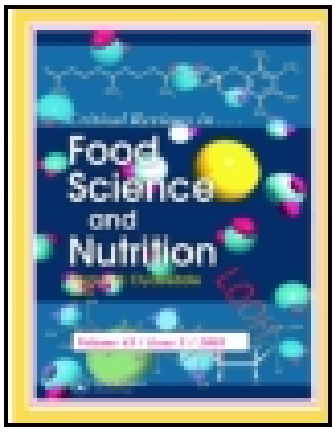

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Factors Influencing Antioxidant Compounds in Rice

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Factors Influencing Antioxidant Compounds in Rice

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Epidemiological and clinical studies suggest that the additive/synergistic effects of several bioactive compounds are responsible for the health benefits of rice. Among the leading contenders are phenolic acids, flavonoids, anthocyanins, proanthocyanidins, tocotrienols, tocopherols, ɤ-oryzanol, and phytic acid, which all possess strong antioxidant activities in vitro. In this review, data related to health effects of rice antioxidants using cultured cells, rodents and humans models are first summarized. The evidence is strong that consumption of rice tocotrienols translates into improved health outcomes. Current research, however, does not strongly support the health-promoting effects of rice tocopherols and phenolic acids. The crucial limitations in studies using rice flavonoids, anthocyanins, proanthocyanidins, ɤ-oryzanol and phytic acid appear to be the appropriateness of the substance tested (i.e., purity), and the scarcity of animal and human interventions. In a second part, rice antioxidants are reviewed with an emphasis on their composition and contents. Taking into account the bioavailability of these compounds, it is evident that a number of factors affect the antioxidant composition of rice, making it difficult to estimate dietary intake. Before harvest, factors including soil type, atmospheric CO_2, chemical inputs, temperature, and degree of ripening are important. After harvest, rice is subjected to processing methods that include drying, parboiling, storage,

irradiation, milling, stabilization, soaking, germination, fermentation, boiling, steaming, roasting, baking, and extrusion. Quantitative knowledge about the effects of these processes is summarized in this review. Surprisingly, a high level of agreement was found among study results, which could be useful in manipulating the growing and processing techniques of rice grains to facilitate efficient and safe consumption of antioxidant compounds.

Keywords phenolics, vitamin E, ɤ-oryzanol, phytic acid, anthocyanins, pre-harvest, processing

INTRODUCTION

Rice has been cultivated and consumed in Asia since the Neolithic Revolution, which occurred approximately 10,000 years ago; today, approximately 2.5 million Asians subsist on rice (Deng et al., 2013; Wu et al., 2013a). Until the 1980s, rice was considered a delicacy in most African countries and was eaten only on special occasions. During the past three decades, however, the demand for this grain has steadily increased. In Cameroon, for example, the per-capita consumption in 2006 was 23 kg rice equivalents compared to 2 kg in 1960 (Goufo, 2008). Of all the staple foods in South America, rice has become the most rapidly growing food source for millions of people (Sharif et al., 2013). In addition to Asia, Africa, and South America, rice consumption continues to rise in Australia, the United States, and in Mediterranean countries in the European Union as consumers shift from protein to more fiber- and antioxidant-based diets (Finocchiaro et al., 2007; Lazarou et al., 2012).

The rice grain at harvest is encased in a protective husk (Ha et al., 2006). The first step in commercial rice milling is to remove the husk and liberate the whole grain (brown rice), which consists of the endosperm (white rice) and the bran layer which include the germ (Liang et al., 2008b; Wang et al., 2011). Consumption of the whole grain and rice bran has been associated with reduced risk of several chronic diseases, including type-2 diabetes, cancers, and cardiovascular diseases. This is particularly true in South Asia where the incidence of breast, colon, and prostate cancers is considerably lower than that in Western countries (Qureshi et al., 2002; Sangkitikomol et al., 2010), and also in Mediterranean countries where the cardiovascular death rate is lower than that in Northern Europe and North America (Lazarou et al., 2012; Goufo

et al., 2014b). Over the last decade there has been an unresolved controversy on the rice components responsible for these health effects. Nevertheless, current knowledge links the health benefits of rice to several bioactive components including dietary fibers and antioxidant compounds. The phytochemical profile of rice has recently been studied extensively, and rice was found to contain significantly high amounts of antioxidant compounds, among which phenolic acids, flavonoids, anthocyanins, proanthocyanidins, tocopherols and tocotrienols (also called tocols or vitamin E), ɔ-oryzanol, and phytic acid (Deng et al., 2013; Goufo et al., 2014b). In the first part of this review, data related to health effects of rice antioxidants using cultured cells, rodents and humans models are first summarized.

