UNIVERSITI PUTRA MALAYSIA

EXTRACTION OF γ-ORYZANOL AND TOCOPHEROLS FROM RICE (*Oryza sativa*) BRAN

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By

ROSNIZAM ISMAIL

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of Requirements for the Degree of Master of Science

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Especially dedicated to……..

Emak….in memory, may Allah bless her wherever she is,
abah, family,
friends and whoever has blown up my spirit..

…thank you for the guidance, encouragement, and love given throughout my life…..

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

EXTRACTION OF γ-ORYZANOL AND TOCOPHEROLS FROM RICE *(Oryza sativa)* BRAN

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July 2008

Chairman : Associate Professor Azizah Abdul Hamid, PhD

Faculty : Food Science and Technology

The objective of this study was to extract γ-oryzanol and tocopherols from rice bran. Different extraction times and sequential extractions utilizing different solvents on γ-oryzanol and tocopherols levels from crude rice bran oil (CRBO) were studied. Rice bran was extracted using hexane with five different extraction times, while sequential extractions was carried out utilizing hexane, hexane/dichloromethane, dichloromethane and dichloromethane/methanol. Extraction of γ-oryzanol and tocopherols was further studied using precipitation and liquid-liquid separation from unsaponified fraction of CRBO. Precipitation was carried out by diluting the sample in hexane overnight at -20˚C. Liquid-liquid separations were carried out using hexane-methanol or hexane-acetonitrile. Solid and liquid portions formed from precipitation were further separated by liquid-liquid separations. The final stage of the extraction process was a combination of precipitation and single-stage chromatographic

separation. Results showed that percentage of extracted oil was not significantly different with the different extraction times used. However, 20 min extraction resulted in the highest γ-oryzanol level ($p<0.05$). On the other hand, 30 min extraction gave the highest tocopherols level ($p<0.05$). Sequential extractions resulted in higher γ-oryzanol and tocopherols levels than that of hexane extraction alone ($p<0.05$). After saponification of CRBO extraction (with hexane, hexane/dichoromethane and dichloromethane), the unsaponified fraction was found to contain 1.4% γ-oryzanol and 0.02% tocopherols. In liquid-liquid separations on unsaponified fraction of CRBO study, the process was able to concentrate 2.1% γ-oryzanol in methanol and 1.8% in acetonitrile, respectively. On the other hand, 0.03% tocopherols was concentrated in methanol and 0.04% in acetonitrile, respectively. In precipitation study, 4.04% γ-oryzanol was concentrated in solid portion. In column chromatography study, combining precipitation and single stage silica chromatography was able to concentrate 10.99% γ-oryzanol and 0.02% tocopherols. This work proposed an alternative method for γ-oryzanol and tocopherols extraction from rice bran.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains.

PENGEKSTRAKAN γ-ORYZANOL DAN TOKOFEROLS DARIPADA DEDAK BERAS *(Oryza **sativa**)*

Oleh

ROSNIZAM ISMAIL

Julai 2008

Pengerusi : Profesor Madya Azizah Abdul Hamid, PhD

Fakulti : Sains dan Teknologi Makanan

Kajian ini dijalankan dengan objektif untuk mengekstrak γ-orizanol dan tokoferol daripada dedak beras. Perbandingan dalam masa pengekstrakan dan pengekstrakan secara sekuel menggunakan pelarut yang berbeza telah dikaji ke atas jumlah γ-orizanol dan tokoferol daripada minyak dedak beras. Dedak beras yang telah distabilkan diekstrak dengan heksana dengan lima masa pengekstrakan yang berbeza. Manakala pengekstrakan sekuel dijalankan dengan menggunakan heksana, heksana/diklorometana, diklorometana dan diklorometana/metanol. Kajian pra-konsentrasi bagi γ-orizanol dan tokoferol selanjutnya telah dikaji dengan kaedah pemendakan dan kaedah pemisahan fasa cecair-cecair daripada bahagian tidak-tersaponifikasi (sebagai sampel) minyak dedak beras. Kaedah pemendakan telah dijalankan dengan mencampurkan sampel dalam heksana dan dibiarkan semalaman pada suhu -20˚C. Manakala kaedah pemisahan fasa cecair-cecair dijalankan dengan mencampurkan sampel dalam heksana-metanol atau heksana-asetonitril. Bahagian

