Research Article

Preparation of Pectin from Fruit Peel of *Citrus maxima*

Uthai Sotanaphun [1*], Amornrut Chaidedgumjorn [2], Nudchanart Kitcharoen [1], Malai Satiraphan [2], Panida Asavapichayont [3] and Pornsak Sriamornsak [3]

[1] *Department of Pharmacognosy,* [2] *Department of Pharmacetuical Chemistry,*
[3] *Department of Pharmaceutical Technology,*
Faculty of Pharmacy, Nakhon Pathom, Thailand
[*] *Corresponding author. E-mail address: uthai@su.ac.th*

Received July 30, 2011; Accepted October 7, 2011

Abstract

The extraction of high-methoxyl pectin from the fruit peel of *Citrus maxima* was studied. The suitable condition was the extraction at 80 ˚C without pH adjustment (pH was about 4.5) in 20 times by volume of water. For up-scale procedure, the peel was pre-soaked in water for overnight to wash out small molecular water-soluble substances. Amberlite XAD-16 polystyrene was used to remove phenolic compounds before concentration and precipitation of pectin. This suggested method was simple and inexpensive. The yield of the obtained pectin was 7.23±0.19%. Its galacturonic acid content and degree of esterification were 74.12±2.07% and 76.30 ±3.38%, respectively.

Key Words**:** *Citrus maxima*; Galacturonic acid; High-methoxyl pectin; Pomelo; Up-scale production

Introduction

Pectin is a polysaccharide widely used in food and pharmaceutical industries. It is used as thickening and gelling agents (May, 1990). Its medical uses are antidiarrhea, detoxification and blood glucose lowering (Voragen et al., 1995). Pectin consists of a linear backbone of linked D-galacturonic acid units and branched region of neutral sugars. The carboxyl group of galacturonic acid can be free or methyl-esterified which classifies pectin into high- and low- methoxyl types depending on their degree of esterification (Voragen et al., 1995; Novosel'skaya et al., 2000; Willats et al., 2001). Fruit peels of Citrus such as orange, lemon and lime, are well recognized as conventional sources of commercial pectin (Rolin, 1993). Classically, two main production steps of pectin include extraction from raw material with water and isolation of pectin from the extracted solution by precipitation with alcohol (May, 1990; Rolin, 1993; Voragen et al., 1995; Kalapathy et al., 2001; Joye and Luzio, 2000). Commercial pectin is extracted at high temperature by hydrolyzing protopectin using acid (May, 1990; Minkov, 1996). In general, higher yield is obtained from high temperature and low pH extraction. In contrast, molecular weight and degree of esterification (DE) will be decreased (Joy and Luzio, 2000). Therefore, the suitable condition for each kind of raw material needs to be optimized. Our previous study showed that pomelo or *Citrus maxima* (Family Rutaceae), one of the popular fruit of Thailand, could be used as a source of high-

methoxyl pectin (Chaidedgumjorn et al., 2009). Its fruit is fairly large and considerable amount of the peel is biological waste. The aim of this study was to investigate the optimum condition for the extraction of high-methoxyl pectin from this waste. The development of a simple up-scale procedure was also suggested.

Experimental

Materials

Mature fruits of *Citrus maxima* (Burm. f.) Merr. cultivar Khao-nam-phueng were harvested from Nakhon-Pathom province, Thailand, during 2005. They were stored at room temperature (30˚C) for 30 days before using in the experiment. Fruit peels (outer green layer and inner white layer) were cut into a cube size approximately 4x4x4 mm^3 and dried at 50˚C in hot-air oven.

Extraction Conditions of Pectin

Dried peel of *C. maxima* (100 g) was extracted with water (2,000 mL x 2 times) at different pHs (2, 3 and 4.5) and temperatures (30, 50, 80 and 100˚C) for 3 hours. The extract was concentrated under reduced pressure to the final volume of 200 mL. It was further dialyzed (D9527, Sigma-Aldrich, St. Louis, Missouri, USA) for 1 hour, repeated 8 times. Pectin was precipitated by adjusting pH to 3.5 and adding double volume of 95% ethanol. After centrifugation at 3,500 rpm for 8 min and washing with 95% ethanol, pectin was collected and dried at 50˚C.

