Influences of Ultrasonic Assisted Pectin Extraction with Hydrochloric and Citric Acid from Kluai Namwa (Musa ABB cv.) on Yields Analyzed by Taguchi Method

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Abstract

Waste of Kluai Namwa is mostly left in Thailand. Pectin extraction methods have been used to be modified. Ultrasound-assisted extraction (UAE) methods were recently known to be involved in green technology. Pectin extraction was determined the conditions of the highest percentage yield of pectin from the banana peel using ultrasound under the limitation of devices. Ultrasound conditions were set under 400 watts-maximum limit with percentage of the amplitude 60 80 and 100. Extraction times were varied at 5 10 and 15 minutes. In this study, the types of acids were compared between hydrochloric and citric acid. The Taguchi method, orthogonal array was used to support systematic experiment and maximizes the desired results. The most important factor affecting the pectin yield was acid types (factor A, 30.03%) followed by exposure time (factor C, 23.18%) and ultrasonic amplitude (factor C, 19.36%). Pectin yield predictive equation was computed by statistical software as - 0.07 - 2.19 Acid type + 0.0529 Ultrasonic amplitude + 0.235 Exposure time, R-Sq (adj) = 82.7%. The results showed that the highest yield was obtained by using the condition which gave 100% of amplitude and 15 minutes of extraction time. The obtained pectin yield was 7.88%. The degree of esterification was 37.78% showed low methoxyl percentage (MeO%) as 6.17%.

Keywords: Ultrasound-assisted extraction, UAE, banana peel, pectin extraction, Taguchi method

Introduction

Banana found in the world is in the Musaceae family. It is a tropical fruit easily found in Thailand which leaves its waste double compared to product in the industries and markets (Guerrero, Aguado, & Curt, 2015). Musa ABB cv., Kluai Namwa is a generally consumed in Thailand such as sweets, chips, and beverages including fresh fruits. Plenty of Kluai Namwa peels were discarded as waste according to that large consumption. There were some conversions of banana processing industrial waste (peel) into hetero polysaccharide as pectin. The main structure of pectin is a backbone of (1⟶4) linked d-galacturonic acid with different degree of methyl esterification (Chaharbaghi et al., 2017; Thakur, Singh, Handa, & Rao,1997). Based on degree of esterification (DE), the pectin is divided into two groups: low methoxyl and high methoxyl pectin. In the low methoxyl pectin, the esterification of acidic groups is less than 50% while it is more than 50% in high methoxylated pectin (Liew, Chin, & Yusof, 2014). Pectin is used as a thickener, a texturiser, an emulsifier, stabilizer and gelling agent in food industry (Kratchanova, Pavlova, Panchev, 2004).

Mostly, industrial pectin extraction is controlled under the extreme condition such as the chemical solvent combination and high temperature setting. The conventional technique can well accelerate the mass transfer between phases but some compounds are loss due to thermal sensitivity. Although the techniques are efficient in facilitating mass transfer between two phases, thermo-labile compounds may be damaged. For example, conventional extraction techniques have been shown to damage some component especially heat-sensitive compound. These uses of conventional solvents were also concerned to the environmental problem. Ultrasound-assisted extraction (UAE), the extraction methods were recently known to be involved in green technology (McDonnell, & Tiwari, 2017). Mineral acids (hydrochloric, nitric, phosphoric, sulfuric acid) and alkali are used in conventional pectin extraction (Maran et al., 2017; Emaga, Ronkart, Robert, Wathelet, & Paquot, 2008; Sun, & Tomkinson, 2002; Kumar, & Chauhan, 2010; Begum, Aziz, Uddin, & Yusof, 2014). Instead of mineral acid, organic acids were used in the extraction techniques such as citric acid, a safe food grade compound considered to be eco-friendly (Oliveira, 2016). The ultrasonic-based tools supported liberation of expected compounds efficiently with its cavitation effect by distracting the cell walls accelerated the solvent transfer to extracted material and caused small degradation. The accomplish results is depending on its functions such as ultrasonic amplitude and duration. This technique was proved to reduce toxic solvent in the environment and improved the duration and energy consumption.

