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NUTRITIONAL ENHANCEMENT OF WHOLE GRAIN BARIO RICE BY PHYTIC ACID DEGRADATION DURING GERMINATION

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By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Doctor of Philosophy

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NUTRITIONAL ENHANCEMENT OF WHOLE GRAIN BARIO RICE BY PHYTIC ACID DEGRADATION DURING GERMINATION

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Chair : Assoc. Prof. Yiu Pang Hung, PhD
Faculty : Agriculture and Food Sciences (Bintulu)

Whole grain rice is suggested to be a better solution against the polished form with more vitamins, minerals and fiber than its processed equivalent. Abundance of phytic acid in whole grain rice demotes the nutritional values of whole grain rice in general, although its beneficial properties were also reported. Various strategies had been conducted to counteract the nutritional limitations in whole grain rice, including germination. These led to the main objective of this study, to determine the nutritional quality in whole grain rice of Bario rice cultivars and later improve the targeted nutritional quality through the germination process.

Thirty rice cultivars named as "Adan" or "Bario" were assessed for nutrient bioaccessibility and antioxidant properties. The studies showed an acceptable level of *in vitro* digestibility in starch (193.77 ± 93.09 mg maltose released/g) and protein (69.01 ± 8.06%), but were poor in antioxidant properties and bioaccessibility of minerals (Phy/Fe 125.37 ± 38.95, Phy/Zn 201.97 ± 80.54 and Phy/Ca 11.58 ± 3.35). Most of the lowland Bario rice with medium grains had relatively better nutrient bioaccessibility while pigmented rice had high amounts of phenolic compounds (0.64 ± 0.21 mg GAE/g) and antioxidant properties. The drawback in the whole grains were related to relatively low mineral content (24.30 ± 0.46 g Fe/100g, 1.30 ± 0.35 g Zn/100g, 13.30 ± 2.79 g Ca/100g) and high phytic acid content (24.29 ± 4.07 g/kg) in general, and also low phenolic content in non-pigmented rice (0.10 ± 0.04 mg GAE/g).

The relationship between intrinsic phytic acid content, nutrient bioaccessibility and antioxidant properties was studied. Results suggested that intrinsic phytic acid could be an important chelating antioxidant (51.92 ± 8.35%, r = 0.32) in non-pigmented rice which also reduced mineral bioaccessibility in the whole grains. Phytic acid content significantly influenced mineral bioaccessibility (r =0.40 [Phy/Fe], r =0.27 [Phy/Zn], r = 0.60 [Phy/Ca]) especially iron and calcium. *In vitro* digestibility of starch and protein were not affected by intrinsic phytic acid content. The contribution of phytic acid to the antioxidant properties of whole grain rice was low, but phytic acid remains as an important antioxidant in non-pigmented rice. These imply that the antinutrient effects from phytic acid were stronger and significantly contributed to the low mineral bioaccessibility in the collection.

Phytate degradation based on changes in phytic acid content, phytase activity and phytate globoids were investigated under various germination treatments in selected rice cultivars. This study showed that germination treatments facilitated the hydrolysis of phytate complexes in whole grain rice significantly, reducing phytic acid content (0.73 - 99.99% loss), accelerating phytase activity (0.38 – 270 U/kg increment) and releasing minerals from phytate globoids. However, the phytate degradation differed with rice cultivars under the same germination conditions. Germination is a suitable treatment for phytate degradation in Bario rice cultivars with the right germination conditions.

The study was continued with optimization of germination conditions by response surface methodology in the cultivar Tuan. Phytic acid content was significantly reduced (1.24 – 99.99% loss) with facilitation of phytase activity (27.43 – 165.3 U/kg increment) during germination and led to increments in mineral bioaccessibility (8.03 – 239.56% Ca, 25.38% Fe, 1.18 – 45.01% Zn). Soaking for 12 hours under acidic condition (pH 2) was the most suitable for improvement of calcium and zinc bioaccessibility at room temperature (25°C). However, longer germination duration (50 hours) was required for higher iron bioaccessibility. Optimized germination conditions of pH 2.7, at 25°C and 12 hours germination successfully reduced phytic acid content and phytic acid to minerals mole ratio, and led to an increase in mineral bioaccessibility in calcium, iron and zinc.