pepejal dan cecair yang terbentuk daripada kaedah pemendakan telah selanjutnya dikaji dengan kaedah pemisahan fasa cecair-cecair. Bahagian terakhir kajian pengekstrakan bahan ini dijalankan dengan gabungan kaedah pemendakan dan kaedah pemisahan kromatografi turus. Keputusan telah menunjukkan peratusan minyak dedak beras yang terekstrak tidak berbeza secara signifikan dengan masa pengekstrakan yang berbeza. Walau bagaimanapun, pengekstrakan selama 20 minit telah memberi jumlah γ-orizanol yang tertinggi daripada masa pengekstrakan yang lain ($p<0.05$). Bagi tokoferol, pengekstrakan selama 30 minit memberi jumlah tertinggi bagi bahan tersebut ($p<0.05$). Pengekstrakan sekuel memberi jumlah lebih tinggi bagi kedua-dua γ-orizanol dan tokoferol daripada pengekstrakan dengan mengunakan heksana sahaja ($p<0.05$). Bahagian tidak-tersaponifikasi minyak dedak beras yang diekstrak dengan menggunakan tiga peringkat pengekstrakan sekuel mengandungi 1.4% γ-orizanol dan 0.02% tokoferol. Dalam kajian pra-konsentrasi γ-orizanol dan tokoferol, kaedah pemisahan fasa cecair-cecair ke atas bahagian tidak-tersaponifikasi minyak dedak beras telah mengkonsentrasikan γ-orizanol kepada 2.1% dalam metanol dan 1.8% dalam asetonitril ($p<0.05$). Tokoferol telah dikonsentrasikan kepada 0.03% dalam metanol dan 0.04% dalam asetonitril ($p<0.05$). Dalam kajian pemendakan, γ-orizanol telah dikonsentrasikan kepada 4.04% iaitu dalam fasa pepejal. Pemisahan fasa cecair-cecair ke atas fasa pepejal (daripada pemendakan) menunjukkan γ-orizanol lebih tinggi berada dalam methanol, sebaliknya, tokoferol berada lebih banyak dalam asetonitril. Dalam kajian pemisahan kromatografi turus, gabungan kaedah pemendakan dan kromatografi turus silica mengkonsentrasikan γ-orizanol kepada

10.99% dan tokoferol kepada 0.02%. Kajian ini mencadangkan satu kaedah alternatif dalam pengekstrakan γ-orizanol dan tokoferol daripada dedak beras.

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I certify that an Examination Committee met on 1st of July 2008 to conduct the final examination of Rosnizam Ismail on his Master of Science Thesis entitled "Extraction of γ-oryzanol and tocopherols from rice (*Oryza sativa*) bran" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirements for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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Date: 16 Oktober 2008

DECLARATION

I declare that the thesis is based on my original work except for quotations and citations, which have been duly acknowledged. I also declare that is not been previously and is not concurrently submitted for any degree at UPM or at any other institution.

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Rosnizam Ismail

Date:

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LIST OF ABBREVIATIONS

°C	Degree Celsius
GC/MS	Gas chromatography/ mass spectroscopy
HPLC	High pressure liquid chromatography
IU	International Unit
TE	Tocopherol Equivalent
ROS	Reactive oxygen species
CVD	Cardiovascular disease
LDL	Low-density lipoprotein
HDL	High-density lipoprotein
CRBO	Crude rice bran oil
NaOH	Sodium hydroxide
BHT	Butylated hydroxytoluene
mg	Milligram
g	Gram
kg	Kilogram
ppm	Part per million
ml	Milliliter
MHz	Mega Hertz
min	minutes
µm	Micrometer
mm	Millimeter

nm	Nanometer
i.d.	Internal diameter
UV/Vis	Ultraviolet/visible
UV	Ultraviolet
SAS	Statistical Analysis System
MeOH	Methanol
$MeCl_2$	Dichloromethane
rpm	Revolution per minute
mol. wt	Molecular weight
$CaCl_2$	Calcium chloride
atm	atmosphere
P’	empirical solvent polarity

CHAPTER 1

INTRODUCTION

Rice (*Oryza sativa*) bran has gained much interest in recent years because it serves as an excellent source of beneficial γ-oryzanol, tocopherols and tocotrienols (Da Silva *et al.*, 2006; Iqbal *et al.*, 2005; Jennings and Akoh, 2000; Dunford and King, 2000). It has been viewed as a potential functional food and commercial source for use as food additive, pharmaceutical and in cosmetic product (Parrado *et al*., 2006; Lloyd *et al.*, 2000). As an under-utilized by-product from paddy-milling, rice bran is potentially an inexpensive raw material. The obstacle hindering the use of rice bran for human consumption is its instability and rapid spoilage.

