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Subcritical Water Extraction of Phenolic Compounds from Mango Seed Kernel Using Response Surface Methodology

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Mango seed kernel, a waste generated from fruit processing, is a problem. But, the antioxidant property of phenolic compounds from mango seed kernel is a good source of functional ingredients. Optimization conditions for extracts yield and total phenolic content extraction from mango seed kernel by using subcritical water-ethanol extraction and conventional techniques such as maceration and soxhlet were investigated using response surface methodology. The studied factors of response surface methodology which influences the extraction yield were: temperature, extraction time and concentration of ethanol in water. The optimum conditions of extracts yield were obtained as follows: temperature at 130 ºC, extraction time for 40 min, concentration of 50 % ethanol. Under these conditions the maximum extracts yield was 71.83%. Subcritical water extraction of total phenolic content (using Folin-Ciocalteu's phenol reagent) was 40.4 mg of tannic acid equivalent per g dry mango seed kernel) much more than six times of that from conventional techniques. It is considered that the subcritical water extraction could be recommended as an alternative technique.

Key Words: Mango seed kernel oil, Subcritical water extraction, Total phenolic content.

INTRODUCTION

Mango (*Mangifera indica* L.) is a fleshy stone fruit which belongs to the family Anacardiaceae. Mango preservative products are major goods exported from Thailand. Therefore, several million tons of mango seed kernel wastes are produced annually from factories. It is found that there are fatty acids and phenolic compounds in mango seed extracted[1]. These composition could be used in many industries such as food preservation, cosmetology and food ingredients[2]. There are several varieties of mango grown in Thailand *i.e.*, Nam-Dok-Mai, Kaew, Chok-Anan, Pimsaen, Rad, Phalun Maha-Chanok, *etc*. Typically, the seed represents from 20 to 60 % of the whole fruit weight and the kernel inside the seed represents from 45 to 75 % of the whole seed. Mango seed kernels (MSK) were shown to be a good source of natural antioxidants which contained various phenolic compounds such as polyphenols, phytosterols and tocopherols, *etc*.[3-5].

Phenolic compounds represent a majority of the natural antioxidants. The most important classes of natural antioxidants include tocopherols, flavonoids and phenolic acids which are common to all plants[6]. The lipid compositions of mango seed kernel consisted about 44-48 % saturated fatty acid (majority steric acid) and 52-56 % unsaturated (majority oleic acid)[7-9]. Soong *et al.*[5] suggested that mango seed kernel could be used as a potential source for functional food ingredients due to its high quality of fat and protein as well as high levels of natural antioxidants. Arogba[10,11] found that the dry mango kernel meal contained gallotannins and condensed tannin-related polyphenols which were tannic acid, gallic acid and epicatechin in the ratio 17:10:1, respectively.

The properties of antioxidants which were extracted from plants depended on the solvent and extraction techniques[12-14]. Conventional extraction techniques based on organic solvents have been applied to the extraction of natural antioxidants from mango seed kernel and found mainly gallic acid, ellagic acid and gallates[4]. These techniques may have undesirable effects on the environmental. Subcritical water extraction (SWE) is an extraction technique using water as the solvent due to its unique dissolving properties, which can be altered by changing the temperatue[15]. With subcritical water extraction temperatures between 100 and 374 ºC (the critical point of water is at 374 ºC and 22 MPa) are generally employed and the pressure must be high enough to keep the water in the liquid state[16]. Subcritical water extraction has been used to polyphenolic compounds from winery by-products[17]; phenolic compounds from flaxshive[18] and flavones, anilines and polyphenols from orange peel[19]; anthocyanins from red grape skin[20]; ginsenosides from American ginseng[21].

The response surface methodology (RSM) is defined as the statistical method that uses quantitative data from appropriate experimental design to reduce number of experimental trials needed to evaluate multiples parameters and their interactions. response surface methodology has been successfully applied for optimizing conditions in food research[18,19] but less for extraction of essential oils[20].

This work was to compare the total phenolic content of subcritical water extraction with conventional extractions. The optimized conditions included temperature, extraction time and percent solvent mixture for subcritical water extraction of mango seed kernel using response surface methodology, which the response variable examined the total yields and total phenolic content.

EXPERIMENTAL

All chemical were obtained from Sigma Chemical Co. Ltd. (USA) unless otherwise specified.

Preparation of plant sample: Mango seeds were procured from the local market. The seeds were washed and cut into two halves. The kernels were removed manually from the seeds and dried in the oven at 60 ºC for 6 h. Then, the mango seed kernels (MSK) were ground into particles ranging from 0.2 to 0.5 mm in size by a hammer mill and being forced through a sieve.