In conclusion, the whole grain rice of Bario rice cultivars had the advantages in *in vitro* digestibility of starch and protein but with limited mineral bioaccessibility and antioxidant properties. The limitations in mineral bioaccessibility were due to the abundance of phytic acid content in the whole grain rice. Phytate degradation in whole grain rice was cultivar and condition dependent. Optimized germination condition could promote phytate degradation and result in improved mineral bioaccessibility in selected whole grain Bario rice. Germinated whole grain rice with better mineral bioaccessibility can be a good substitution of whole grain rice as part of healthy diets.

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PENINGKATAN NILAI PEMAKANAN BERAS PENUH BARIO DENGAN PENGURAIAN ASID FITIK MELALUI PERCAMBAHAN

Oleh

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Beras penuh mempunyai vitamin, mineral dan serat yang lebih tinggi berbanding dengan beras putih. Kandungan asid fitik yang tinggi dalam beras penuh mengurangkan nilai pemakanan secara umum walaupun faedahnya juga telah dilaporkan. Pelbagai strategi telah dilaksanakan untuk mengatasi kekurangan dalam beras penuh, termasuk proses percambahan. Oleh itu, objektif utama kajian ini adalah menentukan nilai pemakanan dalam beras penuh Bario dan seterusnya meningkatkan nilai pemakanan melalui proses percambahan.

Tiga puluh kultivar beras yang dinamakan “Adan” atau “Bario” telah ditentukan bioaksesibiliti nutrien dan sifat antioksida. Koleksi beras ini menunjukkan tahap penghadaman kanji (193.77 ± 93.09 mg maltosa/g) dan protein (69.01 ± 8.06%) yang baik tetapi sifat antioksida dan bioaksesibiliti mineral (Phy/Fe 125.37 ± 38.95, Phy/Zn 201.97 ± 80.54 and Phy/Ca 11.58 ± 3.35) yang rendah. Kebanyakan beras Bario dari lokasi tanaman tanah rendah dan berbentuk sederhana mempunyai bioaksesibiliti nutrien yang lebih tinggi. Manakala beras penuh berpigmen mengandungi fenolik yang tinggi (0.64 ± 0.21 mg GAE/g) dan sifat antioksida yang aktif. Kelemahan yang didapati dalam beras penuh Bario adalah kandungan mineral yang rendah (24.30 ± 0.46 g Fe/100g, 1.30 ± 0.35 g Zn/100g, 13.30 ± 2.79 g Ca/100g), kandungan asid fitik yang tinggi (24.29 ± 4.07 g/kg) dan kandungan fenolik yang rendah (0.10 ± 0.04 mg GAE/g) dalam beras bukan berpigmen.

Hubungan antara kandungan intrinsik asid fitik, bioaksesibiliti nutrien dan sifat antioksida telah dikaji. Keputusan mencadangkan asid fitik dalam beras penuh boleh menjadi agen antioksida yang penting (51.92 ± 8.35%, r = 0.32) dalam beras bukan berpigmen tetapi ia juga mengurangkan bioaksesibiliti mineral. Kandungan asid fitik mempengaruhi bioaksesibiliti mineral (r =0.40 [Phy/Fe], r =0.27 [Phy/Zn], r = 0.60 [Phy/Ca]) terutamanya zat besi dan kalsium. Penghadaman kanji dan protein secara *in vitro* tidak terjejas oleh kandungan asid fitik dalam beras penuh. Sumbangan asid fitik kepada sifat antioksida beras penuh adalah rendah, tetapi asid fitik masih merupakan agen antioksida yang penting dalam beras tidak berpigmen. Ini menunjukkan bahawa asid fitik memberi sumbangan yang lebih berkesan kepada sifat antinutrien dan memberi bioaksesibiliti mineral yang rendah dalam beras penuh Bario.

