



Preparation and Characterization of Pectin Hydroxamates from *Citrus Unshiu* Peels.

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Received: 12 July, 2011; Accepted: 11 August 2011

ABSTRACT

Pectin was extracted from unshiu orange (*Citrus unshiu*) peels and was subjected to chemical modification using hydroxamic acid. The structural and physical properties of the resulting derivatives were investigated as a function of hydroxamic acid content (4.68-9.58%). The extracted unshiu orange pectin showed 66.8% degree of esterification, 787.5 mg/g galacturonic acid, and 92 mg/g neutral sugars, which were composed of arabinose (53%), galactose (35%), glucose (5%), rhamnose (5%), and fructose (2%). Compared to the native pectin, the FT-IR spectra of the hydroxamic acid derivatives showed two new absorption bands at 1,646 cm⁻¹ (C=O) and 1,568 cm⁻¹ (N-H). Specifically, the pectin derivatives with more hydroxamic acids were shown to have enhanced water solubility, upto two-fold higher than that of the native pectin. Thus, the introduction of hydroxamic acid into the pectin structure appears to be a useful tool for improving the solubility of pectin.

KEY WORDS: unshiu orange peel, pectin, hydroxamic acid, FT-IR, water solubility

INTRODUCTION

Jeju native citrus fruits, such as unshiu orange (*Citrus unshiu*), jigak (*Citrus aur-antitum*), sadoogam (*Citrus pseudogulgul*), and yooja (*Citrus junos*) are the most abundantly produced fruits in Korea (1). According to the annual reports of statistics (2), citrus-cultivated areas were reported to be approximately 21,400 hectares producing approximately 662,000 tons of fruit in 2006. Citrus fruits are widely consumed as

fresh fruit and in the form of processed products such as juice, canned fruit and marmalade. The citrus fruit peels are major by-products of citrus fruit processing and are generally treated as waste (3). It would thus be worthwhile to look for a way to utilize these citrus fruit peels.

Pectin exists abundantly in fruit peels and pomace such as citrus and apple, and is extensively used in the food industry as a gelling agent and stabilizer (4-5). Recently, pectin has received significant attention due to its diverse biological effects, such as lowering cholesterol (6) and ability to delay the emptying of the gastric content (7). Additionally, it has

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been reported that chemical modification of pectin, such as sulfation and hydroxamation increased, or produced biological activity, including anticoagulant and antimicrobial effects (8-11). Previous studies focused on hydroxamic acid derivatives because of their protective effects against cellular oxidation (12-14). Thus, galacturonic acid (15) and alginic acids (16) as well as apple pectin (17) were subjected to hydroxamation to increase and/or produce anti-oxidative effects. However, no efforts to prepare and characterize pectin hydroxamates from orange peels have been reported, especially none on the influence on varieties in their structures or their physicochemical properties. A study of the pectin hydroxamates from different plant sources would thus be useful to expand their use in a variety of scientific and commercial applications.

MATERIALS AND METHODS

Extraction of pectin from unshiu orange peels

Pectins were extracted from the domestic unshiu orange peels according to the method of Koubala *et al.* (18). Unshiu orange peels were kindly provided by the First Fruits Company (Seoul, Korea). They were dried in an air convection oven (J-300M, Jisico Co., Ltd., Seoul, Korea) at 80°C and ground using a lab blender (FM 680T, Hanil Co., Seoul, Korea) and passed through a 350 mesh sieve (Testing Sieve, Chung Gye Sang Gong Sa, Seoul, Korea). The dried peel powder was mixed with 85% ethanol at 80°C for 20 minutes. The residue (100 grams), that was insoluble in alcohol, was treated with oxalic acid/ammonium oxalate (0.25%, pH 4.6, 4 L) agitating at 80°C for 1 hour. After pressure filtration, the filtrate was washed with ethanol (96% → 70% → 96%), desolvated using an evaporator (N-1000, Eyela, Tokyo, Japan), and then freeze-dried (FD-8508, Ilsin Lab Co., Seoul, Korea).

Composition analysis of pectin extract

The degree of esterification (%) of the extracted pectin was calculated from the molar

ratio of methanol to galacturonic acid. Anhydrogalacturonic acid content was measured by colorimetric method at 524 nm (DU 650, Beckman Coulter Inc., USA) (19) and the methanol content was determined by an enzymatic method with alcohol oxidase (20).

The free sugar content of the pectin samples was measured using a liquid chromatography (HPAEC-PAD system, Dionex DX500, Sunnyvale, CA, USA) equipped with a CarboPac™ PA1 (4 x 250 mm). The pectin was hydrolyzed with trifluoroacetic acid at 100°C for 4 hours and 20 µl of the sample was injected. The mobile phase and flow rate were 18 mM sodium hydroxide and 1.0 ml/min., respectively.