Purification Methods of Pectin

Dried peel of *C. maxima* (100 g) was extracted with water (2,000 mL x 2 times) at 80˚C and pH 4.5. Five purification methods were compared (Table 1). Dialysis was the reference method (method A).

Table 1 Yield and general properties of pectin prepared from different purification methods: (A) = dialysis, (B) = pretreatment in water, (C) = pretreatment + washing pectin with EtOH, (D) = pretreatment + washing pectin with HCl/EtOH, and (E) = pretreatment + XAD16

Method	Extraction cycle	Yield [Total yield] (% w/w)	Galacturonic acid (%)	Viscosity (centipoises)[1]	pH[2]
A	1st	4.73 ± 0.13^{a}	73.23 ± 1.55^{a}	12.16 ± 0.06^{a}	3.69 ± 0.03^{a}
	2nd	4.10 ± 0.25^{a,b}	73.43 ± 0.63^{a}	8.09 ± 0.40	3.66 ± 0.04^{a}
		[8.84 ± 0.39^{c}]			
B	1st	11.41 ± 0.30	41.05 ± 0.94	2.90 ± 0.00^{b}	4.56 ± 0.02^{b,c}
	2nd	6.60 ± 0.02	54.18 ± 0.05	2.91 ± 0.23^{b}	5.62 ± 0.07^{d}
		[18.01 ± 0.28]			
C	1st	4.40 ± 0.06^{a,d}	61.62 ± 0.45^{b}	11.64 ± 0.19^{a}	5.72 ± 0.42^{d}
	2nd	3.89 ± 0.23^{b,d,e}	62.82 ± 0.06^{b}	8.82 ± 0.01^{c}	4.86 ± 0.01^{b}
		[8.28 ± 0.30^{c,f}]			
D	1st	3.75 ± 0.03^{b,d,e}	69.49 ± 0.61	18.00 ± 0.39	4.32 ± 0.03^{c}
	2nd	3.76 ± 0.06 ^{b,d,e}	72.94 ± 0.29	13.05 ± 0.07	3.76 ± 0.01^{a}
		[7.51 ± 0.03^{f}]			
E	1st	5.61 ± 0.23	65.70 ± 0.23	10.58 ± 0.24	3.83 ± 0.00^{a}
	2nd	3.20 ± 0.78^{e}	71.41 ± 1.57	9.28 ± 0.69^{c}	3.74 ± 0.01^{a}
		[8.81 ± 1.01^{c}]			

[1]Viscosity of the 1% w/w solution at 25˚C. [2]pH at 1% w/w solution. $^{a\text{-}e}$ Same letters within a column indicate no significant difference ($p \geq 0.05$).

