UNIVERSITI PUTRA MALAYSIA

EVALUATION OF AQUEOUS- PIGMENTED RICE EXTRACTS AS BASES FOR ANTIOXIDANT DRINKS

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By

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Chairperson: Assoc. Prof. Sharifah Kharidah Syed Muhammad, PhD
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Pigmented rice, which consists of black rice and red rice, are categorized as functional foods due to their antioxidant content, mainly contributed by anthocyanins for black rice and proanthocyanidins for red rice. However, as most of the antioxidant compounds in pigmented rice are hydrophilic, loss of antioxidants can occur during rice cooking in excess water. Therefore, the antioxidants in pigmented rice were extracted using hot water and the extracts were evaluated as bases for antioxidant drinks. The objectives of this study were to determine the optimum processing conditions for maximum DPPH radical scavenging activity, total flavonoid content, and total phenolic content in aqueous pigmented rice extracts; to evaluate the physical properties of the extracts; to compare the antioxidant capacities of the extracts with that of commercial fruit drinks; to evaluate the physical, antioxidant, and microbiological properties of the aqueous pigmented rice extracts during storage; to determine the effects of ingredients (citric acid, sodium benzoate, and sucrose) and carbonation on degradation kinetics of anthocyanins in aqueous black rice extract during storage. The optimization of the processing conditions for the aqueous pigmented rice extracts were determined using response surface methodology, and the optimized extracts were studied for their physical and antioxidant properties. The extracts were then stored for 12 weeks at room and refrigerated temperatures to evaluate their physical, microbiological, and antioxidant stability during storage. Lastly, high performance liquid chromatography (HPLC) was used to determine the anthocyanin degradation kinetics in aqueous black rice extract during storage. It was found that aqueous black rice extract had significantly higher ($p<0.05$) antioxidant power than aqueous red rice extract, and both rice extracts possessed significantly higher ($p<0.05$) antioxidant power compared to three commercial fruit drinks evaluated in this study. There was no significant difference between aqueous black and red rice extracts in terms of pH, viscosity, and total soluble solids. However, in terms of colour, aqueous black rice extract was significantly darker, more blue, and more red than aqueous red rice extract. The optimum hot water extraction conditions for black rice extract were water/rice (W/R) ratio of 20 ml/g at 92 ^{o}C for 40 minutes, while that for red rice extract were W/R ratio of 20 ml/g at 97 ^{o}C for 30 minutes. In terms of their storage stability, the variations in terms of antioxidant content and physical properties were observed more at storage temperature of 25 ^{o}C than at 4 ^{o}C. The extracts were able to be kept for 4 weeks at room temperature, while those kept at 4 ^{o}C remained microbiologically stable throughout 12 weeks of storage. It was also found that all four anthocyanin compounds in aqueous black rice extract, with and without added ingredients and carbonation, followed first-order reaction kinetic during storage. Cyanidin-3-rutinoside was the most stable anthocyanin compound, followed by peonidin-3-glucoside, cyanidin-3,5-diglucoside, and cyanidin-3-glucoside. Cyanidin-3-glucoside and peonidin-3-glucoside were stabilized by addition of 12% sucrose, while cyanidin-3,5-diglucoside and cyanidin-3-rutinoside were stabilized by addition of 0.3% citric acid. In conclusion, aqueous pigmented rice extracts have the potential of being the bases for antioxidant drinks.