Musa balbisiana pectin was extracted by ultrasound assisted citric acid and optimized. The optimal extraction condition with pH 3.2 was under 323 watts of ultrasound power, and consumed 27 minutes of extraction duration. The mean experimental yield of pectin was 8.99 ± 0.018% (Maran et al., 2017). However, Girma and Worgu (2016) extracted the banana peels by using conventional acidic method and found yield of pectin between 7.5% to 11%.

In recent years, the Taguchi-based optimization technique has produced a unique and powerful optimization discipline that differs from traditional practices (Phadke, 1989). The Taguchi experimental design simplifies the amount of tests under orthogonal array (OA). Orthogonal array allows one to compute the main and interaction effects via a minimum numbers of experiment trials (Ross, 1996). Furthermore, it supports the optimum extraction results efficiently and systematically. This optimization technique using Taguchi design has not been found in any pectin extraction research yet. The Taguchi method uses a loss function to calculate the deviation between the experimental values and the desired values. Then, the signals to noise ratios (SN) are derived from the Taguchi loss function. Actually, there are three types of the qualified ratio analysis, the lower-the-better, the higher-the-better, and the nominal-the-best. The SN ratio is calculated from each level of the process parameters by the SN analysis. Yield extraction was maximized, so then the higher-the-better quality characteristic was used as shown in eq.1:

Higher the better (maximize):

$$SN_L = -10\log\left(\frac{\sum_{i=1}^{n} 1/y_i^2}{n}\right) \qquad \text{eq.1}$$

where y_i is from the data in the experiments at the number of i^{th} and n is the amount of experiment replication.

The aims of this work were the extraction evaluation and optimization with the parameters of ultrasonic amplitude, ultrasonic duration and acid type on maximal pectin recovery from Kluai Namwa peels using (2x3x3) full factorial design with mixed orthogonal array. Thus, ANOVA (analysis of variance) was used to describe the effects of the operating parameters on pectin yield.

Methods and Materials

Sample preparation

The commercial cultivar banana Musa ABB cv., Kluai Namwa were purchased from the local fruit market in Chiang Mai, Thailand. The Kluai Namwa peels were removed from flesh and used for the study. After fruit peels were finely chopped, they were washed in flowing water and soaked in 3%w/v ascorbic acid for 5 minutes before hot water blanching for 15 minutes to improve browning. The wet peels were dried under optimum 60°C in hot air oven until reach the constant weight. After, dried peel was milled and then sieved at 40-mesh size before packing in an airtight dark pouch under dry storage at room temperature prior to analyses. The food grade chemicals and reagents from Sigma Chemicals are employed in this work.

Ultrasonic assisted pectin extraction

An ultrasonic generator device (VCX 400, Sonics Vibra Cell, Newtown, CT, USA) was carried out with 2.00 cm tip probe for these experiments. The probe was immerged 13 mm depth directly into the container of extraction mixture, suspension of 10g dried powder with volume of distilled water. The ultrasonic was operated at 20 kHz and 400 w maximum power which an amplitude controller was connected. The experiments were conducted within the ambient temperature range of 30°C ±2. The solid-liquid ratio and pH were fixed at 1:10g/ml and 2.5. To avoid the solvent evaporation, the reactor was sealed during the experiment. Food grade citric acid was calculated and adjusted in the solvent to obtain the desired pH. The experimental extraction parameters were shown on Table 1. Full factorial design with orthogonal array of Taguchi L18 (2^1 x 3^2) was shown in Table 2. It should be noted that the all computation and graphics in this study were performed using the statistical software Minitab 17, State College, PA: Minitab, Inc.

Table 1 Experimental extraction parameters with their levels.

