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Research paper

Characterization of Oil Extracted from Red Pitaya (*Hylocereus polyrhizus*) Seeds using Supercritical Fluid Extraction

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Abstract

Hylocereus polyrhizus or red pitaya is one of the commercially planted crops in southeast Asia for fruit. The seeds are disposed as a by-product in pitaya juice manufacturing. In this study, supercritical fluid extraction (SFE) was performed to obtain oil from red pitaya seeds. The chemical composition of extracted oil was identified by gas chromatography-mass spectrometry (GC-MS) analysis. The analysis revealed that the extracted oil consist mainly of polyunsaturated fatty acid (PUFA) with linoleic acid as its main constituents. The extracted oil was further analyzed by Fourier-transform infrared (FT-IR) spectroscopy, thermogravimetric analysis (TGA), differential scanning calorimetry (DSC) and tested for its antimicrobial activity to characterize the oil quality. In general, DSC and TGA analysis showed high thermal stability profile of red pitaya seed oil and antimicrobial activity test showed that the extracted oil exhibited slightly inhibitory effect against gram-positive bacteria named *Staphylococcus aureus*.

Keywords: *Characterization; Hylocereus polyrhizus; Red pitaya; Supercritical fluid extraction*

1. Introduction

Pitaya or pitahaya is a beautiful exotic fruit of cactus species of the genus *Hylocereus* that consists of climbing three-ribbed stems and aerial roots. Pitaya has been widely developed as industrial crops, especially in Asian countries such as Malaysia, Vietnam, Taiwan and Philipines. Locally, in southeast Asia, pitaya is more commonly known as 'dragon fruit'. Pitaya comes in a number of varieties; they can be distinguished to each other according to the color of the leathery, slightly leafy skin and flesh of the fruit which contains many tiny black seed. Three varieties of pitaya that have been grown commercially are *Hylocereus polyrhizus* (red-skinned fruit with red flesh), *Hylocereus undatus* (red-skinned fruit with white flesh) and *Hylocereus megalanthus* (yellow-skinned fruit with white flesh). Among all, *H. polyrhizus* or also called as red pitaya have recently drawn much attention from growers worldwide because of their economic value and potential health benefits [1]. The seeds of red pitaya are commonly disposed as a by-product in pitaya juice manufacturing [2]. Thus, the oil produced from this renewable resource could be utilised as a potential new source of speciality oil.

Various traditional methods of extraction described in the literatures, such as soxhlet extraction and pressurized solvent extraction. Each inherent disadvantages including long extraction time, large solvent requirement and high energy inputs [3]. On the other hand, supercritical fluid extraction (SFE) with carbon dioxide (CO_2) as a solvent is a new technology that may be utilized as an alternative to traditional methods. SFE have been recognized as an environmental-friendly process for oil extraction. The use of CO_2 as a solvent offer considerable advantages including non-toxic, chemically stable and cost efficient (available of high purity CO_2 solvent at low cost). SFE extraction produces solvent free extract with higher extraction rate (shorter extraction time) and enhance selectivity [4-7]. Supercritical CO_2 has suitable ability to extract the lipophilic substances such as essential oils from seeds due to its non-polar properties [8].

Furthermore, to our knowledge, there is no information available in current literature on the use of SFE for oil extraction from red pitaya seeds. In this study, the physicochemical properties of the SFE extracted red pitaya seed oil were determined and further characterize using gas chromatography mass spectrometry (GC-MS), Fourier-transform infrared (FT-IR) spectroscopy, thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC). Its antimicrobial activities of the extracted oil were also evaluated to characterize the oil quality. The properties of SFE extracted oil was analyzed and compared with the *n*-hexane extracted oil.

2. Materials and Methods

2.1 Materials

Red pitaya fruit was obtained locally from vicinity of Sepang, Malaysia. Red pitaya seeds were then manually separated from the red fleshed and pulp in the lab. The seeds were cleaned and washed under running water until all the flesh and pulp removed.

Seeds were then dried, crushed into smaller particles in a mill and kept in a dessicator until further analysis.
Liquefied CO2 of purity 99.9% was supplied by Poly Gas Sdn. Bhd. in pressurized deep tube cylinder. The entire standard chemical such as n-hexane with purity of 99.99%, ethanol, methanol and sodium chloride were purchased from Merck (Darmstadt, Germany), respectively. Standard solution of fatty acid methyl esters (FAMEs) FAME 37 Mix was obtained from Sigma (St. Louis, MO, USA). All other chemicals were of analytical grade.