Maceration: The mango seed kernel powder (10 g) were added to 150 mL of hexane for solid to solvent ratio of 1:15 in a 500 mL flask, respectively and mixed on a magnetic stirrer for 2 to 8 h at room temperature (28 ºC). The supernatant was passed through Whatman filter paper (No. 1). All filtrates were evaporated under vacuum at 60 ºC using a rotary evaporator (Buchi, Switzerland). The volume of sample adjust to 25 mL using HPLC grade ethanol and stored in refrigerator until analysis. The experiments were carried out in duplicate.

Soxhlet: Soxhlet extractions were carried out in triplicate using 20 g (dry weight) mango seed kernel powder with 300 mL of 95 % ethanol for 2 to 8 h. Temperature during Soxhlet extraction was set at 70 ºC. The extracted oil was evaporated under vacuum at 60 ºC using a rotary evaporator (Buchi, Switzerland). The extracted sample was evaporated and prepared for analysis same as maceration.

Subcritical water extraction: The mango seed kernel powder (8 g) were filled into an extraction column (Type 304 Stainless) 200 mL and then added 120 mL of solvent with varying water-ethanol concentration into vessel. The extraction vessel was placed in a heating bath to maintain an operating temperature within ± 1 ºC of the set point temperature for each run. Response surface methodology was employed to optimize the operating conditions of subcritical water technique to obtain a high extraction yield. The studied parameters and their concentration ranges were: ethanol concentration (X_1) at 5, 50, 95 % mixture, temperature (X_2) at 100, 130, 160 ºC, extraction time (X_3) 20, 40, 60 min. After the operation finished, the extracted solution were collected and then prepared sample for analysis as maceration. All the experiments were performed in duplicate and each set of yields was average value.

Determination of total phenolic content: The total phenolic content of extracts was determined using Folin-Ciocalteu's phenol reagent (modified from Kahkonen *et al.*[22]). In brief, 1 mL of mango seed kernel extracts was mixed with 1 mL of Folin-Ciocalteu's phenol reagent and allowed to react for 3 min. Then, 0.8 mL of 7.5 % (w/v) sodium carbonate was added. The mixture were agitated and allowed to stand for further 0.5 h in the dark. The absorbance of mango seed kernel extracts and a prepared blank were measured at 765 nm using a spectrophotometer (UV-Vis model 1601, Shimadzu, Japan). The concentration of total phenolic compounds in mango seed kernel extracts was expressed as mg of tannic acid equivalents (TAE) per g dry weight of mango seed kernel using a linear equation. All determination was performed in duplicate.

RESULTS AND DISCUSSION

Comparison of maceration and Soxhlet techniques: The comparison of maceration and Soxhlet techniques at solid to solvent ratio (w/v) of 1:15 and using 95% ethanol was investigated the influence of temperature on extraction efficiency yield of mango seed kernel at various times (Fig. 1).

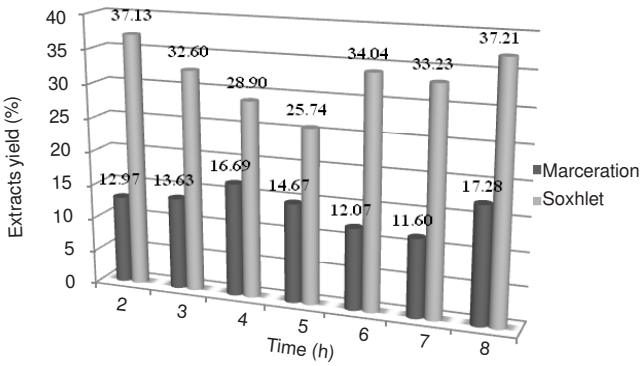

Fig. 1. Comparison of maceration and Soxhlet extractions on efficiency yield

Soxhlet extraction showed the oil yield much more than two times of maceration for all times and used only 2 h to get the best yield of 37.13 %, but hexane could extract highest yield at 12.97 % for 2 h. According to increasing temperature[23,24], it helps to enhance both the solubility of solute and the diffusion coefficient. Heating also might soften the plant tissue. Thus, the oil extraction could be developed by increasing temperature.

Optimization for subcritical water-ethanol extraction by response surface methodology: The experimental values for the total yields from mango seed kernel were plotted in Figs. 2-4 at different combinations of the independent variables along with the predicted response using a second-order polynomial model in terms of valuable xi described by eqn. 1 as following:

$$Y = -505.796 + 1.373X_1 + 6.498X_2 + 5.582X_3 - 0.005X_1X_2 - 0.001X_2X_3 - 0.002X_1X_3 - 0.006X_1^2 - 0.0234X_2^2 - 0.067X_3^2 \quad (1)$$

where Y represents the response variables (% yield), X_1, X_2 and X_3 are ethanol concentration in water (%), temperature (ºC) and extraction time (min), respectively.

