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PHYSICOCHEMICAL CHARACTERISTICS AND ANTIOXIDANT ACTIVITIES OF BANANA PEELS PECTIN EXTRACTED WITH MICROWAVE-ASSISTED EXTRACTION

SUMMARY

Pectin production from banana peels was carried out in microwave-assisted extraction (MAE). The main purpose of this study is to determine the extraction conditions with the best physicochemical characteristics and highest antioxidant activity (AA). The pectin was isolated using the MAE method with two different solvents (citric acid and tartaric acid) at 420 and 613 W for 5 and 10 min. In this study, using MAE with tartaric acid as a solvent strongly improves the yield and AA of banana peel pectin (BPP) compared to those of BPP extracted by citric acid. BPP extracted with MAE and tartaric acid at 420 W for 10 min had the highest yield (15.23±0.52%), while the best AA was obtained at 420 W for 5 min (free radical scavenging activity of 37.17±0.7%). The chemical properties of BPP including equivalent weight, degree of esterification, methoxyl content and total anhydroronic acid, depended on the extraction conditions and BPP could be classified as a low-methoxyl pectin (LMP). The present study also proved that BPP was an ideal alternative source of commercial pectin with the high purity.

Keywords: Banana, microwave, pectin, peel, waste

INTRODUCTION

Banana is considered one of the most important tropical fruits in the Vietnam market. In Vietnam, there are many types of banana that are widely cultivated everywhere, especially Pisang Awak (*Musa acuminata* × *Musa balbisiana*). It can be processed into many kinds of foods, such as chips, crackers, banana muffins, baked bananas, fried banana cakes, etc. Therefore, banana fruit peels make up a large fraction of the waste produced from banana processing. This waste also is an important issue to the processing industries and a challenge for by-product waste management (Kamble *et al.*, 2017). The proper utilization of banana peels can enhance some economic values and reduce environmental pollution (Phaiphan, 2019). In there, using banana peels to produce pectin is one

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of the best solutions because this compound can be used in many fields; for instance, pectin is an additive in food products (thickener, stabilizer and gelling agents) (Emaga *et al.*, 2008) and it is also found in cosmetic and pharmaceutical products (Rolin, 1993).

Pectin is a complex mixture of polysaccharides found in the primary cell wall and intracellular layer of plant cells, mainly in fruits (Mudgil, 2017). The gelling mechanism of pectin strongly depends on the degree of esterification (DE). Pectin is classified into high methoxy pectin (HMP, DE>50%) and low methoxy pectin (LMP, DE<50%) (Mesbahi *et al.*, 2005). Currently, there are many methods to extract pectin from fruit waste. Among them, microwave-assisted extraction (MAE) is the best choice compared to other methods due to improved pectin yield and short extraction times. Many previous studies demonstrated these advantages mentioned above. For instance, Maran *et al.* (2013), Koštálová *et al.* (2016) and Sarah *et al.* (2018) extracted pectin from orange peel, unutilized pumpkin biomass and cocoa peel. In addition, the yield and quality of pectin also depends on solvents. Therefore, the combination of MAE and various solvents can improve the pectin yield and significantly change its characteristics, which adapt to various demands in the food industry. However, until now, there have been no studies on these combinations to extract pectin from banana waste in Vietnam. Based on these reasons, we conducted experiments to isolate pectin from banana peels using MAE, with citric acid and tartaric acid as solvents.

MATERIAL AND METHODS

Materials

Bananas originated from Tien Giang province (Vietnam) and were similar in the uniformity of shape, size and ripeness. Bananas were free of physical damage, and fungal infection. The banana peels were collected from a fried banana store in Ho Chi Minh City and removed from the pulp at the stage 5 of ripeness (more yellow than green). The pectin extraction efficiency obtained the highest level in this stage (Emaga *et al.*, 2008). In addition, all other chemicals used were of analytical reagent grade.

Sample preparation

The sample preparation was performed according to procedure of Khamsucharit *et al.* (2018) with minor modifications. Briefly, banana peels were dried under the sun until their moisture was less than 8%. Next, raw materials were ground into fine powder in a grinder (0.6 mm particle size). Then, the samples were packed in a polyethylene bag and stored at room temperature prior to analysis.

