CHEMICAL ENGINEERING TRANSACTIONS
VOL. 78, 2020
A publication of AIDIC
The Italian Association of Chemical Engineering
Online at www.cetjournal.it
Guest Editors: Jeng Shiun Lim, Nor Alafiza Yunus, Jiří Jaromír Klemeš

ISBN 978-88-95608-76-1; **ISSN** 2283-9216
DOI: 10.3303/CET2078083

Extracting Seed Oil and Phenolic Compounds from Papaya Seeds by Ultrasound-assisted Extraction Method and Their Properties

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Papaya seeds which make up 8 % to 15 % of fresh fruit weight, are rich source of proteins, crude fibers, fatty acid, calcium, and phosphorus. The aim of this study is to determine the composition of papaya seed oil, to evaluate the antioxidant activity of defatted-papaya seed residue, and to optimize the phenolic compound extracted from defatted-papaya seed residue. The papaya seed oil contained high level of oleic acid (73.79 %), and other acids such as palmitic (14.38 %), stearic (3.58 %), linoleic (1.06 %). The oil yield of ultrasound-assisted extraction method reached 30.24 wt% which was higher than other extracting methods. The antioxidant activity of defatted-papaya seed extraction was evaluated using DPPH method. The results showed that the IC_{50} values of 67.81 µg/mL and total polyphenol content of 33.99 mg GAE/g. The optimization of extraction conditions was obtained from the orthogonal quadratic experimental model. The highest total polyphenol concentration reached 37.34 mg GAE/g when extracting the papaya seeds by sonicating for 34.2 min, at 46.23 °C with the solvent/solid ratio of 14:1.

1. Introduction

Papaya is mainly distributed in tropical and subtropical areas. In 2012, worldwide annual production of papaya reached 12.5 Mt (Barroso et al., 2016). This full-nutrition fruit is mainly used for fresh consumption and papain production. Whereas, papaya seeds, accounting for 8 % to 15 % of fresh fruit weight are currently disposed and become biomass in fruit processing units. In addition, this process required labour and capital costs for treatment (Hameed, 2009).

The group of Marfo reported a rich source of proteins (27.8 %), crude fibers (22.6 %), fatty acid, calcium and phosphorus in papaya seeds (Marfo et al., 1986). Because of the high fatty acid contain (28.3 %), papaya seeds can be considered as the new sources of consumption oils. The papaya seed oil is high in beneficial triacylglycerols, with predominant by triolein (>37 %) and monounsaturated fatty acid as oleic acid >70 % (Samaram et al., 2013). Additionally, papaya seed oil exhibits a high stability against oxidation and considerable antioxidant activity.

Nowadays, the most commonly methods to extract oil from papaya seeds are Soxhlet extraction, solvent extraction, ultrasound-assisted extraction, and supercritical fluid extraction. The group of Syed reported the use of Soxhlet extraction for extracting seed oil (Syed et al., 2011). Although this method recorded a high oil extraction recovery, it required expensive capital investment, high operational costs, and undesirable effects on the quality of final product. Besides, the oil obtained using supercritical carbon dioxide is free of organic solvent. The processing time of this method is lower than that of conventional solvent extraction. However, this process required expensive equipment and costly material (Aleksovski et al., 1998). The group of Vilbett reported the ultrasound-assisted extraction of fatty acid from papaya seed with shortened extraction time (Vilbett et al., 2015). With the above reasons, the ultrasound-assisted extraction was selected to investigate further under our conditions to determine the composition, to evaluate antioxidant activity of defatted-papaya seed residue and to optimize the phenolic compound from defatted-papaya seed residue.

Paper Received: 27/05/2019; Revised: 10/08/2019; Accepted: 25/10/2019
Please cite this article as: Doan M.T.N., Huynh M.C., Pham A.N.V., Chau N.D.Q., Le P.T.K., 2020, Extracting Seed Oil and Phenolic Compounds from Papaya Seeds by Ultrasound-assisted Extraction Method and Their Properties, Chemical Engineering Transactions, 78, 493-498 DOI:10.3303/CET2078083

2. Materials and methods

2.1 Materials

Papaya seeds were collected from local markets. Seeds were dried at 60 °C for 32 h until they reached constant weight. The dried seeds were ground into fine powder which was used in the extraction process. Folin-Ciocalteu reagent and gallic acid (3,4,5-trihydroxybenzoic acid), sodium hydroxide, and potassium hydroxide were purchased from Sigma–Aldrich (St. Louis, Mo, USA). 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and quercetin were obtained from Merck (Darmstadt, Germany). Ethanol, methanol, *n*-hexane, and diethyl ether were purchased from Chemsol (Vietnam). These chemicals were used without further purification.