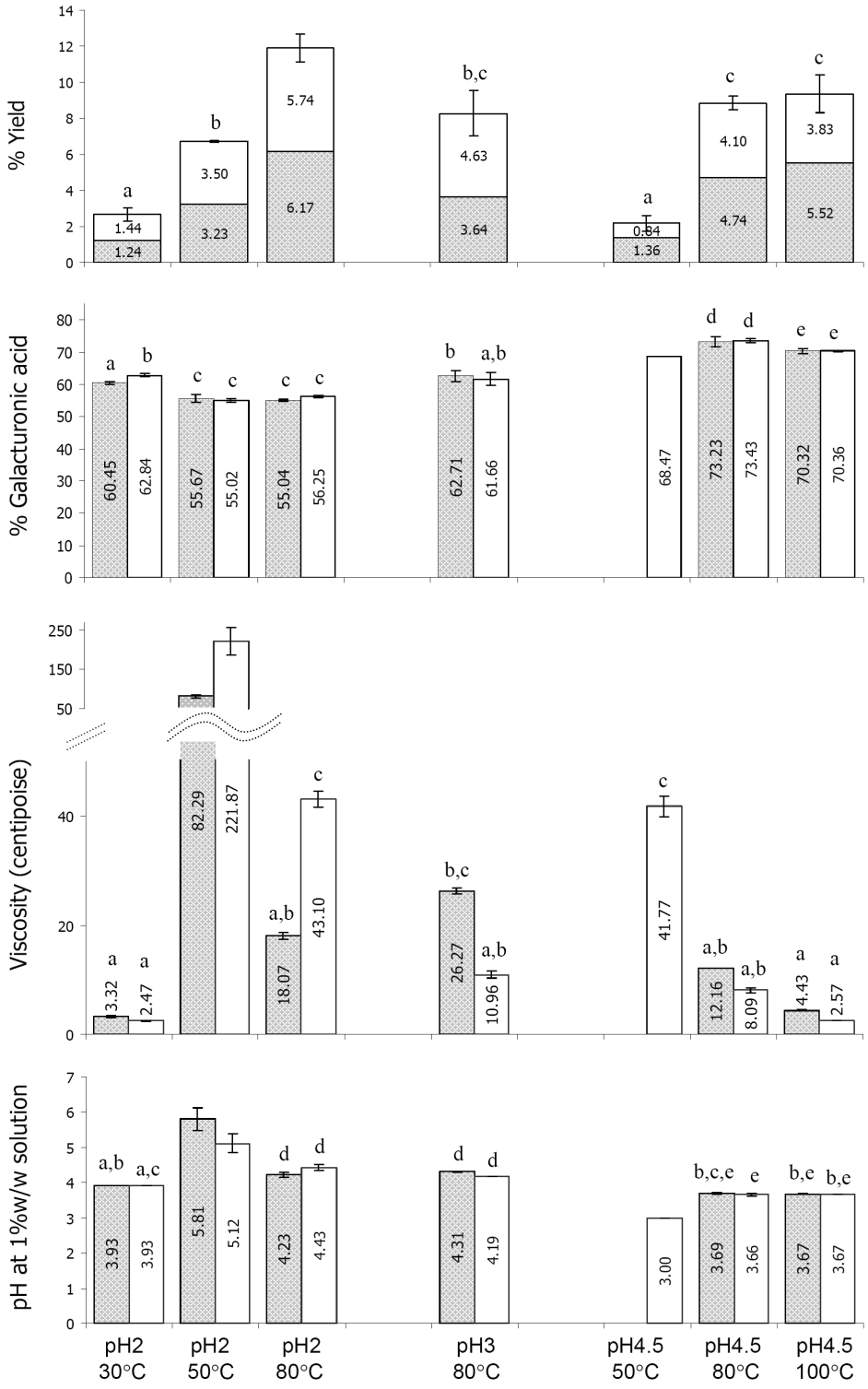

Figure 1 Yield and general properties of pectin extracted from different conditions (■ = first extraction cycle, □ = second extraction cycle. [a-e] Same letters within a graph indicate no significant difference ($p \geq 0.05$).

Quality of pectin from the first extraction cycle at pH4.5 and 50°C could not be determined because it did not dissolve in water.)

For the other four methods, the peel was soaked in water for overnight and filtered before using for the extraction. After precipitation, the extracted pectin was either directly collected by filtration through cheese cloth (method B) or centrifugation and washing. Pectin from method C was washed with 70-95% ethanol, whereas method D used hydrochloric solution (pH 2) and followed with 70-95% ethanol. Amberlite XAD-16 polystryrene resin (06442, Fluka Chemika, Switzerland) was used in method E to purify the extracted solution before pectin precipitation.

