EXTRACTION OF PHENOLIC COMPOUNDS FROM MANGO PEEL USING SUBCRITICAL WATER TECHNIQUE

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ABSTRACT

Mango processing causes huge amount of mango wastes (peel and seed). Development of new value-added product from mango waste not only decreases the environment impact but also increases the profit for mango product factories. In this study, the effect of subcritical water as green technology extraction method on extractability of phenolic substances from mango peel were studied. The effects of pH, ratio of sample to solvent, time and temperature on extractability of phenolic were separately investigated. Finally using surface methodology Box-Behnken design were the effect of process parameters extraction time (20-60 min), ratio of sample to solvent (1:40-1:80 w/v) and temperature (180-220 °C) on extractability of phenolic compound studied. The results of this study have shown that the optimum process condition for phenolic extraction from mango peel were at pH 8, ratio of sample to solvent 1:80 (w/v), 40 min at 220°C. The amount of extracted phenolic at these conditions was 99.69 mg gallic acid equivalents/g dry weight

KEYWORDS: subcritical water extraction, fresh mango peel, phenolic compound, Gallic acid

1. Introduction

Mango is one of very important fruit for economy, Thailand produced and exported fresh mango in 2015 about 37,000 tons/year which have value of 1,690 million baht and packaged mangos products at 26,000 tons/year which have value at 1,268 million baht. In fruit processing industry, production of canned mango causes many mango wastes including mango peels, pulp wastes and seeds). Mango wastes including peel and seed mainly consist of antioxidant, vitamins, minerals and sugar that can be to develop valuable added products. Mango peel wastes have high nutritive value, are rich on antioxidants and phenolic compounds which can exhibit anti-mutagenic and anti-carcinogenic effect. The phenolic

compounds help to decrease free radical such as enzymes inhibition and carcinogens inactivation. The free radicals can damage DNA which causes mutagenic effect [1]. For extraction of phenolic from agricultural waste, different extraction method can be applied. Conventional extraction techniques including soxhlet extraction and maceration are time intensive, use toxic solvent and have low extraction efficiency. Recently, subcritical water extraction (SWE) as a novel technique for extraction of nutrients from agricultural wastes was applied. SWE has the ability to penetrate the cellular wall of plant materials and increases the mass transfer of solvent in the extraction [2]. In addition, this technique uses water as solvent without toxic residue in extract. The aim of this study was to investigate the effect of different extraction conditions (temperature, pH, time and ratio of sample to solvent) on phenolic content from fresh mango peel with subcritical water extraction method. Using surface methodology Box-Behnken design was the optimum process parameters of phenolic substance extraction from mango peel determined.

2. Materials and Methods

2.1 Materials and Chemicals

Ripe mango peels (Mangifera indica L. cultivar Keaw) were obtained from Erawan Food Public Co., Ltd (Thailand). Citric acid, Sodium bicarbonate and Sodium carbonate were purchased from Ajax finechem Pty Ltd. Gallic acid and Folin-Cioculture was obtained from Sigma Chemical Co., Ltd.

2.2 Preparation of material

Preparation of fresh mango peel, frozen fresh ripe mango peels were crushed with grinder until become homogenous fresh mango peel and this sample was used for phenolic extraction by using subcritical water technique.

2.3 Subcritical Water Extraction

Subcritical water extraction (SWE) was carried out in high pressure vessels designed in department. Each vessel consisted of high pressure tube (∅inn=1.5 mm, L = 30 cm) have volume of 50 ml and was suitable for operation at up to 220 °C. As heating media was thermal, oil was used. High pressure vessels were immersed in oil bath with pre-set

temperature for desired time. After that the tubes were cooled in tap water for 30 min, opened the tube and brought the content of tubes on Funnel with filter paper (Whatman no.1).

1) Study the effects of each parameter including pH, ratio of sample to solvent, time and temperature on phenolic extraction

For this study various variables were conducted including pH of solution (2, 3, 4, 5, 6, 7 and 8) extraction time (20, 40, 60 and 80 minute), ratio of sample to solvent (1:40, 1:50, 1:60 and 1:70 g/ml) and temperature (160, 180, 200 and 220 °C) are applied. After extraction, the extracted sample was filtered through filter paper and then the filtered solution was analysed by UV-spectrophotometer as described in 2.4.