Preparation of pectin hydroxamic acids

Pectin hydroxamic acid derivatives were prepared by the method of Hou *et al.* (9). Alkaline hydroxylamine (2 M, pH 12.0, 100 ml) was added to the pectin solution (1%, 100 ml) and stirred at room temperature for 4, 18, 24 or 48 hours. The pH (using an Orion 3-star, Thermo Electron Corporation, Beverly, MA, USA) was adjusted to 6.5 with hydrochloric acid, and the resulting derivatives were precipitated with three volumes of 2-propanol, desolvated using an evaporator, and then freeze-dried. The pectin hydroxamic acid derivatives were designated as T4, T18, T24, and T48, depending on their reaction times (4, 18, 24 and 48 hours, respectively).

Characterization of pectin derivatives

The hydroxamic acid contents of the pectin derivatives were determined, with slight modifications, by the method of Soloway *et al.* (21). First, the derivative solution (0.2 ml) was mixed with hydrochloric acid (0.3 ml, 4 mM), and then 0.5 ml hydrochloric acid (0.1 mM) containing 10% ferric chloride was added. After standing for 10 minutes at room temperature, the absorbance of the resulting solution was measured at 540 nm with acetohydroxamic acid as a standard.

For the confirmation of the incorporation of hydroxamic acid into the pectin polymer, the derivatives were ground and mixed with potassium bromide (KBr) at a ratio of 1:20 and a thin pellet was then prepared for FT-IR analysis (Nicolet FT-IR spectrometer, MAGNA-IR 760 E.S.P, Nicolet Instrument Corp., Madison, WI, USA).

The solubilities of native pectin and derivatives in water were determined by the method of Chang *et al.* (22). The derivative dispersion (10 mg/ml) was agitated at 25°C for 24 hours and centrifuged at 3,500 g for 15 minutes (VS-21SMTi, Vision Scientific Co., Kyunggi-do, Korea). The supernatant was dried at 60°C for 24 hours in a vacuum oven (J-DV01, Jisico Co., Ltd., Seoul, Korea). The solubility was calculated from the weight of the initial dispersed solid and the weight of the residue after drying.

Statistical analysis

All experiments were performed in triplicate. The results were analyzed using Statistical Package for Social Science (SPSS, Version 12.0, 2004, SPSS Inc., Chicago, IL, USA). Analysis of variance (ANOVA) was carried out to determine any significant difference between the samples. Duncan's multiple range test was then used for comparison of means at the level of 0.05.

RESULTS AND DISCUSSION

Extraction of pectin from unshiu orange peels

The yield and chemical composition of the pectin extracted from unshiu orange peels are presented in Table 1. The yield of the pectin in this study was 9%, which was similar to that of citrus pectin extracted with citric acid (23). In general, the yields of the pectin extracted from citrus peels varied from 9% to 30%, depending on the extraction methods (18, 24-25). On the other hand, the galacturonic acid content of the

Table 1 Characterization of the pectin extracted from unshiu orange peels

YIELD (%)		9
Degree of esterification (%)		66.8
Galacturonic acid (mg/g)		787.5
Total neutral sugars (mg/g)		91.5
Individual neutral sugar (mg/g)	Arabinose	48.7
	Fructose	1.5
	Galactose	31.9
	Glucose	5
	Rhamnose	4.5

pectin extract was 788 mg/g which was higher than the 343 mg/g of citrus pectin extracted with citric acid (23).

The degree of esterification (DE) is one of the key factors affecting the practical application of pectin in food products such as gelation, thickening, or consistency adjustment (26).

Pectins are generally classified as high methoxyl (DE > 50%) or low methoxyl (DE < 50%), based on the percentage of the esterified carboxyl groups. The degree of esterification (DE) of the pectin extracted in this study was 67%, confirming that the unshiu orange pectins extracted in this study are high methoxy pectins. Kurita *et al.* (23) and Tamaki *et al.* (27) also reported 65% and 63% of DE of citrus pectins, respectively, which compares well with the results obtained here.

Moreover, the extracted pectin was shown to have 92 mg/g of total neutral sugars which were composed of arabinose (53%), galactose (35%), glucose (5%), rhamnose (5%), and fructose (2%). The pattern of the composition of neutral sugars was similar to that of citric-extracted pectin (23), even though the content of total neutral sugars was lower. Therefore, the yield and chemical composition of the extracted pectin appeared to be affected by the source of pectin and the extraction method.