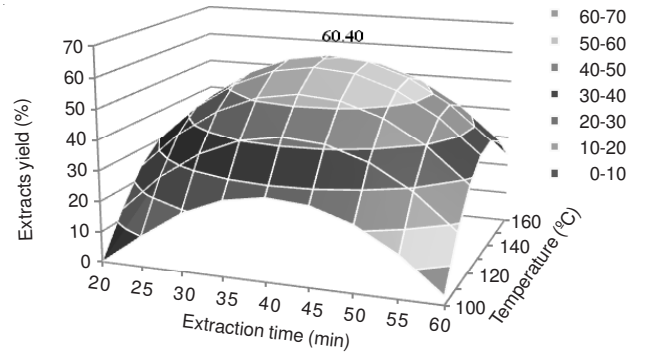

Fig. 2. Extracts yield from subcritical water-ethanol xxtraction by RSM with 5 % ethanol

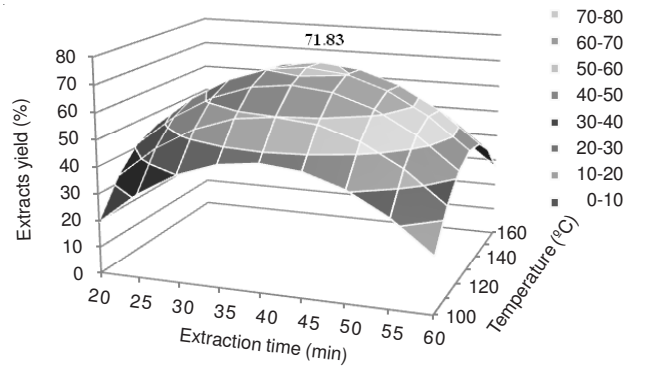

Fig. 3. Extracts yield from subcritical water-ethanol extraction by RSM with 50 % ethanol

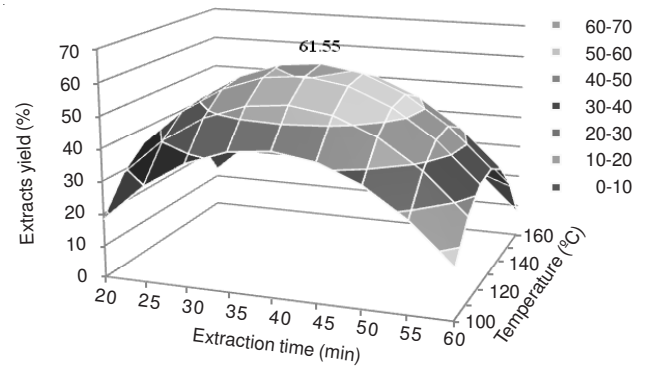

Fig. 4. Extracts yield from subcritical water-ethanol extraction by RSM with 95 % ethanol

From response surface methodology (Figs. 2-4), it showed that the higher temperature could develop the ability of extraction by using ethanol as solvent, but it should not be higher than 130 ºC due to heat could damage the substances before extraction. Moreover, the higher temperature could reduce extraction time to 40 min that much shorter than Soxhlet and marceration (Fig. 1). When comparison of maximum extracts yield from subcritical water extraction, Soxhlet and maceration found that the extracts yield were 71.83 % at 40 min, 37.12 % at 8 h, 17.28 % at 8 h, respectively. Thus, the highest extracts yield and the shortest extraction time from mango seed kernel was subcritical water-ethanol extraction at 50 % ethanol as solvent, temperature at 130 ºC and time 40 min. Because 50 % ethanol was lower density than 95 % ethanol which developed diffusivity of solvent.

Comparison of total phenolic contents from subcritical water-ethanol extraction and conventional methods: In the present study, comparisons of total phenolic content were made between the subcritical water-ethanol extraction and the conventional methods such as maceration and Soxhlet as viewed in Table-1 and Figs. 5-7. From extracts analysis, maceration and Soxhlet demonstrated total phenolic content in Table-1.

TABLE-1
TOTAL PHENOLIC CONTENTS OF EXTRACTS OBTAINED FROM MACERATION AND SOXHLET TECHNIQUES OF MANGO SEED KERNEL

Extraction time (h)	Total phenolic content (mg of TAE/g)	
	Maceration	Soxhlet
2	2.7	5.9
3	4.8	5.4
4	4.8	6.1
5	5.9	5.9
6	5.5	6.1
7	5.7	6.1
8	6.0	6.2

Total phenolic content from mango seed kernel using subcritical water-ethanol extraction showed in Figs. 5-7.

