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Emulsifying and structural properties of pectin enzymatically extracted from pumpkin

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ABSTRACT

The present study investigated the emulsifying and structural properties of pectin enzymatically extracted from pumpkin. Pumpkin pectin fraction A was obtained from raw pumpkin with an enzymatic preparation of cellulase and α-amylase. Pumpkin pectin fraction B was achieved by treating the fraction A solution with pronase to reduce protein content. According to the findings (on protein content, galacturonic acid content, neutral sugar composition, and molecular weight distribution), the pronase treatment could remove protein from the fraction A without considerably influencing any other chemical and molecular properties. Moreover, the fraction A exhibited emulsifying properties in water and oil mixture, whereas the removal of protein in the fraction B resulted in the loss of emulsifying properties. The FT-IR and 1D NMR analysis revealed that the backbone of pumpkin pectin is mainly composed of α-1,4-D-galacturonic acid in which a considerable portion of galacturonic acid residues is present as methyl esters, and some L-rhamnose are involved in the linear region of the backbone through α-1,2-linkages.

1. Introduction

Pectin, the most abundant polysaccharides in the middle lamella and cell wall of many higher plants, has been widely used in the food industry as a gelling agent, thickener, and stabilizer of jam, jelly, and acid milk products (Singthong, Cui, Ningsanond, & Goff, 2004; Tamaki, Konishi, Fukuta, & Tako, 2008). The identification of the molecular structure for pectin is of fundamental importance because the physicochemical and functional properties of pectin are highly dependent on its structure. However, pectin is very heterogeneous in its composition, which renders the structural study of the polysaccharides extremely challenging.

Pectin is considered as an alternation of homogalacturonan regions, generally called "smooth regions", and rhamnogalacturonan regions I, also called "hairy regions" (Waldron, Parker, & Smith, 2003). Homogalacturonan is a repetition of (1 → 4)-linked α-D-galactopyranosyluronic acid (Gal*p*A) residues. Rhamnogalacturonan region I is constituted by an alternating sequence of (1 → 4)-linked α-D-Gal*p*A and (1 → 2)-linked α-L-rhamnopyranose (Rha*p*) residues. Various side chains (arabinan, galactan, and arabinogalactan) can be linked to the rhamnose residues (Vincken et al., 2003; Voragen, Pilnik, Thibault, Axelos, & Renard, 1995).

Citrus peel and apple pomace are the major raw materials used for the production of commercially acceptable pectin. Other raw materials, including pumpkin (Matora et al., 1995), sugar beet (Levigne, Ralet, & Thibault, 2002), sunflower (Iglesias & Lozano, 2004), honey pomelo (Guo et al., 2014), and papaya peels (Koubala, Christiaens, Kansci, Loey, & Hendrickx, 2014) have been considered as possible pectin sources and their potential has been discussed.

Several studies on the extraction of pumpkin pectin have been performed using different extraction methods, such as an acidic extraction method and enzymatic extraction method (Fissore, Ponce, Stortz, Rojas, & Gerschenson, 2007; Matora et al., 1995; Ptichkina, Markina, & Rumyantseva, 2008; Shkodina, Zeltser, Selivanov, & Ignatov, 1998). Matora et al. (1995) observed that the enzyme extraction method (multi-enzyme culture supernatants from various strains of *Bacillus polymyxa*) gave much bigger yields of pumpkin pectin than did the acid extraction method (0.1 M HCl). Moreover, the current acid pectin extraction methods not only do not allow pectin to be extracted fully with no damage to its structure, but also lead to the environmental concerns due to acid usage (Ptichkina et al., 2008).

Different enzymes, like cellulase, hemicellulase, protease, amylase, and so on, have been employed for the enzymatic extraction of pumpkin pectin (Fissore et al., 2007; Shkodina et al., 1998). Shkodina et al. (1998) employed cellulase (*Trichoderma*

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viride) or hemicellulase (*Aspergillus niger*)] for the extraction of pectin from pumpkin pulp (following juice extraction), and reported that the cellullase treatment led to an increase in pectin yields and in galacturonic acid contents, as compared to that of the hemicellulose treatment. The same result was also observed in the literature performed by Fissore et al. (2007), who found that the amounts of galacturonic acid in pumpkin pectin obtained from cellulase were greater than those obtained from hemicellulase.

The emulsifying properties of pectin have been demonstrated in a few literatures (Dickinson, 2003; Funami et al., 2011; Leroux, Langendorff, Schick, Vaishnav, & Mazoyer, 2003; Siew, Williams, Cui, & Wang, 2008). A small percentage of hydrophobic protein (about 2%) in sugar beet pectin plays major role in stabilizing oil-in-water emulsions (Siew et al., 2008). Leroux et al. (2003) also noted that beet pectin and citrus pectin may efficiently decrease the interfacial tension between oil and water phase in the emulsions; however, the emulsion stabilizing features of pectin change due to the contents difference of calcium ions, acetyl groups, and proteins.

As far as the authors are aware, no studies have apparently reported the effect of pronase treatment on the emulsifying properties of pumpkin pectin, nor have elucidated the structural features of pectin enzymatically extracted from pumpkin. In the present study, pectin fraction A was obtained from raw pumpkin using cellulase and α-amylase, and then pumpkin pectin fraction B was produced after pronase treatment with the fraction A. The main objectives of the present study are to (1) evaluate the potential of pumpkin for the enzymatic extraction of pectin and (2) investigate the emulsifying and structural features of pectin enzymatically extracted from pumpkin.