Up-Scale Extraction of Pectin

Dried peel of *C. maxima* (1 kg) was pretreated by soaking in water (20 L) at room temperature (30˚C) for 18 hours (overnight). Then the peel was filtered and pressed, and further extracted with water (20 L) at 80˚C for 2 times (4.5 and 5.5 hours) in an electric ern (Satien Stainless Steel public company limited, Thailand). The combined extract was filtered through an Amberlite XAD-16 polystryrene resin (1 kg) to remove phenolic compounds. The extract was concentrated to about 500 mL and pH adjusted to 3.5. Then 95% ethanol (1 L) was added to precipitate pectin. Pectin coagulation was filtered through cheese cloth, dried at 50˚C and ground to powder.

Analysis of Galacturonic Acid, Degree of Esterification and Viscosity of Pectin Solution.

Galacturonic acid content and degree of esterification were determined by the titration method (USP 26-NF21, 2002). The apparent viscosity of 1.0 %w/w pectin solution was determined using a Brookfield digital viscometer model RV DV-1 (Brookfield Engineering Laboratories, Inc., USA) with an UL adaptor at 25°C and a speed of 20 rpm.

Results and Discussion

The peel (both green and white layers) of *C. maxima* cultivar Khao-nam-phueng, stored for 30 days after harvesting, was used for the extraction of water-soluble pectin or high-methoxyl pectin (Chaidedgumjorn et al, 2009). Some extractive conditions were varied to investigate the effect of pH and temperature. The results are shown in Figure 1. At low pH and high temperature a larger amount of extracted pectin was obtained. The highest yield (11.91%) was the extraction at pH2 and 80˚C for 2 extraction cycles. However its low %galacturonic acid content (55.04-56.25%) indicated that pectin was mixed with other constituents. Purity of pectin or galacturonic acid content depended on pH, whereas the effect of temperature was not clear. A higher %galacturonic acid was obtained with the increasing of pH. In contrast, high viscosity products were obtained when using low pH condition. Extraction at pH2 and 50˚C gave the high viscosity products (82.29-221.87 centipoises at 1% solution), but its %galacturonic acid was low (55.02-55.67%). It was suggested that pectin along with other polymers were extracted at this low pH condition. These unknown polymers might be responsible for the high viscosity property, since the molecular weight of pectin from *C. maxima* was low (Chaidedgumjorn et al, 2009). A little higher pH of this product (pH 5.12-5.81) also confirmed the low purity because pectin is acidic in nature. Viscosity was strongly affected by temperature. The increase in temperature might shorten the polymer chain and decreased its viscosity property as reported for pectin extracted from other sources (Joye and Luzio, 2000). Based on all above information, pH 4.5 and 80˚C was suggested as the suitable condition for the extraction of water-soluble pectin from *C. maxima*. The pH 4.5 is the natural acidity produced by acid composition in the peel. A similar method using natural pH has been reported for the extraction of pectin from lemon peel (Ehrlich, 1997). The yield from this condition with repeated 2 extraction cycles (8.84%) was not different from that previously reported (Huong and Luyen, 1989), but a better quality in terms of viscosity was obtained.

To scale-up the extraction, purification using dialysis was not likely possible. Our preliminary

