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EXTRACTION OF RICE BRAN OIL USING SUBCRITICAL CARBON DIOXIDE SOXHLET

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By

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

EXTRACTION OF RICE BRAN OIL USING SUBCRITICAL CARBON DIOXIDE SOXHLET

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November 2015

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Faculty: Food Science and Technology

Rice bran has been proven that it contains high amount of bioactive compounds such as vitamin E and γ-oryzanol and is recognized as source of healthy oil. To preserve these compounds in the final product, a good extraction method is needed. The current extraction method used in the industry involving hazardous chemicals such as hexane, which is toxic to human and environment. On the other hand, the supercritical fluid extraction method involving high temperature (50 - 80 °C) and is a costly technology. A green extraction method that operates under lower temperature and lower cost is needed. The subcritical carbon dioxide Soxhlet (SCDS) extraction system, which operates at low temperature (28 ± 1 °C), had used to extract rice bran oil in this study. Three samples of rice bran have been used: steam, hot-air stabilization and unstabilized rice bran (control). The extraction yields, contents of tocopherols, tocotrienols and oryzanol, fatty acid profiles; and the oxidative stabilities of the extracted rice bran oils were analyzed. The yields using hexane and SCDS extraction were approximately 22 % and 13 – 14.5 %, respectively. However, the oil extracted using the SCDS system contained approximately 10 times higher oryzanol and tocols compounds and lower free fatty acid levels and peroxide values compared with the hexane-extracted oil. While the steam stabilized rice bran gave the best oil quality relative to the others two samples. The steamed stabilized rice bran was chosen to be extracted using supercritical carbon dioxide extraction (SC-CO_2) at 350 bar and 40 °C. The percentage oil yield extracted using SC-CO_2 was 16.25 % in 200 minutes extraction time. However, the bioactive compounds in SC-CO_2 extracted oil was even lower than hexane extracted oil. SCDS is a relatively new process, understanding and modeling of the extraction behavior is necessary. The extraction kinetics of rice bran oil and its bioactive compounds, namely, α-, β-, γ-, and δ-tocopherol; α-, γ-, and δ-tocotrienol; and γ-oryzanol, are discussed and correlated using the diffusion model (DM), logistic model (LM) and simple single plate model (SSPM). The extraction rate of the oil and active compounds was halved after 7 hours. After 15 hours of extraction, up to 95 % of the extractable active compounds were obtained. The SSPM is recommended for

predicting the oil yield and oryzanol while LM is suitable to predict the tocopherols and tocotrienols in rice bran oil. In short, SCDS is a good extraction method, especially for the thermal labile compounds.

Abstrak tesis yang dikemukaan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Sarjana Sains

