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EFFECT OF PRETREATMENTS ON CHEMICAL AND ANTIOXIDANT PROPERTIES OF SKY FRUIT (*Swietenia macrophylla*) SEED OIL

[*Pengaruh Pra-perlakuan Terhadap Sifat Kimia dan Antioksidan Minyak Biji Buah Tunjuk Langit (Swietenia macrophylla)*]

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ABSTRACT

The objective of this study was to investigate the effect of pretreatments on chemical and antioxidant properties of sky fruit (*Swietenia macrohylla*) seed oil. The seeds were treated with different heat pretreatments (roasting, steaming, and microwaving) and subsequently subjected to oil extraction by using a Bligh and Dyer method. It was found that different pretreatments significantly ($p<0.05$) affected yield and peroxide value of the extracted oils. However, no significant effect of pretreatment was observed on free fatty acid content of the seed oils. The oils exhibited significantly different levels of 1, 1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity due to different heat pretreatments. The results revealed that the mean percentages of DPPH scavenging activity of untreated (control) seed oil (87.69%) and steamed seed oil (83.40%) were significantly higher than those of roasted seed oil (75.71%) as well as the microwaved one (63.98%). In contrast, the pretreatments did not significantly affect total phenolic content (TPC) of the seed oils with the TPC mean values ranging from 0.016 to 0.022 mg/g (as gallic acid). Data gained from this study provided valuable information for edible oil industries in searching for alternative source of edible oil with medicinal benefits.

Keywords: antioxidant, microwaving, roasting, seed oils, sky fruit, steaming

ABSTRAK

Tujuan penelitian ini adalah mengetahui pengaruh perlakuan pendahuluan terhadap sifat kimia dan antioksidan minyak biji buah tunjuk langit (Swietenia macrohylla). Biji diberi beberapa perlakuan pendahuluan panas yang berbeda (pemanggangan, pengukusan, dan pemanasan microwave*), kemudian diekstraksi minyaknya menggunakan metode Bligh dan Dyer. Perlakuan pendahuluan yang berbeda memberikan perbedaan signifikan ($p<0,05$) terhadap rendemen dan nilai peroksida dari minyak yang diekstrak. Namun, perlakuan pendahuluan tidak memberikan pengaruh yang signifikan terhadap kandungan asam lemak bebas minyak biji. Minyak menunjukkan tingkat signifikansi yang berbeda terhadap* scavenging activity *1, 1-difenil-2-pikrilhidrazil (DPPH) karena perlakuan pendahuluan panas yang berbeda. Hasil penelitian menunjukkan bahwa persentase rata-rata* scavenging activity *DPPH dari minyak biji kontrol (87,69%) dan minyak biji yang dikukus (83,40%) jauh lebih tinggi dibandingkan dengan dari minyak biji panggang (75,71%) serta minyak biji* microwave *(63,98%). Sebaliknya, perlakuan pendahuluan tidak secara signifikan mempengaruhi kandungan fenolik total (TPC) dari minyak biji dengan nilai rata-rata TPC berkisar 0,016 sampai 0,022 mg/g (sebagai asam galat). Data yang diperoleh dari studi ini memberikan informasi yang penting untuk industri minyak makan dalam mencari sumber alternatif minyak nabati yang mempunyai manfaat terhadap kesehatan.*

Kata kunci: *antioksidan, perlakuan* microwave*, pemanggangan, minyak biji, buah langit, pengukusan*

