UNIVERSITI PUTRA MALAYSIA

EFFECTS OF GERMINATION TIME AND VARIETIES OF UNMILLED RICE ON BLOOD CHOLESTEROL IN SPRAGUE-DAWLEY MALE RATS

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By

SHAHIN ROOHINEJAD

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

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DEDICATION

This thesis is dedicated to my father who is always giving me his unlimited support and to my mother who is always praying for me well being.

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

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Chair: Professor Dr. Mohd. Yazid Abd Manap, PhD

Faculty: Food Science and Technology

Brown rice (unmilled rice) and pre-germinated brown rice are known to contain various functional compounds such as γ-oryzanol, dietary fiber and γ-aminobutyric acid (GABA) with substantial benefits for human health. The objectives of this study were to investigate the effect of pre-germination time of different unmilled rice varieties on GABA composition, and evaluate the hypocholesterolemic properties of selected pre-germinated unmilled rice varieties containing different GABA levels in rats. The results revealed a high variation in GABA contents in different unmilled rice varieties. GABA contents in Malaysian unmilled rice seeds ranged 0.01 to 0.1 (mg/g). MRQ74 and MR 220 showed the highest GABA contents among thirty five Malaysian unmilled rice varieties analyzed; while MR 232 had the least GABA content. It was also concluded that the effect of pre-germination on GABA content clearly was dependent on unmilled rice variety and pre-germination times, thus reflecting the longer pre-germination time provided higher GABA concentration level. Moreover, it was revealed that the GABA

contents in pre-germinated samples were dependent on the initial GABA content of its source. In this study, the hypocholesterolemic properties of selected pre-germinated unmilled rice varieties containing different GABA levels in rats were investigated. Hypercholesterolemia and elevation of LDL-Cholesterol were successfully ameliorated by most of the unmilled rice and pre-germinated unmilled rice diets. Germinated unmilled rice showed more cardioprotective effect than non-germinated unmilled rice in hypercholesterolemic rats. Moreover, it was found that longer germination times had better effect on lipid profile of hypercholesterolemic rats. In addition to lipid profile, the hypoglycemic effects of unmilled rice and pre-germinated unmilled rice were found to be remarkable. The significant ($p < 0.05$) main effects of unmilled rice variety, germination time and their interaction on lipid and glucose profile were observed in the present study. A negative correlation between GABA content and the serum levels of TC and a positive correlation between GABA content and HDL-C were observed. Conversely, no correlation was shown between GABA content and LDL-C, TG and glucose levels. Although GABA played a role for prediction of lipid profile changes, this study was not capable to show a causative effect for this component. Therefore further extensive investigations are needed to clarify the main chemical(s) responsible for hypocholesterolemic effect of unmilled rice and pre-germinated unmilled rice. This study recommends that the unmilled rice and pre-germinated unmilled rice with excellent functional properties can be used instead of polished rice in Malaysian diet.

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

KESAN MASA CAMBAH DAN KEPELBAGAIAN BERAS YANG TIDAK DIPROSES TERHADAP KOLESTROL DARAH DALAM TIKUS SPRAGUE-DAWLEY

Oleh

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Beras perang (beras yang tidak diproses) dan beras perang pra-cambah adalah diketahui mengandungi pelbagai sebatian berfungsi seperti γ-orizanol, serat diet dan asid γ-aminobutirik (GABA) yang mempunyai banyak manfaat terhadap kesihatan manusia. Objektif kajian ini adalah untuk mengkaji kesan tempoh pra-cambah pelbagai beras yang tidak diproses yang berbeza terhadap komposisi GABA, dan menilai ciri-ciri hipokolesterolemik dalam pelbagai beras yang tidak diproses pra-cambah yang terpilih yang mengandungi tahap GABA yang berbeza dalam tikus. Keputusan menunjukkan bahawa terdapat pelbagai variasi yang berbeza pada kandungan GABA dalam pelbagai beras yang tidak diproses. Kandungan GABA biji beras yang tidak diproses di Malaysia berjulat diantara 0.01 hingga 0.1 (mg/g). MRQ74 and MR 220 menunjukkan kandungan hasilan GABA yang paling tinggi di antara tiga puluh lima variasi beras yang tidak diproses yang dianalisa, manakala MR 232 mengandungi kandungan GABA yang paling

