# Microwave-Assisted Extraction of Pectin from Passion Fruit Peels under Alkaline Conditions

Le Pham Tan QUOC (✉)

**Summary**

In this study, microwave-assisted extraction (MAE) of pectin from passion fruit (*Passiflora edulis* Sims) peels has been investigated together with the alkaline extraction conditions such as NaOH concentration (60-220 mM), solid-to-liquid (SL) ratio (1:20-1:40, g/mL), extraction time (3-11 min), and microwave power (167-502 W) on the yield of crude pectin, purity, and degree of esterification (DE) of pectin. The results indicated that all these factors significantly affected the extraction efficiency and purity of recovered pectin. The best extraction condition was found to be as follows: NaOH concentration of 180 mM, SL ratio of 1:35 g/mL, extraction time of 7 min, and microwave power of 376 W. Under these conditions, the experimental crude pectin yield, purity, and DE value were 14.2%, 87.4%, and 92.6%, respectively. The pectin obtained was considered as high-methoxyl pectin (HMP) and the microstructure of initial peels powder completely changed under microwave heating.

**Key words**

alkaline condition, extraction, microwave, *Passiflora edulis* Sims, pectin

Institute of Biotechnology and Food Technology, Industrial University of Ho Chi Minh City, 12 Nguyen Van Bao Street, Go Vap district, Ho Chi Minh City, Vietnam

✉ Corresponding author: lephamtanquoc@iuh.edu.vn

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## Introduction

Pectin is a complex heteropolysaccharide and it is found in the primary cell wall and higher plant middle lamella (Wandee et al., 2019). It also is an important functional ingredient in the food industry, especially beverage and jam processing. Additionally, it is considered additive in food technology, its role is well known for being used as a gelling agent, thickener and emulsifier (Qin et al., 2019). Besides, the gel strength of pectin is strongly affected by the degree of esterification (DE) and the molecular weight. There are two types of pectin, such as high-methoxyl pectin (HMP, DE>50%) and low-methoxyl pectin (LMP, DE<50%), depending on the DE of the galacturonic acids residue (Quoc, 2019).

For decades, commercial pectin has most often been obtained from apple pomace and citrus peel. However, currently, many studies are pointing out that the high-quality pectin can be found in different parts of other plant materials, for instance, durian ring (Maran, 2015), pistachio green hull (Chaharbaghi et al., 2017), sugar beet pulp (Li et al., 2012), dragon fruit (Rahmati et al., 2019), etc. These materials are alternative sources of commercial pectin, but the usage of them in commercialization remains limited. To produce commercial pectin from other raw materials, new potential species and by-products from the fruit have been found, especially passion fruit peels. Its peel is an agricultural solid waste, rich in high-quality pectin. According to Oliveira et al. (2016), the content of pectin in passion fruit peel peaked at 12.67% with ultrasound-assisted extraction (UAE) and nitrite acid as an extraction solvent. However, the obtained pectin had low purity and low DE (60.36%) whereas the conventional extraction method was only 7.95%. Hence, a pectin extraction method from passion fruit peel is needed to improve the yield and quality of obtained pectin.

Some extraction techniques are also affected by various extraction factors, for instance, temperature, pH, SL ratio, and extraction time. Using convection methods has many disadvantages such as long extraction time, low product quality, large solvent consumption, and thermally unsafe. Now, various modern pectin extraction methods can overcome the aforementioned obstacles and difficulties, for instance, microwave-assisted extraction (MAE) (Rahmati et al., 2019), ultrasound-assisted extraction (UAE) (Oliveira et al., 2016), enzyme-assisted extraction (EAE) (Dranca and Oroian, 2019), etc. Although some previous studies recorded that passion fruit peels could be used as raw material for pectin separation (Seixas et al., 2014; Oliveira et al., 2016), the combination of MAE and the alkaline solution has not been studied.

The major aim of this study was to use a strong alkaline solution (NaOH) for the extraction of pectin from passion fruit peels under the assistance of microwave heating. The factors affecting the yield of pectin such as NaOH concentration, SL ratio, extraction time, and microwave power were also determined. The pectin obtained should be of high DE, purity, and the yield should be as high as possible.

