

## OPTIMIZING THE PECTIN EXTRACTION PROCESS FROM AMBERALLA PEEL BY THE COMBINED OXLALIC ACID AND MICROWAVE AND COMPARISON OF CHARACTERISTICS WITH THE PECTINS OBTAIN TO TRADITIONAL EXTRACTION METHOD

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### Abstract

The extraction of high-methoxyl pectin from amberella peels was studied. The best result was obtained with pH of 4.6, solid-liquid ratio of 1:38, extraction time of 9 min and microwave power of 600W in which the pectin content was 20%. Some characters of pectins were estimated. Pectins obtained by the combined oxalic acid and microwave showed DE value and galacturonic acid 7.3% and 100.6%; respectively, higher than those treated with hydrochloride acid. DA values were the same between both pectin samples. The lightness of pectins extracted by the combined oxalic acid and microwave was more suitable for food application than that of pectins obtain to the hydrochloride treatment. There were significant different in color co-ordinates between the pectins from hydrochloride treatment and that from the combined oxalic acid and microwave treatment. The viscosity and viscosity-average molecular weight of pectins of the combined oxalic acid and microwave were 120% and 161% higher than those of traditional method. Microwave irradiation could be damaged more cell walls of amberella peels to release a large amount of pectins and to reduce treatment time for minimizing the cost of extraction process. The combined oxalic acid and microwave is a good method to get more pectins from amberella peels.

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### 1. INTRODUCTION

Pectin is a polysaccharide found ubiquitously in cell walls of higher plants. This polysaccharide is mainly composed of two moieties of rhamnogalacturonan (hairy region) and homogalacturonan (smooth region). Rhamnogalacturonan consists of neutral sugar such as L-arabinose, D-galactose and D-xylose are covalently linked to the rhamnosyl residues of the backbone (Voragen et al., 1995). Pectins are sorted into high methoxyl and low methoxyl based on their degrees of esterification. High methoxyl pectin has degree of esterification above 50% and vice versa with low methoxyl ones (Voragen et al., 1995). The mechanism of formation of pectins gels is determined by the degree of substitution of methyl esters. In high methoxyl pectins, high concentrations of soluble solids and low pH are

necessary conditions for gel formation. On the other hand, ion  $\text{Ca}^{2+}$  supports to gel formation in case of low methoxyl pectins through “egg box” mechanism. Pectin is widely used as a gelling agent and stabilizer in many food applications such as jam, jelly, fruit preparations, fruit drink concentration, fermented dairy products (Tsoga et al., 2004). Ambarella or golden apple (*Spondias cytherea*) is a tropical fruit belonging to the family of Anacardiaceae which are considered as the vitamin C and mineral sources (Morton, 1987; Ishak et al., 2005). Ambarella peels make up 19% of the total fruit weight and become main byproduct components (Koubala et al., 2008). Citrus peel and apple pomace are considered as main raw materials for commercially pectin production (May, 1990). Therefore, an alternative pectin preparation from ambarella

peels applied for food products could be employed to vary product characters.

Over the past decade, various novel extraction techniques have been introduced such as microwave-assisted extractions (MAE), supercritical fluid extraction (SFE), and high pressurized solvent extraction (HPSE) in order to recover pectin from various waste biomass. Among the these extraction techniques, MAE has been considered as a potential and powerful alternative to conventional extraction techniques, due to its moderate capital cost and its good performance under atmospheric conditions, shorter time, less solvent, higher extraction rate and better products with lower cost (Eskilsson and Björklund, 2000; Maran et al., 2013). In this study, oxalic acid – a mild weak organic acid – was replaced for strong mineral acids which caused to corrosion of equipment and deleterious effects on the environment. The aim of this study was to evaluate the impact of different extraction conditions on the yield and some bio-chemical characteristics of ambarella peel pectins and their suitability as a source of industrial pectin.

