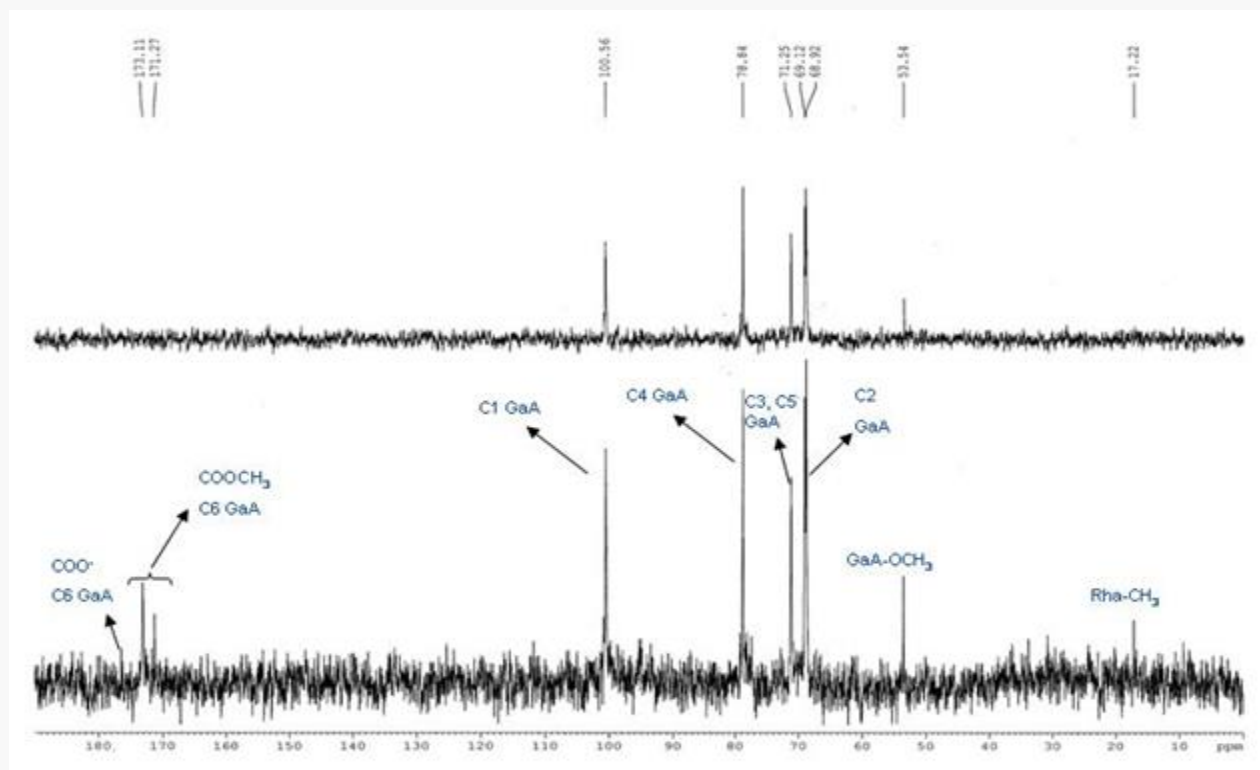


FIG. 3. ^{13}C NMR spectrum shows peaks for the pectic polysaccharides.

Gel permeation chromatography (GPC)

The GPC chromatogram (FIG. 4) for pectin sample presented a wide peak (elution volume of 6.9 mL) with a shoulder (elution volume of 7.5 mL). The peak molar mass (M_{pk}) of polysaccharide was estimated using pullulan (a neutral polysaccharide) standard plot. Considering that pectin is a polyelectrolyte, it is expected that it elutes at a lower volume than a neutral macromolecule with the same molar mass. This is due to chain stiffening and extent, because of electrostatic repulsion of carboxylate groups. The estimated M_{pk} of the main fraction is 9.5×10^5 g/mol. So, the molar mass of pectin is equal or lower than that value for the main fraction. Published molar mass values for pectins ranges from 1.4×10^5 to 1.68×10^6 [31-34]. The shoulder indicates the presence of a fraction of molar mass of 2×10^5 g/mol.

