องค์ประกอบของสารต้านอนุมูลอิสระ กิจกรรมของสารต้านอนุมูลอิสระ และเสถียรภาพระหว่างการเก็บรักษาของรำจากข้าวสี และการประยุกต์ใช้รำจากข้าวสีในขนมปัง

นายธรรณพ เหล่ากุลดิลก

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ANTIOXIDANTS COMPOSITION, ANTIOXIDANT ACTIVITIES AND STORAGE STABILITY OF PIGMENTED RICE BRANS AND THEIR APPLICATIONS AS FOOD INGREDIENT IN WHEAT BREAD

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Thesis Title	ANTIOXIDANTS COMPOSITION, ANTIOXIDANT ACTIVITIES AND STORAGE STABILITY OF PIGMENTED RICE BRANS AND THEIR APPLICATION AS FOOD INGREDIENT IN WHEAT BREAD
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ธรรณพ เหล่ากุลดิลก : องค์ประกอบของสารต้านอนุมูลอิสระ กิจกรรมของสารต้านอนุมูลอิสระ และเสถียรภาพระหว่างการเก็บรักษาของรำจากข้าวสี และการประยุกต์ใช้รำจากข้าวสีในขนมปัง. (ANTIOXIDANTS COMPOSITION, ANTIOXIDANT ACTIVITIES AND STORAGE STABILITY OF PIGMENTED RICE BRANS AND THEIR APPLICATION AS FOOD INGREDIENT IN WHEAT BREAD) อ. ที่ปรึกษาวิทยานิพนธ์หลัก : ศ.ดร. วรรณา ตุลยธัญ, อ. ที่ปรึกษาวิทยานิพนธ์ร่วม: Professor Charles F. Shoemaker, Ph.D., รศ. ดร. ศักดิ์ดา จงแก้ววัฒนา, 153 หน้า.

งานวิจัยนี้ได้หาเอกลักษณ์และวิเคราะห์ปริมาณสารต้านอนุมูลอิสระของตัวอย่างข้าวแดง1 พันธุ์ และข้าวดำ 3 พันธุ์เปรียบเทียบกับข้าวปกติ ในส่วนการขัดสี (milling fractions) ที่แตกต่างกัน พบว่า ในส่วนของรำข้าวมีปริมาณสารต้านอนุมูลอิสระสูงสุด และสูงกว่าส่วนของข้าวกล้องและข้าวขาว 7 และ 62 เท่า ตามลำดับ ปริมาณสารต้านอนุมูลอิสระในข้าวดำสูงกว่าข้าวแดงและข้าวปกติ โดยรำจากข้าวดำเบอร์1 มีสารต้านอนุมูลอิสระสูงสุด สารแอนโทไซยานินหลักในข้าวสีทุกตัวอย่าง คือ cyanidin-3-glucoside และpeonidin-3-glucoside โดยพบว่า cyanidin-3-glucoside ซึ่งพบ 90-95% (ยกเว้นข้าวดำเบอร์ 2 พบ 58%) เป็นสารแอนโทไซยานินส่วนใหญ่ในข้าวสี นอกจากนั้นยังพบว่ากรดฟีนอลิกในข้าวส่วนใหญ่เป็นกรดฟีนอลิกที่ยึดเกาะกับองค์ประกอบอื่น ซึ่งกรดฟีนอลิกที่จำแนกได้ทั้ง 6 ได้แก่ gallic, protocatechuic, hydroxybenzoic, *p*-coumaric, ferulic, และ sinapic acids ข้าวดำมี protocatechuic และ hydroxybenzoic acids สูงกว่าข้าวแดงและข้าวปกติ เมื่อนำตัวอย่างข้าวมาสกัดด้วยเมทานอลและนำไปวิเคราะห์กิจกรรมของสารต้านอนุมูลอิสระพบว่า สารสกัดจากรำข้าวมี DPPH radical scavenging, reducing power และ lipid peroxidation inhibition สูง

เมื่อทดลองนำรำข้าว ได้แก่ รำข้าวแดง รำข้าวดำเบอร์1และรำข้าวปกติมาเสริมในขนมปัง โดยทดแทนแป้งสาลีที่ 5 และ 10% โดยมีขนมปังปกติเป็นตัวอย่างควบคุม พบว่าการเสริมรำข้าวเพิ่ม G'(storage modulus) G" (loss modulus) ของโดขนมปัง และลด deformation ของการทดสอบ creep test ขนมปังเสริมรำข้าวทุกตัวอย่างมีกิจกรรมของสารต้านอนุมูลอิสระสูงกว่าตัวอย่างควบคุม และยังพบสารต้านอนุมูลอิสระจากรำข้าวในขนมปังหลังการอบ โดยเฉพาะอย่างยิ่ง แกมม่าโอรีซา-นอลที่สูญเสียเพียง 16-34% หลังการอบ และยังพบสาร cyanidin-3-glucoside ในขนมปังเสริมรำข้าวดำแต่สูญเสีย 82-89% หลังการอบ การเสริมรำข้าวที่ 5% ไม่ทำให้เกิดผลเสียต่อคุณภาพขนมปัง อย่างไรก็ตามเมื่อเสริมรำข้าวที่ 10% ลด specific volume และเพิ่ม crumb firmness ของผลิตภัณฑ์

การคงสภาพรำข้าวโดยการให้ความร้อนด้วยไอน้ำสามารถยับยั้งเอนไซม์ไลเปส และพบว่ารำข้าวมีกรดไขมันอิสระน้อยกว่า 10% หลังเก็บรักษานาน 2 เดือน การคงสภาพรำข้าวดำทำลายสารแอนโทไซยานิน 80-100% ในระหว่างการเก็บรักษารำข้าวในถุงผ้าในอุณหภูมิห้อง แอลฟ่าโทโคฟีรอลมีค่าอัตราการสลายตัวสูงสุดเมื่อเทียบกับสารต้านอนุมูลอิสระอื่น

ภาควิชา เทคโนโลยีทางอาหาร…….. ลายมือชื่อนิสิต.....

สาขาวิชา เทคโนโลยีทางอาหาร……..ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก……...

ปีการศึกษา 2553……....ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์ร่วม ……...

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KEYWORDS : PIGMENTED RICE / BRAN / ANTIOXIDANT / PHENOLIC ACIDS / ORYZANOL

THUNNOP LAOKULDILOK : ANTIOXIDANTS COMPOSITION, ANTIOXIDANT ACTIVITIES AND STORAGE STABILITY OF PIGMENTED RICE BRANS AND THEIR APPLICATIONS AS FOOD INGREDIENT IN WHEAT BREAD. THESIS ADVISOR : PROF. VANNA TULYATHAN, Ph.D., THESIS CO-ADVISOR : PROF. CHARLES F. SHOEMAKER, Ph.D. AND ASSOC. PROF. SAKDA JONGKAEWWATTANA, Ph.D., 153 pp.

One red rice and 3 black rice samples were identified and quantified their antioxidant compounds compared with normal rice in different milling fractions. Bran was the milling fraction containing major portion of rice antioxidants. On average, bran contained 7 and 62 times of total antioxidants content higher than unmillled and milled fraction, respectively. The antioxidants content in black rice was higher than in red and normal rice. The highest antioxidant content was found in black rice bran no.1. The major anthocyanins of pigmented rice were cyanidin-3-glucoside and peodinin-3-glucoside. Cyanidin-3-glucoside was the major anthocyanin found in all pigmented rice samples, the percentage of which was 90-95% (except black rice no.2; 58%). Most of phenolic acids in rice were bound phenolic. Six phenolic acids were detected in this study including gallic, protocatechuic, hydroxybenzoic, *p*-coumaric, ferulic, and sinapic acids. Black rice contained higher contents of protocatechuic and hydroxybenzoic acids than red and normal rice. The methanolic extracts of rice bran samples showed high antioxidant activities in all measurements which were the determination of DPPH radical scavenging activity, reducing power and lipid peroxidation inhibition. The antioxidant effects of pigmented rice are related to their phenolic pigments in the bran.

Rice brans were studied for their potential as food used by adding into wheat bread. Brans from normal, red and black rice no.1 were selected to substitute with wheat flour at 5 and 10%, compared with traditional bread as a control. Addition of rice bran increased G' (storage modulus) and G" (loss modulus) of bread doughs and also decreased the deformation of creep test. All wheat bread added rice bran (WRB) showed higher antioxidant activities than control. Rice antioxidants were retained in bread after baking especially γ-oryzanol which lost only 16-34% by baking. Cyanidin-3-glucoside was detected in black rice bran added breads and lost by 82-89% after baking. 5%WRB showed no adverse effect on baking qualities. However, addition of rice bran at 10% reduced specific volume and increased crumb firmness of final bread products.

Stabilization of rice bran by using opened steam heating was able to deactivate lipase enzyme in rice bran and after 2 months of storage, stabilized rice bran contained <10% of free fatty acid. Stabilization destroyed rice bran antioxidants especially black rice bran which lost 80-100% of their anthocyanins. During storage at ambient temperature in cloth bags, α-tocopherol showed the highest degradation rate constant as compared to other antioxidants. Black rice bran retained high antiradical and antioxidant activities throughout the storage at ambient temperature.

Department : Food Technology Student's Signature .....

Field of Study : Food Technology Advisor's Signature .....

Academic Year : 2010 Co-Advisor's Signature .....

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CHAPTER I

INTRODUCTION

In recent years, there has been an increasing of interest in natural antioxidants in food. Several studies confirmed the beneficial effects of antioxidants against many chronic diseases such as coronary heart disease, cancer, diabetes and inflammatory. For food industries, antioxidants are used to preserve food by retarding deterioration, rancidity or discoloration due to oxidation. The demand of natural antioxidants was widely expanded because of the adverse effects reported of many synthetic antioxidant compounds. Therefore, new sources of natural antioxidants were desirable for customers and many food industries.

Rice is the major crop of the world and is known to be a source of many antioxidants. However, only small amount of rice antioxidants is intake by human because we almost consume rice in milled form. Major portion of rice antioxidants locate at outer layer of caryopsis which is removed by milling process. Rice bran is a by-product of milling process which is rich in antioxidant compounds. Rice bran may be discarded or used as animal feed. Thus, rice bran has been considered to be the cheap source of natural antioxidants. Moreover, pigmented rice varieties have been developed in many countries because of their high antioxidants containing. Many studies reported that the bran of pigmented rice varieties showed greater antioxidants and free-radical scavenging activity than the bran of non-pigmented rice. However, the literature data of antioxidants composition in pigmented rice are relatively limited. Rare studies reported the antioxidants information of pigmented rice bran. This information is important for further scientific researches. In chapter 3 of this dissertation, we revealed the antioxidants composition and their concentrations in 3 different milling fractions, including bran, unmilled and milled fractions.

Pigmented rice contains many antioxidant compounds; however, the activities of antioxidants in pigmented rice have been rare studied. The activities of antioxidants are influenced by many factors such as structure, polarity, concentration, mechanism of action, lipid composition and temperature. Chapter 4 discussed the data of antioxidant activities of some authentic rice antioxidants compared with some

synthetic antioxidants and also revealed the antioxidant activities of pigmented rice fractions by 3 different antioxidant activities measurement methods.

Despite, rice bran has been incorporated in many bakery products for enhancing dietary fiber and nutrients but very little data on antioxidative properties was studied. Although, pigmented rice bran is a rich source of natural antioxidants, thus its potential as food ingredient has not been studied. Incorporation of pigmented rice bran into bread may produce new health-promoting products. However, the effects of adding rice bran into bread on baking quality and antioxidative property should be considered. Chapter 5 is devoted to study the potential of pigmented rice bran as food used.

Nevertheless, pigmented rice bran may show high potential as food ingredient but the stability of rice bran is the main draw-back. The rapid deterioration of rice bran lipid caused by the activity of naturally occurring lipase enzymes. Lipid deterioration can be prevented by stabilizing rice bran immediately after milling. Heat processes seems to be the only method with commercial potential to inactivate lipase. However, heat may destroyed rice antioxidants in rice bran. The effects of stabilization methods on antioxidative properties and stability of pigmented rice bran were discussed in chapter 6 of this dissertation.

CHAPTER II

LITERATURE REVIEW

2.1 Rice structure and composition

Rice is the second-largest cultivated crop produced worldwide with current annual world production being approximately 583 million metric tons (FAO, 2004). The structure of the rough rice grain is shown in Figure 2.1. The principle parts of the grain are hull, pericarp, seed coat, nucellus, embryo, aleurone layer, and endosperm.

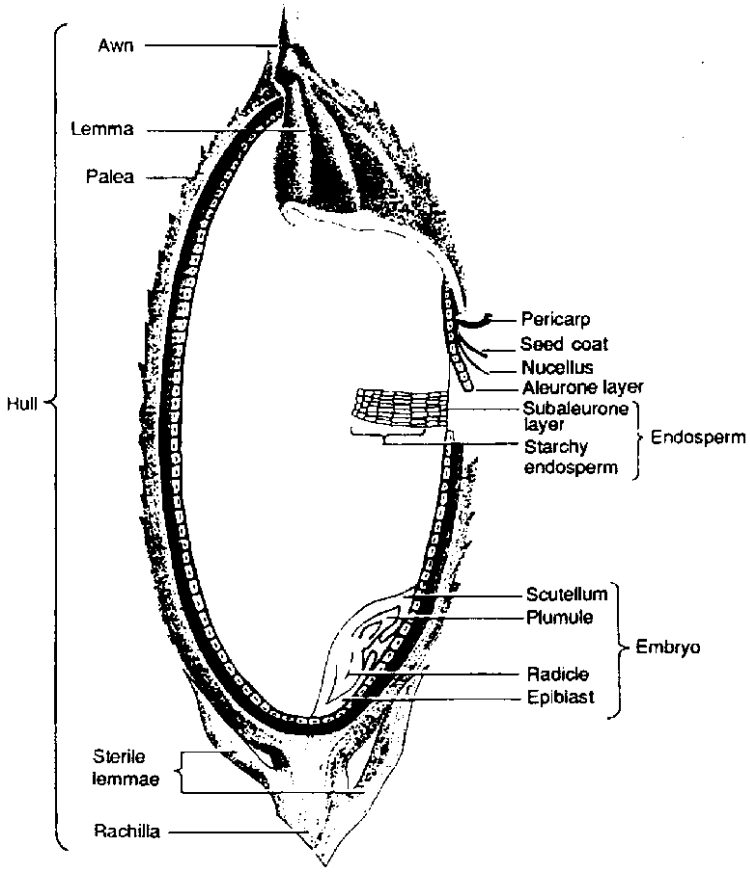


Figure 2.1 Longitudinal section of rice grain

Hull is the outer covering for the caryopsis (brown rice or unmilled rice). It protects the grain from insect and external environment. The hull comprises 18-20% by weight of the rough rice (Juliano and Bechtel, 1985). The hull contains

appreciable silica (silicon dioxide) and small amount of calcium, sodium, magnesium, potassium, manganese, aluminum, iron, copper, and zinc (Juliano and Bechtel, 1985).

After rice dehulling, the outer four layers of the caryopsis are the pericarp, seed coat, nucellus, and aleurone (Figure 2.1). Along with much of the embryo, these layers comprise the bran portion of rice grain. The bran portion is 5-8% of the brown rice weight. The cell of aleurone layers consist of many inclusions called protein bodies. Bran contains high level of protein (13%), fat (16%), and dietary fiber (10%) (Amissah *et al*., 2003).

Further milling of caryopsis removes the subaleurone layer and a small part of the starch endosperm. The subaleurone layer is rich in protein and has fewer lipid bodies than the aleurone layer, but contains a small number of starch granules. The starchy endosperm is rich in starch granules, contain some protein bodies, and almost no lipid bodies.

Rice bran is one of the valuable by-product of milling, constituting approximately 10% weight of raw rice. It is defined by the Rice Millers' Association (RMA) to be the brown outer layer of the brown rice kernel that is removed when milling brown rice to milled or white rice. Rice bran comprises primarily of the pericarp, aleurone, and subaleurone layers of the kernel, and typically includes the embryo or germ and small amount of the starchy endosperm (Marshall and Wadsworth, 1994). It is a rich source of nutrients, which is high in lipid, protein and dietary fiber.

Lipid content in rice bran is approximately 18-22% which contains approximately 75% unsaturated fatty acids. Three major types of lipids in rice bran are oleic, linoleic and palmitic acid (Warren and Farrell, 1990). Rice bran lipid contains more unsaponifiable component than common vegetable oil sources (Orthoefer, 1996). The unsaponifiable component contains a unique complex of naturally occurring antioxidant compounds, of which tocopherols, tocotrienols and γ-oryzanol are the most interested. Protein in rice bran is high in nutrition values (Juliano, 1994) and hypoallergenic (Helm and Burks, 1996). The protein efficiency ratio (PER) of rice bran ranges from 1.59 to 2.04. Available lysine contents of protein concentrates ranged from 54-58.8%. Threonine and isoleucine are limiting

amino acid of rice bran protein. Bran contained 13.4% of water-extractable protein, of which 9.3 and 7.4% were albumins and globulins, respectively.

Rice bran provides health-promoting effects against chronic diseases as well as functional properties in foods such as emulsifying and texture-controlling activities (Hamid-Abdul and Luan, 2000).

2.2 Antioxidants

Halliwell *et al*., (1995) defined an antioxidant as "any substance that, when present at low concentrations compared to those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate," while, Krinsky, (1992) defined antioxidants in aspect of biology as "compounds that protect biological systems against the potentially harmful effects of processes or reactions that cause extensive oxidation."

According to the USDA Code of Federal Regulations, "antioxidants are substances used to preserve food by retarding deterioration, rancidity or discoloration due to oxidation" (Dziezak, 1986). The main justification for using an antioxidant is to extend the shelf life of foodstuffs and to reduce waste and nutritional losses by inhibiting and delaying oxidation. However, antioxidants cannot improve the quality of an already oxidized food product (Sherwin, 1978; Coppen, 1983). Regarding to the mode of action, antioxidants may be classified as free radical scavengers, chealators of metal ions or prooxidants of lipid oxidation or singlet oxygen scavengers (Shahidi and Wanasundra, 1992).

2.2.1 Antioxidant mechanism

A. Inactivation of lipid free radical

Antioxidants can retard lipid oxidation by inactivating or scavenging free radicals therefore inhibiting initiation and propagation reactions of lipid autoxidation (Scheme 2.1). Free radical scavenger or primary chain-breaking antioxidants (AH) are capable of accepting a radical from oxidizing lipids species such as peroxyl ($LOO^{\cdot}$)

and alkoxyl ($LO^{\cdot}$) radicals. Free radical scavenger rapidly donates a hydrogen atom to a lipid radical, or is converted to other stable products (Gordon, 1990).

Because of the rate limiting step of the propagation stage, peroxyl radicals are found in the greatest concentration of all radicals in the systems, thus the free radical scavenger primarily react with peroxyl radicals. Peroxyl radicals also have lower energy than alkoxyl radicals and thus react more readily with the low energy hydrogens of free radical scavenger than with polyunsaturated fatty acids (Liebler, 1993). A free radical scavenger inhibits lipid oxidation by more effectively competing with other compounds (especially unsaturated fatty acids) for peroxyl radicals.

Initiation $LH \longrightarrow L^{\cdot} + H^{\cdot}$ (1)

Propagation $L^{\cdot} + O_2 \longrightarrow LOO^{\cdot}$ (2)

$LOO^{\cdot} + LH \longrightarrow L^{\cdot} + LOOH$ (3)

Termination $LOO^{\cdot} + LOO^{\cdot} \longrightarrow$ (4)

$LOO^{\cdot} + L^{\cdot} \longrightarrow$ non-radical products (5)

$L^{\cdot} + L^{\cdot} \longrightarrow$ (6)

Action of antioxidant

$LOO^{\cdot}$ or $LO^{\cdot} + AH \longrightarrow$ LOOH or LOH + $A^{\cdot}$ (7)

$LOO^{\cdot}$ or $LO^{\cdot} + A^{\cdot} \longrightarrow$ LOOA or LOA (8)

Scheme 2.1 Autoxidation of polyunsaturated lipids and their consequence in quality deterioration of food and action of antioxidant (free radical scavenger)

Antioxidant efficiency is dependent on the ability of the free radical scavenger (antioxidant) to donate hydrogen to the lipid free radical. The reactions are exothermic in nature. The activation energy increases with increasing A–H and L–H

bond dissociation energy. Therefore, the efficiency of the antioxidants increases with decreasing A–H bond strength (Shahidi and Naczk, 2004). So, the transfer of the hydrogen atom to the lipid free radical is more energetically favorable and thus more rapid. The ability of a free radical scavengers to donate a hydrogen atom to a lipid free radical can be predicted from standard one-electron reduction potentials. Any compound that has a reduction potential lower than the reduction potential of a lipid free radical (or oxidized species) is capable of donating a hydrogen to that free radical unless the reaction is kinetically unfeasible. However, the resonance delocalization, and susceptibility to autoxidation influence the effectiveness of the free radical scavenger.

The energy of the resulting free radical scavenger radical ($A^{\cdot}$) is influence on the efficiency of the free radical scavenger. The $A^{\cdot}$ with low energy will decrease catalyzing the oxidation of other molecules. The most efficient free radical scavengers have low energy radicals as a result of resonance delocalization In this regard, phenolic compounds are good hydrogen donors. Efficient free radical scavengers also produce radicals that do not react rapidly with oxygen to form peroxides. Free radical scavenger peroxides (LOOA) can decompose into additional radicals species, which could further promote oxidation. Thus, formation of LOOA can result in consumption of the antioxidant with no net decrease in free radicals numbers (Shahidi and Wanasundra, 1992; and Nawar, 1996).

B. Control of lipid prooxidants

Food lipid oxidation is also catalyzed by transition metals, singlet oxygen and enzyme lipoxygenase. Plant phenolic compounds are not only known act as free radical terminators but they may chelate metal ions and quench singlet oxygen. Control of lipid oxidation catalysts can therefore be a very important factor in controlling oxidative rancidity.

Transition metals accelerate lipid oxidation reactions by hydrogen abstraction and peroxide decomposition, resulting in the formation of lipid free radicals (Kanner, German and Kinsella, 1987). The activity of prooxidative metals is influenced by chelators or sequestering agents. Metal chelating agents may have a dramatic effect on increasing the oxidation stability through blocking the pro-oxidant

metal ions, and thus limiting the formation of chain initiators by preventing metal-assisted homolysis of hydroperoxides (LOOH). The metal chelating characteristics of natural phenolics, such as flavonoids, are also an important factor in their antioxidant activities (Chen and Ahn, 1998).

Singlet oxygen is an excited state of oxygen in which two electrons in the outer orbitals have opposite spin directions. Lipid oxidation can be catalyzed by singlet oxygen to induce the formation of lipid peroxides from unsaturated fatty acids. The singlet oxygen can be deactivated by quenchers. Tocopherols can chemically quench singlet oxygen in reactions that lead to the formation of tocopherol peroxides and epoxides (Bradley and Min, 1992). The physical quenching singlet oxygen by tocopherol base on charge transfer mechanism, which is the charge transfer complex forming of tocopherols with the electron-deficient singlet oxygen molecule.

Lipoxygeneses, the enzyme found in plants and some animal tissue, is the cause of lipid oxidation. Lipoxygenase activity can be controlled by heat to inactivate and plant breeding programs that decrease the concentrations of these enzymes. Moreover, phenolics are capable of indirectly inhibiting lipoxygenase activity by acting as free radical inactivators, but reduce the iron in the active site of the enzyme to the inactive form (Laughton *et al*., 1991).

C. Surface active antioxidants and physical effects

Oil-in-water emulsions, water-in-oil emulsions, the air-lipid interface of bulk oils and solid fats, and the water-lipid interface of biological membranes are often found in food systems. The oxidative reaction are prevalent at interfacial surface, results of increased contact with oxygen, the presence of free radicals, reactive oxygen, prooxidative metals and the migration of the more polar lipid peroxides out of the hydrophobic lipid core toward the more polar interface.

The effectiveness of phenolic antioxidants is often dependent on their polarity. Porter, Black and Drolet (1989) used the term ‘‘antioxidant paradox’’ to describe how polar antioxidants are most effective in bulk lipids while nonpolar antioxidants are most effective in dispersed lipids. In bulk tocopherol-stripped corn oil, Trolox (a water-soluble analog of α-tocopherol) more effectively inhibited lipid peroxide formation than α-tocopherol. However, when tocopherol-stripped corn oil

was emulsified with Tween 20, α-tocopherol inhibited peroxide formation more effectively than Trolox. The observed increase in activity of α-tocopherol compared to Trolox in emulsified oil was attributed to its retention in the oil and possibly to its ability (due to its surface activity) to concentrate at the oil–water interface. The lower activity of Trolox in emulsions was due to its partitioning into the water phase, where it was not able to inhibit autoxidation of the corn oil (Huang *et al*., 1996).

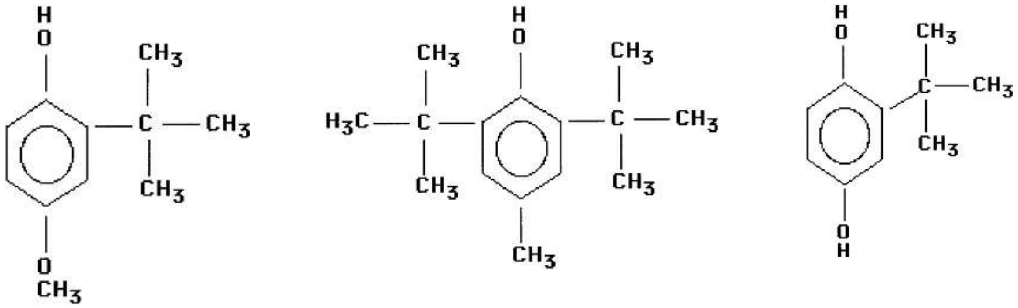
D. Antioxidant interaction

Food systems usually contain many antioxidants, which have different potential functions, including inhibition of prooxidants of different types (e.g., metals, reactive oxygen species, enzymes), inactivation of free radicals and prooxidants in aqueous, interfacial, and lipid phases; and inactivation of compounds at different stages of oxidation. Combinations of chelators and free radical scavenger often result in synergistic inhibition of lipid oxidation. Synergistic interaction most likely occurs by a ‘‘sparing’’ effect provided by the chelator. Since the chelator will decrease oxidation rates by inhibiting metal-catalyzed oxidation, fewer free radicals will be generated in the system. This means that the eventual inactivation of the free radical scavenger through reactions such as termination or autoxidation will be slower, thus making its concentration greater at any given time. The combination of chelator and free radical scavenger thus decreases free radical generation and increases radical scavenging potential. Synergistic antioxidant activity can also be observed by the combination of two or more different free radical scavenger. This occurs when one free radical scavenger reacts more rapidly with free radicals than the other as a result of differences in bond disassociation energies and/or stearic hindrance of free radical scavenger /$LOO^{\cdot}$ interactions (Nawar, 1996).

2.2.2 Synthetic antioxidants

Antioxidants prolong shelf-life of food by protecting them against deterioration caused by oxidation, such as rancidity, color changes and loss of nutrition. Synthetic antioxidants such as BHA (Butylated hydroxyanisole), BHT (Butylated hydroxytoluene) and propyl gallates (Figure 2.2) have been used in food industries. BHA effectively controls the oxidation of animal fats, but is a relatively

ineffective antioxidant in most vegetable oils. BHA provides good carry-through, which is the ability to be added to food, survive processing, and remain stable in food, especially in baked products. BHT is a water-insoluble, white, crystalline solid antioxidant that is more soluble in food oils and fats than BHA. BHT is frequently used in combination with BHA in foods because the two antioxidants are synergistic in their actions. Propyl gallate is a white to light gray, crystalline antioxidant that is partially soluble in water, alcohol, ether, vegetable oils, and lard. It is used as an antioxidant in foods, fats, oils, ethers, emulsions, waxes, and transformer oils (German, 2001). However, in recent years, there has been an enormous demand for natural antioxidants mainly because of adverse toxicological reports on many synthetic antioxidant compounds (Madhavi, Deshpande and Salunkhe, 1996). Therefore, the importance of natural antioxidants has increased greatly.



BHA BHT Propyl gallate

Figure 2.2 BHA, BHT and propyl gallate, the synthetic antioxidants, structure

2.2.3 Rice antioxidants

In recent years there has been an increased interest in antioxidants present in food. In any biological system an important balance must be maintained between the formation of reactive oxygen and nitrogen species (ROS and RNS, respectively) and their removal. ROS and RNS are formed regularly as a result of normal organ functions such as cell aerobic respiration (Bagchi and Puri, 1998; and Gutteridge and Halliwell, 2000). To maintain an oxido/redox balance, organs protect themselves from the toxicity of excess ROS/RNS in different ways, including the use of endogenous

and exogenous antioxidants. Increasing intake of dietary antioxidants may help to maintain an adequate status and therefore the normal physiological function of a living system (Kaur and Kapoor, 2000). Several epidemiogical studies have showed that a dietary intake of foods rich in natural antioxidants correlates with reduced risk of coronary heart disease, cancer, inflammatory and aging related disorder (Garewall, 1997).

Rice is a source of many natural antioxidants which mainly including of phenolic acids, vitamin E, and γ-oryzanol. In addition, anthocyanins are also found in pigmented rice varieties. Consuming of these antioxidants have been reported to associated with reduce the risks of many chronic disease such as cardiovascular disease, diabetes, and cancer (Hudson *et al*., 2000; McPeak, Rukmini and Sastry, 2001; Zhao *et al*., 2004; and Chen *et al*., 2006).

A. Phenolic acids

Phenolic compounds in food originate from one of the main classes of secondary metabolites in plants derived from phenylalanine and, to a lesser extent in some plants, also from tyrosine (van Sumere, 1989; Shahidi, 2000, 2002). Chemically, phenolics can be defined as a class of chemical compounds consisting of a hydroxyl group attached to an aromatic hydrocarbon group.

It is recognized that, phenolic compounds can act as antioxidants by radical scavenging (Sroka and Cisowski, 2003). They inhibit lipid oxidation by rapidly donating a hydrogen atom to the lipid radicals. Phenolic antioxidants are excellent hydrogen or electron donors, and their radical intermediates are relatively stable due to resonance delocalization and generally lack of suitable sites for attack by molecular oxygen. The reaction of a phenol with a lipid free radical forms a phenoxy radical, which is stabilized by delocalization of unpaired electrons around the aromatic ring (Shahidi and Naczk, 1995). The phenol itself is not active as an antioxidant, substitution at the *ortho* and *para* positions with alkyl groups (ethyl or n-butyl) increases the electron density of the OH moiety by inductive effect enhancing its reactivity toward lipid free radicals. The position and the degree of hydroxylation are of primary

importance in determining antioxidant activity (Dziedzic and Hudson, 1984). The introduction of a second hydroxyl group at the *ortho* or *para* position of a phenol also increases its antioxidant activity (Fot *et al*., 1996). The stability of the phenoxy radical is increased by bulky groups at the *ortho* positions. However, the presence of bulky substituents in the 2 and 6 positions reduces the rate of reaction of the phenol with lipid free radicals. This steric effect opposes the increased stabilization of the radical, and both effects must be considered in assessing the overall activity of an antioxidant (Gordon, 1990). Milic, Djilas and Canadanovic-Brunet (1998) showed that the ability of phenolic acids to scavenge lipid alkoxy radicals ($LO^{\cdot}$) depended on their structure and the number and position of the hydroxyl groups. Using an ESR spin trapping technique they showed the antioxidant effect increased in the order of gallic > caffeic > chlorogenic > vanillic > salicylic acids for a hydroperoxide-enriched sunflower oil model system. Phenolic compounds can also quench singlet oxygen (Foley *et al*., 1999) and chelate metal ions (Brown *et al*., 1998). Andjelkovic *et al*., (2006) reported the ion-chelation properties of some phenolics bearing catechol and galloyl functional groups. The order of activity ranked with binding constants of the complex was chlorogenic acid > caffeic acid > gallic acid > hydroxytyrosol > protocatechuic acid.

Phenolic compounds are ubiquitous in cereals. Phenolic acids, such as *p*-hydroxybenzoic, 3,4-dihydroxybenzoic (protocatechuic acid), vanillic, syringic, *p*-coumaric, caffeic, ferulic, sinapic, chlorogenic, and rosmarinic acids (Figure 2.3) are widely distributed in the plant kingdom. Different phenolics belonging to the benzoic acid, cinnamic acid, flavonoid and tannin classes of compounds may be present in free, esterified/etherified or insoluble bound forms (Ribereau-Gayon, 1972; Salunkhe *et al*., 1982). Thus, ferulic acid may be linked to polysaccharides (Faulds and Williamson, 1999; Hartley, Jones and Wood, 1976; Hatfield, Ralph and Grabber, 1999; Iiyama, Lam and Stone, 1990; Ishii, 1997; Ishii and Hiroi, 1990; Markwalder and Neukom, 1976), lignins (Higuchi *et al*., 1967; Iiyama *et al*., 1990) and suberin (Riley and Kolattukudy, 1975); *p*-coumaric acid may be linked to polysaccharides (Hartley *et al*., 1976), lignins (Grabber *et al*., 2000; Nakamura and Higuchi, 1978), and cutin (Kolattukudy, Espelie and Soliday, 1981). In aleurone layer of

cereal grains, ferulic acid is mainly linked to polysaccharides via an ester bond at the *O*-5 position of arabinofuranose (Ishii, 1997). Adom and Liu (2002) reported that ferulic acid was the major phenolic compound in wheat, oat and rice. The ratio of free, soluble-conjugated and bound ferulic acids was 0.1: 1: 100.

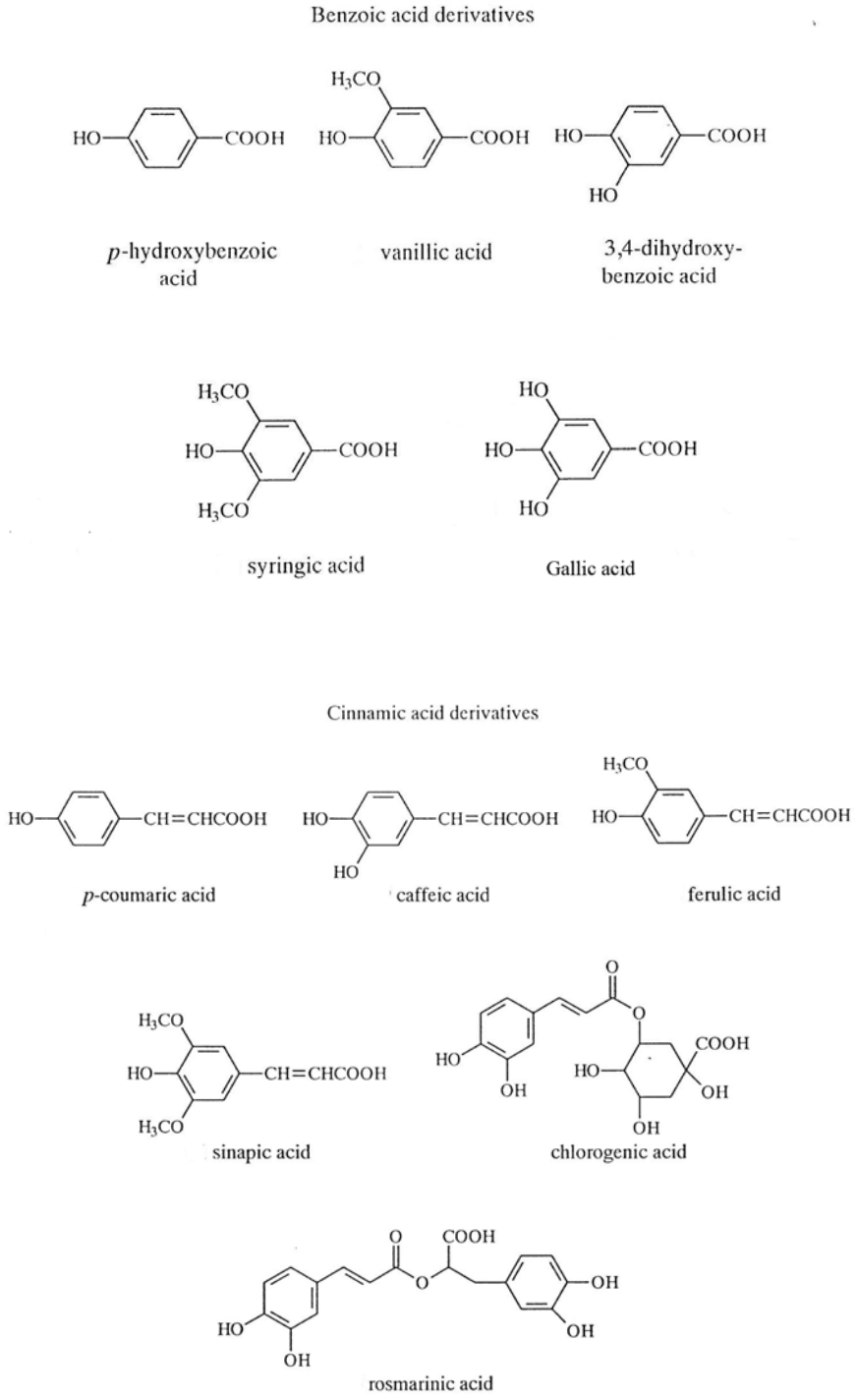


Figure 2.3 Chemical structures of some phenolic acids

Sosulski, Krygier and Hogge (1982) reported rice flour contains 856 mg/kg of phenolic acids and in this respect is similar to oat and wheat flour. Ferulic acid is the major phenolic acid present in rice flour. Bunzel *et al*., (2002) reported almost of the total phenolics in rice are in the insoluble bound form. Moreover, the acids are considered to be present as phenolic–carbohydrate esters because they are released by alkaline solvents. The alkaline extracts of rice endosperm cell walls contain 9.1, 2.5 and 0.56 mg/g of ferulic acid, *p*-coumaric acid and dehydrodimers of ferulic acid, respectively. Yoshizawa *et al*., (1970) reported that rice bran contains a number of sugar–amino acid–phenolic acid conjugates, of which seven contain ferulic acid and at last one an acidic amino acid.

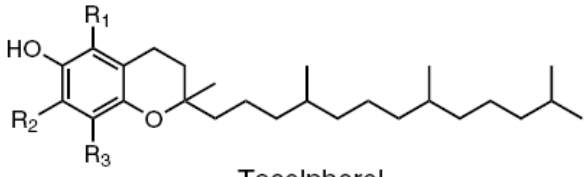
Study of the phenolic acids in rice showed that brown rice contained higher level of phenolic acids content (338 mg/kg dry grain) than milled rice (61 mg,kg dry grain), thereby most of pheolic acids in rice located at the outer layer of grain (Zhou *et al*., 2004). Goffman and Bergman, (2004) reported that the color of rice bran significantly affected the phenolic content and antiradical efficiency. Lloyd, Siebenmorgan and Beers (2000) showed that rice bran collected from various milling breaks of commercial system had varying antioxidant levels. Moreover, long grain rice bran contained more antioxidants than the medium grain rice bran.

B. Vitamin E

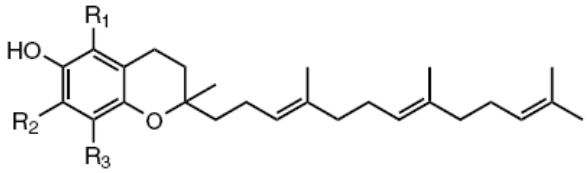
Tocopherols and tocotrienols, known as chromanols, are well recognized for their efficient protection against lipid oxidation in food and biological systems. These components are synthesized by plants and provide essential nutrients for humans and animals. Tocopherols are present in green parts of higher plants, leaves, and oil seeds (Hess, 1993). There are eight structurally different compounds in the tocopherol family (Figure 2.4); four known as tocopherols and four known as tocotrienols. α-Tocopherol is present mainly in the plant cell chloroplasts, while the other three isomers are found outside of these organelles. The basic structure of all eight of these compounds is similar, consisting of a 6-chromanol aromatic ring system containing a

hydroxyl group and a 16-carbon phytol side chain (Bauernfeind, 1997). Tocotrienols differ from tocopherols by the presence of three double bonds in the phytol side chain. Tocopherols and tocotrienols both consist of α, β, γ, and δ isomers, which differ in the number of methyl groups present in the aromatic ring (Figure 2.4). Tocopherols have three chiral centers in the phytol chain, namely, 2′, 4′, 8′, making eight possible stereoisomers.

The most active naturally occurring form of vitamin E is D-α-tocopherol. With regard to vitamin E activity, α-tocopherol is the most potent member of this family. The antioxidant activity decreases from δ to α (Dziezak, 1986). Tocopherols as antioxidants can donate hydrogen atom of the hydroxyl group to the lipid peroxyl radical ($LOO^{\cdot}$) and thus prevent the oxidation of oils. The radical formed from a tocopherol is stabilised through delocalisation of the solitary electron over the aromatic ring structure. This radical forms non-radical products which can be reduced to tocoquinones and to tocopherol dimers. α-Tocopherol has also been associated with retarding the decomposition of hydroperoxides (Frankel, 1996).



Tocolpherol



Tocotrienol

	R_1	R_2	R_3
α	CH_3	CH_3	CH_3
β	CH_3	H	CH_3
γ	H	CH_3	CH_3
δ	H	H	CH_3

Figure 2.4 Tocopherol and tocotrienol structures

Vitamin E is proved for its abilities to prevent and/or slow of the onset of various chronic disease states. Dietary intake of vitamin E has been reported the association with the reduction of the risk of cardiovascular disease. Oxidation of low-density lipoprotein (LDL) is considered to be a major causative factor in development of cardiovascular disease. Tocopherol inhibits biological oxidation of LDL-cholesterol as well as tocotrienol lowers total cholesterol and LDL-cholesterol in serum, therefore reduces the risk of coronary heart disease (Qureshi *et al*., 1997). Tocotrienols present in rice bran inhibit the liver microsomal enzyme HMGCoA reductase (Qureshi and Qureshi, 1992), the key enzyme involved in the endogenous synthesis of cholesterol, and this helps to lower the circulating cholesterol. Tocopherol also possessed the ability to inhibit protein glycoxidation as well as enhances immune response (Ozer, Boscoboinik and Azzi, 1995).

Rice bran is a good source of vitamin E containing up to 150 µg/g (Hargrove, 1994). The major components of vitamin E in rice bran are α-tocopherol, α-tocotrienol, γ-tocopherol and γ-tocotrienol. White rice contains 6.4 µg/g of tocopherols and 5 µg/g of tocotrienols (Sheppard, Pennington and Weihrauch, 1993). The level of tocopherols and tocotrienols found in rice bran were ranged from 41-61µg/g and 155-163µg/g, respectively (Aguilar-Garcia *et al*., 2007). Methanolic extracts of five varieties of long-grained rice (Thai rice) contained 0.35-0.77 mg/g of tocopherol and 0.22-0.46 mg/g of tocotrienol (Chotimarkorn, Benjakul and Silalai, 2008).

C. Gamma-oryzanol

Steryl ferulates were first discovered in rice bran oil in 1954. Since it was isolated from rice bran oil (*Oryza Sativa* L.) and contained a hydroxyl group, it was conveniently named oryzanol. Subsequent studies revealed that oryzanol is not a single compound but instead comprises a variety of ferulic acid esters called α-, β-, and γ-oryzanol. Of these, γ-oryzanol has been the best characterized. The triterpene alcohol components of a typical γ-oryzanol consist primarily of cycloartenol and 24-methylenecycloartanol but they also include other minor sterols, such as campestanol, stigmastanol, β-sitosterol,

cycloartanol, and cholesterol (Graf, 1992). Nearly ten components of γ-oryzanol were identified and having powerful antioxidant activity and occurring naturally only in rice bran. The cycloartenyl ferulate, 24-methylenecycloartanyl ferulate and campesteryl ferulate are the major components (Figure 2.5), accounting for 80% of γ-oryzanol in rice bran oil (Xu and Godber, 1999).

Ferulic acid is found in several cereals and plants. In rice bran ferulic acid is found in ferulic ester forms which possess many health benefits. Ferulic acid is active unit of γ-oryzanol and provides free radical scavenging activity against DPPH radical to γ-oryzanol (Akiyama *et al*., 2005). The metabolic pathway is initiated by two plant enzymes phenylalanine and tyrosine ammonia lyases convert phenylalanine and tyrosine to *trans*-cinnamate and *p*-coumarate, respectively (Graf, 1992). Ferulic acid derives from *p*-coumaric acid through hydroxylation followed by methylation with methionine acting as the methyl donor. Another precursor of γ-oryzanol is phytosterols, which are found mainly in plant cell walls and membranes and are cell membrane components that modulate membrane fluidity (Jiang and Wang, 2005). The difference of ferulic esters in rice bran and other cereals is the dominance of dimethysterols (cycloartenol and 24-methylenecycloartanol) in rice bran, while desmethylsterols (sitosterol, campesterol and their respective saturated forms) in other cereals (Nystrom *et al*., 2007).