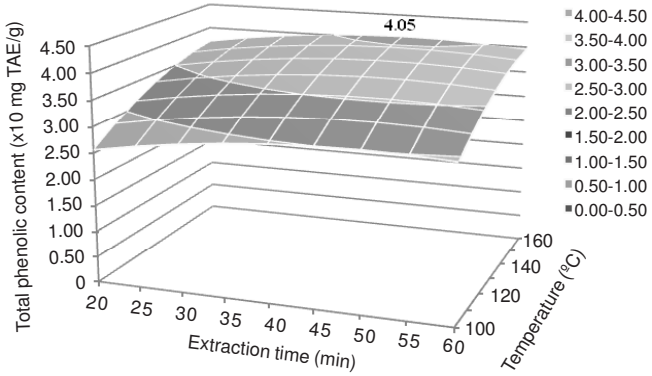

Fig. 5. Per cent yield from subcritical water-ethanol extraction by RSM with 5 % ethanol

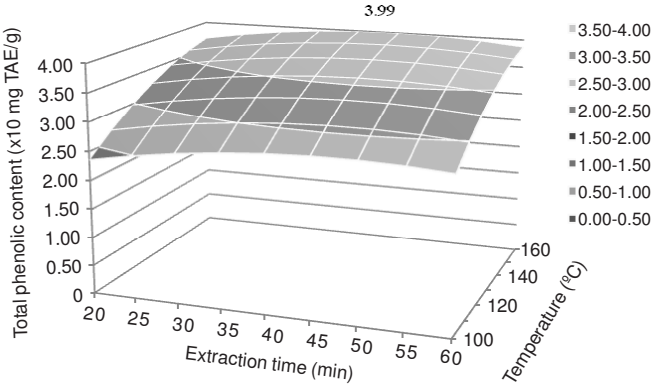

Fig. 6. Per cent yield from subcritical water-ethanol extraction by RSM with 50 % ethanol

From Fig. 5-7, the results indicated that the temperature and time affected the extraction of phenolic compounds from mango seed kernel. The extractios yield increased with increasing

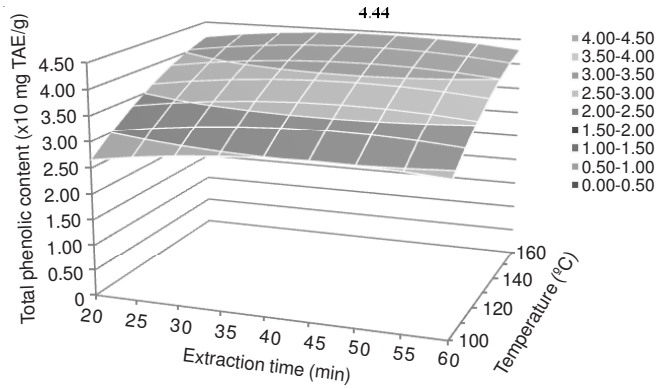

Fig. 7. Per cent yield from subcritical water-ethanol extraction by response surface methodology with 95 % ethanol

temperature due to increased solubility of phenolics in water. In addition, the highest content of total phenolic content in the name as tannic acid equivalent (TAE) was maximum at extraction time for 40 min. Comparing the different extraction methods in Table-1 and Figs. 5-7, they were significant differences between extracted by subcritical water-ethanol extraction and conventional techniques such as maceration and Soxhlet. Total phenolic content in mango seed got the maximum at 160 ºC, using 95 % ethanol as solvent and 40 min operation of subcritical ethanol-water extraction that was 44.4 mg of tannic acid equivalent (TAE) per g dry mango seed kernel. While maceration gave 6.0 mg TAE/g and Soxhlet viewed 6.2 mg TAE/g. Actually, SWE was the most appropriate technique for phonolic compounds from mango seed kernel extraction with only 5 % ethanol and obtained 4.05 mg TAE/g. Because of no residues in product and green technology.

Conclusion

This study showed that the phenolic compounds from mango seed kernel were successfully extracted using subcritical water in the shorter time and required only 5% ethanol. Optimal extraction conditions were established using a series of experimental runs using response surface methodology. The experimental data indicated that the significant variables for achieving maximal extracts yield were the temperature, extraction time, amount of ethanol. Subcritical water extraction is not only an environmental friendly processing technology but a high efficient method for the extraction of phenolic compounds from mango seed kernel. Furthermore, by using subcritical water extraction, it is possible to extract different types of free and bound form phenolics from mango seed kernel. These compounds could be used in the food and nutraceutical industries.

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