Parameters	Symbol	Level 1	Level 2	Level 3
Acid types	A	Citric acid	HCl	-
Ultrasonic amplitude (%)	B	60	80	100
Exposure time (min)	C	5	10	15

Table 2 Full factorial design with orthogonal array of Taguchi L_{18} (2^1 x 3^2)

Experiment no.	Factor A	Factor B	Factor C
1	1	1	1
2	1	1	2
3	1	1	3
4	1	2	1
5	1	2	2
6	1	2	3
7	1	3	1
8	1	3	2
9	1	3	3
10	2	1	1
11	2	1	2
12	2	1	3
13	2	2	1

Table 2 (Cont.)

Experiment no.	Factor A	Factor B	Factor C
14	2	2	2
15	2	2	3
16	2	3	1
17	2	3	2
18	2	3	3

After ultrasonic treatment, the suspension was collected to filter using filter paper (Whatman no-1). Then, it was centrifuged at 5500 rpm for 15 min. The supernatant was precipitated with volume of 95% (v/v) ethanol. Pectin was coagulated and then washed for three times with 95% (v/v) ethanol to remove the mono- and disaccharides. This washed pectin was dried at 50°C in the hot air oven until reach the constant weight (Morthy et al., 2017). The percentages of pectin yield (Py) were calculated as in the eq.2. The pectin extraction experiments were triplicates.

$$Py(\%) = (m_0 / m) \times 100 \qquad \text{eq.2}$$

where m_0 is the pectin weight which dried and reached at constant and m is the weight of dried banana peel powder (g).

Esterification degree

The esterification degree or the degree of esterification (DE) of samples was observed by titrimetric method with modification (Santos, Espeleta, Branco, & de Assis, 2013). The powder sample was weight 0.1 g and transferred to a 250 ml flask, then added with 3 ml of ethanol and dissolved in 40 ml of deionized water. Phenolphthalein was added for couple drops and titrated with 0.1 M NaOH. The titrated base neutralized the free carboxyl acid existed in anhydrogalacturonic acid and accounted to V1. The polymer ester groups were then saponified by adding 10 ml of 0.5 M NaOH and continuously shaken the sample for 15 minutes. After that, the sample was added 10 ml of 0.5 M HCl and shaken until the pink color disappeared. V2 was accounted after vigorous shaking the final solution titrated with 0.1 M NaOH to color change to a slight pink. V1 and V2 were brought to calculate DE as the following eq.3.

$$DE (\%) = (V2 / (V2 + V1)) \times 100 \qquad \text{eq.3}$$

Methoxyl percentage determination

The methoxyl percentage (MeO%) was determined according to the procedure of Gee, McComb, McCready (1958) reported by Kar & Arslan (1999) and Zouambia, Ettoumi, Krea, & Moulai-Mostefa (2017). Then, the amount of methoxyl is 16.32% in 100% of esterified pectin which was calculated as in eq.4.

$$MeO\% = (16.32 / 100) \times DE \qquad \text{eq.4}$$

Taguchi orthogonal array experimental design

Taguchi OA experiments were designed to find the significant factors affecting extraction of pectin from Klaui Namwa peel. There were designed using mixed 3 factors, 2 and 3 levels (2x3x3 or $2^1 \times 3^2$) provided the most suitable mixed OA L18 as shown in Table 1. The ranges of the factors for optimization of extraction process were acid types (hydrochloric acid, HCl and citric acid, CA), ultrasonic amplitude (60%, 80% and 100%) and ultrasonic standing duration or exposure time (5, 10, 15 minutes). Experiments were conducted using Taguchi design which consisted of total 18 runs. Each run was performed in triplicate. The SN analysis was based to calculate the SN ratios for each level of the extraction parameters.

