



## **UNIVERSITI PUTRA MALAYSIA**

CHEMICAL COMPOSITION OF STABILIZED RICE BRAN MILLING FRACTIONS AND CHARACTERIZATION OF THEIR FERULIC ACID RICH EXTRACTS

**RAJA ROHAYA BT. RAJA SULAIMAN** 

**FSTM 2010 7** 



# CHEMICAL COMPOSITION OF STABILIZED RICE BRAN MILLING FRACTIONS AND CHARACTERIZATION OF THEIR FERULIC ACID RICH EXTRACTS

# RAJA ROHAYA BT. RAJA SULAIMAN

# MASTER OF SCIENCE UNIVERSITI PUTRA MALAYSIA

2010



# CHEMICAL COMPOSITION OF STABILIZED RICE BRAN MILLING FRACTIONS AND CHARACTERIZATION OF THEIR FERULIC ACID RICH EXTRACTS

By

# RAJA ROHAYA BT. RAJA SULAIMAN

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

UPM

# **DEDICATION**



To my dearest
loving hubby (for the unbreakable patience and understanding), who would
have loved to see this thesis completed.
For my beloved
Che and Ayah (for the strength and courage), who have devoted themselves
for their children well-beings.
To Kak Inie, Kak Nora, Adique, Imie, Irie, Idie and Idenand also my other
family members and in-laws
Thank you
Thank you
Thank you
Lastly, but not the leastto my precious Nik Muhammad Nafiz Najwan
You are the best gift yet the toughest test



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

CHEMICAL COMPOSITION OF STABILIZED RICE BRAN MILLING FRACTIONS AND CHARACTERIZATION OF THEIR FERULIC ACID RICH EXTRACTS

By

RAJA ROHAYA BINTI RAJA SULAIMAN

September 2009

Chairman: Dr Azizah Hj. Abd. Hamid, PhD

Faculty : Food Science and Technology

The research was carried out to study the characteristics of local stabilized rice bran. Four rice bran-milling fractions, after stabilized by microwave heating, were analyzed for their chemical composition. Its potential as a source of phenolic antioxidants was investigated by assessing the Total Phenolic Compounds (TPC) by spectrophotometry and the effect of stabilization. The fraction with the highest yield of TPC was subjected to High Performance Liquid Chromatography (HPLC) analysis for phenolic compounds profiling. The yield and selectivity of different extraction solvents for phenolic compounds analysis were investigated. Phenolic acid content of the crude extract of the 1<sup>st</sup> Rice Bran Milling Fraction which fractionated by Sephadex LH-20 column chromatography was determined by HPLC. The antioxidant capacity of isolated rice bran extracts were assayed by Ferric Thiocyanate (FTC) and Thiobarbituric Acid Reactive Substances (TBARS). Results showed that all milling fractions especially the 1<sup>st</sup> fraction were rich in fat, protein, carbohydrates and total dietary fibre (TDF). The TPC found in rice bran in



decreasing order was: Fraction 1 > Fraction 3 > Fraction 2 > Fraction 4. The TPC of all fractions were detected at 257-488 mg ferulic acid equivalent/kg. The stabilization gave no losses of phenolic compounds in all bran fractions studied except for Fraction 3. The efficiency of various solvent to extract phenolic acids from rice bran in decreasing order was: 1 % acidified water > ethanol > acetone  $\approx$ methanol > ethyl acetate. The 1<sup>st</sup> rice bran milling fraction contained highest of ferulic acid, followed by p-coumaric, caffeic acid and sinapic acid. Rice bran was found to be potentially a source of phenolic compounds particularly the 1<sup>st</sup> milling fraction. The amount of TPC in fractionated extracts (Fraction A – D) was about 6folds higher than that in stabilized rice bran. Fraction C and D contained higher ferulic acid at 831.51 and 841.54 ppm; respectively, may ideally serve as the ferulic acid-rich extracts. FTC and TBARS methods showed Fraction A - D possessed significantly higher inhibition activities than that of  $\alpha$ -tocopherol. Thus, the fractionation employed was efficient in obtaining the ferulic acid rich-extracts with comparable antioxidative activities. Apart from increasing the utilizations of local raw materials, the first rice bran fraction was found to be an excellent source of energy, fat, mineral and could be a very good source of dietary fiber and other nutrients especially many health-promoting components such as ferulic acid. Therefore, the rice bran fraction can be a potential natural source of antioxidants or as a value-added product to variety of functional foods.