## 2. MATERIALS AND METHODS

*General procedure:* Raw materials were collected from Go Vap market, Vietnam. Sands and other impurities were removed from raw materials. Then, sorted materials were blanched at 100°C/5 min, dried at 70°C/8h, grinded and sieved through a screen in order to obtain ambarella peel powders. Then, appropriate methods were applied to extract pectin from ambarella peel powders. The crude extract was filtered through a vacuum filter and centrifuged at 10,000 rpm for 15 min. The supernatant was concentrated to about half of its volume in a rotary evaporator before being precipitated with ethanol 70% (w/v) for 1 hour and extract to ethanol ratio of 1:4 (v/v). The precipitate was dried in a vacuum oven and ground to obtained crude extract powders.

*Traditional extraction procedure:* Hydrochloride acid was used to acidify solvent for extracting pectin from ambarella peel powders. After conducting the preliminary

experiments, the extraction parameters were as following: pH 1.5, raw material to solvent ratio of 1:40 (%w/v), extraction time of 25 min, extraction temperature of 85°C.

*Novel extraction procedure:* The combined microwave and oxalic acid treatment was applied to obtain pectin from ambarella peel powders. The response surface methodology was used to identify the relationship existing between the response function and process variables and to determine the optimal condition of extraction process. The three independent variables including pH ( $X_1$ ), solid-liquid ratio ( $X_2$ ) and extraction time ( $X_3$ ) were surveyed. Dried amaralla peel powders were extracted under conditions of varied pH (2.6-6.6), solid: liquid ratio (1:20-1:60 w/v) and treatment time (2-9 min) with distilled water (Table 1). Extraction solvent was acidified by oxalic acid. The microwave power was fixed at 600W based on the preliminary experiments.

For verification of the model, the pectin was extracted under optimal conditions and the extracted pectin yield was determined. The experimental and predicted values were compared in order to determine the validity of the model.

**Table 1. Experimental values and coded levels of the independent variables**

Independent variables	Coded variables	Coded variable levels		
		-1	0	+1
pH	$X_1$	2.6	4.6	6.6
Liquid-solid ratio (v/w)	$X_2$	20	40	60
Treatment time (min)	$X_3$	2	6	9

**2.2 Pectin yield:**  $H\% = \text{pectin weight/sample weight} * 100$

### 2.2 Determine some basis characters of pectin

**Galacturonic acid** was determined colorimetrically as described by Anthon and Barrett (2008). Copper was reduced by galacturonic in sample and in standard solution at pH 4.8. Then, reduced copper was quantified by Folin–Ciocalteu reagent. Absorbance was read at 750 nm using a standard curve with galacturonic acid.

**Degree of esterification** would be evaluated by titrated method mentioned by Owens et al (1952). The process briefly described as follows: Weigh 0.5g of crude pectin into 250ml erlen. Then, 5 ml ethanol, 1 g NaCl and a few drops of phenol red indicator are added into this erlen. Next, 100 ml of distilled water were added into this erlen in order to dissolve pectin completely (could be heated slightly to 90°C). Titration of the obtained solutions with 0.1N NaOH until the phenol red turn into pink color that persists on 30 seconds (pH 7.5), recoded the volume of NaOH 0.1N ( $V_1$ ). Next, 25ml of 0.25N NaOH was added into these erlen, vibrated well and let stand for 30 minutes at room temperature. Then, 25ml of HCl 0.25N were added to erlen and mixed well. Titrate the solution contained in erlen with 0.1N NaOH until pink at the previous (pH 7.5), recoded the volume of NaOH 0.1N ( $V_2$ ). DE values were calculated based on the %MeO, % AUA as follows:

$$\%MeO = \frac{V_1 * 0.1 * 31 * 100}{m},$$

$$\%AUA = \frac{(V_1 * 0.1 + V_2) * 176 * 100}{m} \quad \text{and}$$

$$\%DE = \frac{176 * \%MeO * 100}{31 * \%AUA}$$

Where m (mg) is the weight of sample,  $V_1$  (ml) is the volume of NaOH 0.1N using in the first titration and  $V_2$  (ml) is the volume of NaOH 0.1N using to titrate in the next time.

