Bulgarian Journal of Agricultural Science, 25 (No 1) 2019, 192–196

Effect of the assistance of microwave and oxalic acid on the extraction yield of pectin from pomelo (*Citrus maxima*) peel

Le Pham Tan Quoc

Institute of Biotechnology and Food Technology, Industrial University of Ho Chi Minh City, 12 Nguyen Van Bao, Ward 4, Go Vap district, Ho Chi Minh city, Vietnam
Corresponding author: lephamtanquoc@iuh.edu.vn

Abstract

Quoc, L. P. T. (2019). Effect of the assistance of microwave and oxalic acid on the extraction yield of pectin from pomelo (*Citrus maxima*) peel. *Bulgarian Journal of Agricultural Science*, *25*(1), 192–196

Pectin is quite important component in the plant cell and useful in the pharmaceutical and food technology. This study shows the changes of pectin content and its DE values of pomelo peel with changes of extraction factors such as pH, dried peel/solvent ratio, microwave power and extraction time. The results show that the pectin content reached 18.81±0.21%, yield of pure pectin was 91.02±0.21%. The optimal extraction conditions of pectin from pomelo peel were pH of 4.2, material/solvent ratio of 1/39, extraction time of 9 minutes, microwave power of 660 W. The results also proved that using microwave assisted extraction and oxalic acid can increase the content of pectin from pomelo peel.

Keywords: microwave; oxalic acid; pectin; pomelo

Introduction

Nowadays, pectin is used widely for its functionality in nutrition, medicines and pharmacy, especially food science. They used pectin as food additives for gelling, thickening, emulsifying properties in fish, meat, jam, etc. (May, 1997; Barrera et al., 2002). But until now the structure and composition of pectin are still not completely understood because pectin can change during separation from plants, processing and storage of plant material (Novosel'skaya et al., 2000). Pectin is a complex mixture of polysaccharides that makes up about one third of the cell wall dry substance of plants. The highest concentrations of pectin are found in the middle lamella of cell wall, with a gradual decrease as one passes through the primary wall toward the plasma membrane (Kertesz, 1951). Although pectin presents commonly in most of the plant tissues, the number of sources that may be used for the commercial manufacture of pectin do not satisfy the needs of consumers because the gel formation depends on the degree of esterification (DE) and the molecular size. Pectins from various sources don't have the same physicochemical properties. Hence, detection of a large quantity of pectin in a fruit alone is not in itself enough to qualify that fruit as a source of commercial pectin (Thakur et al., 1997).

The commercial pectins are almost produced from citrus peel or apple pomace, both by-products from juice (or cider) manufacturing. Meanwhile, pomelo (*Citrus maxima*) belongs to *Citrus* group and it is widely planted in Vietnam, especially in South Vietnam. The spongy white peel can account up to 30% of the total fruit weight and is a good source for pectin extraction. There are many pectin extraction methods such as conventional method (Methacanon et al., 2014), microwave assisted method (Quoc et al., 2015), ultrasound-assisted methods (Bagherian et al., 2011), etc. with inorganic acid solvents (sulfuric, hydrochloric or nitric acid) and organic acid solvents (tartaric, oxalic or acetic acid). According to Seixas et al. (2014), the microwave extraction has many benefits such as the short extraction time, low solvent consumption and high pectin yield. In addition, using organic acid solvents can improve color quality of pectin (Quoc et al., 2015). Besides,

there are not studies that combine the microwave method and oxalic acid to extract pectin from pomelo peel. For this reason, this study focuses on pectin extraction using pomelo peel as raw material due to its abundant growth in Vietnam region. The following properties would be reported to rate the effectiveness of this synthesis: the pH value, peel/solvent ratio, microwave power and the extraction time.

Materials and Methods

Materials

The ripe "Nam Roi" pomelo from Vinh Long province (Vietnam) was chosen as the raw material. The spongy white peel was removed using a paring knife and cut into small pieces and then they were blanched in steam for 5 minutes. Then, the peel was dried at 70°C until moisture of dried peel were less than 9% and ground into powder (<0.6 mm).