## Materials and Methods

### Materials

Fresh passion fruit (*P. edulis*) was purchased from a local market (Dak Lak, Vietnam). The diameter ranged from 5 to 6 cm and the weight was from 60 to 70 g. No physical damage and no pest contamination of the plants were observed on fruit. Furthermore, all other chemicals used were of analytical reagent grade.

### Sample Preparation

At first, samples were cleaned under tap water and cut to remove the seeds, purple skin, and pulp. Then, the peels were dried in an oven at 80°C until a constant weight was obtained (~5%). After that, they were ground with a grinder (Panasonic MX-V310KRA, China) for 3 min and separated by a sieve (the hole size of 0.8 mm) to collect the specimens that could pass through. After that, the samples obtained were packed in a polyethylene (PE) bag and preserved at room condition (29±2°C). These powdered samples were used for all subsequent experiments.

### Pectin Extraction Process

The extraction process was carried out in a 1000 mL round bottom flask and condenser system, followed by heating in a microwave oven (Whirlpool model MWX201BL, China). 5 g of passion fruit peel powder was soaked in alkaline solution (NaOH) and was extracted with the assistance of microwave heating. The MAE conditions were described as follows: NaOH concentrations ranged from 60 to 220 mM, solid-to-liquid (SL) ratios were from 1:20 to 1:40 (g/mL), extraction time was adjusted from 3 to 11 min and microwave power changed from 167 to 502 W. Then, the mixture obtained was cooled down to room temperature (29±2°C) and filtered by cloth to remove the residue. Ethanol solution (99%, v/v) was added to the filtrate (filtrate-to-ethanol ratio of 1:1, v/v) and the mixture was left for an hour to completely precipitate pectin. The precipitate was separated and washed 2 times with ethanol solution (70%, v/v) to remove impurities. Finally, it was dried at 80°C in a hot air oven for nearly 4 hours. The dried crude pectin was packed and stored in PE bags.

### Determination of Crude Pectin Content

Extraction yield (Y) was determined by formula:

$$Y = m_1 / m_0 \times 100\% \quad (1)$$

$m_1$: The obtained crude pectin weight (g)

$m_0$: The initial dried sample weight (g)

### Determination of the Purity of Pectin

The purity of pectin was determined according to the procedure of Quoc (2019): 0.15 g of the crude pectin obtained was added and soaked in 100 mL of 0.1 N NaOH for 7 hours. Then, 50 mL of 1 N $CH_3COOH$ was added to the mixture. After 5 min, 50 mL of 2 N $CaCl_2$ was also added to the mixture and kept for 1 hour. The solution was boiled for 5 min, filtered by Whatman filter paper (No. 4) in vacuum filtration. The received residue was dried

until the constant weight was obtained. Calcium pectate obtained was washed by hot water until the $Cl^-$ ion was not detected in the solution and dried for 2 hours at 105°C. The purity of pectin was calculated according to the following formula below:

$$P = \frac{m_{calcium\ pectate} \times 0.92}{m_{crude\ pectin}} \times 100\% \quad (2)$$

P: The purity of pectin (%)

$m_{calcium\ pectate}$: The obtained weight of calcium pectate (g)

$m_{crude\ pectin}$: The weight of crude pectin (g)

0.92: Coefficient of pectin was 92% in volume of calcium pectate

### Determination of the Degree of Esterification (DE) of Pectin

The degree of esterification (DE) was conducted by a titrimetric method based on the procedure of Pinheiro et al. (2008) with some minor changes. Firstly, the dried pectin (0.15 g) was transferred to a 250 mL Erlenmeyer flask and wetted with 99% ethanol solution (v/v). Then, the sample was dissolved in 20 mL of distilled water at 40°C and slightly stirred for 2 hours. The solution obtained was titrated with 0.1 N NaOH in the presence of phenolphthalein until the solution turned faint pink and the result was recorded as the initial titer ($V_1$). After that, 10 mL of 0.1 N NaOH solution was added to a neutralized polygalacturonic acid sample after determination of the free carboxyl groups and the mixture was stirred for 2 hours to saponify the esterified carboxyl groups of the polymer. Afterwards, 10 mL of 0.1 N HCl was added and excess HCl was titrated with 0.1 N NaOH. The endpoint of reaction was reached when a faint pink color persisted for at least 30 sec. This titration volume was recorded as the saponification titer ($V_2$). The DE value was calculated according to the formula below:

$$DE = \frac{V_2}{V_1 + V_2} \times 100\% \quad (3)$$

### Scanning Electron Microscope (SEM)

The structural morphology of the sample was determined by a scanning electron microscope (Hitachi S-4800, Japan) at 10 kV. SEM micrographs were undertaken at various magnifications.

### Data Analysis

All experiments were carried out with three replications and the results were expressed in the form of a mean±standard deviation (SD). The experimental data was analyzed by the one-way analysis of variance (ANOVA) method which was performed using Fisher's least significant difference (LSD) procedure to compare treatment means at $P < 0.05$. Statistical analysis was performed using Excel (version 14.0, Microsoft Corp, USA) and Statgraphics Centurion XV (version 15.1.02, Statgraphics Technologies, Inc., USA).

## Results and Discussions

### Effect of NaOH Concentration on the Extraction Yield of Pectin, Purity, and DE Value

The influence of different alkaline concentrations on the pectin yield, purity, and DE value was presented in Table 1, while extraction parameters including SL ratio of 1:30 (g/mL), extraction time of 7 min, and microwave power of 376 W remained unchanged. The statistical data recorded that there were significant differences of yield, purity, and DE values ($P < 0.05$) between alkaline concentrations.

**Table 1.** Effect of alkaline concentration on the extraction yield of pectin, purity, and DE value

| NaOH concentration (mM) | Yield (%) | Purity (%) | DE (%) |
|---|---|---|---|
| 60 | 6.7±0.44$^a$ | 82.8±0.21$^b$ | 93.5±0.52$^a$ |
| 100 | 7.8±0.25$^b$ | 82.9±0.74$^b$ | 94.7±0$^b$ |
| 140 | 10.5±0.06$^c$ | 84.8±0.32$^c$ | 94.8±0$^b$ |
| 180 | 13.4±0.4$^d$ | 86.7±0.53$^d$ | 94.8±0$^b$ |
| 220 | 14±0.36$^e$ | 81.4±0.15$^a$ | 94.7±0.03$^b$ |

Different lowercase letters in the same column denote significant difference ($P < 0.05$) between NaOH concentrations

An increase in NaOH concentrations can lead to an increase in the yield and purity of pectin, whereas DE values only ranged from 93.5 to 94.8% for all concentrations. However, at the highest NaOH concentration (220 mM), the purity of the obtained pectin was lower than that of other alkaline concentrations. The best yield, purity, and DE value at NaOH concentration of 180 mM were 13.4%, 86.7%, and 94.8%, respectively. The maximum yield and DE value in this study were higher than those of the study of Seixas et al. (2014) (13% and 64.15%, respectively). They also extracted pectin from passion fruit peel by MAE with nitric acid as solvent. Compared to the acid, extraction with NaOH solutions at the same method can provide higher yields and DE value. This notice is similar to the results of Wandee et al. (2019), who isolated pectin from pomelo peel by MAE with HCl and NaOH as the solvent.

Thus, based on the results achieved, the NaOH concentration of 180 mM was selected for the next experiments in this study.

### Effect of SL Ratio on the Extraction Yield of Pectin, Purity, and DE Value

To determine the most appropriate SL ratio to isolate pectin, SL ratios ranged from 1:20 to 1:40 were tested, whereas other parameters, such as NaOH concentration, extraction time, and microwave power remained unchanged, as shown in Table 2. Table 2 shows a steady increase in pectin yield in the range of ratios from 1:20 to 1:40, the yields obtain the best values at SL ratios of 1:35 and 1:40. Besides, DE values slightly fluctuate from 93.6 to 94.7% during all tests, while the maximum purity is 88% at SL ratio of 1:35. This also proves that SL ratio of 1:35 is the best choice to extract pectin from passion fruit peel by MAE.