γ-Oryzanol is concentrated in rice bran. Raw rice bran contains about 2-4% of γ-oryzanol which is 30-40 times higher than tocopherol content (Chen and Bergman, 2005; Aguilar-Garcia *et al*., 2007). It is a potential antioxidant in food. Because of the high concentration of γ-oryzanol in rice bran oil, it is well known for good stability in frying oil and can be added as natural antioxidants to enhance oil stability. Nystrom *et al*., (2007) reported that in frying condition, γ-oryzanol was more stable than tocopherol. Moreover, addition of rice bran oil has been shown to inhibit oxidation in many foods, such as whole milk powder and refrigerated cooked beef (Nanua, McGregor and Godber, 2000; Kim *et al*., 2003).

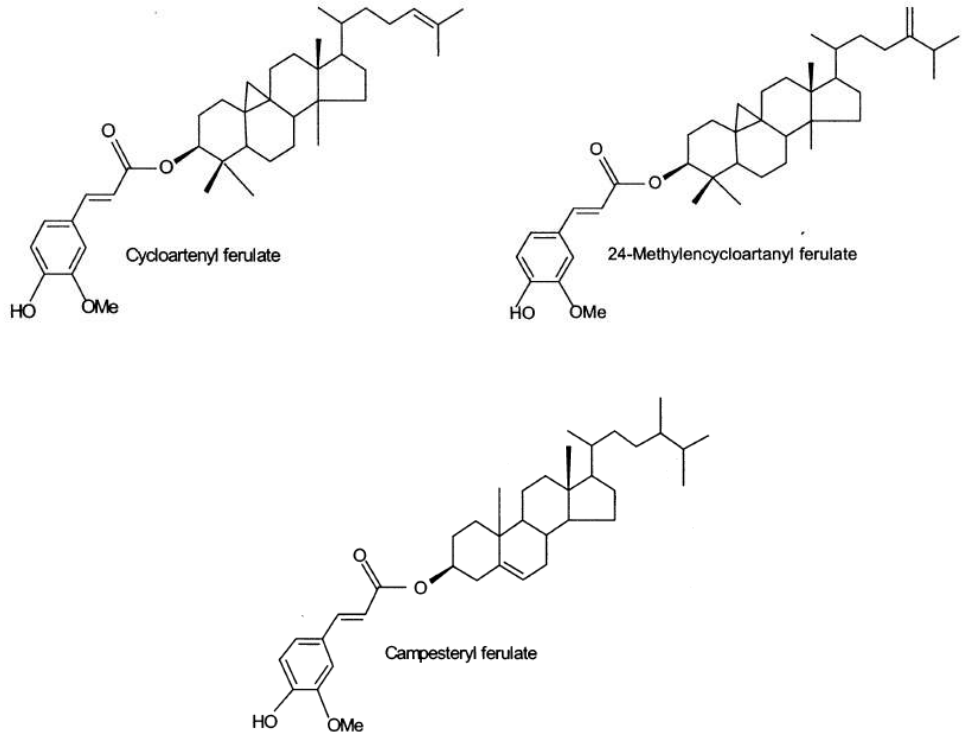


Figure 2.5 Molecular structures of campesteryl, cycloartenyl, and 24-methylenecycloartanyl ferulates.

The antioxidant activity of γ-oryzanol has also been demonstrated by the inhibition of lipid peroxidation in the retina (Fukushi, 1996). Several studies have been done on the biological benefits of γ-oryzanol including decreasing total cholesterol and low density lipoproteins (LDL) and increasing high density lipoproteins (HDL) in serum, decreasing platelet aggregation, anti-inflammatory and alleviate menopausal disorders (Cicero and Gaddi, 2001; Seetharamaiah and Chandrasekhara, 1989; Xu, Hua and Godber, 2001; Yoshino *et al*., 1989). The most importance benefit of γ-oryzanol is its ability to lowering human serum cholesterols.

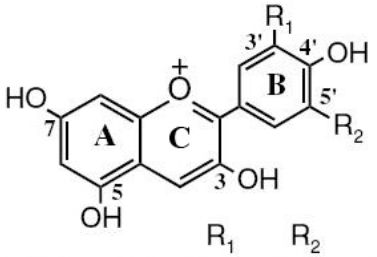
Cholesterol in human diet has been indicated as contributing to the high death rate from coronary heart disease. Epidemiological data suggested a relationship between serum cholesterol level and coronary heart disease. Oxidation of cholesterol produces the cholesterol oxide products which are toxic agents linked to atherosclerosis (Paniangvait *et al*., 1995). Xu *et al*.,

(2001) reported that γ-oryzanol, extracted from rice bran, was a more potent antioxidant of rice bran in the reduction of cholesterol oxidation than vitamin E component. The higher antioxidant activities of γ-oryzanol components may be due to their structure, which is similar to that of cholesterol. Besides, γ-oryzanol also decreases hepatic cholesterol biosynthesis and contains ability to reduce cholesterol absorption (Rong, Ausman and Nicolosi, 1997).

D. Anthocyanins

Anthocyanins are water-soluble flavonoids which are plant secondary metabolites responsible for the blue, purple, and red color of many cereal grains including rice. They occur primarily as glycosides of their respective anthocyanidin-chromophores. The most commonly anthocyanidin aglycones in plant foods are cyanidin (cy), delphinidin (dp), petunidin (pt), peonidin (pn), pelargonidin (pg), and malvidin (mv). These pigments are based on the same flavyrium (2-phenybenzopyrilium) skeleton hydroxylated in 3, 5, and 7 positions, and differing in the number and position of hydroxyl and methoxyl groups in the B ring (Escribano-Bailon, Santos-Buelga and Rivas-Gonzalo, 2004) (Figure 2.6). Anthocyanin often occur in plant foods with a sugar moiety such as glucose, galactose, arabinose, xylose, rhamnose and rutinoside which are located at the position 3 and less frequently 7 of the chromane rings (Escribano-Bailon *et al*., 2004).

There are several hundred known anthocyanins (Andersen and Jordheim, 2006). They vary in 1) the number and position of hydroxyl and methoxyl groups on the basic anthocyanidin skeleton; 2) the identity, number and positions at which sugars are attached; and 3) the extent of sugar acylation and the identity of the acylating agent.



	R_1	R_2
Pelargonidin	H	H
Cyanidin	OH	H
Delphinidin	OH	OH
Peonidin	OCH_3	H
Petunidin	OCH_3	OH
Malvidin	OCH_3	OCH_3

Figure 2.6 Common anthocyanin structures. Sugar moieties are generally on position 3 of the C-ring.

Although the most commonly consumed rice varieties have whitish pericarp, there are several colored varieties that have black or red caryopsis. These pigmented rice varieties contain anthocyanins which are located in the aleurone layer as a mixture of anthocyanins (Hu *et al*., 2003). Total anthocyanin content in ten pigmented rice varieties varied from 0-493 mg/100g grain (Ryu, Park and Ho, 1998). Cyanidin-3-glucoside and peodinin-3-glucoside are the major anthocyanins in the pigmented rice. The major anthocyanin in Korean pigmented rice was cyanidin-3-glucoside (Cho, Yoon and Hahn, 1996). Cyanidin-3-glucoside (85%) and peonidin-3-glucoside (15%) were the major anthocyanins in pigmented brown rice (*Oryza sativa* L. japonica) (Yawadio, Tanimori and Morita, 2007). Abdel-Aal, Young and Rabalski, (2006) reported that the most abundant anthocyanins in black and red rice were cyanidin-3-glucoside.

Anthocyanins have been known for the potential health effect, reducing the risks of chronic diseases such as cardiovascular disease, cancer, virus inhibition and Alzheimer's disease (Andersen and Jordheim, 2006). Anthocyanins and other flavonoids are regarded as important nutraceuticals mainly due to their antioxidant effects, which give them a potential role in prevention of the various diseases associated with oxidative stress. Phenolic compounds isolated from black rice possess marked health benefits in

preventing diabetic complications by inhibiting aldose reductase (Yawadio *et al*., 2007). The activity was ranked in the following order: cyanidin-3-glucoside > quercetin > ferulic acid > peodinin-3-glucoside > tocopherol.

2.3 Pigmented rice

Pigmented rices have been developed in many country including United State and Thailand. Many studies have been reported the health benefits of pigmented rice antioxidants against chronic disease such as cancer and cardiovascular disease (Zhao *et al*., 2004; and Chen *et al*., 2006). Hu *et al*., (2003) reported that black rice contains pigments, which are mainly located (about 85%) in the aleurone layer as a mixture of anthocyanins. Pigmented rice varieties have the potential to promote human health because they contain various antioxidative compounds that have the ability to inhibit the formation or to reduce the concentrations of reactive cell-damaging free radicals (Adom and Liu, 2002; Hu *et al*., 2003; Hyun and Chung, 2004; and Oki *et al*., 2002).These compounds include anthocyanins; which were cyanidin-3-glucoside and peonidin-3-glucoside (Hu *et al*., 2003; Park, Kim and Chang, 2008; and Kim *et al*., 2008); cyanidin and malvidin (Hyun and Chung, 2004); *p*-coumaric, 4, 7-dihydroxyvanillic acid, protocatechuic acid methyl ester, syringaldehyde, and vanillin (Asamarai *et al*., 1996; Goffman and Bergman, 2004; Miyazawa *et al*., 2003); ferulic and sinapinic acids (Tian, Nakamura and Kayahara, 2004). Furthermore, pigmented rices also accumulated vitamin E and γ-oryzanol in their caryopsis (Parrado *et al*., 2003).

High concentration of antioxidants in pigmented rice is associated with the reduction of the risk of chronic diseases. The supplementation of atherogenic diets with black rice reduced the oxidative stress (Xia *et al*., 2003). The pigmented fraction from black rice has been reported to its antioxidant activity and its potential to prevent DNA scission and deterioration of human LDL induced by reactive oxygen species (Hu *et al*., 2003). Therefore, pigmented rice varieties with high antioxidants may be used as health-promoting foods. In addition, its bran also has been reported the greater antioxidant and free-radical scavenging activities than bran of non-pigmented rice (Nam *et al*., 2006).

Limited study has been done on the antioxidant and antiradical capacity of different milling fractions of rice grain in relation to their distribution of phenolic acids, α-tocopherol and γ-oryzanol, especially in black rice varieties which are rich sources of antioxidant (tocopherol, tocotrienol and γ-oryzanol) levels. γ-Oryzanol concentration was significantly higher in outer bran layers of the grain. Chotimakorn *et al*., (2008) reported the effect of rice variety (long grain non-pigmented rice) and antioxidant content components of rice bran had significant effect on its antioxidation properties. In contrast, Li, Pickard and Beta (2007) reported that the scavenging activity and total phenolic content of wheat bran was generally twice as high as that of whole meal.

CHAPTER III

ANTIOXIDANT COMPOSITIONS OF PIGMENTED RICE IN DIFFERENT MILLING FRACTIONS

3.1 Introduction

Rice is the staple food in many Asian countries. Because of the expansion of healthy food product markets, some pigmented rice has been developed in many countries, including Asia and US. Many researches reported the health benefits of pigmented rice. Lu *et al*., (2008) and Xia *et al*., (2006) found that pigmented rice has the capability of preventing atherosclerosis in mouse model and human study. Moreover, pigmented rice was reported to have a higher antioxidant activity than normal rice (Choi, Jeong and Lee, 2007).

Pigmented rice is rich in antioxidant compounds especially anthocyanins, which possess anti-oxidative, anti-inflamatory, anti-cancer, anti-neurodegenerative and hypoglycemic activities (Hu *et al*., 2003; Zhao *et al*., 2004; Hyun and Chung, 2004; and Tsuda *et al*., 2003). Other antioxidants also found in pigmented rice, including phenolic acids, γ-oryzanol and tocopherol. Rice phenolic acids have antioxidative, antimutagenic, anticancer properties and play a role in maintaining health (Birosova, Mikulasova and Vaverkova, 2005; Gomes *et al*., 2003). Ferulic acid, found abundant in rice, is not presented in significant quantities in fruit and vegetables (Bunzel *et al*., 2002). Several studies have been reported the biological benefits of γ-oryzanol including the decreasing of total cholesterol and low density lipoproteins (LDL) and the increasing of high density lipoproteins (HDL) in serum, decreasing platelet aggregation, anti-inflammatory and alleviate menopausal disorders (Cicero and Gaddi, 2001; Seetharamaiah and Chandrasekhara, 1989; Xu *et al*., 2001; Yoshino *et al*., 1989). The most importance benefit of γ-oryzanol is the ability of lowering human serum cholesterols. γ-Oryzanol has higher antioxidative activity in cholesterol than vitamin E that may be due to the similarity of γ-oryzanol structure and cholesterol (Xu *et al*., 2001). Besides, γ-oryzanol also decreases hepatic cholesterol biosynthesis and contains

the ability to reduce cholesterol absorption (Rong *et al*., 1997). Tocopherol have vitamim E activity and it is the most important lipid-soluble antioxidant, and that it protects cell membranes from oxidation by reacting with lipid radicals produced in the lipid peroxidation chain reaction (Jensen and Lauridsen, 2007). Vitamin E deficiency causes the increasing of oxidation of cellular membranes. Olson and Munson (1994) give the following disorders associated with vitamin E deficiency: reproduction disorders, abnormalities of muscle, liver, bone marrow, brain function, and defective embryogenesis diathesis.

Rice contains many antioxidants but they are mainly located in the outer layer of the caryopsis. The milling process produces 3 different fractions which contain different amounts of antioxidant. Most previous studies focused on some antioxidants in unmilled rice or bran of normal rice. Zhou *et al*., (2004) have been studied only the phenolic acids in brown and milled rice of normal rice, while Rohrer and Siebenmorgen, (2004) have reported only vitamin E and oryzanol in kernel thickness fractions of normal rice. Nam *et al*., (2006) reported that pigmented rice bran had greater antioxidant activity than normal rice bran. The anthocyanin composition of pigmented rice has been studied (Ryu *et al*., 1998; Park *et al*., 2008) but no comparison study between milling fraction was found.

However, relatively little research on antioxidant compounds in different milling fraction of pigmented rice has been reported, especially research on their antioxidant activities.

The objective of this chapter was to identify most of antioxidant constituents in different pigmented rice and determine their concentrations in different milling fractions.

3.2 Methodology

3.2.1 Materials

Unmilled, milled, and bran fractions of normal rice (California long grain rice) obtained from Pacific International Rice Mills, Inc. (USA). Unmilled fractions of aromatic red rice and black rice no.1 (black japonica rice) obtained from SunWest Foods, Inc. (USA). The two unmilled black rice samples which were black rice no.2 (black japonica rice) and black rice no.3 (Hong Kong black rice) obtained from Lundberg Family Farms (USA). All pigmented rice samples were milled by laboratory rice miller (Ricepal32, Yamamoto Co., Ltd.) 2 times by level 3 and 4 respectively to remove the bran layer. The bran of each were combined. The 3 milling fractions of rice including milled rice (white rice), unmilled rice (brown rice) and the bran samples were collected for further analysis. All samples were stored immediately at -18^{o}C after milling and kept at this condition until analysis (Appendix A).

3.2.2 Quantitative analysis of antioxidants in rice fraction samples

- *Chemicals*

Standard α-tocopherol, ferulic acid, 2,2-diphenyl-1-picryhydrazyl ($DPPH^{\cdot}$), butylated hydroxytoluene (BHT), Folin-Ciocalteu reagent, *p*-coumaric acid , sinapic acid and *p*-hydroxybenzoic acid were purchased from Sigma-Aldrich Chemical Co., (St. Louis, MO, USA). HPLC grade acetonitrile, formic acid, trifluoroacetic acid (TFA), protocatechuic acid and gallic acid were purchased from Acros Organics (NJ, USA). HPLC grade of methanol was purchased from Fisher Chemical (NJ, USA). γ-Oryzanol was purchased from Wako Chemicals USA, Inc. (VA, USA). Cyanidin-3-glucoside and peonidin-3-glucoside were purchased from ChromaDex Inc. (CA, USA).

- *Determination of anthocyanin components*

Anthocyanins of rice fraction samples were determined according to the method described by Kim *et al*., (2008) with some modifications. Briefly, 3 g of the

defatted rice fraction sample was extracted twice by mixing with methanol (30 ml) acidified with HCl (1.0 N, 85:15 v/v) and shaking at 4^{o}C for 24 hr. The crude extracts were filtered with Whatman No.1 filter paper. The extracts were centrifuged at 12,000 g at 5^{o}C for 20 minutes. The extracts were kept in a refrigerator at 4^{o}C (in the dark) for 2 days to precipitate large molecules and then centrifuged at 12,000 g at 5^{o}C for 20 minutes. The upper layer was concentrated and filtered through 0.45 μm syringe filter before injected to HPLC.

The HPLC instrument (LC-10AT, Shimadzu) connected with UV/vis detector (SPD-10A, Shimadzu) was used for analysis. TosoHaas super-ODS, C18 2μm 4.6 x 100 mm column was used to separate the anthocyanin components. The mixture of water, methanol and formic acid (75:20:5 v/v) was used as a mobile phase with isocratic elution at 0.5 ml per minute flow rate. UV/vis detector was set at 530 nm and sample loop was 5μl. Anthocyanins in samples were identified by comparing retention times with those of the authentic standard compounds (Appendix B). All identified anthocyanins were quantified with external standards by using HPLC analysis as described above. The standard curves of anthocyanins were plotted as peak area against concentrations of external standards by duplicate injection of the 6 series dilution working solution of standard mixture.

- *Determination of α-tocopherol and γ-oryzanol content*

α-Tocopherol and γ-oryzanol in rice fraction samples were determined according to method of Aguilar-Garcia *et al*., (2007) with some modifications. Rice fraction samples (100 mg) were extracted twice with methanol (6 ml) and centrifuged at 825 g for 10 minutes. The supernatant was combined and then evaporated to 4 ml, then made up to exactly 5.0 ml with HPLC grade methanol in a volumetric flask. This solution was filtered through 0.45 μm syringe filter before subjected to HPLC analysis.

α-Tocopherol and γ-oryzanol were analyzed by HPLC using a Shimadzu (LC-10AT) equipped with UV/vis detector. The C18 column (Inertsil ODS-3, 5μm, 250x4.6mm) was used to separate these compounds. Mobile phase was the mixture of methanol and acetonitrile (15:85 v/v) at flow rate of 2 ml/min with isocratic mode.

The sample loop was set at 20µl. The UV/vis detector was set at 292 and 325nm for tocopherol and oryzanol, respectively. A preliminary identification of the peaks was done by comparison with pure standards retention times. The γ-oryzanol was quantified against the standard curve (Appendix C), which related the known quantity of total γ-oryzanol to the total peak area of the UV absorbance.

- *Determination of total and bound phenolic acids*

Total and bound phenolic acids were determined according to the method reported by Tian *et al*., (2005) with some modifications. For total phenolic acids determination, 2 g of rice samples were extracted with hexane (4 x 50 ml) to remove fat. The residue was hydrolyzed with 1 M NaOH (2 x 100 ml, 2h each). The supernatants were pooled and acidified with 4 N HCl to pH 1 and then extracted (4 times) with ethyl acetate (200 ml each). The ethyl acetate fractions were evaporated to dryness, and then dissolved with methanol (5 ml, 15%) and analyzed by HPLC. For bound phenolic acid determination, defatted rice sample was extracted with ethanol (70%) to remove free phenolic acids before hydrolysis and performed by following the same method as total phenolic acids determination, mentioned above.

The extract was separated by using HPLC connected with C18 column (5µm, 4.6 x 250 mm) and UV/vis detector. The mixture of acetonitrile (B) and pure water with TFA (0.1%) was used as a mobile phase at the flow rate of 1.5 ml/min. Gradient elution was performed as follows: 0-15 min, linear gradient from 5-9% solvent B; from 15-30 min 9% solvent B; 30-37 min, linear gradient from 9-13% solvent B; from 37-55 min, linear gradient from 13-18% solvent B; from 55-60 min, linear gradient from 18-20% solvent B. The detector was set at 250 nm to detect hydroxybenzoic acid and at 325 nm to detect hydroxycinammic acid. The separated phenolic acids were identified by comparing their retention times with authentic compounds and were quantified using an external standard method (Appendix D).

3.3 Results and discussion

3.3.1 Antioxidants content

a) Unmilled rice fraction

Rice contains several antioxidant compounds. Among these, phenolic acids, tocopherol and γ-oryzanol are the best known antioxidants in rice. In pigmented rice, anthocyanins are responsible for its color as well as antioxidative properties. Our study determined the contents of these substances in rice fraction samples by using HPLC analysis.

Unmilled rice (known as brown rice) is the milling fraction which obtained after rice dehulling. It includes of milled and bran fractions. Thus, comparison of antioxidants contents between unmilled rice samples would be the comparing between rice cultivars.

Table 3.1 shows the antioxidants contents in unmilled rice fractions. Anthocyanin was not found in normal rice but presented in high concentration in pigmented rice. The anthocyanin contents of black rice (197.79-288.90 μg/g) were much greater than red rice (3.42 μg/g). Abdel-Aal *et al*., (2006) reported that red rice contained about 100 times of total anthocyanins lower than black rice. The highest content of anthocyanins was found in black rice no.1 (288.90 μg/g) which contained the highest of total antioxidant contents. According to the study by Kallithraka *et al*., (2005), black rice contained lower anthocyanin content than grape (731.7 μg/g fresh weight).

Phenolic acids content of normal rice and red rice were 302.13 and 308.63 μg/g, respectively. Black rice samples showed higher concentration of phenolic acids (431.80-474.06 μg/g) than the former. α-Tocopherol content was not detected in all samples while γ-oryzanol was found to be the highest concentration among 4 antioxidants in rice (370.04-545.22 μg/g). The results showed that black rice cultivars had higher content of antioxidants than red and normal rice, due to the high containing of anthocyanins in black rice.

b) Milled rice fraction

All rice samples were milled with the same milling conditions. However, the amount of the obtaining milled rice and rice bran fractions of each cultivar were not similar because of the different of milling quality. In our study, milling quality of rice samples was ignored.

In milled rice fractions, most of antioxidants were removed. Normal milled rice contained 23.40 μg/g of phenolic acids and 22.23 μg/g of γ-oryzanol which were removed by milling about 92% and 94%, respectively (Table 3.2). The results confirmed with the previous studies reported that most of antioxidants in rice located at outer layer of the grain (Ko *et al*., 2003; Kong and Lee, 2009). No anthocyanins were detected in red milled rice but the grain color was not perfectly white as compared to normal milled rice. It was indicated that red mill rice contained very small amount of anthocyanins which was unable to detect by our analysis condition. Red milled rice retained 13% of phenolic acids and 14% of γ-oryzanol compared to unmilled rice. All black rice samples retained some anthocyanins in their milled rice fractions. Black milled rice contained anthocyanins 16%, 3%, 7% compared to their unmilled rice fraction for black rice no.1, 2 and 3, respectively. Milling process also removed phenolic acids and γ-oryzanol of black rice samples. Black milled rice lost 92% of phenolic acids and 83-88% of γ-oryzanol compared to unmilled rice fraction. α-Tocopherol was not detected in all milled rice samples due to its low concentration (Table 3.2).

Total antioxidant contents of milled rice were shown in Table 3.2. Total antioxidant contents, ranked in descending order, as follows: black rice no.1, black rice no.3, red rice, black rice no.2 and normal rice. This result suggested that the consumption of milled pigmented rice may intake higher content of rice antioxidants than normal (non-pigmented) milled rice.

c) Bran fraction

The contents of anthocyanin, phenolic acids, α-tocopherol and γ-oryzanol in rice bran are shown in Table 3.3. Total antioxidant contents, ranked in descending order, as follows: black bran no.1, black bran no.3, black bran no.2, normal bran and red bran, the values of which were 9.93, 8.26, 6.42, 5.85 and 3.59 mg/g, respectively. Black rice bran samples contained total anthocyanins in the ranged of 1.14-2.56 mg/g which was higher than those reported in seed coat of black soy bean (0.22-1.87 mg/g) (Xu *et al*., 2007) and purple wheat bran (1.16 mg/g) (Li *et al*., 2007). Total phenolic acids found in rice bran samples in the ranged of 1.53-3.29 mg/g which was slightly lower than those reported in wheat bran (3.36-3.97 mg/g) (Kim *et al*., 2006). Rice bran samples contained γ-oryzanol in the ranged of 1.86-4.06 mg/g. γ-Oryzanol is not presented in common cereal in high level, with the exception of rice. Thus rice bran takes an advantage over other cereals for this reason. α-Tocopherol is an importance natural antioxidant in rice bran. Rice bran contains vitamin E up to 150 mg/kg (Saunders, 1990). The content of α-tocopherol in rice bran sample was ranged in 16.34-71.01μg/g (16.34-71.01 mg/kg). α-Tocopherol has been considered the greatest value of vitamin E homolog due to its high level of antioxidant activity (Packer, 1995). According to the report by Aguilar-Garcia *et al*., (2007), our study showed lower α-tocopherol content in rice bran which may due to the different of genotype or sample preparation method.

Although both milled and unmilled fractions of normal rice contained the lowest value of total antioxidant content comparing with other rice cultivars but its bran showed higher content of those than red bran. The high total antioxidant containing of normal bran may contributed from the industrial rice milling which was performed in normal rice. The industrial rice miller possesses higher performance than laboratory rice miller used for pigmented rice samples. Bran from normal rice was finer and may contain lower impurity such as broken rice than other brans. Major antioxidants found in normal rice bran were γ-oryzanol and phenolic acids which were 62.9% and 35.9% of total antioxidant content, respectively, while the minor was α-tocopherol which was found only 1.2% of total antioxidants. Anthocyanins were not detected in normal bran. γ-Oryzanol and

phenolic acids were the major antioxidants for red rice bran containing 51.8% and 42.5% of total antioxidant contents, respectively. Red bran contained small amount of anthocyanins and α-tocopherol which were 5.2% and 0.5% of total antioxidant contents, respectively. For black rice bran, 17.7-25.8% of total antioxidant was anthocyanins which are responsible for the black color of the bran. In addition, black rice bran showed high content of phenolic acids and γ-oryzanol, the percentages of which were 33.1-43.2% and 38.7-41.1% of total antioxidant content, respectively. These results showed that γ-oryzanol (1.86-4.06 mg/g) and phenolic acids (1.53-3.29 mg/g) were the major antioxidants in all bran samples. Compared to others antioxidants, α-tocopherol content was lowest (16.34-71.01 μg/g) in all rice bran samples. These results suggest that rice bran is a good source of phenolic acids and γ-oryzanol. γ-Oryzanol was about 50 times higher than that of α-tocopherol. Xu *et al*., (2001) reported that γ-oryzanol is a more potent antioxidant than α-tocopherol in reduction of cholesterol oxidation.

Table 3.1 Antioxidant contents (μg/g) of unmilled rice fraction samples[1]

Antioxidants	Unmilled rice fraction samples				
	Normal	Red	Black no.1	Black no.2	Black no.3
Anthocyanins	Tr[2]	3.42±0.23	288.90±9.64	197.79±12.13	277.44±9.12
Phenolic acids	302.13±22.78	308.63±30.93	474.06±23.05	431.80±23.09	446.98±17.25
α-Tocopherol	Tr	Tr	Tr	Tr	Tr
γ-Oryzanol	370.04±12.47	459.37±25.11	545.22±19.98	513.76±14.10	493.25±5.81
Total	672.17	771.42	1308.18	1143.35	1217.67

[1] Mean ± SD, [2]Tr = trace

Table 3.2 Antioxidant contents (μg/g) of milled rice fraction samples[1]

Antioxidants	Milled rice fraction samples				
	Normal	Red	Black no.1	Black no.2	Black no.3
Anthocyanins	Tr[2]	Tr	46.08±3.54	6.36±1.06	19.32±1.53
Phenolic acids	23.40±3.98	39.69±2.20	37.29±1.98	32.59±3.47	33.83±3.54
α-Tocopherol	Tr	Tr	Tr	Tr	Tr
γ-Oryzanol	22.23±4.13	63.87±5.40	93.90±3.39	61.31±1.42	67.13±0.80
Total	45.63	103.56	177.27	100.26	120.28

[1] Mean ± SD, [2] Tr = trace

Table 3.3 Antioxidant contents (μg/g) of rice bran fraction samples[1]

Antioxidants	Bran fraction samples				
	Normal	Red	Black no.1	Black no.2	Black no.3
Anthocyanins	Tr[2]	188.13±7.65	2562.39±34.41	1135.17±43.39	1657.70±20.31
Phenolic acids	2101.33±175.09	1525.94±102.69	3289.41±115.77	2771.87±102.29	3182.88±228.58
α-Tocopherol	71.01±4.41	16.34±3.90	24.03±1.87	26.89±3.59	24.90±0.45
γ-Oryzanol	3681.02±72.42	1859.31±48.87	4057.60±56.92	2482.94±39.52	3390.01±94.72
Total	5853.36	3589.72	9933.43	6416.87	8255.49

[1]Mean ±SD, [2]Tr= Trace

Figure 3.1 shows the total antioxidants content of each milling fraction of 5 rice cultivars. Between 3 milling fractions, bran contained the highest content of antioxidants. On average, bran fraction contained about 7 times of total antioxidants content higher than unmilled fraction and 62 times higher than milled fraction. It was shown that bran was the most valuable source of rice antioxidants. The results show that unmilled fraction consisted of 9 times total antioxidant contents higher than its milled fraction.

In conclusion, most of antioxidants were located at the outer layer of rice grain and were removed by milling process. Among 3 milling fractions, bran fraction contained the greatest content of antioxidants, especially, black rice bran which contained high level of anthocyanin contents, as well as, other general rice antioxidants such as phenolic acids, α-tocopherol and γ-oryzanol.

In the next section, the anthocyanins compounds and phenolic acids of each milling fraction were identified.

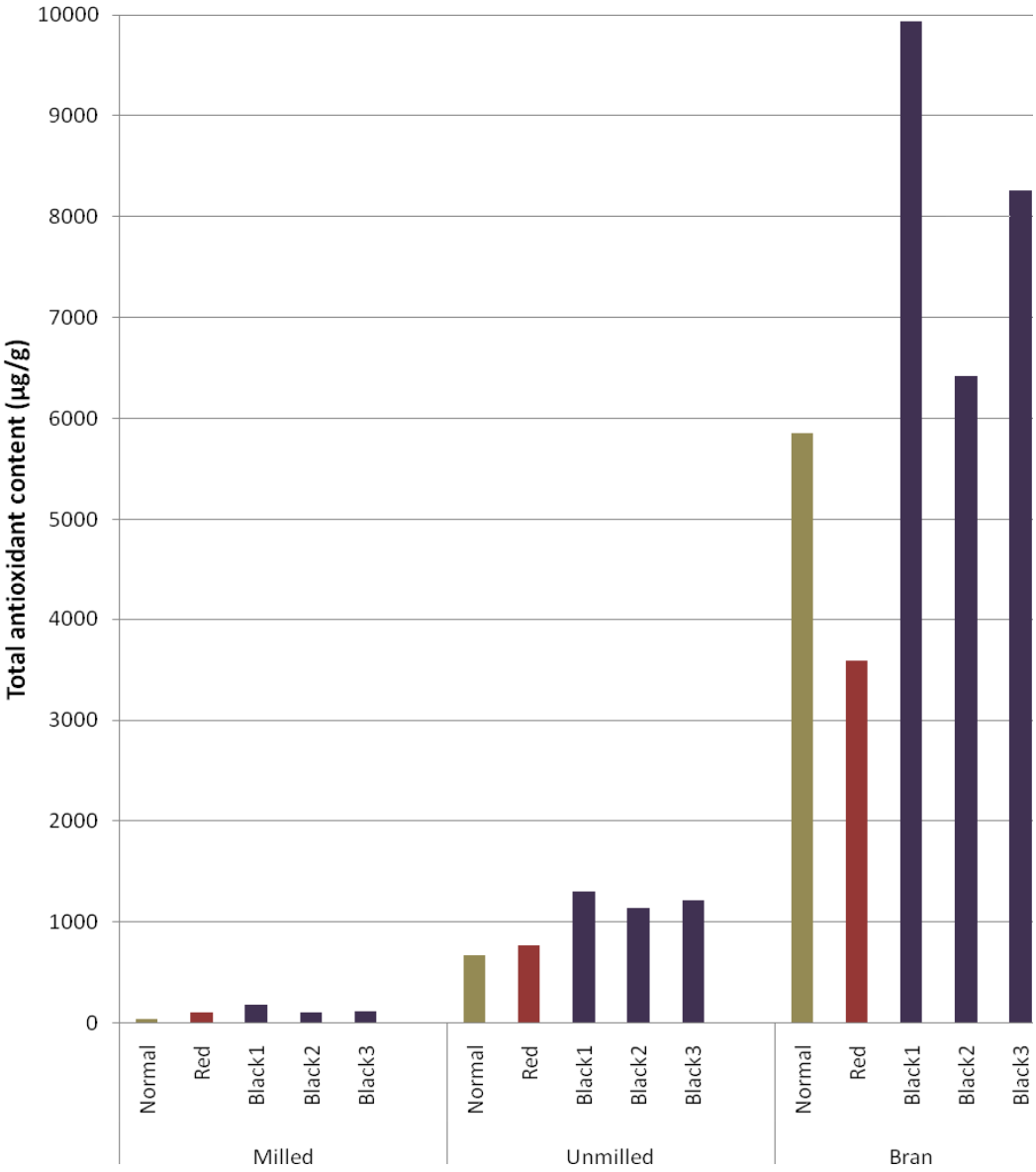


Figure 3.1 Antioxidant content of pigmented rice in different milling fraction

3.3.2 Antioxidant composition

a) Anthocyanins

Anthocyanins were found only in pigmented rice samples. Black and red rice contained a limited number of anthocyanins (Abdel-Aal *et al*., 2006). The HPLC analysis separated pigmented rice anthocyanins into 2 major compounds which were cyanidin-3-glucoside and peonidin-3-glucoside (Figure 3.2). The result was similar to those reports by Ryu *et al*., (1998); Park *et al*., (2008); and Kim *et al*., (2008). Some studies reported other anthocyanins such as malvidin, pelargonidin-3, 5-

diglucoside, cyanidin-3, 5 diglucoside, and cyanidin-3-rutinoside which were found in pigmented rice in lesser amount (Zhang *et al*., 2006; and Abdel-Aal *et al*., 2006). Cyanidin-3-glucoside and peonidin-3-glucoside are based on the same flavyrium skeleton hydroxylated in 3, 5, and 7 positions but differing in the numbers and positions of hydroxyl and methoxyl groups in B ring (Escribano-Bailon *et al*., 2004) (Figure 3.3). Despite, the color of anthocyanins is influenced by the hydroxylation and methoxylation, increasing the number of methoxyl groups enhances the redness (Delgado-Vargas, Jimenez and Parades-Lopez, 2000), red rice contained the same anthocyanins pattern as black rice. The differ of red and black rice anthocyanin was the much lower concentration of both anthocyanins in red rice compared to black rice. The result was confirmed with the study of anthocyanin composition in cereal reported by Abdel-Aal *et al*., (2006). The amount of each anthocyanin component in different milling fractions is shown in Figure 3.4.

Cyanidin-3-glucoside was the major anthocyanin in all pigmented rice fraction samples. The ratio of cyanidin-3-glucoside and peonidin-3-glucoside of red rice bran and black rice bran (no.1 and 3) were about 95:5 and 90:10, respectively. Peonidin-3-glucoside was found in high level only in black rice no.2 sample (58:42). The ratio of these anthocyanins in rice depended on rice cultivar, It has been reported that the percentage of cyanidin-3-glucoside in 10 black rice samples were in the ranged of 80-100% (Escribano-Bailon *et al*., 2004).

Anthocyanins were highly concentrated in bran fraction. However, the content of anthocyanins in red rice bran (188.13 μg/g) was comparable to unmilled black rice (197.79 to 288.90 μg/g) (Table 3.1 and 3.3). Comparison of anthocyanins content between milling fraction found that on average, bran fractions contained about 7 and 58 times of anthocyanins content higher than unmilled and milled fractions, respectively.

The highest total anthocyanins content was found in black rice bran no.1 sample (2562.39 μg/g) while the lowest was in red unmilled rice sample (3.42 μg/g) (Table 3.1 and 3.3). Unmilled black rice samples contained total anthocyanins in the ranged of 197.79-288.90μg/g and milled black rice samples contained anthocyanins in ranged of 6.36-46.08 μg/g.

Anthocyanins are associated with the reducing risks of chronic disease such as cancer, cardiovascular disease, Alzhemer's disease (Anderson and Jordheim, 2006). Our results showed that black rice bran samples were the valuable source of anthocyanins, the contents of which were 1.14-2.56 mg/g. Black rice bran anthocyanins was higher than those reported in grapes (0.86-1.91 mg/g fresh weight) (Kallithraka *et al*., 2005).

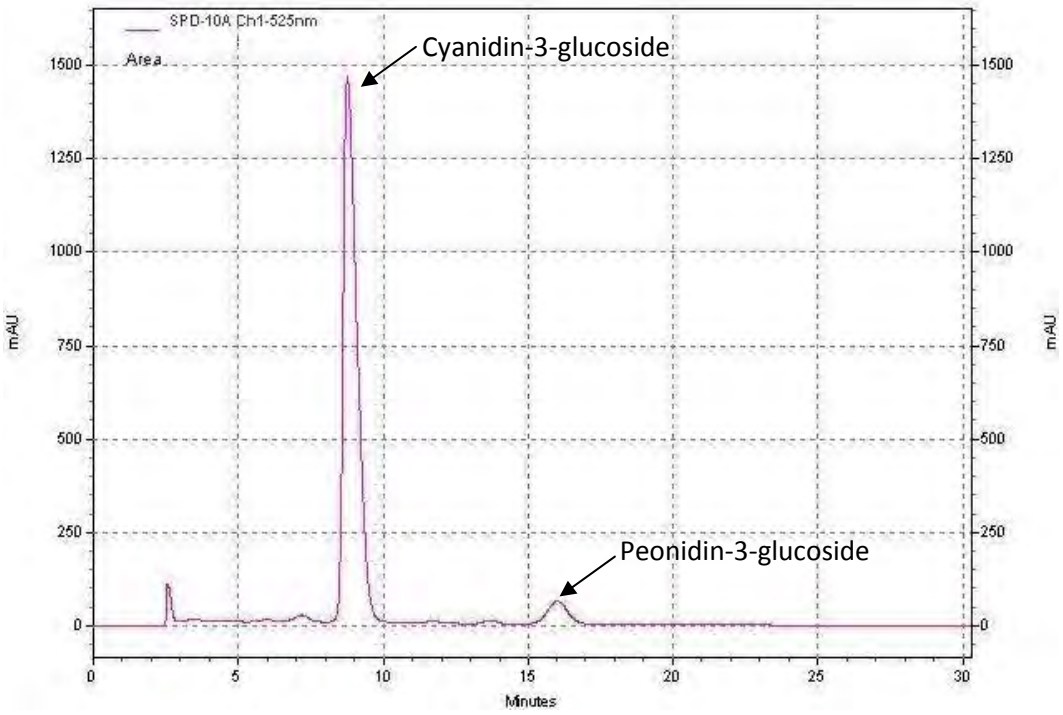
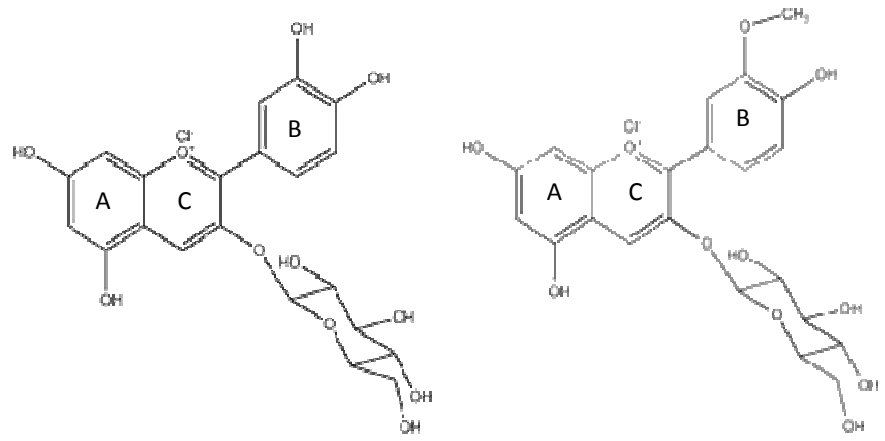


Figure 3.2 Chromatogram of anthocyanin compounds in pigmented rice



Cyanidin-3-glucoside Peonidin-3-glucoside

Figure 3.3 Molecular structures of cyanidin-3-glucoside and peonidin-3-glucoside

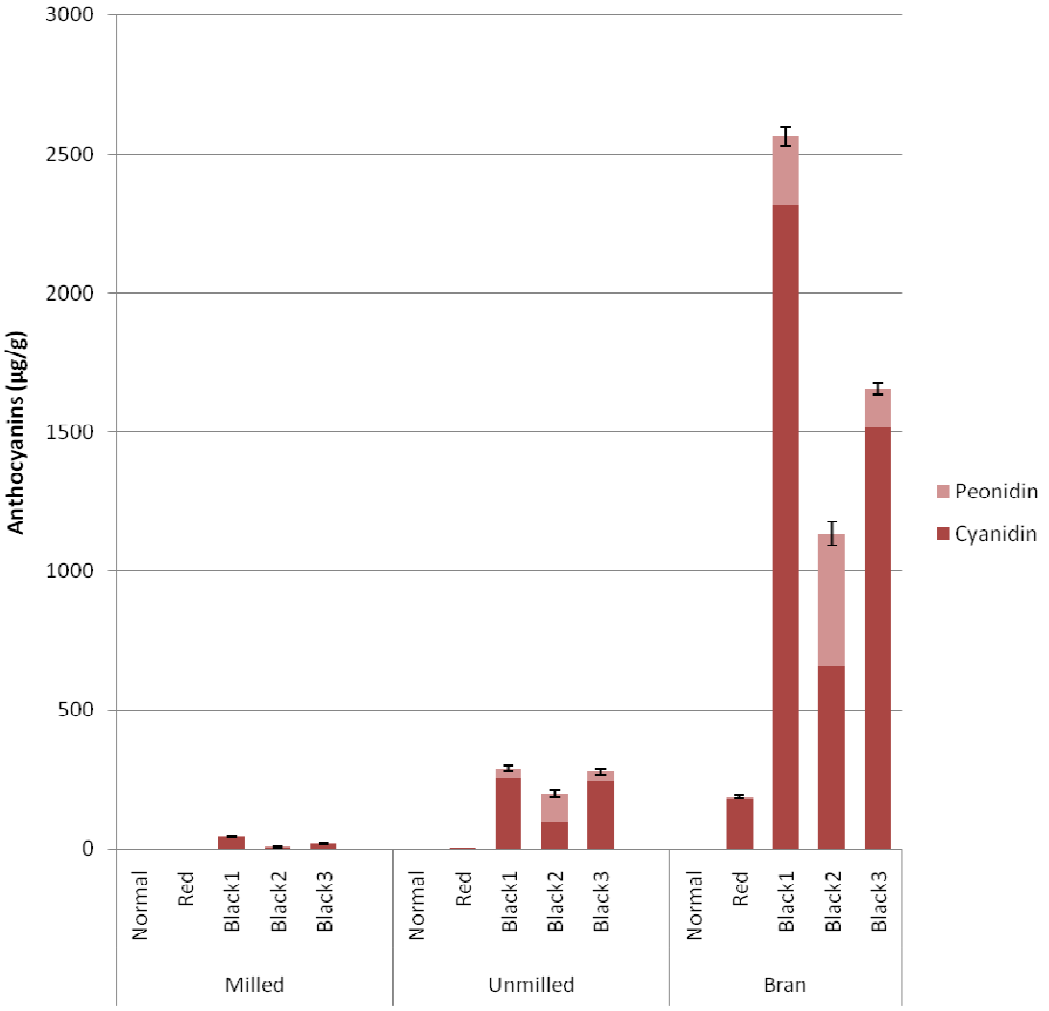


Figure 3.4 The content of anthocyanin compounds in rice fraction samples

b) Phenolic acids

HPLC analysis separated rice phenolics into 6 known phenolic acids including gallic acid, protocatechuic acid, hydroxybenzoic acid, *p*-coumaric acid, ferulic acid and sinapic acid (Figure 3.5). These phenolic acids were found in all samples but in different amount. For our study, some small unknown peaks were presented in the chromatograms. Other phenolic acids such as chlorogenic, caffeic, vanillic acids, have been found in rice (Tian *et al*., 2005).