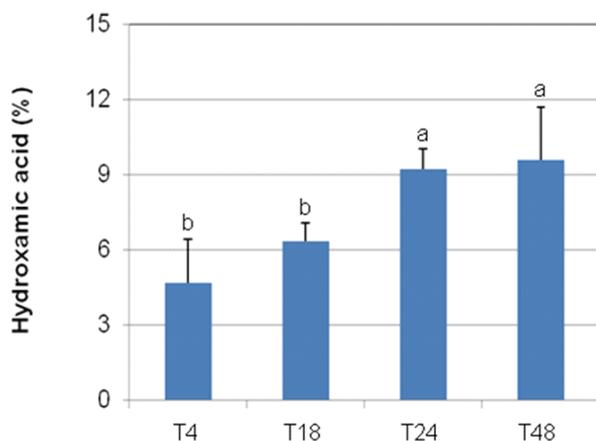


Figure 1 Hydroxamic acid contents of pectin derivatives as a function of reaction time with alkaline hydroxylamine

Characterization of pectin hydroxamic acid derivatives

Hydroxamation is the generation of amine groups in a polymer structure by introducing hydroxylamine into the carboxyl groups of the polymer chains. In this study, pectin hydroxamates with varying hydroxamic acid contents were prepared by controlling the reaction time. Figure 1 shows that as the reaction time increased from 4 hours to 48 hours, the hydroxamic acid content of the derivative increased from 4.68% to 10.43%, respectively. Notably, the hydroxamic acid percentage gradually increased as the hydroxamation process continued ($p < 0.05$). In addition, the structure of the pectin derivatives was investigated using FT-IR. Figure 2 shows

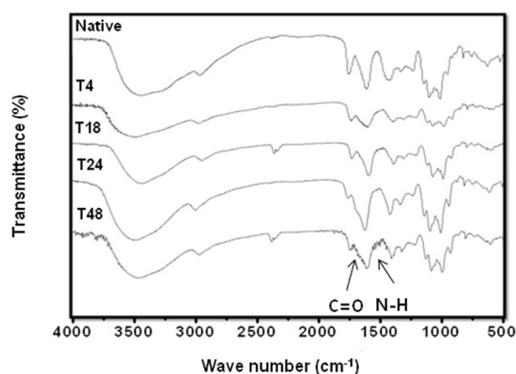


Figure 2 FT-IR spectra of pectin and its hydroxamic acid derivatives

two new bands at $1,646 \text{ cm}^{-1}$ (C=O) and $1,568 \text{ cm}^{-1}$ (N-H), respectively (28). Therefore, the FT-IR spectra and hydroxamic acid analysis confirmed that the pectin hydroxamic acid derivatives were successfully produced from unshiu orange pectins.

The effect of hydroxamation on the water solubility of pectin was investigated (Figure 3). Chemical modification by hydroxamation caused a distinct increase in the water solubility of pectin. In addition, water solubility significantly improved in response to the content of hydroxamic acid ($p < 0.05$). The water solubility of the native pectin increased from 47% to about 88% when 9.6% of hydroxamic acid was incorporated into the carboxyl groups in the pectins. It appears that an increased incorporation of ionic groups into the pectin structure would be responsible for the enhanced water solubility. The positive relationship between water solubility and the introduction of an anionic group into the polymer chains has been reported for several chemical modifications of polymers including carboxymethylation (29), oxidation (30), and sulfation (11). It is generally recognized that improved water solubility is desirable for pharmaceutical formulations since it provides for ease of administration, as well as, enhanced physiological activity such as anti-tumor (29), cholesterol-lowering (30), and anti-oxidative properties (17). The increased water solubility

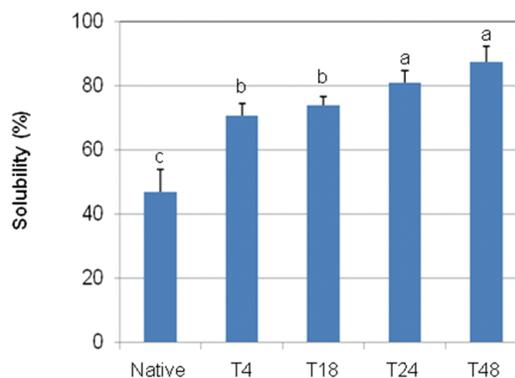


Figure 3 Effect of the degree of hydroxamation on solubility

of the hydroxamated pectin may partly contribute to the potential physiological activities which should be investigated further.

CONCLUSION

Domestic unshiu orange peels were utilized to produce pectin (67% DE, 790 mg/g galacturonic acid, 92 mg/g total neutral sugars), and its derivatives with different contents of hydroxami hydroxamic acid (4.68% - 9.58%) were successfully prepared. The introduction of hydroxamic acid groups into the pectin structure was confirmed by FT-IR analysis and resulted in improved water solubility of the pectins. Further research should be carried out to investigate the effects of the hydroxamic acid content of different biological properties of the pectins.

ACKNOWLEDGEMENTS

This work was carried out with the support of the "Cooperative Research Program for Agriculture Science & Technology Development (Project No. PJ907142)" Rural Development Administration, Republic of Korea.

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