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Optimization of low power ultrasound-assisted extraction of phenolic compounds from mandarin (*Citrus reticulata* Blanco cv. Sainampueng) peel

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ABSTRACT

Mandarin peel is a good source of phenolic compounds, which can be extracted by the ultrasound-assisted extraction (UAE) method. This research was to optimize the UAE conditions for maximum mandarin peel extract (MPE) relating to the extract yield, total phenolic content and the content of a mandarin peel rich flavonoid, hesperidin, using a response surface method comparing with the maceration extraction (MAE) method. The results showed that the selected factors (temperature, time and power) have a significant influence on the extraction yield, total phenolic content and hesperidin content. The extraction at 48 °C and 56.71 W for 40 min was considered the optimal UAE condition since it provided the maximum yield (26.52%), total phenolic (15,263.32 mg Eq gallic/100 g DW) and hesperidin (6435.53 mg/100 g DW). At the same extraction temperature and time, UAE showed greater extraction efficiency than MAE with 1.77 times higher yield than that of MAE.

1. Introduction

Mandarins (*Citrus reticulate* Blanco cv. Sainampueng) are a major citrus fruit produced in Thailand with an annual production of over 185,000 tons in 2012 (Office of Agricultural Economics., 2013). Mandarin peel accounts for half the total dry weight of the fruit and contains a rich source of natural antioxidants such as phenolic compounds (e.g., flavonoids and phenolic acids) (Oboh & Ademosun, 2012; Singanusong, Nipornram, Tochampa, & Rattanatraiwong, 2015; Tripoli, Guardia, Giammanco, Majo, & Giammanco, 2007). Reports data demonstrate that phenolic compounds have diverse beneficial properties to health including anti-inflammatory, antiallergic, antiviral, anticancer and antioxidant (Babbar, Oberoi, Uppal, & Patil, 2011; Procházková, Boušová, & Wilhelmová, 2011; Tripoli et al., 2007). The benefits of phenolic compounds in food also include inhibiting lipid oxidation and rancidity (Procházková et al., 2011; Simitzis, Symeon, Charismiadou, Ayoutanti, & Deligeorgis, 2011; Zia ur, 2006). The flavonoid hesperidin is a major phenolic compound found in large quantity in mandarin peel, and has also been discussed as a component of citrus peel extract and its use in food (Ma et al., 2008; Morand et al., 2011; Simitzis et al., 2011; Zia ur, 2006), as have its medical benefits (Hosseinimehr & Nemati, 2006; Jain & Parmar, 2011; Kuntić, Filipović, & Vujić, 2011; Mahmoud, Ashour, Abdel-Moneim, & Ahmed, 2012).

The extraction of mandarin peels is primarily for the purpose of separating the phenolic compounds from plant tissues. Various methods have previously been investigated, including MAE, Soxhlet extraction, enzyme-assisted extraction and heat extraction methods. These methods also show some disadvantages such as low efficiency, long extraction time, high temperature requirements or the requirement in terms of the difficulty to operate the costly equipment and accessories required. More recently developed extraction methods have shown improvement in efficiency for phenolic compounds extraction, including microwave-assisted extraction, supercritical water extraction (Cheigh, Chung, & Chung, 2012) and accelerated solvent extraction (Hossain, Barry-Ryan, Martin-Diana, & Brunton, 2011). However, the equipment required by these methods is difficult to operate and is expensive.

The ultrasound-assisted extraction (UAE) principally uses the shear force created by the action of cavitation bubbles generated during the propagation of the ultrasonic waves. The collapse of those bubbles disrupts the plant cell wall, thereby increasing the release of extractable compounds (Chemat, Zill, & Khan, 2011; Li, Fabiano-Tixier, Tomao,

Abbreviations: UAE, ultrasound-assisted extraction; MPE, mandarin peel extract; MAE, maceration extraction; BBD, Box-Behnken Design; RSM, Response Surface Methodology

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Cravotto, & Chemat, 2013; Singanusong et al., 2015). This method becomes popular for the extraction of phenolic compounds from mandarin alike fruits because it is inexpensive along with low instrumental requirements and simplicity of operation. However, previous studies reported that using UAE at high temperature and at high ultrasonic power and intensity degrades the phenolic compounds and some other substances (Guo et al., 2014; Zhang, He, & Hu, 2011). For example, using ultrasonic power higher than 100 W causes the degradation of (all-*E*)-astaxanthin, and degradation increased as both treatment time and ultrasonic power increased (Zhao et al., 2006). Using UAE at 150 W for longer than 15 min disrupts the total polyphenols extracted from grape seeds (Da Porto, Porretto, & Decorti, 2013). When the ultrasonic power was higher than 160 W the oil yields from pomegranate seeds decreased (Tian, Xu, Zheng, & Martin Lo, 2013).

The purpose of this research was to investigate the effects on MPE yield, total phenolic and hesperidin content of the process independent variables of temperature, time and power of UAE when using low power ultrasonic intensities less than 60 W. These variables were calculated to determine the optimizing extraction conditions by using the Response Surface Methodology for determination of the optimal extraction conditions that provided the maximum MPE yield, total phenolic and hesperidin content, and comparing the efficiency of UAE at optimized conditions against the MAE method.