Elemental analysis and metals determination

The percentages of C, H and N determined for pumpkin pectin were 34.24%, 6.38% and 0.46%, respectively. The amount of protein (2.7%) was obtained using a conversion factor equal to 5.85. Azero and Andrade reported the value of 5.4% of protein for pectin obtained from citrus peel what agrees with the value determined for pumpkin pectin. Quantification of metals in the sample from the solutions was analyzed by inductively coupled plasma atomic emission spectrometry (ICP)

relating to the average intensity value of the signal with the equation of the standard curve [35]. The highest metal content was found to be for calcium (2842 ppm), followed by potassium (987 ppm) and sodium (477 ppm). The calcium content suggests that part of the acid content in the pectin sample may be in form of salt as calcium pectinate. The presence of these metals in pectin may be relevant as they are part of the human diet.

TABLE 1. Assignments to the peaks of the ^{13}C spectrum of pectic polysaccharides.

Polymer	Carbon	Shift (ppm)	Shift (ppm)
Galacturonan	C-6 free	176	175.4
Galacturonan	C-6 ester	171	171.4
Arabinan	C-1	106	107.8
Galacturonan	C-1	101	100.8
Arabinan	C-4	84	84.7
Arabinan	C-4	83	83.0
Arabinan	C-2	81	81.6
Galacturonan	C-4	79	81.1
Galactan	C-4	78	78.4
Arabinan	C-3	77	77.4
Galacturonan	C-3	71	72.0
Galacturonan	C-5	73	74.2
Galacturonan	C-2	68	71.3
Arabinan	C-5	67	67.7
Galactan	C-6	62	61.5
Arabinan	C-5	61	62.0
Galacturonan	OCH ₃	53.5	53.6
Rhamnose	CH ₃	17.5	17.7

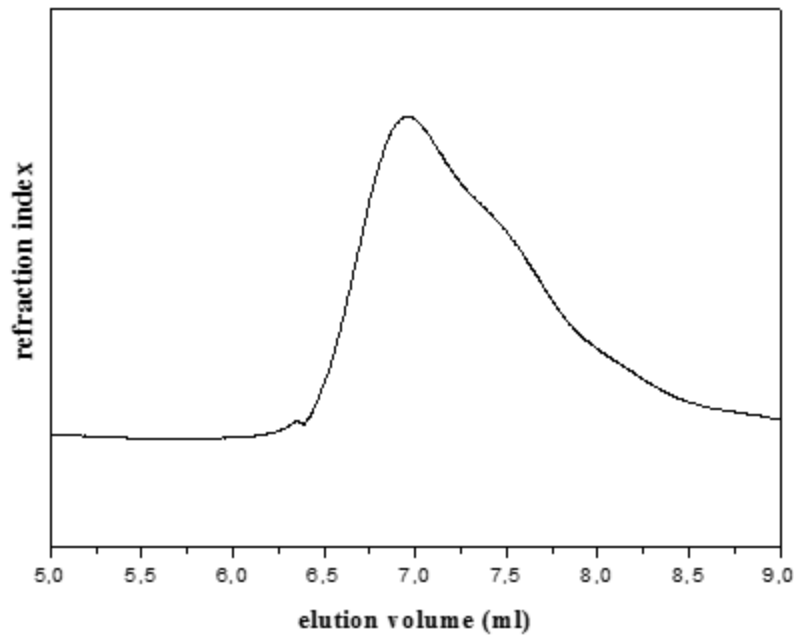


FIG. 4. GPC chromatogram for pumpkin pectin. $M_w=9.5 \times 10^5$ g/mol.