Numerous factors can affect the antioxidant composition of the rice grain. Before harvest, the levels of antioxidants in rice are predominantly driven by genetic factors (Kiing et al., 2009; Ahn et al., 2010). Other pre-harvest factors that influence the synthesis of antioxidants in rice include environmental variables (e.g., temperature, location, light, precipitation, biotic stresses); cultural practices (e.g., irrigation, fertilization, harvesting) also affect antioxidant contents. After harvest, rice is generally dried, stored for some time, and milled. With consumersô growing interest in nutraceuticals and the expansion of health-food markets, several techniques have been applied to add value to the rice grain. These include germination, fermentation, parboiling, and stabilization. Most dietary intake studies, especially epidemiological investigations, require measurement of nutrient contents in foods as directly consumed (Russel, 2010). Until recently, rice was simply consumed after boiling in water. However, rice-based products, the production of which involves processing techniques such as extrusion, baking, and roasting, are becoming popular. All of these factors may have a significant effect on the contents of antioxidants

contained in rice. The second part of this paper summarizes the major changes (percent increase or decrease) in the composition of rice antioxidants associated with pre- and post-harvest factors. This information may be useful for calculating antioxidant contents in processed grains for any rice variety grown worldwide.

RICE ANTIOXIDANTS

The antioxidant composition of rice has recently been reviewed (Goufo et al., 2014b). At least 29 phenolic acids have been identified to date in rice, of which ferulic, *p*-coumaric, and sinapic acids predominate (Goufo et al., 2014b). There are notable differences in the levels of these compounds among cultivars of different colors. For example, in the whole grain, levels of ferulic acid range from 4.16 to 47.39 mg/100 g, while levels of *p*-coumaric acid range from 0.43 to 20.28 mg/100 g. At least 33 flavonoids have been identified in rice; the primary flavonoids are tricin and apigenin (Goufo et al., 2014b). Tricin levels in rice bran can be as high as 193 mg/100 g (Shalini et al., 2012). In the whole grain, apigenin content ranges from 0.20 to 2.85 mg/100 g (Goufo et al., 2014b). Anthocyanins are another class of flavonoids and exhibit maximum absorbance at 510 nm (Chen et al., 2006; Hiemori et al., 2009; Rattanachitthawat et al., 2010; Wu et al., 2013b). About 18 anthocyanins have been identified in rice, of which cyanidin-3-*O*-glucoside is the major component with levels ranging from 130 to 2640 mg/100 g in the bran of pigmented rice varieties. Next in dominance is peonidin-3-*O*-glucoside (11.4ï 534.1 mg/100 g), while cyanidin-3-*O*-rutinoside and cyanidin-3-*O*-galactoside are found in trace amounts (Goufo et al., 2014b). Anthocyanidins are intermediates in the synthesis of proanthocyanidins, which

consist mainly of flavon-3-ols units (catechin, epicatechin, and their 3-*O*-gallates and epigallates) (Finocchiaro et al., 2007; Hirawan et al., 2011; Kim et al., 2013). Catechin (1.57ï 3.16 mg/100 g) and epicatechin (0.57ï 6.14 mg/100 g) are particularly abundant in the husk of pigmented rice varieties (Goufo et al., 2014b). Of the eight tocols, ɔ-tocotrienol is generally the most abundant in rice whole grain (8.60ï 46.95 mg/kg), followed by Ŭ-tocopherol (2.40ï 43.81 mg/kg) (Goufo et al., 2014b). ɔ-Oryzanol is a mixture of at least 25 steryl ferulates; 24-methylenecycloartanyl *trans*-ferulate (40.47ï 259.05 mg/kg), cycloartenyl *trans*-ferulate (20.90ï 133.1 mg/kg), campesteryl *trans*-ferulate (20.00ï 76.04 mg/kg), and ɓ-sitosteryl *trans*-ferulate (8.60ï 85.00 mg/kg) are the main steryl ferulates found in rice whole grain. Other steryl ferulates are present at very low concentrations (usually < 2 mg/kg) (Goufo et al., 2014b). Phytic acid, also known as phytate phosphorus or myo-inositol-1.2.3.4.5.6-hexakisphosphate, is the most abundant form of phosphorus in the whole grain (1.07 g/100 g) and in the bran (4.94 g/100 g), representing 65ï 73% of the total phosphorus content (Frank et al., 2007; Depar et al., 2013; Goufo et al., 2014b).