2.2 Methods

2.2.1 Extraction of papaya seed oil by ultrasound-assisted extraction and Soxhlet extraction method

Papaya seed oil was extracted by ultrasound-assisted extraction with *n*-hexane as solvent (Samaram et al., 2013). The mixture was sonicated for 30 min at 50 °C in supersonic bath. For Soxhlet extraction, 10 g of seed powder was placed in a cellulose paper bag and extracted using *n*-hexane in a 250 mL by Soxhlet extractor for 8 h (Syed et al., 2011). Then the solvent was separated by using rotary evaporator (RE-801, Yamato Scientific) and the fatty oil was collected from the liquid phase.

2.2.2 Extraction of phenolic compounds by ethanol

Phenolic compounds were extracted from the defatted-papaya seed residue by ethanol. The mixture of ethanol and defatted-papaya seed residue was sonicated for 30 min at 50 °C (Wang et al., 2008). The antioxidant activity of the phenolic compounds in ethanol was evaluated.

2.2.3 Determination of phenolic concentration and antioxidation values

This method was based on the reaction between Folin-Ciocalteu reagent and hydroxyl radical in the phenolic compounds whereby the reagent solution turns from yellow to dark blue (Phung T.K.Le et al., 2018). Gallic acid was used to prepare standard solutions. The total phenolic concentration of the samples and the standard solutions were determined by measuring the absorbance at 765 nm.
The free radical scavenging activity of the extracts based on the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical was determined following the method described by the group of Phung (Phung T.K.Le et al., 2018). The IC_{50} value was used to assess strong or weak inhibition of the sample. This IC_{50} value was defined as the concentration of sample at which it could inhibit 50 % of free radicals. In general, the higher the activity pattern is, the lower the IC_{50} value obtains.

2.2.4 GC – MS for analyzing extracted

The GC-MS analysis of each sample was carried out on a SCION SQ 456 GC-MS system. The column used was Rxi-5MS capillary column (30 m × 0.25 mm i.d., 0.25 µm) (Restek, France). Helium was used as the carrier gas. Sample was ionized at 250 °C scanning speed at 1 s/scan.

2.2.5 Response surface experimental design

Experimental design was performed using orthogonal quadratic model with three variables. The values of variables were listed in Table 1.

Table 1: Variables employed in an orthogonal quadratic model for optimisation

Variables	Unit	Survey range		
		Lower	Center	Upper
Sonicating time (X_1)	min	15	30	45
Temperature(X_2)	°C	30	40	50
Solvent/Solid ratio(X_3)	mL/g	10	15	20

The values were set up based on preliminary studies (data not shown) and the previous studies (Vilbett et al., 2015). Ethanol was used as solvent for extracting phenolic compounds from the defatted papaya seed residue. The center means the center point of the design. The lower and the upper mean the factor intervals of variation.

3. Results and discussion

3.1 Recovery yields

The yields of oil base on weight powder seed by ultrasound-assisted extraction and Soxhlet extraction. The extracting yield by Soxhlet method was 30.24 wt%. As shown in Figure 1, the yields of oil recovery by ultrasound-assisted extraction method reached 25.32 wt% after sonicating for 30 min at 50 °C. This value is higher than the yield extracted by enzyme extraction (24.19 wt%) (Puangsri et al., 2005). Based on the above results, the ultrasound-assisted extraction performance is lower than the Soxhlet extraction. However, the time of extraction process is significantly reduced, from 8 h by Soxhlet extraction to 1 h by ultrasound-assisted extraction. Thus, the ultrasound-assisted extraction is potential method for oil extraction, in both small and industrial scales.

