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The Effect of Extraction Conditions on Total Phenolic Content of Mango Peels and Mango Seed Kernel Employing Ultrasound-Assisted Extraction Method

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ABSTRACT

Objective – A greener approaches focusing on recovering the wastes of peels and seed kernels of mango as the low-cost source for high value-added products which constitute rich source of phenolic content.
Methodology/Technique – Ultrasonic-assisted solvent extraction (UAE) method is employed for the isolation of phenolic compound due to its ability to increase the extraction efficiency. The total phenolic content (TPC) of the mango peels and kernels are evaluated based extraction time, extraction temperature and ethanol concentration by employing One-Factor-at-Time (OFAT) screening method.
Findings – Maximum TPC for the mango peels are obtained at 15 minutes' extraction period, 40oC extraction temperature and 60% of ethanol concentration. Meanwhile for the mango seed kernel, the maximum TPC are achieved at 25 minutes' extraction period, 60ºC extraction temperature and 60% of ethanol concentration. Among of the parameters tested, ethanol concentration exhibits significant influence towards TPC in both peels and seed kernel of mango followed by extraction temperature and extraction time.
Novelty – UAE method is employed for the isolation of phenolic compound due to its ability to enhance the extraction kinetics through acoustic cavitation produced in the solvent. Factors affecting the sonication process were also studied and the TPC of peels and kernel were compared.

Type of Paper: Empirical

Keywords: Total Phenolic Content; Mango Polyphenol; Ultrasound-Assisted Extraction; Mango Peels; Extraction Conditions

1. Introduction

Mango (Mangifera indica L.) is belongs to the family of Anacardiaceae, which cultivated in more than 100 tropical and subtropical countries and amongst the most prominent palatable organic products with ranking

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fourth in the world fruit production (Food and Agriculture Organization Corporate Statistical Database (FAOSTAT), 2011).

Mango is amongst of the fruits with high content of various polyphenolic compounds. The amount of the polyphenol compounds being found in the all parts of mango, not only in the pulp but also in the peels, seed, flower, leaf and bark (Masibo and He, 2008). The phenolic compounds work mainly as antioxidants, a property that enables them to protect human cells against damage due to oxidative stress leading to lipid peroxidation, DNA damage, and many degenerative diseases. It has ability to scavenge free radicals and exhibits antimutagenic, anticarcinogenic, antiglycemic, anticholesterol, anti-inflammatory and antimicrobial properties (Noratto *et al*., 2010). Thus, mango phenolics have been the molecule of interest due to various biological activities that enables them to be used as ingredients in cosmetics, pharmaceuticals, nutraceuticals and food products.

Some of the mango varieties demonstrate incredible sensorial properties and they are exceedingly esteemed for transforming into products such as juice, nectar and mash. A major problem related to the mango processing industry is the production of vast amounts of by-products, specifically the peels and seeds which constitute about 35% and 60% of the total fruit weight, respectively (Ribeiro *et. al*., 2008; Maisuthisakul and Gordin 2009). These by-products are usually discarded although known to have significant potential benefit due to its powerful antioxidant properties and high content of phenolic compound (Maisuthisakul and Gordin, 2009). Hence, the reutilization of this great mass of natural waste product represents an important opportunity from both environmental and socioeconomic points of view.

The extraction of phenolic compounds from mango by-products could be achieved by various methods of extraction. Among of these methods, ultrasonic-assisted extraction (UAE) is regarded as one of the method of interest due to its simple operation, inexpensive and efficient alternative to either conventional techniques such as maceration and Soxhlet extraction which known to be solvent and time consuming or other high cost modern techniques such as microwave-assisted extraction (MAE) or supercritical fluid extraction (SFE) (Wang and Weller, 2006). The main benefits of ultrasound in solid–liquid extraction include the increase of extraction yield and faster kinetics, because it enhances the extraction efficiency by acoustic cavitations produced in the solvent by the passage of an ultrasound wave (Ghafoor *et al*., 2009; Zou *et al*., 2013). Ultrasound method can be used with any solvent for extracting a wide variety of natural compounds (Wang and Weller, 2006). Ethanol and methanol are common solvent used for phenolic compound extraction but ethanol is preferred due to the methanol toxicity (Ignat *et al.*, 2011).

Thus, looking forward for a future use of those wasted by-products, this work aims to study the extraction process of phenolic compounds from mango peels and seed kernels employing ultrasound technique in order to determine the significant operational conditions of this process that may lead to a maximum extraction yield.