Pectin extraction process

The extraction process was based on the procedure described by Quoc *et al.* (2015) with small changes. Pectin from banana peels (5 g) was extracted using a microwave apparatus (Sanyo EM-S2182W, China) combined with citric acid (0.1 M) and tartaric acid (0.1 M), the extraction conditions were a solid-to-liquid

ratio of 1:20 (g/mL), the microwave powers of 420 and 613 W for 5 and 10 min. Next, the extract was cooled and filtered using filter paper (20-25 µm pore size) to remove the residue. Then, alcohol (96%, v/v) and the obtained filtrate were mixed gently together (filtrate-to-alcohol ratio of 1:3, v/v) and the mixture was left for approximately 20 h to completely precipitate the pectin. The pectin was then filtered through cloth and purified with an alcohol (70%, v/v) solution. Pectin product was dried at 60^{o}C in a hot air oven until its weight stabilized. Finally, it was packed and stored for further experiments.

Determination of pectin content

The percentage of pectin yield (Y) was estimated following the formula below:

$$Y = \frac{m_1}{m_0} \times 100\%$$

m_1: Weight of the obtained pectin (g); m_0: Weight of the initial sample (g).

Determination of moisture of BPP

To determine the moisture content, BPP (0.5 g) was dried in a moisture analyzer at 105^{o}C until a constant weight was achieved. Then, the weight of the sample was recorded. The percentage of moisture content was calculated based on the moisture lost.

Determination of color parameters of BPP

The color parameters of BPP were expressed by L^*, a^* and b^* values and they were measured using a colorimeter (CS-10, China).

Determination of equivalent weight (EW) of BPP

The EW of BPP was determined according to the method reported by Owens *et al.* (1952) with minor modifications. Pectin (0.5 g) was placed in a 250 mL conical flask and wetted with 5 mL of ethanol. 100 mL distilled water and a few drops of phenolphthalein as indicator were added. The solution was titrated with 0.1 N NaOH until the color of the indicator changed to pink and kept for at least 30 s. The neutralized solution was used to determine the methoxyl content. The EW was calculated following the formula below:

$$EW\ (g/mol) = \frac{weight\ of\ sample \times 1000}{mL\ of\ alkali \times Normality\ of\ alkali}$$

Determination of the methoxyl content (MeO) and total content of anhydrouronic acid (AUA) of BPP.

The MeO and AUA were determined following the procedure of Nguyen and Pirak (2019) with slight corrections. 25 mL of 0.1 N NaOH was added into the obtained mixture from the EW analysis. Then, the mixture was shaken continuously and kept for 30 min at room temperature. Next, 25 mL of 0.1 N HCl was added into the mixture, which was also titrated by a 0.1 N NaOH solution until the color changed to pink, as before, with phenolphthalein as an indicator. This can be calculated by using the following formula:

$$MeO(\%) = \frac{normality\ of\ NaOH \times mL\ of\ alkali \times 31 \times 100}{weight\ of\ sample \times 1000}$$

$$AUA\ (\%) = \frac{176 \times 0.1 \times 100}{weight\ of\ sample \times 1000} \times (x + y)$$

where 31 is the molecular weight of the methoxyl group; x and y are the volumes (mL) of the NaOH solutions from the EW determination and MeO determination, respectively.

Determination of degree of esterification (DE) of BPP

According to Owens *et al.* (1952), the DE was estimated by the following formula:

$$DE\ (\%) = \frac{176 \times \%MeO}{31 \times \%AUA} \times 100$$

Determination of antioxidant activity (AA) of BPP

The AA of BPP was evaluated using the DPPH (2,2-Diphenyl-1-picrylhydrazyl) free radical scavenging method, as described by Venzon *et al.* (2015) with minor changes. A DPPH solution (0.1 mM) was dissolved in ethanol. Next, 1.5 mL of 0.1 g/mL of sample solution was mixed with 1.5 mL of DPPH solution. The obtained solution was kept for 30 min in the dark and then its absorbance was measured at 517 nm. The AA was calculated as the reduction of absorbance of the solution (%).