sedikit. Kesimpulannya, kesan pra-cambah terhadap kandungan GABA adalah bergantung kepada kepelbagaian beras perang dan tempoh pra-cambah. Oleh itu, semakin lama tempoh pra-cambah, semakin tinggi tahap kepekatan GABA. Malah, ia menunjukkan bahawa kandungan GABA dalam sampel-sampel pra-cambah bergantung kepada sumber kandungan awal GABA. Dalam kajian ini, ciri-ciri hipokolesterolemik pada pelbagai beras yang tidak diproses pra-cambah yang terpilih yang mengandungi tahap kepekatan GABA yang berbeza telah dikaji. Hiperkolesterolimia dan peningkatan kolesterol-LDL memberi tindak balas yang menggalakkan terhadap kebanyakan beras yang tidak diproses dan beras yang tidak diproses pra-cambah. Beras yang tidak diproses yang bercambah menunjukkan kesan kardioprotektif yang lebih tinggi berbanding beras yang tidak diproses yang tidak bercambah. Tambahan lagi, didapati bahawa tempoh percambahan yang lama mempunyai kesan yang baik terhadap profil lemak bagi tikus hyperkolesterolemia. Selain mempunyai kesan yang baik terhadap profil lemak, kesan hipoglisemik oleh beras yang tidak diproses dan beras yang tidak diproses pra-cambah juga didapati luar biasa. Kesan signifikan utama ($p < 0.05$) pada kepelbagaian beras yang tidak diproses, tempoh percambahan dan interaksinya pada lipid dan profil glukos telah diperhatikan di dalam kajian ini. Satu korelasi negatif di antara kandungan GABA dan tahap serum pada TC dan satu korelasi positif di antara kandungan GABA dan HDL-C telah diperhatikan. Dengan kata lainnya, tiada korelasi telah ditunjukkan di antara kandungan GABA dan LDL-C, TG dan tahap glukos. Walaupun GABA memainkan peranan penting untuk jangkaan terhadap perubahan profil lipid, kajian ini tidak mampu menunjukkan satu kesan penyebab kepada komponen ini. Oleh itu, kajian yang lebih lanjut di masa hadapan diperlukan untuk memastikan bahan kimia utama yang menyebabkan kesan hipokolestrolemik pada beras yang tidak diproses dan beras yang

tidak diproses pra-cambah. Kajian ini mencadangkan bahawa, beras yang tidak diproses dan beras yang tidak diproses pra-cambah ini yang mempunyai ciri-ciri yang sangat baik boleh digunakan dalam diet rakyat Malaysia mengantikan beras yang telah diproses**.**

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The world is moved along, not only by the mighty shoves of its heroes, but also by the aggregate of the tiny pushes of each honest worker.

Helen Keller

I certify that a Thesis Examination Committee has met on 19 February 2009 to conduct the final examination of Shahin Roohinejad on his thesis entitled "Effects of Germination Time and Varieties of Unmilled Rice on Blood Cholesterol in Sprague-Dawley Male Rats" 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 thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of **Master of Science**. The members of the Supervisory Committee were as follows:

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DECLARATION

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

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SHAHIN ROOHINEJAD

Date:

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LIST OF ABBREVIATIONS

GABA	Gamma aminobutyric acid
FNPA	Free non-protein amino acid
IFT	Institute of Food Technologists
AOAC	Association of Official Analytical Chemists
MARDI	Malaysian Agriculture Research and Development Institute
PMV	Penyakit Merah Virus
HYVs	High yielding varieties
HPLC	High performance liquid chromatography
BR	Brown rice
NG	Non-germinated
NGBR	Non-germinated brown rice
PG	Pre-germinated
PGBR	Pre-germinated brown rice
GBR	Germinated brown rice
UR	Unmilled rice (Malaysian varieties)
PGUR	Pre-germinated unmilled rice (Malaysian varieties)
GAD	Glutamate decarboxylase
FAO	Food and Agriculture Organization of the United Nations
USDA	United States Department of Agriculture
TAA	Total amino acid
TEAA	Total essential amino acid
Asp	Aspartic acid

Glu	Glutamic acid
Ser	Serine
Gly	Glycine
His	Histidine
Arg	Arginine
Thr	Threonine
Ala	Alanine
Pro	Proline
Tyr	Tyrosine
Val	Valine
Met	Methionine
Iso	Isoleucine
Leu	Leucine
Phe	Phenylalanine
Lys	Lysine
RCBD	Randomized Complete Block Design
PITC	Phenylisothiocyanate
TC	Total cholesterol
LDL	Low density lipoprotein
HDL	High density lipoprotein
TG	Triglyceride
CHD	Coronary heart disease
CNS	Central nervous system
CVD	Cardiovascular disease

CHAPTER 1

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important crops for more than a third of the world's population in addition to wheat and corn. World production of rice has risen steadily from about 200 million tons of paddy rice in 1960 to 645 million tons in 2007. Rice accounts for over 22% of global energy intake. More than 90% of the world's rice is grown and consumed in Asia. In Malaysia, rice is a strategic crop with annual consumption of approximately 85 kg per capita. Rice is classified according to the degree of milling that makes a brown rice (unmilled rice) different from white rice.