*Keywords:* rice bran, stabilization, proximate composition, total phenolic compounds (TPC), fractionation, ferulic acid-rich extract, FTC, TBARS, antioxidant activity.



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

KOMPOSISI KIMIA BAHAGIAN KISARAN DEDAK BERAS YANG DISTABILKAN DAN PENCIRIAN EKSTRAK YANG KAYA ASID **FERULIK** 

Oleh

RAJA ROHAYA BINTI RAJA SULAIMAN

September 2009

Pengerusi: Dr Azizah Hj. Abd. Hamid, PhD

Fakulti

: Sains Makanan dan Teknologi

Penyelidikan ini dijalankan bagi mengkaji ciri-ciri dedak beras tempatan yang distabilkan. Empat fraksi kisaran dedak beras, setelah distabilkan melalui pemanasan mikrogelombang, dianalisis bagi komposisi kimia mereka. Potensi sebagai sumber antiopengoksida fenolik dikaji melalui penentuan Jumlah Sebatian Fenolik (JSF) dengan kaedah spektrofotometer serta kesan ke atas penstabilan. Fraksi dengan hasil JSF yang tertinggi dianalisis dengan Kromatografi Cecair Prestasi Tinggi (KCPT) bagi sebatian fenolik. Hasilan dan kecenderungan pelbagai pelarut pengekstrakan ke atas analisis sebatian fenolik telah diselidik. Kandungan asid fenolik bagi ekstrak kasar fraksi kisaran-pertama dedak beras yang telah diperingkatkan melalui kromatografi turus Sephadex LH-20 telah ditentukan KCPT. Kaedah FTC dan TBARS telah digunakan untuk mengkaji keupayaan antipengoksida ekstrak dedak beras. Keputusan menunjukkan semua fraksi kisaran terutama fraksi pertama adalah kaya lemak, protein, karbohidrat dan Jumlah Serabut Diet (JSD). JSF dedak beras yang ditemui mengikut turutan menurun: Fraksi 1 > Fraksi 3 > Fraksi 2 > Fraksi 4.



JSF kesemua fraksi dikesan sebanyak 257 - 488 mg bersamaan asid ferulik /kg.

Penstabilan itu tidak menyebabkan kehilangan sebatian fenolik dalam semua fraksi dedak kecuali Fraksi 3. Kecekapan pelbagai pelarut untuk mengekstrak asid fenolik daripada dedak beras dalam turutan menurun adalah: air berasid 1 % > etanol > asiton ≈ metanol > etil asetat. Fraksi kisaran-pertama dedak beras mengandungi asid ferulik yang tertinggi, diikuti oleh asid p-kumarik, asid kafeik, dan asid sinapik. Dedak beras didapati berpotensi sebagai sumber sebatian-sebatian fenolik terutama Fraksi kisaran-pertama. Amaun JSF ekstrak terperingkat (Fraksi A - D) adalah 6 kali lebih tinggi daripada dedak yang distabilkan. Fraksi C dan D mengandungi lebih tinggi asid ferulik iaitu 831.51 dan 841.54 ppm, adalah sesuai sebagai ekstrak kayaasid ferulik. Kaedah FTC dan TBARS menunjukkan Fraksi A - D mempunyai aktiviti perencatan lebih tinggi dan signifikan daripada α-tocopherol. Oleh itu, pemeringkatan yang digunakan adalah berkesan dalam mendapatkan ekstrak kayaasid ferulik dengan aktiviti antipengoksida yang setanding. Selain meningkatkan penggunaan bahan-bahan mentah tempatan, fraksi pertama dedak beras didapati sebagai sumber terbaik tenaga, lemak, mineral dan mungkin satu sumber serat pemakanan yang amat bagus dan nutrien lain terutama banyak komponen-komponen yang meningkatkan kesihatan seperti asid ferulik. Oleh itu, fraksi dedak beras ini mungkin berpotensi sebagai satu sumber antipengoksida semulajadi atau sebagai satu nilai tambah produk untuk pelbagai jenis makanan berfungsi.