The major portion of phenolic acids in all samples existed in bound form (Table 3.4 and Figure 3.6-3.10). The phenolic acids in all rice fraction samples

were dominated by ferulic acid with lesser amount of gallic, protocatechuic, hydroxybenzoic, *p*-coumaric and sinapic acids (Figure 3.6-3.10). Ferulic acid and *p*-coumaric acid were reported to be the major phenolic acids in rice (Sosulski *et al*., 1982). Several studies have reported the occurrence of bound ferulic acid in cereal (Sosulski *et al*., 1982; Liu, 2007; Klepacka and Fornal, 2006). This observation was confirmed in the present study. Ferulic acid was reported to be the major phenolic compound in corn, wheat, oats and rice grains (Adom and Liu, 2002).

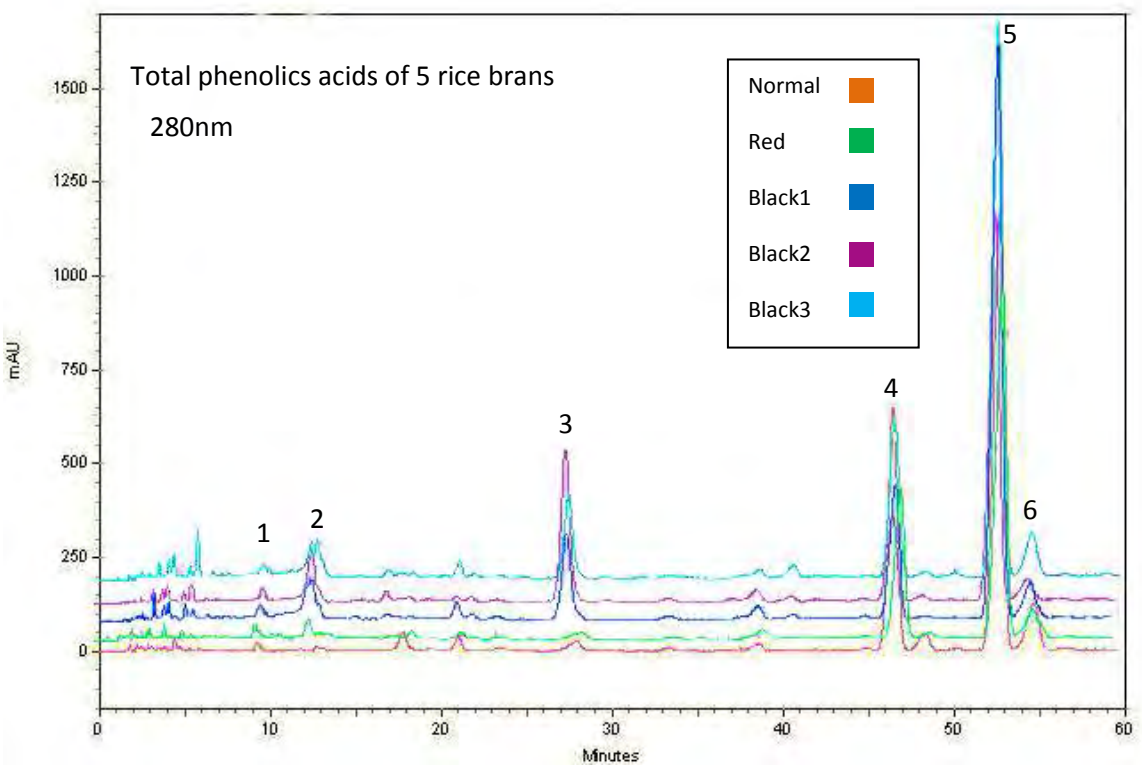


Figure 3.5 Chromatogram of total phenolic acids in rice fraction samples (1=gallic acid, 2= protocatechuic acid, 3= hydroxybenzoic acid, 4= *p*-coumaric acid, 5= ferulic acid and 6= sinapic acid)

Major phenolic acid in cereal is in bound form which unable to extract easily by solvent used in the measurement of total phenolic by Folin-Ciocalteu reagent assay. Thus, hydrolysis of rice samples has been done in our determination

to release bound phenolic acid from polysaccharide. The contents of free and bound phenolic acids of 5 rice varieties in different milling fraction are shown in Figure 3.6-3.10. The bound phenolic acids, which bound to polysaccharides and lignin in cell wall (Bunzel *et al*., 2002), was the major type of phenolic found in all rice samples. Black rice samples showed the greater level of free phenolic acids than normal and red rice. The percentage of free phenolics acid in unmilled fraction samples were 9, 12, 39, 44 and 31% of total phenolic acids for normal, red, black no.1, 2 and 3, respectively. Zhou *et al*., (2004) reported that the level of free phenolic acids, as the proportion of total phenolic acids were 11-15% in brown rice (non-pigmented rice).

Table 3.4 Percentage of free and bound phenolic acids in pigmented rice bran fraction

Phenolic acids	Percentage of free/bound phenolic acids				
	Normal	Red	Black no.1	Black no.2	Black no.3
Gallic acids	16.7/83.3	56.7/43.3	91.5/8.5	52.4/47.6	82.2/17.8
Protocatechuic acid	47.6/52.4	26.2/73.8	54.6/45.4	65.0/35.0	77.1/22.9
Hydroxybenzoic acid	20.1/79.9	23.8/76.2	25.4/74.6	33.5/66.5	36.1/63.9
p-Coumaric acid	3.7/96.3	14.6/85.4	10.0/90.0	6.2/93.8	21.1/78.9
Ferulic acid	4.0/96.0	15.4/84.6	7.9/92.1	16.8/83.2	8.4/91.6
Sinapic acid	13.4/86.6	12.6/87.4	27.3/72.7	3.2/96.8	9.4/90.6
Total	6.0/94.0	16.6/83.4	23.6/76.7	29.2/70.8	27.2/72.8

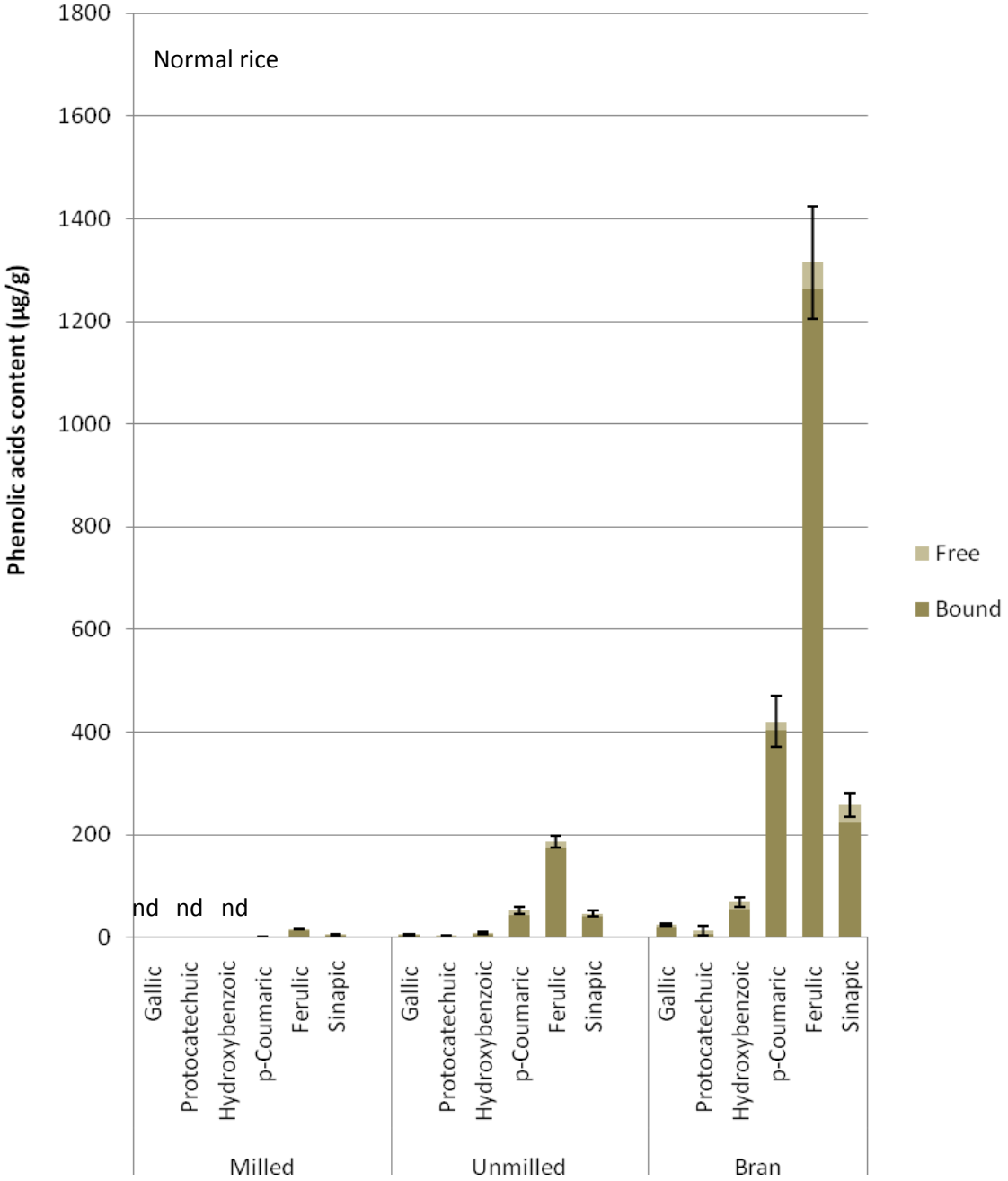


Figure 3.6 The content of phenolic acids in normal rice fractions

p-Coumaric, ferulic and sinapic acids were present in normal rice as the major compounds. Unmilled and bran fractions had very low concentrations of gallic, protocatechuic and hydroxybenzoic acids (Figure 3.6). Gallic, protocatechuic and hydroxybenzoic acid were not detected in normal milled rice. Normal rice bran contained phenolic acids 7 and 90 times greater than unmilled

rice and milled rice, respectively. After milling, normal milled rice retained 7.7% of phenolic acids content comparing with unmilled rice.

Red rice samples showed the same phenolic acids profile as normal rice except protocatechuic acid which seemed to be higher in red rice bran over the normal bran. The content of gallic, protocatechuic and hydroxybenzoic acid in milled red rice were not detected (Figure 3.7). However, the level of free phenolic acids in red rice (16.6%) was higher than normal rice (6.0%) (Table 3.4). Milling removed 87.1% of phenolics acid of red rice.

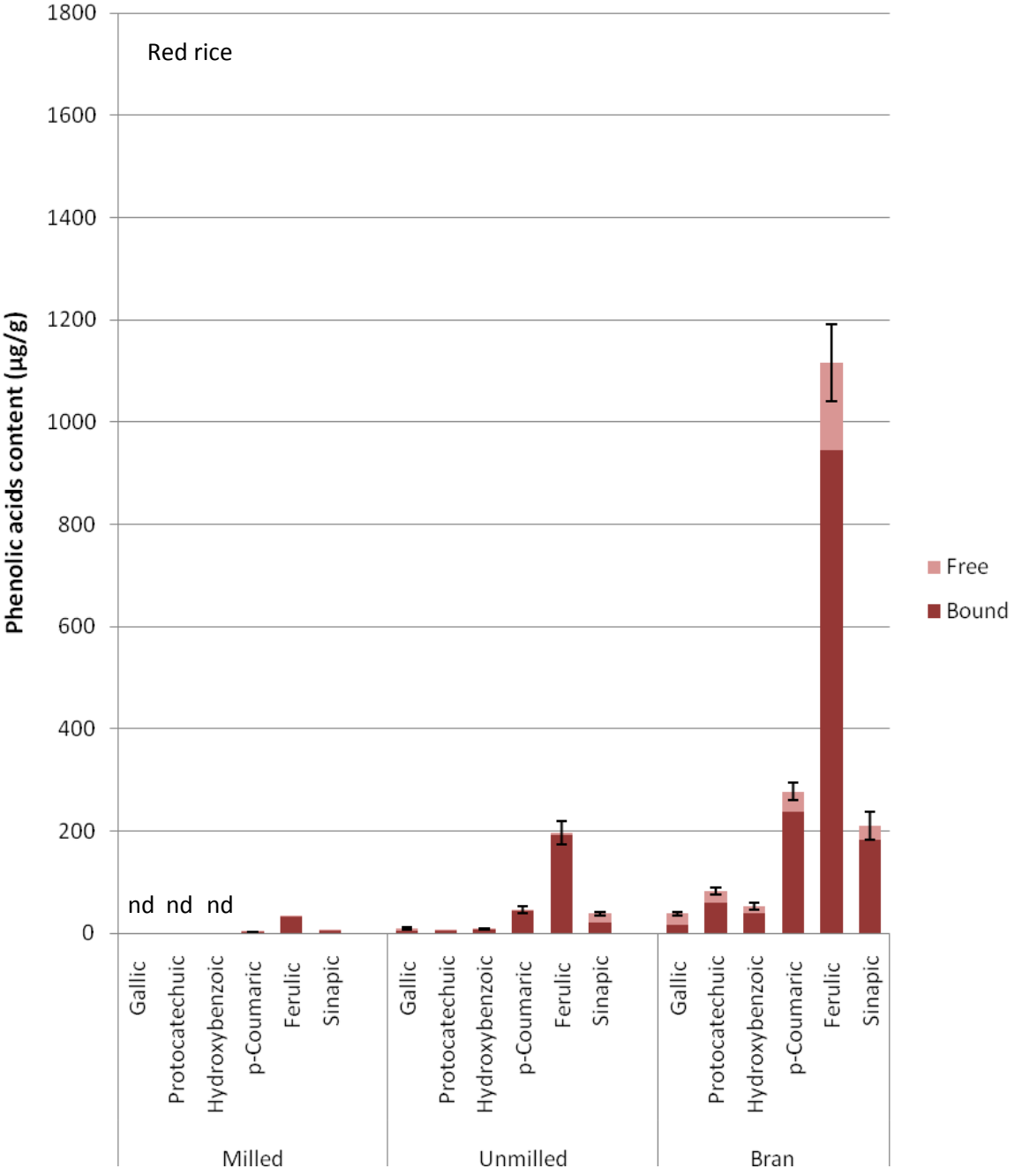


Figure 3.7 The content of phenolic acids in red rice fractions

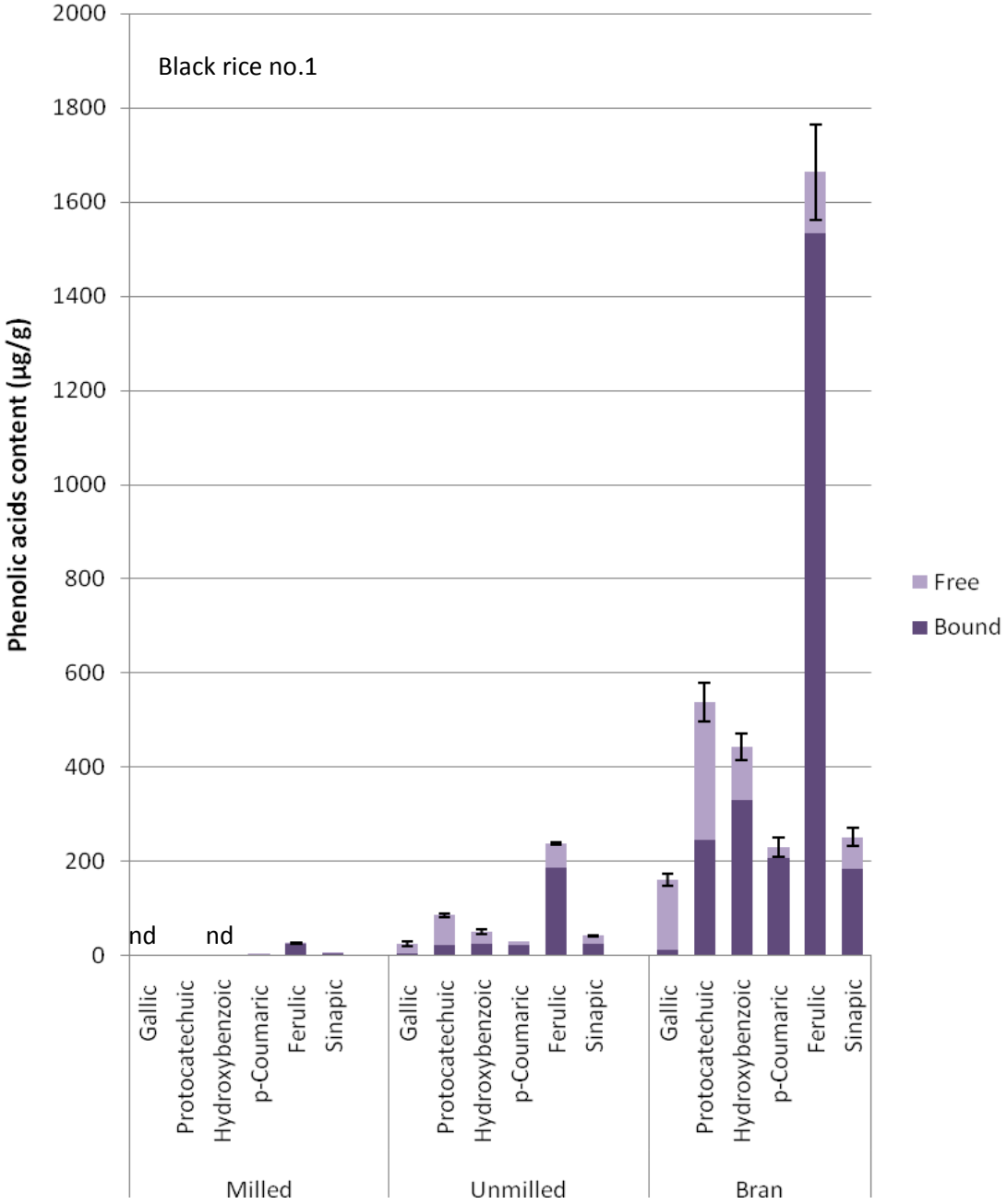


Figure 3.8 The content of phenolic acids in black rice no.1 fractions

All black rice showed similar profile of phenolic acids (Figure 3.8-3.10), however, *p*-coumaric acid was not the major phenolic acid in black rice but protocatechuic and hydroxybenzoic acids were found in higher level than *p*-coumaric acid. Protocatechuic and hydroxybenzoic acids contents in black rice were also greater than red and normal rice (Figure 3.11). The higher level of

protocatechuic and hydroxybenzoic acid in black rice may involve with the metabolism of anthocyanins. Both protocatechuic and hydroxybenzoic acids are the phenolics in the benzoic acid families. Benzoic acid derivatives are produced via the loss of a two-carbon moiety from phenyl propanoids (Shahidi, 2000). The hydroxylation of hydroxybenzoic acid leads to the formation of protocatechuic acid (dihydroxybenzoic acid). In some plant, anthocyanins acylated with some phenolic acids such as hydroxybenzoic acid and *p*-coumaric acid (Saito *et al*., 1998; Markham and Ofman, 1993). The factors affect the phenolic content in plant has been reported by Raskin (1992). After infection or UV irradiation, many plants increase their benzoic acid derivative content, which may induce the biosynthesis of defense substance. The degradation of anthocyanins may involve the increased of some phenolic acids content in rice. At pH values higher than 7, anthocyanins are instable and degraded to aldehyde and phenolic acids (Castaneda-Ovando *et al*., 2009). Regarding to anthocyanin structure, cyanidin-3-glucoside degraded to protocatechuic acid (Hiemori, Koh and Mitchell, 2009) which may be the cause of higher level of protocatechuic acid in black rice.

Protocatechuic acid was found in milled fraction of black rice no.1 in small amount. The average content of free phenolic acids in the black rice group was significantly higher than that of the red and normal rice. Free phenolic acids contents of black rice bran were 23.6-29.2% (Table 3.4), while red and normal bran were 16.6 and 6.0%, respectively. Free gallic, protocatechuic and hydroxybenzoic acids of black rice bran were higher than the other phenolic acids. The average percentage of free gallic, protocatechuic and hydroxybenzoic acids in black brans were 57.5% which was higher than red and normal bran (35.6 and 28.1%).

Our study indicated that pigmented rice, especially black rice contained higher level of protocatechuic and hydroxybenzoic acids and showed higher content of free phenolic acid than normal rice.

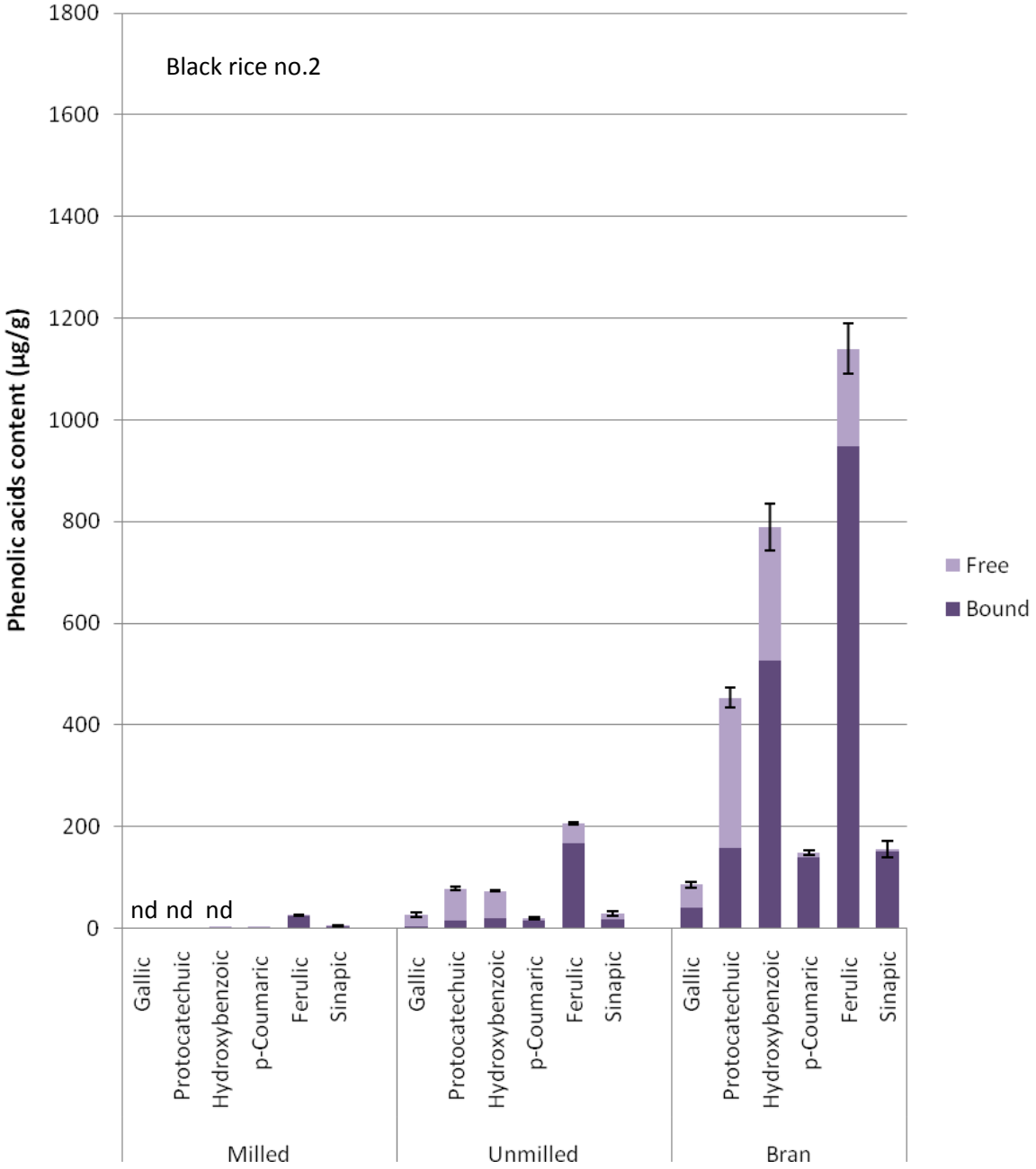


Figure 3.9 The content of phenolic acids in black rice no.2 fractions

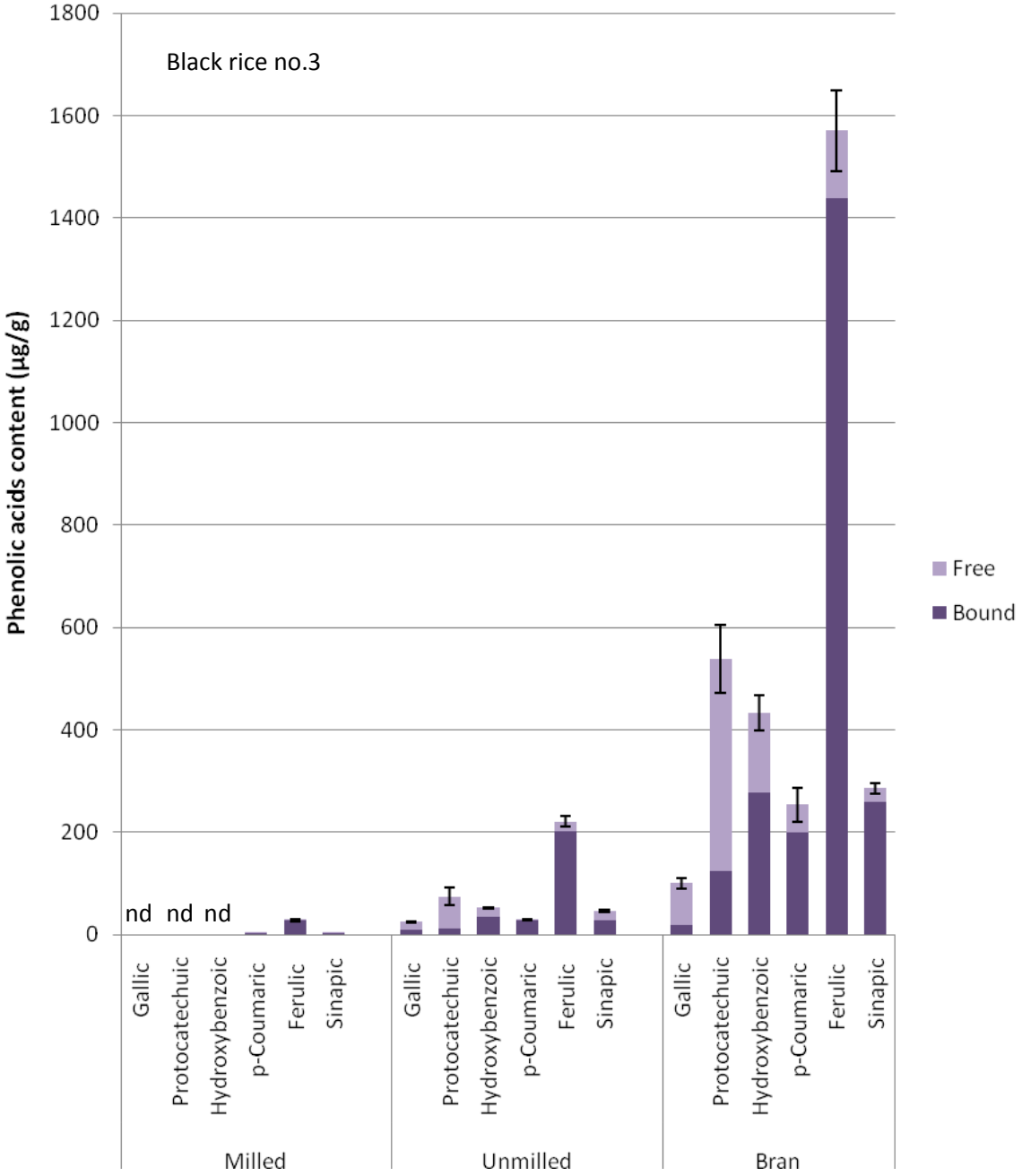


Figure 3.10 The content of phenolic acids in black rice no.3 fractions

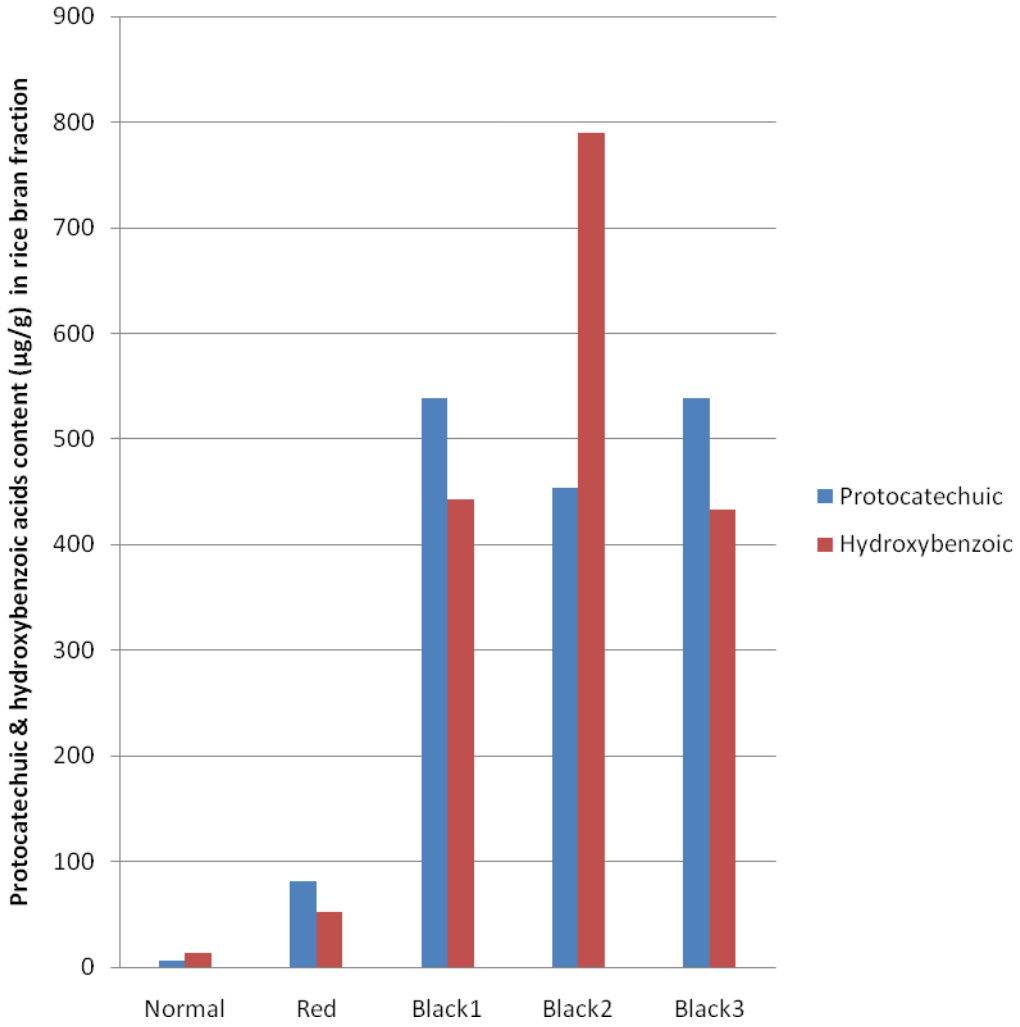


Figure 3.11 The content of protocatechuic and hydroxybenzoic acids in rice bran fractions

4.3 Conclusion

Pigmented rices contained many antioxidants including anthocyanins, phenolic acids, α-tocopherol and γ-oryzanol. However, most of which were located in bran fraction. Thus, bran was the most valuable source of rice antioxidants. The antioxidants content in black rice was higher than in red and normal rice. The highest antioxidant content was found in black rice bran no.1.

The major anthocyanins of red and black rice were cyanidin-3-glucoside and peonidin-3-glucoside. Red rice showed smaller content of anthocyanins than

black rice. Cyanidin-3-glucoside was the major anthocyanin found in all pigmented rice samples, the percentage of which was 90-95% (except black rice no.2; 58%).

Most of phenolic acids in rice were bound phenolic, which bound with polysaccharide in cell wall. HPLC analysis separated all rice phenolic acids into 6 known compounds including gallic, protocatechuic, hydroxybenzoic, *p*-coumaric, ferulic, and sinapic acids. Black rice contained higher contents of protocatechuic and hydroxybenzoic acids than red and normal rice.

CHAPTER IV

ANTIOXIDANT ACTIVITIES OF SELECTED AUTHENTIC RICE ANTIOXIDANTS AND METHANOLIC EXTRACTS OF PIGMENTED RICE IN DIFFERENT MILLING FRACTIONS

4.1 Introduction

Consumers are concerned about the safety of their food and about potential effects of synthetic additives on their health. The suspicion of synthetic antioxidants may act to promote carcinogenicity (Namiki, 1990) has led to increasing of natural antioxidants interest.

The main antioxidative components in grains are classified as phenolic and flavonoid compounds and other substances such as tannins, ferulic acid, caffeic acid, *p*-hydroxybenzoic acid, protocatechuic acid, sinapic acid, isoferulic acid and syringic acid (Martinez-Tome, 2004). In addition, rice contained γ-oryzanol (Xu and Godber, 1999). Beneficial effects of antioxidants on promoting health are believed to be achieved though several mechanisms, such as directly reacting with and quenching free radicals, reducing peroxides, chelating metals and stimulating the antioxidative defense enzyme system (Zhou, 2004). Pigmented brown rice have been reported to possess health benefits in preventing diabetic complication by inhibiting the key enzyme (aldose reductase) involved in their development (Yawadio *et al*., 2007). Our results from the previous chapter showed that pigmented rice is a potential source of antioxidants such as phenolic acids, anthocyanins, tocopherol and γ-oryzanol. Pigmented rice with high level of antioxidants showed potential for use as an excellent dietary source of antioxidants for disease prevention and health promotion.

Traditionally, rice is consumed in its polished form. The milling process of rice aimed at the refinement of white rice (polished or milled rice) and the removal of bran fraction as by-product. Most of the antioxidants are in the bran fraction as confirmed in our study (Chapter 3). Literature data on antioxidant content and antioxidant activity in pigmented rice bran are very limited. In the present study, we proposed to evaluate the free radical scavenging ability and antioxidant activity of the

milling fraction of pigmented rice and therefore understand its value as a potential source of natural antioxidants in food.

The objectives of this research were (1) to compare the antioxidant activities of some authentic rice antioxidants with some synthetic antioxidants, and (2) to determine the antioxidant activities of pigmented rice in 3 milling fractions by 3 antioxidant activity measurements.

4.2 Methodology

4.2.1 Materials

Unmilled, milled, and bran fractions of normal rice (California long grain rice) obtained from Pacific International Rice Mills, Inc. (USA). Unmilled fractions of aromatic red rice and black rice no.1 (black japonica rice) obtained from SunWest Foods, Inc. (USA). The two unmilled black rice samples which were black rice no.2 (black japonica rice) and black rice no.3 (Hong Kong black rice) obtained from Lundberg Family Farms (USA). All pigmented rice samples were milled by laboratory rice miller (Ricepal32, Yamamoto Co., Ltd.) 2 times by level 3 and 4 respectively to remove the bran layer. The bran of each were combined. The 3 milling fractions of rice including milled rice (white rice), unmilled rice (brown rice) and the bran samples were collected for further analysis. All samples were stored immediately at -18^{o}C after milling and kept at this condition until analysis (Appendix A).

Four authentic antioxidants including cyanidin-3-glucoside (ChromaDex Inc.), peonidin-3-glucoside (ChromaDex Inc.), α-tocopherol (Sigma-Adrich Chemical Co.,) and γ-oryzanol (Wako Chemicals USA, Inc.), and two synthetic antioxidants including BHT (Sigma-Adrich Chemical Co.,) and EDTA (Sigma-Adrich Chemical Co.,), were evaluated for their antioxidant activities by 3 assays.

4.2.2 Evaluation of antioxidant activity

- *Samples preparation and extraction*

Rice fraction samples were finely grounded and defatted twice with hexane (1:20 w/v) for 30 minutes. The defatted rice fraction was extracted with 100% methanol (1:20 w/v) in an electrical shaker overnight at room temperature and then filtered through Whatman No.1 filter paper. The extracts were stored under freezer at -18 ^{o}C until used for further analysis. All analysis was performed within 2 weeks after extraction.

- *Determination of DPPH radical scavenging activity*

The free radical scavenging capacity of selected antioxidants and rice fraction samples extract was estimated following a previously reported procedure using the stable 2,2'-diphenyl-1-picrylhydrazyl radical (DPPH·) (Brand-Williams , Cuvelier and Berset, 1995). Briefly, different dilutions of the extracts were prepared. An aliquot of the solution or diluted extract (1.0 ml) was vigorously mixed with 1.0 ml of freshly prepared 0.004% DPPH in methanol and held in the dark for 30 minutes at room temperature. The absorbance was then read at 517 nm (UV-160, Shimadzu) against blanks. DPPH free radical-scavenging ability was calculated by using the following formula:

Scavenging ability (%) = [$\text{Absorbance}_{517\text{nm of control}} - \text{Absorbance}_{517\text{nm of sample}}$/ $\text{Absorbance}_{517\text{nm of control}}$] × 100.

The scavenging activity of rice fraction extracts was expressed as 50% effective concentration, EC_{50} (μg/ml or mg/ml) which was obtained by interpolation from linear regression analysis. A lower EC_{50} value indicates a higher antiradical activity (Brand-Williams *et al*., 1995).

- *Determination of reducing power*

The reducing power of the extract was determined by the method of Yen and Duh (1993) with some modifications. An aliquot (2.5 ml) of the extract was mixed with sodium phosphate buffer (2.5 ml, 2M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (0.5 ml, 1%). The mixture was incubated at 50^oC for 20 minutes. Trichloacetic acid (10%, 5 ml) was added to the mixture, which was then centrifuged at 6000g for 10 minutes to stop the reaction. Aliquot (5 ml) from the upper layer of the solution was mixed with deionized water (5 ml) and ferric chloride solution (1 ml, 1%). The absorbance at 700nm was then measured using UV/vis spectrophotometer (UV-160, Shimadzu). Higher absorbance of the reaction mixture indicated higher reducing power.

- *Determination of lipid peroxidation inhibition*

Lipid peroxidation inhibition of rice fraction sample extracts and selected antioxidants was measured according to the method reported by Lingnert, Vallentin and Eriksson (1979). Briefly, linoleic acid (5 mM) was emulsified with the aid of an equal amount of Tween 20 in sodium phosphate buffer (0.1M, pH7). An aliquot (4 ml) of linoleic acid solution was mixed with 200µl of rice fraction extract in test tubes. The tubes were placed in darkness at 37^oC for 8 hours to accelerate the oxidation and then were added with methanol (6 ml, 60%). The progress of autoxidation is monitored by UV absorbance at 234 nm (A_{max} of conjugated diene peroxides from lionoleic oxidation). The absorbance at 234nm was measured against blanks. Controls without antioxidant were run parallel. BHT and α-tocopherol were used for comparison. The inhibition of lipid peroxidation was calculated according to the following equation:

$$\%\text{Inhibition of lipid peroxidation} = [\text{Absorbance}_{234\text{nm of control}} - \text{Absorbance}_{234\text{nm of sample}}/\text{Absorbance}_{234\text{nm of control}}] \times 100$$

4.3 Results and discussion

4.3.1 Antioxidant activity of some commercial and authentic rice antioxidants

Antioxidant activities determination of selected antioxidants including BHT, EDTA, α-tocopherol, γ-oryzanol, cyanidin-3-glucoside and peonidin-3-glucoside, were performed by DPPH radical scavenging activity, reducing power and lipid peroxidation inhibition assays. Stock solutions of all antioxidants were made by dissolving in methanol. The stock solutions were diluted to many concentrations for using in antioxidant activity determinations. Because of the limiting amount of peonidin-3-glucoside in our research, the highest concentration of which was limited at 20 μg/ml.

Both DPPH radical scavenging activity and lipid peroxidation inhibition of antioxidants were expressed as 50% effective concentration, EC_{50} (μg/ml) which was obtained by interpolation from linear regression analysis. While reducing power of antioxidants was expressed as concentration at absorbance (700nm) = 0.1. Thus, lower EC_{50} or concentration indicated higher activity.

a) DPPH radical scavenging

The determination of DPPH radical scavenging activity of selected antioxidants (Figure 4.1) showed that BHT, α-tocopherol, γ-oryzanol, cyanidin-3-glucoside and peonidin-3-glucoside were the primary antioxidants which donates hydrogen atoms to free radicals to terminate free radical chain reaction. EDTA with no DPPH radical scavenging activity was not the primary antioxidant. The DPPH radical scavenging activity among these antioxidants was in the following order: α-tocopherol ≈ cyanidin-3-glucoside > peonidin-3-glucoside > BHT > γ-oryzanol > EDTA. BHT, a synthetic phenolic compound, is one of the most widely used synthetic antioxidant in food industry. It is fat soluble but not effective in frying because of its volatility. Regrading to the EC_{50} value, DPPH radical scavenging activity of BHT was lower than α-tocopherol and both anthocyanins. α-Tocopherol, cyanidin-3-glucoside and peonidin-3-glucoside, which were all found in pigmented rice showed high (low EC_{50}) radical scavenging activity. The

antioxidant activity of α-tocopherol was higher than BHT mainly due to the heterocyclic ring hydrogen donor part. α-Tocopherol works as antioxidant by donating hydrogen atom of hydroxyl group to the free radical, the radical formed from α-tocopherol is stabilised through delocalisation of the solitary electron over the aromatic ring structure (Frankel, 1996).

The antioxidant activity of anthocyanin plays a vital role in prevention of neuronal and cardiovascular illnesses, cancer, and diabetes (Konczak and Zhang, 2004). Our results showed that cyanidin-3-glucoside and peonidin-3-glucoside possess high activity against DPPH molecule. Cyanidin with catechol group is able to capture free radicals by donation of phenolic hydrogen atom (Rice-Evans, Miller and Paganga, 1996). Cyanidin had higher antioxidant activity than peonidin due to the *o*-dihydroxyl substitution in cyanidin is most susceptible to oxidation than not substitution in peonidin (Castaneda-Ovando *et al*., 2009). Bagchi *et al*., (1998) reported that anthocyanins had higher antioxidant activity than vitamin E, but from our study, anthocyanins were effective as α-tocopherol.

γ-Oryzanol, a unique antioxidant occurring only in rice, is a mixture of ferulic acid esters of triterpene alcohol and sterol. Because of the ferulic acid structure, γ-oryzanol posses the antioxidant activity. The DPPH radical scavenging activity of γ-oryzanol was lower than other antioxidants, but it's high content makes it be an importance antioxidant in rice. Besides, consuming γ-oryzanol decreases hepatic cholesterol biosynthesis and reduce cholesterol absorption (Rong *et al*., 1997).