Fourier transform infrared spectroscopy (FTIR) spectra of BPP

The FTIR spectra of BPP were recorded on a Bruker Tensor 27 (Germany) spectrophotometer in the 4 000–500 cm^{-1} region with a resolution of 1 cm^{-1}. The sample was incorporated with potassium bromide (KBr) and pressed into a pellet prior to analysis according to the procedure of Jiang *et al.* (2012).

Scanning electron micrograph

The micromorphology of the samples and residues after the extraction process were evaluated by a scanning electron microscope (SEM) (Jeol JSM-6400, Japan). Samples were examined at 5 kV and the vacuum pressure was 0.04 Pa with various magnifications.

Data analysis

All experiments were performed in triplicate except for FTIR spectral analysis, which was performed one time. The data was analyzed using analysis of variance (ANOVA) by Statgraphics Centurion XV software (version 15.1.02, Statgraphics Technologies, Inc., USA), expressed as the mean value±standard deviation and compared using the Fisher's least significant difference (LSD) procedure at a 5% confidence level.

RESULTS AND DISCUSSION

Physical properties of BPP

Yield of BPP

Table 1 illustrates that the pectin yield of banana peels isolated with MAE fluctuated from 5.28% to 15.23%. Using tartaric acid as a solvent significantly enhances the pectin yield compared to citric acid. In addition, an increase in extraction time can lead to an increase in the efficiency of extraction for both solvents, especially tartaric acid. This observation is similar to that of Sarah *et al.* (2018). The microwave can break down the parenchyma cells, enhance the penetration of the solvent and lead to the improvement of the pectin yield (Kratchanova *et al.*, 2004). The highest pectin yields in this study are from 14.74% to 15.23% at 420 W and 613 W for 10 min. These results were higher than those recorded by Girma and Worku (2016), who also isolated pectin from banana peels (pectin yield of 11.31%) using conventional extraction, or lower than those reported by Maran *et al.* (2013), who used microwave to extract pectin from orange peels (pectin yield of 19.24%). These results indicate that pectin yield strongly depends on the raw material, methods and extraction conditions.

Moisture of BPP

The moistures of BPP that were obtained were quite low, ranging from 3.39 to 3.83% for samples extracted using tartaric acid and from 4.99 to 6.69% for samples extracted using citric acid (Table 1).

Table 1. Yields, moisture and color parameters of BPP at various extraction conditions

Physical properties	Solvents	Microwave power (W)	Extraction time (min)	
			5	10
Pectin yield (%)	Citric acid	420	5.28 ± 0.68^{a}	5.86 ± 0.02^{ab}
		613	5.51 ± 0.39^{a}	6.31 ± 0.21^{b}
	Tartaric acid	420	11.28 ± 0.24^{c}	15.23 ± 0.52^{e}
		613	12.38 ± 0.62^{d}	14.74 ± 0.15^{e}
Moisture (%)	Citric acid	420	4.99 ± 0.6^{b}	5.77 ± 0.06^{bc}
		613	6.69 ± 1.31^{c}	5.94 ± 0.19^{bc}
	Tartaric acid	420	3.39 ± 0.9^{a}	3.83 ± 0.49^{a}
		613	3.45 ± 0.48^{a}	3.42 ± 0.17^{a}
L^*	Citric acid	420	10.51 ± 0.7^{bc}	6.98 ± 1.3^{a}
		613	7.45 ± 1.25^{ab}	12.68 ± 3.73^{c}
	Tartaric acid	420	23.92 ± 2.3^{d}	32.47 ± 1.62^{e}
		613	25.98 ± 1.41^{d}	29.72 ± 1.65^{e}
a^*	Citric acid	420	3.44 ± 1.17^{ab}	3.47 ± 0.87^{abc}
		613	4.42 ± 0.88^{bc}	2.82 ± 0.73^{ab}
	Tartaric acid	420	5.17 ± 0.22^{c}	2.72 ± 0.38^{ab}
		613	3.87 ± 1.96^{abc}	2.29 ± 0.7^{a}
b^*	Citric acid	420	5.69 ± 0.91^{a}	6.91 ± 0.5^{a}
		613	5.11 ± 0.9^{a}	5.31 ± 1.31^{a}
	Tartaric acid	420	13.88 ± 0.03^{c}	10.13 ± 1.21^{b}
		613	15.28 ± 2.34^{c}	11.3 ± 1.35^{b}