Rice bran represents 10% of paddy (rough rice) weight fraction and reported to contain up to 17% protein, 23% lipid, 27% dietary fibre, vitamins (thiamine, riboflavin, niacin, tocopherols and tocotrienols), minerals (calcium, phosphorus and magnesium) and other bioactive compounds, in particular, γ-oryzanol (Parrado *et al*., 2006; Da Silva *et al.*, 2006; Shin *et al.*, 1997, Salunkhe *et al.*, 1992; Saunders, 1990; Fedeli, 1983).

Up to 28%, crude rice bran oil (CRBO) can be extracted from bran, making rice bran oil the richest oil from cereal (Da Silva *et al.*, 2006). Several countries including Japan and India used the oil as speciality oil (Saunders, 1990; Prabhakar *et al.*, 1986; Bhattacharyya *et al.*, 1983). Furthermore, CRBO is one of the nature's richest sources

of potent antioxidant including tocopherols, tocotrienols and γ-oryzanol, which are unique compared to other plants oil (Da Silva *et al.*, 2006; Lloyd *et al.*, 2000; Jennings *et al.*, 2000; Das *et al.*, 1998). These compounds in CRBO are believed to be responsible for hypocholesterolemic effect and reduction of aortic fatty streak (Pszczola, 2001; Kahlon *et al.*, 1996; Rogers *et al.*, 1993).

γ-oryzanol has been shown to be a potent antioxidant and inhibits cholesterol oxidation *in vitro* better than that of tocopherols and tocotrienols. 24-methylene cycloartanyl ferulate exhibited the highest cholesterol antioxidation compared to other γ-oryzanol members in rice bran oil (Xu *et al.*, 2001). It was reported that γ-oryzanol exhibited hypocholesterolemic action, decreased cholesterol absorption and plasma cholesterol level (Wilson *et al*, 2007; Vissers *et al.*, 2000). In technological uses, the compound contributed to the rice bran oil stabilization at frying temperature and use as special cosmetic ingredient (Miller *et al.*, 2003).

Tocopherols is well known as an active free radical and reactive singlet oxygen scavenger that prevent peroxidation of lipid either *in vivo* or *in vitro* systems. Tocopherols has been repeatedly shown to have anticancer and anti-thrombosis properties; able to reduce cardiovascular disease (CVD) and offer protection against coronary heart disease by arresting free radical damages (Sylvester, 2002; Pszczola, 2001; Cicero and Gaddi, 2001; Eintenmiller and Landen, 1999). Both γ-oryzanol and tocopherols are liposoluble compounds that are extracted in CRBO.

Various methods were used to separate or isolate both γ-oryzanol and tocopherols either from rice bran or other samples. Extraction methods used commonly involved solvent extraction followed by various concentration techniques such as adsorption column chromatography, preparative HPLC, chemical separation (such as esterification and transesterification and aminoalkylation), hydrogenation, crystallization, molecular distillation, supercritical extraction and others. Some methods involved sophisticated and expensive equipment and have certain drawbacks (Abidi, 2001; Sumner Jr. *et al.*, 2001; Akihisa *et al.*, 2000; Qureshi *et al.*, 2000; Xu *et al.*, 1999; Cheruckuri *et al.*, 1999; Das *et al.*, 1998; Hunt, 1997; Fizet, 1996; Baldwin *et al.*, 1990; Takagi *et al.*, 1984).

Column chromatography was reported to be effective in separating desired compounds. Selective separation of the compounds of interest from rice bran may involve several complex and intensive unit operations. Beside, many procedures were time demanding procedures which enhance oxidation compounds and thermal degradation of the target compounds (Dunford *et al.*, 2003; Guiochon and Lin, 2003; Diack *et al.*, 1994; Dondi and Guiochon, 1992).