2. Material and Methods

2.1 Sample preparation and pretreatment

The peels and seeds wastes of the mangoes collected from local fruit stalls and restaurants were washed and sun-dried. Then, samples were freeze-dried at -50 ^{o}C for at least 24 hours, ground into powdered-form and sieved at 500-µm-screen size before being stored in an air-tight container. The samples were stored at 4 ^{o}C and protected from light to minimize the phenolic degradation prior to extraction process. The peels and seeds were treated and analysed separately to compare the phenolic content in both parts of mango.

2.2 Phenolic compounds extraction via ultrasound-assisted extraction (UAE)

The ultrasound-assisted extraction (UAE) was carried out in an ultrasonic device with an ultrasound power of 200 W and frequency of 40 kHz, equipped with a temperature controller and a digital timer. An amount of 2.0 g of the mango peels and seed kernels powdered samples were precisely weighed and placed in separate beakers. Both of the samples were subsequently mixed with specific concentration of ethanol solvent at a liquid-to-solid ratio of 30:1. Each of the beakers containing the mixture was placed at the centre of the water bath sonicator. This is to allow the ultrasound wave produced by the sonicator evenly distributed around the beaker, thus enhance the extraction process. After the extraction complete, the mixture was centrifuged at 4000 rpm for 10 min to separate out the solid (pellet) and supernatant liquid. The supernatant was then collected in a capped sample bottle and the pellet was re-extracted under the same conditions to increase the recovery of the product. The supernatant from the first and second extraction were combined and evaporated by a rotary evaporator at 35 °C prior to be weighed and stored at 4 °C in a refrigerator for subsequent analysis.

2.3 Total phenolic content (TPC) determination

The total phenolic content in the extracted phenolic compound samples of peels and seed kernels of the mango was determined with the Folin-Ciocalteu colorimetric method proposed by Singleton and Rossi (1965) with some alterations. A sample of 0.1 mL was mixed with 7.9 mL dionised water prior to be added with 0.5 mL Folin-Ciocalteu phenol reagent. In between of 30 sec to 5 min, 1.5 mL of sodium carbonate (20%, w/v) was then added to the mixture and shook to allow a well mixing. Then, the solution was left at 40 °C for 30 min and the absorbance was measured at 765 nm using spectrophotometer (UV-1800, SHIMADZU). Each measurement was carried out in triplicate and the TPC in the solution was expressed as mg of Gallic acid equivalent (GAE) per g dry weight of samples.

2.4 Parametric studies by One-Factor-At-a-Time (OFAT)

One-Factor-At-A-Time (OFAT) approach is used to observe the effect of single process variables whereas each of the selected parameter is sequentially varied while keeping the other parameters fixed. This method assumes no interactions between effecting variables and that the response variable is a function of only single varied parameter. The total phenolic content (TPC) of mango peel and seed kernel samples were evaluated under three extraction parameters which are extraction time, extraction temperature and ethanol (solvent) concentration. The extraction time was varied within the range of 5 to 60 minutes, while the extraction temperature was tuned within the range of 30 °C to 80 °C and the ethanol concentration was varied from 10% to 80%.

3. Result and Discussions

3.1 Single Factor Experiment

The influence of several factors (extraction time, extraction temperature and ethanol concentration) on the total phenolic content (TPC) of the extract of mango peel and seed kernel samples were detected and analyzed accordingly. Based on the tabulated results, the values of TPC were higher in the peels compared to the seed kernels of mango due to higher content of total polyphenolic composition that naturally occurs in the peels (Masibo and He, 2008).

3.1.1 Effect of extraction time

The effects of extraction time on TPC in both samples of mango peel and seed kernel were shown in Figure 1. The extraction time was varied from 5,15,25,40 and 60 minutes while the extraction temperature and ethanol concentration were kept constant at 30°C and 40% respectively. Highest TPC of 93.57 mg GAE/ g crude sample in the mango peels extract was obtained at 15 minutes while the mango seed kernels requires longer time for a lower maximum value of TPC (3.71 mg GAE/g crude sample) which is at 25 minutes. This may be due to the presence of crude fats in the seed kernels that might hinder the extractability of phenolic compound (Jahurul et al., 2013). The TPC values in both of the peels and seed kernels samples increase as the time increases until achieve the respective optimum values at 15 and 25 minutes, then dropped as the time were extended. This can be explained by the Fick's second law of diffusion, which stated that a final equilibrium between the solute concentrations in the solid matrix (plant matrix) and in the bulk solution (solvent) might be reached after a certain time, leading to deceleration of the extraction yield (Silva et al., 2007). The deceleration might cause by the phenolic decomposition due to prolonged exposure to ultrasonic irradiation and other unfavorable surrounding conditions (Naczk and Shahidi, 2004; Zou et al., 2014). Shorter extraction period which were within 15 to 25 minutes are obtained through UAE method implies that ultrasound might accelerate the establishment of an equilibrium for dissolution of the target compounds between the plant cell wall and the extraction solvent in a short time (Tarabaki and Nateghi, 2011). Hence, this is a significant advantage of UAE compared to conventional extraction methods.