2) Box-Behnken design application for phenolic extraction using SWE method

Response surface methodology (RSM) by using Box-Behnken was applied to find out optimum process parameters for highest phenolic extraction. 3 independent variables including temperature, pH, ratio of sample to solvent and extraction were applied time as shown in Table 1.

Definition: The phenolic content (mg GAE / g dry weight) is Y
The ratio of sample to solvent (w/v) is X_1
The extraction time (min) is X_2
Temperature (°C) is X_3

Table 1 Factors and variables in extraction by subcritical water method

Variables	Codes	Ranges and levels		
		-1	0	1
Ratio of sample to solvent (w/v)	X_1	1:40	1:60	1:80
Extraction time (min)	X_2	20	40	60
Temperature (°C)	X_3	180	200	220

2.4 Measurement of Phenolic Content

The total phenolic content was determined by using the Folin-Ciocalteau method. 0.1 ml of extracted sample solution was mixed with 7.9 ml of distilled water and 0.5 ml of Folin-Ciocalteau reagent. After 5 min, 1.5 ml of 7.5% sodium carbonate was added and stirred

vigorously on a vortex mixer. The mixture was maintained for 60 min in the dark at room temperature. After that the absorbance at 725 nm was measured and converted to the phenolic content using calibration curve. Different concentration of Gallic acid (160, 182, 213, 256, 320, 427 and 640 mg/l) were used for calibration curve of phenolic compound. The final results of extracted sample were represented as milligrams of gallic acid equivalents per g dry weight (mg GAE/g dry wt) [2].

2.5 Measurement of Moisture Content and Dry Matter by Oven Dryer Method

1) Fresh mango peels were added in pre-weight glass dishes and inserted in hot air oven dryer at 120 °C for 6 hours after that the weight of glass dished and dried mango was measured. The samples were placed again in oven dryer for 30 min until obtained exact weight.

2) The moisture content of mango peel calculated using equation (1).

$$\text{The moisture content (\%)} = \frac{(w_1 - w_2) \times 100}{w_1} \quad (1)$$

When w_1 = sample weight before drying

w_2 = sample weight after drying

3) The sample weight was calculated as percentage of dry weight of mango peel as substituting from (2).

$$\text{Dry matter (\%)} = 100 - (\%\ \text{moisture content}) \quad (2)$$

3. Results and Discussion

3.1 Determination of Moisture Content and Relative Humidity (% dry matter) of Frozen Fresh Mango Peel

Moisture content and dry matter in fresh mango peel was 81.83% and 18.16% respectively. That means 5.55 g fresh mango peels have 1 g dry matter.

3.2 Determination of phenolic content of fresh mango peels before extraction

50 g of fresh mango peels was mixed with 450 ml distilled water. The mixture was immediately filtered and centrifuged. The analytical measurement of phenolic substance has indicated that the phenolic content in mango peels was only 26.08 mg GAE/g dry wt.

3.3 Effect of pH on Phenolic Extraction

The effect of difference pH value (at 2, 3, 4, 5, 6, 7 and 8) of solvent (distilled water) on phenolic compounds extraction at temperature of 180 °C, extraction time of 60 min, ratio of sample to solvent 1:50 using subcritical water were shown in Figure 1. The pH value had a significant effect on phenolic extraction. The lowest phenolic extraction was 49.50 mg GAE/g dry wt and observed at pH 5. The maximum phenolic extraction of 72.09 mg GAE/g dry wt could be achieved at pH 8 because bonded phenolics can be hydrolysed using a strong base or acid. This study shows that phenolics in fresh mango peels were extracted more readily in base solvent [3].