Isolation of pectin

Five grams of pomelo peel were soaked in oxalic acid solution 0.25% with dried peel/acid ratio of 1/19, 1/29, 1/39, 1/49, 1/59 (w/v) and pH values of 3.8, 4.2, 4.6, 5, 5.4. Then mixture was placed in the microwave for 3, 6, 9, 12 minutes at the power 195, 379 and 660 W. After that, the mixture was cooled down to room temperature and filtered using filter fabric. Then, ethanol solution (96%) was used to precipitate pectin (the pectin solution/alcohol ratio was 1/3, v/v) for 60 minutes at pH value of 3.5. The precipitated pectin was washed with ethanol to remove the mono- and disaccharides. At the end, the mixture was dried at 70°C for 4 hours and then stored in bags.

Determination of pectin content

According to Nguyen (2001), crude pectin (0.15 g) was added in 250 mL flask, then adding 100 mL of 0.1 N NaOH. Crude pectin was soaked in NaOH solution for 7 hours, then added 50 mL of 1 N CH_3COOH and 50 mL $CaCl_2$ after 5 minutes and kept it in 1 hour. The solution was boiled for 5 minutes, filtered by filter paper and dried for 1 hour. Calcium pectate was washed with hot water until not having Cl^- ion in the solution. After washing, it was dried for about 2 hours at 105°C. The pure level of pectin was calculated according to the following formula below:

$$P = \frac{W \times 0.92 \times 100}{M}$$

P (%): the pure level of pectin
W (g): weight of calcium pectate
M (g): weight of crude pectin
0.92: pectins have 92% in volume of calcium pectate

The yield of pectin was determined by formula:

$$Y = \frac{Mo}{M}$$

Y (%): yield of pectin
M_o (g): weight of pure pectin ($M_o = P \times M$)
M (g): the weight of dried pomelo peel

Determination of degree of esterification (DE)

This method was slightly modified from titrimetric method of Pinheiro et al. (2008). Pectin (0.5 g) was added in 250 mL flask and dissolved in 5 mL ethanol, 1 g NaCl and some drops of phenolphthalein. Adding 100 mL of warm deionized water dissolved pectin. The solutions were titrated with 0.1 N NaOH and the result was recorded as V_1. Then 25 mL of 0.25 N NaOH was added into this solution which was stirred at room temperature for 30 minutes. After that, 25 mL of 0.25 N HCl was added and the solutions were shaken until the pink color disappeared. The solution was titrated again with 0.1 N NaOH and the final result was recorded as V_2. The DE value was calculated according to the formula below:

Color evaluation

Color parameter consists of L^* (lightness), a^* (redness and greenness), b^* (yellowness and blueness) values were recorded and were carried out by using a Chroma Meter CR-410 (Minolta, Japan).

Data analysis

The experimental data was analyzed by the one-way analysis of variance (ANOVA) method and significant differences among the means from triplicate analyses at ($p<0.05$) were determined by Fisher's least significant difference (LSD) procedure using Statgraphics software (Centurion XV). The values obtained were expressed in the form of a mean ± standard deviation (SD).

Results and Discussion

Effect of pH on the extraction yield of pectin and DE value

The experimental process is performed with microwave-assisted extraction at a microwave power of 379 W, dried peel/acid solution ratio of 1/29 and extraction time of 6 minutes. Changes of pH value are showed in Table 1.

The results show that there are significant differences of yield, purity and DE values ($p < 0.05$) from changes of pH values. The highest yield, purity and DE value peaked at pH value of 4.2. High pH led to a lower yield and purity of dried

Table 1. Effect of pH on the extraction yield and DE value

pH	Yield (%)	Purity (%)	DE (%)
3.8	12.34±0.21[a]	78.07±0.43[a]	88.75±0.54[a]
4.2	15.84±0.25[b]	89.60± 0.30[b]	90.67±0.63[b]
4.6	13.51±0.30[c]	86.11±0.38[c]	89.23±0.76[c]
5	11.12±0.26[d]	84.58±0.50[d]	90.78±0.68[b]
5.4	12.05±0.31[a]	79.86±0.48[a]	90.53±0.60[b]

Different superscript letters in the same column denote significant differences ($p < 0.05$).

