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Optimisation of ultrasound-assisted extraction of oil from papaya seed by response surface methodology: Oil recovery, radical scavenging antioxidant activity, and oxidation stability

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ABSTRACT

The present study aimed to investigate the effects of ultrasound-assisted extraction (UAE) condition on the yield, antioxidant activity and stability of the oil from papaya seed. The studied ultrasound variables were time, temperature, ultrasound power and solvent to sample ratio. The main goal was to optimise UAE condition providing the highest recovery of papaya seed oil with the most desirable antioxidant activity and stability. The interaction of ultrasound variables had the most and least significant effects on the antioxidant activity and stability, respectively. Ultrasound-assisted extraction provided a relatively high oil recovery (~73%) from papaya seed. The strongest antioxidant activity was achieved by the extraction at the elevated temperature using low solvent to sample ratio. The optimum ultrasound extraction was set at the elevated temperature (62.5 °C) for 38.5 min at high ultrasound power (700 W) using medium solvent to sample ratio (~7:1 v/w). The optimum point was practically validated.

1. Introduction

Antioxidants are a series of bioactive compounds commonly used to preserve the quality of food products by protecting them against oxidation rancidity (Nakatani, 1997). They also protect the human body from many chronic cardiovascular diseases, cancers, and ageing by capturing free radicals (Bordbar et al., 2013). Previous studies proved that typical herbal plants (such as rosemary, chamomile, rose-hip, hawthorn, lemon verbena and green tea) as well as some seeds extracts (such as winter melon oil and papaya seed extracts) could be great sources of natural antioxidants due to the high antioxidant activities (Bimakr et al., 2012; Yoo, Lee, Lee, Moon, & Lee, 2008; Zhou et al., 2011). Papaya seeds are agricultural biomass wastes potential for valuable by-products (i.e. protein, dietary fibre and papaya seed oil) (Puangsri, Abdulkarim, & Ghazali, 2005). Papaya seed oil is rich in monounsaturated fatty acid and contains functional compounds (Lee, Lee, & Su, 2011; Samaram, Mirhosseini, Tan, & Ghazali, 2013). However, the extraction type and condition can significantly affect the yield, quality and antioxidant activity of papaya seed oil (Rossetto et al., 2008; Zhou et al., 2011).

Extraction is the first key step to isolate natural bioactive compounds from plants and materials. Different extraction methods (i.e. solvent extraction, expelling extraction process, supercritical fluid extraction (SFE) and microwave assisted extraction (MAE)) have been developed for the recovery of bioactive compounds and essential oils (Wang & Weller, 2006). However, several disadvantages like extra solvent amount in solvent extraction, low yield in expelling process, massive investment in supercritical fluid extraction (SFE) and the requirement for the aqueous phase in microwave assisted extraction indicates the demand of comprehensive extraction method to recover different target compounds in economic condition (Lee et al., 2011; Samaram et al., 2013; Wang & Weller, 2006). Ultrasound-assisted extraction (UAE) is a new simple technique for the recovery of oil and bioactive compounds from different sources. The intensity of ultrasound power creates extra vibration in sample molecules and facilitates the recovery of target compounds from solid material to the liquid solvent phase. Therefore, high yield in short extraction time beside the utilisation of low solvent amount are remarkable advantages of ultrasound-assisted extraction technique. Moreover, ultrasound-assisted extraction is adjustable to be utilised with polar and non-polar solvents in various temperatures (Bimakr et al., 2012; Wang & Weller, 2006).

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Previous researchers investigated the effects of ultrasound extraction variables (i.e. ultrasound power, time, temperature and solvent to solid ratio) on the recovery of oil from different plant source (Bimakr et al., 2012; Zhang et al., 2009). Moreover, the ultrasound-assisted extraction under the recommended optimum condition resulted in extra yield for almond powder oil (Zhang et al., 2009), winter melon oil (Bimakr et al., 2012) and phenolic compounds from wheat bran (Wang, Sun, Cao, Tian, & Li, 2008). However, there is no published report testing different ultrasound extraction conditions for the recovery of oil from papaya seeds. It should be noted that the oil recovery from papaya seeds was carried out by supercritical fluid extraction (SFE) and solvent extraction in a preliminary study. Our preliminary study revealed that supercritical fluid extraction of papaya seed oil under different experimental conditions resulted in very poor recovery and excessive amount of impurities. On the other hand, the solvent extraction of papaya seed oil using different solvents under various experimental conditions resulted in low quality oil containing pigment and other undesirable impurities in the final extract.

It was hypothesised that ultrasound-assisted extraction (UAE) could be a reliable extraction technique for the recovery of qualified oil from papaya seeds. In fact, the enhancement of extraction yield along with maintaining bioactive and heat sensitive components is the main advantage of ultrasound-assisted extraction as compared to the conventional extraction methods. In addition, the ultrasound facilities and utilisation procedure are inexpensive and simple (Bimakr et al., 2012; Samaram et al., 2013). Therefore, the current study was designated to investigate the effects of the ultrasound extraction variables on the yield, antioxidant activity (DPPH), and oxidation stability of papaya seed oil. The main goal of the present work was to develop the optimum ultrasound extraction condition resulting in papaya seed oil with the optimum desirable yield and reliable antioxidant activity and stability.