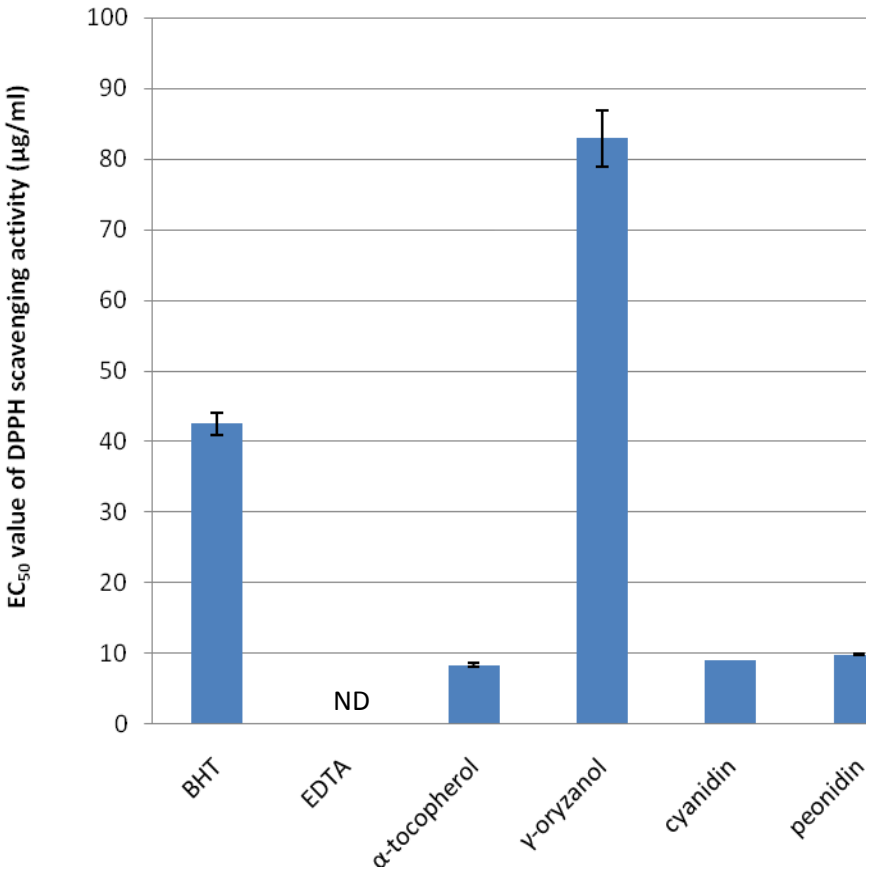


Figure 4.1 EC_{50} value of DPPH radical scavenging activity of some commercial and authentic rice antioxidants (shorter bar represents higher activity; ND= not detectable)

DPPH radical is a long-lived nitrogen radical, which is not similar to the highly reactive and transient peroxyl radicals in lipid peroxidation. Thus, this method may be useful for screening antioxidants, but antioxidant effectiveness must always be studied by other methods because their activity is dependent on a variety of factors including polarity, solubility and metal-chelating activiy (Huang, Ou and Prior, 2005; Gordon, 2001).

b) Reducing power

Any compound that has a reduction potential lower than the reduction potential of free radical is capable of donating a hydrogen to that free radical (Decker, 2007). Reducing power of some selected antioxidants is shown in Figure 4.2. Among the selected antioxidants, BHT showed the highest reducing power. Reducing power of EDTA was not detected, which comfirmed with the result of DPPH radical scavenging activity. EDTA is a secondary antioxidant which chelates the metal ion such as Cu^{2+} and Fe^{3+}. Cyanidin-3-glucoside showed higher reducing power than α-tocopherol; and γ-oryzanol showed the lowest reducing power.

c) Lipid peroxidation inhibition

Lipid peroxidation inhibition was dose dependent with α-tocopherol concentration (Figure 4.3C), whereas the other antioxidants were not (Figure 4.3A, B, D, and E). The lipid peroxidation inhibition curves of BHT, EDTA and cyanidin-3-glucoside were a sigmoid curve or S-shaped which fitted with polynomial equations (Table 4.1). In the concentration range investigated, low concentration of BHT, EDTA, γ-oryzanol, and cyanidin-3-glucoside inhibited lipid peroxidation at low level. The inhibitions highly increased with increasing their concentrations and reached maximum inhibition at high concentration of 8 µg/ml for BHT, 15 µg/ml for EDTA, 100 µg/ml for γ-oryzanol and 160 µg/ml for cyanidin-3-glucoside. Beyond these concentrations, the increasing of antioxidants concentration did not increase lipid peroxidation inhibition.

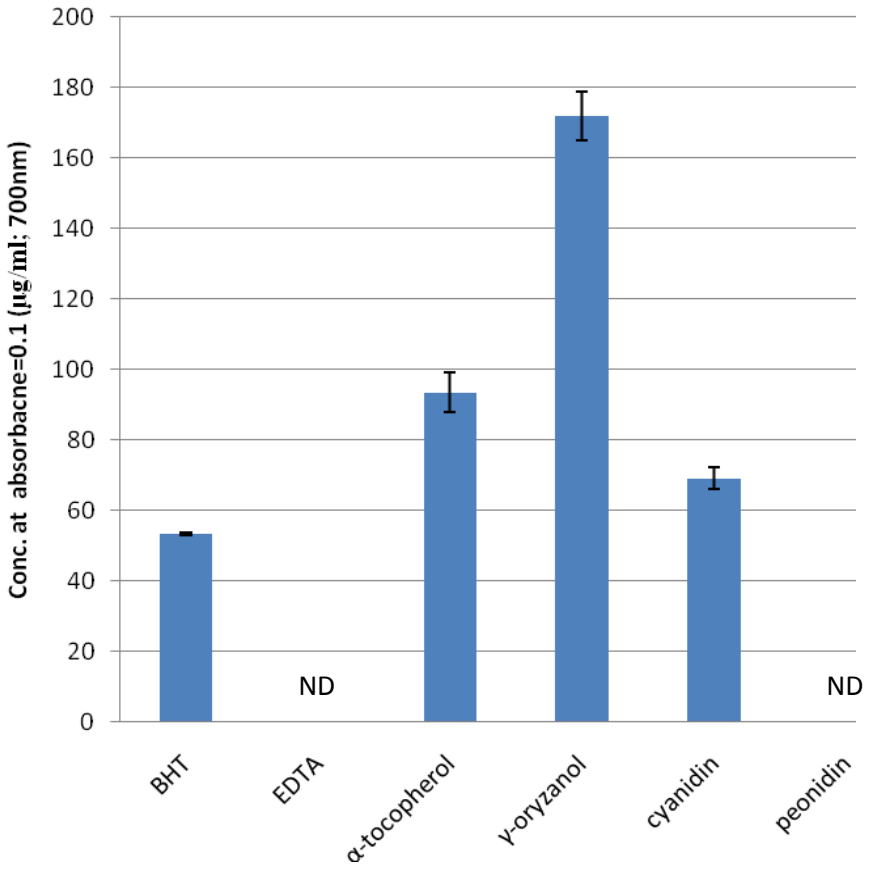


Figure 4.2 Reducing power (express as concentration of the solution at absorbance = 1.0 at 700nm) of some commercial and authentic rice antioxidants (shorter bar represents higher activity; ND= not detectable)

Table 4.1 shows the EC_{50} value of lipid peroxidation inhibition of selected antioxidants. EDTA with no DPPH radical scavenging activity and reducing power, showed the great lipid peroxidation inhibition, which confirmed that it is the secondary antioxidant. Metal chelating properties of EDTA can retard lipid oxidation by blocking the pro-oxidant metal ions, and thus limiting the formation of chain initiators by preventing metal-assisted homolysis of hydroperoxides (Yanishlieva-Maslarova, 2001). In oil-in-water emulsion system, cyanidin-3-glucoside had the lowest lipid peroxidation inhibition, because of its high polarity, while lipid soluble antioxidants as α-tocopherol and γ-oryzanol showed the higher lipid peroxidation inhibition. The solubility of antioxidants influences their activity

(Huang *et al*., 2005). McClements and Decker, (2000) reported that the higher polarity of antioxidants had the lower efficiency in inhibiting lipid oxidation because they are located predominantly in aqueous phase, away from the place where lipid oxidation occurs.

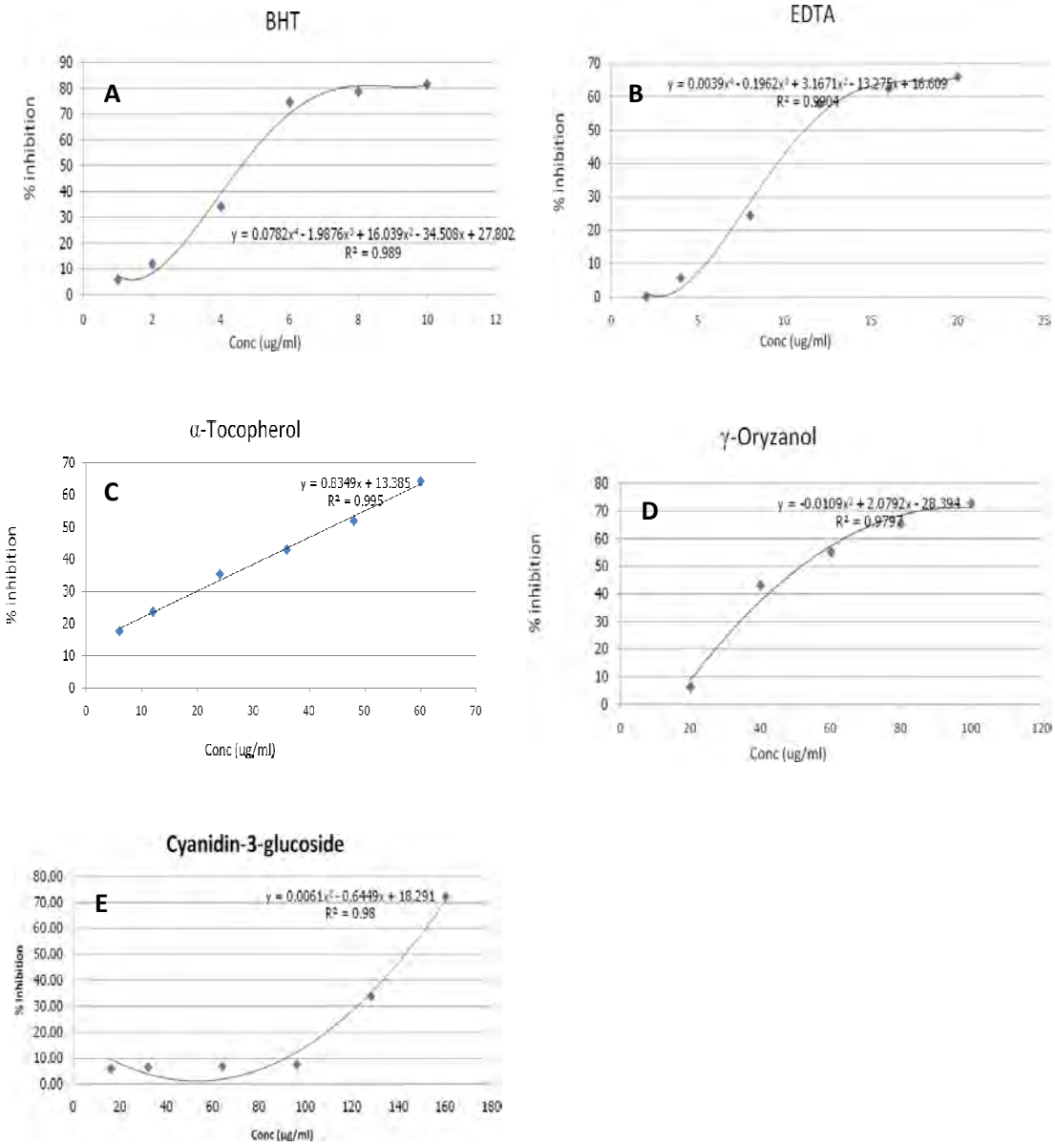


Figure 4.3 Relationship between %inhibition and concentration of some commercial and authentic rice antioxidant solutions

Table 4.1 EC_{50} values of lipid peroxidation inhibition and relationship between %inhibition and concentration of some commercial and authentic rice antioxidant solutions

Antioxidants	EC_{50} (μg/ml)[1]	Equation type	Equations
BHT	4.60	Quartic polynomial	$y = 0.0782x^4 - 1.9876x^3 + 16.039x^2 - 34.508x + 27.802$ $R^2 = 0.989$
EDTA	11.04	Quartic polynomial	$y = 0.0039x^4 - 0.1962x^3 + 3.1671x^2 - 13.275x + 16.609$ $R^2 = 0.990$
α-Tocopherol	43.86	Linear	$y = 0.8349x + 13.385$ $R^2 = 0.995$
γ-Oryzanol	51.73	Quadratic polynomial	$y = -0.0109x^2 + 2.0792x - 28.394$ $R^2 = 0.980$
Cyanin-3-glucoside	142.26	Quadratic polynomial	$y = 0.0061x^2 - 0.6449x + 18.291$ $R^2 = 0.980$

[1]Lower values represent higher activity

4.3.2 Antioxidant activities of methanolic extract of rice fractions

Rice fraction samples from chapter 3, including milled, unmilled and bran fractions of 5 rice cultivars were extracted using methanol. The extracts were determined for their antioxidant activities by 3 assays.

a) DPPH radical scavenging activity

Using methanol as the extraction solvent, many antioxidants are also extracted, including tocopherols, tocotrienols, γ-oryzanol, free phenolic compound and anthocyanins (Chotimarkorn *et al*., 2008). These antioxidants also

exhibit an antioxidative effect in many methods. Goffman and Bergman (2004) reported that phenolic compounds were the main compounds in rice responsible for DPPH radical scavenging activity.

Bran extracts of all rice cultivars showed highest DPPH radical scavenging activity (Figure 4.4). The cultivar with the highest DPPH radical scavenging activity was black rice no.1, followed by black rice no.3, black rice no.2, red and normal rice. The DPPH radical scavenging activity of black rice no.1 was approximately 6 times higher than normal bran extract. This can be due to the high antioxidant content especially the anthocyanins of pigmented rice (Figure 3.1, 3.4 and 4.4).

Because of the very high DPPH radical scavenging activity of cyanidin-3-glucoside, peonidin-3-glucoside and α-tocopherol (Figure 4.1), thus, the extract contains high concentration of anthocyanins and α-tocopherol has high DPPH radical scavenging activity. However, DPPH free radical scavenging of all extract was less than of BHT, a synthetic antioxidant (Figure 4.4).

DPPH has a UV-vis absorption maximum at 517 nm. The antioxidant reduces it and the color fades to colorless. This method has been used extensively to evaluate reducing substance base on the reduction of methanolic DPPH radical solution in the presence of a proton-donating substance. However, DPPH is a long-lived radical which's not similar to peroxyl radical in lipid peroxidation (Huang *et al*., 2005). Thus, the antioxidation activity should be performed in several methods.

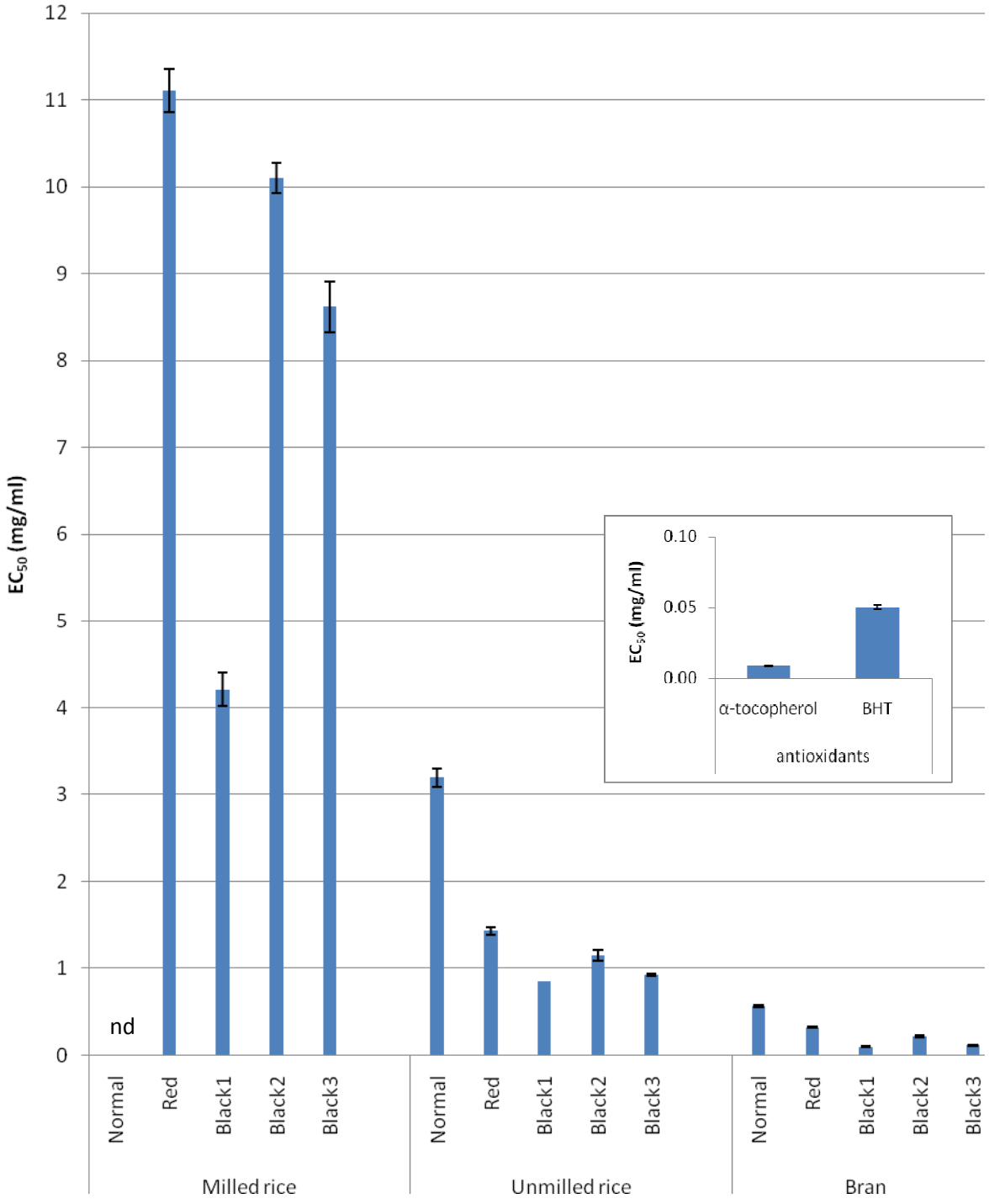


Figure 4.4 EC_{50} value of DPPH radical scavenging activity of rice fraction extracts, α-tocopherol and BHT, the standard antioxidants (shorter bar represents higher activity)

b) Reducing power

In the reducing power assay, the presence of reducing agents in the extract would result in reducing ferric/ferricyanide complex to the ferrous form. The ferrous form can be monitored by measuring the formation of Perl's prussian blue color at 700 nm. The reducing power of the extracts was expressed as absorbance of the extract at 700nm. Higher absorbance of the mixture indicated higher reducing power.

Figure 4.5 shows the reducing power of rice fraction extracts. There was not very much different of the reducing power among fractions of normal rice, while pigmented rice showed the obvious defferent. The result showed that anthocyanins in pigmented rice samples affected the reducing power of the extracts. It was confirmed by the results of the DPPH radical scavenging (Figure 4.4). The highest reducing power was observed in the bran extracts from all pigmented rice cultivars (Figure 4.5). Pigmented rice bran extracts showed higher reducing power than normal rice bran. The highest reducing power was found in black rice bran no.1 whereas the lowest was found in normal rice bran extract.

A similar trend for reducing power of bran extracts was observed among the pigmented rice bran fractions (Figure 3.3and 4.5). A high content of anthocyanin was reflected in the high reducing power of the extracts. Because of anthocyanin content in pigmented bran, it can be concluded that there is dependence with respect to anthocyanin concentration of rice bran. This finding supported the fact that anthocyanins play an important role in reducing power of pigmented rice bran (Figure 4.2).

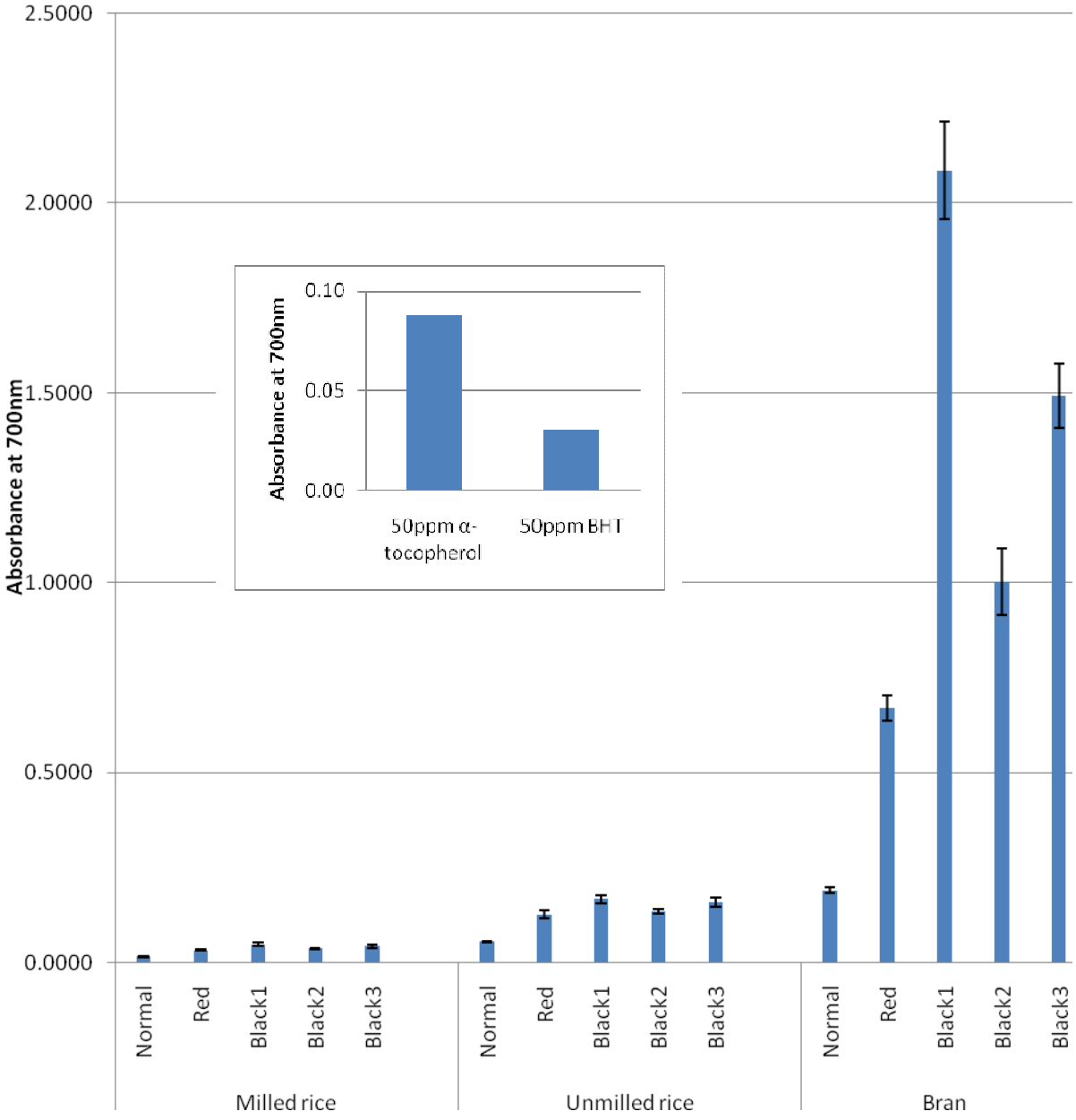


Figure 4.5 Reducing power of rice fraction extracts

c) Lipid peroxidation inhibition

Linoleic acid - buffer emulsion was exposed to the air at 37^{o}C to induce the lipid peroxidation. The efficiency of antioxidant (% inhibiton of lipid peroxidation) was determined by measuring the occurring of conjugated diene.

Lipid peroxidation inhibition of milled rice was ranged from 4.24-27.98% while unmilled and bran extracts were ranged from 46.69-72.88% and 63.36-85.48%, respectively (Figure 4.6). These results indicated that the metanolic-extracts of pigmented rice possessed components acting as electron donors, which can terminate lipid peroxidation chain reactions, possibly through conversion of lipid peroxy radicals to more stable products.The results showed that lipid peroxidation inhibition was not dose dependent. Bran with 7 times total antioxidant content higher than unmilled fraction (Table 3.1 and 3.4), showed similar level of lipid peroxidation inhibition as unmilled fraction. For milled and unmilled rice fraction extracts, black rice no.1 had the highest value of lipid peroxidation inhibition while the lowest was normal rice. In contrary, among the bran extracts, normal rice had the highest value of lipid peroxidation inhibition. In bran fraction, despite black rice bran samples contained higher antioxidants content than normal bran, but they showed the lower of lipid peroxidation inhibition than that of normal bran. The results were similar to the study by Nam *et al*., (2006) who reported that several pigmented rice extracts acted as pro-oxidants in the linoleic peroxidation assay. This phenomenon was often found in phenolic antioxidants. Cillard *et al*., (1980) reported that often phenolic antioxidants lose their activities at high concentrations and behave as pro-oxidants. Food with excess amount of antioxidant was not necessary to retard oxidation. Antioxidants are capable to retard oxidation only in small amount (Halliwell *et al*., 1995). Thus, antioxidants should be added to foodstuffs as early as possible to achieve maximum protection against oxidation (Coppen, 1983).

From the present study, it can be concluded that the results of the DPPH radical scavenging activity and reducing power experiment cannot predict the antioxidant activity against the formation of lipid peroxidation product (conjugated diene) as with the case of normal rice bran extract.

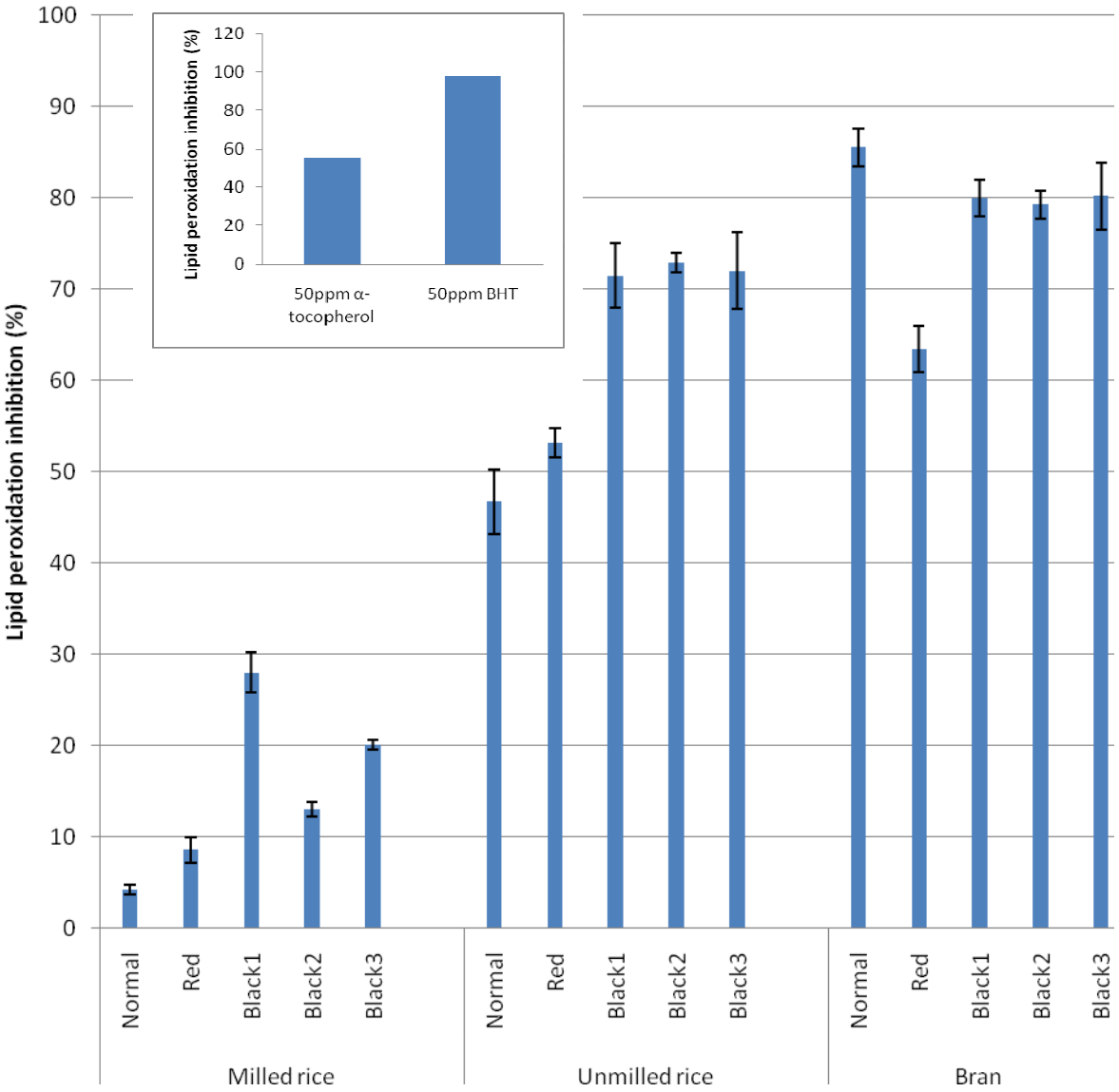


Figure 4.6 Lipid peroxidation inhibition of rice fraction extracts

4.3.3 Correlation of antioxidant contents and antioxidant activities

The results of HPLC analysis of rice antioxidants from chapter 3 and the different antioxidant assays used in the present investigation were compared and correlated with each other. Correlation between the results of different assays is shown in Table 4.2. There are linear regressions and a significant ($p<0.01$) relationship between DPPH radical scavenging activity and reducing power and % inhibition of linoleic acid oxidation. The DPPH assay and reducing power were highly correlated ($r = 0.978$). The positive correlations were observed between DPPH assay or reducing power *vs* lipid peroxidantion inhibiton in lower correlation coefficient ($r = 0.584$ and 0.560, respectively). The results suggested that the DPPH assay and reducing power have similar predictive activity of antioxidant activities in pigmented rice.

Many methods have been adapted to assess antioxidants in food stuffs. DPPH radical scavenging method and reducing power method are based on single electron transfer (SET) reaction whereas inhibition of linoleic acid oxidation is based on hydrogen atom transfer (HAT) reaction (Huang *et al*., 2005). Many studies found the similar tendency between methods which were based on the same SET reaction (Balogh, Hegedus and Stefanovits-Banyai, 2010; Pantelidis *et al*., 2007; Ercisli and Orhan, 2008). Balogh *et al*., (2010) reported that DPPH assay and FRAP (ferric ion reducing antioxidant power assay) (SET *vs* SET) were highly correlated ($r = 0.941$) wherese lower correlation was found in methods which were based on different reactions (SET *vs* HAT) such as DPPH assay *vs* photochemiluminescence assays ($r = 0.851$). The explanations attribute the close correlation between these assays to the similarity of the chemistry behind them (Huang *et al*., 2005).

The results from the correlation between the measurements of antioxidant activity may be explain why the highest DPPH radical activity and reducing power of extract from black rice bran no. 1 showed lower lipid peroxidation inhibition than normal bran extract. Semilar result was found by Balogh *et al*., (2010) who reported that the berry extract with highest DPPH or FRAP (SET) had lower total radical-scavenger capacity as assay by chemiluminescence (HAT).

Table 4.2 Correlation coefficient (r) between antioxidant activities and antioxidant contents in pigmented rice fractions*

	DPPH radical scavenging	Reducing power	Lipid peroxidation inhibition
DPPH radical scavenging	-	0.978	0.584
Reducing power	0.978	-	0.560
Lipid peroxidation inhibition	0.584	0.560	-
Total antioxidant	0.929	0.911	0.700
Total phenolic acids	0.929	0.924	0.714
Peonidin-3-glucoside	0.607	0.656	0.575
Cyanidin-3-glucoside	0.962	0.963	0.573
Gallic acid	0.955	0.982	0.649
Ferulic acid	0.880	0.862	0.726
Protocatechuic acid	0.940	0.947	0.586
Hydroxybenzoic acid	0.840	0.888	0.530
Sinapic acid	0.820	0.788	0.719
p-Coumaric acid	0.590	0.566	0.658
γ-Oryzanol	0.742	0.744	0.663

*All factor was significantly correlated each other at $p<0.01$, n= 24-30 (Pearson's correlation)

The highly correlation between antioxidant contents and antioxidant activites were found in this study (Table 4.2). The contents of total antioxidant, total phenolic acids, cyanidin-glucoside, gallic acid and protocatechuic acid correlated extraordinarily well with DPPH radical scavenging activity and reducing power ($r > 0.9$). These results suggested that these antioxidants highly influenced the DPPH radical scavenging activity and reducing power of the rice fraction extracts. Cyanidin-3-glucoside contents which highly correlated with DPPH radical scavenging activity and reducing power ($r = 0.962$ and 0.963, respectively) but showed low correlation with inhibition of lipid peroxidation ($r = 0.573$), that comfirmed the lower inhibition of lipid peroxidation of black bran extract than normal bran (Figure 4.1 and 4.7). The contents of total antioxidant, total phenolic acids, ferulic acid and sinapic acid relatively high correlated with %lipid peroxidation inhibition ($r > 0.7$). Phenolic acids may attribute to the inhibition of lipid peroxidantion of pigmented rice extracts.

4.4 Conclusion

The observation of antioxidant activities of some commercial and authentic antioxidants concluded that α-tocopherol, cyanidin-3-glucoside and peonidin-3-glucoside had higher DPPH radical scavenging activity than BHT and EDTA. However, in oil-in-water emulsion system, polarity of antioxidants influenced the inhibition of lipid peroxidation. Lipid soluble antioxidants including BHT, α-tocopherol and γ-oryzanol had higher lipid peroxidation inhibition than cyanidin-3-glucoside, a water soluble antioxidant.

Pigmented rice bran extracts showed high DPPH radical scavenging activity, reducing power and lipid peroxidation inhibition. This study provides evidence that clearly demonstrate that pigmented rice bran is a rich source of antiradical and antioxidant compounds. The antioxidant effects of pigmented rice are related to their phenolic pigmented in the bran. Suggesting that it may have potential for utilization as a novel cereal product, rich in natural antioxidants.

CHAPTER V

PHYSICOCHEMICAL AND ANTIOXIDATIVE PROPERTIES OF WHEAT BREAD ADDED PIGMENTED RICE BRAN

5.1 Introduction

Bread is an important staple food in many countries. The attempt to enhance the nutrition value into the bread has been widely studied. Adding wheat bran and germ into dough was successful in term of enhanced dietary fiber (Sidhu, Al-Hooti and Al-Saqer, 1999). Nowadays antioxidants are interested in the aspect of antioxidant activities and health benefits. The health benefit effect of antioxidants such as phenolics consuming are associated with their antioxidative, anti-inflammatory, anticarcinogenic and antimutagenic properties (Smith *et al*., 1985).

Rice bran is a by-product of rice milling process, which is comprised primarily of pericarp, aleurone, subaleurone and layers of the kernel; and typically includes the embryo or germ and small amount of the starchy endosperm. Rice bran is known for its valuable source of various antioxidants. γ-Oryzanol is an antioxidant abundance in rice bran. Raw rice bran contains about 2-4% of γ-oryzanol which is 30-40 times higher than tocopherol content (Chen and Bergman, 2005; Agular-Garcia *et al*., 2007). Pigmented rice contains pigments in their pericarp. These pigments are anthocyanins which possess antioxidant activity. Fortification of rice bran into bread dough may improve oxidative stability of bread and also enhance the nutrition values. Addition of rice bran powder to rice flour dough for enhancing oxidative stability of fried dough during storage has been studied by Chotimarkorn and Silalai, (2008). However, rice lacks of gluten protein, the wheat protein structure of bread dough, which may affect dough properties and bread quality. Rice bran has been incorporated into many bakery products such as doughnuts, pancake and muffin (Saunder, Sloan and James, 1988). Sekhon *et al*., (1997) found that the bread volume decreased with the increasing level of rice bran while muffin volume was increased. Moreover, baking at high temperature may destroy rice bran antioxidants. Fortifying bread with antioxidants to increase the bioavailability of the nutrient such as vitamin E in the flour has been studied. Park, Seib and Chung (1997) reported that added

vitamin E were retained in proofed dough 96% and no further loss during baking and 7 days of storage. However, in the study of Ranhotra, Gelroth and Okot-Kotber (2000), they found that one-third of added vitamin E lost during baking.

Wheat dough exhibits both viscous and elastic properties, behavior that is known as viscoelasticity. It has been already established that some dough rheological test can predict dough behavior in a bakery at early stages of the manufacturing process (Bollain and Collar, 2004; Collar and Bollain, 2004; Dobraszczyk and Roberts, 1994). Dynamic oscillatory measurements have been applied to bread dough system. Khatkar, Bell and Schofield (1995) reported that dynamic rheological properties of wheat gluten related to bread quality. However, Janssen, Vliet and Vereijken (1996) reported that dynamic rheological properties of dough were not clearly related with bread quality thus either creep-recovery test or stress relaxation experiment was considered to perform, simultaneously (Carson and Sun, 2001; Wang and Sun, 2002).

The aims of this chapter were to describe the effects of rice bran on wheat bread dough rheological properties and quality characteristics of baked bread. The second aim was to compare the bioactive compounds content and antioxidantive properties of wheat/rice bran (WRB) breads with normal wheat bread (control).

5.2 Methodology

5.2.1 Materials

Normal rice bran (California long grain rice) obtained from Lundberg Family Farms. Aromatic red rice and black rice no.1 (black japonica rice) obtained from SunWest Foods, Inc. (USA). All pigmented rice was milled by laboratory rice miller 2 times in level 3 and 4 respectively. All rice bran samples were stored at -18^{o}C within 1 hour after milling and kept it at this condition until analysis. The ingredients used in the formula bread were all purpose wheat flour enriched, bleached, persisted (Gold Medal Flour, General Mills, Inc., MN, USA), bread machine yeast (ACH Food Companies, Inc., TN, USA), unsalted sweet cream butter (Safeway Inc.**,** CA, USA), and sugar (Safeway Inc.**,** CA, USA).

5.2.2 Bread baking method

Bread samples were made using an automatic bread maker machine (Model 5891, Sunbeam Programmable Bread Maker). The program of normal white bread was chosen for bread making. This program adopts the following sequential process: first kneading (5 minutes), rest (5 minutes), second kneading (20 minutes), first rising (15 minutes), third kneading (10 seconds), second rising (30 minutes), and baking (50 minutes). Bread machine formula was obtained from preliminary trials. Rice bran was substituted with wheat flour for 5% and 10%. For dough rheological test, all ingredients were mixed in the bread machine chamber except yeast. The formulations of breads are shown in Table 5.1.

Table 5.1 The formulations of breads

Ingredients (g or ml)	White bread (Control)	5% WRB bread[1]	10% WRB bread[2]
Wheat flour	463	440	417
Rice bran	0	23	46
Sugar	40	40	40
Salt	5	5	5
Butter	20	20	20
Yeast	8	8	8
Water	264	264	264
Total	800	800	800

[1] 5% WRB bread is wheat flour plus 5% rice bran bread

[2] 10% WRB bread is wheat flour plus 10% rice bran bread

5.2.3 Bread quality evaluation

Crust and crumb color of bread was measured with a LabScan XE, Hunter Lab colorimeter (Hunter Associates Laboratory, Inc., VA, USA). Color readings were expressed by Hunter values for *L*, *a* and *b* which were obtained from the average value of 6 times reading.

Specific volume (cm^3/g bread) was measured by the rapeseed displacement procedure in a volume measuring apparatus, 1 hour after baking.

Texture analyzer instrument (Texture Analyzer; TA-XT2, Stable Micro systems, UK) equipped with 31.75 mm dia. probe was used to determine the firmness of bread crumbs. Bread was sliced in 2 cm thickness and was placed on the plate. The crumb was compressed for 50% of the original height at a cross head speed of 1 mm/s. The average peak force of compression from 4 measurements was reported as firmness (g).

5.2.4 Rheology analysis

Bread dough was prepared by automatic bread maker machine and then was immediately sealed in airtight plastic container. The experiment was done at 25^{o}C. The dough was placed on the parallel plate. The gap was adjusted to 2 mm and the edges were trimmed by knife. The dough was rested between the plates for 5 minutes before testing. Silicon oil was used to coat outer edges to prevent drying of the sample. The tests conducted in rheometer (AR 1000N, TA instruments, USA) were (a) frequency sweep test from 0.1 to 120 Hz and (b) creep test by applying a constant stress at 50 Pa for 60 s on sample and allowing the sample to recover the strain in 180 s after removal load.

5.2.5 Determination of antioxidants in rice bran bread

- *Determination of total phenolics content*

Total phenolic content of WRB bread was determined by using Folin-Ciocatuen reagent. According to the method reported by Goffman and Berman (2004) with some modification. Briefly, 200 mg of bread crumb was extracted with 5 ml of methanol (99.9%) overnight (vortex 2 times on first and final) and then were centrifuged at 3822 g for 5 minutes. Four mL of supernatant was filtered by 1 μm syringe filter. The extracts were diluted with deionized water. Folin-Ciocatuen reagent (500 μL) and ethanolamine (1 mL, 0.5M) were added to 1.2 mL of the diluted solution, mixed and allow to stand at room temperature for 30 minutes and the absorbance at 600nm was measured using a UV-vis spectrophotometer (Shimaszu,model UV-160). The results were expressed as mg gallic acid equivalents per g of sample.

- *Determination of anthocyanin components*

Anthocyanin of WRB bread was determined according to the method reported by Kim *et al*., (2008) with some modifications. Ten grams of bread crumb was defatted with 50 mL hexane 10 min for 3 times. Defatted samples were extracted with 50 mL of methanol acidified with HCl (85:15 v/v, 1 N) 3 times then were filtered with Whatman filter paper No.1. The extracts were centrifuged at 12,000 g at 5^{o}C for 20 minutes. Stored the extracts at 4 ^{o}C for 2 days to precipitate large molecules then centrifuged at 12,000 g at 5^{o}C for 20 minutes. Supernatants were concentrated and filtered through 0.45 μm syringe filter before injected in HPLC.

The HPLC instrument (Shimadzu, model LC-10AT) connected with UV-vis detector (Shimadzu, model SPD-10A) was used to identify and quantify the anthocyanins in rice fractions. TosoHaas super-ODS, C18 2μm 4.6 x 100 mm column was used to separate the anthocyanins. The mixture of water, methanol and formic acid (75:20:5 v/v) was used as a mobile phase with isocratic elution at 0.5 ml per minute flow rate. UV-vis detector was set at 530 nm and sample loop was 5μL.

Identification and quantification of anthocyanins followed the method as described previously in chapter 3.

- *Determination of γ-oryzanol content*

γ-Oryzanol in WRB bread was determined according to the method reported by Aguilar-Garcia *et al*., (2007) with some modifications. Bread crumb was extracted (2 times) with methanol (99.9%) and centrifuged at 825g for 10 minutes. The supernatant was evaporated and made up to 10ml with HPLC grade methanol. This solution was filtered through 0.45 μm syringe filter before injected to HPLC.

The HPLC instrument was connected with UV-vis detector and C18 column (Inertsil ODS-3, 5μm, 250x4.6mm) was used to determine γ-oryzanol. The mobile phase is the mixture of methanol and acetonitrile (15:85 v/v). The HPLC was set the flow rate at 2 mL per minute at isocratic mode. The sample loop was set at 20μl. UV-vis detector was set at 325nm. Identification and quantification of γ-oryzanol followed the method as described previously in chapter 3.

5.2.6 Determination of antioxidant activity

- *Extraction method*

To determine antioxidant activity, bread crumb samples were extracted by methanol. Each bread was extracted by methanol 1:10 (w/v) overnight and then filtered through Whatman No.1 filter paper and kept at -18 ^{o}C until analysis

- *Determination of 2,2'-diphenyl-1-picrylhydrazyl(DPPH) radical scavenging activity*

DPPH radical scavenging activity of WRB bread was determined according to the method reported by Brand-Williams *et al*., (1995) with some modifications. Bread extract was diluted in many dilutions by methanol. An aliquot of 1.0 mL of each

dilution was vigorously mixed with 1.0 mL of 0.004% DPPH in methanol and allowed to stand in the dark for 30 minutes. The absorbance at 517 nm was read against blanks using a spectrophotometer (UV-160, Shimadzu). DPPH free radical-scavenging ability was calculated by using the following formula:

Scavenging ability (%) = [$Absorbance_{517nm\ of\ control} - Absorbance_{517nm\ of\ sample}$/ $Absorbance_{517nm\ of\ control}$] × 100.

The scavenging activity of the bread extract was expressed as 50% effective concentration, EC_{50} (mg/ml) and was obtained by interpolation from linear regression analysis.

- *Determination of reducing power*

The reducing power of WRB bread extract was measured according to the method reported by Yen and Duh (1993) with slight modifications. The extract (2.5 ml) was mixed with sodium phosphate buffer (2.5 ml, 2 M, pH 6.6) and potassium ferricyanide solution (0.5 ml, 1%w/v). The mixture was incubated at 50^{o}C for 20 minutes. Trichloacetic acid (5 ml, 10%v/v) was added into the mixture and centrifuged at 6,000 g for 10 minutes. The supernatant (5 ml) was mixed with water (5 ml) and ferric chloride solution (1.0 mL, 1%w/v). The absorbance of the resulting solution was measured at 700 nm using an UV- spectrophotometer.