2.2 Supercritical Fluid Extraction

SFE using CO2 as a solvent was carried out in a 60 mL extraction vessel using SFE system (OV-SCF) supplied by Taiwan Supercritical Technology Co., Ltd. 20 g of dried, ground red pitaya seeds were placed into the extraction vessel (4.5 cm internal diameter and 14.5 cm in height). CO2 was fed from a gas cylinder equipped with a cooler circulator to keep CO2 liquefied. The liquefied CO2 was pressurized at 4750 psi using an air-booster pump and fed into the vessel under heating temperature of 47ºC. The precision of temperature and pressure of the extraction system were ±0.5ºC and ±1 psi, respectively. The extracted oil was collected during extraction in glass vial and stored in a sealed dark container until needed for further analysis.

2.3 Solvent Extraction

A solvent extraction using n-hexane as a solvent was performed as a reference extraction in order to compare the results on the oil properties. 20 g of dried red pitaya seed was ground, soaked in the n-hexane and left overnight. The solution was then evaporated to discard the n-hexane from the oil in a rotary evaporator. In order to make sure all of the oil were extracted from the seeds, the processes were repeated three times.

2.4 Measurement of Physicochemical Properties

The specific gravity and refractive index were determined using a specific gravity bottle and refractometer at room temperature of 25ºC, respectively. Acid, iodine and saponification values were determined according to standard Test Methods modified from American Oil Chemist's Society (AOCS) as well as Malaysian Palm Oil Board (MPOB) Test Methods [9].

2.5 Analysis and Characterization

The composition of fatty acids in the red pitaya seeds oil (RPSO) was determined by means of GC-MS analysis using Agilent Technologies 7890A gas chromatograph coupled to Agilent Technologies 5975 mass spectrometer and equipped with HP-88 fused silica capillary column (100 m x 250 µm i.d; film thickness 0.25 µm; Agilent, USA). 1 µL aliquot of sample was injected into a split mode GC. The temperature of the oven was maintained at 150ºC for 5 min and then the temperature was increased to 240ºC with a ramping rate of 4ºC min-1 and held for 15 min. The temperature of injector and detector were 250ºC and 260ºC, respectively. Carrier gas, helium with total flow rate of 25 mL min-1 was utilized.
The structural analysis was performed using a Fourier-transform infrared (FT-IR) spectrophotometer, Perkin Elmer model 1725x with the wave number in the region of 4000 and 400 cm-1.
Thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) were used to study the thermal properties of the extracted oil. TGA (model TGA 7, Perkin-Elmer) were carried out in nitrogen atmosphere with flow rate of 20 mL min-1 and heating rate of 10ºC min-1 with operating temperature of 50ºC to 600ºC. On the other hand, the DSC (model DSC 822E, Mettler Toledo) measurements were carried out in nitrogen atmosphere and flow rate of 20 mL min-1 with the samples were subjected to the following temperature program: 70ºC isotherm for 1 min to eliminate the thermal history of the samples, then cooled at 5ºC min-1 to -40ºC. The samples were held at -40ºC isotherm and heated at 5ºC min-1 to reach 70ºC [10].
Antimicrobial test was carried out to determine the ability of RPSO in treating bacteria named Staphylococcus aureus. Sterile agar (at 45ºC) was poured into sterile petri dishes, which had been inoculated with the test organism. The test was carried out by placing 6 mm diameter of paper disc containing sample onto a plate which microbes are growing. The microbe culture was standardized to 0.5 McFarland standards which is approximately 10^8 cells. Streptomycin standard was used for bacteria. The plates were inverted and incubate at 30-37ºC for 18-24 hours, 24-48 hours or until sufficient growth has occurred. After incubation, each plate was examined. The diameters of the zones of complete inhibition were measured, including the diameter of the disc. Zones were measured to the nearest whole millimeter, using sliding calipers or a ruler, which was held on the back of the inverted petri plate.

3. Results and Discussion

3.1 Physicochemical Properties

The RPSO extracted by the SFE and n-hexane were characterized by measuring their iodine value, saponification value and acid value. Both extraction methods produced oils that were yellow in color, but the yellow color of SFE extracted oil was much lighter. Based on result listed in Table 1, the specific gravity and refractive index values showed no significant difference between the oil extracted by both methods. The iodine values obtained were relatively high (106.80 and 111.20 g of I2/100 g of oil) designating high percentages of unsaturated fatty acid (UFA) in the oils. Saponification value measures the chain length (molecular weight) of fatty acids in the oil. The saponification values of the extracted oil were 132.6 and 129.1 mg of KOH/g of oil showing that the oils consist mainly of unsaturated fatty acids. 3.00 and 2.42 of NaOH/g of oil were recorded for the acid values of oil extracted by SFE and n-hexane, respectively. These values were found within desirable limits for edible oil [11].