2. Materials and methods

2.1. Material

N-hexane (reagent grade) was purchased from fisher scientific (Pittsburgh, PA, USA). 1, 1-diphenyl-2-picrylhydrazyl (DPPH) reagent was supplied by Sigma–Aldrich (St. Luis, MO, USA). Other reagent grade chemicals such as acetic acid, chloroform, potassium iodide, sodium thiosulfate, ethanol (96%), p-anisidine (p-AV) and starch indicators were supplied by Merck (Darmstadt, Germany). Ripened papaya fruits (*Sekaki*variety) were purchased from a hypermarket (Selangor, Malaysia). Ripened fruits were chosen according to Lam colour index (Yon, 1994). Fruits were cleaned and cut into halves in order to collect the seeds. Subsequently, the seeds were collected, washed and dried at 45 °C oven for 2 days. Dried papaya seeds were ground and sieved to achieve the powder with uniform particles. The seed powder was kept in 4 °C till further use.

2.2. Ultrasound-assisted extraction (UAE)

In the current study, ultrasound-assisted extraction (UAE) was applied for the recovery of oil from papaya seeds. An ultrasonic water bath (Power sonic 420; 40 kHz frequency; maximum power, 700 W; internal dimension, 500 × 300 × 150 mm) was employed for extraction purpose. The extraction was carried out under different experimental conditions: time (x_1, 5–30 min), temperature (x_2, 25–50 °C), ultrasound power (x_3, 235–700 W) and solvent to sample ratio (x_4, 6:1–10:1, v/w) (Table 1). The range and conditions of ultrasound-assisted extraction (UAE) were accomplished according to the previous studies (Chua et al., 2009; Zhang et al., 2009). Then, a preliminary study was carried out to choose the appropriate range for each extraction variable. During extraction, the temperature was continuously adjusted and maintained by adding hot or cold water at the desired level. N-Hexane was used as the solvent. The extraction was performed in duplicate for each treatment. Average of two individual applications was considered for further data analysis.

2.3. Determination of extraction yield

The extraction yield was obtained by dividing the amount of the extracted oil to the initial amount of seed powder (5 g). A 0.0001 g analytical balance (Mettler Toledo GmbH, Greinfensee, Switzerland) was used for accurate balancing. The extraction yield was obtained from the following formula (Bimakr et al., 2012):

$$\text{Extraction yield } (\%) = [\text{Oil amount}/\text{Initial sample amount})] \times 100 \quad (1)$$

2.4. Measurement of antioxidant activity

Antioxidant activity was determined based on DPPH radical scavenging activity (2, 2-diphenyl-2-picrylhydrazyl radical) (Bordbar et al., 2013). For DPPH assay, papaya seed oil was diluted 6 times in n-hexane. Then, 1 ml DPPH solution (20 mmol) was added to 500 μL diluted papaya seed oil in micro tubes. Samples were incubated in the dark place for 40 min. The absorbance readings of diluted samples were recorded at 517 nm by using a spectrophotometer (Spectro UV–VIS double beam UVD 2950, Labomed, Inc., Culver City, CA, USA). Results were compared with the absorbency of the blank. It should be noted that the stronger antioxidant activity would be indicated by lower absorbency of the reacted mixture. The analysis was performed in duplicate for each sample. DPPH antioxidant activity was calculated according to the following formula (Bordbar et al., 2013):

$$\text{DPPH}\% = [(\text{blank}_{\text{absorbance}} - \text{sample}_{\text{absorbance}})/\text{blank}_{\text{absorbance}}] \times 100 \quad (2)$$

2.5. Oil stability test

Peroxide value (PV) and p-anisidine value (AV) were determined based on AOCS official methods (Cd 8-53, 2003; Cd 18-90, 2009). The stability analysis test was performed in triplicate for each sample. Totox value (TV) was calculated based on the following equation (Naghshineh, Ariffin, Ghazali, Mirhosseini, Kuntom, & Mohammad, 2009; Serjouie, Tan, Mirhosseini, & Che Man, 2010):

$$\text{TV} = 2\text{PV} + \text{AV} \quad (3)$$

2.6. Statistical design and data analyses

The current research investigated the effects of ultrasound extraction variables namely time (x_1), temperature (x_2), ultrasound power (x_3) and solvent to sample ratio (x_4) on the yield (Y_1), DPPH antioxidant activity (Y_2), PV (Y_3), AV (Y_4) and TV (Y_5) of papaya seed oil. In this study, the cube style-central composite design (SCCD) was applied to generate 30 extraction treatments by involving five levels for each extraction variable. A coded SCCD including all axial, cube and star points is shown by Table 1. In this design, the randomized run order was created by considering 16 factorial points, 8 star points and 6 repeated centre points. As shown in Table 1, 8 star points (runs 11, 12, 13, 14, 15, 17, 18 and 19) were considered by Minitab software. These star points are outside of the ranges selected for independent variables to ensure the adequacy and reli-