- *Determination of lipid peroxidation inhibition*

Lipid peroxidation inhibition of WRB bread extract was measured according to the method reported by Lingnert *et al*., (1979) with some modifications. Prepare 5 mM of linoleic acid in 0.1M sodium phosphate buffer (pH7) by using Tween20 at the same amount of linoleic acid. Linoleic acid solution (4 ml) was mixed with the bread extract (200 µl). The mixture was incubated at 37^{o}C for 8 hours and then was added with methanol (6ml, 60%v/v). The absorbance at 234nm was measured against blanks. The inhibition of lipid peroxidation was calculated by using the following formula:

%Inhibition of lipid peroxidation = [$Absorbance_{234nm\ of\ control}$ − $Absorbance_{234nm\ of\ sample}$/$Absorbance_{234nm\ of\ control}$] × 100

5.2.7 Statistical analysis

Statistical package for the social sciences (SPSS, version 17.0, 2008) was used to conduct an Oneway ANOVA, to find out if the effects of different bread samples were significant. Dancan's multiple range test ($p<0.05$) was used to detect differences among treatment means.

5.3 Results and discussion

5.3.1 Rheological properties of WRB bread doughs

The variation of G' (storage modulus) and G'' (loss modulus) with frequency sweep from 120 to 0.1 Hz for control (all wheat) and rice bran added wheat dough is shown in Figure 5.1 and 5.2. The value of moduli increased with an increased in frequency for both control and rice bran added dough samples. In all samples, the G' value was more than G'', in whole range of frequencies which showed that the dough was more elastic than viscous. Similar observations on dynamic rheological studies on dough have been reported (Fu, Mulvaney and Cohen 1997; and Masi *et al*., 1998). Both G' and G'' was higher for rice bran added dough than the control. The G' and G" was increased in the same level resulted in the similar tanδ (G"/G') for all samples. The higher of both moduli showed that dough became harder with rice bran added. Similar result was found in the study by Sudha, Vetrimani and Leelavathi (2007) who reported that the resistance to extension values and the extensibility of wheat flour dough decreased with increased defatted rice bran level, indicating the dough becoming harder.

Many reports have found lower values of G′ corresponded with better baking quality (Weipert, 1988; Amemiya and Menjivar, 1992; Schober, Clarke and Kuhn, 2002). Others, however, have found that a higher value of G′ for

glutens and doughs relates to better baking performance (Attenburrow *et al*., 1990; Janssen *et al*., 1996). These conflicting results arise because most of the tests were carried out at rates and deformation conditions very different from those experienced by the dough during baking expansion.

Rice bran consists of many components such as lipid, carbohydrate and protein (Hargrove, 1994). Lipid affected dough rheological properties by acting as a plasticizer decrease G' and G" (Fu *et al.*, 1997). While fiber disrupts the starch-gluten matrix and force gas cells to expand in a particular dimension (Gan *et al*., 1992) affecting dough viscoelastic behavior by influencing water absorption. Water exerts a strong influence on dynamic viscoelastic behavior of dough (Masi *et al*., 1998). Dough with higher moisture content showed lower G' and G" because water can act as a lubricant. High fiber containing of rice bran may increased the water absorption. Thus, the water on dough system was decreased by increasing rice bran which effected on dough rheological properties by increasing G' and G" of dough. Rice protein may also affect dough properties. Oazvald *et al*., (2009) reported that glutelins, the major storage protein in rice can be incorporated with wheat glutenin and changed the functional properties of the dough.

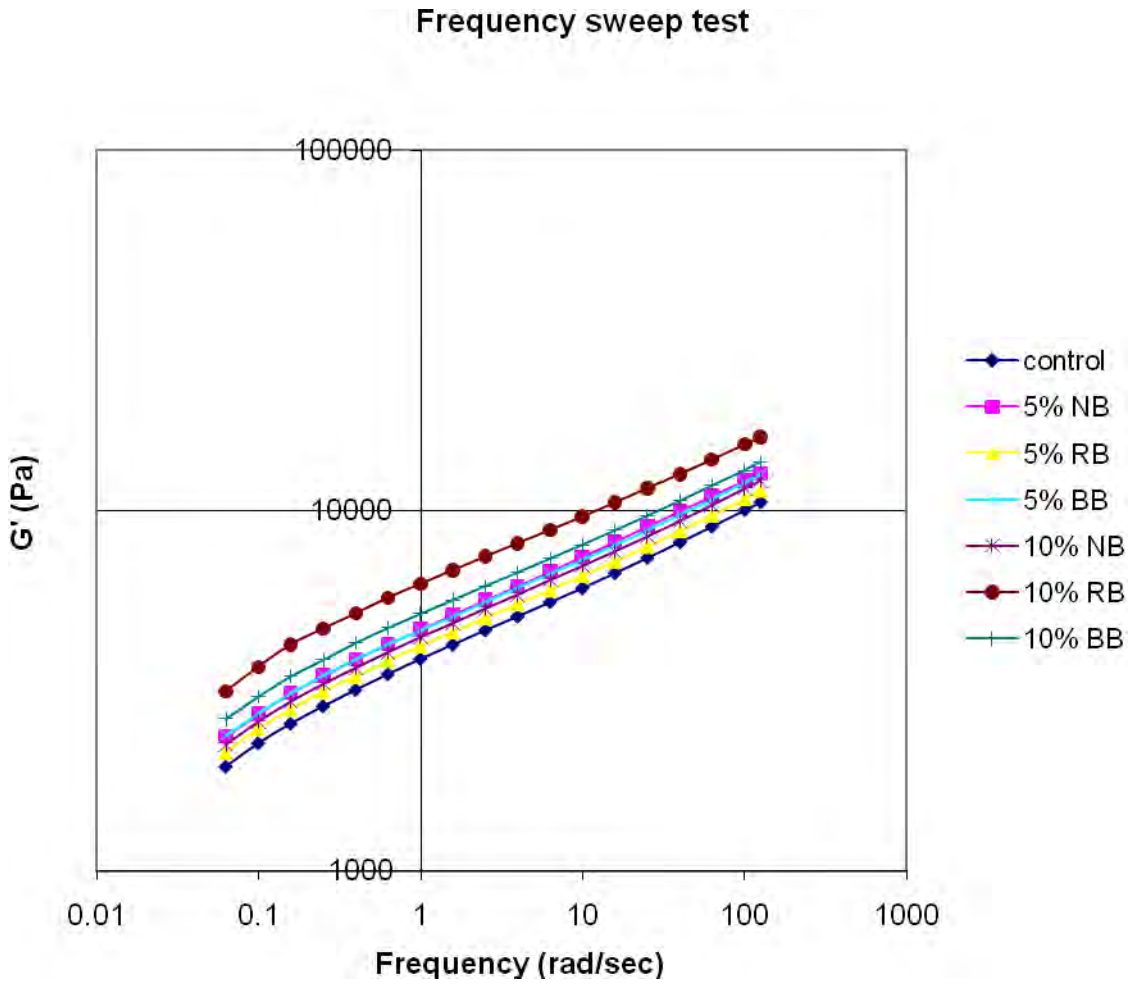


Figure 5.1Storage modulus (G') of WRB bread doughs (Control = all wheat, NB= normal rice bran, RB= red rice bran, and BB= black rice bran; each line was made from the mean of 6 measurements; 2 replications)

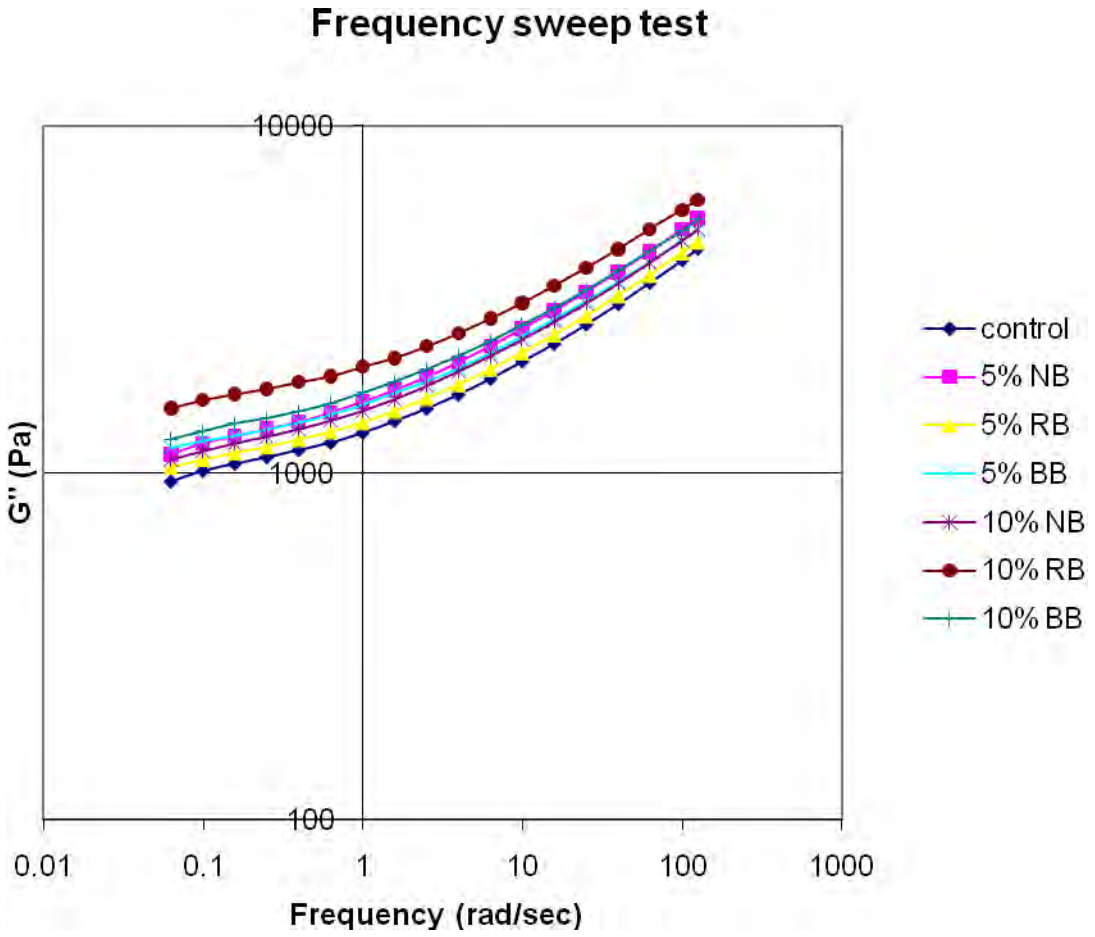


Figure 5.2 Loss modulus of WRB bread doughs (Control = all wheat, NB= normal rice bran, RB= red rice bran, and BB= black rice bran; each line was made from the mean of 6 measurements; 2 replications)

The creep curve for all the samples are shown in Figure 5.3. The curves exhibited a viscoelastic behavior combining both viscous fluid and elastic component. Substituted rice bran dough showed smaller deformation while loading constantly stress than the control. This result was higher with adding more rice bran. Doughs with 10% red and black rice bran showed very low deformation during creep phase. The instantaneously recovered elastic strain after removal load was high for the control while rice bran dough was lower.

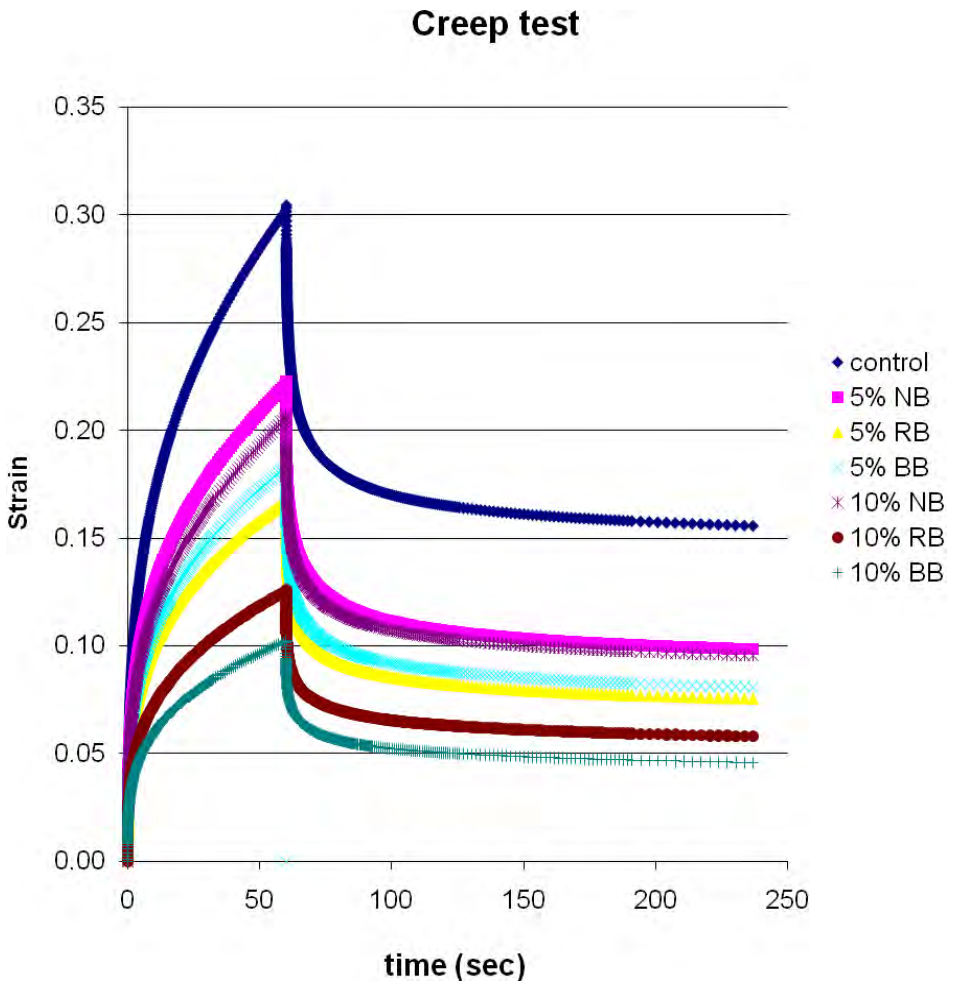


Figure 5.3 Creep curves of WRB bread doughs (Control = all wheat, NB= normal rice bran, RB= red rice bran, and BB= black rice bran; each line was made from the mean of 6 measurements; 2 replications)

5.3.2 Quality characteristics of baked bread prepared from flour with substituted with rice bran

Crust and crumb color of the bread samples are shown in Table 5.2. Addition of normal rice bran at 5% did not affect color of crust and crumb but increasing normal rice bran addition to 10% in WRB bread, decreased the yellowness (*b*) and lightness (*L*) of crust, while increased redness (*a*) of the crumb. Normal rice bran has light-brown color and the browning reaction occurred during

baking may affecte the crust color. Red and black rice contained anthocyanins in their pericarp thus its bran contains almost the pigment of the grain (Figure 3.4, Chapter 3). Substitution wheat flour with red rice bran decreased redness (*a*) and yellowness (*b*) of the crust, meanwhile, the lightness (*L*) was not affected. Crumb color of red bran bread was significantly ($p<0.05$) affected. Redness (*a*) of crumb increased while yellowness (*b*) and lightness (*L*) decreased. The greater effect of rice bran addition on wheat bread color was found in bread added with black rice bran. Crust color was very dark when added with black rice bran, Lightness (*L*) decreased from 41.86 to 35.81 and 24.63 by fortified with 5 and 10% black rice bran to breads, respectively. Crust redness (*a*) was decreased when added with black rice bran, conversely crumb redness was increased. The redness (*a*) values of the crumb may reflect the anthocyanins content in black rice bran. In crust, anthocynins were destroyed by heat during baking but some anthocyanins were remained in crumb caused the higher redness of crumb.

The addition of bran fractions is known to reduce bread quality, resulting in a lower specific volume and denser crumb firmness. Dough rheological properties tests showed that doughs became stronger with added with rice bran (Figure 5.1 and 5.2). Doughs that are too strong do not allow proper development of the bubbles and result in the formation of dense, unpalatable loaves of small volume (Belton, 2003). However, there was no significantly difference ($p>0.05$) between specific volume of 5% rice bran added samples and control (Table 5.2). Specific volume was significantly decreased by adding normal rice bran at 10%. Addition of red and black rice bran at 10% into bread showed smaller adverse effect on loaf volume than normal bran. Published results also suggested that loaf volume depressing effect of fibrous materials is the results of reduced gas retention (Pomeranz *et al*., 1977). Rice bran contained 8-12% crude fiber (Hargrove, 1994). The decreasing of functional quality of final bread has been ascribed to a dilution of functional gluten protein (Pomeranz *et al* 1977; Gan *et al*., 1992). Wang, Vliet and Hamer (2004) also reported that not only the amount of gluten is reduced by bran but the properties of gluten are affected, the gluten becomes stiffer and less extensible. Noort *et al*., (2010) proposed that ferulic acids monomers of bran bound to insoluble cell wall material may be able to interact with gluten proteins,

resulting in adverse effects on functionality of gluten network. Physical properties of bran may affected loaf volume. de Kock *et al*., (1999) reported that smaller particle size of wheat bran showed higher depression in loaf volume. In contrary, some studies reported that reducing the particle size of bran reduced its negative effects (Lai, Hoseney and Davis, 1989). The reduction of baking quality may be also due to the present of lipase and lipoxygenase enzymes in rice bran. Hydrolysis of triacylglycerol, by lipase enzyme lead to the accumulating of free fatty acids which are oxidized by lipoxygenase. This resulted in poorer baking quality and reduced loaf volume (Tait and Galliard, 1988; de Kock *et al*., 1999).

Crumb firmness of WRB bread is shown in Table 5.2. The results showed that crumb firmness was not clearly related with specific volume. Crumb firmness tended to increase with adding rice bran. However, addition of rice bran at 5% to 10% showed no adverse effect on crumb firmness of the bread ($p>0.05$). Crumb of 10% normal bran bread was significantly firmer than that of the control. Previous study has been reported the adverse effects of wheat bran addition on crumb firmness (Gan *et al*., 1992).

These results indicated that wheat breads added with 5% rice bran had no adverse effects on loaf volume and crumb firmness. Crust and crumb color were influenced by rice bran especially pigmented rice bran. Bread with black rice bran became darker.

Although the quality characteristics of WRB bread containing rice bran 5-10% are lower than that of bread from 100% wheat flour (control), the decreasing in quality is minor, and at 5% rice bran addition, the bread appears to be of acceptable quality and are nutrition rich in antioxidants such as phenolics, γ-oryzanol and anthocyanins (data are presented in the following section).

Table 5.2 Crumb firmness, specific volume, crust and crumb color of fortified rice bran bread samples[1, 2]

Wheat/% rice bran type[3]	Crumb firmness (g)	Specific volume (cm^3/g bread)	Crust color			Crumbs color		
			L	*a*	*b*	*L*	*a*	*b*
Control	611.1±17.11[b]	4.51±0.03[a]	41.86±3.61[ab]	12.72±0.91[a]	18.10±0.45[a]	60.92±4.09[a]	2.95±0.75[c]	16.37±1.70[a]
W/5% NB	649.4±27.86[ab]	4.50±0.04[a]	39.91±3.31[b]	13.54±0.49[a]	17.88±0.14[a]	61.87±4.54[a]	3.66±1.10[c]	17.58±1.26[a]
W/10% NB	698.9±12.45[a]	4.20±0.04[d]	31.08±2.36[c]	11.55±0.79[ab]	12.29±0.33[b]	61.73±1.87[a]	4.30±0.57[b]	17.99±0.64[a]
W/5% RB	615.7±19.66[b]	4.48±0.04[ab]	40.47±2.18[a]	10.39±0.33[b]	17.27±0.54[a]	53.59±2.83[b]	5.36±0.28[b]	11.01±0.88[b]
W/10% RB	621.1±27.44[b]	4.39±0.03[bc]	39.36±1.81[ab]	7.52±1.06[c]	10.46±0.17[c]	41.02±1.44[c]	7.23±0.65[a]	10.36±1.10[b]
W/5% BB	620.2±18.67[b]	4.50±0.04[a]	35.81±2.40[bc]	8.06±1.39[c]	11.07±0.31[c]	31.87±1.54[d]	5.55±0.30[b]	4.59±0.30[c]
W/10% BB	664.3±31.54[ab]	4.38±0.04[c]	24.63±1.88[d]	5.09±0.30[d]	4.46±0.14[d]	25.18±1.95[d]	5.87±0.61[ab]	3.39±0.17[c]

[1]Mean ± SD

[2]Values within the same column with difference superscripts are significant difference ($p<0.05$)

[3]Control= all wheat, NB= normal rice bran, RB= red rice bran, and BB= black rice bran

5.3.3 Antioxidants and antioxidant activities of rice bran breads

Total phenolics, anthocyanins and γ-oryzanol contents of rice bran breads are shown in Table 5.3. Total phenolics content of the control (0% rice bran) bread was 2.55 mg/g which was lower than those of all WRB breads (2.89-3.62 mg/g). The results showed that rice bran fortification was capable to enhance the total phenolics to wheat bread. Addition of 10% normal rice bran increased total phenolics content to 3.14 mg/g. The bread formulated with 10% black rice bran showed highest content of phenolics when compare to the bread formulated with 5% and 10% normal and red bran, respectively. Addition of 5 and 10% black bran increased total phenolics content by 20.4 and 42.0 % in wheat bread, respectively, while 5 and 10% of normal and red bran increased the total phenolics content by 13.3-16.9% and 23.1-23.9%, respectively.

In this study, WRB bread dough was baked at 350^{o}F for 50 minutes to make bread. After this baking condition, red bran breads lost all of anthocyanins content while black bran breads lost 82.0-88.6% of cyanidin-3-glucoside. Peonidin-3-glucoside was not detected in the extract. The results showed that approximately 12-18% of cyanidin-3-glucoside was retained in black bran bread. Cyanidin-3-glucoside was very stable against heat at acidic pH (pH 2) and stability decreased as pH increased to 9 (Cabrita, Fossen and Anderson, 2000). Hiemori *et al*., (2009) reported that the degradation of rice cyanidin-3-glucoside was about 80% after pressure cooking.

γ-Oryzanol was more stable to baking than anthocyanins (Table 5.3). It highly retained in WRB breads and baking caused loss of 15.8-33.8 %. Nystrom *et al*., (2007) reported that in frying condition, γ-oryzanol was more stable than tocopherol.

Table 5.3 Antioxidant contents in WRB bread (dry basis) baked with added different rice bran types[1,2]

Wheat/%rice bran type[3]	Total phenolic (mg gallic acid eqv./g)	Anthocyanins (μg/g)		γ-Oryzanol (μg/g)
		Cyanidin-3-glucoside	Peonidin-3-glucoside	
Control	2.55±0.13[c]	N/A[4]	N/A	ND
W/% NB	2.98±0.12[ab]	N/A	N/A	74.72±6.90[d] (18.14%)
W/10% NB	3.14±0.27[a]	N/A	N/A	147.42±8.52[b] (19.24%)
W/5% RB	2.89±0.06[ab]	ND	ND	59.07±4.38[d] (15.82%)
W/10% RB	3.16±0.01[a]	ND	ND	99.27±5.95[c] (29.26%)
W/5% BB	3.07±0.08[a]	11.82±1.03 (82.0%)	ND	71.82±7.29[d] (33.78%)
W/10% BB	3.62±0.12[a]	14.98±1.29 (88.6%)	ND	179.55±9.56[a] (17.23%)

[1]Mean ± SD, The number in brackets showed % losing after baking, ND= not detectable

[2]Values within the same column with difference superscripts are significant difference ($p<0.05$)

[3]Control= all wheat, NB= normal rice bran, RB= red rice bran, and BB= black rice bran

[4]N/A = not available

Table 5.4 shows antioxidant activity of WRB breads in 3 different assays. DPPH radical scavenging activity and lipid peroxidation inhibition of the control sample were not detected. However, the control wheat bread extract possessed reducing power activity. The phenolics compound in wheat flour and the products from Maillard reaction formed during baking may contribute to its reducing power. Maillard products, such as reductones (from dideoxytriulose), have antioxidant activity. Moreover, imines, amino deoxy sugars, Amadori products and dihydrocyclic derivatives, the products of Maillard reaction, also had reducing property (Pokorny, 2001).

Table 5.4 Antioxidant activities of WRB bread with different rice bran types[1]

Wheat/%rice bran type[2,3]	DPPH radical scavenging (EC_{50}; mg/ml)[4]	Reducing power (absorbance at 700nm)	% Lipid peroxidation inhibition
Control	ND	0.490 ± 0.020^{d}	ND
W/5% NB	40.32 ± 1.32^{a}	0.578 ± 0.034^{cd}	8.36 ± 0.66^{d}
W/10% NB	30.01 ± 0.79^{b}	0.635 ± 0.029^{bc}	18.12 ± 0.89^{c}
W/5% RB	41.60 ± 1.54^{a}	0.611 ± 0.048^{bc}	7.41 ± 0.54^{d}
W/10% RB	26.38 ± 1.43^{c}	0.617 ± 0.015^{bc}	18.80 ± 1.13^{c}
W/5% BB	18.65 ± 0.94^{d}	0.713 ± 0.047^{b}	31.72 ± 0.94^{b}
10% BB	7.50 ± 0.64^{e}	0.847 ± 0.022^{a}	38.68 ± 1.05^{a}

[1]Mean ± SD

[2]Control= all wheat, NB= normal rice bran, RB= red rice bran, and BB= black rice bran

[3]Values within the same column with difference superscripts are significant difference ($p<0.05$)

[4]The lower values represent higher activity, ND= not detectable

Bread added with 5% of rice bran showed DPPH radical scavenging activity, reducing power and lipid peroxidation inhibition higher than that of the control bread, the results showed that only 5% of rice bran fortification can improve the antioxidative properties of wheat bread. Black rice breads (W/5%BB and W/10%BB) showed highest antioxidant activity when compared to the bread added with red and normal bran. All WRB breads showed DPPH radical scavenging activity, the highest activity of which was found in 10% black rice bran bread. Black rice bran contained high concentration of many antioxidants such as anthocyanins, phenolic compounds, α-tocopherol and γ-oryzanol which had activities to scavenge free radical (Table 3.3, Chapter 3). The higher content of antioxidants retaining in black rice bran bread especially anthocyanin (Table 5.3) resulted in the highest DPPH radical scavenging activity.

Addition of black rice bran in breads increased reducing power in the higher level than those of normal and red rice bran breads. The 5% black bran bread extract showed 38.1% higher reducing power than the control bread (0% bran) while addition of 5% normal and red bran increased the reducing power 20.7% and 15.1%, respectively. Compared with the control bread, WRB bread with 10% black, red and normal bran showed 60.8%, 38.1% and 21.7% higher reducing power than control, respectively. Overall, among the WRB bread extracts studied, W/10% BB was found to have the highest reducing power and DPPH radical scavenging activity (Table 5.4).

The antioxidant activity of rice bran bread samples was confirmed by the measurement of lipid peroxidation inhibition in linoleic acid emulsion model (Table 5.4). The extract of control bread showed no inhibition effect against conjugated diene formation during incubation for 8 hr, while all fortified rice bran bread extracts showed peroxidation inhibition, ranged from 7.41% to 38.68%. Black bran bread extracts had higher level of lipid peroxidation inhibition than red and normal bran bread. The extract of 5% black bran bread inhibited 31.72% of lipid peroxidation, whereas the lipid peroxidation inhibition of 5% red and normal bran bread extracts were 7.41% and 8.36%, respectively. For 10% rice bran fortification, black bran bread inhibited 38.6% of lipid peroxidation, while red and normal bran inhibited 18.80% and 18.12% of lipid peroxidation, respectively. The

results indicated that incorporation of black rice bran into bread gives it high added value and provides an additional source of antioxidant in the diet.

These results indicated that fortification of rice bran especially black rice bran into wheat bread can enhance the antioxidative properties of bread. Moreover, baking processing retained high level of rice bran antioxidant in the formulated bread.

5.4 Conclusion

The rheological properties of bread dough were affected by addition of rice bran. The G' and G" values of doughs were increased with adding rice bran. Creep test showed that the deformation of dough added with rice bran was lower than control which caused by the higher of G' and G" by adding rice bran.

Addition of rice bran into wheat bread enhanced antioxidative properties of bread. Rice bran bread contained higher content of total phenolics than that of wheat bread (control). Fortification of black rice bran enhanced antioxidant content in wheat bread in higher level than normal and red rice bran. Rice bran antioxidants including anthocyanins and γ-oryzanol, were partially retained after baking.

Bread with addition of rice bran had stronger antioxidant activities than that of control bread, and the increasing of level of rice bran addition further enhanced more antioxidant capacity of the breads. Black rice bran fortified bread had higher antioxidant activities than normal and red rice bran. Pigmented rice bran (red and black rice bran) affected crumb color of wheat bread. Increasing pigmented bran concentration from 5% to 10% substitution had no adverse affect on bread crumb firmness. Loaf volume was decreased at 10% of rice bran addition.

The recommendation level of rice bran would be 5% addition to reduce the adverse effects on baking quality characteristics. Among 3 types of rice bran, black rice bran was the best to fortify into wheat bread for improving the antioxidant contents and antioxidant activities of bread, however, crumb of black bran bread was much darker (low *L* value) than other rice bran bread crumb.

CHAPTER VI

THE STABILITY STUDY OF RICE BRAN DURING 2 MONTHS STORAGE

6.1 Introduction

From chapter 3 and 4, it was concluded that rice bran was the most valuable milling fraction containing high level of many antioxidants and also high in antioxidant activities of its extract. However, rice bran was generally stored at ambient condition before using for oil industry. Thus, rice bran should be stable at least 2 months for using as rice bran oil raw material (Sayre *et al*., 1982).

The main draw-back to the use of rice bran as a food ingredient such as edible oil is the rapid deterioration of rice bran lipid. This deterioration is due to the activity of naturally occurring enzymes including lipase. Lipase is an important concern in the food industry because lipid hydrolysis can cause deterioration of food quality (Ashie, Simpson and Smith, 1996). During the milling process, rice bran lipids come into contact with lipase which rapidly hydrolyzes the ester bonds of triacylglycerol, producing free fatty acids and glycerol. The free fatty acid content of raw rice bran stored at 32^{o}C 85%RH rapidly increased 47% after 28 days (Randall *et al*., 1985).

Lipid deterioration can be prevented by stabilizing rice bran immediately after milling. Heat processes seem to be the only method with commercial potential to inactivate lipase. Another benefit of heat treatment is the destruction of microorganism and insect. Dry heat can prevent lipase activity by lowering the bran moisture content but the activity can be active again if the bran moisture increased to atmospheric equilibrium (Sayre *et al*., 1982). However, the stabilizations of rice bran by heating caused the decreasing of antioxidant contents. The stabilized rice bran using high temperature steam-injected expander decreased 26% of γ-oryzanol (Lloyd *et al*., 2000). Shin *et al*., (1997) proposed that vitamin E vitamers are stable to high temperature in the absence of oxygen. However, antioxidant contents in stabilized rice bran may decrease again during storage. They also reported that γ-oryzanol content of stabilized rice bran contain in rice

sack rapidly decreased during storage at ambient temperature. γ-Oryzanol and ferulic acid in rice bran oil are reported to decreased blood cholesterol but γ-oryzanol is more effective lowered LDL-cholesterol and raised HDL-cholesterol levels (Wilson *et al*., 2007).

This chapter was proposed to determine the potential health properties of pigmented rice bran, using normal rice bran (non-pigmented) as control, by measuring their content of antioxidants and anthocyanins during storage.

The objectives were to study the effects of the thermal stabilization on antioxidant contents and to determine the stability of rice bran antioxidants during 2 months storage at ambient temperature.

6.2 Methodology

6.2.1 Materials

Normal rice bran and black rice bran were selected for using in this experiment. Both brans were separated into 2 groups, first group was packed in cloth bags without stabilization (raw) (control) and the second group was heated by steaming to deactivate lipase (stabilized) before packed in cloth bags. These bran samples were stored in the dark place at ambient temperature (23-27^{o}C) for 2 months and were analyzed every 20 days.

6.2.2 Stabilization method

Rice bran samples were heated at 100^{o}C by opened steam heating for 30 minutes. After cooling to room temperature, rice bran samples were dried to <14% moisture content by vacuum oven at 40^{o}C, then packed in cloth bags and stored for 2 months.

6.2.3 Rice bran and lipid qualities determination

- *Lipase activity*

Lipase activity of rice bran was determined by using the method of Rose and Pike (2006) with some modifications. Rice bran was grounded and then partially defatted with 3 vol of hexane (1:10 wt/vol) for 30 min. Residual hexane was allowed to evaporate at room temperature and 1 g of the grounded, defatted rice bran was weighed into each of two test tubes: one blank (A_i) and one sample (A_f). Olive oil (0.6 ml) and distilled water (0.15 ml) were added to both tubes and mixed. Hexane (5 ml) was added, the tube was vortexed and then incubated at 32^{o}C in water bath for 4 hours (no incubation step for the blank). The tube was centrifuged at 1000 g for 3 minutes. Hexane was decanted into a 100 ml round-bottomed flask, and the extraction was repeated twice. The hexane extracts were pooled, evaporation was performed on a rotary vacuum evaporator at 40°C, and the residue was redissolved in 4 ml of isooctane. Free fatty acid content was quantified according to the method of Kwon and Rhee (1986) described below. Lipase activity was expressed as units/gram (U/g), where 1 U was defined as the micromoles of fatty acid liberated per hour. Lipase activity was calculated as follows:

$$\text{Lipase activity} = 1000 \quad \frac{(4 + v)(A_f - A_i)}{\varepsilon t l s}$$

where 1000 is the conversion factor from mol/L to μequiv/mL, 4 is the volume of isooctane used to redissolve lipids (mL), v is the volume of olive oil added (mL), Af is the absorbance of sample after incubation at 715 nm, Ai is the absorbance of blank at 715 nm, ε is the molar absorptivity of oleic acid at 715 nm ($M^{-1} \cdot cm^{-1}$), t is the incubation time (h), l is the path length (1 cm for a standard cuvette), and s is the sample weight (g). Olive oil was chosen as the substrate because it allows for quantification of true lipase activity, it is widely used and inexpensive, and it contains approximately 70% oleic acid, which was the fatty acid used to create the standard curve.

- *Free fatty acid content*

Free fatty acid content of rice bran was determined according to the method of Kwon and Rhee (1986) with some modifications. Rice bran (1 g) was extracted by hexane (5 ml) twice for 30 minutes in test tube. The tube was centrifuged at 1000 g for 3 minutes and then collected the hexane layer. The hexane was pooled and evaporated at 40^{o}C and dissolved by 4 ml isooctane and transfer to a new tube. Cupric acetate (1 ml, 5%) was added to the tube and then vortexed for 1 minute. The absorbance at 715 nm was measured with UV-vis spectrophotometer (Shimadzu, model UV-160). Free fatty acid content was calculated by using standard curve making from pure oleic acid as the standard.

- *Conjugated diene value*

Rice bran oil was extracted twice with 5 vol of hexane for 30 minutes. The hexane fractions were pooled and then evaporated at 40^{o}C by rotary evaporator. Conjugated diene value of crude oil was analyzed by the method described by White, (1995). Crude oil was diluted to the concentration around 0.05-0.1 g/L (up to conjugated diene content) by isooctane. Absorbance at 233 nm was measured using UV-vis spectrophotometer. The conjugated diene value should be in the range of 0.2-0.8% for the accuracy results. The percentage of conjugated diene was calculated using the following equation:

Conjugated dienoic acid (%) = 0.84 (A_s/bc-k_0)

where A_s is the observed absorbance at 233 nm, b is the cell path length (cm), c is the concentration of sample in g/L of the final dilution, and k_0 is the absorptivity of acid (0.03).

6.2.4 Quantitative analysis of antioxidants in rice bran during storage

- *Determination of total phenolics content*

Total phenolics content of rice bran was determined with the Folin-Ciocalteu reagent according to the method reported by Goffman and Berman (2004) with some modifications. Briefly, rice bran samples (200 mg) were extracted with methanol (5 ml) overnight (vortex 2 times at the beginning and final) and then were centrifuged at 3822g for 5 minutes. Supernatant (4 ml) was filtered by 1 μm syringe filter. The extracts were diluted with deionized water. The diluted solution (1.2 ml) was transferred to a test tube and mixed with Folin-Ciocalteu reagent (500 μl) and ethanolamine (1 ml, 0.5 M). After 30 minutes at room temperature, absorbance at 600 nm was measured with UV-vis spectrophotometer (Shimadzu, model UV-160). The total phenolic content were expressed as mg gallic acid equivalents (mg of GAE) per g of rice bran.

- *Determination of anthocyanin components*

Anthocyanins in the black rice bran were determined according to the method described by Kim *et al*., (2008) with some modifications. Briefly, defatted rice bran (3 g) was extracted twice by mixing with 30 ml of methanol acidified with 1.0 N HCl (85:15 v/v) and shaking at 4^{o}C for 24 hr. The crude extract was filtered with Whatman No.1 filter paper. The extract was centrifuged at 12,000 g at 5^{o}C for 20 minutes. The extracts were kept in a refrigerator at 4^{o}C for 2 days to precipitate large molecules and then centrifuged 12,000 g at 5^{o}C for 20 minutes. The upper layer was concentrated and filtered through 0.45 μm syringe filter before injected to HPLC.

The HPLC instrument (LC-10AT, Shimadzu) connected with UV/vis detector (SPD-10A, Shimadzu) was used for analysis. TosoHaas super-ODS, C18 2μm 4.6 x 100 mm column was used to separate the anthocyanin components. The mixture of water, methanol and formic acid (75:20:5 v/v) was used as a mobile phase with isocratic elution at 0.5 ml per minute flow rate. UV/vis detector was set at 530 nm and

sample loop was 5µl. Identification and quantification of anthocyanins followed the method as described previously in chapter 3.

- *Determination of α-tocopherol and γ-oryzanol content*

α-Tocopherol and γ-oryzanol in rice bran were determined according to the method of Aguilar-Garcia *et al*., (2007) with some modifications. Rice bran samples (100 mg) were extracted twice with 6ml methanol and centrifuged 10 minutes at 825 g. The supernatant was combined and then evaporated to 4 ml, then made up to exactly 5.0 ml with HPLC grade methanol in a volumetric flask. This solution was filtered through 0.45 µm syringe filter before subjected to HPLC analysis.

α-Tocopherol and γ-oryzanol were analyzed by HPLC using a Shimadzu (LC-10AT) HPLC equipped with UV/vis detector. The C18 column (Inertsil ODS-3, 5µm, 250x4.6mm) was used to separate these compounds. Mobile phase was the mixture of methanol and acetonitrile (15:85 v/v) at flow rate of 2 ml/min with isocratic mode. The sample loop was set at 20µl. The UV/vis detector was set at 292 and 325nm for tocopherol and oryzanol, respectively. Identification and quantification of α-tocopherol and γ-oryzanol followed the method as described previously in chapter 3.

5.2.5 Evaluation of antioxidant activities

- *Samples preparation and extraction*

Rice bran samples were finely grounded and defatted twice with hexane (1:20 w/v) for 30 minutes. The defatted rice bran was extracted with 100% methanol (1:20 w/v) in an electrical shaker overnight at room temperature and then filtered through Whatman No.1 filter paper. The extracts were stored under freezer at -18 ^{o}C until used for further analysis.

- *Determination of DPPH radical scavenging activity*

The free radical scavenging capacity of rice bran extract was estimated following a previously reported procedure using the stable 2,2'-diphenyl-1-picrylhydrazyl radical (DPPH·) (Brand-Williams *et al*., 1995). Briefly, different dilutions of the extracts were prepared. An aliquot of the diluted extract (1 ml) was vigorously mixed with 1.0 ml of freshly prepared 0.004% DPPH in methanol and held in the dark for 30 minutes at room temperature. The absorbance was then read at 517 nm (UV-160, Shimadzu) against blanks. DPPH free radical-scavenging ability was calculated by using the following formula:

Scavenging ability (%) = [$\text{Absorbance}_{517\text{nm of control}} - \text{Absorbance}_{517\text{nm of sample}}$/ $\text{Absorbance}_{517\text{nm of control}}$] × 100.

The scavenging activity of rice bran extracts were expressed as 50% effective concentration, EC_{50} (mg/ml), and were obtained by interpolation from linear regression analysis. A lower EC_{50} value indicates a higher antiradical activity (Brand-Williams *et al*., 1995). BHT and α-tocopherol were used for comparison.

- *Determination of reducing power*

The reducing power of the extract was determined by the method of Yen and Duh (1993) with some modifications. An aliquot (2.5 ml) of the extract was mixed with sodium phosphate buffer (2.5 ml, 2M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (0.5 ml, 1% w/v). The mixture was incubated at 50^{o}C for 20 minutes. Trichloacetic acid (5 ml, 10% w/v) was added to the mixture, which was then centrifuged at 6000g for 10 minutes to stop the reaction. Aliquot (5 ml) from the upper layer of the solution was mixed with deionized water (5 ml) and ferric chloride solution (1 ml, 1% w/v). The absorbance at 700 nm was then measured using UV/vis spectrophotometer. Higher absorbance of the reaction mixture indicated higher reducing power.

- *Determination of lipid peroxidation inhibition*

Lipid peroxidation inhibition of rice bran extract was measured according to the method reported by Lingnert *et al*., (1979). Brifly, linoleic acid (5 mM) was emulsified with the aid of an equal amount of Tween 20 in sodium phosphate buffer (0.1M, pH7). An aliquot (4 ml) of linoleic acid solution was mixed with 200µl of rice bran extract in test tube. The tubes were placed in darkness at 37^{o}C for 8 hours to accelerate the oxidation and then were added methanol (6 ml, 60%). The progress of autoxidation is monitored by UV absorbance at 234 nm (A_{max} of conjugated diene peroxides from lionoleic oxidation). The absorbance at 234nm was measured against blank. Controls without antioxidant were run parallel. BHT and α-tocopherol were used for comparison. The inhibition of lipid peroxidation was calculated according to the following equation:

$$\%\text{Inhibition of lipid peroxidation} = [\text{Absorbance}_{234\text{nm of control}} - \text{Absorbance}_{234\text{nm of sample}}/\text{Absorbance}_{234\text{nm of control}}] \times 100$$

6.3 Results and discussion

6.3.1 Effects of stabilization

6.3.1.1 Effects of stabilization on lipase activity and lipid quality

Lipase is the endogenous enzyme in rice bran. It catalyzes the hydrolysis of triglyceride to free fatty acids and glycerol. The stabilization of rice bran by opened steam heating at 100^{o}C for 30 minutes was used to deactivate lipase enzyme. The effects of stabilization on lipase activity and lipid quality are shown in Table 6.1. The lipase activity of normal and black rice bran showed losses of 37 and 36%, respectively, due to the denaturization of lipase by heating. However, opened steam heating (100^{o}C, 30 minutes) was not an effective method to totally deactivate lipase enzyme. Extrusion of rice bran at 105^{o}C deactivated 88% of lipase activity (Randall *et al*., 1985) while extrusion at 110^{o}C had the residue activity only 2.3% (Saunder and Heltved, 1985). Free fatty acid contents of stabilized normal and black rice bran were decreased by 25 and 31%, respectively.

Some preformed free fatty acids apparently in the raw bran may be leached out and/or oxidized which account for the initial reduction. Conjugated diene value of normal bran was increased by 13% after stabilization, this may be the result of the long drying process which enables to contact the air and allowed oxidation of rice bran lipid. The decreasing of resistance against oxidation may caused by losses of antioxidants by heat and evaporation and/or improvement of oxygen accessible (Pokorny, 2001).