Results

Pectin yields (Py) were obtained based on the experimental design using Taguchi technique and the SN ratios provided optimization. Thus, the ‘‘larger-the-better’’ equation was used for the calculation of the SN ratio. Table 3 shows the SN ratios values for the pectin yield percentage. At the end of extraction, the average value of the pectin yield was calculated to be 4.2%. Similarly, average value of SN ratio for pectin yield was calculated to be 6.51 dB. Analysis of the effect of each control factor (A, B, C) on pectin yield was performed with a ‘‘SN response table’’. The response tables of SN for pectin yield are shown in Table 3. This table, which is made by using the Taguchi technique shows the optimal pectin yield from the optimal levels. The level values of control factors given in Table 4 are shown in graph forms in Figs 1. Optimal parameters of the control factors for maximizing the pectin yield can be easily determined from this graph. The best level for each control factor was found according to the highest SN ratio in the levels of that control factor. Thus, the levels and SN ratios with the best yield were specified as factor A (Level 1, SN = 11.801), factor B (Level 3, SN = 11.794) and factor C (Level 3, SN = 11.869). In other words, an optimum yield was obtained with a citric acid solution (A2), at ultrasonic amplitude (B3) 100% and at exposure time (C3) 15 min (Figure 1). The appearances of extracted pectin were shown on figure 2 to compare the amount of dried pectin related to the coagulated one.

Table 3 The results of experiments, SN ratios values and predicted means.

Experiment no.	Control factors			Pectin Yield (Py, %)	SN-ratio (dB)	Predicted Py (%)
	A Acid type (At)	B Ultrasonic amplitude (Ua, %)	C Exposure time (Et, min)			
1	CA	60	5	1.77	5.62	1.681
2	CA	60	10	2.89	9.22	3.039
3	CA	60	15	4.22	11.83	4.160
4	CA	80	5	2.10	5.92	2.001
5	CA	80	10	4.34	13.22	4.444
6	CA	80	15	5.40	14.69	5.396
7	CA	100	5	3.70	11.22	3.889
8	CA	100	10	6.6	15.90	6.347
9	CA	100	15	7.88	18.56	7.944
10	HCl	60	5	1.36	2.02	1.449
11	HCl	60	10	1.52	3.63	1.371
12	HCl	60	15	1.94	6.43	1.999
13	HCl	80	5	1.13	1.58	1.229

Table 3 (Cont.)

Experiment no.	Control factors			Pectin Yield (Py, %)	SN-ratio (dB)	Predicted Py (%)
	A Acid type (At)	B Ultrasonic amplitude (Ua, %)	C Exposure time (Et, min)			
14	HCl	80	10	2.34	6.90	2.236
15	HCl	80	15	2.69	8.55	2.694
16	HCl	100	5	1.88	5.62	1.691
17	HCl	100	10	2.46	8.31	2.712
18	HCl	100	15	3.88	11.15	3.816

Table 4 SN response table for pectin yield factor.

Levels	Control factors: Pectin yield (%)		
	A	B	C
Level 1	**11.801**	6.458	5.331
Level 2	6.020	8.480	9.533
Level 3	-	**11.794**	**11.869**
Delta	0.459	5.336	6.533
Rank	2	3	1

Bold values show the optimal levels of control factors.