Penguraian asid fitik berdasarkan perubahan dalam kandungan asid fitik, aktiviti enzim fitase dan globoid fitik semasa proses percambahan telah dikaji dalam kultivar yang terpilih. Kajian ini menunjukkan bahawa percambahan membantu hidrolisis kompleks asid fitik, dengan mengurangkan kandungan asid fitik (0.73 - 99.99%), mempercepatkan aktiviti fitase (0.38 – 270.00 U/kg) atau penguraian mineral dari globoid. Walau bagaimanapun, perubahan tersebut adalah berbeza dalam setiap kultivar beras yang diuji. Justeru, percambahan merupakan kaedah yang sesuai untuk penguraian asid fitik dalam kultivar beras Bario dengan keadaan percambahan yang betul.

Kajian ini diteruskan dengan pengoptimuman keadaan percambahan dalam kultivar "Tuan". Kandungan asid fitik telah dikurangkan dengan ketara (1.24 – 99.99%) melalui aktiviti fitase (27.43 -165.30 U/kg) semasa percambahan dan seterusnya meningkatkan bioaksesibiliti mineral (8.03 – 239.56% Ca, 25.38% Fe, 1.18 – 45% Zn). Percambahan dalam kaadaan berasid (pH 2) untuk 12 jam adalah keadaan yang paling sesuai untuk peningkatan bioaksesibiliti zat kalsium dan zink pada suhu bilik (25°C). Namun demikian, tempoh percambahan yang lebih panjang (50 jam) diperlukan untuk bioaksesibiliti zat besi yang tinggi . Kaadaan percambahan yang telah dioptimumkan pada pH 2.7, 25°C dan 12 jam berjaya mengurangkan kandungan asid fitik, nisbah asid fitik kepada mineral dan memberi peningkatan dalam bioaksesibiliti mineral dalam zat kalsium, besi dan zink.

Kesimpulannya, beras penuh Bario mempunyai kelebihan dalam penghadaman kanji dan protein, tetapi bioaksesibiliti mineral dan sifat antioksida yang terhad. Kelemahan dalam bioaksesibiliti mineral adalah disebabkan kandungan asid fitik yang tinggi dalam beras penuh. Penguraian asid fitik dalam beras penuh Bario adalah bergantung kepada kultivar dan keadaan percambahan. Keadaan percambahan yang optimum dapat membantu penguraian asid fitik dan memberi bioaksesibiliti mineral yang lebih baik dalam beras penuh Bario terpilih. Beras penuh bercambah yang mempunyai bioaksesibiliti mineral yang lebih tinggi boleh dijadikan alternatif kepada beras penuh untuk pemakanan yang sihat.

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This thesis was submitted to the Senate of University Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

AACC	American Association of Cereal Chemistry
AAE	Ascorbic Acid Equivalent
AAS	Atomic Absorption Spectrometer
ANOVA	Analysis of Variance
AOAC	Association of Analytical Communities
BERNAS	National Paddy and Rice Board
BRRI	Bangladesh Research Rice Institute
CCD	Central Composite Design
DNMRT	Duncan New Multiple Range Test
DNA	Deoxyribose Nucleic Acid
DPPH	2,2-diphenyl-1-picrylhydrazyl
EDTA	Ethylenediaminetetraacetic acid
EDX	Energy Dispersive X-Ray
FAO	Federal Agriculture Organization
FTIR	Fourier Transform Infrared Spectroscopy
GABA	Gamma Amino Butyric Acid
GAE	Gallic Acid Equivalent
GC-MS	Gas Chromatography Mass Spectrometer
HPIC	High Performance Ion Chromatography
HPLC	High Performance Liquid Chromatography
ICP-AES	Inductively Coupled Plasma Atomic Emission Spectrometer
ICP-MS	Inductively Coupled Plasma Mass Spectrometer
Ins	Inositol
IRRI	International Rice Research Institute

ISGA	International Sprouted Grains Association
IUB	International Union of Biochemistry
IUPAC	International Union of Pure And Applied Chemistry
kDa	kilo Dalton
kV	kilo Volts
M	Molar
MANOVA	Multivariate Analysis Of Variance
mM	mili molar
mRNA	messenger Ribonucleic Acid
MyIPO	Malaysia Intellectual Property Organization
NCFFN	National Coordinating Committee on Food and Nutrition
NMR	Nuclear Magnetic Resonance
PCA	Principal Component Analysis
PDA	Photodiode array
Phy/Fe	Phytic acid to iron mole ratio
Phy/Zn	Phytic acid to zinc mole ratio
Phy/Ca	Phytic acid to calcium mole ratio
PTFE	Polytetrafluoroethylene
QBSD	Quadrant Back Scattering Detector
SAHN	Sequential Agglomerative Hierarchical Nesting
SAS	Statistical Analysis Software
SEM	Scanning Electron Microscope
USDA	U.S. Department of Agriculture
UV-Vis	Ultraviolet Visible light
WHO	World Health Organization