INTRODUCTION

Swietenia macrophylla or big leaf mahogany is a tree from family Meliaceae. *Swietenia macrophylla*'s wood, leaves and fruits can be used for many purposes (Falah *et al*., 2008). *Swietenia macrophylla* produces a fruit that commonly known as sky fruit, because the fruit seems to point upwards to the sky. Sky fruit has been processed commercially to a wide range of health foods and healthcare products. Besides, sky fruit was used as an old folk medicine for treatment of diabetes and hypertension by chewing and then swallowing the seeds. Sky fruit seed was reported to contain different types of limonoids such as swietenine, swietenine acetate and anti-diabetic, antimicrobial, antidiarrhoeal benefits (Chen *et al*., 2010; Maiti *et al*., 2008; Falah *et al*., 2008; Maiti *et al*., 2007). The seed is rich in fat content and swietenolide, a bitter principle (Chakrabarty and Chowdhuri, 1956). Apart from that, the seed oil consisted of six types of fatty acid which included palmitic acid (12.50%), stearic acid (16.42%), arachidic acid (0.56%), oleic acid (25.30%), linoleic acid (33.87%) and linolenic acid (11.32%) (Chakrabarty and Chowdhuri, 1956). The abundance of poly-unsaturated fatty acid (PUFA) especially linoleic and oleic acids shows that sky fruit seed oil has the ability to provide benefit to

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human health. However, little attention has been paid on the information of PUFA in sky fruit seed. PUFA is known to be highly prone to oxidation. Oxidation of oil produces rancid flavour and decreases quality of oil. Different heat pretreatments used in industry such as roasting, microwaving and steaming might provide different effects on the seed oil quality. Previously, it has been found that roasting could provide better resistance for honeydew seed oil towards oxidative deterioration (Chin, 2012). Microwaving pretreatment and steaming pre-treatment increased the yield and quality of seed oil (Uquiche *et al*., 2008; Danso-Boateng, 2011). To this far, the effect of heat pretreatments on sky fruit seed oil is yet to be studied. Therefore, this study was conducted to investigate the effect of pretreatments on chemical and antioxidant properties of sky fruit seed oils.

MATERIALS AND METHODS

Sample preparation

100 g of sky fruit seeds (each batch) after roasting by using convection oven (model Master 450, Garland, Freeland, Pennsylvania) at 200°C for 20 minutes, steaming at 100°C for 20 minutes, or microwaving by using micro oven (model Dimension 4, The Genius, National, Japan) at 2450 MHz for 15 minute were used to prepare the sky fruit seed oils.

Extraction of oil

The oils from untreated (control) and treated seeds were extracted by using a Bligh and Dyer method with a slight modification. 100 g of sky fruit seeds were mixed with solvent of methanol (200 ml) and chloroform (100 ml) in a ratio of 2 : 1. The mixture was finely grounded by using Waring blender (Waring Commercial, HGB2WTS3, USA). The mixtures were filtered to separate the seeds and solvent. Same amount of methanol and chloroform solvent were mixed filtered seeds. Next, the mixture was transferred into a blue capped bottle and agitated by using an incubator shaker (model Innova 40R, USA) at rpm 200 at 25°C for an hour. After the agitation process, the mixture was filtered to separate the seeds and solvent. The agitation and filtering process were conducted consistently until a clear solvent was obtained from the residual seeds. The mixture of solvent and oil was evaporated by using rotary evaporator (model N-1000, Eyela, Tokyo Rikakikai Co. Ltd., Tokyo, Japan) at 65°C until almost all solvents were evaporated. The oil obtained after evaporation was centrifuged (model Hettich Zentrifugen Universal 32, Germany) at rpm 4000 for 10 minutes. The supernatant of oil layer was stored at -20°C for further analysis (Yanty *et al*., 2008; modified by Chin, 2012).

Free fatty acid (FFA)

1 g of oil was dissolved with 25 ml diethyl ether with 25 ml alcohol and 1 ml 1 % phenolphthalein solution. The solution was titrated with aqueous 0.1 M of sodium hydroxide by shaking continuously until pink colour solution which persists for 15 seconds was formed. The amount of aqueous 0.1 M of sodium hydroxide used was recorded. The free fatty acid value was calculated as follows: Free fatty acid as oleic acid % = (volume of titration x normality of NaOH x 28.2)/weight of sample (AOCS, 1976).