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

PENILAIAN EKSTRAK AKUEUS- BERAS BERPIGMEN SEBAGAI ASAS MINUMAN ANTIOKSIDAN

Oleh

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Beras berpigmen, yang terdiri daripada beras hitam dan beras merah, dikategorikan sebagai makanan berfungsi disebabkan kandungan antioksidannya, yang disumbangkan terutama oleh antosianin beras hitam dan proantosianidin beras merah. Walau bagaimanapun, oleh kerana kebanyakan sebatian antioksidan dalam beras berpigmen adalah hidrofilik, kehilangan antioksida boleh berlaku semasa memasak nasi di dalam air yang berlebih. Oleh itu, antioksidan dalam beras berpigmen ini diekstrak menggunakan air panas dan ekstrak tersebut dinilai sebagai asas minuman antioksidan. Objektif daripada kajian ini adalah untuk menentukan keadaan pemprosesan yang optimum untuk aktiviti memerangkap radikal DPPH, jumlah kandungan flavonoid dan jumlah kandungan fenolik maksimum dalam ekstrak akueus beras berpigmen; untuk membandingkan kapasiti antioksidan ekstrak dengan minuman buah-buahan komersial; untuk menilai ciri fizikal daripada ekstrak akueus beras berpigmen; untuk menilai ciri fizikal, antioksidan, dan mikrobiologi ekstrak akueus beras berpigmen semasa penyimpanan; untuk menentukan kesan bahan (asid sitrik, natrium benzoate, dan sukrosa) dan pengkarbonan pada kinetik degradasi empat sebatian utama antosianin dalam ekstrak akueus beras hitam. Keadaan pemprosesan yang optimum untuk ekstrak akueus beras berpigmen dikaji menggunakan kaedah gerak balas permukaan dan ciri fizikal dan antioksidan daripada ekstrak yang optimum kemudian dikaji. Ekstrak akueus beras berpigmen kemudian disimpan selama 12 minggu dalam suhu bilik dan suhu dingin untuk menilai stabilitas fizikal, mikrobiologi, dan antioksidan ekstrak selama penyimpanan. Akhirnya, *high performance liquid chromatography* (HPLC) digunakan untuk menentukan kinetic degradasi antosianin dalam ekstrak akueus beras hitam. Ia telah didapati bahawa ekstrak akueus beras hitam mempunyai lebih tinggi ($p<0.05$) kuasa antioksidan daripada ekstrak akueus beras merah, dan kedua-dua ekstrak beras mempunyai lebih tinggi ($p<0.05$) kuasa antioksidan berbanding tiga minuman buah-buahan komersial yang dinilai dalam kajian ini. Tidak terdapat perbezaan yang signifikan di antara ekstrak akueus beras hitam dan merah dari segi pH, kelikatan, dan jumlah pepejal larut. Walau bagaimanapun, dari segi warna, ekstrak akueus beras hitam adalah ketara lebih gelap, lebih biru, dan lebih merah daripada ekstrak

akueus beras merah. Syarat-syarat pengekstrakan air panas optimum bagi beras hitam adalah nisbah air/beras sebanyak 20 ml/g pada 92 °C selama 40 minit, manakala bagi beras merah adalah nisbah air/beras 20 ml/g pada 97 ºC untuk 30 minit. Dari segi kestabilan penyimpanan, variasi dari segi kandungan antioksidan dan ciri fizikal lebih diperhatikan pada suhu penyimpanan 25 °C daripada di 4 °C. Ekstrak dapat disimpan selama 4 minggu pada suhu bilik, manakala yang disimpan di 4 °C stabil dari aspek mikrobiologi sepanjang 12 minggu penyimpanan. Ia juga telah didapati bahawa kesemua sebatian antosianin dalam ekstrak akueus beras hitam, dengan dan tanpa bahan tambah, mengikuti kinetik tindakbalas tertib-pertama semasa penyimpanan. Cyanidin-3-rutinosida adalah sebatian antosianin yang paling stabil, diikuti oleh peonidin-3-glukosida, cyanidin-3,5-diglukosida, dan cyanidin-3-glukosida. Cyanidin-3-glukosida dan peonidin-3-glukosida lebih stabil dengan penambahan 12% sukrosa manakala cyanidin-3,5-diglukosida dan cyanidin-3-rutinosida lebih stabil dengan penambahan 0.3 % asid sitrik. Kesimpulannya, menurut hasil kajian ini, ekstrak akueus beras berpigmen mempunyai potensi menjadi asas untuk minuman antioksidan.

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I certify that a Thesis Examination Committee has met on 10 November 2014 to conduct the final examination of Adyati Putriekasari Handayani on her thesis entitled "Evaluation of Aqueous- Pigmented Rice Extracts as Bases for Antioxidant Drinks" 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 degree of Master of Science.