Data followed by the different superscript letters in the same physical property are significantly different ($p\leq0.05$)

In general, all moistures are less than 10%, which is a safe moisture level for preserving the pectin powder to avoid the growth of microorganisms and the effect of pectinase on pectin quality (Mohamadzadeh *et al.*, 2010; Nguyen and Pirak, 2019). The moistures obtained in this study are in agreement with those from pectin of unripe banana peels (Kamble *et al.*, 2017) or white dragon fruit peels (Nguyen and Pirak, 2019), etc.

Color parameters of BPP

As seen in Table 1, the color parameters (L^*, a^* and b^*) of BPP had a significant difference (p<0.05). The BPP extracted using tartaric acid showed higher levels of lightness and yellowness than the BPP extracted with citric acid. The highly colored pectin was attributed to the presence of impurity components trapped inside the pectin during the precipitation process, such as phenolic compounds (Baississe *et al.*, 2010), water-soluble pigments (Nguyen and Pirak, 2019), etc. In general, as the extraction time increased, the lightness of pectin also increased for most of the samples. This trend can be explained by the fact that the phenolic compounds or water-soluble pigments are destroyed the longer the samples are kept at high temperatures, leading to the lightness of the samples. According to Khamsucharit *et al.* (2018), L^*, a^* and b^* of pectin from banana peels of different varieties in Thailand ranged from 77.33 to 85.3, 2.09 to 3.88 and 5.37 to 8.59, respectively. The L^* values of all samples in this study are significantly lower, whereas the b^* values of samples extracted using tartaric acid are higher than those mentioned above. Therefore, the color of pectin also depends on the nature and source of the fruits.

Chemical properties of BPP

EW of BPP

The EW of BPP at different conditions ranged from 188.77 to 200.82 g/mol for samples extracted using citric acid and from 222.56 to 229.71 g/mol for samples using tartaric acid (Table 2). Generally, the obtained results revealed that extraction conditions (solvents, microwave power and extraction time) dramatically influenced the EW of BPP. Compared to some previous studies, EWs of pectin from banana peels extracted using a conventional method and MAE were 925.01 g/mol (Girma and Worku, 2016) and from 1 500 to 2 300 g/eq (Rivadeneira *et al.*, 2020), respectively. Hence, the EWs in this study were quite low compared to those studies because the produced pectin had high partial degradation (Nguyen and Pirak, 2019). Additionally, Ramli and Asmawati (2011) also indicated that the EW values also depend on the amount of free acid in the samples.

MeO of BPP

Table 2 shows the MeO values of BPP. These values vary from 0.57 to 1.24% (for samples extracted with citric acid) and from 0.27 to 0.39% (for samples extracted with tartaric acid). In general, there was a tendency for the MeO values to increase slightly when extraction time increased and the microwave power does not significantly affect the MeO values. In addition, the obtained results are quite low compared to those recorded from other studies.

According to Aina *et al.* (2012), the MeO values range from 0.2 to 12%; they completely depend on extraction conditions and source of materials. For instance, the MeO values of pectin from mango peels, lemon peels, grapefruit peels, sweet orange peels and banana peels were 8.89% (Girma and Worku, 2016), 4.46, 5.79, 3.9% (Aina *et al.*, 2012) and 3.86-5.97% (Khamsucharit *et al.*, 2018), respectively. With the low MeO values (<7%), the pectin product can form gels with low concentrations of sugars compared to commercial pectins, which have MeO values ranging from 8 to 11% and can form high sugar gels (>65% sugar) (Castillo-Israel *et al.*, 2015).