*Katakunci:* dedak beras, penstabilan, komposisi proksimat, Jumlah Sebatian Fenolik (JSF), pemeringkatan, ekstrak kaya-asid ferulik, FTC, TBARS, aktiviti antipengoksida.



### **ACKNOWLEDGEMENTS**

This work was carried out at the Department of Food Science, Faculty of Food Science and Technology, University of Putra Malaysia.

I wish to express my sincerest gratitude to my supervisor, Assoc. Professor Dr. Azizah Hj. Abd. Hamid, whose encouraging guidance, stimulating discussions and kind understanding have led my work. Apart from scientific stuffs, many thanks are also due to open-mindedness to discuss anything anytime during all these years.

My special thanks also are due to my co-supervisors, Assoc. Professor Dr. Azizah Osman and Assoc. Professor Dr. Nazamid Saari for their support and excellent ideas during the course of this study.

I want to express my greatest appreciation to En. Azman Abu Yamin, En. Abdul Halim, Pn. Jamaliah, and other staffs of Biochemistry Laboratory for their reliable help with chromatographic media and instruments.

Sincere thanks are due to my helpful colleagues particularly at the Nutraceutical and Enzymology Laboratory, and from Dept. of Food Science and Dept. of Food Technology, FSTM, who have made the period when I worked on the thesis very inspiring.

I also owe thanks to all my relatives, friends and colleagues outside and inside UPM. My best and warmest thanks go to my family: Hubby, Ayah, Che, and the others for their support and understanding during the course of this work. Special thanks also due to them for the 'doa restu' that showered me during all the years.



I certify that an Examination Committee met on **8.9.2009** to conduct the final examination of **Raja Rohaya Binti Raja Sulaiman** on her **Master of Science** thesis entitled "**Chemical Composition Stabilized Rice Bran Milling Fractions And Characterization Of Their Ferulic Acid Rich Extracts**" 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:

Suhaila Mohamed, Ph.D.
Professor
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Chairman)

Amin Ismail, Ph.D. Associate Professor Faculty of Medicine and Health Sciences Universiti Putra Malaysia (Internal Examiner)

Faridah Abas, Ph.D. Faculty of Food Science and Technology Universiti Putra Malaysia (Internal Examiner)

Independent Examiner, Ph.D.
Professor
Name of faculty/institute
Name of university
(External Examiner)

**BUJANG KIM HUAT, PhD** 

Professor/Deputy Dean School of Graduate Studies Universiti Putra Malaysia

Date:



This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Masters of Science. The members of the Supervisory Committee were as follows:

### Azizah Hj. Abd. Hamid, PhD

Associate Professor Faculty of Food Science and Technology Universiti Putra Malaysia (Chairman)

### Azizah Osman, PhD

Professor Faculty of Food Science and Technology Universiti Putra Malaysia (Member)

### Nazamid Saari, PhD

Professor Faculty of Food Science and Technology Universiti Putra Malaysia (Member)

HASANAH MOHD GHAZALI, PhD

Professor and Dean School of Graduate Studies Universiti Putra Malaysia

Date: 12 August 2010



### **DECLARATION**

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at Universiti Putra Malaysia or other institutions.