Table 6.1 Lipase activity, free fatty acid content and conjugated diene value of raw and stabilized rice bran[1]

Samples	Normal bran			Black bran		
	Raw	Stabilized	+/-	Raw	Stabilized	+/-
Lipase activity (U/g)	2.13±0.09	1.34±0.12	-37%	2.79±0.07	1.78±0.05	-36%
Free fatty acid content (%)	6.75±0.23	5.08±0.34	-25%	9.17±0.69	6.28±0.12	-31%
Conjugated diene value (%)	0.54±0.02	0.61±0.02	+13%	0.84±0.08	0.84±0.07	-

[1]Mean ± SD

6.3.1.2 Effects of stabilization on antioxidants

Heating is known as the process which can destroy the antioxidant compounds in food. The effects of stabilization on rice bran antioxidants are shown in Table 6.2. Total phenolic content of black and normal rice bran decreased 15 and 16%, respectively. Anthocyanins showed high instability against heat. Black rice bran showed losses of 80% cyanidin-3-glucoside and all of peonidin-3-glucoside. Average concentration of cyanidin-3-glucoside in raw black rice bran (1735.3 μg/g) was five times higher than that in stabilized black bran (349.8 μg/g). Instability of anthocyanins during heat processing was reported, with losses of up to 92.5% in the thermal production of pekmez[1] (Alasalvar, Al-Farsi and Shahidi, 2005). Black bran lost 6% of α-tocopherol, while normal bran lost 43% of α-tocopherol. Despite, the destruction of α-tocopherol in normal bran was greater than black bran but stabilized normal bran still contained higher content of α-tocopherol (42.31 μg/g) than stabilized black bran (20.54 μg/g). γ-Oryzanol content was decreased by 13 and 10% after stabilization for normal and black bran, respectively. The lower thermal destruction of γ-oyzanol was also found in extruded rice bran which lost only 10.8% at 140^{o}C for 6 minutes (Shin *et al*., 1997). The result confirmed that γ-oyzanol was relative stable to heat (Okada and Yamaguchi, 1983). Heating of rice brans destroyed many antioxidants, therefore antioxidant activities of rice bran extracts will be evaluated in the next section.

[1] Pekmez is a molasses-like syrup obtained after condensing juices of fruit must, especially grape, fig or mulberry.

Table 6.2 Total phenolics, cyanidin-3-glucoside, peonidin-3-glucoside, α-tocopherol and γ-oryzanol contents of raw and stabilized rice bran[1]

Samples	Normal bran			Black bran		
	Raw	Stabilized	+/-	Raw	Stabilized	+/-
Total phenolics content (mg/g)	10.99±0.89	9.20±0.54	-16%	12.48±0.88	10.63±0.64	-15%
Cyanidin-3-glucoside (μg/g)	N/A[2]	N/A	N/A	1735.31±35.23	349.83±43.21	-80%
Peonidin-3-glucoside (μg/g)	N/A	N/A	N/A	221.38±14.28	0.00	-100%
α-Tocopherol (μg/g)	74.73±4.23	42.31±4.04	-43%	21.83±3.28	20.54±2.89	-6%
γ-Oryzanol (μg/g)	2764.18±113.17	2394.87±132.29	-13%	2859.33±142.99	2579.98±120.87	-10%

[1]Mean ± SD

[2]N/A = not available

6.3.1.3 Effects of stabilization on antioxidant activity of the extracts

This experiment was performed on antioxidant activity in rice bran extracts in 3 analysis methods. Anthocyanin pigments and phenolic compounds possess antioxidant activity, therefore destruction of anthocyanins and phenolic compounds by thermal processing may cause the great effect on antioxidant activity of black rice bran. The effects of stabilization on antioxidant activities of rice bran extracts are shown in Table 6.3. Boiling is generally regarded as being destructive to antioxidant compositions (Krishnaswamy and Raghuramulu, 1998). As verified by our antioxidant activity assays, the results from black and normal rice bran showed that heat stabilized brans lost about 22-46% DPPH radical scavenging activity values, about 4-8% reducing power values and about 5-8% lipid peroxidation inhibition values (Table 6.3). The DPPH radical scavenging method measured the disappearance of DPPH radical, which is a useful index to estimate total free radical scavenging capacity of a given medium such as methanol extract (Brand-Williams *et al*., 1995). The free radical scavenging capacity of the samples were expressed as EC_{50} in mg of antioxidant per mg of DPPH. The lower EC_{50} value indicated a high free radical scavenging capacity. Thermal stabilization of rice bran samples decreased antioxidant capacity of the methanol extracts of normal and black rice bran (Table 6.3). The antioxidant capacity ranged from 0.13 to 0.55 mg of antioxidant per mg of DPPH radical. Raw black bran showed the highest antioxidant capacity (EC_{50}= 0.13) and the lowest antioxidant capacity was found in stabilized normal bran (EC_{50}= 0.55).

Reducing power assay is also a simple method for preliminary assessment of the antioxidant activity of a test compound. The antioxidant activity of a plant extract is correlated with its reducing power, which is generally associated with the presence of reductones (Pin-Der-Duh, 1998). The reducing powers of the sample extracts also corroborated with those obtained with their DPPH radical scavenging activities. The decreased in the absorbance (A_{700nm}) caused by the extracts, is indicative of their decreased reducing power (Table 6.3). Thermal stabilization of rice brans decreased the reducing power of rice bran and the raw black bran showed the highest reducing power (A_{700nm} = 2.09)

Table 6.3 Antioxidant activities of raw and stabilized rice bran extracts[1]

Samples	Normal bran			Black bran		
	Raw	Stabilized	+/-	Raw	Stabilized	+/-
EC_{50} value of DPPH radical scavenging (mg/ml)[2]	0.45±0.03	0.55±0.02	+22%	0.13±0.01	0.19±0.01	+46%
Reducing power (absorbance at700nm)	0.39±0.06	0.36±0.06	-8%	2.09±0.08	2.01±0.09	-4%
Lipid peroxidation inhibition (%)	85.48±1.76	78.76±3.54	-8%	79.25±2.09	75.35±2.53	-5%

[1]Mean ± SD

[2]Lower values represent higher activity

6.3.2 Storage stability of rice bran

Lipase activity of raw normal bran tended to increase during storage, whereas the activity of stabilized normal bran was decreased (Figure 6.1). Increasing of lipase activity of raw normal rice bran may due to the increasing of microflora which produced lipase during storage. Various bacteria and molds can grow and produce lipase in stored rice bran (Loeb and Mayne, 1952). The lipase from microflora was to be the reason of the dramatically increasing of free fatty acid content of raw normal bran increased throughout the 2 months storage while the decreasing of fatty acid content of stabilized normal bran was observed. The rate of hydrolytic rancidity of normal bran was very high in the first 20 days and was slow after 40 days of storage (Figure 6.2). The lipase activity of rice bran was not related with free fatty acid content because the concentration of substrate was decreasing during storage by conversion of triglyceride to free fatty acid in brans. The rate of action of lipase decreased in bran system. Lipase activity of raw black rice bran decreased throughout the storage which may caused by the antimicrobial activity of black rice bran inhibit the growth of lipase producing microflora in black bran. Phenolic compounds have been reported for their antimicrobial activities by disrupting of cell membrane (Kanatt, Chander and Sharma, 2010).

Lipase activity in raw and stabilized black bran decreased during storage (Figure 6.1). The free fatty acid content of raw black bran increased in slower rate than those of raw normal bran (Figure 6.2) because black bran contains high level of phytate and tannin which capable to deactivate lipase enzyme (Knuckles, 1988; Griffith, 1979). Goffman and Bergman, (2003) reported that red rice bran showed low values for both hydrolytic rancidity and esterase activity because of it contained tannin which inhibit the lipase activity.

Stabilization of normal and black rice bran by opened steam heating was able to retard the hydrolytic rancidity properly, and the free fatty acid contents in stabilized brans were less than 10% at the end of storage which was the accepted level of oil industry (Sayre *et al*., 1982). Refined crude oil with more than 10% of free fatty acid is considered uneconomical and less than 5% free fatty acid is desirable for economic refining purposes (Sayre *et al*., 1982). At the end of

storage, free fatty acid contents of raw normal and black bran were 33% and 15%, respectively.

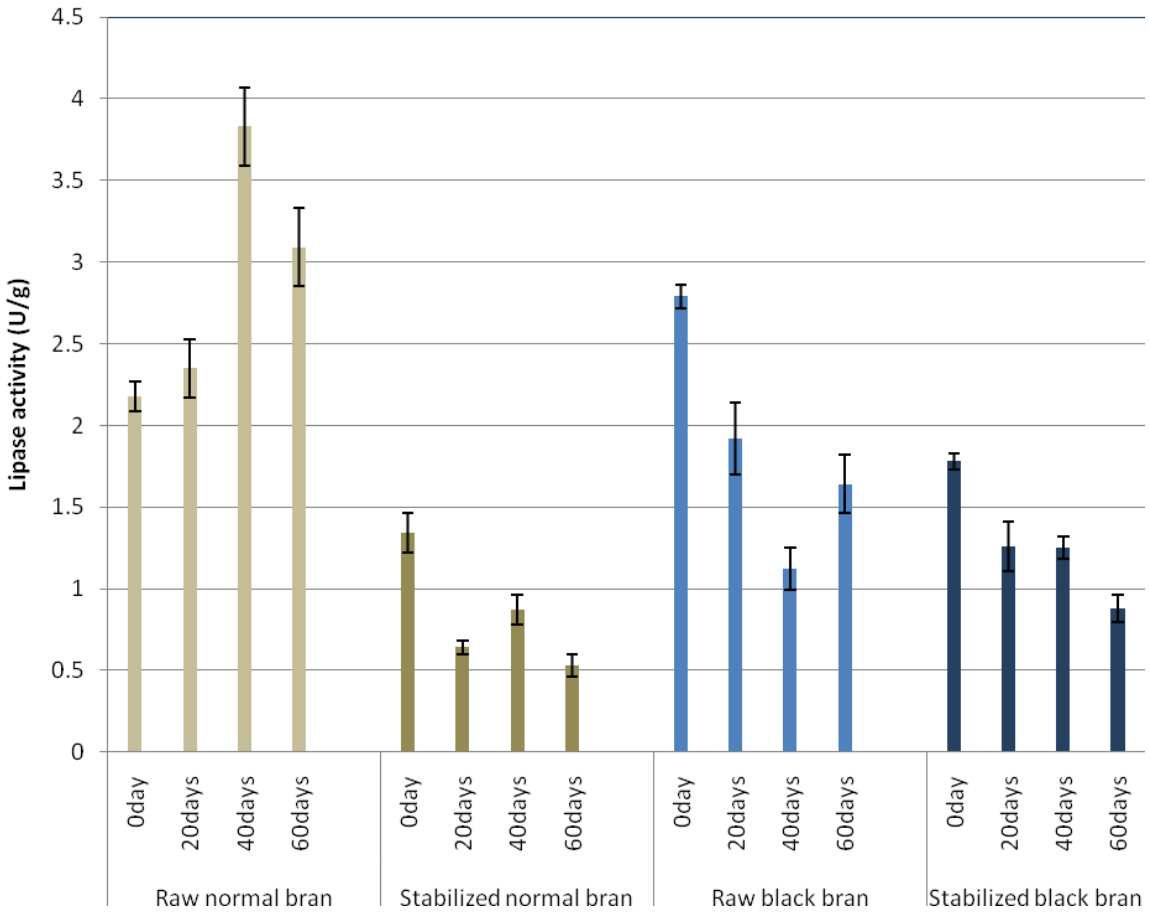


Figure 6.1 Lipase activity of rice bran during storage

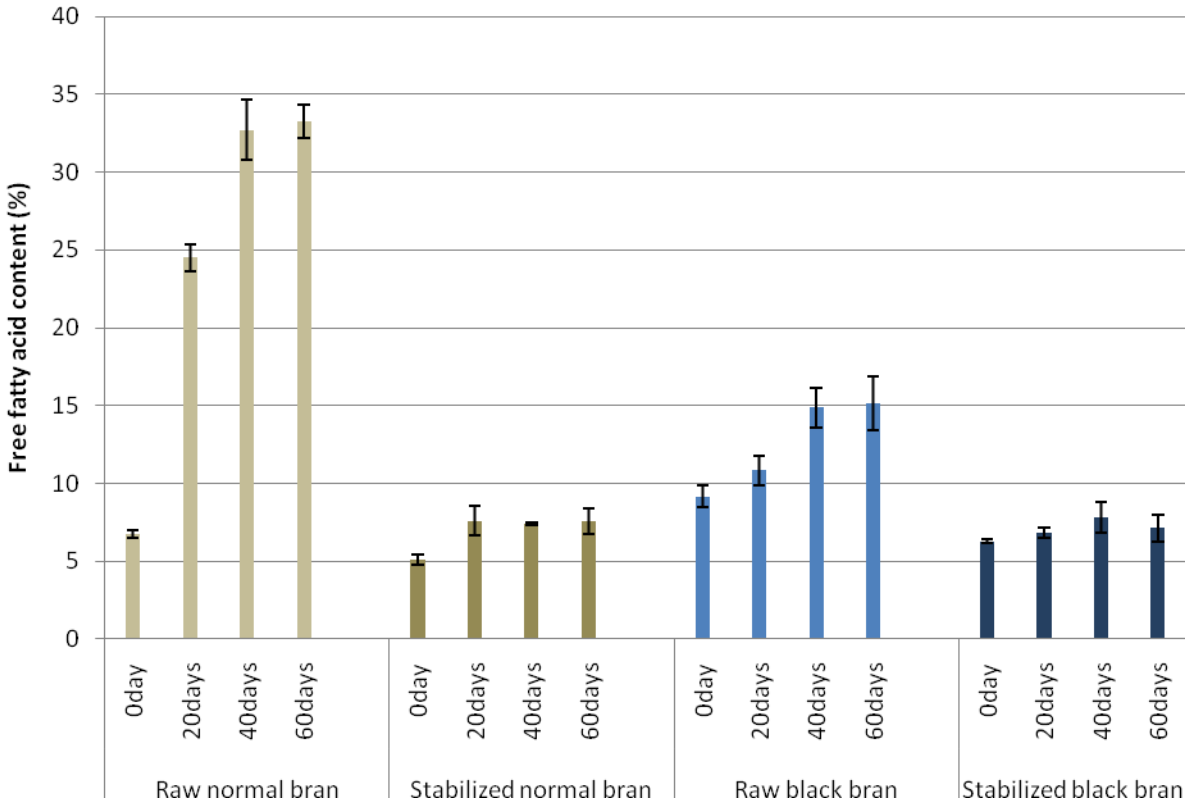


Figure 6.2 Free fatty acid content of rice bran during storage

Endogenous rice bran antioxidants may prolong shelf life of rice bran by inhibiting the rancidification during storage. In this study, conjugated diene value was used to indicate the lipid oxidation level of food by measuring conjugated diene occurring by oxidation of rice bran lipid. Figure 6.3 shows conjugated diene value of rice bran during storage. The low price and simplicity such as cloth bag often used to store by-product as rice bran. The increasing of the conjugated diene value was found in all bran samples due to the keeping in cloth bags which oxygen is permeable and allow reacting with rice bran lipid. The rate of conjugated diene formation was slow in the first 20 days for black bran samples; the antioxidants in black bran may retard the oxidation in this period. While, the rate of conjugated diene formation of stabilized normal bran was higher than raw bran, it was due to the destruction of antioxidants by heating process as well as the higher level of conjugated diene value at the beginning of storage. However, lipid oxidation is also

dependent on moisture content, temperature, humidity, and microbial growth during storage (Galliard, 1986).

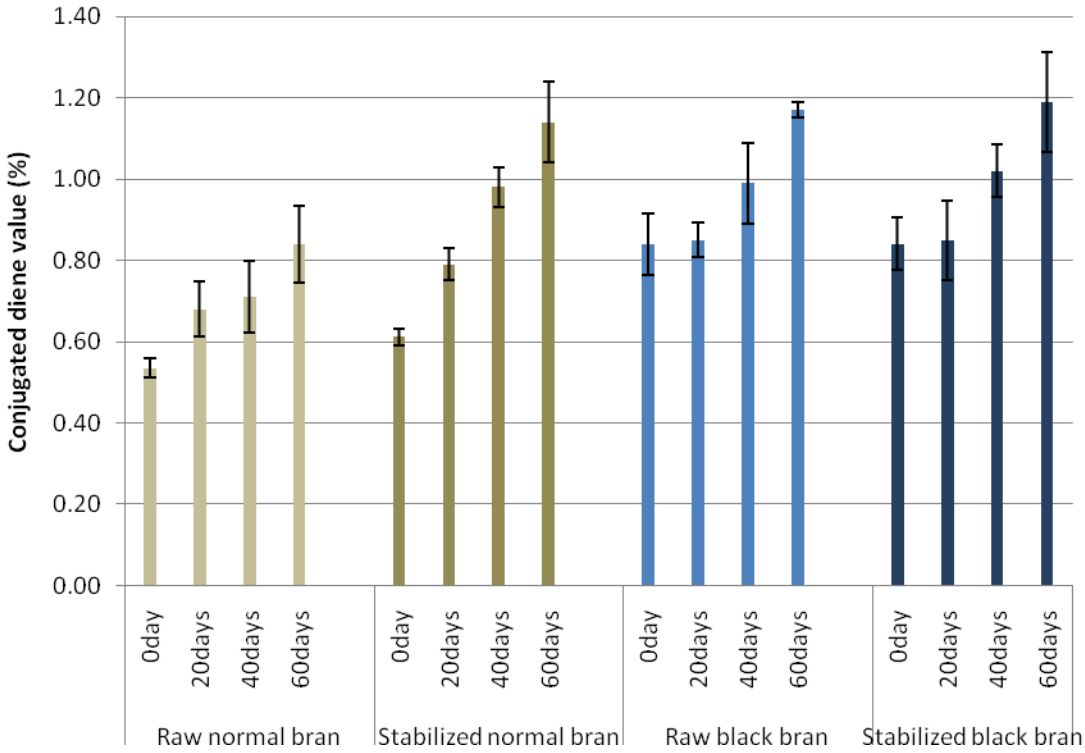


Figure 6.3 Conjugated diene value of rice bran during storage

Figure 6.4 shows the storage stability of total phenolics, α-tocopherol and γ-oryzanol of rice brans. During 2 months storage at ambient temperature, all compounds exhibited similar trends of degradation. Rice bran lost 33-43% of α-tocopherol after 40 days storage, which was similar to the study by Shin *et al*., (1997) which reported that rice bran lost 44% of vitamin E during 35 days storage. In 2 months (60 days), the concentration of α-tocopherol decreased dramatically (37.8%, 41.2%, 36.7% and 44.9% for raw normal bran, stabilized normal bran, raw black bran and stabilized black bran, respectively), indicating its rapid degradation. The role of α-tocopherol as a physical and chemical quencher of singlet O_2 during photooxidation (Kamal-Eldin and Appelquist, 1996) may account for this rapid drop. However, after 40 days the decrease (drop) in α-tocopherol in black bran

group seemed to stabilize, this is possibly due to its remaining low concentration. In general, the reduction observed for total phenolics and γ-oryzanol were less than those observed for α-tocopherol.

Figure 6.5 depicts the changes in anthocyanins in the black bran rice groups during storage. The results demonstrated the relatively stability of anthocyanins in pigmented rice bran. The stability of anthocyanins is dependent on several factors, such as chemical structure, pH, temperature, light intensity, presence of copigments, metallic ions enzymes, O_2, ascorbic acid, sugar and their degradation products (Francis, 1989). Among these factors, it has been demonstrated that pH and temperature mainly affected the stability of anthocyanins (Cevollos-Casols and Cisneros-Zevallos, 2004). From the results, anthocyanins in rice bran samples were relatively stable during ambient temperature storage (23-27^{o}C)

Degree of degradation during rice brans storage was determined from the formation of conjugated dienes (express as % of conjugated dienoic acid). The formation of conjugated dienes, composed of hydroperoxides (usually from polyunsaturated fatty acids) and respective degradation products, was monitored by measuring their characteristics absorbance at 233 nm (Owusu-Apenten, 2004). If lipids are protected in the presence of antioxidants, conjugated diene formation will be retarded. All rice bran groups showed increasing of conjugated diene values during ambient storage (Figure 6.3 and 6.6). At conjugated diene value of 1.19% which was the highest conjugated diene value recorded during 2 months storage in cloth bag (stabilized black bran) at ambient temperature, α-tocopherol decreased by 36.71%, total phenolics decreased by 21.17%, and γ-oryzanol decreased by 12.43% (Figure 6.6). At the conjugated diene value >1.14%, for the raw black bran, 55.06% of α-tocopherol, 80.37% and 89.14% of total phenolics and γ-oryzanol, respectively, remained, while stabilized normal bran, 58.78% of α-tocopherol, and 89.13% and 85.45% of total phenolics and γ-oryzanol, remained. It should be noted that approximately 45% of α-tocopherol was used up in 2 months for the raw black bran sample.

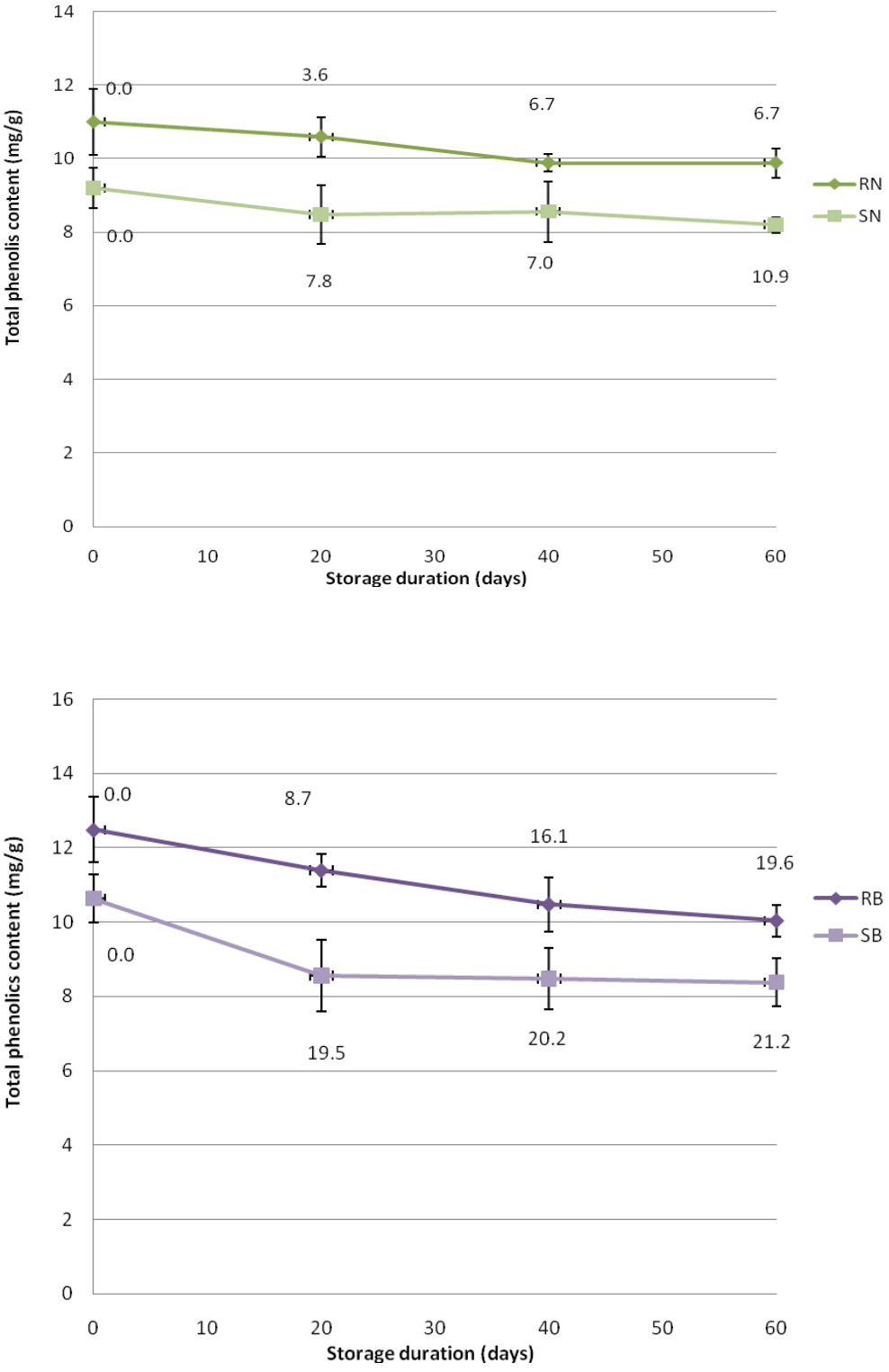


Figure 6.4 (a) Total phenolic content of rice bran during storage (label = % of losses; RN= raw normal bran, SN=stabilized normal bran, RB= raw black bran, SB= stabilized black bran)

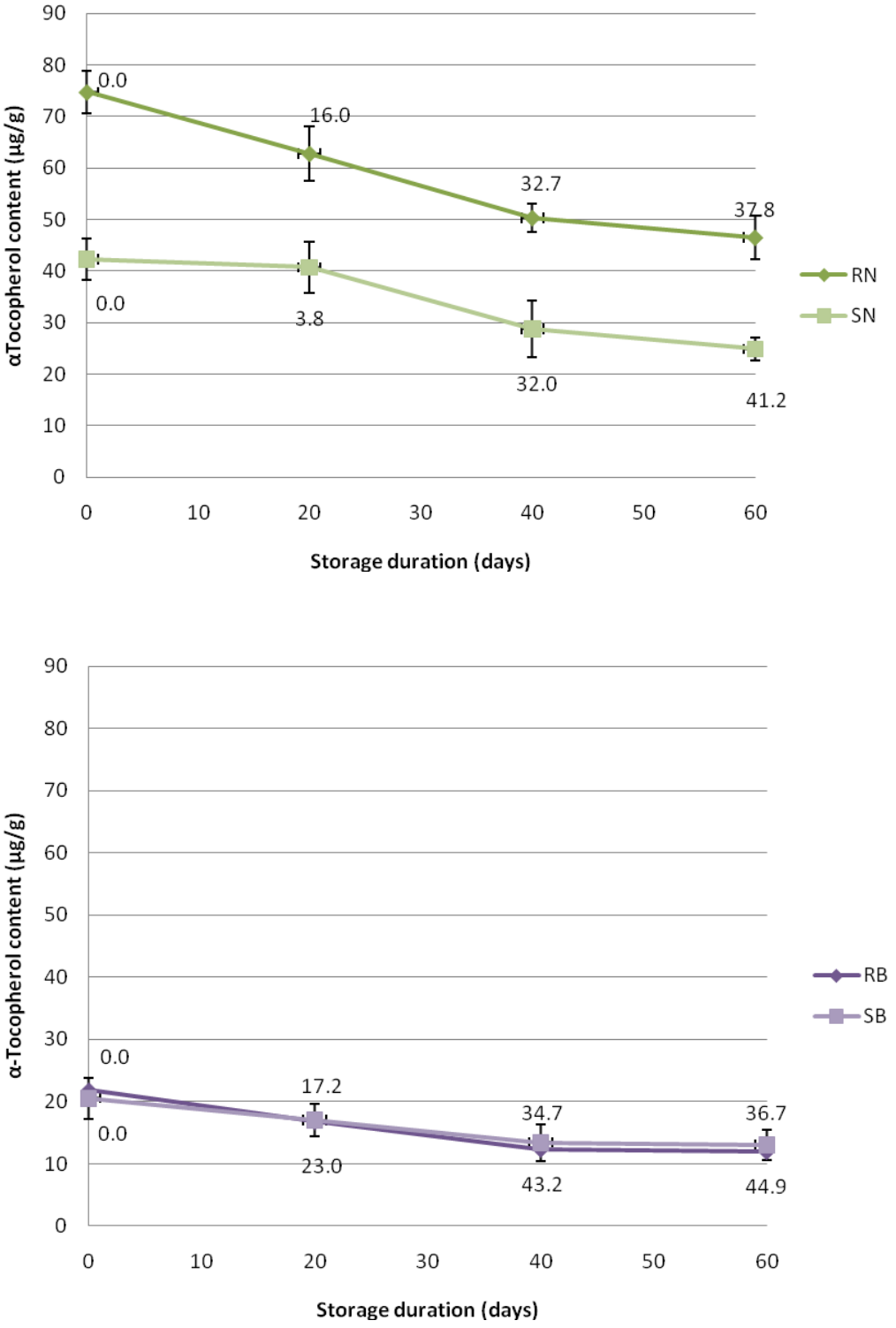


Figure 6.4 (b) α-Tocopherol content of rice bran during storage (label = % of losses; RN= raw normal bran, SN=stabilized normal bran, RB= raw black bran, SB= stabilized black bran)

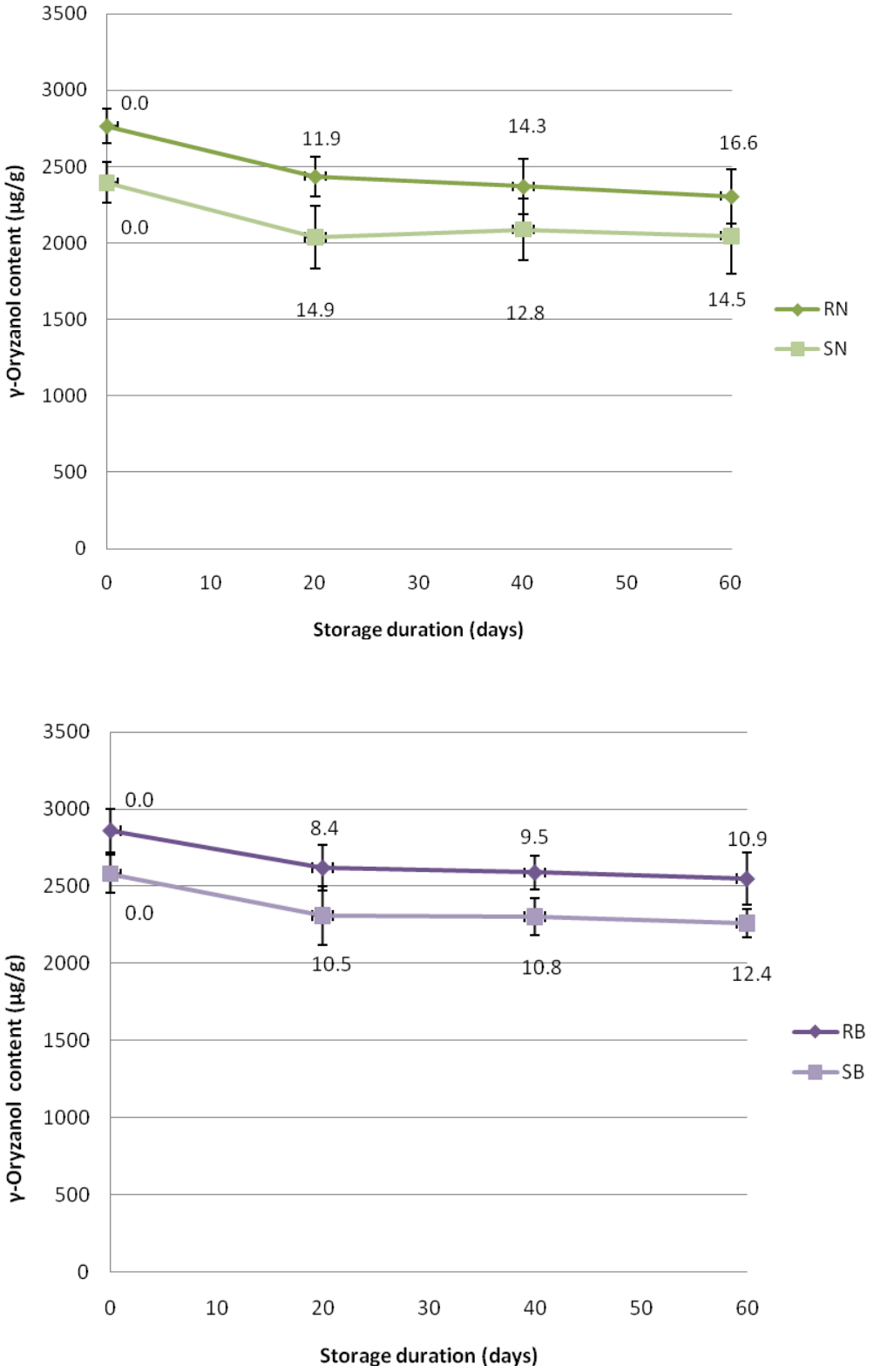


Figure 6.4 (c) γ-Oryzanol content of rice bran during storage (label = % of losses; RN= raw normal bran, SN=stabilized normal bran, RB= raw black bran, SB= stabilized black bran)

The above finding indicated that high amounts of antioxidants are retained during rice brans storage, with the degree of reduction being in the following order: α-tocopherol > phenolics ≈ γ-oryzanol > anthocyanin.

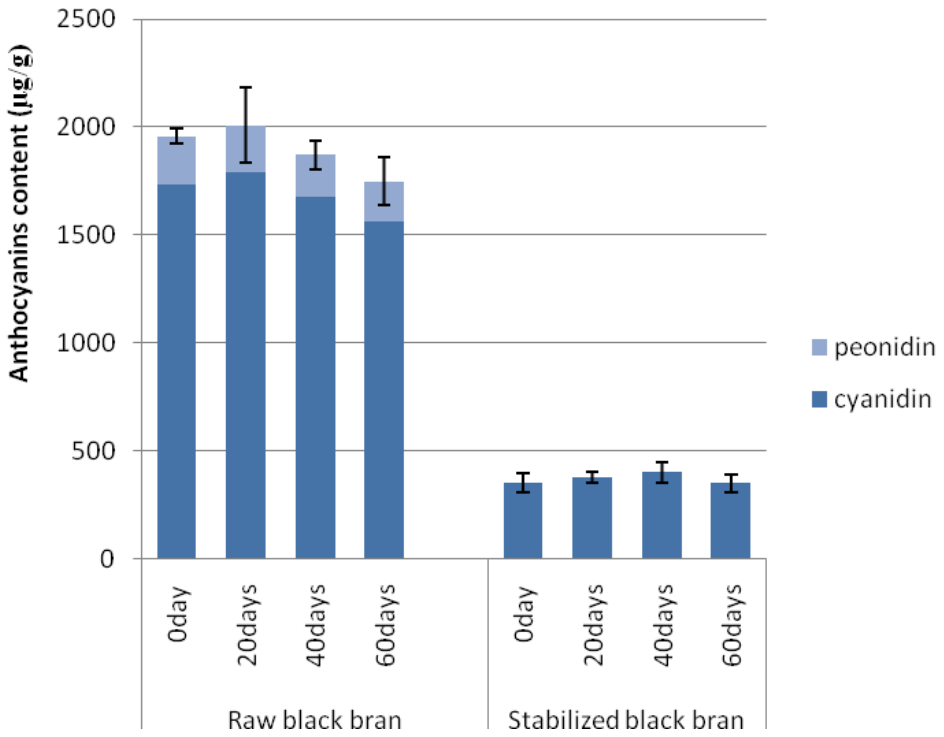
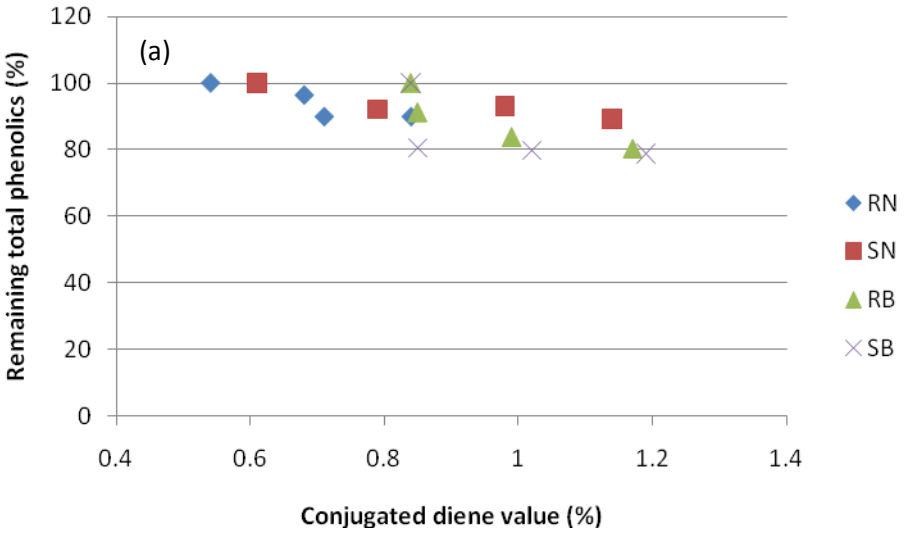
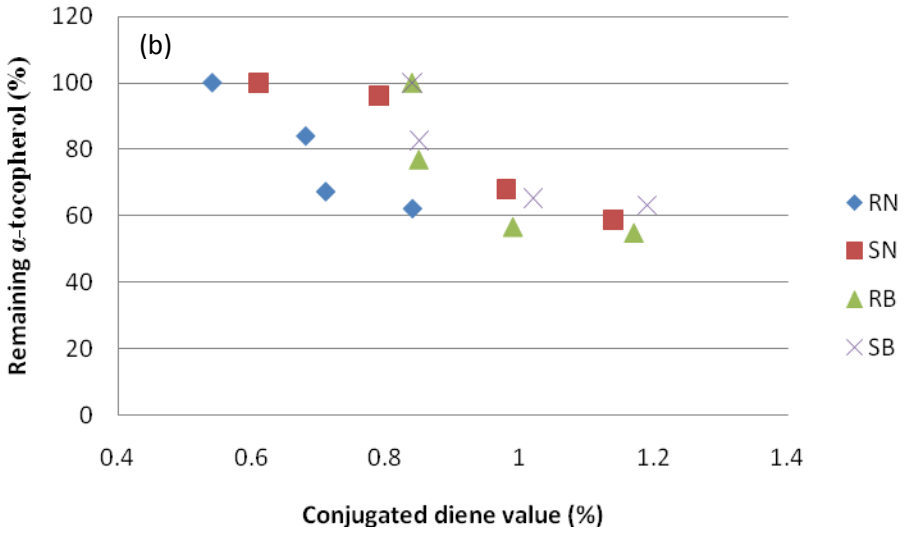
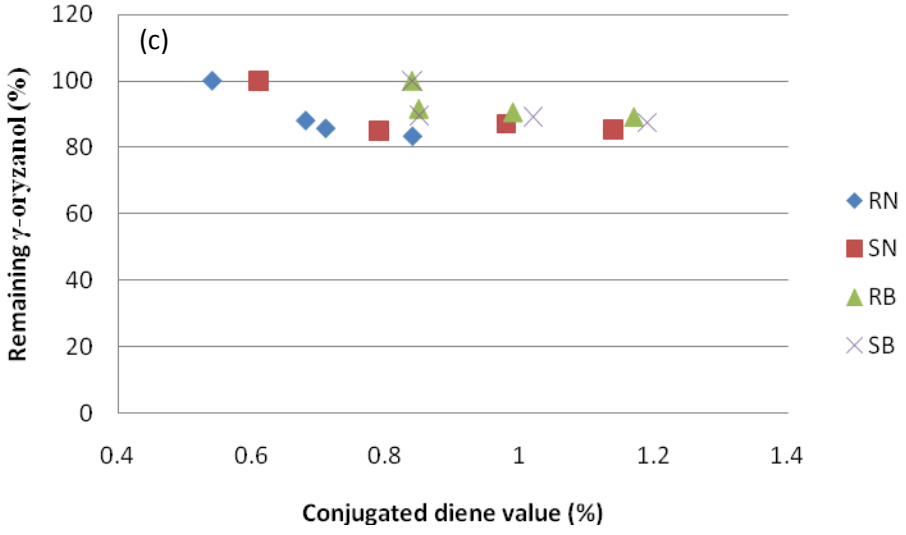


Figure 6.5 Anthocyanins contents of rice bran during storage


(a)
Remaining total phenolics (%)
120
100
80
60
40
20
0
0.4
0.6
0.8
1
1.2
1.4
Conjugated diene value (%)
RN
SN
RB
SB


(b)
Remaining α-tocopherol (%)
120
100
80
60
40
20
0
0.4
0.6
0.8
1
1.2
1.4
Conjugated diene value (%)
RN
SN
RB
SB


(c)
Remaining γ-oryzanol (%)
120
100
80
60
40
20
0
0.4
0.6
0.8
1
1.2
1.4
Conjugated diene value (%)
RN
SN
RB
SB

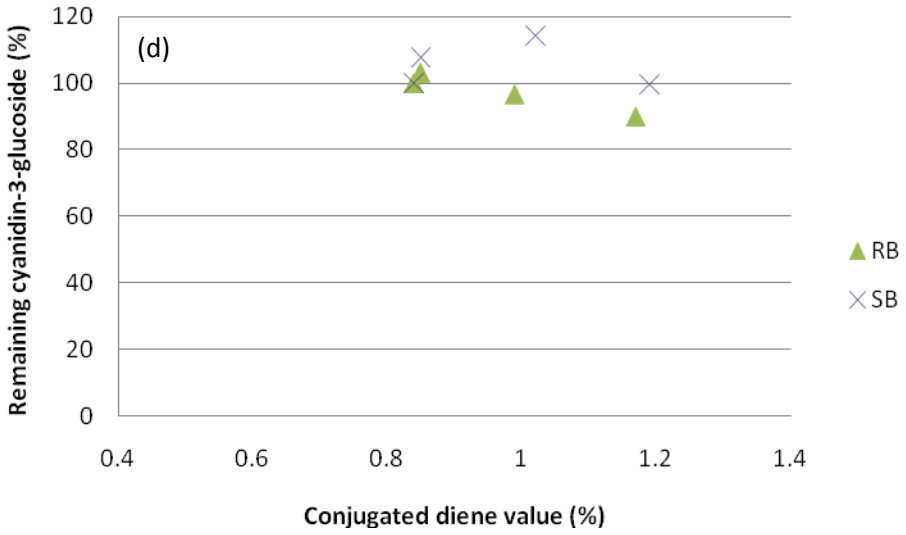


Figure 6.6 Changes in the level of antioxidants (a) α-tocopherol, (b) total phenolics, (c) γ-oryzanol and (d) cyanidin-3-glucoside, at various conjugated diene values during storage at ambient temperature. (RN = raw normal bran, SN = stabilized normal bran, RB = raw black bran, and SB = stabilized black bran)

The decrease in antioxidants of all types of rice bran followed pseudo-first-order kinetics, except cyanidin-3-glucoside (Table 6.4) in stabilized black rice bran. The goodness of fit of kinetic model was evaluated by the correlation coefficient (r). The rate constants ranged from 1.7 x 10^{-3} to 3.7 x 10^{-3} day^{-1} for total phenolics, from 8.0 x 10^{-3} to 10.5 x 10^{-3} for α-tocopherol, 1.8 x 10^{-3} to 2.9 x 10^{-3} day^{-1} for γ-oryzanol and 0.84 x 10^{-3} day^{-1} for cyanidin-3-glucoside. The degradation rate constant of α-tocopherol was highest. The estimated $t_{1/2}$ of α-tocopherol in rice bran ranged 66 to 87 days at ambient temperature. Other antioxidants were more stable in rice bran than α-tocopherol. It is reasonable to predict that antioxidants remained in rice bran may attribute to its antioxidative activity.

Table 6.4 Rate constants (-k) and $t_{1/2}$ for degradation of antioxidants in rice bran held at ambient temperature[1]

Samples[2]	Antioxidants	-k x 10^{-3} ($days^{-1}$)	$t_{1/2}$ (days)	r
RN	Total phenolics	1.9	365	0.895
	α-Tocopherol	8.2	84	0.985
	γ-Oryzanol	2.9	239	0.920
	Cyanidin-3-glu	-	-	-
SN	Total phenolics	1.7	408	0.948
	α-Tocopherol	9.7	71	0.960
	γ-Oryzanol	2.2	315	0.752
	Cyanidin-3-glu	-	-	-
RB	Total phenolics	3.7	187	0.988
	α-Tocopherol	10.5	66	0.961
	γ-Oryzanol	1.8	385	0.893
	Cyanidin-3-glu	1.9	365	0.840
SB	Total phenolics	3.6	192	0.820
	α-Tocopherol	8.0	87	0.965
	γ-Oryzanol	2.0	346	0.859
	Cyanidin-3-glu	0.3	2310	0.100

[1] data were fitted to pseudo-first order kinetics

[2] RN = raw normal bran, SN = stabilized normal bran, RB = raw black bran, and SB = stabilized black bran

In accordance with antioxidants depletion results, it was found that the antioxidant activity of rice bran, as evaluated by DPPH radical scavenging activity, reducing power and lipid peroxidation inhibition, decreased during storage at ambient temperature (Figure 6.7-6.9). Black rice bran groups showed higher free radical scavenging activity (lower EC_{50}), reducing power (higher A_{700}), but lower lipid peroxidation inhibition than those of the normal bran. Therefore, although data indicated that rice brans have high free radical scavenging activity and reducing power but they did not necessary relate to the ability to inhibit lipid

peroxidation, as already observed (Alamed *et al*., 2009). Lipid peroxidation is dependent on many factors including fatty acid composition, oxygen concentration, temperature, surface area, moisture, prooxidants, antioxidants, etc. (Fennema, 1996). Iron, for example, is one of the major minerals in rice bran (Hargrove, 1994). Rice bran contains 190-530 ppm iron. Iron possesses prooxidant activity. Some phenolic compounds are able to chelate iron while others like ferulic acid and coumaric acid which do not have a galloyl moiety, do not bind iron (Andjelkovic *et al*., 2006) although they are good free radical scavengers. Phenolics without chelating activity do not inhibit lipid peroxidation in rice bran.