HEALTH EFFECTS OF RICE ANTIOXIDANTS

From the literature, it is evident that rice antioxidants influence the cellular redox status of animal and human plasma, which could offer protection against chronic diseases associated with oxidative stress or could potentially reduce the burden of these diseases. Oxidative stress is defined as disequilibrium between oxidants and antioxidants, which arises from a failure of endogenous antioxidants (mainly superoxide dismutases, catalase, glutathione peroxidase, peroxiredoxins, and glutathione) to slow or stop the production of reactive oxygen species (ROS)

(Lazarou et al., 2012; Xiao and Kai, 2012; Chen et al., 2013; Fardet and Chardigny, 2013; Huang et al., 2013; Landete, 2013; Trigueros et al., 2013). Most ROS are free radicals derived from oxygen and include hydroxyl ($OH^{\bullet}$), superoxide ($O_2^{\bullet-}$), nitric oxide ($NO^{\bullet}$), peroxyl ($RO_2^{\bullet}$), peroxynitrite ($ONOO^-$), singlet oxygen ($^1O_2$), and hydroperoxide (H_2O_2). All of these are produced during normal aerobic respiration or in response to injury or irritation (González et al., 2011; Lazarou et al., 2012; Pinent et al., 2012; Zhu et al., 2012; Fardet and Chardigny, 2013; Huang et al., 2013; Trigueros et al., 2013). If the generation of free radicals exceeds the scavenging capacity of cells, the excess free radicals can react with biological macromolecules, such as proteins, lipids, and DNA, and produce oxidative damage. Oxidative damage plays an important role in the molecular mechanisms behind many diseases, such as cancer, atherosclerosis, and diabetes (Cordero et al., 2010; González et al., 2011; Lazarou et al., 2012; Pinent et al., 2012; Xiao and Kai, 2012; Chen et al., 2013; Fardet and Chardigny, 2013; Huang et al., 2013; Landete, 2013; Marrazzo et al., 2013; Trigueros et al., 2013; Weed, 2013).

Scientific knowledge about the role of various rice antioxidants in preventing and treating specific diseases has accumulated rapidly during the last decade. In earlier studies using pure commercial antioxidants found in rice, results usually failed to substantiate epidemiological claims or establish causality, probably because the food matrix affects the bioavailability of antioxidants (Juliano et al., 2005; Visioli et al., 2011; Berger et al., 2012; Lavecchia et al., 2013). Attention has recently shifted to using antioxidant-rich extracts from rice; results from experimental studies using those extracts on cultured cell lines, animals and humans are summarized in the following paragraph. Rice antioxidants may exert health-promoting effects in a variety of ways.

Rice Antioxidants May Directly Scavenge ROS, Acting as Chain-Breaking Antioxidants

The free hydroxyl group on the phenolic ring (phenolic acids, flavonoids, anthocyanins, proanthocyanidins, and ɔ-oryzanol) and on the chromanol ring (tocols) is responsible for the antioxidant property of rice phytochemicals (Deng et al., 2013; Landete, 2013). The hydrogen atom from the ring can be donated to ROS, reducing and neutralizing these free radicals. The phenolic compound or tocol that loses a hydrogen atom becomes a free radical that is immediately made non-reactive by resonance delocalization throughout the ring structure (Visioli et al., 2011; Xiao and Kai, 2012; Goufo et al., 2014b). These antioxidant activities may have implications in several oxidative stress-related diseases.

Chronic treatment with a ɔ-oryzanol-rich extract from rice (composition not disclosed) at test doses selected to reflect the daily ingestion in humans, significantly attenuated the elevated nitrite levels in the sciatic nerve tissues of streptozotocin-treated diabetic rats (Ghatak and Panchal, 2012). The ɔ-oryzanol-rich extracts from five purple rice varieties were also effective in inhibiting the combined lipopolysaccharide-IFN-Ŭ-mediated induction of nitric oxide production in vitro in RAW 264.7 macrophage cells. There was a strong correlation between the rice ɔ-oryzanol content of the extracts and the inhibitory effect. The anti-inflammatory effect of all extracts was stronger than that of commercial pure ɔ-oryzanol. However, the ɔ-oryzanol content of the extracts was quite low, only 5ï 9% (w/w) (Saenjum et al., 2012). Ghatak and Panchal (2012) recently demonstrated that a rice extract containing ɔ-oryzanol possesses potent nitric oxide scavenging activities in vitro and inhibits nitrite formation by directly competing with