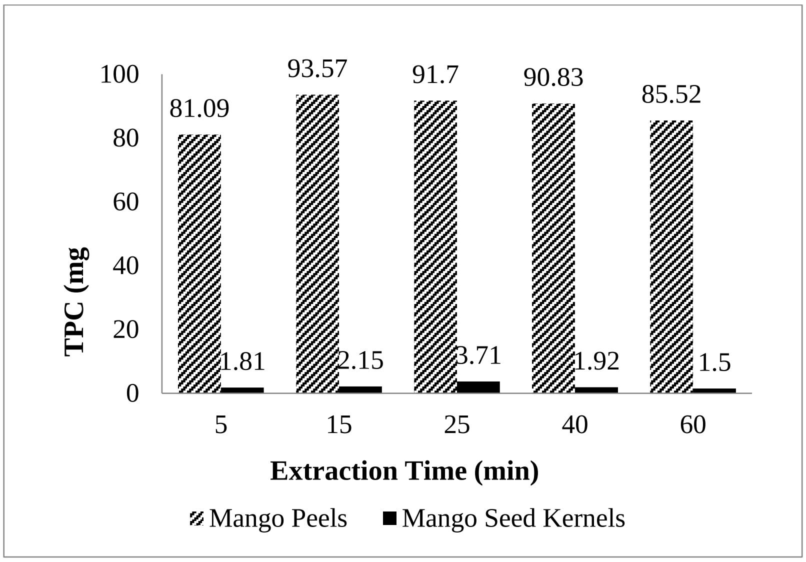

Figure 1. Effect of extraction time on the TPC

3.1.2 Effect of extraction temperature

The effects of extraction temperature on TPC in both samples of mango peel and seed kernel were presented in Figure 2. The extraction temperature was tuned within the range of 30, 40, 50, 60 and 80 °C while the ethanol concentration was fixed to 40% and the optimum extraction times for both peel and seed kernel samples determined earlier were kept constant at 15 and 25 min respectively. Maximum value of TPC was obtained at 40°C for the sample of peels which contains 114.02 mg GAE/g crude sample. Meanwhile, for the seed kernel extract, the highest TPC value of 4.53 mg GAE/ g crude sample was observed at 60°C which requires higher extraction temperature due to the existence of crude fats that rich with fatty acids which possibly complicates the dissolution of the targeted compound. Based on Figure 2, the TPC in the peels extract was improved when the extraction temperature was raised from 30 to 40 °C, and then slightly decreased when the temperature was increased up from 50 to 80 °C. Meanwhile, the value of TPC in the seed kernels extract was escalated when the temperature was increased from 30 to 60 °C then finally reduced when the temperature achieved 80°C. The TPC values for both samples were improved until a certain time and lessen as the temperature arose. In brief, an increasing of extraction temperature could assists the diffusivity of the solvent into the plant matrix because heat will enhance the solubility of compounds from the cells, resulted in the dissolution of components (Dong *et al.*, 2010). However, exposure to higher temperature could lead to the degradation of the thermal sensitive compound, which could explain the reduction of TPC in the samples at higher temperature.