Anthocyanins with vicinal phenolic hydroxy groups can chelate several multivalent metals such as iron (Fennema, 1996). Cyanidin possess vicinal phenolic hydroxy groups while peonidin possess one *p*-hydroxy group on the B-ring (Figure 6.10). Consequently, cyanidin is both an antioxidant and antiradical compounds (Faria *et al*, 2005).

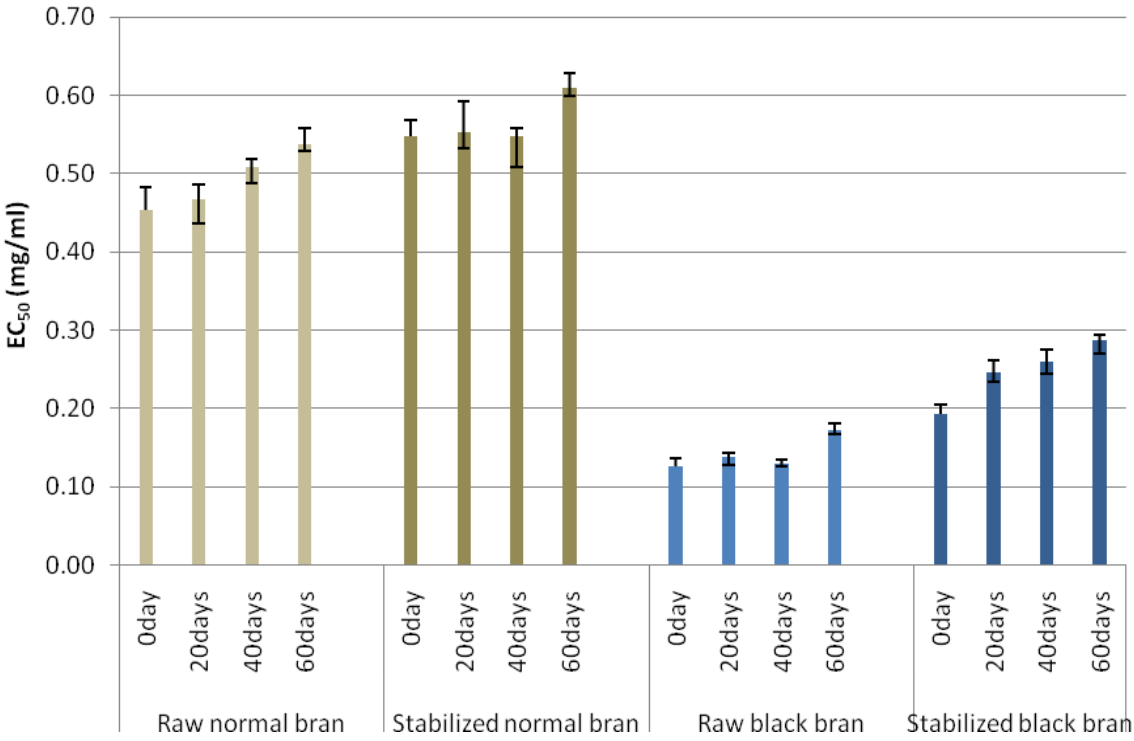


Figure 6.7 DPPH radical scavenging activity of rice bran during storage

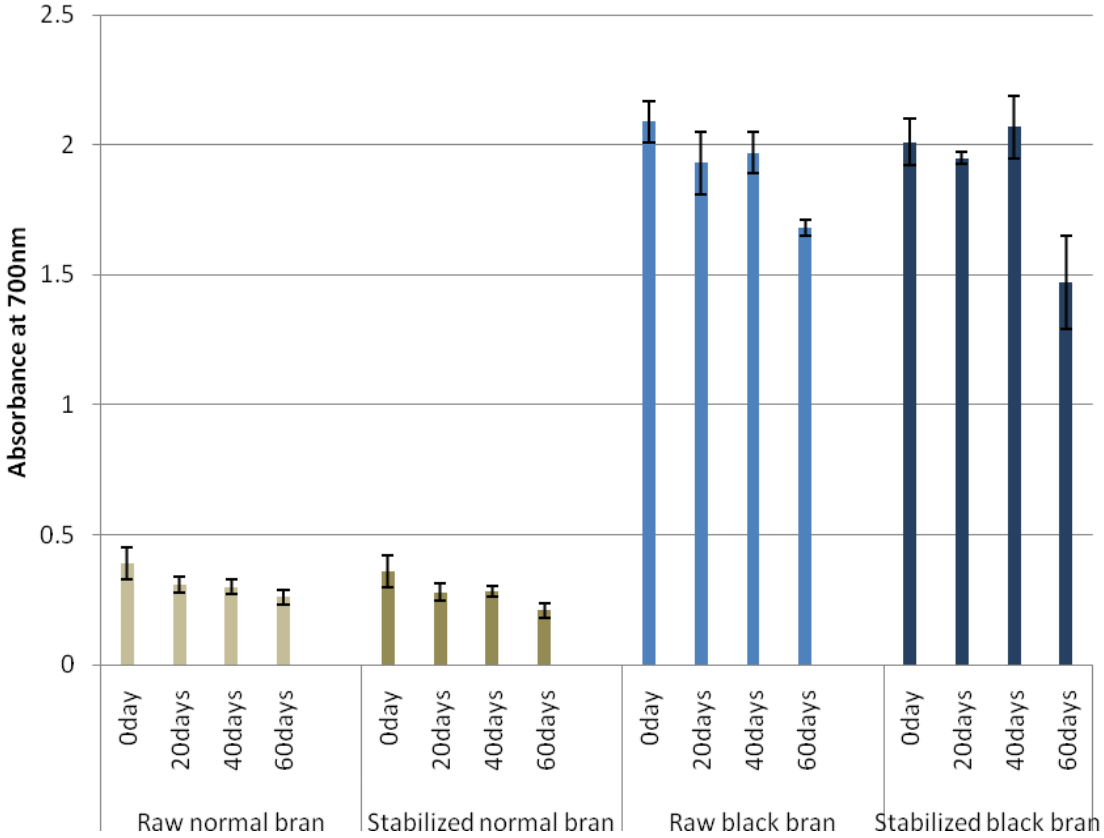


Figure 6.8 Reducing power of rice bran during storage

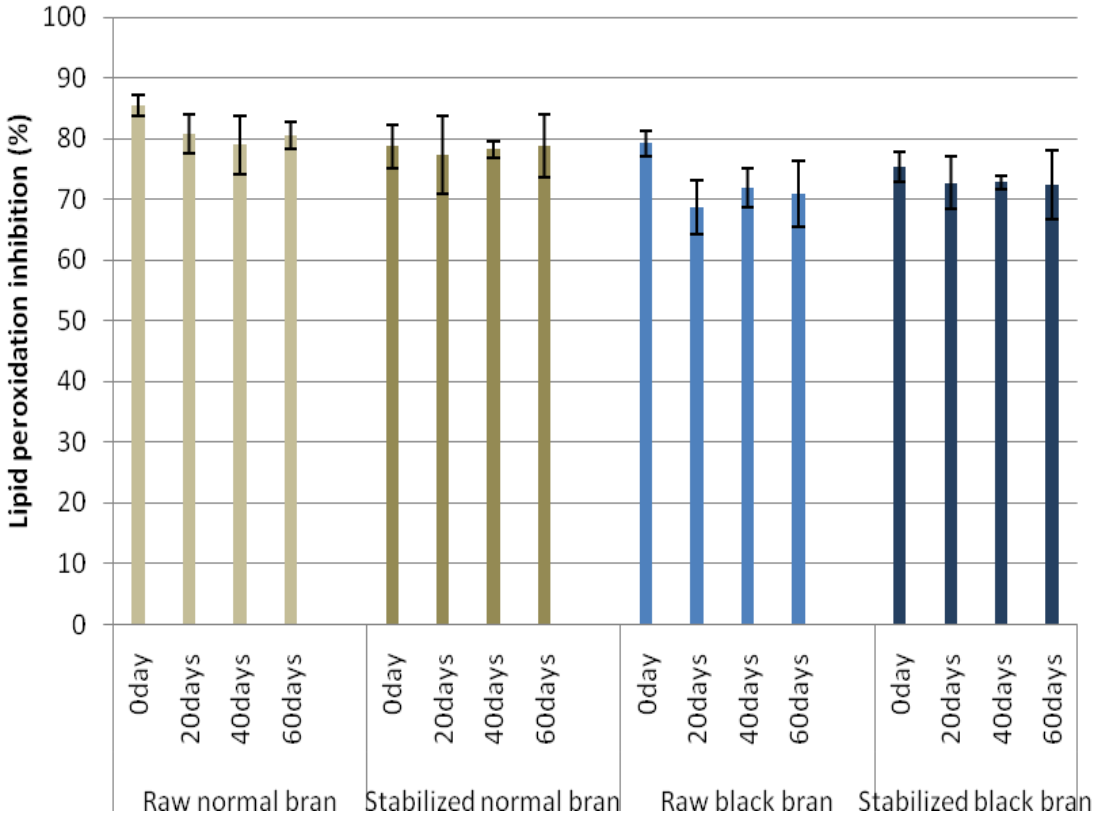


Figure 6.9 Lipid peroxidation inhibition of rice bran during storage

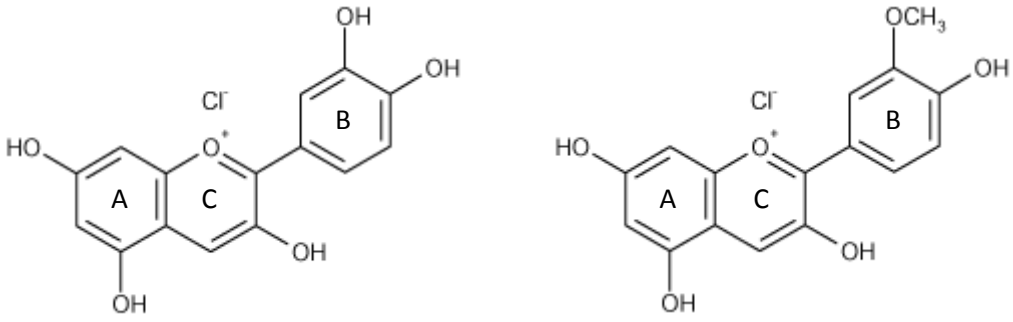


Figure 6.10 Molecular structures of cyanidin and peonidin

The difference in lipid peroxidation inhibition among the rice bran groups (Figure 6.9) may be contributed from the different of phenolic structure and concentration.

This study showed that storage for 60 days at ambient temperature reduces antioxidants and antioxidative ability of rice bran. Results obtained clearly indicate differences in the antiradical activities of black rice bran and the normal rice bran (non-pigmented) extracts. Black rice bran still retained high antiradical and antioxidant activities throughout the storage.

4.5 Conclusion

In conclusion, stabilization of normal and black rice bran by steaming partially deactivated lipase activity and decreased free fatty acid content, but conjugated diene value was increased in normal bran.

Stabilization by using opened steam heating was able to deactivate lipase enzyme in rice bran and after 2 months of storage, stabilized rice bran contained <10% of free fatty acid which was the accepted level for rice bran oil industry. However, stabilization destroyed many rice bran antioxidants especially black rice bran which lost 80-100% of their anthocyanins. The methanolic extracts of stabilized rice bran showed lower in DPPH radical scavenging activity than raw rice bran, while reducing power and lipid peroxidation inhibition was slightly decreased.

During storage, lipase activity of raw normal rice tended to increase whereas lipase activity of other samples was decreased. Conjugated diene value of all rice bran samples kept in cloth bags was increased during storage. α-Tocopherol was the antioxidant in rice responsible for lipid oxidation inhibition and lost 38-45% after storage. Phenolics, anthocyanins and γ-oryzanol had more oxidative stability than α-tocopherol, on average they lost <20% after storage.

DPPH radical scavenging activity of all rice bran extracts was decreased throughout the storage. Reducing power and lipid peroxidation were slightly

decreased during storage. High antioxidant activity of stored rice bran extracts was due to high retaining of rice bran antioxidants.

The results suggested that for our storage condition, the stabilization of rice bran was necessary to deactivate lipase enzyme and to retard the free fatty acid formation during storage. The stabilization process destructed rice bran antioxidants however the antioxidant activity of rice bran extracts was slightly decreased. For black rice bran, anthocyanins were much lost by stabilization process; raw black bran was consideration to be the invaluable source of antioxidants, besides its free fatty acid content was only 15% after 2 months of storage.

CHAPTER VII

SUMMARY

The risk of several chronic health conditions, including atherosclerosis, is lowered by regular consumption of foods containing antioxidants. Pigmented rice is concerned to be a rich source of antioxidants which concentrated in its bran. Our studies are separated into 4 experiments to study the potential of pigmented rice bran as a natural source of antioxidants and food ingredient.

Chapter 3 revealed the antioxidants of pigmented rice in different milling fractions. Bran had the highest value of antioxidants containing. The total antioxidants content in bran was about 7 and 62 times higher than unmilled and milled fraction, respectively. Phenolic acids and γ-oryzanol were the major antioxidants in all rice samples. Anthocyanin was found anbudance in all black rice samples while red rice contained lower amount of anthocyanins. All pigmented rice samples contained 2 anthocyanin compounds which were cyanidin-3-glucoside and peonidin-3-glucoside. Cyanidin-3-glucoside was found to be the major anthocyanin in pigmented rice. According to the identification of phenolic acids in rice, the major portion of phenolic acids was bound phenolic acids. However, pigmented rice contained higher concentration of free phenolic acids compared to non-pigmented rice. Six known phenolic acids, including gallic acid, protocatechic acid, hydroxybenzoic acid, *p*-coumaric acid, ferulic acid and sinapic acid were separated from rice samples using HPLC analysis. Phenolic acids of normal rice were dominated by ferulic acid and *p*-coumaric acid. For black rice samples, the major phenolic acids were protocatechuic acid and hydroxybenzoic acid which may involve with the degradation of anthocyanins. The results of chapter 3 suggested that bran of pigmented rice especially black rice was the richest of rice antioxidants. The activities of rice antioxidants and the methanolic extract of rice fraction samples were determined as described in Chapter 4. Authentic rice antioxidants including cyanidin-3-glucoside, peonidin-3-glucoside, α-tocopherol and γ-oryzanol and synthetic antioxidants including BHT and EDTA were determined their antioxidant activities by using the 3 methods which were DPPH radical scavenging, reduing power and lipid peroxidation inhibition. Cyanidin-3-glucoside possessed high activities of DPPH radical scavenging and reducing power but showed low lipid peroxidation inhibition. The polarity of antioxidants influenced

their activity in each method. High polarity antioxidant such as anthocyanins had low activity in oil-in-water emulsion system in the determination of lipid peroxidation inhibition. The correlation of antioxidant contents and antioxidant activities suggested that anthocyanins in pigmented rice played a role in DPPH radical scavenging activity and reducing power. The methanolic extracts from bran fractions had higher antioxidant activities than unmilled and milled fractions. The extract of black bran showed higher DPPH radical scavenging activity and reducing power than normal bran. However, normal bran showed higher lipid peroxidation inhibition than black bran extracts. Although, the methanolic extracts of all rice bran had lower antioxidant activities than BHT but pure anthetic rice antioxidants such as cyanidin-3-glucoside, peonidin-3-glcoside and α-tocopherol possessed higher activity of DPPH radical scavenging than BHT.

The results from chapter 3 and 4 concluded that rice bran especially black rice brans are the rich source of rice antioxidants which possess high antioxidant activities. The potential of rice bran as food used was determined as described in Chapter 5. Wheat breads were substituted by normal or red or black (no.1) rice bran at 5 and 10%. The wheat rice bread (WRB) dough showed higher of G' and G" than the control sample. The deformation of WRB dough from creep test was also lower than the control. These results confirmed that dough became harder with rice bran added. Wheat breads added with 5% rice bran had no adverse effects on loaf volume and crumb firmness. Crust and crumb color were influenced by rice bran especially pigmented rice bran. WRB bread with black bran was much darker than the control bread. Rice antioxidants from rice bran were retained in final bread products. γ-Oryzanol was highly retained in baked products and lost approximately 16-34% after baking. Addition of rice bran also increased total phenolic content of final baked products. Furthermore, WRB bread extracts showed higher DPPH radical scavenging activity, reducing power and lipid peroxidation inhibition than the control. These results indicated that pigmented rice bran had the potential to apply for human food.

Although rice bran contains abundance of antioxidants and also is proved for their potency as food ingredient; however, rice bran lipid is susceptible for deteriorization by natural occurring lipase enzyme. Stabilization of rice bran by opened steam heating for 30 minutes was used in our study to deactivate lipase. Lipase activity of rice bran decreased by 36-37%, after stabilization. Stabilization process

destructed rice antioxidants, especially anthocyanins of black rice bran which lost 80-100% after stabilization which caused the huge decreasing (46%) of DPPH radical scavenging activity of the extracts.

During storage at ambient temperature, lipase activity of raw normal rice tended to increase whereas lipase activity of other samples were decreased. α-Tocopherol was the antioxidant in rice responsible for lipid oxidation inhibition and lost 38-45% after storage. Phenolics, anthocyanins and γ-oryzanol were more oxidative stability than α-tocopherol, they lost <20% after storage. After 2 months storage, stabilized rice bran contained <10% of free fatty acid which was the accepted level for rice bran oil industry.

REFERENCES

Abdel-Aal, E-S. M., Young, J. C., and Rabalski, I. 2006. Anthocyanin composition in black, blue, pink, purple, and red cereal grains. J. Agric. Food Chem. *54* (13): 4696–4704.

Adom, K. K., and Liu, R. H. 2002. Antioxidant activity of grains. J. Agric and Food Chem. 50: 6182-6187.

Aguilar-Garcia, C., Gavino, G., Baragano-Mosqueda, M., Hevia, P., and Gavino, V. C. 2007. Correlation of tocopherol, tocotrienol, γ-oryzanol and total polyphenol content in rice bran with different antioxidant capacity assays. Food Chemistry, 102: 1228-1232.

Akiyama, Y., Hori, K., Takahashi, T., and Yoshiko, Y. 2005. Free radical scavenging activities of γ-oryzanol constituents. Food Sci Technol Res. 11: 295-297.

Alamed, J., Chaiyasit, W., McClements, D. J. and Decker, E. A. 2009. Relationships between free radical scavenging and antioxidant activity in foods. J. Agric. Food Chem. *57*(7): 2969–2976.

Alasalvar, C., Al-Farsi, M., and Shahidi, F. 2005. Compositional characteristics and antioxidant components of cherry laurel varieties and pekmez. Journal of Food Science 70: S47-52.

Amemiya, J. I., and Menjivar, J. A. 1992. Comparison of small and large deformation measurements to characterize the rheology of wheat flour doughs. J. Food Eng. 16: 91-108.

Amissah, J. G. N., Ellis, W. O., Oduro, I., and Manful, J. T. 2003. Nutrient composition of bran from new rice varieties under study in Ghana. Food Control 14: 21-24.

Anderson, O. M., and Jordheim, M., 2006. The anthocyanins, Flavonoids: Chemistry, Biochemistry, and Applications. Boca Raton FL: CRC Press,

Andjelkovic, M., Camp, J. V., De Meulenaer, B., Depaemelaere, G., Socaciu, C., Verloo, M., and Verhe, R. 2006. Iron-chelation properties of phenolic acids bearing catechol and galloyl groups. Food Chemistry 98: 23-31.

Asamarai, A. M., Addis, P. B., Epley, R. J., and Krick, T. P. 1996. Wild rice hull antioxidants. J. Agric. Food Chem. 44: 126–130.

Ashie, N. A., Simpson, B. K., and Smith, J. P. 1996. Mechanisms controlling enzymatic reactions in foods. Crit. Rev. Food Nutr. 36: 1-30.

Attenburrow, G. E., Barnes, D. J., Davies, A. P. and Ingman, S. J. 1990. Rheological properties of wheat gluten. Journal of Cereal Science 12: 1-14.

Bagchi, D., Garg, A., Krohn, R. L., Bagchi, M., Bagchi, B. J., Balmoori, J. 1998. Protective effects of grape seed proanthocyanidins and selected antioxidants against TPA-induced hepatic and brain lipid peroxidation and DNA fragmentation, and peritoneal macrophage activation in mice. General Pharmacology 30(5): 771-776.

Bagchi, K., and Puri, S. 1998. Free radicals and antioxidants in health and disease. East Mediterr Health J. 4(2): 350-360.

Balogh, E., Hegedus, A., and Stefanovits-Banyai, E. 2010. Application of and correlation among antioxidant and antiradical assays for characterizing antioxidant capacity of berries. Scientia Horticulturae 125: 332–336.

Bauernfeind, J. C. 1997. The tocopherol content of food and influencing factors. CRC Crit. Rev. Food Sci. Nutr. 8: 337-382.

Belton, P. S. 2003. The molecular basis of dough rheology. In S. P. Cauvain (ed.), Bread making Improving quality, pp. 273-287. Cambridge, England: Woodhead Publishing Limited,

Birosova, L., Mikulasova, M., and Vaverkova, S. 2005. Antimutagenic effect of phenolic acids. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub. 149(2): 489–91.

Bollain, C., and Collar, C. 2004. Dough viscoelastic response of hydrocolloid/enzyme/surfactant blends assessed by uni- and biaxial extension measurements. Food Hydrocolloids 18: 499-507.

Bradley, D. G., and Min, D. B. 1992. Singlet oxygen oxidation of foods. Crit. Rev. Food Sci. Nutr. 31(3): 211-236.

Brand-Williams, W., Cuvelier, M. E., and Berset, C. 1995. Use of a free radical method to evaluate antioxidant activity LWT 28: 25–30.

Brown, J. E., Khodr, H., Hider, R. C., and Rice-Evans, C. V. 1998. Structural dependence of flavonoid interaction with Cu2+ ions: implication for their antioxidant properties. Biochemical Journal 330: 1173-1178.

Bunzel, M., Allerdings, E., Sinwell, V., Ralph, J., and Steinhart, H. 2002. Cell wall hydroxycinnamates in wild rice (*Zizania quatic* L.) in soluble dietary fibre. European Food Research and Technology 214: 482-488.

Cabrita, L., Fossen, T., and Andersen, O. M. 2000. Colour and stability of the six common anthocyanidin 3-glucosides in aqueous solutions. Food Chemistry 68: 101-107.

Carson, L., and Sun, X. S. 2001. Creep-recovery of bread and correlation to sensory measurements of textural attributes. Cereal Chemistry 78(1): 101-104.

Castaneda-Ovando,A., Pacheco-Hernandez, M. L., Páez-Hernandez, M. E., Rodríguez, J. A., and Galan-Vidal, C. A. 2009. Chemical studies of anthocyanins: A review. Food Chemistry 113(4): 859-871.

Cevallos-Casals, B. A., and Cisneros-Zevallos, L. 2004. Stability of anthocyanin-based aqueous extracts of Andean purple corn and red-fleshed sweet potato compared to synthetic and natural colorants. Food Chemistry 86: 69-77.

Chen, M.-H., and Bergman, C. J. 2005. A rapid procedure for analyzing rice bran tocopherol, tocotrienol and γ-oryzanol contents. Journal of Food Composition and Analysis 18: 319-331.

Chen, P-N., Kue, W-H., Chiang, C-L., Chiou, H-L., Hsieh, Y-S., and Chu, S-C. 2006. Black rice anthocyanins inhibit cancer cells invasion via repressions of MMPs and u-PA expression. Chem Biol Interact. 163 (3): 218-229.

Chen, X., and Ahn, D. U. 1998. Antioxidant activities of six natural phenolics against lipid oxidation induced by Fe2+ or ultraviolet light. J. Am. Oil Chem. Soc. 75: 1717-1721.

Cho, M. H., Yoon, H. H., and Hahn, T. R. 1996. Thermal stability of the major color component, cyanidin 3-glucoside, from a Korean pigmented rice variety in aqueous solution. Agricultural Chem and Biotechnol 39: 245-248.

Choi, Y., Jeong, S-H., and Lee, J. 2007. Antioxidant activity of methanolic extracts from some grains consumed in Korea. Food Chemistry 103: 130-138.

Chotimarkorn, C., and Silalai, N. 2008. Oxidative stability of fried dough from rice flour containing rice bran powder during storage, LWT. 41: 561-568.

Chotimarkorn, C., Benjakul, S., and Silalai, N. 2008. Antioxidant components and properties of five long-grained rice bran extracts from commercial available cultivars in Thailand. Food Chemistry 111: 636-641

Cicero , A. F. G., and Gaddi, A. 2001. Rice bran oil and γ-oryzanol in the treatment of hyperlipoproteinaemias and other conditions. Phytotherapy Research.15: 277- 289.

Cillard, J., Cillard, P., Cormier, M. and Girre, L. 1980. α-Tocopherol prooxidant effect in aqueous media: increased autooxidation rate of linoleic acid. J. Am. Oil Chem. Soc.57: 252-255.

Collar, C., and Bollain, C. 2004. Impact of microbial transglutaminase on the viscoelastic profile of formulated bread doughs. European Food Research and Technology 218: 139-146.

Coppen, P. P. 1983. Use of antioxidants, In J. C. Allen and R. J. Hamilton (eds.), Rancidity in Foods, pp. 67–87. London: Applied Science Publishing Company,

de Kock, S., Taylor, J., and Taylor, J. R. N. 1999. Effect of heat treatment and particle size of different brans on loaf volume of brown bread. LWT. 32: 349-356.

Decker, E. A. 2007. Antioxidants mechanisms, In C. C. Akoh, and D. B. Min (eds.), Food lipids: chemistry, nutrition, and biotechnology, pp. 475-492. New York: Taylor & Francis Group, LLC,

Delgado-Vargas, F., Jimenez, A.R., and Parades-Lopez, O. 2000. Natural pigments: carotenoids, anthocyanins, and betalains-characteristics, biosynthesis, processing and stability. CRC Crit. Rev. Food Sci. Nutr. 40:173–289.

Dobraszczyk, B. J., and Roberts, C. A. 1994. Strain hardening and dough gas cell-wall failure in biaxial extension. Journal of Cereal Science 20: 265-274.

Dziedzic, S. Z., and Hudson, B. J. F.1984. Phenolic acids and related compounds as antioxidants for edible oils. Food Chemistry 14: 45–51.

Dziezak, J. D. 1986. Preservatives: antioxidants. Food Technol 9: 94–102.

Ercisli, S., and Orhan, E., 2008. Some physico-chemical characteristics of black mulberry (Morus nigra L.) genotypes from Northeast Anatolia region of Turkey. Sci. Hortic.116: 41–46.

Escribano-Bailón, M. T., Santos-Buelga, C., and Rivas-Gonzalo, J. C. 2004. Anthocyanins in cereals: review. Journal of Chromatography A 1054: 129-141.

Faria, A., Oliveira, J., Neves, P., Gameiro, P., Santos-Buelga, C., de Freitas, V. and Mateus, N. 2005. Antioxidant properties of prepared blueberry (*Vaccinium myrtillus*) extracts. J. Agric Food Chem. 53: 6896-6902.

Faulds, C.B., and Williamson, G. 1999. The role of hydroxycinnamates in the plant cell wall. J. Sci. Food and Agric. 79: 393-395.

Fennema, O. R. 1996. Food Chemistry 3rd edition. New York: Marcel Dekker Inc.,

Foley, S., Navaratnam, S., McGarvey, D. J. L., and, E. J., Truscott, T. G., and Rice-Evans, C. A. 1999. Singlet oxygen quenching and the redox properties of hydroxycinnamic acids. Free Radical Biology and Medicine 26: 1202-1208.

Food and Agricultural Organization of United Nations, http://www.fao.org/docrep/007/j3877e/j3877e02.htm (accessed December 2006)

Fot, M., Piatelli, M., Buratta, M.T. and Ruberto, C. 1996. Flavonoids, coumarins and cinnamic acids as antioxidants in a micellar system. Structure-activity relationship. J. Agric. Food Chem. 44: 497-501.

Francis, F. 1989. Food colourants: Anthocyanins. Critical Reviews in Food Science and Nutrition 28: 273–314.

Frankel, E. N. 1996. Antioxidants in lipid foods and their impact on food quality. Food Chemistry 57: 51–55.

Fu, J., Mulvaney, S. J., and Cohen, C. 1997. Effect of added fat on rheological properties of wheat flour dough. Cereal Chemistry 74(3): 304-311.

Fukushi, J. 1996. Edible rice bran oil III: antioxidant effects of gamma oryzanol. Hokkaidoritus Elsei Kenkyushoho 16: 111.

Galliard, T. 1986. Hydrolytic and oxidative degradation of lipids during storage of wholemeal flour: Effects of bran and germ components. Journal of Cereal Science 4:179-192.

Gan, Z., Galliard, T., Ellis, P. R., Angold, R. E., and Vaughan, J. G. 1992. Effect of the outer bran layers on the loaf volume of wheat bread. Journal of Cereal Science 15: 151-163.

Garewal, H. S. 1997. Antioxidants and disease prevention. Florida: CRC Press LLC,

German, B. 2001. Antioxidants. In A. L. Branen, P. M. Davidson and S. Salminan (eds.), Food additives, pp. 523-542. New York: Rlarcel Ilekker, Inc.,

Goffman, F. D., and Bergman, C. 2003. Relationship between hydrolytic rancidity, oil concentration, and esterase activity in rice bran. Cereal Chemistry 80(6): 689-692.

Goffman, F. D., and Bergman, C. J. 2004. Rice kernel phenolic content and its relationship with antiradical efficiency, Journal of the Science of Food and Agriculture 84: 1235-1240.

Gomes, C. A., da Cruz, T. G., Andrade, J. L., Milhazes, N., Borges, F., and Marques, M. P. 2003. Anticancer activity of phenolic acids of natural or synthetic origin: a structure-activity study. J Med Chem. 46(25): 5395-5401.

Gordon, M. H., 2001. The development of oxidative rancidity in food. In J. Pokorny, N. Yanishlieva and M. Gordon (eds.), Antioxidants in food, pp. 4-20. Cambridge, UK: Woodhead Publishing Limited/CRC Press,

Gordon, M.H. 1990. The mechanism of antioxidant action *in vitro. I*n B. J. F. Hudson (ed.), Food Antioxidants, pp. 1-18. London: Elsevier,

Grabber, J. H., Ralph, J., and Hatfield, R. D. 2000. Cross-Linking of Maize Walls by Ferulate Dimerization and Incorporation into Lignin. J. Agric. Food Chem. 48: 6106-6113.

Graf, E. 1992. Antioxidant potential of ferulic acid. Free Radical Biology & Medicine 13: 435-448.

Griffith, D.W. 1979. The inhibition of digestive enzymes by extracts of field beans (*Vicia fabia*). J. Sci. Food Agric 30: 458–462.

Gutteridge, J. M. C., and Halliwell, B., 2000. Free radicals and antioxidants in the year 2000 – A historical look to the future. Ann. N. Y. Acad Sci. 899: 136-147.

Halliwell, B., Aeschbach, R., Lolinger, J. and Aruoma, O.A., The characterization of antioxidants, Food Chem. Toxic. 33, 601-617, 1995.

Hamid-Abdul, A., and Luan, Y. S. (2000). Functional properties of dietary fibre prepared from defatted rice bran. Food Chemistry 68(1): 15–19.

Hargrove, Jr. R. 1994. Processing and utilization of rice bran in the United State. In W.E. Marshall and J. I. Wadsworth. (eds.), Rice Science and Technology, pp. 384-385. New York: Marcel Dekker, Inc.,

Hartley, R.D., Jones, E.C., and Wood, T.M. 1976. Lignin-carbohydrate linkages in plant cell walls. 3. Carbohydrates and carbohydrate esters or ferulic acid released from cell walls of *Lolium multiflorum* by treatment with cellulolytic enzymes. Phytochemistry 15: 305–307.

Hatfield, R.D., Ralph, J., and Grabber, J.H. 1999. Review. Cell cross-linking by ferulates and diferulates in grasses. Journal of the Science of Food and Agriculture 79: 403–407.

Helm, R. M., and Burks, A. W. 1996. Hypoallergenicity of rice protein. Cereal foods world 41(11): 839-843.

Hess, J. L. 1993. Vitamin E: α-Tocopherol in antioxidants. In R.G. Alscher and J.L. Hess (eds.), Antioxidants in Higher Plants, pp. 111-134. Boca Raton, FL: CRC Press,

Hiemori, M., Koh, E., and Mitchell, A. E. 2009. Influence of cooking on anthocyanins in black rice (*Oryza sativa* L. *japonica* var. SBR). J. Agric. Food Chem. 57: 1908-1914.

Higuchi, T., Ito, Y., Shimada, M., and Kawamura, I. 1967. Chemical properties of milled wood lignin grasses. Phytochemistry 6: 1551–1556.

Hu, C., Zawistowski, J., Ling, W., and Kitts, D. D. 2003. Black Rice (*Oryza sativa* L. *indica*) Pigmented fraction suppresses both reactive oxygen species and nitric oxide in chemical and biological model systems. J. Agric. Food Chem. 51(18): 5271–5277.

Huang, D.J., Ou, B.X., and Prior, R.L. 2005. The chemistry behind antioxidant capacity assays. J. Agric. Food Chem. 53: 1841–1856.

Huang, S.-W., Hopia, A., Schwarz, K., Frankel, E. N., and German, J. B. 1996. Antioxidant activity of α-tocopherol and Trolox in different lipid substrates: Bulk oils vs. oil-in-water emulsions. J. Agric. Food Chem. 44: 444-452.

Hudson, E. A., Dinh, P. A., Kokubun, T., Simmomds, M. S. J., and Gescher, A. 2000. Characterization of potential chemopreventive phenols in extracts of brown rice that inhibit the growth of human breast and colon cancer cells. Cancer Epidemiol Biomakers Prev. 9: 1163-1170.

Hyun, J. W., and Chung, H. S. 2004. Cyanidin and malvidin from Oryza sativa cv. Heungjinjubyeo mediate cytotoxicity against human monocytic leukemia cells by arrest of G(2)/M phase and induction of apoptosis. J. Agric. Food Chem. 52: 2213–2217.

Iiyama, K., Lam, T.B., and Stone, B.A. 1990. Phenolic acid bridges between polysaccharides and lignin in wheat internodes. Phytochemistry 29: 733–737.

Ingold, K.U., Peroxyradicals. Accounts Chem. Res. 2(1): 1-9.

Ishii, T. 1997. Structure and functions of feruolylated polysaccaharides, review. Plant Sci. 127: 111–127.

Ishii, T. and Hiroi, T. 1990. Linkage of phenolic acids to cell-wall polysaccharides of bamboo shoot. Carbohydr. Res. 206: 297–310.

Janssen, A. M., Vliet, V., and Vereijken, J. M. 1996. Rheological behavior of wheat glutens at small and large deformations. Comparisons of two glutens differing in breadmaking potential. Journal of Cereal Science 23: 19-31.

Jensen, S. K., and Lauridsen, C. 2007. Alpha-tocopherol stereoisomers. Vitam. Horm 76: 281–308.

Jiang, Y., and Wang , T. 2005. Phytosterols in cereal by-products. J. Am. Oil Chem. Soc. 82(6): 439-444.

Juliano, B. O. 1994. Rice: chemistry and technology. The American association of cereal chemists, Minnesota, USA: Inc. St. Paul,

Juliano, B. O., and Bechtel, D. B. 1985. The rice grain and its gross composition. In B.O. Juliano (ed.), Rice Chemistry and Technology, 2nd ed. pp.17-57. St Paul, MN: Am. Assoc. Cereal Chem.,

Kallithraka, S., Mohdaly, A. A-A., Makris, D. P., and Kefalas, P. 2005. Determination of major anthocyanin pigments in Hellenic native grape varieties (*Vitis vinifera* sp.): association with antiradical activity. Journal of Food Composition and Analysis 18(5): 375-386.

Kamal-Eldin, A., and Appelqvist, L-A. 1996. The chemistry and antioxidant properties of tocopherols and tocotrienols. Lipids 31: 671–701.

Kanatt, S. R., Chander, R., and Sharma, A. 2010. Antioxidant and antimicrobial activity of pomegranate peel extract improves the shelf life of chicken products. International Journal of Food Science and Technology 45: 216-222.

Kanner, J., German, J. B., and Kinsella. J. E. 1987. Initiation of lipid peroxidation in biological systems. Crit. Rev. Food Sci. Nutr. 25: 317-364.

Kaur, C., and Kapoor, H. C. 2000. Antioxidants in fruits and vegetables--the millennium's health. International Journal of Food Science & Technology 36(7): 703 – 725.

Khatkar, B. S., Bell, A. E., and Schofield, J. D. 1995. The dynamic rheological properties of glutens and gluten sub-fractions from wheats of good and poor bread making quality. Journal of Cereal Science 22: 29-44.

Kim, J., Suh, M., Yang, C., and Lee, H. G. 2003. Effect of gamma-oryzanol on the flavor and oxidative stability of refrigerated cooked beef. Journal of Food Science 68(8): 2423-2429.

Kim, K-H., Tsao, R., Yang, R., and Cui, Y. S. 2006. Phenolic acid profiles and antioxidant activities of wheat bran extracts and the effect of hydrolysis conditions. Food Chemistry 95: 466-473.

Kim, M-K., Kim, H-A., Koh, K., Kim, H-S., Lee, Y. S., and Kim, Y. H. 2008. Identification and quantification of anthocyanin pigments in colored rice. Nutrition Research and Practice 2(1): 46-49.

Klepacka, J., and Fornal, L. 2006. Ferulic acid and its position among the phenolic compounds of wheat. Crit. Rev. Food Sci. Nutr. 46: 639-647.

Knuckles, B. E.1988. Effect of phytate and other myo-inositol phosphate esters on lipase activity. Journal of Food Science 53: 250–252.

Ko, S-N., Kim, C. J., Kim, H., Kim, C. T., Chung, S-H., Tae, B. S. and Kim, I. H. 2003. Tocol levels in milling fractions of some cereal grains and soybean. J. Am. Oil Chem. Soc. 80(6): 585-589.

Kolattukudy, P. E., Espelie, K. E., and Soliday, C. L. 1981. Hydrophobic layers attached to cell walls and associated with waxes. Encycl. Plant Physiol. 13B: 225.

Konczak, I., and Zhang, W. 2004. Anthocyanins-more than natures colours. Journal of Biomedicine and Biotechnology 5: 239-240.

Kong, S., and Lee, J. 2009. Antioxidants in milling fractions of black rice cultivars Food Chemistry 120 (1): 278-281.

Krinsky, N.I. 1992. Mechanism of action of biological antioxidants. Proc Soc Exp Biol Med 200: 248–254.

Krishnaswamy, K., and Raghuramulu, N. 1998. Bioactive phytochemicals with emphasis on dietary practice. Ind. J. Medical Res. 108: 167-181.

Kwon, D.Y., and Rhee, J. S. 1986. A simple and rapid colorimetric method for determination of free fatty acids for lipase assay. J. Am. Oil Chem. Soc. 63:89-92.

Lai, C. S., Hoseney, R. C., and Davis, A. B. 1989. Effects of wheat bran in breadmaking. Cereal Chemistry 66: 217-219.

Laughton, M. J., Evans, P. J., Moroney, M.A., Hoult, J. R. S. , and Halliwell, B. 1991. Inhibition of mammalian 5-lipoxygenase and cyclo-oxygenase by flavonoids and phenolic dietary additives. Biochem. Pharm. 42(9): 1673-1681.

Li, W., Pickard, M. D., and Beta, T. 2007. Effect of thermal processing on antioxidant properties of purple wheat bran. Food Chemistry 104: 1080-1086.

Liebler, D. C. 1993. The role of metabolism in the antioxidant function of vitamin E. Crit. Rev. Toxicol. 23(2):147.

Lingnert, H., Vallentin, K., and Eriksson, C. E. 1979. Measurement of antioxidative effect in model system. Journal of Food Processing and Preservation 3: 87-103.

Liu, R. H. 2007. Whole grain phytochemicals and health. Journal of Cereal Science 46: 207-219.

Lloyd, B. J., Siebenmorgen, T. J., and Beers, K. W. 2000. Effects of commercial processing on antioxidants in rice bran. Cereal Chemistry 77(5): 551-555.

Loeb, J. R., and Mayne, R. Y. 1952. Effect of moisture on the microflora and formation of free fatty acids in rice bran. Cereal Chemistry 29: 163–175.

Lu, Z., Kou, W., Du, B., Wu, Y., Zhao, S., Brusco, O. A., Morgan, J. M., and Capuzzi, D. M. 2008. Effect of Xuezhikang, and extract from red yeast Chinese rice, on coronary events in a Chinese population with previous myocardial infarction. The American Journal of Cardiology 101: 1689- 1693.

Madhavi, D. L., Deshpande, S. S., and Salunkhe, D. K. 1996. Food Antioxidants. New York: Marcel Dekker,

Markham, K. R., and Ofman, D. J. 1993. Lisianthus flavonoid pigments and factors influencing their expression in flower colours. Phytochemistry 34: 679-685.

Markwalder, H.U. and Neukom, H. 1976. Diferullic acid as possible crosslink in hemicelluloses from wheat germ. Phytochemistry 15: 836–837.

Marshall, W. E., and Wadsworth, J. I. 1994. Rice Science and Technology. New York: Marcel Dekker, Inc.,

Martinez-Tome, M. Murcia, M. A., Frega, N., Ruggieri, S., Jiménez, A. M., Roses, F., and Parras, P. 2004. Evaluation of Antioxidant Capacity of Cereal Brans. J. Agric. Food Chem. 51: 4690-4699.

Masi, P. Cavella, S., and Sepe, M. 1998. Characterization of dynamic viscoelastic behavior of wheat flour doughs at different moisture contents. Cereal Chemistry 75(4): 428-432.

McClements, D. J. and Decker, E. A. 2000. Lipid oxidation in oil-in-water emulsion: Impart of molecular environment on chemical reaction in heterogeneous systems. Journal of Food Science 65: 1270-1282.

McPeak, P., Rukmini, C., and Sastry, R. C. 2001. Supportive therapy for diabetes, hyperglycemia and hypoglycemia. US Patent 6,303,586 B1.

Milic, B.Lj., Djilas, S.M. and Canadanovic-Brunet, J.M. 1998. Antioxidative activity of phenolic compounds on the metal-ion breakdown of lipid peroxidation system, Food Chemistry 61: 443-447.

Miyazawa, M., Oshima, T., Koshio, K., Itsuzaki, Y., and Anzai, J. 2003. Tyrosinase inhibitor from black rice bran. J. Agric. Food Chem . 51: 6953–6956.

Nakamura, Y. and Higuchi, T. 1978. Ester linkage of *p*-coumaric acid in bamboo lignin. III. Dehydrogenerative polymerization of coniferyl *p*-hydroxybenzoate and *p*-coumarate. Cell. Chem. Technol. 12: 209–221.

Nam, S. H., Choi, S. P., Kang, M. Y., Koh, H. J. Kozukue, N., and Friedman, M. 2006. Antioxidative activities of bran extracts from twenty one pigmented rice cultivars. Food Chemistry 94: 613-620.

Namiki, M. 1990. Antioxidants/antimutagens in food. CRC Crit. Rev. Food Sci. Nutr. 29:273–300.

Nanua, J. N., McGregor, J. U., and Godber, J. S. 2000. Influence of high oryzanol rice bran oil on the oxidative stability of whole milk powder. Journal of Dairy Science 83: 2426-2431.

Nawar, W. W. 1996. Lipids. In O. Fennema, (ed.), Food Chemistry, 3rd ed. pp. 225. New York: Dekker,

Noort, M. W. J., van Haaster, D., Hemery, Y., Schols, H. A., and Hamer, R. J. 2010. The effect of particle size of wheat bran fractions on bread quality – Evidence for fibre – protein interactions. Journal of Cereal Science 52: 59-64.

Nystrom, L., Achrenius, T., Lampi, A-M., Moreau, R. A., and Piironen, V. 2007. A comparison of the antioxidant properties of steryl ferulates with tocopherol at high temperatures. Food Chemistry 101: 947-954.

Okada, T., and Yamaguchi, N. 1983. Antioxidative effect and pharmacology of oryzanol. Journal Japan Oil Chemistry Society 32: 305-310.