oxygen in the reaction with nitric oxide. The ⍺-oryzanol in the study by Saenjum et al. (2012) may have used the same mechanism (i.e., scavenging peroxynitrite produced by the cells). Extracts from rice that are rich in tocotrienols (Siddiqui et al., 2010) and phenolic acids (Jung et al., 2005) are also reported to inhibit the increase in total nitric oxide levels in both serum and urine of C57BL/KsJ-*db/db* type 2 diabetic mice, compared with the control group. However, caution is recommended in interpreting these results because of the single dose used in the two studies (200 mg/kg of body weight/day), which largely exceeded what could be expected from dietary exposure. Huang et al. (2005) found that the isovitexin-rich extract from the rice husk (96% purity) could reduce the amount of hydroperoxide production that was induced by lipopolysaccharide in RAW 264.7 macrophage cells. It was also found that an anthocyanin-rich extract from rice (composition not provided) was able to inhibit DNA damage induced by hydrogen peroxide on human mononuclear leukocytes in vitro (Sangkitikomol et al., 2010). But it was not clear whether those reductions occurred by virtue of the antioxidant activity of these compounds or due to their inducible nitric oxide synthase (iNOS) suppression potential.

Another key mediator of glucose-induced oxidative injury, the superoxide anion, was also inhibited in vitro in RAW 264.7 macrophage cells by the ↄ-oryzanol-rich extract from rice (Saenjum et al., 2012). The study demonstrated that ⍺-oryzanol could scavenge superoxide anion radicals in vitro; this result, however, contradicted the findings of Juliano et al. (2005).

Rice Antioxidants May Bind Pro-oxidant Metals to Suppress Oxidative Reactions

Transition metal ions are important in the production of ROS (Lin et al., 2002; Huang et al., 2005; Canan et al., 2012).

The bathophenanthroline test was used in vitro to demonstrate that phytic acid forms a chelate with iron, making it catalytically inactive and resulting in the inhibition of iron-mediated hydroxyl radical production (Canan et al., 2012). Interestingly, the phytic acid-rich extract from rice (90% purity) showed three-fold higher activity compared with the commercial phytic acid (98% purity) (Canan et al., 2012). Although there is evidence that the antioxidant capacity of phytic acid depends on the chelation of metal ions, the mechanism for the interaction between rice phytic acid and minerals in vivo has not been reported.

Many phenolic compounds also participate in metal chelation through their *O*-dihydroxyphenyl groups. For example, excess hydrogen peroxide induced in vitro by cadmium in A2780 ovarian cells was significantly suppressed when an isovitexin-rich extract from rice (composition not disclosed) was added prior to cadmium treatment. Isovitexin was shown to form complexes with cadmium, thereby preventing heavy-metalï induced cell injury (Lin et al., 2002).

Rice Antioxidants May Protect Against Lipoprotein-Cholesterol Oxidation

Unless ROS are quenched by the mechanisms described above, oxidative damage leads to lipid and protein dysfunction. Low-density lipoproteins (LDL) are plasma proteins that carry cholesterol and triglycerides and are particularly susceptible to oxidation by free radicals (Qureshi et al., 2001). Lipoprotein disorders are among the most common metabolic disorders

encountered in clinical practice (Qureshi et al., 2002; Iqbal et al., 2003; Xiao and Kai, 2012; Fardet and Chardigny, 2013). LDL oxidation results in increased plasma levels of triglycerides and of total, free, and esterified cholesterol (Iqbal et al., 2003; Wilson et al., 2007; Fardet and Chardigny, 2013).

There is evidence that rice antioxidants play a role in reducing oxidized lipids in plasma and urine. Dietary supplementation with a single dose of an anthocyanin-rich extract from rice (composition not given) prevented the development of fructose-induced insulin resistance in rats (Guo et al., 2007). In particular, the treatment reduced the plasma triglyceride, total cholesterol, and LDL cholesterol concentrations to levels that were comparable with those observed in rats treated with the drug pioglitazone. After fructose-induced insulin resistance had been established, four weeks of treatment with the anthocyanin-rich extract ameliorated the glucose intolerance and hyperlipidemia, but the extract failed to reverse the fructose-induced hyperinsulinemia as pioglitazone did (Guo et al., 2007). An anthocyanin-rich extract from rice (43.2% purity) also improved the lipid profile in apolipoprotein-E-deficient rats (Xia et al., 2006) and in dyslipidemic rats (Yang et al., 2011). In the latter case, however, only the serum triglyceride level was lowered; there were no differences in the serum levels of total cholesterol, LDL cholesterol, or high-density lipoprotein (HDL) cholesterol between the treated and the control groups (Yang et al., 2011). In most studies, the authors speculated that anthocyanin compounds, which are potent free radical scavengers, could have reduced the extent of LDL oxidation in vivo when rats were preventively fed the anthocyanin rich-extract (Xia et al., 2006; Guo et al., 2007).