pomelo peel pectin, whereas DE values change insignificantly. According to Kertesz (1951), H^+ ions increased sharply with the decrease of pH values, protopectin in cell was hydrolyzed rapidly. In addition, pectin was released easily from raw material at low pH value due to the separation of linkage of pectin with hemicellulose (Rombouts and Thibault, 1986). The yield of this study was similar with other studies such as yield of pectin extraction from cocoa husks at pH value of 4.6 (Ramli and Asmawati, 2011), mango peel at pH value less than 2 (Sudhakar and Maini, 2000), lower than result of Quoc et al. (2015) who also extracted pectin from pomelo peel by tartaric acid at pH value of 1.5 and higher than result of Crandall and McCain (2000) who extracted pectin from soybean byproduct at pH value of 2.4. Pectin in this case called high methoxyl pectin (HMP) (DE higher than 50%), it will form gel with sucrose at pH lower than 3.5 (Mesbahi et al., 2005). Hence, the suitable pH value of 4.2 was chosen for the next survey.

Effect of dried peel/acid solution ratio on the extraction yield of pectin and DE value

Yield, purity and DE value have the significant differences ($p<0.05$) at various dried peel/acid solution ratios. The yield, purity and DE value reached the highest values at peel/acid solution ratio of 1/39 (Table 2) and this ratio is chosen for subsequent experiments. Purity and DE value were approximate 90%. An increase of volume of acid solution can lead to an increase in the diffusion rate of yield, purity and DE value of pectin. This shows that the solvent quantity is an important factor to affect the yield and properties of pectin. The effect of microwave and the suitable solvent quantity cause excessive swelling of the materials, the cell walls were ruptured and pectin was released easily into solvent (Guo et al., 2001; Maran et al., 2013). However, the increase of volume of acid solution was exceeded 1/39 (w/v), it barricaded the penetration of pectin into solution and decreases the extraction yield (Maran et al., 2013). The volume of acid solution in this study were lower than that of study of Quoc et al. (2015) (1/40, w/v) and were higher than that of study of Maran et al. (2013) (1/16.9, w/v); they extracted pectin from pomelo peel and orange peel by tartaric and sulfuric acid, respectively.

Table 2. Effect of dried peel/acid solution ratio on the extraction yield and DE value

Peel/solvent ratio (w/v)	Yield (%)	Purity (%)	DE (%)
1/19	6.83±0.25[a]	84.36±0.23[a]	86.30±0.62[a]
1/29	15.81±0.3[b]	89.57±0.31[b]	90.64±0.85[b]
1/39	17.23±0.35[c]	89.93±0.41[b]	90.11±0.56[b]
1/49	14.93±0.36[b]	87.5±0.27[c]	88.12±0.71[c]
1/59	13.83±0.32[d]	82.88±0.35[d]	85.03± 0.75[d]

Different superscript letters in the same column denote significant differences ($p < 0.05$).

Effect of microwave power on the extraction yield of pectin and DE value

Table 3 shows that the extraction efficiency was improved by raising microwave power from 195 W to 660 W and the yield, purity and DE value have the significant differences ($p<0.05$). The optimal yield, purity and DE value were 18.1%, 90.72%, 91.25% at the highest microwave power (660 W), respectively.

The yield, purity and DE value of pectin increase slowly with the increase of microwave powder. Energy from microwave irradiation can loose, destroy the cell wall matrix and cut off the soft tissues. The skin tissues are rapidly and extensively opened up (Kratchanova et al., 2004; Bagherian et al., 2011). In addition, the temperature of acid solution also increases, then pectin was released and dissolved easily in solvent (Hoshino et al., 2009). Thus, the microwave power of 660 W was the optimum value in this study. However, this microwave power in this case is higher than that of study of Seixas et al. (2014) (628 W), who extracted pectin from passion fruit peel by tartaric, acetic and nitric acid; it was lower than that of study of Bagherian et al. (2011) (900 W), who extracted pectin from grapefruit by hydrochloric acid.

