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Enhancement of anti-radical activity of pectin from apple pomace by hydroxamation

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ABSTRACT

Apple pomace was utilized to extract pectin of which structure was modified by hydroxamation to improve its antioxidant effect. As the pectin obtained from apple pomace was treated with alkaline hydroxylamine for 4–48 h, the hydroxamic acid content of the pectin derivative increased from 2.68 to 10.43%. Compared to native pectin, the FT-IR spectra of the derivative showed two new absorption bands at 1646 cm^{-1} (C=O) and 1568 cm^{-1} (N–H), confirming that the hydroxamic acid derivative from apple pomace pectin was successfully obtained. The pectin derivatives were also shown to have enhanced anti-radical activities against DPPH in a dose-dependent way. Moreover, the hydroxamation improved the scavenging effect of the pectin which was even 3-fold higher than that of the native one. Thus, the introduction of hydroxamic acid into the pectin structure seemed to be a useful tool for improving biological activities of pectins.

1. Introduction

Apple pomace which represents around 25% of the weight of a fresh apple, is a primary by-product generated from apple juice extraction (Hang & Woodams, 1984). Instead of disposing apple pomace as wastes, it is found to be a promising source of pectin, which is widely used as a gelling and thickening agents in the food industry (Theuwissen & Mensink, 2008). Beyond the traditional use of pectin, it has received increasing attentions with the well-being trend in the current society. Previous studies reported that native pectin has diverse health benefit such as cholesterol lowering effect (Fernandez, Sun, Tosca, & McNamara, 1994) and gastric emptying delay (Schwartz et al., 1988).

In addition to characterizing the intrinsic biological properties of pectin, it would be worthwhile to improve or develop new functional properties of pectin through its structural modification. Thus, pectin was chemically modified for better anticoagulant and antimicrobial activities (Bae et al., 2009; Pienta et al., 1995). However, any experiment has not been carried out yet to modify the structure of apple pomace pectin by hydroxamation for elucidating its response to reactive oxygen species. It is widely recognized that reactive oxygen species such as oxygen ions, free radicals, and peroxides are generated during normal cellular functions and also known to cause oxidative damage in cellular components, inducing the occurrence of diseases such as aging, cardiovascular diseases, and neurological disorders (Ames, 1983; Diaz, Frei, Vita, & Keaney, 1997; Gey, 1990; Harman, 1995; Smith et al., 1996). Therefore, a lot of efforts to protect cells against reactive oxygen species have been made by searching for materials with better antioxidant activity from natural and synthetic sources. Synthetic antioxidants such as BHA (butylated hydroxyanisole) and BHT (butylated hydroxytoluene), are shown to be more effective than natural antioxidants such as α-tocopherol and ascorbic acid (Bjelakovic, Nikolova, Gluud, & Simonetti, 2007). However, due to the consumer preferences for natural products as well as less vulnerability to side effects, there is a need to develop new antioxidant materials which are based on natural sources.

In this study, pectin from apple pomace was extracted and subjected to hydroxamation, producing pectin hydroxamic acid derivatives with different content of hydroxamic acid. Then, their structures and anti-radical activities against DPPH (1,1-diphenyl-2-picrylhydrazyl) were investigated and compared with those of native pectin.

2. Materials and methods

2.1. Extraction of pectin from apple pomace

Apple pomace was kindly provided by First Fruits Co. (Seoul, Korea). The pomace was dried in an air convection oven at 80 °C

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and ground to pass a 350 mesh sieve. Extraction of pectin from apple pomace was carried out according to the method of Koubala et al. (2008). The dried pomace powder was treated with 85% ethanol at 80 °C for 20 min and alcohol insoluble residue (100 g) was stirred with oxalic acid/ammonium oxalate (0.25%, pH 4.6, 4 L) at 80 °C for 1 h. After pressure filtration, the filtrate was serially coagulated and washed by 96% → 70% → 96% ethanol before desolvation by evaporator and freeze-drying.

2.2. Preparation of pectin hydroxamic acid derivatives

In order to produce pectin hydroxamic acid derivatives (Hou, Lee, Hsu, & Lin, 2003), the pectin solution (1%, 100 mL) was treated with alkaline hydroxylamine (2 M, pH 12.0, 100 mL) at room temperature for 4, 18, 24, and 48 h. After the pH was adjusted to 6.5 using HCl, the pectin derivatives were precipitated with three volumes of 2-propanol. The pectin hydroxamic acid derivatives were designated as T4, T18, T24, and T48, depending on their reaction times (4, 18, 24, and 48 h, respectively).