Oxidation of isolated LDL and cellular membranes (plasma, mitochondrial, and endomembrane systems) is commonly estimated by measuring the levels of thiobarbituric acid

reactive substance (TBARS) and oxidized malondialdehyde. Rice extracts that are rich in ɔ-oryzanol (Ghatak and Panchal, 2012), anthocyanins (Guo et al., 2007; Hou et al., 2013), tocotrienols (Siddiqui et al., 2010), phytic acid (Young et al., 2012), and cyanidin-3-*O*-glucoside (Um et al., 2013) all significantly reduced plasma and hepatic TBARS levels to a point near or lower than control levels in vivo using mice models. However, none of these studies has demonstrated a direct relationship between TBARS reduction and antioxidant activity of these phytochemicals. It has also been proposed that rice phenolic acids enhance the binding capacity of the LDL receptor to LDL (Jung et al., 2005; González et al., 2011; Xiao and Kai, 2012; Fardet and Chardigny, 2013). In the case of ɔ-oryzanol, in vivo LDL oxidation could be prevented through the ferulate moiety of the component (Juliano et al., 2005). However, all those hypothetical mechanisms remain to be demonstrated.

Rice Antioxidants May Inhibit the Activity of Enzymes Associated with Hyperlipoproteinemias

Endogenous cholesterol is synthesized in the liver and transported in the blood plasma by lipoproteins (Qureshi et al., 2002; Iqbal et al., 2003; Trigueros et al., 2013). Hyperlipoproteinemias (hypercholesterolemia and hyperlipidemia) are disorders associated with increased plasma concentrations of total cholesterol, LDL cholesterol, and triglyceride (Fardet and Chardigny, 2013; Chen et al., 2013; Trigueros et al., 2013).

β-Hydroxy-*β*-methylglutaryl coenzyme-A reductase (HMGCoA-R) is the rate-limiting enzyme in the mevalonate pathway of endogenous cholesterol synthesis (Chen et al., 2013; Fardet and Chardigny, 2013). The lipid-lowering property of the tocotrienol-rich extract from

rice (10 mg/kg body weight/day; composition not given) that was fed to rats with hypercholesterolemia (carcinogen 7,12-dimethylbenz[Ŭ]anthracene-induced) was demonstrated not to be related to its antioxidant activity. Instead, it was related to the reduction in the enzymatic activity and protein mass of HMGCoA-R (Iqbal et al., 2003). In mice that were deficient in apolipoprotein-E (2/2), the decreases in atherosclerotic lesions, serum triglycerides, total cholesterol, and LDL cholesterol (HDL cholesterol was not affected) was due to the ability of tocotrienols to increase the controlled degradation of reductase protein and decrease the efficiency of translation of HMGCoA-R mRNA (Qureshi et al., 2001). The tocotrienol-rich extract used for the study contained 88.2% tocotrienols, 9.9% tocopherols, and 1.9% unidentified compounds (Qureshi et al., 2001). The same effects were observed in hypercholesterolemic human subjects (males and females) who consumed the tocotrienol-rich extract for five weeks, except that the levels of HDL cholesterol and apolipoprotein A1 increased compared with the baseline values (Qureshi et al., 2002). Tocotrienols differ from tocopherols in having three double bonds in the isoprene side chain. This lack of saturation in the side chain has been demonstrated to be essential for inhibition of HMGCoA-R (Iqbal et al., 2003; Cordero et al., 2010).

Induced liver damage is associated with higher levels of the hepatic lipogenic enzymes glucose-6-phosphate dehydrogenase (G6PDH) and malic enzyme (ME). G6PDH and ME are involved in supplying NADPH for the synthesis of hepatic triglycerides. Um et al. (2013) reported that hypolipidemic effects of the cyanidin-3-*O*-glucoside-rich extract from rice (150 mg/kg body weight/day; 19% purity) in rats with hyperlipidemia induced by a high-fat/cholesterol diet were partly mediated through the inhibition of the activities of G6PDH and

ME. The activities of these enzymes and fatty acid synthase were also shown to be regulated by a phytic acid-rich extract from rice (single dose and composition not given) in high-fat diet-C57BN/6N mice (Young et al., 2012). In the latter case, the lipid-lowering property of the extract was also associated with increased fecal lipid excretion (Young et al., 2012).