Table 1: Physicochemical properties of RPSO extracted by SFE and *n*-hexane.

Properties	SFE	*n*-Hexane
Specific gravity (kg/m^3) (25ºC)	0.857	0.858
Refractive index (25ºC)	1.467	1.467
Iodine value (g of I_2/100 g of oil)	106.8	111.2
Saponification value (mg of KOH/g of oil)	132.6	129.1
Acid value (mg of NaOH/g of oil)	3.00	2.42

3.2 Fatty acid Composition of Red Pitaya Seed Oil

The fatty acid (FA) compositions of RPSO are summarized in Table 2. The content of FA in RPSO (SFE) were compared with those RPSO obtained by solvent extraction using n-hexane. The principal FA recorded for both RPSO were palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2) and linolenic acid (C18:3). It was observed that in both RPSO, polyunsaturated fatty acids (PUFAs) were higher than monounsaturated fatty acids (MUFAs) and saturated fatty acids (SFAs). Both RPSO were characterized by the highest content of linoleic acid (SFE 50.87%, n-hexane 49.04%).

Table 2: The fatty acid (FA) composition of RPSO extracted by SFE and *n*-hexane.

Fatty acids (wt%)	SFE	*n*-Hexane
Palmitic acid (C16:0)	15.62	16.98
Stearic acid (C18:0)	4.30	7.71
Oleic acid (C18:1)	26.92	24.06
Linoleic acid (C18:2)	50.87	49.04
Linolenic acid (C18: 3)	1.12	1.11
Other	1.17	1.10
ΣSFA	19.92	24.69

ΣMUFA	26.92	24.06
ΣPUFA	51.99	50.15

Many health benefit properties of linoleic acid were recently investigated. Linoleic acid is not only an essential fatty acid (EFAs) but the only fatty acid that cannot be synthesized in the body [12]. EFAs are necessary for proper skin function. Deficiencies include epidermal hyper-proliferation, abnormal lipid barrier structure and function, and altered production of anti-inflammatory compounds; resulting in a plethora of skin problems including atopic eczema, acne and psoriasis [13]. The second major fatty acid present in the RPSO was oleic acid (SFE 26.92%, n-hexane 24.06%). Oleic acid strengthens the cell membrane integrity and helps in repairing cells and tissues damage [14]. From the data obtained, it was disclosed that there were no significant difference between the FA content of the RPSO obtained by SFE and n-hexane extraction. Since CO2 and n-hexane were both non-polar solvents, so they extracted the chemical compounds from the solid plant materials in a same way.

3.3 Fourier-Transform Infrared (FT-IR) Analysis

A typical FT-IR spectrum of the RPSO extracted by SFE and n-hexane were compared. As shown in Figure 1, the spectrum shows the characteristic absorption bands at 1750-1735 cm-1, which correspond to C=O absorption band of aliphatic esters. The presence of a strond band in the C-O stretching region, 1300-1100 cm-1 confirmed the presence of ester bond in a compound. This is due to the ester functional group that present in fatty acid of triacylglycerol (TAG) molecule. The absorption band of C-H in the 3000-2840, 1464-722 cm-1 region indicated that long chain of aliphatic ester compound was present. Overall, it was found that both the RPSO extracted by SFE and n-hexane gave similar FT-IR spectrum pattern, indicating the presence of similar functional groups in both oils.

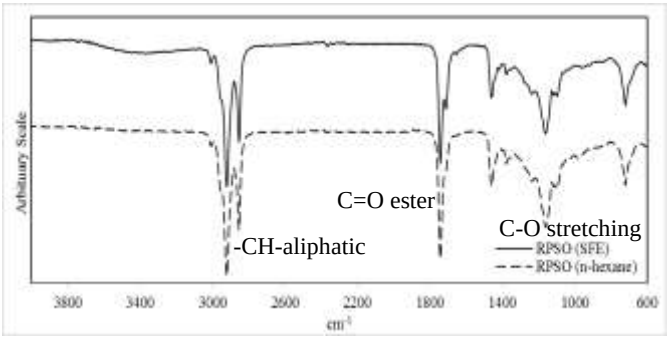

Figure 1: FT-IR spectrum of RPSO extracted by SFE and *n*-hexane.