Effect of extraction time on the extraction yield of pectin and DE value

Extraction time is the most important factor that influences directly the extraction process. The effect of extraction time on the yield, purity and DE value of pectin have a significant difference ($p < 0.05$) (Table 4). The yield, purity

Table 3. Effect of microwave power (W) on the extraction yield and DE value

Microwave power (W)	Yield (%)	Purity (%)	DE (%)
195	14.38±0.35[a]	87.54±0.14[a]	88.46±0.31[a]
379	17.27±0.93[b]	89.96±0.09[b]	90.12±0.17[b]
660	18.10±0.48[c]	90.72±0.13[c]	91.25±0.21[c]

Different superscript letters in the same column denote significant differences ($p < 0.05$).

and DE value increased slightly and peaked at 9 minutes. The absorption of microwave energy in the extraction system promoted the thermal accumulation of the extraction solution leads to the dissolution of pectin into the solution until 9 minutes. However, extraction time was extended for long time; pectin can be degraded by high temperature under the influence of microwave (Xianzhe et al., 2011). Thus, the yield, purity and DE value of pectin decrease slowly, when the extraction time extended beyond 9 minutes. Conversely, the extraction time was shorten, the links between pectin with other components such as cellulose and hemicellulose could not be separated and yield of pectin decreased (Kertesz, 1951).

Table 4. Effect of extraction time on the extraction yield and DE value

Extraction time (minute)	Yield (%)	Purity (%)	DE (%)
3	12.56 ± 0.26^{a}	88.75 ± 0.33^{a}	88.84 ± 0.61^{a}
6	17.98 ± 0.27^{b}	90.54 ± 0.26^{b}	91.29 ± 0.77^{b}
9	18.81 ± 0.21^{c}	91.02 ± 0.21^{b}	91.83 ± 0.52^{b}
12	17.34 ± 0.15^{b}	90.51 ± 0.35^{c}	89.96 ± 0.66^{c}

Different superscript letters in the same column denote significant differences ($p < 0.05$).

Color parameters of pectin in the optimal extraction conditions

Table 5 shows that there are significant differences ($p < 0.05$) of color parameters of two kinds of pectin (commercial pectin and experimental pectin). The L^* and a^* value of experimental pectin were higher than these of commercial pectin whereas b^* value was lower than that of commercial pectin. When compared to commercial pectin, the pomelo peel pectinaceous material was found to be slightly darker in color. Experimental pectin is light cream in color (Fig. 1) and it is quite difficult to distinguish between commercial and experimental pectin by naked eyes.

Table 5. Color parameters of commercial pectin and experimental pectin

Type	L^*	a^*	b^*
Commercial pectin	89.37 ± 0.03^{a}	-0.35 ± 0.01^{a}	12.07 ± 0.06^{a}
Experimental pectin	77.28 ± 0.07^{b}	-0.89 ± 0.01^{b}	13.85 ± 0.01^{b}

Different superscript letters in the same column denote significant differences ($p < 0.05$)

Fig. 1. Experimental pectin

Conclusions

The results in this study show that the combination between microwave assisted extraction and oxalic acid affected strongly the yield of pectin and its properties (purity of pectin and color parameters). The optimal extraction conditions of pectin from pomelo peel were pH of 4.2, material/solvent ratio of 1/39, extraction time of 9 minutes, microwave power of 660 W. The yield, DE value and pure pectin peaked at 18.81%, 91.83% and 91.02%, respectively. This pectin can be used in food industry, especially in beverage and jam processing.

References

Bagherian, H., Ashtiani, F. Z., Fouladitajar, A., & Mohtashamy, M. (2011). Comparisons between conventional, microwave- and ultrasound-assisted methods for extraction of pectin from grapefruit. *Chemical Engineering and Processing: Process Intensification*, *50*(11-12), 1237-1243.

Barrera, A. M., Ramırez, J. A., González-Cabriales, J. J., & Vázquez, M. (2002). Effect of pectins on the gelling properties of surimi from silver carp. *Food Hydrocolloids*, *16*(5), 441-447.

Crandall, P. G., & McCain, A. M. (2000). Isolation and characterization of pectinaceous substances from soybean byproducts. *Journal of Food Processing and Preservation*, *24*(5), 407-422.