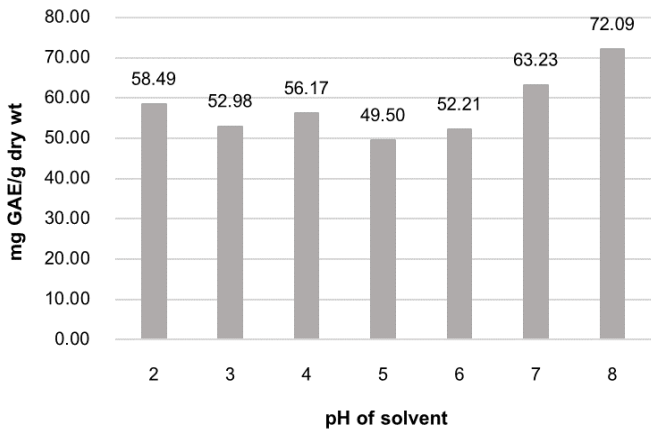

Figure 1 Effect of pH on phenolic content

3.4 Effect of Extraction Time on Phenolic Extraction

The effects of different extraction time of 20, 40, 60, 80 and 100 min on the phenolic compound extractability from fresh mango peels is shown in Figure 2. At the ratio of sample to solvent 1:50, temperature 180 °C and pH 8 with increasing extraction time increased the extracted phenolic. At extraction time 40 min, phenolic compound could be extracted 78.42 mg GAE/g dry wt. Further increasing of extraction time longer than 40 min resulted negative

effect on the amount of extracted phenolic [4]. This maybe because of partial destruction of phenolic at high temperature for longer time.

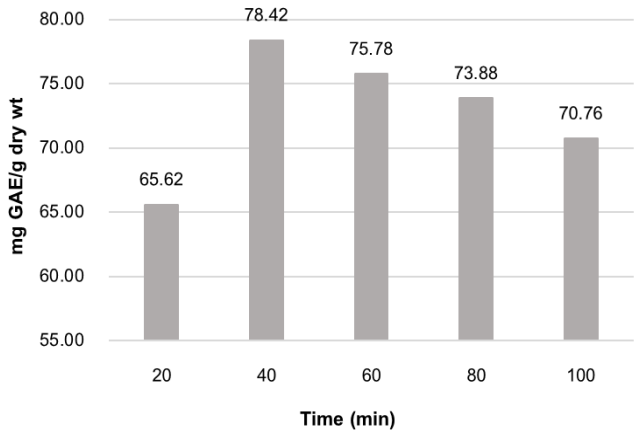

Figure 2 Effect of extraction time on phenolic content

3.5 Effect of Ratio of sample to solvent on the Phenolic Extraction Solution

The effect of ratio of sample to solvent (w/v) at ratio's 1:20, 1:30, 1:40, 1:50, 1:60 and 1:70 on phenolic extraction in fresh mango peel at 40 min, 180 °C and pH 8 is shown in Figure 3. The results showed that at high amount of solvent ratio, the phenolic substance can be extracted better. The efficiency of extraction was low when the volume of water wasn't enough for mass transfer [5]. At a ratio of sample to solvent 1:70 could be a highest amount of phenolic compounds (81.28 mg GAE/g dry wt) observed.

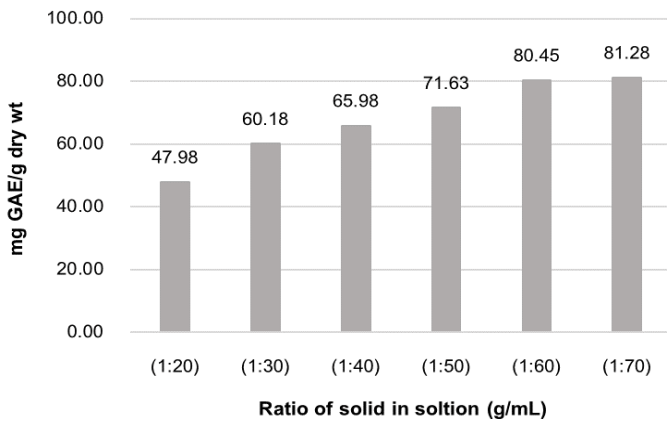

Figure 3 Effect of ratio of sample to solvent on phenolic content

3.6 Effect of Temperature on Phenolic Extraction

The effect of different extraction temperature of 160, 180, 200 and 220 °C on phenolic extraction at 40 min for the extraction time, the ratio of sample to solvent 1:60 and pH 8 is demonstrated in Figure 4. The results show that phenolic extract increased when the temperature increased [6]. The maximum extracted phenolic was 89.9 mg GAE/g dry wt at 220 °C, Alvarez et.al. [7] reported that increasing temperature from 100 to 220 °C, the amount of total extracted phenolic compound increased. In contrast at higher temperature above 220 °C, the amount of phenolic compounds will decrease significantly. This maybe because of thermal degradation of phenolic compounds.