**Acetyl value (AcOH)** of pectin samples was determined by the colorimetric method based on hydroxamic acid reaction (Kliemann, 2009).

### SEM analysis

In order to understand the extraction mechanism, amberella peel powder after extraction by conventional method (hydrochloride acid extraction) and novel technique (combined microwave and oxalic acid extraction) were subjected to SEM. Both pectin samples were dried at 70°C in 4 hours for scanning by SEM. All samples were tested under high vacuum conditions at a

voltage of 3.0 kV (50  $\mu$ m, 2000 $\times$ magnification).

### Intrinsic viscosity and viscosity-average molecular weight

Intrinsic viscosity is denoted as  $[\eta]$  shown the individual polymer molecules in isolation form which frequently occurred in diluted solution. Moreover, this parameter is also useful to understand deeply about the biopolymer characters because of its relation with molecular weight (Khouryieh et al., 2007). First, intrinsic viscosity is determined by extrapolation technique based on the Martin curve as following equation:

$$\ln\left(\frac{\eta_{sp}}{C}\right) = \ln[\eta] + K[\eta]C \quad (1)$$

$$\text{and } \eta_{sp} = \frac{\eta - \eta_0}{\eta} \quad (2)$$

Where  $[\eta]$ ,  $\eta_{sp}$ , C,  $\eta$ ,  $\eta_0$  and K are the intrinsic viscosity (L/g), specific viscosity, pectin concentration (g/L), viscosity of pectin solution (Pas), viscosity of solvent (Pas) and Martin's constant, respectively. Second, the viscosity-average molecular ( $M_v$ ) is estimated by using the Mark-Houwink-Sakurada equation:  $[\eta] = kM_v^\alpha$  (3)

Where  $[\eta]$ ,  $M_v$ , k and  $\alpha$  are intrinsic viscosity (L/g), viscosity-average molecular weight, given constants of solute-solvent system and temperature, respectively. In this study, k and  $\alpha$  were  $2.34 \times 10^{-5}$  and 0.8224, respectively (Xingfeng et al., 2012).

Dried pectin powders were hydrated with the phosphate buffer (0.1M, pH=7) with concentration ranged from 0.2 to 1% w/w overnight at 25°C. Then, the viscosity was measured by Oswald capillary tube. The apparent viscosity was determined by using a conical concentric cylinder at the angular velocity of 100 rpm at 25°C using a temperature-controlled circulating water bath.

### CIELAB colorimetric parameters

All samples was tested about colors in terms of the CIE  $L^*$ ,  $a^*$ ,  $b^*$  values by using a Minolta CR-420 Colorimeter, with illuminant D65 and 10° observer.  $L^*$  displays lightness,  $a^*$  = red (+)

to green (-) axis,  $b^*$  = yellow (+) to blue (-) axis. Chroma indicating color intensity or saturation calculates by  $(a^{*2} + b^{*2})^{1/2}$ . Hue angle shows the properties of color and it is measured by using the equation:  $H^* = \tan^{-1}(b^*/a^*)$ . CIE total color difference between two samples is calculated through the following equation:  $\Delta E_{ab}^* = (\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{1/2}$  (Pérez-Magarino and Gonzalez-Sanjose, S.M.L). Calibration was implemented on a standard white ceramic plate prior to the sample analysis ( $L^* = 91.0$ ,  $a^* = +0.3165$ ,  $b^* = +0.3326$ ).

Pectin obtained to both traditional and combined microwave and oxalic acid extraction were grinded and sorted by sieving. Particle size of 250  $\mu\text{m}$  was selected to measure color. Ten random measurements per sample were taken and average values  $\pm$  SD were shown in this study.

### 3. RESULTS AND DISCUSSIONS

3.1. Optimize the conditions for extracting pectin from amberella peel powders with the combined microwave and oxalic acid treatment.