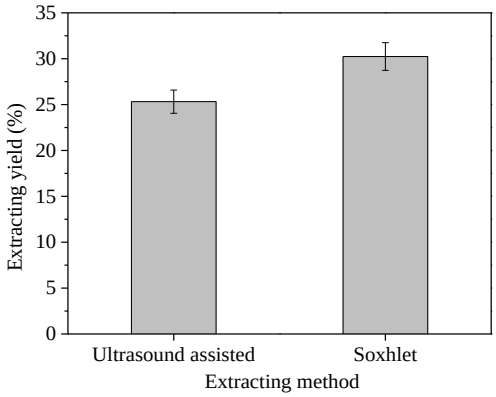

Figure 1: Oil extraction yields by ultrasound-assisted extraction and Soxhlet extraction method

3.2 Chemical properties

The acid value of the papaya seed oil was determined by KOH. The acid value of the extracted oil is 3.66 ± 0.42 mg KOH/g of papaya oil, which is relevant to 1.84 ± 0.21 % of free fatty acid. These results also confirmed previous study (Malacrida et al., 2011) (<4 mg KOH/g oil). The saponification value of papaya seed oil was 211.70 ± 2.25 mg KOH/g oil. This value is similar to the results obtained by the group of Marfo (197 ± 3.03 mg KOH/g oil) (Marfo et al. (1986), and Malacrida (196.4 ± 1.37 mg KOH/g oil) (Malacrida et al., 2011). Comparing with other oil products on the market, the papaya seed oil has similar saponification value. It means the carbon chain of the fatty acid in papaya seed oil has similar average length as the circuit carbon in the following oils like soybeans (192 mg KOH/g oil), cotton seeds (195 mg KOH/g oil), sesame and olive seed oil (192 mg KOH/g oil) (O'brien, 2008)

3.3 Fatty acid compositions

The fatty acid compositions of papaya seed oil were determined by GC-MS. The analysis showed that the main constituents of papaya seed oil are unsaturated fatty acids, of which the glyceride portion is predominantly made up of palmitic, oleic, and stearic acids. Table 2 showed that total long chain fatty acid, which was unsaturated, accounted for over 80 % of the composition.

Table 2: Fatty acid compositions (% of a total fatty acid) as determined by GC-MS of seed oil

Content of fatty acid	Amount (%)
Hexadecanoic acid (palmitic acid) (C16:0)	14.38
cis–9,12–Octadecenoic acid (linoleic acid) (C18:2)	1.06
cis –9–Octadecenoic acid (oleic acid) (C18:1)	73.79
Stearic acid (C18:0)	3.58

Oleic acid (C18:1) has the highest percentage (73.79 %) of total fatty acid in the oil extracted by ultrasound-assisted extraction. The high concentration of oleic acid (C18:1), one of the drug neurotransmitters, can enhance the nutrient absorption of human body. Other acids constitute less than 10 % of the composition. These results were also consistent with previous reports and it also indicated that there was no relationship between ultrasound and the fatty acid compositions of papaya seed oil. Thus, the ultrasound-assisted

extraction can be considered as a simple yet cost-effective method for extracting fatty acid from papaya seeds with high quality oil and high nutrition value.

3.4 Results of total phenolic content (TPC) and antioxidant activities

3.4.1 Antioxidant activities in wasted seed extraction after defatted

The antioxidant activities in defatted-papaya seed residue was evaluated. The antioxidant activity of the residue by DPPH free radical capture method, comparing with vitamin C, was presented in Figure 2.

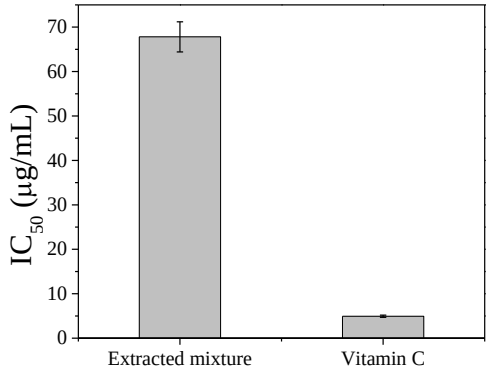

Figure 2: IC50 value of the sample and vitamin C

From Figure 2, the IC_{50} value of defatted-papaya seeds residue was much higher than that of vitamin C (~13 times). However, our IC_{50} value (67.81 µg/mL) was much lower than that of the defatted-papaya seeds extracted by Soxhlet method (Norshazila et al., 2010) and (Zhou et al., 2011) (340 µg/mL and 248.63 µg/mL, respectively). This might be due to the properties difference of papaya seeds as well as pre-treatment and defatted processes.