**Table 2.** Effect of SL ratio on the extraction yield of pectin, purity and DE value

| SL ratio (g/mL) | Yield (%) | Purity (%) | DE (%) |
|---|---|---|---|
| 1:20 | 8.6±0.25[a] | 82.9±0.5[a] | 93.7±0.04[b] |
| 1:25 | 12.3±0.11[b] | 84.0±0.2[b] | 94.6±0.03[c] |
| 1:30 | 13.4±0.43[c] | 86.8±0.15[c] | 94.7±0[d] |
| 1:35 | 14.1±0.07[d] | 88.0±0.36[d] | 93.8±0[b] |
| 1:40 | 14.2±0.08[d] | 86.1±0.32[c] | 93.6±0[a] |

Different lowercase letters in the same column denote significant difference ($P < 0.05$) between SL ratios

Compared to other studies, the optimal SL ratio in this study is higher than that in the study of Rahmati et al. (2019), who isolated pectin from dragon fruit peels at SL ratio of 1:66.57 by MAE. On the contrary, it is lower than the results of Dranca and Oroian (2019), who extracted pectin from apple pomace at SL ratio of 1:10 by EAE. At the same time, the DE value of passion fruit pectin is also two times higher than that of dragon fruit pectin and similar to that of apple pomace pectin (96.02%). Basically, pectin in this study has the high purity (88%), higher than that of pectin in pomelo peels (80.88%) (Quoc et al., 2015). These differences could be explained due to the difference in materials and extraction methods. In addition, an increase in the volume of solvent leads to a decrease in viscosity of materials and an increase in the diffusion rate (Cacace and Mazza, 2003). Hence, this can improve the yield as well as its purity and the extraction process is finished when the diffusion process achieves the equilibrium. Besides, the amount of solvent also affects ethanol consumption to precipitate pectin.

### Effect of Extraction Time on the Extraction Yield of Pectin, Purity, and DE Value

Table 3 illustrates the effect of extraction time on the yield, purity, and DE value of pectin while the other extraction parameters stayed unchanged and were as follows: NaOH concentration of 180 mM, SL ratio of 1:35 (g/mL), and microwave power of 376 W. The received results point out that at the extraction time of 7 min, the highest yield, purity, and DE value were 14.2%, 88.1%, and 92,7%, respectively. From the optimal extraction time of 7 min, there was a strong decrease in the level of purity with longer extraction time while the yields and DE values changed insignificantly during the rest of the scale.

The extraction time may dramatically influence the extraction efficiency of natural products and their chemical-physical properties. The extraction time in this study is shorter than that of other methods, for instance, extracting pectin from apple pomace by EAE (18.25 hours) (Dranca and Oroian, 2019), passion fruit peel by UAE (10 min) (Oliveira et al., 2016), durian rinds by heating method (43 min) (Maran, 2015), etc. In addition, it also depends on the kind of materials and particle size of the sample because a larger specific surface area of the extraction sample will result in quicker extractions (Liu et al., 2006). In this case, the dimension of powdered samples is smaller than 0.8 mm, which leads to shortening the extraction time. However, extending the extraction time in the microwave field under high temperatures can cause the degradation of the pectin and its properties (Maran, 2015). Hence, a suitable extraction time of 7 min was chosen for the next experiment.