2. Materials and methods

2.1. Materials

Raw pumpkin (*Cucurbita mixta*), harvested in ON, Canada, was used in the present study. Cellulase from *Trichoderma viride* (5 U/mg) and α-amylase from *Bacillus licheniformis* (3500 U/mL) were obtained from Sigma (Oakville, ON, Canada). Pronase from *Streptomyces griseus* (7.0 U/mg) was provided by Roche Molecular Biochemicals (Indianapolis, USA). Standards for rhamnose, arabinose, galactose, glucose, xylose, mannose, and galacturonic acid were purchased from Sigma (Oakville, ON, Canada). All chemicals were of reagent grade unless otherwise specified.

2.2. Enzymatic extraction for the production of pumpkin pectin fractions A and B

Pumpkin pectin fractions A and B were extracted from raw pumpkin using the procedure described by Fissore et al. (2007) with some modifications. Raw pumpkin was first cut, and the seeds and skin were removed. The remainder was reduced to fragments (about 4 cm × 0.5 cm), freeze-dried, and ground to make dried pumpkin powder. Fifty g of pumpkin powder were suspended in 1.5 L of 50 mmol/L sodium citrate buffer (pH 5.2), and then cellulase (600 mg) and α-amylase (500 mg) were added. Our preliminary study indicated that starch contents of dried pumpkin powder were 7.93 g/100 g dry solids; therefore, α-amylase was employed to remove starch existed in pumpkin powder. The mixture was stirred at 30 °C for 20 h and centrifuged at 15,000× *g* for 30 min. The supernatant was collected. The remaining pellet was resuspended in 100 mL of distilled water and subjected to a further centrifugation. The supernatants were combined and treated with two volumes of absolute ethanol (Sigma, Oakville, ON, Canada). The resulting precipitate was collected, solubilised in distilled water, freeze-dried, and ground. This procedure produced pumpkin pectin fraction A.

To produce pumpkin pectin fraction B, 0.5 g/100 mL solution of pumpkin pectin fraction A was stirred with pronase for 60 min at 50 °C to reduce protein content. The solution was heated at 85 °C for 30 min, cooled, and centrifuged at 15,000× *g* for 20 min. The supernatant was collected and then treated with two volumes of absolute ethanol (Sigma, Oakville, ON, Canada). The resulting precipitate was collected, solubilised in distilled water, freeze-dried, and ground. The resulting protein-reduced pectin fraction was termed as pumpkin pectin fraction B. The details of enzymatic extraction for pumpkin pectin fractions A and B are summarised in Fig. 1.

2.3. Moisture content, protein content, galacturonic acid content, degree of esterification, and degree of acetylation

Pumpkin pectin fraction A and fraction B were analysed for their moisture contents using AACCI Approved Method 44-15A (AACCI, 2000). The protein contents of pumpkin fraction A and fraction B were analysed by NA2100 Nitrogen and Protein Analyser (Strada Rivoltana, Milan, Italy) using the factor of 5.7 to convert measured nitrogen to protein. The galacturonic acid contents of pumpkin pectin fraction A and fraction B were determined by the *m*-hydroxybiphenyl method (Blumenkrantz & Asboe-Hansen, 1973). Standard galacturonic acid solutions (10–100 μg/mL) were used to construct the standard curve for the determination. The degree of esterification (DE) and degree of acetylation for the fractions A and B were determined by the titrimetric method of Food Chemical Codex (FCC, 1981, pp. 283–286) and the method of Matora et al. (1995), respectively.

2.4. Neutral sugar composition

The neutral sugar composition of pumpkin pectin fraction A and fraction B was determined by modifying the procedure of Wood, Weisz, and Blackwell (1994). Each sample was hydrolysed in 1 mol/L H_2SO_4 at 100 °C for 4 h and diluted 20 times. The diluted samples were passed through a 0.45 μm filter and injected to a high-performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) (Dionex-5500, Dionex Corporation, Canada). Separation of each neutral sugar was performed on a CarboPac PA1 column (250 × 4 mm I.D., Dionex Corporation, Canada) and a guard column (3 × 25 mm, Dionex Corporation, Canada). The solvents were A: 100 mmol/L NaOH, B: 300 mmol/L NaOH, and C: water (distilled water filtered through Nanopure Infinity, Model: D8971, Dubuque, IA, USA). Elution was with 8% A and 92% C for 7 min, then with a gradient to 100% eluent C for 28 min. The column system was cleaned after each analysis with 100% eluent B for 15 min. After cleaning, the initial conditions were maintained for 10 min between each injection of sample. The solvent flow rate was 1.0 mL/min and the injection volume was 50 μL. A post-column delivery system of 600 mmol/L NaOH with a flow-rate of 0.5 mL/min was added to the HPAEC-PAD system. Standard solutions of the individual neutral sugar (rhamnose, arabinose, galactose, glucose, xylose, and mannose) were used at varying concentrations (10–50 μg/mL) for identification and quantification. The instrument was controlled and data were processed using Dionex AI 450 software (Dionex Corporation, Canada).

2.5. Molecular weight distribution

The molecular weight distribution of pumpkin pectin fraction A and fraction B was evaluated by high performance size exclusion chromatography (HPSEC) equipped with a refractive index (RI)