Rice Antioxidants May Prevent Cholesterol Absorption in the Gut and Increase Fecal Cholesterol Excretion

A ɔ-oryzanol-rich extract from rice improved the lipoprotein pattern in mildly hypercholesterolemic men (38ï 64 years old) who consumed it in vehicles for four weeks. Plasma total cholesterol and LDL cholesterol decreased after two weeks of consumption. However, plasma HDL cholesterol and apolipoprotein A1 and cholesterol biosynthesis remained unchanged (Berger et al., 2005). The blood cholesterol-lowering activity of ə-oryzanol was demonstrated to occur predominantly through prevention of cholesterol absorption in the gut (Berger et al., 2005). Although the mechanism by which ɔ-oryzanol suppress cholesterol absorption is not clearly understood, it may enhance the growth of beneficial gut bacteria such as *Bifidobacterium* and *Lactobacillus* (Vitaglione et al., 2008). ɔ-Oryzanol may also have an action similar to that of dietary fibers. Soluble fibers attract water and slow digestion, thereby lowering absorption of dietary cholesterol (Gualberto et al., 1997; Vitaglione et al., 2008). It is also possible that ɔ-oryzanol is metabolized into free ferulate and free sterol via intestinal cholesterol esterase in the gastrointestinal tract. Free ferulate might be absorbed and act as an antioxidant in plasma, while free sterol inhibits cholesterol absorption in the gastrointestinal tract, thereby

lowering blood cholesterol levels (Berger et al., 2005; Wilson et al., 2007). However, it is unlikely that ɔ-oryzanol alone was responsible for the observed effect, as the high percentage of unsaturated fatty acids in the ɔ-oryzanol-rich extract could have lowered LDL independently of rice sterols (Chen et al., 2013; Fardet and Chardigny, 2013).

Wilson et al. (2007) showed that in hamsters fed a chow-based hypercholesterolemic diet, ɔ-oryzanol has a greater effect on lowering plasma LDL cholesterol and raising plasma HDL cholesterol levels than does ferulic acid. Its advantage lies in increased fecal excretion of cholesterol and its metabolites (Wilson et al., 2007). However, the study needs to be evaluated with precaution, due to the limited information on the composition of the ɔ-oryzanol-rich extract. A phytic acid-rich extract from rice also improved the plasma lipid patterns in high-fat-hyperlipidemia-induced C57BN/6N mice by increasing the fecal excretion of coprostenol and cholesterol (Young et al., 2012). The enzymatic activity of hepatic cholesterol-7Ŭ-hydroxylase, the rate-limiting enzyme in the biosynthesis of bile acids, may be elevated in the presence of these antioxidant compounds (Chen et al., 2013; Fardet and Chardigny, 2013), which could help stimulate the conversion of cholesterol to bile acids, resulting in the elimination of cholesterol from the body.

Rice Antioxidants May Down-Regulate the Key Inflammatory Transcription Factor Nuclear Kappa B (NF-κB)

Inflammation is part of the body's immune response and thus is beneficial. However, if oxidative damage is uncontrolled, inflammation can become self-perpetuating (chronic) and lead

to tissue damage (González et al., 2011). During inflammation, immune cells such as macrophages, neutrophils, monocytes, lymphocytes (T cells and B cells), natural killer cells, mast cells, and dendritic cells are activated (Huang et al., 2005; Xia et al., 2006; Min et al., 2010; Yang et al., 2011; Shalini et al., 2012); these immune cells release excess levels of ROS that function, in part, to kill invading pathogens (Min et al., 2010; González et al., 2011). Intracellular ROS production is associated with several cellular events controlled by NF-əB, including activation of NAD(P)H oxidase, matrix metalloproteinases (e.g., MMP-9 and MMP-2), xanthine oxidase, lipooxygenases (LOX), nitric oxide synthase (iNOS), and cyclooxygenase-2 (COX-2) (Chen et al., 2006; Min et al., 2010; Oka et al., 2010; Kim et al., 2012; Shalini et al., 2012; Norazalina et al., 2013). In addition, immune cells release various pro-inflammatory mediators such as cytokines (e.g., tumor necrosis factors [TNF-Ŭ], interleukin [IL-6, IL-1ɓ]), chemokines (e.g., monocyte chemoattractant protein-1 [MCP-1]), C-type lectin receptors (e.g., P-selectin, collectin), cell adhesion molecules (CAMs e.g., integrins, Ig gene superfamily), prostaglandins (e.g., PGE_2), leukotrienes (e.g., LTB_4), and nitric oxide (Chen et al., 2006; Kim et al., 2012; Shalini et al., 2012; Norazalina et al., 2013). Prostaglandins and leukotrienes are generated from arachidonic acid by the activity of COX-2 and arachidonate-5-LOX, respectively (Huang et al., 2005; Kim et al., 2012).