Peroxide value (PV)

Peroxide value defined as the miliequivalents of active oxygen per kilogram of oil (meq O_2/kg) was determined. 1 g of oil was weighed in a 250 ml conical flask and dissolved with 10 ml of chloroform and 15 ml of acetic acid at a ratio of 2 : 3.1 ml of concentrated potassium iodide was added and kept in dark for 5 minutes. After 5 minutes, 75 ml of distilled water was added. Then, 1 ml of 2% starch indicator was added and the mixture was titrated with 0.01 N sodium thiosulphate till colorless end point. The blank sample was conducted without sample. The peroxide value was determined as [(volume of titration of sample – volume of titration of blank) x normality of $Na_2S_2O_3$ x 1000]/weight of oil (modified from Egan *et al*., 1990).

Free radical scavenging activity (DPPH)

1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical was used to measure the antioxidant activity of sky fruit seed oil. 3 ml of oil extract in methanol (300 mg/ml) was mixed with 3 ml of methanolic solution containing DPPH radicals (0.2 mM). The mixture was vortexed (model MS1 minishaker, IKA Works, Guangzhou) for 1 minute at 2000 rpm and allowed to stand for 30 minutes in the dark. After 30 minutes, the absorbance was read against a blank by using a spectrophotometer (model 4001/4, GENESYS 20 Thermospectronic, Thermo Electron Corp., USA) at 517 nm. Percentage inhibition activity (%) was calculated as [(absorbance of control–absorbance of sample) /absorbance of control] x 100%. α-tocopherol was used as reference compound and was found to exhibit 74.65 $\pm$ 2.13% inhibition of DPPH radical activity (modified from Marina *et al*., 2008).

Total phenolic content (TPC)

The total phenolic content in the methanolic extracts was determined by the Folin-Ciocalteu method by Singleton and Rossi. 2 g of oil was measured into a test tube and then 6.0 ml of methanol was added. The test tube was vortexed (model MS1 minishaker, IKA Works, Guangzhou) and then centrifuged (Hettich Zentrifugen Universal 32, Germany) at 3000 rpm for 10 minutes and the supernatant was collected. This procedure was repeated twice. All the three extractions were combined and the final volume was made up to 20 ml with methanol. 0.1 ml aliquot of methanolic extract was placed in a volumetric flask (10 ml). 0.5 ml of Folin-Ciocalteau was added. 3 minutes later, 1.5 ml of saturated sodium carbonate was added. The flask was filled with water up to 10 ml. After 2 hours reaction at room temperature, the absorbance was read at 765 nm by using a spectrophotometer (model 4001/4, GENESYS 20 Thermo-spectronic, Thermo Electron Corp., USA). Gallic acid was used as the standard in the range 0 to 500 mg/L (modified from Malacrida and Jorge, 2012). The total phenolic content of extract was calculated and expressed in gallic acid equivalent (mg GAE/g).

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Experimental design and data analysis

Experiments were arranged based on a completely randomized design with a one-way treatment structure. A one-way ANOVA with Tukey's Multiple Comparisons statistical analysis was applied on a triplicate data in order to determine any significant differences at at level of $p<0.05$ using a Minitab (Release 16) (Minitab Inc., USA) statistical software package.