RAJA ROHAYA RAJA SULAIMAN

Date: 8 September 2009



# TABLE OF CONTENTS

			Page
DEDICATION			ii
ABSTRACT			iii
ABSTRAK			V
ACKNOWLEDGE	MENTS	3	vii
APPROVAL		,	viii
DECLARATION			X
LIST OF TABLES			xiii
LIST OF FIGURE			xiv
LIST OF ABBREV		NS	xvii
CHAPTER 1 INTRODU	UCTION	N	1
2 REVIEW	OF TH	E LITERATURE	4
2.1		ical Aspects, History and Trade Overview of Rice	4
2.2	Rice F	e · · · · · · · · · · · · · · · · · · ·	6
2.2		Rice Bran Production	6
		Composition of Rice Bran	10
		Properties of Rice Bran	16
		Rice Bran Stabilization	18
		Uses of Rice Bran	20
		Health Claims of Rice Bran	21
2.3	Rice Bran Potentials as A Source of Phenolic Compounds		
	2.3.1	Chemistry and Biosynthesis of Phenolic Compounds	24
		Analysis of Phenolic Compounds	30
	2.3.3	Ferulic Acid	37
	2.3.4	Ferulic Acid Utilizations	39
2.4	Antio	xidants	40
	2.4.1	Phenolic Antioxidants	41
	2.4.2	Evaluation of Antioxidant Status	42
		OMPOSITION OF STABILIZED RICE BRAN	52
MILLIN			52
3.1		uction	52 54
3.2		ials and Methods	54 54
		Materials Methods	54 56
		Methods Statistical Analysis	56 62
2 2		Statistical Analysis ts and Discussions	62 63
3.3	3.3.1		63
	3.3.1	Macronutrients Composition of Stabilized Rice Bran Fractions	U3



	3.3.2	Vitamins and Minerals of Stabilized Rice Bran	72	
	222	Fractions  Aming Acid Composition of Stabilized	78	
	3.3.3	Amino Acid Composition of Stabilized Rice Bran Fractions	/8	
3.4	Concl		81	
		IPOUNDS OF RICE BRAN AS MEASURED BY	82	
		COMETRY AND HIGH PERFORMANCE LIQUID		
CHROMA		RAPHY (HPLC)		
4.1	Introduction			
4.2		ials and Methods	84	
		Materials	84	
		Methods	85	
		Statistical Analysis	89	
4.3		s and Discussions	90	
		TPC as Measured by Spectrophotometer	90	
	4.3.2	Phenolic Acids in the 1 <sup>st</sup> Rice Bran Milling Fraction	97	
		as Measured by HPLC		
4.4	Concl	usions	106	
5 FRACTIO	NATIO	N AND ANTIOXIDATIVE PROPERTIES OF	107	
RICE BR	AN CR	UDE EXTRACTS		
5.1	Introd	uction	107	
5.2	Mater	ials and Methods	109	
	5.2.1	Materials	109	
	5.2.2	Methods	110	
	5.2.3	Statistical Analysis	113	
5.3	Result	s and Discussions	115	
	5.3.1	Analysis of Crude Extract from the 1 <sup>st</sup> Rice Bran Milling Fraction	115	
	5.3.2	Determination of Ferulic Acid in Fractionated Rice	119	
	3.3.2	Bran Extracts	11)	
	5.3.3	Antioxidative Properties of Fractionated	121	
	0.0.0	Rice Bran Extracts	121	
5.4	Concl		131	
6 CONCLUS	SIONS .	AND RECOMMENDATIONS FOR	132	
FUTURE I				
6.1	GENE	ERAL CONCLUSION	132	
6.2		OMMENDATIONS FOR FUTURE RESEARCH	134	
BIBLIOGRAPHY			135	
APPENDICES			170	
BIODATA OF THE STUDENT				
LIST OF PUBLICATIONS				
LIST OF PUBLICA		,	186	