The results of experimental design are presented in Table 2. The pectin yield achieved from the ambarella peel powders ranged from  $7.50 \pm 0.24$  to  $19.08 \pm 0.05\%$ . After omitting insignificantly coefficients, the regression equation for pectin yield (Y) is as follows:

$$Y = 17.05 + 3.3X_3 - 0.71X_1^2 - 0.77X_2^2 - 0.82X_3^2 + 0.51X_1X_2 \quad (1)$$

In order to fit model, coefficient of determination ( $R^2$ ), which is a measure of degree of fit, was 0.98 (table 3). This indicates that only 2% of the total variation is not explained by the model. The highest yield was obtained when extraction conditions were pH 4.6, material/solvent ratio of 1:38 and extraction time of 9 min. Pectin extraction yield was 20% in this case.

**Table 2. Measured pectin yield in the combined microwave and oxalic acid treatment**

	Coded independence variables			Pectin yield (%)
	X1	X2	X3	
1	-1	-1	-1	8.40 $\pm$ 0.12
2	+1	-1	-1	7.50 $\pm$ 0.24
3	-1	+1	-1	7.63 $\pm$ 0.39
4	+1	+1	-1	11.34 $\pm$ 0.51
5	-1	-1	+1	18.50 $\pm$ 0.15
6	+1	-1	+1	17.34 $\pm$ 0.15
7	-1	+1	+1	16.60 $\pm$ 0.33
8	+1	+1	+1	18.24 $\pm$ 0.83
9	-1	0	0	15.10 $\pm$ 0.28
10	0	0	0	15.33 $\pm$ 0.47
11	0	-1	0	15.27 $\pm$ 0.64
12	0	+1	0	15.19 $\pm$ 0.98
13	0	0	-1	10.60 $\pm$ 0.63
14	0	0	+1	19.08 $\pm$ 0.05
15	0	0	0	17.42 $\pm$ 0.28
16	0	0	0	17.95 $\pm$ 0.82
17	0	0	0	17.91 $\pm$ 0.78
18	0	0	0	17.63 $\pm$ 0.12
19	0	0	0	16.82 $\pm$ 0.83
Traditional procedure	8.61 $\pm$ 0.45			

**Table 3. Analysis of variance of the regression model in experiments of the combined oxalic acid and microwave treatment**

Source of variation	DF	SS	MS	F
Total	19	4508.30	237.28	48.73
Constant	1	4240.57	4240.57	
Total corrected	18	267.73	14.87	
Regression	9	262.34	29.15	
Residual	9	5.38	0.60	
Listed F-value <sup>a</sup>	43.82			
R <sup>2</sup>	0.98			

SS: sum of squares; DF: degrees of freedom; MS: mean square; F: F-value.<sup>a</sup> F-value at 95% of confidence level

**Table 4. Significance of regression coefficients of the fitted second-order polynomial model for response (Y)**

	Regression coefficient	Standard error	P
X <sub>1</sub>	0.26	0.182288	0.183817
X <sub>2</sub>	0.15	0.182288	0.436698
X <sub>3</sub>	3.30	0.182288	2.17E-08
X <sub>1</sub> <sup>2</sup>	-0.71	0.259926	0.023589
X <sub>2</sub> <sup>2</sup>	-0.70	0.259926	0.024261
X <sub>3</sub> <sup>2</sup>	-0.82	0.259926	0.011742
X <sub>1</sub> *X <sub>2</sub>	0.51	0.151907	0.008019
X <sub>1</sub> *X <sub>3</sub>	-0.16	0.151907	0.314416
X <sub>2</sub> *X <sub>3</sub>	-0.28	0.151907	0.095642