Oki, T., Masuda, M., Kobayashi, M., Nishiba, Y., Furuta, S., Suda, I., and Sato, T. 2002. Polymeric procyanidins as radical-scavenging components in red-hulled rice. J. Agric. Food Chem. 50: 7524-7529.

Olson, R. E., and Munson, P. L. 1994. Fat-soluble vitamins, In P. L. Munson, R. A. Mueller, and G. R. Breese, (eds.), Principles of Pharmacology, chap. 58. New York: Chapman & Hall,

Orthoefer, F. T. 1996. Rice bran oil: Healthy lipid source. Food Technol 50(12): 62-64.

Oszvald, M., Tomoskozi, S., Tamas, L., and Bekes, F. 2009. Effects of wheat storage proteins on the functional properties of rice dough. J. Agric. Food Chem. 57(21): 10442-10449.

Owusu-Apenten, R. 2004. Introduction to Food Chemistry. New York: CRC Press,

Ozer, N. K., Boscoboinik, D., and Azzi, A. 1995. New roles of low density lipoproteins and vitamin E in the pathogenesis of atherosclerosis. Biochem Mol Biol Int, 35(1): 117–24.

Packer, L., 1995. Nutrition and biochemistry of the lipophilic antioxidants, vitamin E and carotenoids. In A.S.H. Ong, E. Niki, L. Packer (eds.), Nutrition, Lipids, Health, and Disease, pp. 8-35. IL, USA: American Oil Chemists' Society, Champaign,

Paniangvait, P., King, A. J., Jones, A. D., and German, B. G. 1995. Cholesterol oxides in foods of animal origin. Journal of Food Science 60(6): 1159-1166.

Pantelidis, G.E., Vasilakakis, M., Manganaris, G.A., and Diamantidis, G. 2007. Antioxidant capacity, phenol, anthocyanin and ascorbic acid contents in raspberries, blackberries, red currants, gooseberries and cornelian cherries. Food Chemistry 102: 777-783.

Park, H., Seib, P. A., and Chung, O. K. 1997. Fortifying bread with a mixture of wheat fiber and Psyllium husk fiber plus three antioxidants. Cereal Chemistry 74(3): 207-211.

Park,Y. S., Kim, S-J., and Chang, H-I. 2008. Isolation of anthocyanin from black rice (Heugjinjubyeo) and screening of its antioxidant activities. Kor. J. Microbiol. Biotechnol. 36: 55-60.

Parrado, J., Miramontes, E., Jover, M., Marquez, J. C., Angeles Mejias, M., Collantes De Terran, L., et al. 2003. Prevention of brain protein and lipid oxidation elicited by water-soluble oryzanol enzymatic extract derived from rice bran. European Journal of Nutrition 42: 307–314.

Pin-Der-Duh, X .1998. Antioxidant activity of burdock (Arctium lappa Linne): its scavenging effect on free radical and active oxygen. J. Am. Oil Chem. Soc. 75: 455-461.

Pokorny, J. 2001. Natural antioxidant functionality during food processing, In J. Pokorny, N. Yanishlieva, and M. Gordon, (eds.), Antioxidants in Food, pp. 323-347. Cambridge, UK.: Woodhead Publishing Limited/CRC Press,

Pomeranz, Y., Shogren, M.D., Finney, K.F., and Betchel, D.B. 1977. Fiber in breadmaking: Effects on functional properties. Cereal Chemistry 54: 25–41.

Porter, W. L., Black, E. D., and Drolet, A. M.1989. Use of polyamide oxidative fluorescence test on lipid emulsions: contrast in relative effectiveness of antioxidants in bulk versus dispersed systems. J. Agric. Food Chem. 37: 615–624.

Qureshi A. A., Bradlow B. A., Salser W. A., and Brace L. D. 1997. Novel tocotrienols of rice bran modulate cardiovascular disease risk parameters of hypercholesterolemic human. J. Nutr. Biochem. 8: 290-298.

Qureshi,N., and Qureshi, A. A. 1992 Tocotrienols: novel hypocholesterolemic agents with antioxidant properties. In L. Packer, and J. Fuch (eds.), Vitamin E in Health and Disease, pp. 245-267. New York: Marcel Dekker,

Randall, J. M., Sayre, R. N., Schultz, W. G., Fong, R. Y., Mossman, A. P., Tribelhorn, R. E., and Saunders, R. M. 1985. Rice bran stabilization by extrusion cooking for extraction of edible oil. Journal of Food Science 50: 361-364, 368.

Ranhotra, G. S., Gelroth, J. A., and Okot-Kotber, B. M. 2000. Stability and dietary contribution of vitamin E added to bread. Cereal Chemistry 77(2): 159-162.

Raskin, J. 1992. Protein–polyphenol interactions: nutritional aspects, in Proc. 16th Int. Conf. Groupe Polyphenols, 16, Part II, 11–18.

Ribereau-Gayon, P. 1972. Plant Phenolics, Edinburgh, U.K.: Oliver and Boyd,

Rice-Evans, C. A., Miller, N. J., and Paganga, G. 1996. Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radical Biology and Medicine 20(7): 933-956.

Riley, R. G. and Kolattukudy, P. E. 1975. Evidence for covalently attached *p*-coumaric and ferulic acids in cutins and suberins. Plant Physiol 56:650–654.

Rohrer, C. A., and Siebenmorgen, T. J. 2004. Nutraceutical concentrations within the bran of various rice kernel thickness fractions. Biosystems Engineering 88(4): 453-460.

Rong, N., Ausman, L.M., and Nicolosi, R. J. 1997. Oryzanol decreases cholesterol absorption and aortic fatty steaks in hamsters. Lipids 32(3): 303-309.

Rose, D. J., and Pike, O. A. 2006. A simple method to measure lipase activity in wheat and wheat bran as an estimation of storage quality. J. Am. Oil Chem. Soc. 83(5): 415-419.

Ryu, S. N., Park, S. Z. and Ho, C-T. 1998. High performance liquid chromatographic determination of anthocyanin pigments in some varieties of black rice. Journal of Food and Drug Analysis 6: 729-736.

Saito, N., Toki, K., Suga, A., and Honda, T. 1998. Acylated pelargonidin 3,7-glycosides from red flowers of Delphinium hybridum. Phytochemistry 49: 881-886.

Salunkhe, D.K., Jadhav, S.J., Kadam, S.S., and Chavan, J.K. 1982. Chemical, biochemical, and biological significance of polyphenols in cereals and legumes. CRC Crit. Rev. Food Sci. Nutr. 17: 277–305.

Saunders, R. M. 1990. The properties of rice bran as a foodstuff. Cereal Foods World 35: 632.

Saunders, R. M., Sloan, S., and James, C. 1988. Extruded full fat rice bran in muffins. LWT. 21: 245-247.

Saunders, R.M., and Heltved, F. 1985. Fluorimetric assay of lipase in rice bran, and its application to determination of conditions for rice bran stabilization. Journal of Cereal Science 3: 79-86.

Sayre, R. N., Saunders, R. M., Enochian, R. V., Schultz, W. G., and Beagle, E. C. 1982. Review of rice bran stabilization systems with emphasis on extrusion cooking. Cereal Foods World 27(6): 317-322.

Schober, T. J. Clarke, C. I., and Kuhn, M. 2002. Characterization of functional properties of gluten proteins in spelt cultivars using rheological and quality factor measurements. Cereal Chemistry 79: 408-417.

Seetharamaiah, G. S., and Chandrasekhara, N. 1989. Studies on hypocholesterolemic activity of rice bran oil. Atherosclerosis 78: 219–223.

Sekhon, K. S., Dhillon, S. S., Singh, N., and Singh, B. 1997. Functional suitability of commercially milled rice bran in India for use in different food products. Plant Foods for Human Nutrition 50: 127-140.

Shahidi, F. 2000. Antioxidants in food and food antioxidants. Nahrung 44:158–163.

Shahidi, F. 2002. Antioxidants in plants and oleaginous seeds, In M.J., Shahidi, F., and Ho, C-T. (eds.), Free Radicals in Food: Chemistry, Nutrition and Health Effects, pp. 162-175. Washington, D.C.: Morello, ACS Symposium Series 807. American Chemical Society,

Shahidi, F. and Naczk, M. 1995. Food Phenolics: Sources, Chemistry, Effects, Applications, Lancaster, PA: Technolomic Pub. Co. Inc.,

Shahidi, F. and Naczk, M. 2004. Phenolics in Food and Nutraceuticals. Boca Raton, Florida: CRC Press,

Shahidi, F., and Wanasundara, J. P. K. 1992. Phenolic antioxidants. Crit. Rev.Food Sci. Nutr.32: 67.

Sheppard, A., Pennington, J. A. T., and Weihrauch, J. L. 1993. Analysis and distribution of vitamin E in vegetable oils and foods. In F.J. Packer (ed.), Vitamin E in Health and Disease, pp. 9-31. New York: Marcel Dekker Inc.,

Sherwin, E. R. 1978. Oxidation and antioxidants in fat and oil processing. J. Am. Oil Chem. Soc. 55: 809–814.

Shin, T., Godber, J. S., Martin, D. E., and Wells, J. H. 1997. Hydrolytic stability and changes in E vitamers and oryzanol of extruded rice bran during storage. Journal of Food Science 62: 704-708.

Sidhu, J. S., Al-Hooti, S. N., and Al-Saqer, J. M. 1999. Effect of adding wheat bran and germ fractions on the chemical composition of high-fiber toast bread. Food Chemistry 67: 365-371.

Smith, P. K., Krohn, R. I., Hermanson, G. T., Mallia, A. K., Gartner, F. H., Provenzano, M. D., Fujimoto, E. K., Goeke, N. M., Olson, B. J., and Klenk, D. C. 1985. Measurement of protein using bicinchoninic acid. Analytical Biochemistry 150(1): 76-85.

Sosulski, F., Krygier, K., and Hogge, L. 1982. Free, esterified, and insoluble-bound phenolic acids. 3. Composition of phenolic acids in cereal and potato flours J. Agric. Food Chem. 30: 337–340.

Sroka, Z., and Cisowski, W. 2003. Hydrogen peroxide scavenging, antioxidant and anti-radical activity of some phenolic acids. Food and Chemical Toxicology 41: 753-758.

Sudha, M. L., Vetrimani, R., and Leelavathi, K. 2007. Influence of fibre from different cereals on the rheological characteristics of wheat flour dough and on biscuit quality. Food Chemistry 100: 1365-1370.

Tian, S., Nakamura, K., and Kayahara, H. 2004. Analysis of phenolic compounds in white rice, brown rice, and germinated brown rice. J. Agric. Food Chem. 52: 4808-4813.

Tait, S. P. C., and Galliard, T. 1988. Oxidation of linoleic acid in doughs and aqueous suspensions of wholemeal flours: Effect of storage. Journal of Cereal Science 8: 55-67.

Tian, S., Nakamura, K., Cui, T., and Kayahara, H. 2005. High-performance liquid chromatographic determination of phenolic compounds in rice. Journal of Chromatography A 1063: 121-128.

Tsuda, T., Horio, F. Uchida, K., Aoki, H., Osawa, T. 2003. Dietary cyanidin-3-o-β-D-glucoside-rich purple corn color prevents obesity and ameliorate hyperglycemia in mice. J Nutr. 133: 2125-2130.

van Sumere, C.F. 1989. Phenols and phenolic acids. In J. B. Harborne (ed.), Methods in Plant Biochemistry, Volume1, Plant Phenolics, pp. 29-74. London: Academic Press,

Wang, F. C. and Sun, X. S. 2002. Creep-recovery of wheat flour doughs and relationship to other physical dough tests and breadmaking performance. Cereal Chemistry 79(4): 567-571.

Wang, M-W., van Vliet, T., and Hamer, R. J. 2004. How gluten properties are affected by pentosans. Journal of Cereal Science 39: 395-402.

Warren, B. E., and Farrell, D. J. 1990. The nutritive value of full-fat and defatted Australian rice bran. I. Chemical composition. Anim. Feed Sci. Technol. 27: 219–228.

Weipert, D. 1988. The benefits of basic rheometry in studying dough rheology. Cereal Chemistry 67: 311-317.

White, P. J. 1995. Conjugated diene, anisidine value, and carbonyl value analyses. In K. Warner and N.A.M. Eskin, (eds.), Methods to assess quality and stability of oils and fat-containing foods, pp. 159-178. USA: AOCS press,

White, P.J. 1995. Analyses for conjugated diene, anisidine, and carbonyl values. In K. Warner, N. A. M. Eskin (eds.), Methods to Access Quality and Stability of Oils and Fat-Containing Foods, pp. 159-179. Illinois: AOCS press,

Wilson, T. A., Nicolosi, R. J., Woolfrey, B., and Kritchevsky, D. 2007. Rice bran oil and oryzanol reduce plasma lipid and lipoprotein cholesterol concentrations and aortic cholesterol ester accumulation to a greater extent than ferulic acid in hypercholesterolemic hamsters, J. Nutr. Biochem. 18(2): 105-112.

Xia, M., Ling, W. H., Ma, J., Kitts, D. D., and Zawistowski, J. 2003. Supplementation of diets with the black rice pigment fraction attenuates atherosclerotic plaque formation on apolipoprotein E deficient mice. J Nutr. 133(3): 744-751.

Xia, X., Ling, W., Ma, J., Xia, M., Hou, M., Wang, Q., Zhu, H., and Tang, Z. 2006. An anthocyanin-rich extract from black rice enhances atherosclerotic plaque stabilization in apolipoprotein E deficient mice. The Journal of Nutrition 136: 2220-2225.

Xu, J. R., Zhang, M. W., Liu, X. H., Liu, Z. X., Zhang, R. F., Sun, L., and Qiu, L. J. 2007. Correlation between antioxidation and the content of total phenolics and anthocyanin in black soybean accessions. Agricultural Sciences in China 6(2): 150-158.

Xu, Z., and Godber, J. S.1999. Purification and identification of components of gamma-oryzanol in rice bran oil. J. Agric. Food Chem. 47: 2724-2728.

Xu, Z., Hua, N., and Godber, J. S. 2001. Antioxidant activity of tocopherols, tocotrienols, and γ-oryzanol components from rice bran against cholesterol oxidation accelerated by 2,2'-azobis(2-methylpropionamidine) dihydrochloride, J. Agric. Food Chem 49: 2077-2081.

Yanishlieva-Maslarova, N. V. 2001. Inhibiting oxidation. In J. Pokorny, N. Yanishlieva, and M. Gordon, (eds.), Antioxidants in Food: Practical Applications, pp. 22–70. Cambridge, UK: Woodhead Publishing Limited/CRC Press,

Yawadio, R., Tanimori, S., and Morita, N. 2007. Identification of phenolic compounds isolated from pigmented rices and their aldose reductase inhibitory activities. Food Chemistry 101: 1616-1625.

Yen, G. C., and Duh, P. D. 1993. Antioxidative properties of methanolic extracts from penut hulls. J. Am. Oil Chem. Soc. 70: 383-386.

Yoshino, G., Kazumi, T., Amano, M., Takiewa, M., Yamasaki, T., Takashima, S., Iwai, M., Hatanaka, H., and Baba, S. 1989. Effects of gamma-oryzanol and probucol on hyperlipidemia. Current Therap Res. 45: 975-982.

Yoshizawa, K., Komatsu, S., Takahashi, I., and Otsuka, K. 1970. Phenolic compounds in the fermented products. I. Origin of ferulic acid in sake. Agric. Biol. Chem. 34:170–180.

Zhang, M-W., Guo, B-J., Chi, J-W., Wei, Z-C., Xu, Z-H., Zhang, Y., and Tang, X-J. 2006. Separation, purification and identification of antioxidant compositions in black rice. Agricultural Sciences in China 5(6): 431-440.

Zhao, C., Giusti, M. M., Malik, M., Moyer M. P., and Magnuson, B. A. 2004. Effects of commercial anthocyanin-rich extracts on colonic cancer and nontumorigenic colonic cell growth. J. Agric. Food Chem. 52 (20): 6122–6128.

Zhou, Z., Robard, K., Helliwell, S., and Blanchard, C. 2004. The distribution of phenolic acids in rice. Food Chemistry 87: 401-106.

APPENDICES

APPENDIX A

Rice and rice fractions samples

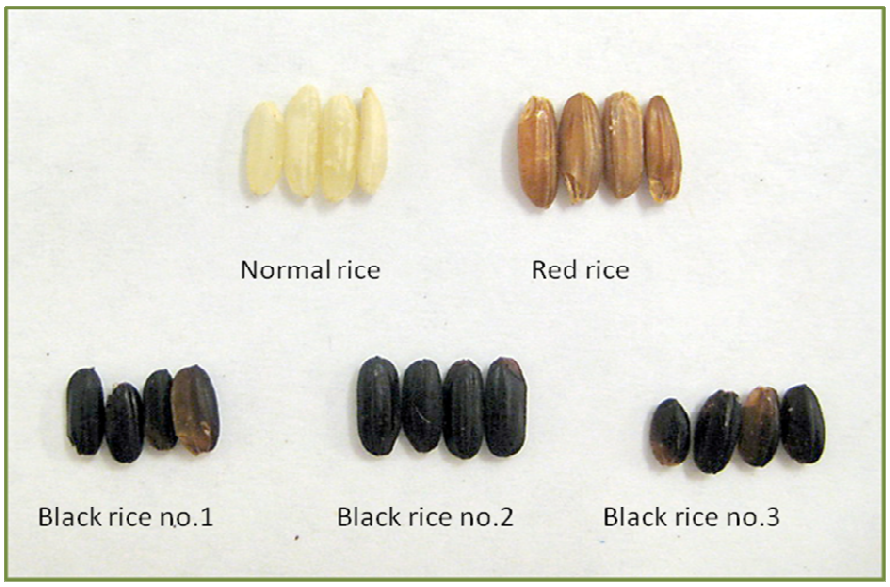


Figure 1 Rice samples

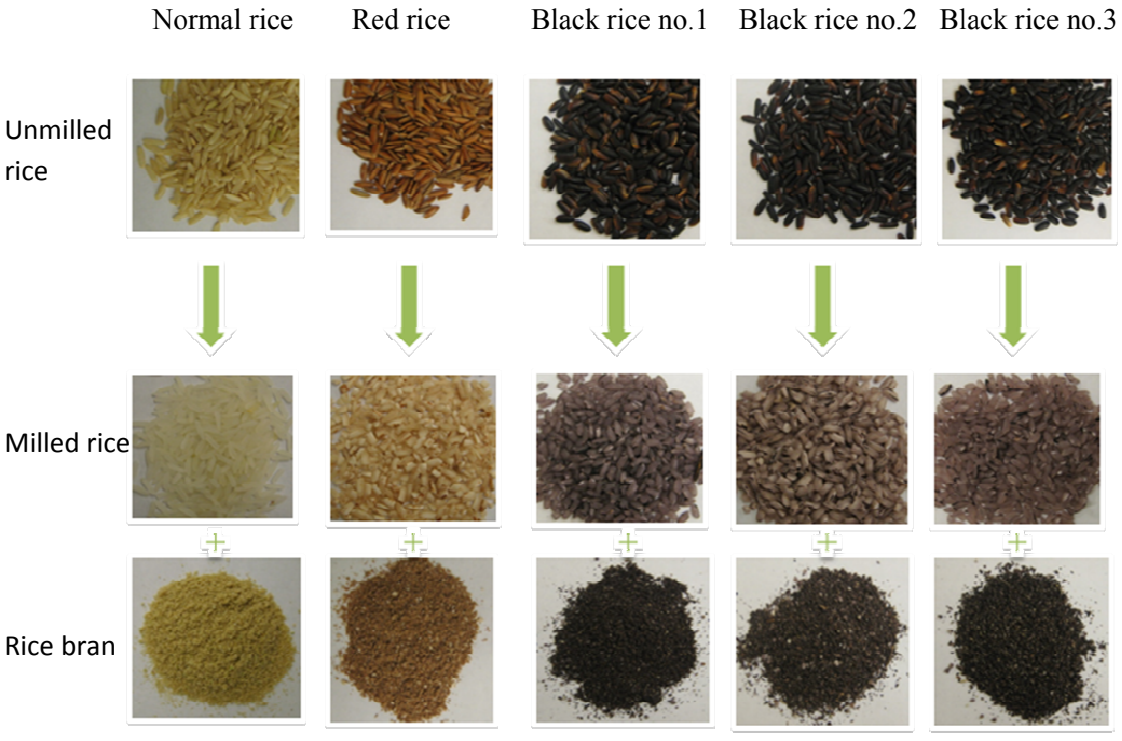


Figure 2 Rice milling fractions

APPENDIX B

Chromatogram of standard anthocyanins for HPLC analysis

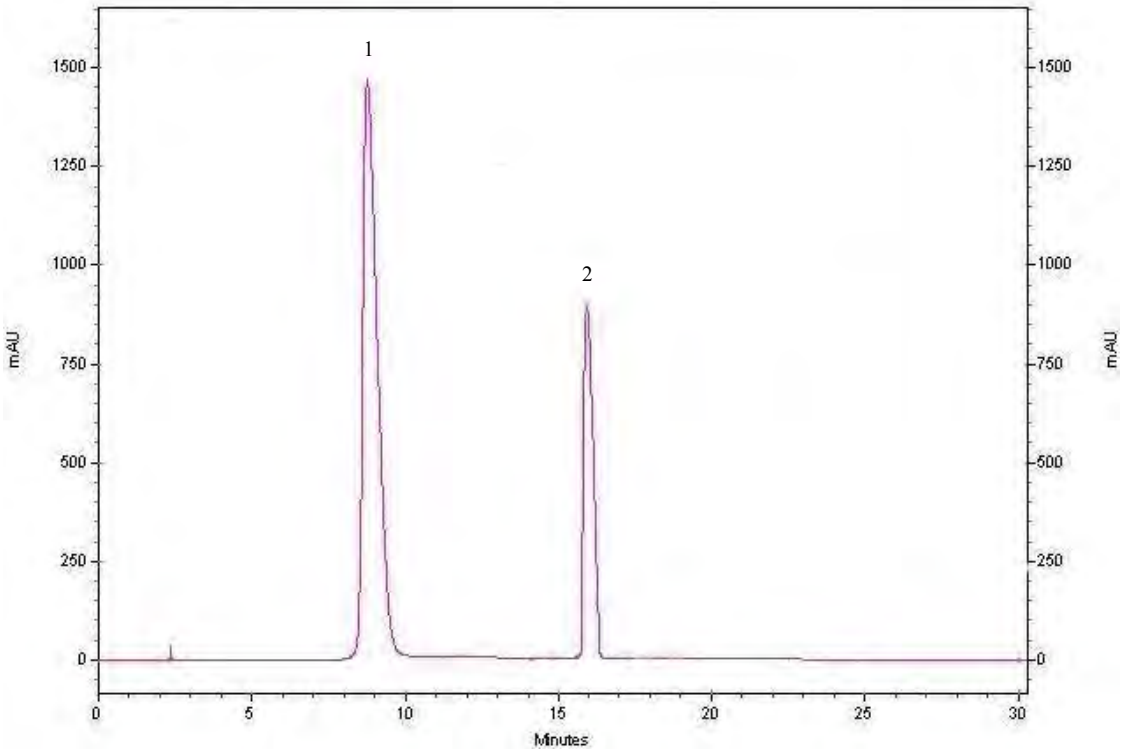


Figure 1 Chromatogram of standard anthocyanins (1= cyanidin-3-glucoside and 2= peonidin-3-glucoside)

Table 1 Retention times, equations of standard curves (peak area *vs* concentration) and regression coefficients (R^2) of standard curve for anthocyanins

Anthocyanins	Retention times (min)	Equations of standard curve	R^2
Cyanidin-3-glucoside	8.8	Y = 109445X-343849	0.9992
Peonidin-3-glucoside	16.0	Y = 45300X+221231	0.9988

APPENDIX C

Chromatogram of standard α-tocopherol and γ-oryzanol for HPLC analysis

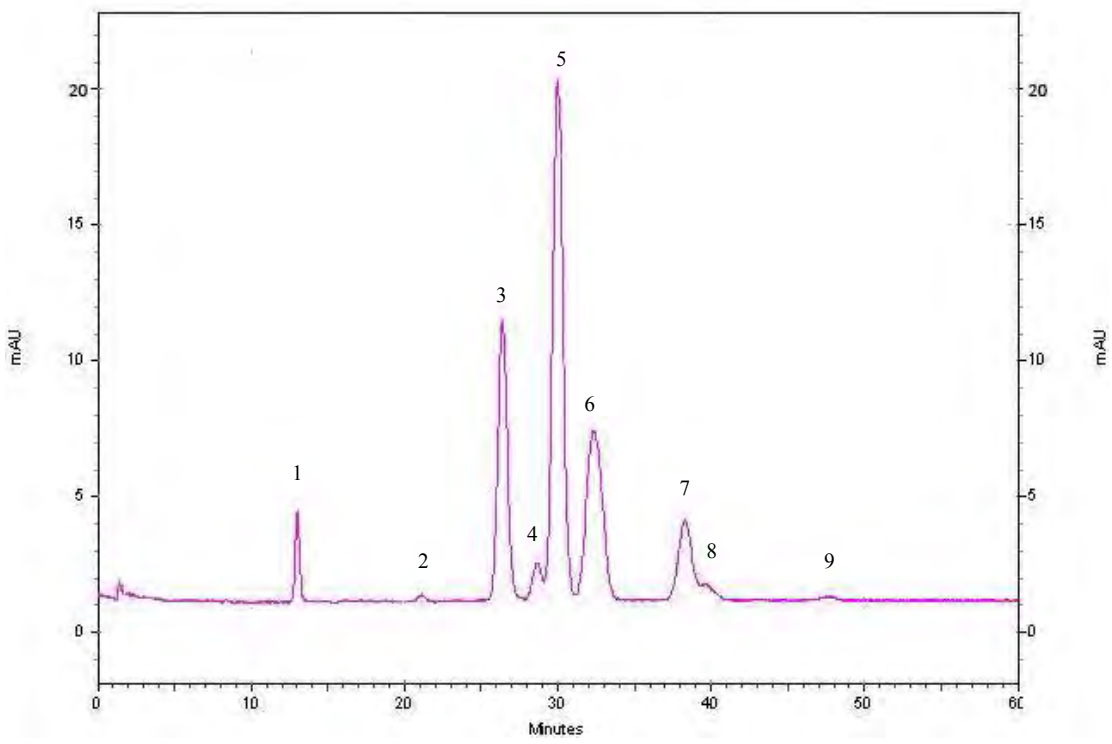


Figure 1 Chromatogram of standard of α-tocopherol and γ-oryzanol (1= α-tocopherol and 2- 9= γ-oryzanol)

Table 1 Retention times, equations of standard curves (peak area *vs* concentration) and regression coefficients (R^2) of standard curve for α-tocopherol and γ-oryzanol

Antioxidant compounds	Retention times (min)	Equations of standard curve	R^2
α-Tocopherol	12.9	Y = 8026.6X+8174.5	0.994
γ-Oryzanol	20.9, 26.0, 28.2, 29.3, 32.1, 38.2, 39.5, 47.6	Y = 36149X+58107	0.9988

APPENDIX D

Chromatogram of standard phenolic acids for HPLC analysis

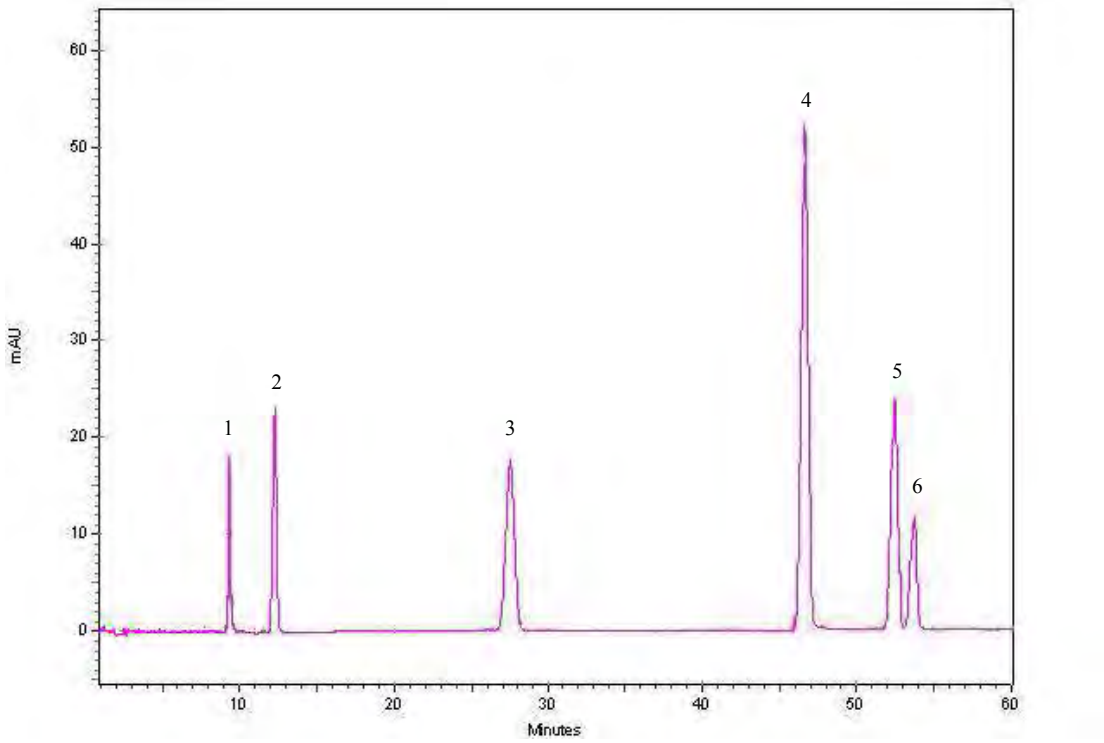


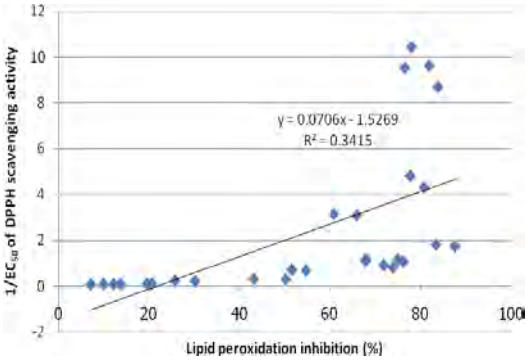
Figure 1 Chromatogram of standard phenolic acids (1=gallic acid, 2= protocatechuic acid, 3= hydroxybenzoic acid, 4= *p*-coumaric acid, 5= ferulic acid and 6= sinapic acid)

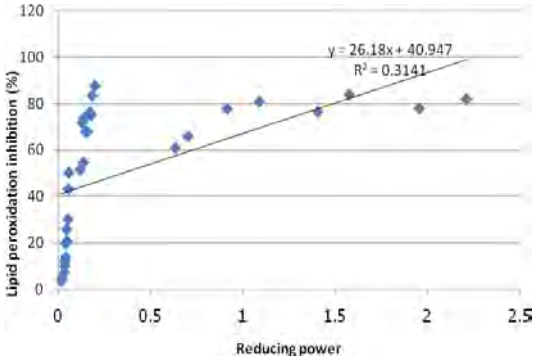
Table 1 Retention times, equations of standard curves (peak area *vs* concentration) and regression coefficients (R^2) of standard curve for phenolic acids

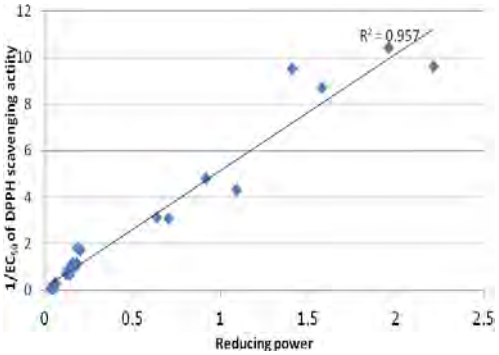
Phenolic acids	Retention times (min)	Equations of standard curve	R^2
gallic acid	9.3	Y = 5324X-9171.6	0.9999
protocatechuic acid	12.4	Y = 4116X-10466	0.9999
hydroxybenzoic acid	27.6	Y = 40809X-13116	0.9998
p-coumaric acid	46.5	Y = 142807X-51036	0.9999
ferulic acid	52.4	Y = 139213X-68893	0.9999
sinapic acid	53.7	Y = 114297X-76779	0.984

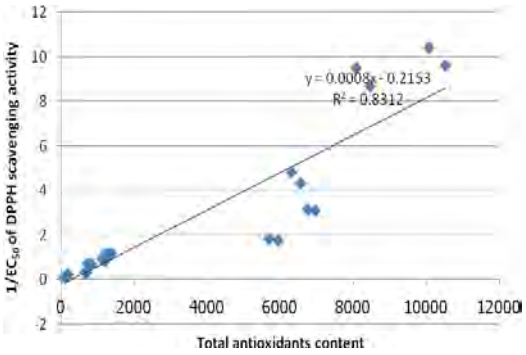
APPENDIX E

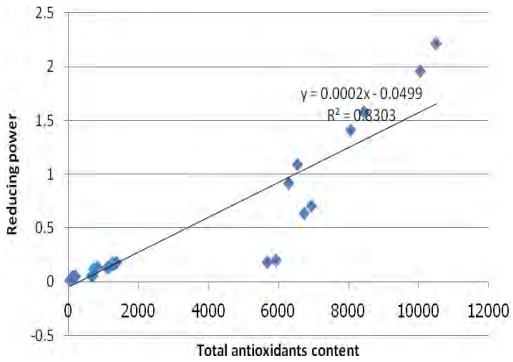
Correlation curves for antioxidant contents and antioxidant activities

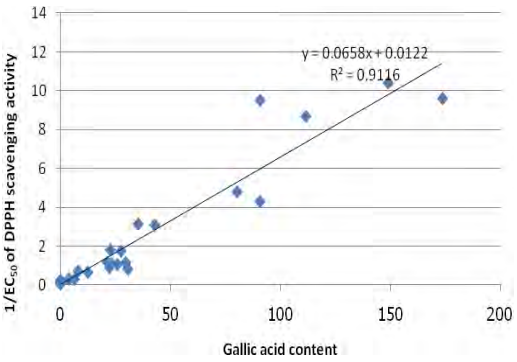


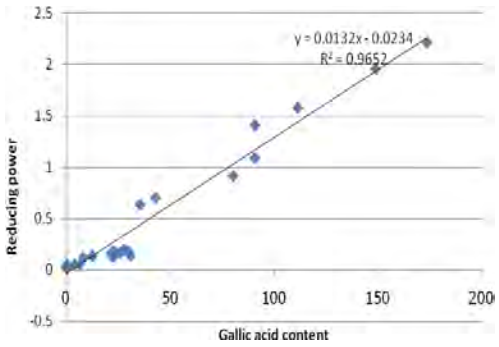


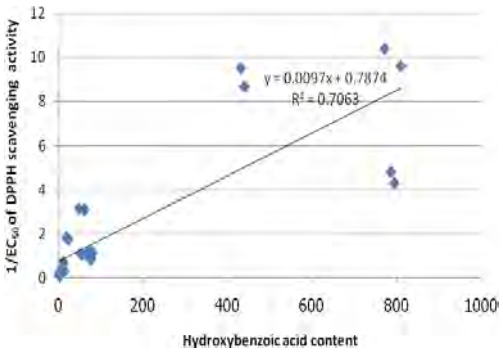


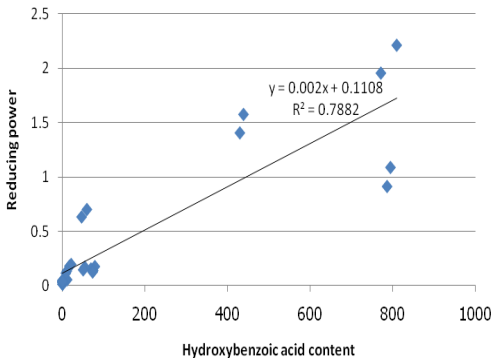


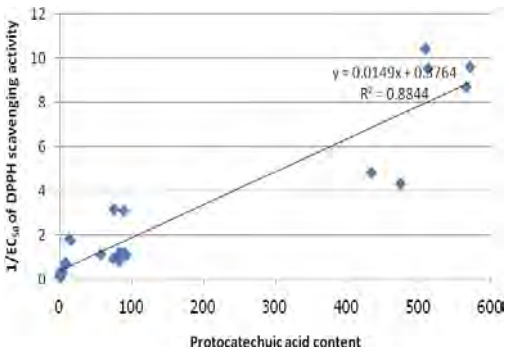


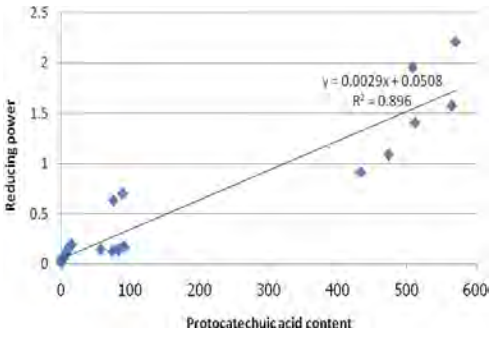


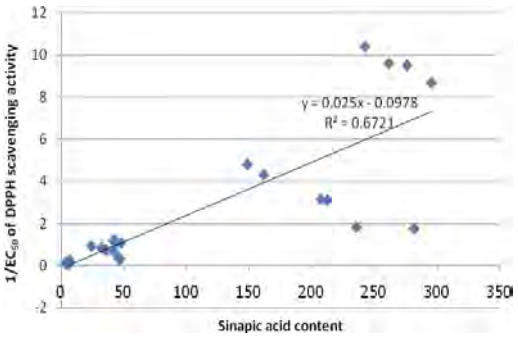

y = 0.0132x - 0.0234
R² = 0.9652
Reducing power
Gallic acid content

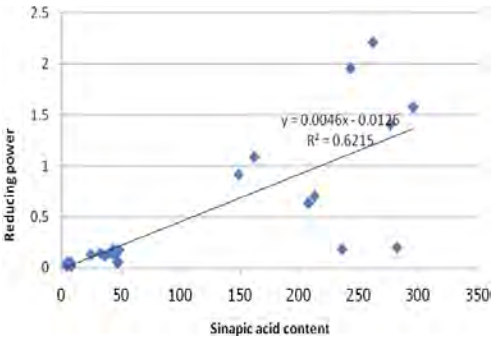

y = 0.0097x + 0.7874
R² = 0.7063
1/EC50 of DPPH scavenging activity
Hydroxybenzoic acid content

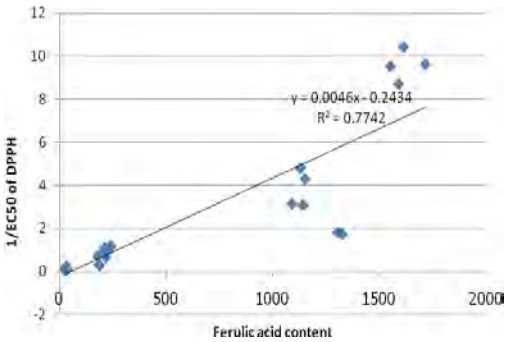

y = 0.002x + 0.1108
R² = 0.7882
Reducing power
Hydroxybenzoic acid content


y = 0.0149x + 0.9764
R² = 0.8844
1/EC50 of DPPH scavenging activity
Protocatechuic acid content


y = 0.0029x + 0.0508
R² = 0.896
Reducing power
Protocatechuic acid content


y = 0.025x - 0.0978
R² = 0.6721
1/EC50 of DPPH scavenging activity
Sinapic acid content


y = 0.0046x - 0.0126
R² = 0.6215
Reducing power
Sinapic acid content


y = 0.0046x - 0.2434
R² = 0.7742
1/EC50 of DPPH
Ferulic acid content

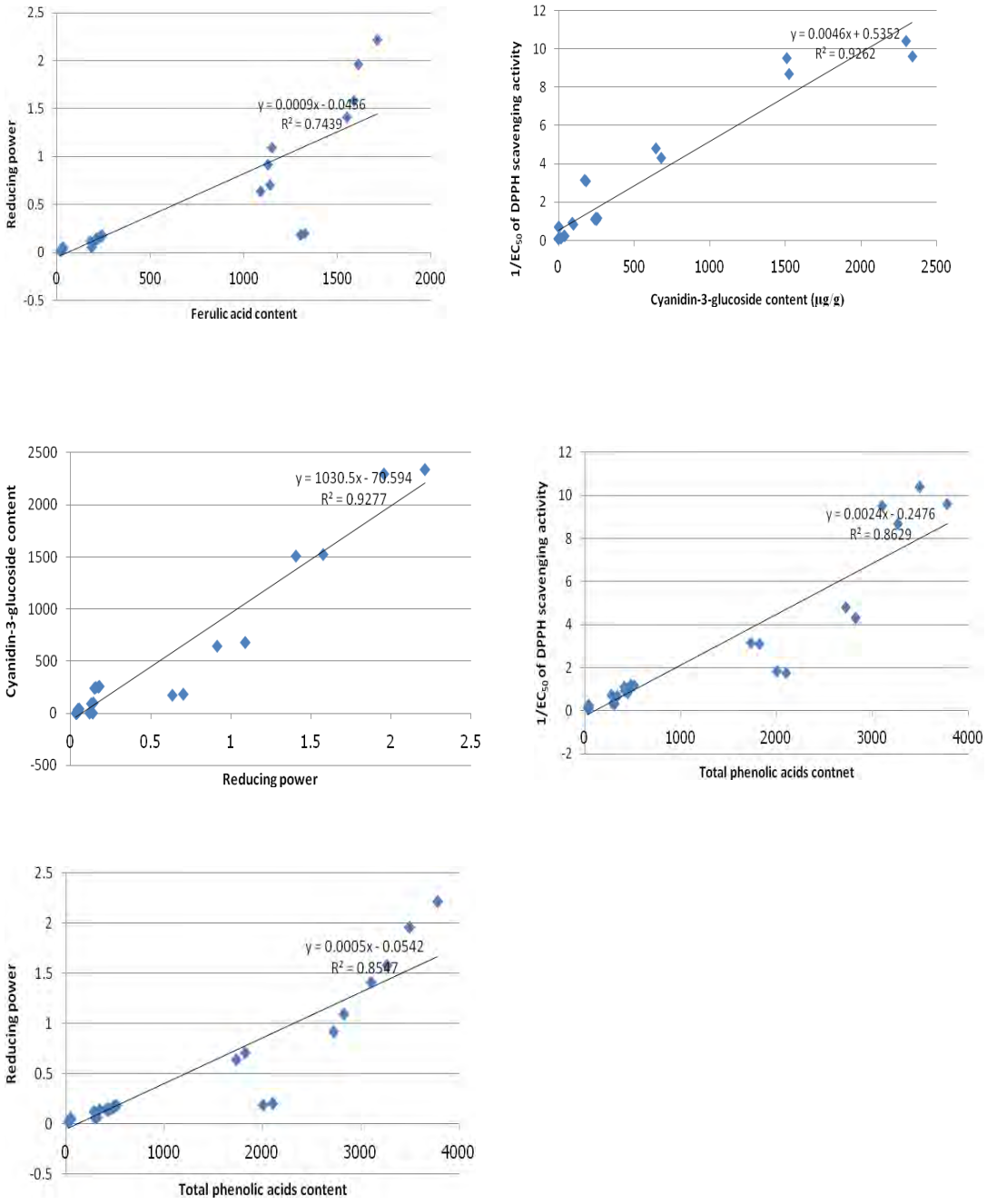


Figure 1. Correlation curves of antioxidant contents *vs* antioxidant activities

BIOGRAPHY

Mr. Thunnop Laokuldilok was born on August 30th, 1980 in Chiang Mai, Thailand. He obtained B.Sc. Degree in Product Development Technology from Chiang Mai University in 2001 and M.Sc. degree in Food Technology from Chulalongkorn University in 2005. His research was published in Journal of Food Biochemistry[1]. In 2006, he received a scholarship from The Royal Golden Jubilee Ph.D. Program under The Thailand Research Fund for further his Ph.D. at the Department of Food Technology, Faculty of Science, Chulalongkorn University. During 2007-2009, he was doing his research under supervision of Professor Dr. Charles F. Shoemaker at Food Science Department, University of California, Davis, California, USA.

[1]Tulyathan, V., Laokuldilok, T., and Jongkaewwattana, S. 2007. Retention of iodine in fortified parboiled rice and its pasting characteristics during storage. Journal of Food Biochemistry 31(2): 217-229.