Guo, Z., Jin, Q., Fan, G., Duan, Y., Qin, C., & Wen, M. (2001). Microwave-assisted extraction of effective constituents from a Chinese herbal medicine Radix puerariae. *Analytica Chimica Acta*, *436*(1), 41-47.

Hoshino, M., Tanaka, M., Terada, A., Sasaki, M., & Goto, M. (2009). Separation and characterization of pectin from juice processing residue extracted by sub-critical water. In: *Proceedings of the 5th annual IFSR*, 223-228.

Kertesz, Z. I. (1951). *The Pectic Substances*. Interscience Publishers, Inc., New York.

Kratchanova, M., Pavlova, E., & Panchev, I. (2004). The effect of microwave heating of fresh orange peels on the fruit tissue and quality of extracted pectin. *Carbohydrate Polymers*, *56*(2), 181-185.

Maran, J. P., Sivakumar, V., Thirugnanasambandham, K., & Sridhar, R. (2013). Optimization of microwave assisted extraction of pectin from orange peel. *Carbohydrate Polymers*, *97*(2), 703-709.

May, C. D. (1997). Pectins. In: A. Imeson (Ed.), *Thickening and gelling agents for food*. Blackie Academic & Professional, London, pp. 232-261.

Mesbahi, G., Jamalian, J., & Farahnaky, A. (2005). A comparative study on functional properties of beet and citrus pectins in food systems. *Food Hydrocolloids*, *19*(4), 731-738.

Methacanon, P., Krongsin, J., & Gamonpilas, C. (2014). Pomelo (*Citrus maxima*) pectin: Effects of extraction parameters and its properties. *Food Hydrocolloids*, *35*, 383-391.

Nguyen, V. M. (2001). *Biochemical Practice*. National University Hanoi Publisher. Ha Noi, Vietnam (in Vietnamese).

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

Quoc, L. P. T., Huyen, V. T. N., Hue, L. T. N., Hue, N. T. H., Thuan, N. H. D., Tam, N. T. T., Thuan, N. N. & Duy, T. H. (2015). Extraction of pectin from pomelo (*Citrus maxima*) peels with the assistance of microwave and tartaric acid. *International Food Research Journal*, *22*(4), 1637-1641.

Ramli, N., & Asmawati (2011). Effect of ammonium oxalate and acetic acid at several extraction time and pH on some physicochemical properties of pectin from cocoa husks (*Theobroma cacao*). *African Journal of Food Science*, *5*(15), 790-798.

Rombouts, F. M., & Thibault, J. F. (1986). Feruloylated pectic substances from sugar-beet pulp. *Carbohydrate Research*, *154*(1), 177-187.

Pinheiro, E. R., Silva, I. M. D. A., Gonzaga, L. V., Amante, E. R., Reinaldo, F. T., Ferreira, M. M. C., & Amboni, R. D. M. C. (2008). Optimization of extraction of high-ester pectin from passion fruit peel (*Passiflora edulis* f. *flavicarpa*) with citric acid by using response surface methodology. *Bioresource Technology*, *99*(13), 5561-5566.

Seixas, F. L., Fukuda, D. L., Turbiani, F. R., Garcia, P. S., Carmen, L. D. O., Jagadevan, S., & Gimenes, M. L. (2014). Extraction of pectin from passion fruit peel (*Passiflora edulis* f. *flavicarpa*) by microwave-induced heating. *Food Hydrocolloids*, *38*, 186-192.

Sudhakar, D. V., & Maini, S. B. (2000). Isolation and characterization of mango peel pectins. *Journal of Food Processing and Preservation*, *24*(3), 209-227.

Thakur, B. R., Singh, R. K., Handa, A. K., & Rao, M. A. (1997). Chemistry and uses of pectin–a review. *Critical Reviews in Food Science & Nutrition*, *37*(1), 47-73.

Zheng, X., Fangping, Y. I. N., Chenghai, L. I. U., & Xiangwen, X. U. (2011). Effect of process parameters of microwave assisted extraction (MAE) on polysaccharides yield from pumpkin. *Journal of Northeast Agricultural University (English Edition)*, *18*(2), 79-86.

Received: 09.02.2018; ***Accepted:*** 13.04.2018