examination indicated that a number of water-soluble small molecular molecules could be removed before pectin extraction by soaking the peel in water at room temperature (30°C) overnight. This method was based on the principle that solubility of small molecules in water, in general, is better than large molecules. A similar method (washing the material with water before pectin extraction) has been reported for beet root (Wang and Chang, 1994). However, even the peel was pretreated in water (method B in Table 1), phenolic compounds, especially flavonoids, were still present in the extracted pectin. Washing pectin with aqueous ethanol is a method that often used for removing non-polar to polar small molecules (Voragen et al., 1995). In this study, extracted pectin was washed with 70% and 95% ethanol until it gave negative test to phenolics and flavonoids (detected with $FeCl_3$ TS. and Shibata's test, respectively). As shown in Table 1, its galacturonic content was improved (comparing method C to method B), but it was still lower than the typical requirements of 65% (May, 1990) and far from pharmaceutical standard at 74% (USP 26-NF21, 2002). Washing pectin with acidic aqueous ethanol was the other reported method (Ali et al., 2003). Pectin from this method possessed acceptable %galacturonic acid (method D in Table 1). However, it needed repeating washing-centrifugation process which was not suitable for the production scale. The efficiency of polymeric resin to remove phenolic compounds from pectin was proven (Schieber et al, 2003). In this study, Amberlite XAD-16 polystyrene was used. Pectin solution was passed over the resin which was covered with cheese cloth and placed on a flour sieve. This technique was better than packing the resin in a column because it was not clogged by some gelling particles in pectin solution. One kilogram of Amberlite XAD-16 was sufficient for pectin solution that prepared from 1 kilogram of the peel. Purity of pectin from this method was closed to the requirement value. Its viscosity and pH were also not different from that prepared from dialysis method (Table 1, comparing method E to method A). Moreover, the advantage of Amberlite XAD-16 is that it can be re-used by re-activation with alcohol.

The concentration technique before pectin precipitation is also concerned. Concentration under reduce pressure is time consuming and uses expensive instrument. Concentration by boiling a volume of pectin solution in a wide tray over a hot-plate for not more than 1 hour could be used as a simple and fast method. But viscosity of pectin was found to decrease. The up-scale production was tested on 1 kilogram of the peel. In order to achieve the same solution concentrations as those of the laboratory scale by measuring total soluble solids (approximate 1.0 and 0.5°Brix for the first and second extraction cycles), we found that extraction time needed be increased from 3 and 3 hours each to 4.5 and 5.5 hours, respectively.

Conclusion

This study investigated the effects of temperature and pH on the extraction of pectin from the fruit peel of *Citrus maxima*. The up-scale extraction process also suggested and concluded in Figure 2. Data of three production batches revealed some variation in yield and quality. The extraction yield was 7.23±0.19%. The viscosity of 1% solution was 4.52±1.36 centipoises. The galacturonic acid content was 74.12±2.07%, and the degree of esterification was 76.30±3.38%, clearly indicated that the extracted pectin was high-methoxyl pectin.

Acknowledgement

This work was financially supported by Silpakorn University Research and Development Institute, under the research program "Production of pectin from pomelo (*Citrus maxima*) peel and application in pharmaceutical industry".