The anti-inflammation effects of rice antioxidants have been attributed to their suppression of the activation of NF-əB. An oral administration of a cyanidin-3-*O*-glucoside-rich extract from rice (composition not given) significantly inhibited the number of leukocytes and the levels of TNF-Ŭ, IL-1ɓ, and PGE_2 in the exudates of the air pouches in carrageenan-treated mice, as well as inhibiting the gene expression of COX-2 and iNOS. The compound also inhibited the

phosphorylation and degradation of IəB-Ǔ, the nuclear translocation of NF-əB (Min et al., 2010). Treatment with a tricin-rich extract from rice (99% purity) in vitro also resulted in down-regulation of lipopolysaccharide-elicited production of TNF-Ǔ, IL-6, PGE_2, and nitric oxide in human peripheral blood mononuclear cells (hPBMCs). Tricin was found to be a potential blocker of both the mRNA and protein-level expression of isoforms of iNOS, COX-2, MMP-9, and MMP-2 and an inhibitor of NF-əB activation (Shalini et al., 2012). When macrophage RAW 264.7 cells were stimulated with lipopolysaccharide in the presence of an isovitexin-rich extract from the rice husk (96% purity) in vitro, a concentration-dependent inhibition of COX-2 expression was observed (Huang et al., 2005), suggesting that isovitexin could inhibit NF-κB activation.

ə-oryzanol has been widely used in cosmetics to prevent and treat skin allergic inflammation (Juliano et al., 2005; Oka et al., 2010). When a ə-oryzanol-rich extract from rice (composition not given) was injected intradermally with anti-DNP IgE into the dorsal skin of rats, the passive cutaneous anaphylaxis reaction induced by DNP-human serum albumin was attenuated. It was demonstrated in RBL-2H3 mast cells in vitro that ə-oryzanol exerts its anti-allergic effect by attenuating mast cell degranulation through the capture of IgE, which usually binds to and activates mast cells (Oka et al., 2010).

Rice Antioxidants May Stimulate the Endogenous Antioxidant Defense System during the Earliest Phases of Inflammation

Implication of oxidative stress in the pathogenesis of several diseases is suggested by the altered expression of antioxidant enzymes such as superoxide dismutases, catalase, and glutathione peroxidase (González et al., 2011; Berger et al., 2012; Zhu et al., 2012; Landete, 2013; Marrazzo et al., 2013). As the substrate of glutathione peroxidase, glutathione is considered as a first line of defense against free radicals in mammals, directly reacting with reactive electrophiles to eliminate oxygen species (González et al., 2011).

The major anthocyanin in rice, cyanidin-3-*O*-glucoside (100 mg/kg body weight/day; purity not given), increased the novo biosynthesis of glutathione in hepathocytes of hyperglycemia-mediated oxidative liver injury diabetic db/db mice. The mechanism was protein kinase Aï cAMP response element binding protein-dependent induction of glutamateï cysteine ligase catalytic subunit expression (Zhu et al., 2012). The same finding was reported by Hou et al. (2013), using an anthocyanin rich-extract from rice (41.9% purity). In addition to glutathione, the activities of superoxide dismutase and glutathione peroxidase were restored or maintained in carbon tetrachloride-damaged liver mice (Hou et al., 2013). The increased glutathione level may also be attributed to the reduced depletion of glutathione, due to the direct free radical scavenging activity of antioxidant compounds, although there is no direct evidence for this. The reduction in the levels of intracellular glutathione in streptozotocin-induced diabetic rats was also limited in the sciatic nerves by treatment with a ɔ-oryzanol-rich extract from rice. However, no significant increase in the levels of superoxide dismutase and catalase was observed in treated vs. control diabetic rats (Ghatak and Panchal, 2012).

Although most cytokines possess pro-inflammatory properties, some (e.g., IL-4, IL-13 and adiponectin) are classified as anti-inflammatory (Ohara et al., 2009; González et al., 2011).

Plasma adiponectin levels are decreased in the obese and insulin-resistant states in both rodents and humans. Ohara et al. (2009) found that serum adiponectin concentration increased in vivo in c57BL/6J mice and ex vivo in 3T3-L1 adipocytes supplemented with a ɔ-oryzanol-rich extract from rice (composition not provided) under NF-kB activated conditions, suggesting that enhancement of adiponectin secretion by ɔ-oryzanol did not occur by inhibiting the NF-kB activation pathway, but by suppressing the activated NF-kB.

Rice Antioxidants May Modulate Signal Transduction Pathways and Gene Expression of Enzymes Involved in the Metastatic Cascade of Cancer Cells

If sufficient amounts of superoxide dismutase, catalase, and glutathione are not available to decompose ROS, ROS can react with and damage DNA, a process associated with increased risk of cancer (Chen et al., 2006; Kong et al., 2009; Hou et al., 2013; Weed, 2013). Cancer occurs when there is an imbalance between cell multiplication and programmed cell death (apoptosis) leading to uncontrolled, rapid proliferation of cells within a tissue (Weed, 2013). Degradation and penetration of the cell extracellular matrix by tumor cells under the action of matrix metalloproteinases (MMPs) and urokinase-type plasminogen activator (u-PA) are key steps in the metastatic cascade of cancer cells (Chen et al., 2006). Transcription of MMPs or u-PA genes is regulated by upstream sequences that include motifs corresponding to NF-əB and plasminogen activator 1 (AP-1) binding sites.