CHAPTER 1

INTRODUCTION

Rice is the primary source of nutrition in many developing countries including Malaysia. It is a readily available medium for transporting the essential nutrients to the local people to tackle nutritional-related diseases. In spite of that, nutritional limitations that underlies within rice grains are contributing to the growing health problems in developing countries (Potrykus, 2003). Rice is consumed mainly as polished rice containing little nutrients, where beneficial nutrients especially those chemical compounds with biomedical values are reduced significantly during the milling process (Champagne *et al.*, 2004).

Whole grain rice can be a substitute to its processed equivalent for daily consumption with more vitamins, minerals and fiber. Positive associations between whole grain rice consumption and lower incidence of many chronic diseases such as heart disease, obesity, cancer and Type 2 diabetes have been reported (Panlasigui and Thompson, 2006; Dinesh Babu *et al.*, 2009; Slavin, 2013). However, whole grain rice is also reported to be low in minerals, starch and protein bioavailability and its antioxidant potential were not as optimistic as reported. The low availability of vitamins, minerals and health promoting phytochemicals in whole grain rice may not be sufficient for effective nutritional consumption (Grusak, 2002).

Rice improvement programs have included nutritional targets for global health development, aside from milling and cooking quality which dictates higher commercial value. Understanding of associations between rice constituents with desired nutritional quality is important to facilitate the production of rice with higher nutritional quality. Phytic acid which is abundantly present in whole grain rice has been considered to be the major contributor to nutritional drawbacks of the whole grain rice. Its strong ability to complex with multi-charged metal ions, proteins and starches reduces the availability of these nutrients (Yoon *et al.*, 1983; Raboy, 2009). Nevertheless, phytic acid also exhibits beneficial properties such as good anti-cancer agent, inhibitor for renal stone development and also behaves as an antioxidant agent (Thompson, 1993). Therefore, phytic acid could play a significant role in the overall nutritional value of whole grain rice depending on its complexing capability with nutrients in different mediums including its formation, release and breakdown.

Strategies have been made to rectify the nutritional limitations present in rice through plant breeding, food processing and nutritional programme, by increasing the targeted nutrients, reducing nutrient inhibitors and increasing enhancers for the release of nutrients. Modern technology enables alteration at certain compound levels through genetic engineering and marker-assisted breeding (Grusak, 2002; Mackey, 2002). These efforts contribute significantly in the production of rice varieties with a relatively high concentration of minerals, vitamins and antioxidants, but low in inhibitors such as phytic acid (Lucca *et al.*, 2001a, 2001b; Glahn *et al.*, 2002). Low phytic acid (*lpa*) mutant rice lines have been generated by chemical mutation through molecular balance mechanisms, in which the seeds contained normal levels of total phosphorus, but reduced levels of phytic acid phosphorus (Raboy *et al.*, 2000). These forms of research require long experimental periods in the selection of suitable rice lines for safe release of nutritionally improved rice varieties. Conversely, food-processing techniques offer a more practical and consumer friendly method to counteract the nutritional limitations in rice.

Traditional food processing and preparation practices provide a good potential for nutritional improvement in rice. The traditional food processing methods are easily adaptable to poor resource settings and could be optimized to provide better consumer acceptance. These include thermal processing, mechanical processing, soaking, fermentation, and germination. Thermal processing is not an effective approach to tackle the influence of phytic acid in whole grain rice, due to the heat stable nature of phytic acid. Mechanical processing reduces significantly the phytic acid levels but also removes the nutrient rich bran layer. Soaking, germination, and fermentation which involve incubation of rice in water or inoculums in different time frames may bring down the phytic acid content (Nergiz and Gökgöz, 2007; Liang *et al.*, 2009; Luo *et al.*, 2009). Among these practices, germination outstand the latter two with its advantages in low nutrient losses due to leaching compared to soaking and less off-flavor generation compared to fermentation.