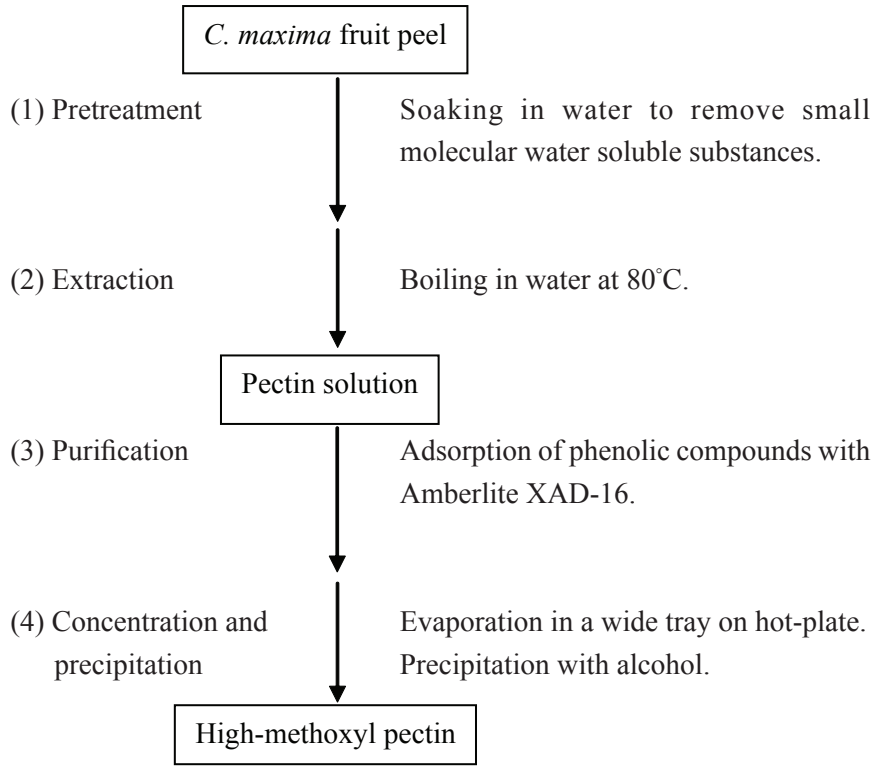

Figure 2 Flow diagram of the up-scale extraction of pectin from *C. maxima* fruit peel

References

Ali Sahari, M., Ali Akbarian, M., and Manuchehr, H. (2003) Effect of variety and acid washing method on extraction yield and quality of sunflower head pectin. *Food Chemistry* 83(1): 43-47.

Chaidedgumjorn, A., Sotanaphun, U., Kitcharoen, N., Asavapichayont, P., Satiraphan, M., and Sriamornsak, P. (2009) Pectins from *Citrus maxima*. *Pharmaceutical Biology* 47(6): 521–526.

Ehrlich, R. M. (1997) Method for making pectin and pectocellulosic products. *United States Patent* 5,656,734. Aug 12.

Huong, D. M., and Luyen, D. V. (1989) Optimization of pectin extraction from dried peel of *Citrus grandis*. *Polymer Bulletin* 22(5-6): 599-602.

Joye, D. D., and Luzio, G. A. (2000) Process for selective extraction of pectins from plant material by differential pH. *Carbohydrate Polymers* 43(4): 342-337

Kalapathy, U., and Proctor, A. (2001) Effect of acid extraction and alcohol precipitation conditions on the yield and purity of soy hull pectin. *Food Chemistry* 73(4): 393-396.

May, C. D. (1990) Industrial pectins: sources, production and applications. *Carbohydrate Polymers* 12(1): 79-99.

Minkov, S., Minchev, A., and Paev, K. (1996) Modelling of the hydrolysis and extraction of apple pectin. *Journal of Food Engineering* 29(1): 107–113.

Novosel'skaya, I. L., Voropaeva, N. L., Semenova, L. N., and Rashidova, S. Sh. (2000) Trends in the science and application of pectins. *Chemistry of Natural Compounds* 36(1): 1-10.

Rolin, C. (1993) Pectin. In *Industrial Gums: Polysaccharides and Their Derivatives*.

(Whistler, R. L., and BeMiller, J. N., eds.), pp. 258-293. Academic Press, New York.

Schieber, A., Hilt, P., Streke, P., Endreb, H.U., Rentschler, C., and Carle, R. (2003) A new process for the combined recovery of pectin and phenolic compounds from apple pomace. *Innovative Food Science & Emerging Technologies* 4(1): 99-107.

United States Pharmacopeial Convention. (2002) *The United States Pharmacopeia-The National Formulary (USP 26-NF21)*. pp. 1401-1402. Webcom Limited, Toronto.

Voragen, A. G. J., Pilnik, W., Thibault, J. F., Axelos, M. A. V., and Renard, C. M. G. C. (1995) Pectins. In *Food Polysaccharides and Their Applications*. (Stephen A. M., edt.), pp. 287-369. Marcel Dekker, New York.

Wang, C. C. H., and Chang, K. C. (1994) Beet pulp and isolated pectin physiochemical properties as related to freezing. *Journal of Food Science* 59(6): 1153-1154.

Willats, W. G. T., McCartney, L., Mackie, W., and Knox, J. P. (2001) Pectin: cell biology and prospects for function analysis. *Plant Molecular Biology* 47(1-2): 9-27.