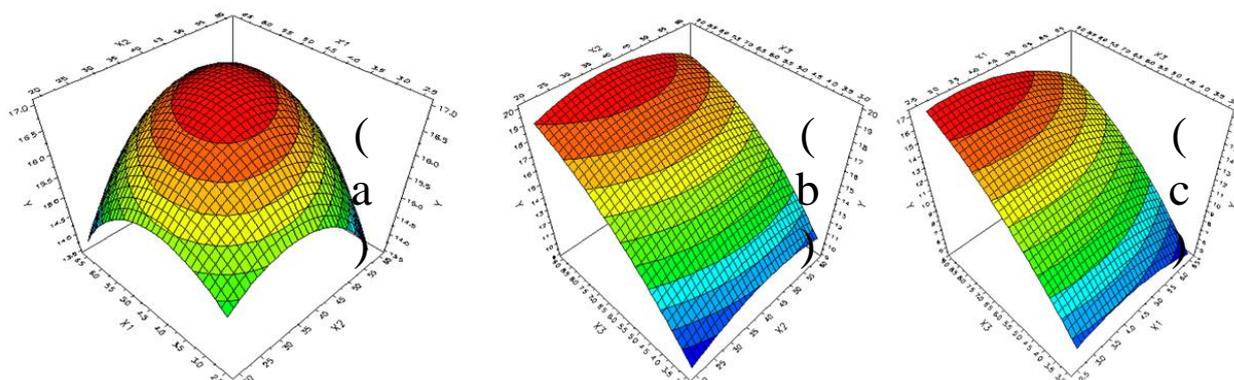


Fig 1. The contour plots for the effects of pH, solid-liquid ratio and microwave irradiation time on pectin extraction from amberella peels

The effects of extraction conditions of ambarella peel powders on pectin yield by the regression coefficients of fitted second-order polynomial are presented in Table 4. It was evident that the linear terms (extraction time), and two quadratic terms (pH, solid-liquid ratio and extraction time) and cross-product terms (pH\*solid-liquid ratio) were significant ( $P < 0.05$ ). The results indicated that the effects of solid-liquid ratio and extraction time were the major contributing factors to pectin extraction from ambarella peel powders. However, pH values had no significant effects ( $P > 0.05$ ) on the pectin extraction from ambarella peel powders within the experimental range.

Additional independent experiments were conducted with the recommended optimum conditions in order to verify the model fitting. The results revealed that the experimental pectic extraction yield (20.1%) was not significantly different from the predicted pectin yield (20.0%).

Fig 1b, c showed that treatment time displayed a linear effect on the response, and the extracted pectin increased with an increase of microwave irradiation time. However, pH and solid-liquid ratio demonstrated a quadratic effect on the response (fig 1a).

It can be seen that pectin extraction yield from the novel procedure is 2.3 times higher than that of the traditional procedure. The reason could be explained that microwave radiation liberates the cell wall matrix. Therefore, the peel tissues are rapidly and extensively opened

when the microwave treatment are applied (Kratchanova et al., 2004). This leads to an increased interaction between extracting solution and raw material in the extraction process. As a result, it can be increased in pectic extraction yield (Bagherian et al., 2011).

### 3.1 Some basis characters of pectins

Table 5. Chemical analysis of the pectins obtained by the traditional extraction and the combined microwave and oxalic acid extraction

Characteristics	Traditional extraction	The combined microwave and oxalic acid extraction
Degree of esterification (%)	74.9 <sup>a</sup> ± 1.3	80.4 <sup>b</sup> ± 1.1
Degree of acetylation (%)	0.43 <sup>a</sup> ± 0.09	0.41 <sup>a</sup> ± 0.03
Galacturonic acid content (%)	35.8 <sup>a</sup> ± 0.30	73.8 <sup>b</sup> ± 0.65

The degree of esterification (DE) of extracted pectin with the combined microwave and oxalic acid was higher than that of the hydrochloride extraction (table 5). In addition, both kinds of pectin possessed the DE exceeded 50%. Thus, they have been belonged to high methoxyl pectin group. A higher DE leads to more rapid setting. Moreover, rapid set pectins (DE above 72%) are also formed gel at lower soluble solids than do slow set pectins (i.e. pectin with a DE of 58–65%). Therefore, high methoxyl pectins in this study could be considered as the slow set pectins.