AUA of BPP

As seen in Table 2, the AUA values of pectin are quite high, from 94.69 to 96.1% for citric acid and from 78.5 to 81.08% for tartaric acid. These results also showed that the microwave power and extraction time did not affect the AUA values. According to Food Chemical Codex (1996), the AUA values are the important factor to determine the purity of the extracted pectin and it is not lower than 65%. All obtained AUA values in this study fall within this range. This proved that all extracted pectin samples had a high purity and they can be used for food additives or pharmaceutical purposes. Compared to other reports, these results were higher than those of pectin from mango peels (70.65%) (Girma and Worku, 2016), Saba banana peels (39.68-57.32%) (Castillo-Israel *et al.*, 2015), etc.

Table 2. Some chemical properties of BPP at various extraction conditions

Chemical properties	Solvents	Microwave power (W)	Extraction time (min)	
			5	10
EW (g/mol)	Citric acid	420	194.31 ± 1.9^{b}	200.57 ± 3.26^{c}
		613	188.77 ± 5.06^{a}	200.82 ± 2.14^{c}
	Tartaric acid	420	224.55 ± 0.58^{d}	229.71 ± 1.21^{e}
		613	222.56 ± 2.05^{d}	226.25 ± 1.02^{de}
MeO (%)	Citric acid	420	0.97 ± 0.13^{c}	1.3 ± 0.22^{c}
		613	0.57 ± 0.14^{b}	1.24 ± 0.06^{c}
	Tartaric acid	420	0.27 ± 0.09^{a}	0.33 ± 0.16^{a}
		613	0.35 ± 0.09^{ab}	0.39 ± 0.09^{ab}
AuA (%)	Citric acid	420	96.1 ± 1.61^{b}	95.16 ± 2.64^{b}
		613	96.54 ± 3.2^{b}	94.69 ± 0.61^{b}
	Tartaric acid	420	79.9 ± 0.7^{a}	78.5 ± 0.61^{a}
		613	81.08 ± 0.89^{a}	80.02 ± 0.54^{a}
DE (%)	Citric acid	420	5.73 ± 0.66^{c}	7.75 ± 1.11^{d}
		613	3.36 ± 0.71^{b}	7.44 ± 0.41^{d}
	Tartaric acid	420	1.91 ± 0.66^{a}	2.39 ± 1.11^{ab}
		613	2.46 ± 0.65^{ab}	2.78 ± 0.65^{ab}

Data followed by the different superscript letters in the same chemical property are significantly different ($p\leq0.05$)

DE of BPP

The results in Table 2 show that the DE values were quite low and ranged from 3.36 to 7.75% for samples extracted with citric acid and from 1.91 to 2.78% for samples extracted with tartaric acid. All these values are less than 50%. Therefore, the extracted pectin was classified as LMP (Mesbahi *et al.*, 2005).

This result contrasts with those of other studies, which showed that the DE of BPP was classified as HMP (Castillo-Israel *et al.*, 2015; Khamsucharit *et al.*, 2018). These differences can be explained by the different modes of extraction and source of initial materials.

Antioxidant activity (AA) of BPP

As seen in Table 3, it was observed that the AA of BPP strongly depends on extraction conditions. The highest AA values ranged from 35.52 to 37.17% at 420 W for 5 min (both citric and tartaric acid). Some previous studies demonstrated that pectin from plants had AA and its AA mechanism is similar to that of phenolic compounds by donating their electron to the oxidants (Serrano-Cruz *et al.*, 2013; Hung *et al.*, 2020). The obtained results also revealed that an increase in microwave power or irradiation time can lead to a significant changes in AA. This can be explained by the fact that the heat-sensitive bioactive compounds were easily degraded by the higher microwave power and longer irradiation time compared with the initial condition (420 W, 5 min). Furthermore, solvents also affect the AA of pectin because their pectin had a different amount of free carboxylic groups, resulting in different exchanges of electrons donated and finally leading to changes in the AA (Hung *et al.*, 2020). Considering that published studies on the AA of BPP are nonexistent, it is difficult to compare our results to any other findings. However, the AA of pectin has been reported for other materials, such as orange pomace (Venzon *et al.*, 2015), white dragon fruit peels (Nguyen and Pirak, 2019) and pomelo peels (Hung *et al.*, 2020), etc.