2. Materials and methods

2.1. Plant materials

Fresh mandarins, (*Citrus reticulata* Blanco cv. Sainampueng) were harvested 10 months after bloom in December 2012 and 2 further harvestings in January and February 2013, a total of 3 replicates, from the same farm in Fang district, Chiang Mai Province where the most available and famous mandarins were cultivated in Thailand. The peels were cleaned with tap water and cut into 1 cm^2 pieces which were dried in a hot air oven at 60 °C until reaching a moisture content of 9–10%. The dried peels were ground with a blade mixer, sieved through a 300 μm (50 Mesh) sieve and the powder kept in an amber glass bottle at -20 °C until used.

2.2. Ultrasound-assisted extraction

One g of the dried ground mandarin peel was thoroughly mixed with 20 mL of 80% acetone (Singanusong et al., 2015) and placed in a 120 mL amber glass bottle. The bottle was immersed in an ultrasonic bath (model 175DAE, Crest Co. Ltd., Malaysia) that was used as the ultrasound source for this experiment. The liquid level in the immersed bottle was lower than that of the liquid in the bath in order to achieve a maximum ultrasonic energy. The bath was a rectangular container (16.4 cm × 13.3 cm × 10.2 cm) with transducers at a frequency of 38.5 kHz annealed to the bottom. The power of the ultrasonic source used in this experiment was set at three levels, referred to here as level 1 (30.34 W), level 4 (44.85 W) and level 7 (59.36 W). At each power level the temperature was varied at 30, 40 and 50 °C and the time varied at 20, 30 and 40 min; 17 treatments in all were applied, as discussed below in the Box-Behnken Design (BBD) discussion. The operating extraction conditions with the different temperature, time and power combinations are shown in Table 1.

2.3. Maceration extraction

The dried ground mandarin peel was extracted using similar conditions of UAE except without application of ultrasonic power during the extraction process.

2.4. Experimental design

The Response Surface Methodology (RSM) was applied to obtain the optimal conditions for maximum extraction of the phenolic compounds and hesperidin content. The BBD was used to determine the optimal UAE conditions. The three independent variables of extraction temperature (°C, X_1), extraction time (min, X_2) and extraction power (W, X_3) at three levels (−1, 0, +1) were investigated. The coded and decoded values of the independent variables and their levels are shown in Table 1. A total of 17 different experiment combinations, including five replicates of the center points, were analyzed using the software Design-Expert 6.0 for statistical analysis of variance, regression coefficients and regression equation. The second-order polynomial model was fitted to each response giving an Eq. (1) in terms of the code factors, as follows:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 \quad (1)$$

where Y is the predicted response variable; β_0 is the intercept; β_1, β_2 and β_3 are the linear coefficients of X_1, X_2 and X_3, β_{11}, β_{22} and β_{33} are the squared coefficients of X_1, X_2 and X_3, and β_{12}, β_{13} and β_{23} are the interaction coefficients of X_1, X_2 and X_3. All measurements were replicated three times and the experimental results were expressed as means.

2.5. Yield determination

The yield was determined following the method of Tian et al. (2013). After extraction, the supernatant was filtered under vacuum through Whatman paper No. 4. A 100 mL volume of the liquid extract was evaporated by rotary evaporator at 50 °C for 5 min and dried in a freeze dryer at -75 °C for 10 h. The dried extracts were kept in an amber glass bottle at −20 °C until used. The yields of MPE were calculated by comparing the weight of the dried mandarin peel extract with the weight of the original dried ground mandarin peel as in Eq. (2).

$$\text{Yield of MPE (\%)} = (W_o/W_p) \times 100 \quad (2)$$

W_o is the weight of the freeze dried mandarin peel extract (g)
W_P is the weight of original dried ground mandarin peel (g)

2.6. Total phenolic content

The total phenolic content of the freeze dried MPE was determined according to the Folin-Ciocalteu colorimetric method (Anagnostopoulou, Kefalas, Papageorgiou, Assimopoulou, & Boskou, 2006). The total phenolic content was expressed as mg gallic acid equivalent/100 g dried weight (DW).

2.7. HPLC analysis

A 200 mg sample of the freeze dried mandarin peel extract was diluted with methanol 10 mL. The diluted solution was quantitatively analyzed for hesperidin content by HPLC Agilent 1100 chromatograph using a UV–Visible detector at 280 nm and a C18 reversed-phase column (Agilent TC-C18 250 mm × 4.6 mm, 5 μm) operated at 37 °C. The mobile phase included two solvents; 0.5% acetic acid (A) and 100% acetonitrile (B). The linear solvent gradient in the volume ratios were calculated as follows: 10–30% B over 20 min, with the solvent gradient subsequently increased to 35% B at 25 min and maintained at 35% B for 5 min. The flow rate was 1 mL/min and the injection volume was 20 μL (Khan, Abert-Vian, Fabiano-Tixier, Dangles, & Chemat, 2010). Identification of hesperidin was based on the retention times compared with the standard (Sigma-Aldrich, Germany). Analysis was performed at least three times and only the mean values were reported. The hesperidin content was calculated from the peak area according to the