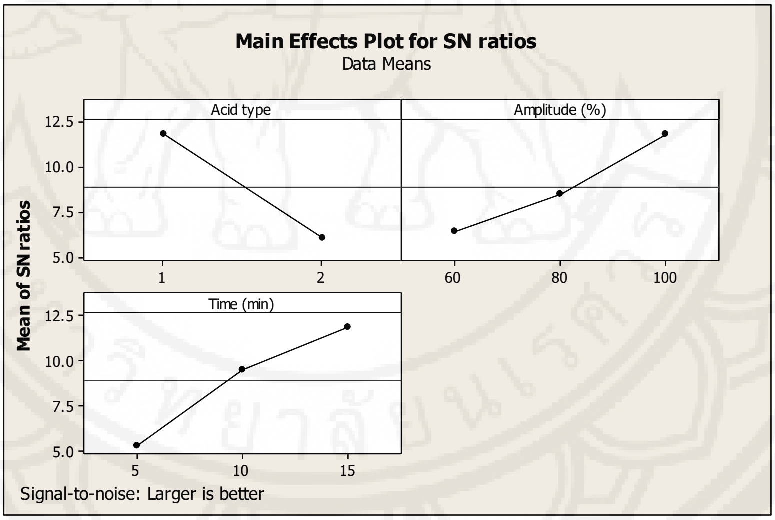

Figure 1 Effect of process parameters on average SN ratio for pectin yield

Figure 2 *Kluai Namwa* pectin extracted; (a) Before drying (b) After drying

The ANOVA results for the pectin yield are shown in Table 5. This analysis was at 95% confidence level and 5% significance level. The comparing F-values of each factor determined the significance of control factors in ANOVA. The percentage of contribution rate is shown in the last column indicates the influence degree on the process performance. The contributions of the A, B and C factors on the pectin yield were observed to 30.03%, 19.36% and 23.18% respectively. (Table5) The interaction percent contributions of the AB, AC, BC and ABC were 4.29%, 4.18%, 2.11%, and 0.44% subsequently.

Table 5 Results of ANOVA for pectin yield

Variance source	Degree of freedom (DF)	Sum of square (SS)	Mean Square (MS)	F ratio	Prob>F	Contribution rate (%)
A	1	64.616	64.616	65.97	< 0.0001	30.03
B	2	41.658	20.829	21.26	< 0.0001	19.36
C	2	49.890	24.945	25.47	< 0.0001	23.18
AB	2	9.253	4.503	4.72	0.0151	4.29
AC	2	9.005	1.136	4.60	0.0167	4.18
BC	4	4.542	0.239	1.16	0.3449	2.11
ABC	4	0.957	0.980	0.24	0.9113	0.44
Error	36	35.263				
Total	53	215.185				

S = 0.989716 R-Sq = 83.61% R-Sq(adj) = 75.87%

Table 6 The predictive equation and R^2 value

Predictor	Coef	SE Coef	T	P
Constant	-0.067	1.166	-0.06	0.955
Acid type	-2.1889	0.3687	-5.94	0.000
Amplitude (%)	0.05292	0.01129	4.69	0.000
Time (min)	0.23450	0.04515	5.19	0.000

S = 0.782106 R-Sq = 85.7% R-Sq(adj) = 82.7%

Regression analyses are used for the modeling and analyzing of several variables where there is relationship between a dependent variable and one or more independent variables. In this study, the dependent variable is pectin yield (Py), whereas the independent variables are acid type (At), Ultrasonic amplitude (Ua) and exposure Time (Et).

$$\text{Pectin yield} = -0.07 - 2.19\ \text{Acid type} + 0.0529\ \text{Ultrasonic amplitude} + 0.235\ \text{Exposure time} \qquad \text{eq.5}$$

In obtaining predictive equations for the pectin yield, regression analysis was used. The predictive equation was made for linear models. R^2 values of the equations which were obtained by linear regression model were found to be 82.7% as shown in table 6 which performed good correlation in this model. The predictive equations which were obtained by the linear regression model of pectin yield as in eq.5. The pectin yield exhibited a higher tendency with increasing the ultrasonic amplitude and exposure time when the citric acid was more effective type.

Discussion

The effects of acid types, ultrasonic amplitude and exposure time on pectin yield were analyzed by ANOVA via the Taguchi experimental design method which is the statistical method to determine the individual interaction of the control factors. Thus, the most important factor affecting the pectin yield was acid types (factor A, 30.03%) followed by exposure time (factor C, 23.18%) and ultrasonic amplitude (factor B, 19.36%). The interactions showed less influences due to the percent contribution.

Thus, the changes in the pectin yield which were obtained depending on the difference of the acid type. Commonly, the conventional pectin extraction process, acids have been used to adjust pH of the extraction solvent resulting in the tendency of decreasing pectin yield with increasing pH. The desired low pH roles in degradation of pectin from plant tissue to be solubilized while some pectin might still be attached to the cell wall components in weaker acid solution. The higher pectin yield from banana peels found at the pH range from over 1.5 to 2 (Girma & Worku, 2016).