Table 3. AA (%) of BPP at various extraction conditions

Solvents	Microwave power (W)	Extraction time (min)	
		5	10
Citric acid	420	35.52 ± 1.97^{f}	8.40 ± 2.03^{a}
	613	24.44 ± 0.41^{d}	27.26 ± 0.20^{e}
Tartaric acid	420	37.17 ± 0.70^{f}	16.86 ± 1.77^{c}
	613	11.61 ± 1.26^{b}	18.24 ± 0.6^{c}

Data followed by the different superscript letters are significantly different ($p\leq0.05$)

FTIR analysis of BPP

FTIR spectra of BPP extracted using citric and tartaric acid as solvents are illustrated in Figure 1. The spectral features of all samples from 950 to 1 200 cm^{-1} were assigned to carbohydrates, especially sugar composition. The intense peaks corresponding to the characteristics of pectin polysaccharides were also shown in these spectral regions, which can be assigned to C=O stretching, C–O stretching, C–C stretching, C–H stretching and C–O bending (Wongkaew *et al.*, 2020). In addition, the absorptions of antisymmetric and symmetric stretching frequencies of the ionic carboxyl groups (COO^{-}) in all samples were from 1 600 to 1 650 cm^{-1} and from 1 400 to 1 450 cm^{-1} for both solvents (Posé *et al.*, 2012). The strong absorptions recorded at approximately 1 700 cm^{-1} determined the presence of the C=O stretching vibration of ester carbonyl groups (George *et al.*, 2015, Hung *et al.*, 2020). These findings are similar to the report of Posé *et al.* (2012). According to Coates (2000) and Hung *et al.* (2020), the intense absorptions of around 3 000 cm^{-1} for all samples showed the distension of –CH, $–CH_2$, $–CH_3$ and

methyl esters of galacturonic acid, while the peaks ranging from 3 300 to 3 450 cm^{-1} indicated the distension of a –OH group. In general, some major components obtained from FTIR spectra were in agreement with those of pectin from other materials, such as orange pomace (Venzon *et al.*, 2015), pomelo peels (Hung *et al.*, 2020) and unripe banana peels (Kamble *et al.*, 2017), etc.

Figure 1. The FT-IR spectra of BPP obtained using various extraction conditions

Morphology of the pectins

At a low magnification, the shape of BPP particles was also similar to pectin from other materials (data not shown), such as jackfruit waste (Begum *et al.*, 2017) and *Akebia trifoliata* var. *australis* peel (Jiang *et al.*, 2012). The pectin particles had lamellate, lumpish and irregular powder shapes and a rough surface. At a higher magnification (3 000×), Figure 2 shows the structure on the surface of BPP quite clearly. The structure of pectin extracted using various solvents was

significantly different. All BPP particles extracted using citric acid were significantly ruptured and more porous on the surface, while the morphology of the pectins extracted using tartaric acid was slightly ruptured and showed a slight tendency to be wrinkled at 613 W for 10 min.