Brown rice (BR) or unmilled rice (UR) is the whole grain of rice, from which the germ and outer layers containing the bran have not been removed. BR grains are rich in more nutritional components, such as dietary fibers, phytic acids, vitamins B and E and gamma amino butyric acid (GABA) than the ordinary milled rice grains. These bio-functional components are found in the germ and bran layers (Champagne *et al*., 2004).

Malaysian Agriculture Research and Development Institute (MARDI) have released 35 unmilled rice (UR) varieties in order to maintain the annual production of UR. The major concern is releasing the varieties resistant against pests and diseases providing higher harvest yield at shorter maturation period. However, there is a lack of information regarding the nutritional values of UR varieties such as GABA.

GABA is a well known non-protein-based amino acid that is widely distributed in both animals and plants. It is one of the major inhibitory neurotransmitters in the central

nervous system. GABA is primarily synthesized by the decarboxylation of L-glutamic acid (Glu) in the presence of glutamate decarboxylase (GAD). The alteration of GABA and GAD levels in the brain was found to result in many neurological disorders such as seizures, Parkinson's disease, stiff-man syndrome and schizophrenia (Bao *et al*., 1995). Recently, the interest in utilization of GABA as a nutritional compound has enormously increased. Previous researchers indicated that plant extracts containing high concentration level of GABA significantly ($p < 0.05$) influenced the blood pressure (Nakagawa and Onota, 1996) and alcohol-related symptoms (Cha and Oh, 2000). With all the health-giving benefits of GABA, various processes such as fermentation, enzymatic treatment, gaseous treatment and (pre) germination have been suggested to enhance the GABA concentration.

The term 'pre-germination' is used to describe the soaking or steeping process of cereal in water. Pre-germination is one the most common methods to enhance GABA and other nutrients such as dietary fiber, inositols, ferulic acid and gamma oryzanol through endogenous enzyme activation in BR (Ohtsubo *et al*., 2005). The pre-germination treatment of rice results in chemical alteration of molecules stored in both the embryo and endosperm into novel substances. The number of pre-germinated brown rice-contained products is increasing in Asian food market because they contain higher amount of GABA and other nutritional compounds than the ordinary polished rice-contained products. Pre-germinated brown rice (PGBR) plays an important role in improving public nutrition, thereby promoting people's health (Ito and Ishikawa, 2004).

Recently, several researchers have studied the effect of BR and PGBR on lipid profile (Kim *et al*., 2006; Oh *et al*., 2005), blood glucose (Ito *et al*., 2005), cancer (Oh and Oh,

2004) and blood cholesterol (Oh *et al*., 2003). Hypercholesterolemia is a representative life-style related condition caused by an excessive consumption of fat-rich diet associated with a sedentary life-style. It is known as a metabolic derangement contributing to many diseases notably cardiovascular disease (CVD). Previous studies also suggested the potential role of GABA in hypocholesterolemic subject (Lee *et al*., 2007; Miura *et al*., 2006).

Although the administration of the germinated brown rice (GBR) containing high GABA content has been reported to prevent ethanol-induced increases in liver triglyceride and total cholesterol concentrations, the mechanism is still unknown (Cha and Oh, 2000; Nakagawa and Onota, 1996). However, there is little information on the effect of the pre-germination time and different unmilled rice varieties on hypercholesterolemia.

1.1 Problem statement

Although the effect of pre-germination on GABA content was demonstrated in previous studies, the level of GABA in Malaysian unmilled rice (UR) and pre-germinated unmilled rice (PGUR) are still unknown. PGBR has been reported to have cardioprotective effect; however, it is still not clear whether different varieties have the same cholesterol-lowering effect and increasing the pre-germination time cause more lipid profile reduction. Even though various studies have shown the cholesterol-lowering effect of unmilled rice varieties, the hypocholesterolemic effect of Malaysian unmilled and pre-germinated unmilled rice varieties containing different GABA levels are still unknown.

1.2 Objectives of the study

1.2.1 General objective

To study the hypocholesterolemic properties of pre-germinated rice varieties containing different GABA levels on Sprague Dawley male rats.

1.2.2 Specific objectives

(1) To investigate the effect of pre-germination time of different unmilled rice varieties on GABA contents.

(2) To evaluate the hypocholesterolemic properties of selected pre-germinated unmilled rice varieties containing different GABA levels in rats.

1.3 Hypothesis

1.3.1 Hypothesis in the laboratory experiment (chapter 3)

There is no (significant) difference among mean of GABA content of non-germinated unmilled rice (μ_{NG}) and pre-germinated unmilled rice (μ_{PG}). It means:

H_0: $\mu_{NG} = \mu_{PG}$

H_A: $\mu_{NG} \neq \mu_{PG}$