3.4 Thermal Properties

The thermal properties of RPSO were estimated by TGA and DSC measurements. Figure 2 shows the TGA curves of RPSO extracted by SFE and n-hexane, respectively. High thermal stability profiles were found in both RPSO with decomposition temperature greater than 400ºC. The TGA data of RPSO extracted by SFE with decomposition of 423.74ºC was in agreement with RPSO extracted by n-hexane with decomposition temperature of 416.87ºC where at least 98% weight loss of the both RPSO occurred at only single step heating cycle due to the decomposition of the fatty compounds. Greater thermal stability of RPSO represent higher amount of long fatty acid chains, as well as degree of unsaturation. DSC is a suitable technique to characterize such phase transitions as crystallization and melting of vegetable oils that require the intake or release of thermal enthalpy. The DSC thermograms obtained upon cooling and heating are presented in Figure 3. Crystallization of oils was well known to be influenced by chemical composition and more interpretable than those obtained upon heating [15]. Starting the DSC analysis with cooling cycle for RPSO, there were two exothermic peaks were observed at 5.89ºC and -5.70ºC for RPSO (SFE), whereby only one exothermic peak was observed at -5.01ºC for RPSO (n-hexane). Every oil has own unique characteristic of fatty acids and TAG. One extra exothermic peak of RPSO (SFE) may be due to the presence of different amount of TAG compounds as compared to RPSO (n-hexane). As for heating cycle, two endothermic events were observed for both RPSO (SFE) (-7.36ºC and 17.47ºC) and RPSO (n-hexane) (-4.24ºC and 10.28ºC), with slight shifts of both peaks ascribable to slightly differences in fatty acid opposition in some samples.

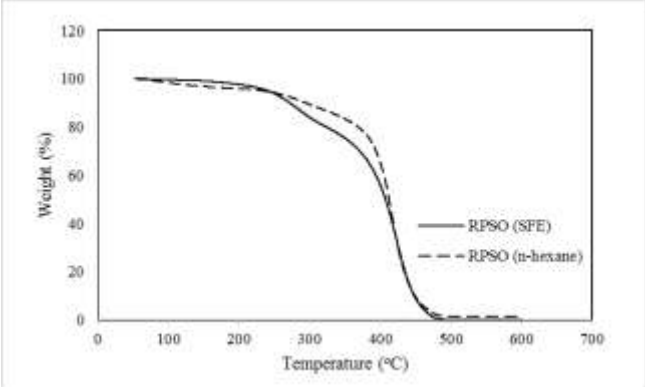

Figure 2: TGA curves of RPSO at a heating rate of 10ºC min^{-1}.

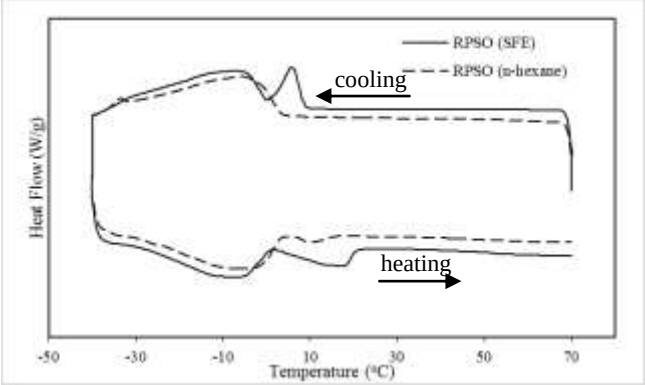

Figure 3: DSC thermograms of RPSO at a cooling rate and heating rate of 5ºC min^{-1}, respectively.

3.5 Antimicrobial Activities

Both RPSO extracted by SFE and n-hexane exhibited significant antibacteria activity against the gram positive bacteria under study, showing slightly inhibitory properties against Staphylococcus aureus, both with similar inhibition zone of 8 mm, respectively. Messaoud et al. (2012) suggests that the antimicrobial effect of plant extracts may be attributed to both their essential oils and phenolic compounds, possibly in combination, which are known to cause damage to cell membranes, causing leakage of cellular materials and ultimately the microorganism death [16]. S. aureus usually involved in skin infections, which means that RPSO has a potential use as a functional ingredient in topical products, protecting the skin against this microorganism.

4. Conclusion

In summary, results showed negligible difference in fatty acid composition for both oil extracted by SFE and n-hexane, respectively. The extracted oil was rich in unsaturated fatty acids mainly linoleic acid and oleic acid, which are related to oil health benefits. The RPSO showed high thermal stability and its ability to inhibit the growth of bacteria namely Staphylocccus aureus, indicating superior quality. As a by-product from the juice industry, the physicochemical properties and characteristics of RPSO showed

great potential for applications in the pharmaceutical, food and cosmetic industries.

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