The galacturonic acid contents of the traditional extraction and the combined microwave and oxalic acid treatment were 35.81% and 73.8%, respectively. According to Food Chemical Codex, Food and Agriculture Organisation and European Union, the commercial pectin must contain at least 65% galacturonic acid (Willats et al., 2006). Base on the experimental data, it can be seen that the pectin from the novel extraction meets this requirement, whereas pectin from traditional extraction does not.

Degree of acetylated pectin (DAc) is defined as the ratio of acetylated galacturonic acid groups to total galacturonan units (Imerson, 2010). Acetyl groups are presented in low amounts in pectins from apple and citrus, but are presented in much higher amounts in pectins from sugar beet and potato (Visser and Voragen, 1996). In our study, both pectins consist of low amounts of acetyl groups. The acetyl values of pectin obtain by traditional and novel techniques were not significantly different with each other. These values are consistent with that of passion fruit rind pectin (0.3–0.5%) (Yapo & Koffi, 2006), lemon pectin (0.26%) and apple pectin (0.72%) (Imerson, 2010).

### 3.2 Color parameters of pectins

**Table 6. Measured CIELAB (D65/10°) coordinates and  $\Delta E^*$  for pectins**

Parameters	Hydrochloride extraction	The combined microwave and oxalic acid extraction
L*	43.50 <sup>b</sup> ± 0.63	50.25 <sup>a</sup> ± 1.08
a*	8.87 <sup>a</sup> ± 0.21	10.19 <sup>b</sup> ± 0.33
b*	22.10 <sup>a</sup> ± 1.67	20.27 <sup>b</sup> ± 0.91
C*	76.86 <sup>a</sup> ± 0.02	80.03 <sup>b</sup> ± 0.01
H*	44.68 ± 1.61	51.02 ± 0.93
$\Delta E^*$	17.21 ± 0.17	24.18 ± 0.15

Because food color is one of the important quality indices to measure the consumers' purchasing needs, the color change of pectins was evaluated after hydrochloride acid or microwave and oxalic acid treatments. Lightness (L\*) values in both kind of pectins were significantly different (table 6). The table 6 shows that pectin collected from the novel

procedure was brighter than that of the traditional one. Consequently, the pectin in the novel procedure is easy to use in food application. Parameter a\* had positive values in both pectins, revealing a certain reddish tone in them, even though they were relatively small values. Parameter b\* was different between the two kinds of pectins and toward to the yellowish tone. Both H\* and C\* values depend on the changes in the a\* and b\* values. Therefore, these values are quite different on both of pectins because of the difference of a\* and b\* values. The color different ( $\Delta E^*$ ) is a measure of the distance in color space between two color. In this study, there is a huge different in  $\Delta E^*$  values of both pectins. It might be that the long time thermal exposure of pectin treated with hydrochloride acid has been contributed in color variation.

### 3.1 Intrinsic viscosity and viscosity-average molecular weight

**Table 7. The different of intrinsic viscosity and viscosity-average molecular weight between samples treated with hydrochloride acid and oxalic acid**

Extraction method	Martin equation	R <sup>2</sup>	[ $\eta$ ] (L/g)	M <sub>v</sub> (Da)
Oxalic acid and microwave treatment	y = -0.806x + 0.737	0.997	2.09	1047161
Hydrochloride acid treatment	y = -0.724x - 0.052	0.972	0.95	401196