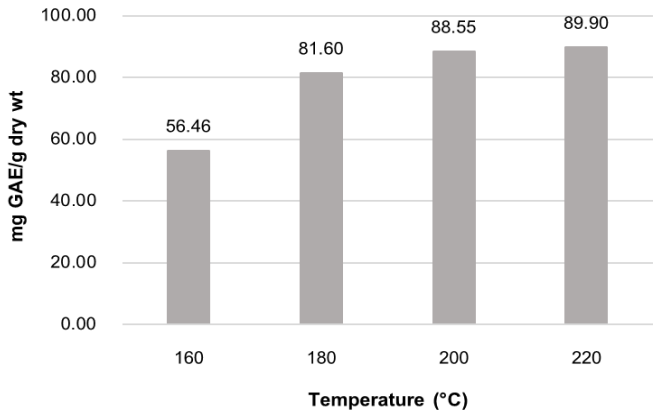

Figure 4 Effect of temperature on phenolic content

3.7 The Optimal Conditions for Phenolic Extraction

Determination of the optimum conditions for phenolic extraction by using subcritical water method with Box-Behnken design and considering 3 independent factors of sample to solvent ratio, extraction time and temperature was carried out. The process parameters for Box-Behnken are demonstrated in Table 2. To determine the phenolic content was second polynomial equation and the coefficient value of variable in the excel program applied. The coefficient value of variable obtained according to (3).

$$Y = 85.077 + 8.688X_1 - 1.134X_2 + 4.047X_3 - 0.026X_1X_1 - 0.109\ X_2X_2 - 1.073X_3X_2 + 2.094X_1X_2 + 2.575X_1X_3 - 5.610X_2X_3 \quad (3)$$

Table 2 The data results of phenolic extraction with subcritical water method by Box-Behnken design

Run	Independent variables			Y (mg GAE/g dry wt.)	
	X_1	X_2	X_3	Experimental	Predicted
1	-1	0	-1	73.53	73.87
2	-1	1	0	73.11	73.08
3	-1	-1	0	82.16	79.53
4	-1	0	1	74.50	76.81
5	0	-1	-1	73.09	75.37
6	0	-1	1	94.37	94.69
7	0	1	-1	84.64	84.32
8	0	1	1	83.48	81.20
9	1	0	-1	88.41	86.10
10	1	-1	0	92.69	92.72
11	1	1	0	92.02	94.64
12	1	0	1	99.69	99.34
13	0	0	0	84.47	85.08
14	0	0	0	85.30	85.08
15	0	0	0	85.46	85.08

In Figure 5 is the effect of temperature, extraction time and ratio of sample to solvent on phenolic extraction demonstrated. These experimental results indicated that the phenolic content of fresh mango peels could be extracted at subcritical condition of water. Increasing extraction time from 20 min to 60 min improved the extractability of phenolic substances. Furthermore, the ratio of samples in solution from 1:40 to 1:80 increased the phenolic extraction drastic. In general, it showed increasing of extraction temperature up to 200 °C an increasing of phenolic substance extraction during SWE treatment of mango peel.