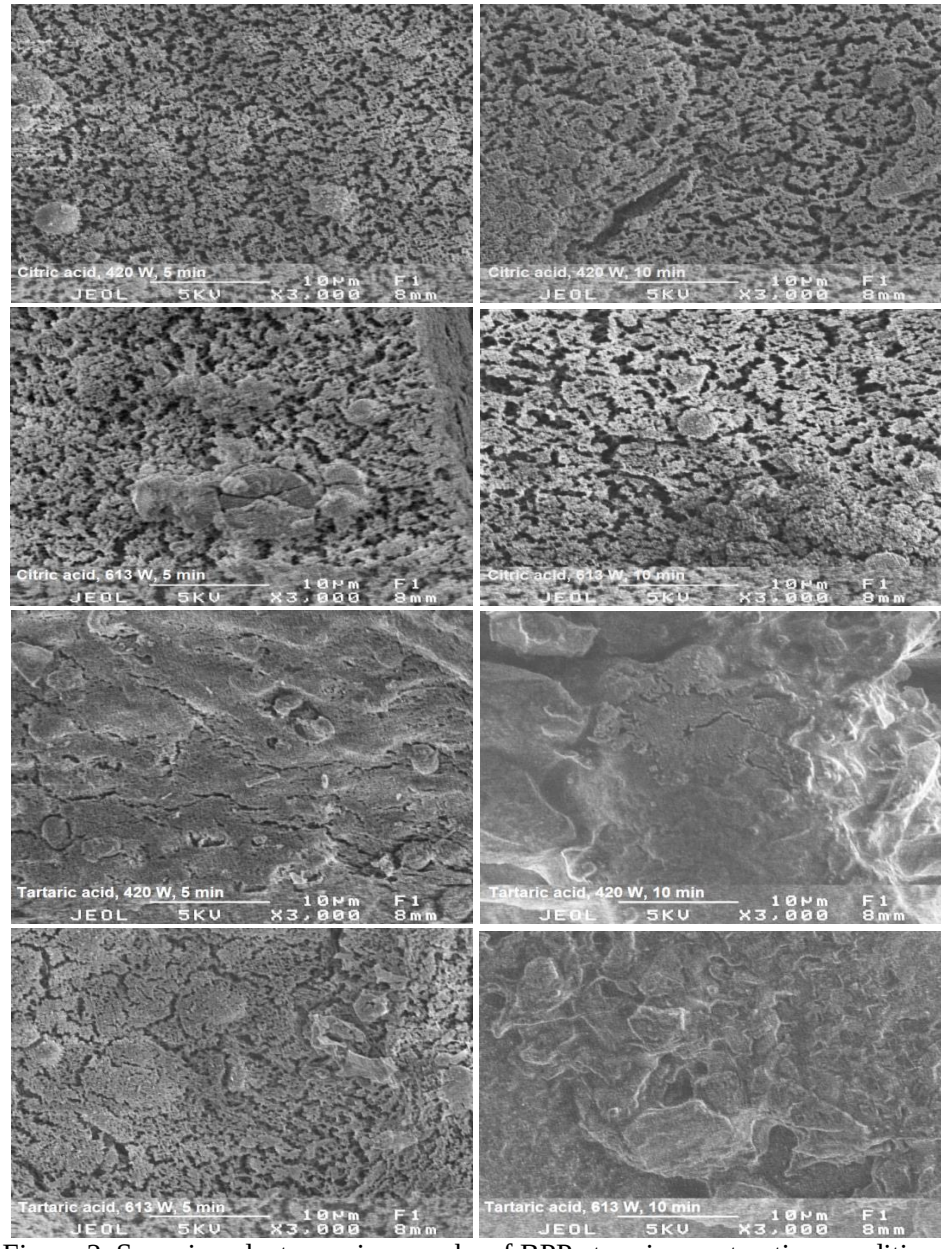

Figure 2. Scanning electron micrographs of BPP at various extraction conditions

Also, the microwave treatment caused a partially collapsed and ruptured morphology in the microstructure of the extracted pectin (Rahmati *et al.*, 2012) because of the rapid rise in temperature (Liew *et al.*, 2016) and the high microwave power can lead to loosening of the cell wall matrix (Kamal *et al.*, 2020). In addition, sources of raw materials as well as extraction conditions (pH, time, solid to solvent ratio, etc.) strongly influence the morphology of the obtained pectin.

CONCLUSIONS

In this study, pectin was successfully extracted from banana peels using MAE and citric/tartaric acid as solvents. The result revealed that the physical and chemical properties of pectin were significantly affected by the modes of extraction (solvent, microwave power and irradiation time). The results also indicated that pectin extracted from both solvents was of LMP with high purity. Besides, using different solvents make significant changes in the morphology of the pectin. The product obtained is still an ideal pectin source that can be used in the food industry in the future.

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