3.4.2 Result of total phenolic content

The total phenolic in defatted-papaya seed residue which was extracted by methanol solvent and ethanol solvent was determined. As shown in Figure 3, the total phenolic concentrations in the defatted-papaya residue by ethanol and methanol was 34.00 mg GAE/g extraction (or 2.18 mg GAE/g seed) and 16.34 mg GAE/g extraction, respectively. The results proved that ethanol was a more effective solvent to extract phenol compounds from papaya seed wastes. Comparing with methanol and other organic solvents; ethanol, a low-toxicity compound, is a suitable solvent to extract polar antioxidants such as phenolic compounds. The results were also like the study of Quy D.D. et al. (2014). Hence, ethanol was used in the consequence experiments.

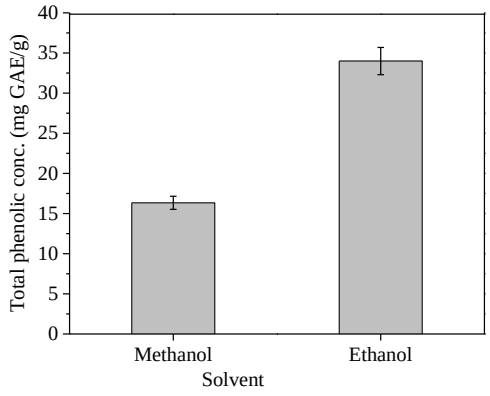

Figure 3: Total phenolic content of the extraction in methanol and in ethanol

3.5 Optimization the extraction process of phenolic compound

Experiments were conducted based on the matrix of the experimental designed, built by Design Expert software 7.1.5 (2017). The obtained results were used to calculate the regression equation and optimum conditions for extracting process. The significant of the coefficients was determined based on the p-value. If its

p-value is less than 0.0500, the coefficient is significant. Based on the ANOVA results, as presented in Table 3, these significant coefficients are X1, X2, X12, X22, X32. The regression equation is shown in Eq(1), with the R2 =0.9837.

$$Y = -59.89 + 2.24X_1 + 1.44X_2 - 0.033X_1^2 - 0.0157X_2^2 - 1183.2X_3^2 \quad (1)$$

where X_1, X_2 and X_3 are the survey variables – ultrasound time, temperature, solvent/solid ratio.

Table 3: Analysis of variance (ANOVA) by Design Expert

Source	Sum of Squares	Degrees of Freedom	Mean square	*F* Value	*p*-Value
Model	685.18	9	76.13	47.06	< 0.0001
X_1	158.83	1	158.83	98.19	< 0.0001
X_2	67.21	1	67.21	41.55	0.0004
X_3	5.79	1	5.79	3.58	0.1004
X_1X_2	0.42	1	0.42	0.26	0.6247
X_1X_3	0.45	1	0.45	0.28	0.6137
X_2X_3	0.005	1	0.005	0.003	0.9571
X_1^2	376.81	1	376.81	232.94	< 0.0001
X_2^2	16.54	1	16.54	10.23	0.0151
X_3^2	59.10	1	59.10	36.54	0.0005
Lack of fit	10.92	5	2.18	10.74	0.0873

As shown in Figure 4, total phenolic concentration reached the highest value by sonicating the sample for 30 to 35 min with the solvent/solid ratio was fixed around 13:1 to 15:1 at 50 °C.