**Table 3.** Effect of extraction time on the extraction yield of pectin, purity, and DE value

| Extraction time (min) | Yield (%) | Purity (%) | DE (%) |
|---|---|---|---|
| 3 | 9.1±1.18[a] | 84.6±0.61[a] | 90.8±0.23[a] |
| 5 | 11.6±0.41[b] | 86.5±0.25[c] | 91.8±0.64[b] |
| 7 | 14.2±0.19[c] | 88.1±0.2[d] | 92.7±0.12[c] |
| 9 | 14.3±0.83[c] | 85.6±0.64[b] | 92.7±0.12[c] |
| 11 | 14.4±0.18[c] | 85.6±0.31[b] | 92.3±0.55[bc] |

Different lowercase letters in the same column denote significant difference ($P < 0.05$) between extraction times

### Effect of Microwave Power on the Extraction Yield of Pectin, Purity, and DE Value

Microwave powers were changed to determine the best power level, whereas all other factors were fixed. The results are shown in Table 4. Theoretically, microwave radiation penetrates through the cell wall and it also is the major cause that leads to the complete disruption and collapse of the primary cell walls of the passion fruit peels, which results in an increase of pectin yield. These results are in agreement with those of Seixas et al. (2014) and Rahmati et al. (2019).

**Table 4.** Effect of microwave power on the extraction yield of pectin, purity, and DE value

| Microwave power (W) | Yield (%) | Purity (%) | DE (%) |
|---|---|---|---|
| 167 | 7.1±0.64[a] | 88.4±0.32[d] | 91.9±0.23[a] |
| 334 | 10.3±0.21[b] | 87.8±0.21[c] | 92.5±0.64[b] |
| 376 | 14.2±0.1[c] | 87.4±0.47[c] | 92.6±0.12[b] |
| 418 | 14.4±0.2[c] | 86.2±0.25[b] | 92.6±0.12[b] |
| 502 | 14.6±0.27[c] | 85.0±0.21[a] | 92.6±0.55[b] |

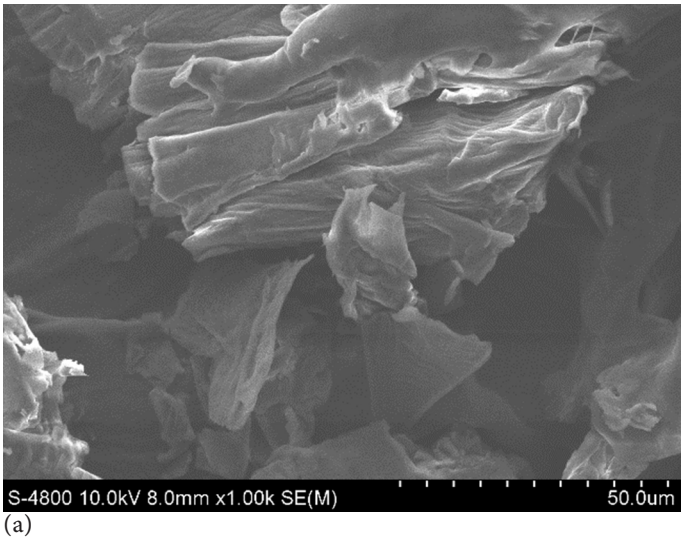
Different lowercase letters in the same column denote significant difference ($P < 0.05$) between microwave powers

An increase in microwave power can lead to an increase in the extraction temperature, resulting in a significant increase in pectin yield (Seixas et al., 2014) and a slight decrease in purity. At the same time, the DE values stay almost unchanged and range from 91.9 to 92.6%. These values are still higher than 50%, therefore, passion fruit pectin is considered to be high methoxyl pectin (HMP). The maximum yield, purity, DE value were 14.2%, 87.4%, and 92.6% at a microwave power of 376 W, respectively. The trend of these results is similar to other studies, for instance, according to Bagherian et al. (2011), extraction yield increased with