PENGEKSTRAKAN MINYAK DEDAK PADI DENGAN MENGGUNAKAN SOXHLET SUBGENTING KARBON DIOKSIDA

Oleh

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Dedak padi mengandungi sebatian bioaktif seperti vitamin E dan γ-oryzanol yang tinggi dan dikenali sebagai sumber minyak yang berkhasiat. Sebuah kaedah pengekstrakan yang baik diperlukan bagi mengekalkan sebatian berkenaan dalam hasil akhir. Kaedah pengekstrakan semasa yang digunakan dalam industri melibatkan bahan kimia berbahaya seperti heksana, yang mendatangkan toksik kepada manusia dan alam sekitar. Manakala, kaedah pengekstrakan supergenting cecair melibatkan suhu yang tinggi (50 - 80 °C) dan memerlukan penggunaan teknologi yang tinggi. Sebuah kaedah pengekstrakan mesra alam sekitar yang beroperasi di bawah suhu dan kos yang lebih rendah amat diperlukan. Sistem pengekstrakan Soxhlet Subgenting Karbon Dioksida (SCDS), yang beroperasi pada suhu rendah (28 ± 1 °C), telah digunakan untuk mengekstrak minyak dedak padi dalam kajian ini. Tiga sampel dedak padi telah digunakan: dedak padi yang distabilkan dengan stim, dedak padi yang distabilkan dengan udara panas dan dedak padi yang tidak distabilkan (kawalan). Hasil pengekstrakan, kandungan tokoferol, tokotrienol dan γ-oryzanol, profil asid lemak dan kestabilan oksidatif daripada minyak dedak padi telah dianalisa. Hasil pengekstrakan menggunakan heksana dan sistem pengekstrakan SCDS adalah kira-kira 22% dan 13-14.5% masing-masing. Walau bagaimanapun, minyak yang diekstrak dengan sistem SCDS mengandungi kira-kira 10 kali lebih tinggi kandungan sebatian bioaktif, serta tahap asid lemak bebas dan nilai peroksida yang lebih rendah berbanding minyak yang diekstrak dengan heksana. Selain itu, dedak padi yang distabilkan dengan stim menghasilkan minyak dengan kualiti yang terbaik berbanding dua sampel yang lain. Dedak padi yang distabilkan dengan stim dipilih untuk diekstrak menggunakan pengekstrakan supergenting karbon dioksida (SC-CO_2) pada 350 bar dan 40 °C. Peratusan hasil minyak diekstrak menggunakan SC-CO_2 adalah 16.25% dalam 200 minit masa pengekstrakan. Namun, sebatian bioaktif dalam minyak yang diekstrak dengan menggunakan SC-CO_2 adalah lebih rendah berbanding minyak yang diekstrak dengan heksana. SCDS adalah satu proses pengekstrakan yang baru, pemahaman dan pemodelan terhadap tingkah laku pengekstrakan berkenaan adalah diperlukan. Kinetik pengekstrakan dan sebatian bioaktif minyak dedak padi, iaitu, α-, β-, γ-, dan δ-tokoferol;

α-, γ-, dan δ-tokotrienol; dan γ-oryzanol, telah dibincangkan dan dikolerasikan menggunakan model resapan (DM), model logistik (LM) dan model plat mudah tunggal (SSPM). Kadar pengekstrakan minyak dan sebatian bioaktif adalah separuh selepas 7 jam. Selepas 15 jam pengekstrakan, hampir 95% daripada sebatian bioaktif yang boleh diekstrak telah diperolehi. SSPM adalah disyorkan untuk meramal tingkah laku hasil minyak dan oryzanol manakala LM adalah sesuai bagi meramal kandungan tokoferol dan tokotrienol dalam minyak dedak padi. Pendek kata, SCDS adalah kaedah pengekstrakan yang baik, terutamanya bagi sebatian labil haba.

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I certify that a Thesis Examination Committee has met on 2 November 2015 to conduct the final examination of Chia Siew Lian on her thesis entitled "Extraction of rice bran oil using subcritical carbon dioxide Soxhlet" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
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LIST OF ABBREVIATIONS

b	Parameter in logistic model
B	Volume of sodium thiosulfate added into blank
D	Diffusion coefficient of the solute in the sphere
D_m	Matrix diffusivity
E	Extractable degree
f_i	Modelled value
FLD	Fluorescence detector
h	Position of bed
H	Height of the bed
mEq	Milliequivalent
q	Concentration of the solute remaining in the sphere
q_o	Initial concentration of the extractable material
r	Radius of the sphere
t	Time
T_c	Critical temperature
t_m	Time where maximum extraction rate achieved
v/v	Volume per volume
w/v	Weight per volume
x_o	Concentration of the targeted species
Y	Extraction yield
δ	Thickness of the particles in the plate-shaped
$\bar{y}$	Mean of experimental value

CHAPTER 1

INTRODUCTION

1.1 Background

The global production of rice (*Oryza sativa*) in 2014 was 674.4 million tons (FAO, 2014), leading to about 67.44 million tons of rice bran as it contributes to approximately 10 % of the weight of rice (Sharif et al., 2014). Rice bran has been considered as a by-product in rice processing and commonly used as animal feed or discarded as waste, especially in Malaysia. In fact, rice bran contains rich amount of unsaponifiable, vitamins and nutraceutical compounds. Its oil also contains high valuable compounds such as tocopherol, tocotrienol and oryzanol, which exhibit anticancer, anti-inflammation, and cholesterol-lowering properties (Muhammad et al., 2013; Murase & Iishima, 1963; Nakayama et al., 1987; Patel & Naik, 2004; Rong et al., 1997; Sen et al., 2006). Hence, rice bran not only can be utilized as an alternative source of functional food, its oil has immense potential in the high-end nutraceutical, pharmaceutical and cosmeceutical industries (Patel & Naik, 2004).