Rheology measurement: Interaction with calcium ions

The flow curves of pectin are shown in FIG. 5. Pectin solution exhibited shear-thinning behavior as well as in presence of 1% calcium ions, showing high viscosity values at low shear rates, with viscosity values decreasing with the increase of shear rate. That increase in viscosity values for pectin/calcium solution is due to the gel formation. Cross-links are formed between Ca^{2+} and the negatively charged carboxyl groups of HG, leading to the formation of structures known as “Egg-boxes” junction zones in which non-methoxylated galacturonic acid residues blocks interact strongly with calcium ions which have specific positions in well-adapted cavities (Braccini and Pérez, 2001). Increasing shear rates values we can observe disruption of the pectin/calcium gel network achieving values similar to the pectin solution around 81 s^{-1} .

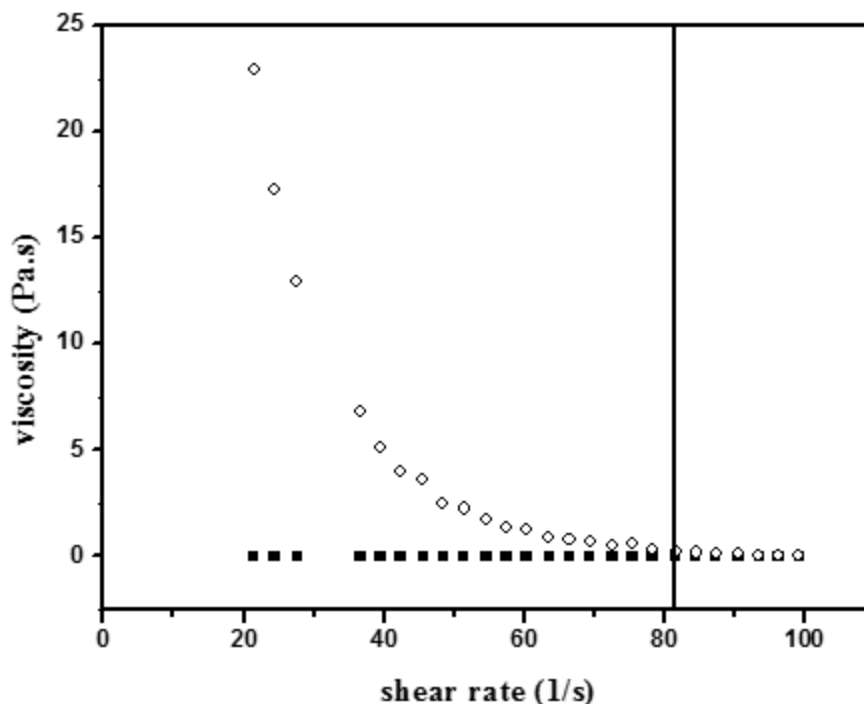


FIG. 5. Flow curves of continuous shear of 1% pectin with (○) and without (■) 1% calcium ions at 25°C.

Conclusion

IR spectroscopy identified the polysaccharide bands. ^1H NMR was effective to quantify the DM of the pectin sample. DM was 44% for the sample by ^1H , and was characterized as low-methoxyl pectin. By ^{13}C NMR spectroscopy, different groups of known polymers were identified in the chains of pectic polysaccharides obtained. The peak molecular mass was determined by GPC as a value of 9.5×10^5 g/mol. The rheological study of continuous shear of pectin solution presented shear thinning behavior and high viscosity values for pectin/calcium solution showing the formation of interactions between the pectic chains and calcium ions in a gel network structure. It was observed in pectin/calcium rheological studies as well as the low-methoxyl determined DM that pumpkin pectin can be used for bioactive encapsulations using ionotropic gelation procedures. Elemental analysis, protein content and metals K, Na and Ca and were also determined for pumpkin pectin sample. The isolated pectin may be used for applications both in food and pharmaceutical formulations.

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