RESULTS AND DISCUSSION

Seed oil yield

The yield of sky fruit seed oil with different pretreatments is shown in Table 1. The oil yield from microwaved sky fruit seed was 29.50% and significantly ($p<0.05$) different from roasting and steaming seed oils. Microwaving pretreatment showed the highest oil yield compared to roasting, steaming and untreated sky fruit seeds. Microwave heating vapourizes the water of the seed microstructure and increasing its interior pressure which leads to cell membrane rupture. Thus, efficiency of the extraction of oil from oil seeds was improved by enabling the passage of oil from the cell membrane (Uquiche *et al*., 2008). In a study of microwaved hazelnut seeds indicated that microwave pretreatment affected cell walls and membrane cell and obtained high extraction yield of oils compared to untreated hazelnut seeds (Uquiche *et al*., 2008). The rupture of cell membrane resulted in permanent pores which allowing the oil to move through the permeable cell walls (Azadmard-Damirchi *et al*., 2010). Oil yield of roasting pretreatment sky fruit seed was 24.82% which was lower than the oil yield from untreated seed (28.46%). However, previous studies showed that roasted cashew nut and roasted honeydew seed have high oil yield compared to untreated cashew nut and untreated honeydew seed (Chandrasekara and Shahidi, 2011; Chin, 2012). Steamed sky fruit seed showed the lower oil yield which was 23.29%. Similar trend also found in steamed honeydew seed. The oil yield of steamed honeydew seed was 14.97% lower than microwaved honeydew seed (18.85%) and roasted honeydew seed (21.59%). This was due to the increase of moisture in the seed during steaming which caused difficulty in filtration to separate the seed with solvent. Some of the oil was lost during extraction process (Chin, 2012).

Table 1. Yield of sky fruit seed oil with different seed pretreatments

Pretreatment	Yield (%)
Control	28.46 ± 0.88^{a}
Microwaving	29.50 ± 1.64^{a}
Roasting	24.82 ± 1.10^{b}
Steaming	23.29 ± 1.41^{b}

Values are mean ± standard deviation from three replications (n = 3)
Values with different superscripts letter are significantly different at $p<0.05$

Free fatty acid (FFA)

There was no significant different on free fatty acid between untreated fresh sky fruit seed oil with other sky fruit seed oils in different pretreatments. The free fatty acid content of extracted oils was in the range of 1.48 – 1.85% as shown in Table 2. The free fatty acid content in this study was higher than Chakrabarty and Chowdhuri's study (1956) which was 0.6%. Roasting and steaming pretreatments seed oil (1.63% and 1.85% respectively) showed higher free fatty acid compared to untreated fresh sky fruit seed oil (1.57%) and microwaving seed oil (1.48%). High value of free fatty acid in oil from roasted seed and steamed seed were due to greater liberation of free fatty acids affected by heat application (Oneya *et al*., 2005). Microwaving pretreatment sky fruit seed oil showed the lowest free fatty acid value (1.48%). It was due to inactivation of lipolytic enzyme by microwave heat (Thanonkaew *et al*., 2012). The lower the free fatty acid value, the better the storage and shelf life of the oil will be (Jafari *et al*., 2012).

Table 2. Free fatty acid of sky fruit seed oil with different seed pretreatments

Pretreatment	Free Fatty Acid (% in Oleic Acid)
Control	1.57 ± 0.17^{a}
Microwaving	1.48 ± 0.16^{a}
Roasting	1.63 ± 0.04^{a}
Steaming	1.85 ± 0.16^{a}

Values are mean ± standard deviation from three replications (n = 3)

Peroxide value (PV)

Table 3 depicts the peroxide value of sky fruit seed oil with different seed pretreatments. Peroxide value of steaming pretreatment sky fruit seed oil was 1.85 meq O_2/kg and significantly ($p<0.05$) different with untreated, roasting, and microwaving seed oils. Steaming pretreatment sky fruit seed oil has the lowest peroxide value which was 1.98 meq O_2/kg. The peroxide value of fresh steamed sky fruit seed oil was not much different with fresh steamed honeydew seed oil (1.93 meq O_2/kg) (Chin, 2012). Peroxide value for microwaving pretreatment sky fruit seed oil was 2.96 meq O_2/kg which higher than untreated seed oil (2.61 meq O_2/kg). The high peroxide value was due to the presence of reactive radicals that formed by exposure to microwaves (Uquiche *et al*., 2008). Roasting pretreatment sky fruit seed oil has the highest free fatty acid which was 3.25 meq O_2/kg. Previous study showed that roasted seed decreased the peroxide value due to the non enzymatic reaction and caramelization products formed during roasting. Maillard reaction products formed through the interaction of proteins and reducing sugars helps in reducing the peroxide value in roasted seed oil (Lee *et al*., 2004). However, the finding was in contrast with the present finding.