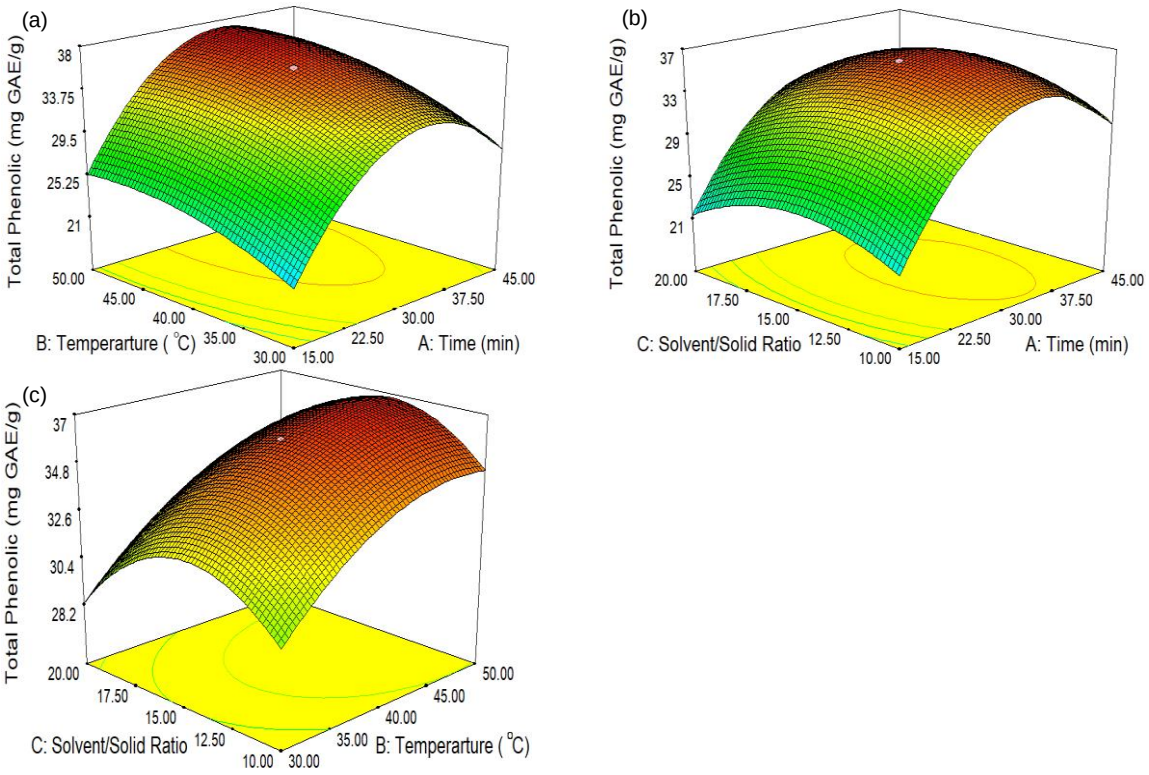

Figure 4: Effects of ultrasound extraction variables on total phenolic concentration (a: time and temperature; b: time and solvent to sample ratio; c: temperature and solvent to sample ratio)

The value decreased and reached lowest value when the sample was sonicated at 30 °C for 15 min. By increasing the sonicating time to 45 min and the temperature to 50 °C, the total phenolic concentration dropped significantly. Extracting phenolic compound process was not effective when increasing sonicating

time and temperature, due to the decomposition of sensitive compounds caused by heat and solvent evaporation.

The highest total phenolic concentration could reach 37.34 mg GAE/g by extracting the papaya seed residue at 46.23 °C for 34.2 min and solvent/solid ratio of 14:1 The additional experiments were conducted based on the optimum conditions, which were suggested by the software in order to verify the calculated optimum values. The total phenolic concentration was 37.54 ± 0.43 (mg GAE/mg), which showed equivalence to the calculated values.

4. Conclusions

This paper reported the extracting methods to extract seed oil from papaya seed and defatted-papaya residue, as well as their properties. The papaya seed oil was successfully extracted by ultrasound-assisted extraction method and the phenolic compounds from defatted-papaya residue were extracted by ethanol. In papaya seed oil, oleic acid was the dominant compound, followed by palmitic and stearic acids. The analytical results showed that papaya seed oil had the potential to become the new oleic-rich oil, suitable for dietary oil products. The experimental design with orthogonal quadratic model was established to optimize the phenolic compounds extraction process. Total phenol content was 37.34 mg GAE/g. The antioxidant activity of the extraction from defatted-papaya seed residue has good result with IC_{50} of 67.81 µg/mL. In addition, the bioactive and phenolic content in papaya seed are also significant, potentially applied to make nature – based products for human beings.

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