In table 7, the Martin equations of samples treated with hydrochloride acid and oxalic acid were illustrated with high values of R<sup>2</sup> (more than 0.97) shown that intrinsic viscosity of pectin was fitted and Martin equation could be used to calculate the viscosity-average molecular weight. Pectin solutions behaved like Newton fluids if their concentrations below 1% (Endress, Döschl-Volle, & Dengler, 1996). Moreover, in diluted solutions all high molecular particles separate each other. Therefore, intrinsic viscosity which depends on molecular weight and chain dimension of biopolymer could be measured in the low range of pectin concentration from 0.2 to 1% (Khouryieh, Herald, Aramouni, & Alavi, 2007). In table 7, intrinsic viscosity and

viscosity-average molecular weight of pectin obtained to the samples treated with oxalic acid 2.2 and 2.6 times higher than those treated with hydrochloride acid, respectively. The reasons could be explained that the long duration for

treating with hydrochloride with elevated temperature might be impelled the degradation of high molecular pectin into low molecular ones. These phenomena might be not met in the situation of oxalic acid ones.

### 3.2 SEM analysis

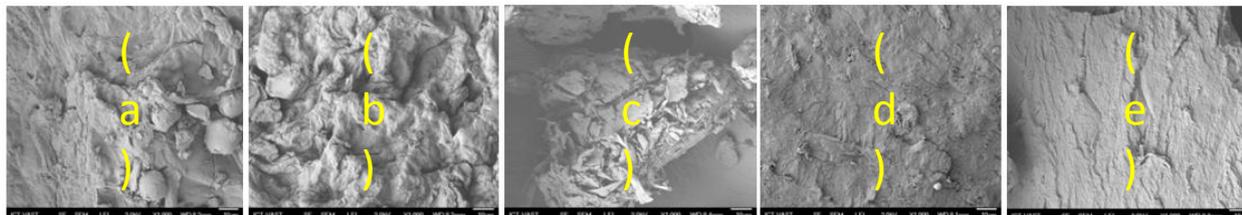


Fig 2. (a)-raw materials, (b)-residual from hydrochloride treatment, (c)-residual from microwave and oxalic treatment, (d)-pectin from hydrochloride treatment and (e)-pectin from microwave and oxalic treatment

There was no rupture of cell walls or significant destruction to the microstructure of the sample without treatment (raw materials). After hydrochloride acid treatment the cell wall of amberella became thinner and the microstructure disorganized. In traditional method (hydrochloride treatment), the solvent transferred into the sample and released the compounds by permeation and solubilisation. Consequently, little destruction of the microstructure of sample occurs and a few of slight ruptures took place on the surface of the sample. However, the combined microwave and oxalic acid destroyed more cell walls and created more channel to release pectin. Huge perforations have been seen on the external surface of the particle treated with the combined microwave and oxalic acid. Extreme thermal stresses and localized high pressures in case of microwave heating were ruptured cell walls more rapidly than in traditional extraction. The rate of mass transfer of cell constituents through the cell walls was enhanced in this phenomenon. Similar effects were reported by Pare and Belanger (1997) and Chen and Spiro (1995) for the microwave extraction of rosemary leaves in hexane. Fig 2c exhibited that microwave can rupture the structures of cells of amberella efficiently under the chosen optimal condition. Therefore, the pectin can be released easily into the extraction solution more than conventional method.

There are different in pectin shapes when various extraction methods are applied. Microwave was broken the cluster formation of the pectin through its swelling effect. Meanwhile, pectin obtained from traditional method still remained as the cluster formation (Liu et al., 2006). That is a reason why the surface of pectin in conventional method is more smoothly than that in novel method. For this reason, the pectin collected from the novel method absorbed water faster than that from the traditional method.

### 4. CONCLUSION

The extraction of pectin with the combined microwave and oxalic acid was found to be more efficient and less polymer chain disruptive process than that of hydrochloride acid treatment. The physico-chemical parameters of the extracted pectin are dependent both on extraction process. The optimal conditions for pectin extraction using the combined microwave and oxalic acid technique were determined as follows: material-to-liquid ratio 1:38, pH 4.6 and extraction time 9 min. Under these conditions, the highest pectin yield was 20%, which was significant higher to the traditional extraction method.

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