2.3. Characterization of apple pectin extract

The content of galacturonic acid was determined with a colorimetric method described by Filisetti-cozzi and Carpita (1991). Analysis of methanol content was also performed according to the method of Klavons and Bennett (1986) and the degree of esterification (%) was calculated from the molar ratio of methanol to galacturonic acid. Free sugar content in pectin samples was measured using liquid chromatography (HPAEC-PAD system, Dionex DX500, Sunnyvale, CA, USA) equipped with a CarboPac™PA1 (4 × 250 mm) where pectin was hydrolyzed with trifluoroacetic acid at 100 °C for 4 h and 20 μL of sample was injected. The eluent and flow rate were 18 mM sodium hydroxide solution and 1.0 mL/min, respectively.

2.4. Determination of hydroxamic acid content in pectin derivatives

Hydroxamic acid contents were determined with acidic ferric chloride solution with slight modifications (Soloway & Lipschitz, 1952). Derivative solution (0.2 mL) was mixed with 4 mM hydrochloric acid (0.3 mL) and then 0.1 mM hydrochloric acid containing 10% ferric chloride (0.5 mL) was added. After standing for 10 min at room temperature, the absorbance of the resulting solution was measured at 540 nm with acetohydroxamic acid as a standard.

2.5. Structural characterization of the pectin derivatives

The ground sample was blended with potassium bromide (KBr) at a ratio of 1:20 and a thin pellet was prepared for FT-IR analysis (Nicolet FT-IR spectrometer, MAGNA-IR 760 E.S.P, Nicolet Instrument Corp., Madison, WI).

2.6. DPPH radical scavenging activity

The DPPH radical scavenging activities of native pectin and its hydroxamic acid derivatives were examined by the method of Yang, Guo, and Yuan (2008). Pectin solutions (1 mL) with different concentrations (1, 2, 5 mg/mL) were added to 50 μM DPPH in ethanol (1 mL). After incubation at 60 °C for 30 min, the absorbance of the resulting solution was determined at 517 nm. The DPPH scavenging activity was calculated using the following formula.

$$\text{Scavenging activity}\ (\%) = \{(C - CB) - (S - SB)\}/(C - CB) \times 100$$

where, C, CB, S, and SB were the absorbance of control, control blank, sample, and sample blank, respectively.

2.7. Statistical analysis

All experiments were carried out in triplicate. For statistical analysis, one-way analysis of variance was applied to decide a significance of difference among samples at the level of 5%. Duncan's multiple range test was then carried out for mean comparisons.

3. Results and discussion

3.1. Pectin extraction and characterization

The yield, degree of esterification, galacturonic acid content, and total neutral sugars in the pectin obtained from apple pomace are summarized in Table 1. The yield of pectin from apple pomace was 10% which was lower than that of golden apple pectin (22%) isolated by the similar extraction method with oxalic acid/ammonium oxalate (OAAO) (Koubala et al., 2008). It seemed that the yield of pectin varied depending on its sources, showing 16–22% from apples, 9–30% from limes, and 17–25% from mango, respectively (Berardini et al., 2005; Koubala et al., 2008; Kratchanova, Benemou, & Kratchanova, 1991). The degree of esterification (DE) of the extracted pectin was 70%, having about 76% of the galacturonic acid content. Rascón-Chu et al. (2009) and Canteri-Schemin, Fertonani, Waszczynskyj, and Wosiacki (2005) reported 57% and 69% of DE of apple pomace pectin, respectively which were favorably compared to our results. The content of total neutral sugar detected in this pectin extract was 16%, which was composed of arabinose, glucose, galactose, xylose, rhamnose, and fructose. The similar pattern of neutral sugar content and composition was observed in OAAO-extracted ambarella pectin (Koubala et al., 2008) while lower than the values reported by Rascón-Chu et al. (2009) for 'golden delicious' apple pectin. Thus, the source of pectin and its extraction method appeared to play a significant role in the yield and composition of the pectin isolated.

3.2. Structural characterization of pectin hydroxamic acid derivatives

When hydroxylamine is inserted into carboxyl groups in pectin polymer chains, amide groups are generated, producing hydroxamic acid derivatives with a general structure of R-CO–NHOH. Thus, the presence of hydroxamic acid in the pectin derivative can be investigated by the determination of hydroxamic acid content and

Table 1
Characterization of the pectin extracted from apple pomace.

Yield (%)	9.5
Degree of esterification (%)	70.5
Galacturonic acid (mg/g)	758.8
Total neutral sugar (mg/g)	158.5
Individual neutral sugar (mg/g)	
Arabinose	66.9
Fructose	4.1
Galactose	30.8
Glucose	32.6
Rhamnose	10.0
Xylose	14.0