Germination is the natural process of plants to provide nutrients to seedlings. The mechanism of germination activates hydrolytic enzymes for the breakdown of starch, non-starch polysaccharides and proteins, and leads to an increase in readily absorbable oligosaccharides and amino acids (Yang *et al.*, 2001; Rimsten *et al.*, 2003; Shu *et al.*, 2008). The germination process also improves protein and carbohydrate digestibility, increases bioactive compounds, denatures amylase inhibitors and other anti-nutritional factors in whole grains (Egli *et al.*, 2002; Tian *et al.*, 2004; Komatsuzaki *et al.*, 2007). Nevertheless, nutrient density within the germinated grains is highly dependent on the germination conditions such as temperature, pH and time during processing. Optimization of the germination process could maximize the nutritional values output in whole grain cereals and legumes. However, this may vary among grains and varieties.

Sarawak, the land of hornbills is blessed with a great diversity of rice. Bario rice is one of the famous traditional premium rice identified by the Department of Agriculture, Sarawak for its soft texture, fine elongated grains and exquisite taste and aroma. Its unique characteristics dictated a geographical indication award from the Intellectual Property Corporation of Malaysia (MyIPO) in 2009. It is originated from the Kelabit's highlands, known as "Bario" and widely adopted by lowland rice growers in northern Sarawak regions. Bario rice cultivars are reported to be good source of protein and thiamine, moderate glycemic index and also low in fat content (Nicholas *et al.* 2014). However, the information on phytic acid level, nutrient bioaccessibility and antioxidant properties in the Bario rice cultivars is lacking.

Hence, the general objectives of the study were to determine nutritional value of Bario rice in the whole grain, and, identify germination condition for nutritional improvement of the grains.

The specific objectives of this study were:

a) To determine the nutrient bioaccessibility and antioxidant properties in the Bario rice cultivars.
b) To identify the effect of the intrinsic phytic acid content on nutrient bioaccessibility and antioxidant properties in the rice.

c) To study the effect of germination on phytic acid content, phytase activity and phytate globoids in the rice.
d) To optimize the germination conditions for mineral bioaccessibility improvement in the rice.

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BIODATA OF STUDENT

Lee Huei Hong was born in 24 August 1985 and raised up in Kuala Lumpur, Malaysia. She attended her primary education in S.R.J.K. (C) Lai Chee, Kuala Lumpur (1991), secondary education in S.M.K.P. Jalan Ipoh, Kuala Lumpur (1997), followed by pre-university education in S.M.K. Maxwell, Kuala Lumpur (2003). She pursued her Bachelor study in Bioindustry Sciences in Universiti Putra Malaysia Bintulu Sarawak Campus (UPMKB), and graduated with first class honors in 2009.

Her interest in scientific research leads her to continue her postgraduate study in the Faculty of Agriculture and Food Sciences, UPMKB. She was offered the PhD studentship by the School of Graduate Studies, UPM and received a scholarship award (National Science Fellowship Batch1/2009) from the Ministry of Science, Technology and Innovation (MOSTI), Malaysia. She was also supported by the special graduate research assistant scheme offered by UPM and through research grant from the Ministry of Education.

Throughout her PhD candidature, she actively participated in various research activities at national and international levels. These include outbound research attachment at International Rice Research Institute (IRRI), Philippines and rice conferences at state level (SARICON 2011), national level (NRC2011) and international level (IRC2014). Exhibitions participated include Malaysia Innovations Expo 2013 (MIExpo 2013) and BioMalaysia and Bioeconomy Asia Pacific Conference and Exhibition 2013. The patent filed, awards received and published journal articles and conference proceedings from her PhD program are presented in the next page.

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Awards

Young Rice Scientist Award on "Dietary roles of intrinsic phytate in whole grains of Bario rice", given by International Rice Research Institute (IRRI) at 4th International Rice Congress 2014, Bangkok, Thailand, 30 Oct 2014).

Bioinnovation Award in Agriculture/Agro-based Industry (Bronze Medal) on "Multiplex Assay for Detection of Bario Rice Samples", given by the organizers of BioMalaysia and Bioeconomy Asia Pacific Conferences and Exhibition 2013 (21 Oct 2013).