Rice bran is naturally unstable. The lipase content hydrolyzes the lipid in the bran into glycerol and free fatty acid, resulted in off-flavor, rancidity, degradation of nutritional values and changes in functional properties (Malekian et al., 2000; Thanonkaew et al., 2012). In addition, the lipoxygenase and peroxidase in the brans also lead to an increase in the peroxide and thiobarbituric acid value but a decrease in the iodine value (Orthoefer, 2005). Consequently, inactivation of the enzymes is vital to warrant the high commercial value of rice bran. Heat stabilization, by either wet or dry heating, has been one of the common methods (Orthoefer, 2005). The different stabilization methods produce varied quality of the rice bran oil.

Only after stabilization process, will the rice bran be subjected to extraction process to obtain its oil. At present, rice bran oil extraction has been conducted via various methods, such as solvent extraction, supercritical fluid extraction, cold-press and subcritical water extraction (Kuk & Dowd, 1998; Lai et al., 2009; Pourali, Salak Asghari, et al., 2009; Shen et al., 1996; Thanonkaew et al., 2012). The most commonly employed method in the industry is solvent extraction using hexane. This could be attributed to its low cost, high solvation property and simpler operation procedure (Wang & Weller, 2006). However, hexane is not environmental friendly; its residual is dangerous to human health (Wang & Weller, 2006). Having considered the adverse health effect brought by the solvent, researchers have proposed using green technology such as supercritical fluid extraction (SFE). The quality of rice bran oil extracted using SFE was found better than that from hexane extraction. However, the setup cost is high and the extraction process involves relatively high temperature and pressure. As SFE may sometime be operated up to 80 ºC, degradation of heat-sensitive compounds is expected.

In order to preserve the heat sensitive compounds, extraction need to be done under lower temperature and pressure. In subcritical carbon dioxide extraction, the carbon

dioxide exists in the liquid-vapor equilibrium condition and is very close to the critical point. Subcritical CO_2 has been used in floral fragrance extraction from *Quisqualis indica* to produce organoleptically superior end product (Rout et al., 2008). It has also been applied in various fruits and plants extraction such as nutmeg, chaste tree fruits, celery, thyme, chili, clove, turmeric and ginger (Mele et al., 2013; Naik et al., 1989; Spricigo et al., 1999). These studies have shown that liquid CO_2 is able to produce extracts with higher retention of volatile and thermally labile compounds compared to supercritical CO_2 (SC-CO_2) and solvent extraction method.

In this study, subcritical carbon dioxide Soxhlet (SCDS) is proposed to extract rice bran oil. The main advantage of this technique is that the entire extraction process is operated at a lower temperature (28±1 °C) and pressure (68- 70 bar) relative to SFE. The rice bran is stabilized by steam and drying heating methods. The performance of the extraction and stabilization methods is assessed by the level of oxidative stability of the extracted rice bran oil and the components in the oil such as tocopherol, tocotrienol, oryzanol contents, and the fatty acid profile. The hexane and supercritical carbon dioxide extraction methods are used as the controls. The extraction kinetics of the rice bran oil and the oil composition in the SCDS extraction are then modeled using the semi-empirical mathematical equations. The most suitable modeling equation is also proposed.

1.2 Objectives

Overall, the research is undertaken with the aim to produce high quality rice bran oil using SCDS method. In particular, this study attempts

1. To investigate the effect of rice bran stabilization methods and extraction methods on yield and chemical composition of rice bran oil.
2. To correlate the extraction kinetics of oil yield, tocopherols, tocotrienols and γ-oryzanol of the oxidatively stable rice bran oil containing the greatest amount of bioactive compounds into semi-empirical equations.

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