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

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ACE	Angiotensin I-converting enzyme
ACN	Acetonitrile
AE	Antioxidant extractability
$AlCl_3$	Aluminium chloride
ANOVA	Analysis of variance
ASE	Accelerated solvent extraction
AUC	Areas under the curves
BRD	Black rice drink
$C_6H_8O_7$	Citric acid
Ca	Calcium
CCD	Central composite design
CE	Catechin equivalent
cGMP	Cyclic guanosine monophosphate
CH_3COONa	Sodium acetate
CO_2	Carbon dioxide
Cy	Cyanidin
DM	Diabetes mellitus
DNA	Deoxyribonucleic acid
Dp	Delphinidin
DPPH	2,2-diphenyl-1-picrylhydrazyl
DRBC	Dichloran rose bengal chloramphenicol
EMB	Eosin methylene blue
eNOS	Endothelial nitric oxide synthase
ESR	Electron spin resonance
EU	European union
Fe	Ferrum
$Fe(III)(TPTZ)_2$	Ferric 2,4,6-tripyridyl-s-triazine
$FeCl_3.6H_2O$	Ferric chloride hexahydrate
$FeSO_4$	Ferrous sulfate
FRAP	Ferric reducing ability power
GAE	Gallic acid equivalent
GC-TOFMS	Gas chromatography-time-of-flight mass spectrometry
GMP	Good manufacturing practice
GPC	Gel permeation chromatography
GTP	Guanosine-5'-triphosphate
H-NMR	Hydrogen-1 nuclear magnetic resonance
H_2CO_3	Carbonic acid
H_2O	Water
H_2O_2	Hydrogen peroxide
H_3PO_4	Phosphoric acid
HCl	Hydrochloric acid
HCOOH:MeOH	Formic acid:methanol
HDPP	High density polypropylene
HPLC	High performance liquid chromatography
HSV	Herpes simplex virus
HTST	High temperature short time
HWE	Hot water extraction

IC_{50}	Half maximum inhibitory concentration
IL	Interleukin
IQ	2-amino-3-methylimidazo [4,5-f] quinoline
IR	Infrared
KHN	Khao Hom Nin
kHz	Kilohertz
KOH	Potassium hydroxide
LC-MS	Liquid chromatography-mass spectrometry
LDL	Low-density lipoprotein
LDL-TC	Low-density lipoprotein total cholesterol
MeOH	Methanol
Mg	Magnesium
MMP	Matrix metalloproteinase
Mn	Manganese
mPas	Millipascal second
mRNA	Messenger ribonucleic acid
Mv	Malvidin
Na_2CO_3	Sodium carbonate
NaCl	Sodium hydroxide
$NaNO_2$	Sodium nitrate
NaOH	Sodium hydroxide
NBT	Nitro-blue tetrazolium
NO	Nitric oxide
PET	Polyethylene terephthalate
Pg	Pelargonidin
pKa	Acid dissociation constant
Pn	Peonidin
Pt	Petunidin
RAS	Reninangiotensin system
ROS	Reactive oxygen species
RRD	Red rice drink
RSM	Response surface methodology
RT-PCR	Reverse transcription-polymerase chain reaction
Se	Selenium
S_N1	Nucleophilic substitution 1 reaction
SOD	Superoxide dismutase
TAG	Triacylglycerol
TC	Total cholesterol
TE	Trolox equivalent
TFA	Trifluoroacetic acid
TFC	Total flavonoid content
TMAC	Total monomeric anthocyanin content
TNF	Tumor necrosis factor
TPC	Total phenolic content
TPTZ	2,4,6-tripyridyl-s-triazine
u-PA	Urokinase-type plasminogen activator
USA	United States of America
UV-Vis	Ultraviolet-visible
v/v	Volume per volume

W/R	Water/rice
wt./vol	Weight per volume
Zn	Zinc
μg	Microgram
μL	Microlitre
μM	Micromolar

CHAPTER 1

INTRODUCTION

Consumers' concerns about convenient foods and healthy diets have become significantly greater recently. In Malaysia and Taiwan, there are four important factors affecting food choice, which are health, natural content, weight control, and convenience (Prescott, 2002). Consumers have started to become aware of health and nutrition, together with westernisation of food habits and increasing occurence of non-chronic non-communicable diseases (Malaysian Ministry of Health, 1996). Based on these facts, production of functional food and beverages is now increasing.