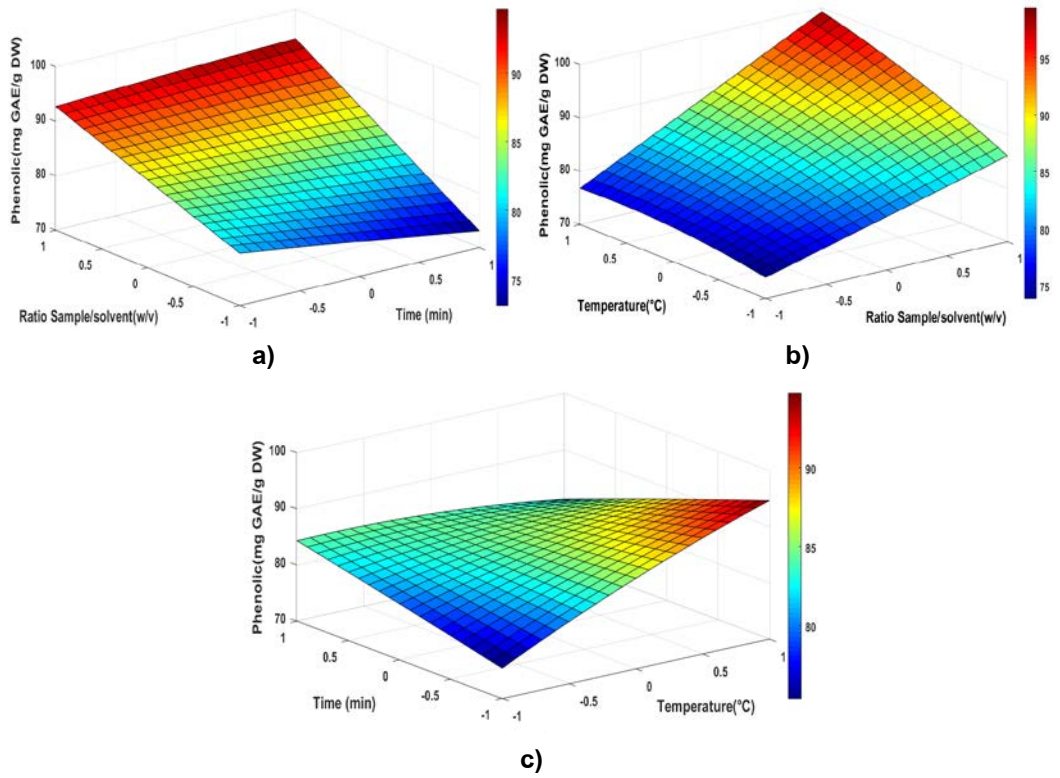

Figure 5 RSM showing the effect of temperature, time and ratio of sample to solvent on phenolic compound at fixed a) 200 °C, b) 40 min and c) 1:60 g/ml

From the polynomial 2nd equation in (3), the R-square value was equal to 0.95 which obtained from Box-Behnken design. The ratio of sample to solvent (X_1) and temperature (X_3) has positive effect on increasing of phenolic extraction significantly ($p < 0.05$). The maximum value of phenolic extraction in fresh mango peel was obtained from subcritical water method. The optimum conditions were 220 °C, 40 min, and 1:80 (w/v). The phenolic compound concentration at optimum condition was 99.34 mg GAE/g dry wt. The predictive value that computed from equation in (3) was similar to experimental results as shown in Table 2.

3.8 Comparison of phenolic compounds extraction with and without SWE treatment

The extracted phenolic compounds from fresh mango peel using subcritical water were about 4 times higher than from sample without SWE treatment (Figure 6). This indicated that

SWE technique is an effective method to enhance extractability of phenolic compounds from mango peel.

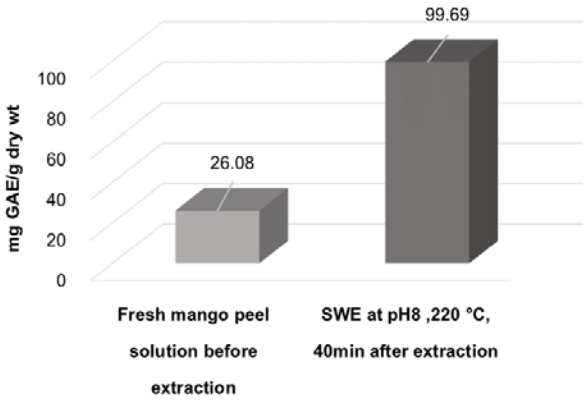

Figure 6 Comparison of phenolic contents before and after SWE treatment

4. Conclusions

The effect of different process parameters such as pH, temperature, time and ratio of sample to solvent on extractability of phenolic compounds from mango peels using SWE technique was studied. The suitable pH for phenolic extraction were at pH 2 and pH 8. Increasing temperature and time increased the extractability of phenolics. Similarly, with increasing ratio of sample to solvent up to 1:70 increased the extractability of phenolics. The optimum process parameters obtained using Box-Behnken design was at pH 8, 220 °C, 40 min and ratio of sample to solvent 1:80. The amount of extracted phenolic for optimum condition was 99.69 mg GAE/g dry wt. SWE technique can be as an alternative technology for fast extraction with high amount of phenolic and can apply in the food, pharmaceutical and beverage industries.

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