The study of acid types was treated under the same pH instead of concentration. Normally, at the same concentration, strong acids have lower pH than weak acids due to higher proton or hydrogen ions releasing into the solution at once. In addition, pH of a solution is related to its concentration of hydrogen ions - the higher the concentration of hydrogen ions $H+$ the lower the pH. Although, citric acid is accounted to be a weak acid which do not fully ionized unlike strong acid as hydrochloric acid. Citric acid solution could form an equilibrium mixture of acid dissociation give a constant Ka which is able to be calculated its pH and also acid concentration. The reason of great present hydrogen ions and dissociated acid equilibrium of citric acid comparing to the strong ionized hydrochloric acid solution at the equal pH would support the better yield which was able to extract pectin from the plant tissue gradually.

However, the results in the experiment was found that the pectin yield was higher when the citric acid was used. It was related to some researches which studied the various acid types at desired pH control. Raji et al. (2017) evaluated pectin yield made for organic and mineral acids (tartaric, citric, acetic, lactic, hydrochloric, nitric, phosphoric, and sulfuric) from melon peel. The efficient yields obtain from the maximum to minimum were citric acid (14.3 ± 0.28%) > Tartaric acid > Hydrochloric acid (9.5 ± 0.35%) >Acetic acid > Lactic acid > Nitric acid > Phosphoric acid > Sulfuric acid. The most of organic acids performed the high yields and citric acid was the most effective to the melon peel pectin extraction. Canteri-Schemin, Fertonani, Waszczynskyj, and Wosiacki, (2005) found that apple pomace pectin with citric acid extraction had the highest average value (13.75%) and it was greater than the other ordering acid; hydrochloric acid, nitric acid, phosphoric acid. The effects of different acids on extraction yield of pectin of jackfruit peel were as follows: citric acid (17.9%) > hydrochloric acid > lactic acid > nitric acid > sulfuric acid > tartaric acid (Xu et al., 2018). These reports support the result of higher pectin extraction yield with citric acid in this research. In recent published researches, the organic acids, especially citric acid, has been more interesting and effective in the optimization process of pectin extraction yield known as the natural and safe food ingredient which is in economic and environmental considerations.

The effect of higher ultrasonic amplitude and exposing time increased pectin yield as shown on the experiment results. According to the liberation of expected compounds by the ultrasonic-based device, it supports the mechanism of extraction due to the cavitation effect by distracting the cell walls. This technique accelerated the solvent move into the broken down cell wall as in small degradation. However, the limitation of ultrasonic power of 400w operated at 100% amplitude was a maximum condition.

In case of temperature condition as mentioned in the method, the experiments conducted within the temperature range of 30°C ±2. The slightly increasing temperature with increasing of ultrasound amplitude and time (15min-max) was expected. On the other hand, temperature factor in the conventional is more effective which could be risen up to about 90°C. The pectin yield increases with the increasing temperature since high temperature would lead to break down of pectin molecules as pectin is composed of α-(1-4) linked units of galacturonic acid or methyl ester. At too high temperature would result in lower molecular size which is not perceptible with alcohol in case of mango peel conventional extraction (Girma et al., 2016). These found evidences would support the advantage of ultrasound-assisted extraction which gave the high yield result under the moderate temperature and short duration.

Conclusion and Suggestion

The maximum pectin yield of the experiments was 7.88% when using the condition of citric acid extracted solution and ultrasonic power of 400w operated at 100% amplitude for 15 minutes. The degree of esterification (%DE) and methoxyl percentage (MeO%) were calculated as 37.78% and 6.17%. In this study, the Taguchi method was used to determine optimal the pectin extraction method assisted by Ultrasonic device under difference acid type conditions. With this technique, the obtained Kluai Namwa pectin yield increased with the increase of ultrasonic amplitude and exposure time under the organic citric acid. The most important factor affecting the pectin yield was acid types (factor A, 30.03%) followed by exposure time (factor C, 23.18%) and ultrasonic amplitude (factor C, 19.36%). According to this analysis, the most influence of extraction came from the type of acid followed by time and amplitude accordingly. Pectin yield predictive equation was - 0.07 - 2.19 Acid type + 0.0529 Ultrasonic amplitude + 0.235 Exposure time, R-Sq = 85.7%. This experiment showed the most important factor. The acid type could be changed to others to observed the effect of obtained yield. In addition, ultrasonic amplitude was limited to the maximum power of the apparatus.

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