Table 3. Peroxide value of sky fruit seed oil with different seed pretreatments

Pretreatment	Peroxide Value (meq O_2/kg)
Control	2.61 ± 0.55^{ab}
Microwave	2.96 ± 0.03^{ab}
Roasting	3.25 ± 0.53^{a}
Steaming	1.98 ± 0.01^{b}

Values are mean ± standard deviation from three replications (n = 3)
Values with different superscripts letter are significantly different at $p<0.05$

Free radical scavenging activity (DPPH)

Results of the present study showed that percentage of inhibition activity of untreated sky fruit seed oil (87.69%) was significantly ($p<0.05$) higher than roasted sky fruit seed oil (75.71%) and microwaved sky fruit seed oil (63.98%) but not

significantly difference from steamed sky fruit seed oil (Table 4). The result revealed that heat treatment did not increase the percentage of inhibition activity in the oil. From previous study, roasted sesame seed oil has high antioxidant activity compared to raw sesame seed oil due to the formation of sesamol from the degradation of sesaminol (Jannat *et al*., 2010). However, sesamol does not present in the sky fruit seed.

Table 4. Inhibition of DPPH radical activity (%) of sky fruit seed oil with different seed pretreatments

Pretreatment	Inhibition of DPPH Radical Activity (%)
Control	87.69 ± 2.46[a]
Microwave	63.98 ± 2.75[c]
Roasting	75.71 ± 3.43[b]
Steaming	83.40 ± 2.09[a]

Values are mean ± standard deviation from three replications (n = 3)
Values with different superscripts letter are significantly different at p<0.05

Total phenolic content (TPC)

Table 5 shows the total phenolic content of sky fruit seed oil with different seed pretreatments. There was no significant different between untreated sky fruit seed oil with other sky fruit seed oils in terms of total phenolic content. Steaming pre-treatment on sky fruit seed oil achieved the highest total phenolic content with the value 0.022 mg GAE/g. Untreated sky fruit seed oil had a similar total phenolic content with microwaved sky fruit seed oil which was 0.017 mg GAE/g. Roasting pretreatment sky fruit seed oil had the lowest total phenolic content which only 0.016 mgGAE/g. The total phenolic content for roasting seed oil seemed to be not much different from the total phenolic content for microwaving and untreated seed oil. Phenolic compounds are desirable in edible oils, but only a small amount of phenolic compounds are transferred to the oil (Vujasinovic *et al*., 2012). Steaming pretreatment on sky fruit seed oil showed increased of total phenolic content compare to untreated seed oil. The increasing in total phenolic content might due to the result from the transformation of bound to free polyphenols by the heat treatment and the formation of high-molecular weight phenolic compounds or the production of new phenolic compounds (Bae *et al*., 2012).

Table 5. Total phenolic content of sky fruit seed oil with different seed pretreatments

Pretreatment	Total phenolic content (mg GAE/g)
Control	0.017 ± 0.004[a]
Microwave	0.017 ± 0.002[a]
Roasting	0.016 ± 0.001[a]
Steaming	0.022 ± 0.002[a]

Values are mean ± standard deviation from three replications (n = 3)

CONCLUSION

This study documented the influence of different pre-treatments namely roasting, steaming, and microwaving on chemical and antioxidant properties of sky fruit seed oils. Different pretreatments significantly affected yield, peroxide value and free radical scavenging activity of the extracted oils. However, no significant effect of pretreatments was reported on free fatty acid and total phenolic content. Eventhough steamed sky fruit seed produced low yield of oil, it desirably possessed low free fatty acid value and high inhibition activity towards free radical and high total phenolic content.

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