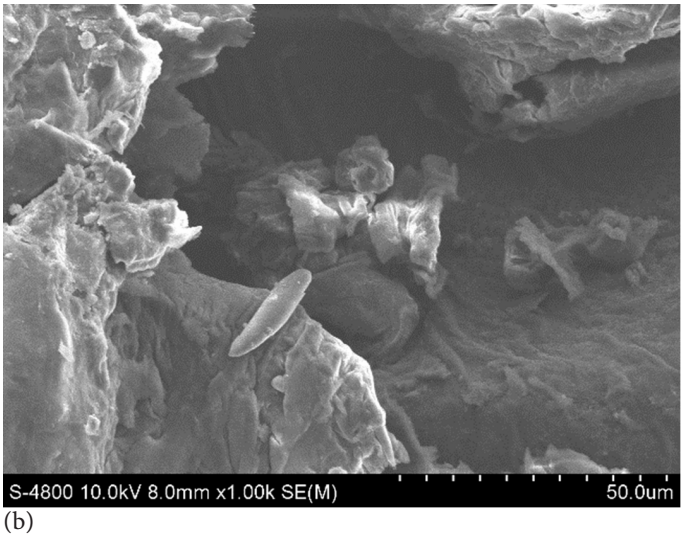
increasing microwave power. They have studied the influence of microwave power and time on the extraction and characterization of pectin from grapefruit. Besides, Rahmati et al. (2019) reported that the DE value of pectin seemed to be insignificantly increased by increasing microwave power in the pectin extraction from the dragon fruit peels. This finding seems to be similar to our results.

### Morphology Change of Extraction Samples and Pectin Powder

SEM analysis of passion fruit peel powder before and after extraction under microwave heating investigated morphological changes. The micrograph of initial dried powder was compact, undisturbed, and smooth in shape (Fig. 1a). Meanwhile, the image of materials exposed to microwave radiation was illustrated in Fig. 1b. Under the effect of microwave radiation, the cell wall and internal tissue of passion fruit peels were damaged and ruptured seriously. The microstructure of samples was folded, wrinkled, and degraded; the surface was flaky in shape. These phenomena were completely similar to those in the study of Rahmati et al. (2019), who used dragon fruit peels as the initial materials.



(a)



(b)

**Figure 1.** Scanning electron microscopy for the passion fruit peel powder before (a) and after (b) extraction under microwave heating

Fig. 2 illustrates a dry form pectin structure extracted by microwave under alkaline extraction. With the effect of microwave heating, the microstructure of pectin was smooth and compact with a little wrinkle on the surface. It is completely different from that of dry pectin extracted from mangosteen rind by heating method (Wathoni et al., 2019) or passion fruit peels by Celluclast (Liew et al., 2015). These results also indicated that the morphology of pectin depended on the extraction method and the initial materials.

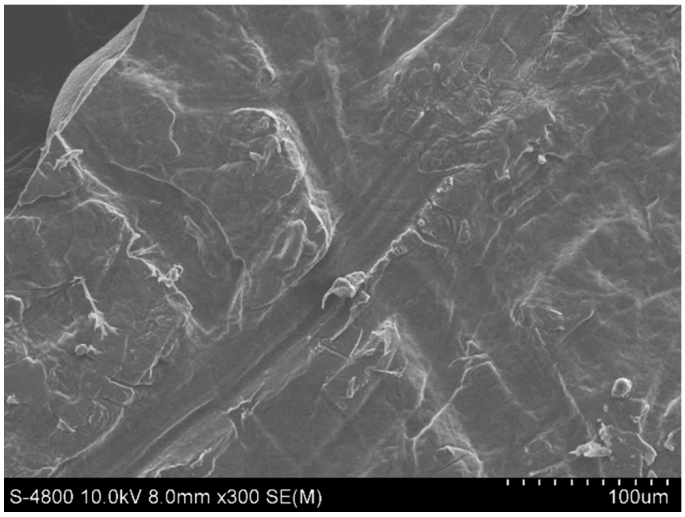


**Figure 2.** Scanning electron microscopy of the dry pectin

## Conclusion

In general, pectin was successfully extracted from passion fruit peels by MAE under alkaline conditions and had a specific characteristic. All extraction conditions (NaOH concentration, SL ratio, time, and microwave power) significantly influenced pectin extraction from passion fruit peels. The maximum extraction yields and purity of pectin under optimum conditions (NaOH concentration of 180 mM, SL ratio of 1:35 (g/mL), time of 7 min and microwave power of 376 W) were 14.2% and 87.4%, respectively. Pectin was classified as high-methoxyl pectin. Essentially, the passion fruit peel is the potential new source to be developed as an additive for food applications.

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