Functional food is defined as "Food that may provide health benefits beyond basic nutrition", or "Food similar in appearance to conventional food that is intended to be consumed as part of a normal diet, but has been modified to subserve physiological roles beyond the provision of simple nutrient requirements" (Bech-Larsen and Grunert, 2003) in some countries, including 11 Asian countries and regions (China, Indonesia, Japan, Malaysia, Myanmar, the Philippines, Singapore, South Korea, Taiwan, Thailand, and Vietnam) (ILSI-SEAR, 2004). Pigmented rice can be categorized as functional food due to its various health benefits. Previous investigations had shown that anthocyanins and proanthocyanidins contained in black and red rice possessed anti-inflammatory and anti-oxidative activities (Hu *et al.*, 2003). Significant inhibition of atherosclerotic plaque formation was also shown by dietary supplementation of black rice pigments to rabbits (Ling *et al.*, 2002). In one study conducted by Kamei *et al.* (1998), it was shown that anthocyanins inhibited cell invasion of different cancer cells. Due to their anthocyanin content, both black rice and red rice also show good potential to be natural food colorants (Abdel-Aal *et al.*, 2006). Itani and Ogawa (2004) mentioned that red rice started to become more popular in Japan as a functional food due to its high polyphenol content.

Unfortunately, since major antioxidant compounds contained in red and black rice are water-soluble components, they tend to be soluble in the cooking water used to cook the rice, since these types of rice are generally cooked in a pot using excess water (absorption method) (Hiemori *et al.*, 2009). Therefore, it is a good option to retain those hydrophilic compounds by using hot water extraction method and transform the extracts into a new variety of functional drinks. The drinks will show natural deep dark purple colour for black rice drink and red brown colour for red rice drink due to the presence of anthocyanins and proanthocyanidins. Currently, in the market, berries and blackcurrants are the most common sources of anthocyanins used as natural colorants for beverages, but these fruits are not normally planted in Malaysia. Pigmented rice is basically underutilized worldwide as the presence of bran resulted in its cooked rice tasting harsh. Black rice is mostly found in China, followed by Sri Lanka, Indonesia, India, The Philippines, Bangladesh, Malaysia, Thailand, and Myanmar (Chaudhary, 2003), while red rice is considered as a common food crop in China, Bhutan, Sri Lanka, India, The Philippines, and other Asian countries (Srinivas, 1976; Villareal *et al.*, 1989; Perera and Jansz, 2000; Ling *et al.*, 2001; Itani and Ogawa, 2004), with up to 9 times lower in price than fresh berries in the market. In Malaysia itself, black rice is currently planted in Sarawak.

Therefore, it is easier and cheaper to obtain these two types of rice as sources of anthocyanins than berries and blackcurrants.

The objectives of the first part of the study were to determine the optimum processing conditions (extraction temperature, time, and water/rice ratio) for maximum DPPH radical scavenging activity, total flavonoid content, and total phenolic content in aqueous pigmented rice extracts, to compare the antioxidant capacities of the extracts with that of commercial fruit drinks, and to determine the physical properties of the extracts. The second part of the study involved the evaluation of the physical, antioxidant, and microbiological properties of the aqueous pigmented rice extracts during storage. Lastly, the third part of the study was to determine the effects of ingredients (citric acid, sodium benzoate, and sucrose) and carbonation on degradation kinetics of four major anthocyanin compounds (cyanidin-3-glucoside, cyanidin-3,5-diglucoside, peonidin-3-glucoside, cyanidin-3-rutinoside) in aqueous black rice extract.

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