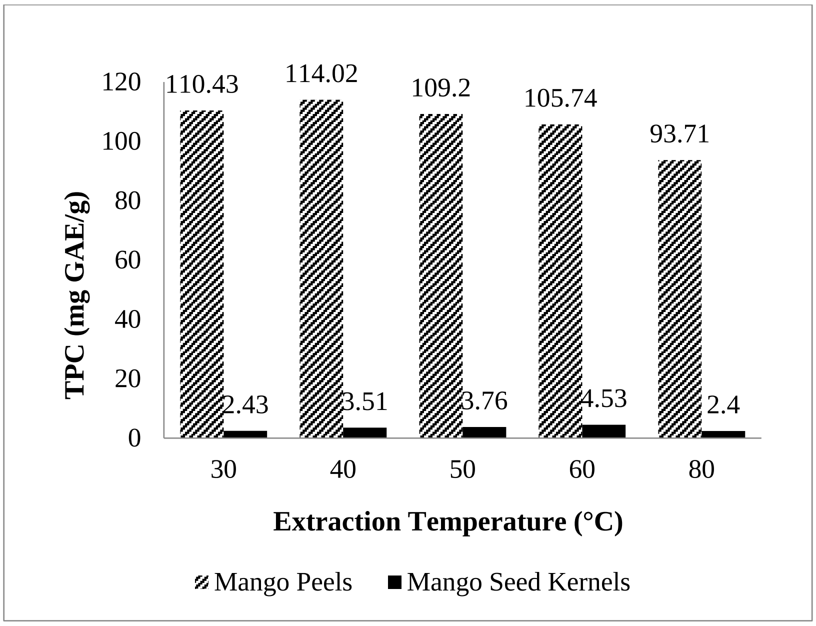

Figure 2. Effect of extraction temperature on the TPC

3.1.3 Effect of ethanol concentration

The effects of ethanol concentration on TPC in both samples of mango peel and seed kernel were evaluated and indicated in Figure 3. The range of ethanol concentration used was varied from 10,20,40,60 and 80%. The time and temperature of the extraction we kept constant at the optimum values obtained in previous which were at 15 minutes and 40°C for the peel samples as well as 25 minutes and 60°C for the seed kernel samples. Figure 3 exhibits both of the peel and seed kernel extracts yielded maximum amount of TPC in 60% ethanol concentration. Maximum TPC values of 116.28 mg GAE/g crude and 6.33 mg GAE/g crude were obtained in both peel and seed kernels samples respectively. The TPC values showed an increment when the concentration of ethanol solvent increased from 10% to 60 % and then dropped at 80 % concentration. These results were in accordance with previous studies, which reported that binary-solvent system was favourable over a mono-solvent system for phenolic compound extraction (Nawaz *et al.,* 2006). Aqueous solution of ethanol and water mixture could be the best solvent since both of the substances were highly polar and would only extract those compounds, which have similar polarity with the solvent. Based on the results, 40% to 60% ethanol concentrations were considered as the optimum ratio of ethanol-water mixture binary- solvent that able to extract maximum yield of phenolic as compared to 10% and 80% ethanol concentrations which approach the mono-solvent characteristics.

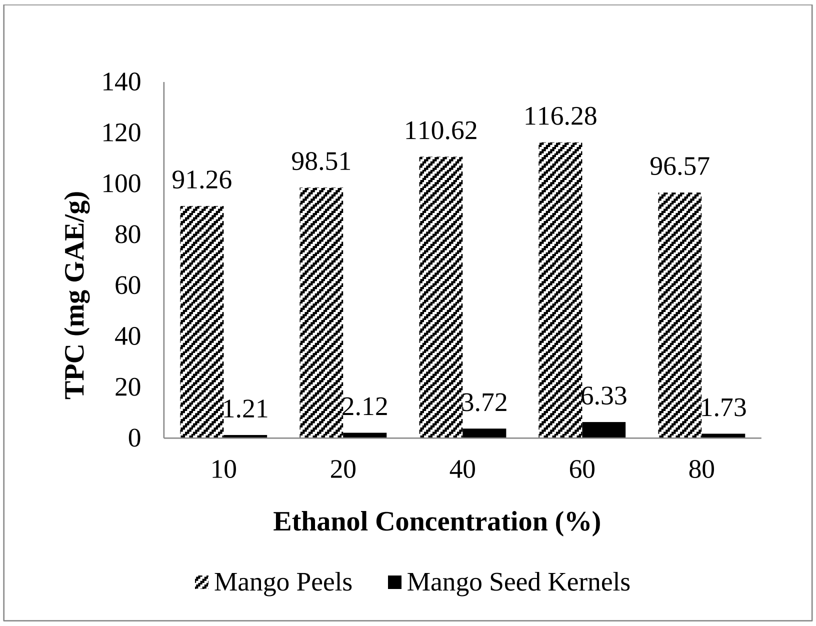

Figure 3: Effect of Ethanol Concentration on the TPC

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