In vivo, the cyanidin-3-*O*-glucoside-rich extract from rice (98% purity) markedly inhibited the invasion and motility of SKHep-1 cells from human hepatocellular carcinoma injected

subcutaneously in immunodeficient ALB/c *nu*/*nu* mice. This anti-metastatic effect was mediated by reduced expression of MMP-9 and u-PA and was linked not to NF-əB, but to AP-1 activities (Chen et al., 2006). However, the study involved the intake of only one portion of food containing the tested polyphenol (100 mg/kg of body weight/day). Pre-treatment with the tricin-rich extract from rice in vitro inhibited the lipopolysaccharide-induced activation of MMP-2 and MMP-9 in human peripheral blood mononuclear cells. Tricin was found to exert its effect via inhibition of COX-2 activity, with little effect on COX-2 protein expression (Shalini et al., 2012). In contrast, apigenin, the flavonoid analogue of tricin, has been shown to down-regulate COX-2 expression instead (Shalini et al., 2012). The ə-oryzanol-rich extract from rice (composition not provided) was also reported to reduce tumor size in BALB/c mice that had CT-26 colon cancer cells transplants on their backs (Kim et al., 2012). Tumor inhibition was associated with the following biomarkers: induction of cytolytic activity of splenic natural killer cells; partial restoration of nitric oxide production and phagocytosis in peritoneal macrophages; inhibition of new blood-vessel formation (angiogenesis) in tumor tissues, causing hypoxia-induced tumor cell death; increase in the release of the pro-inflammatory cytokines TNF-Ŭ, IL-1ƀ, and IL-6 from macrophages; inhibition of the pro-angiogenic vascular endothelial growth factor; and down-regulation of COX-2 and 5-LOX (Kim et al., 2012). A phytic acid-rich extract from rice (composition not given) was also found to slow the progression of colorectal cancer in azoxymethane-induced rats by inhibiting COX-2 (Norazalina et al., 2013).

Rice cycloartenyl *trans*-ferulate (purity not given), the major component of ɔ-oryzanol, also inhibited the growth of human colorectal adenocarcinoma SW480 cells in vitro and caused regression of SW480 xenografts in vivo in mice that had received transplants of SW480 solid

tumor (Kong et al., 2009). Cycloartenyl *trans*-ferulate triggered both the death receptor and the mitochondrial apoptosis pathways, and the mechanisms involved enhanced activation of caspase-8 and caspase-3 (Kong et al., 2009). A tocotrienol-rich extract from rice (53% tocotrienols, 30.5% tocopherols, and 16.5% unidentified compounds) was also found to elevate the expression of death receptors and to induce caspase-3-dependent apoptosis in human malignant mesothelioma MM H28 cells (Nakashima et al., 2010). A phytic acid-rich extract from rice (composition not given) had the same effect on the human colorectal cancer cell line HT-29 (Nurul-Husna et al., 2010). Compared with commercial rice phytic acid, the phytic acid-rich extract exhibited higher sensitivity toward the cell line. The phytic acid-rich extract also suppressed in vivo the activity of ß-catenin, which is a key regulator of the cadherin-mediated cellï cell adhesion system (Norazalina et al., 2013).

Alkaline phosphatase (ALP) and glutathione-*S*-transferase (GST) are used as markers to monitor the severity of carcinogenesis. The anti-tumor effect of a tocotrienol-rich extract from rice was investigated in rats treated with the chemical carcinogen 7,12-dimethylbenz [Ů]anthracene. The reduction in the severity and extent of neoplastic transformation in the mammary glands was highly correlated with decreased ALP and GST activities (Iqbal et al., 2003).

The major DNA modification appears to be the oxidation of guanine to 8-oxy-2Nj deoxyguanosine. The hepatic and urinary levels of 8-oxy-2Nj deoxyguanosine were significantly lowered in carbon tetrachloride-intoxicated mice by the anthocyanin-rich extract from rice, to levels even lower than those of the control group (Hou et al., 2013). However, only a small portion (41.9%) of the extract was anthocyanin. The extract also contained other unidentified

phytochemicals (37.9%), carbohydrates (5.5%), proteins (5.2%), and lipids (3.9%) (Hou et al., 2013). Therefore, it is difficult to attribute the hepatoprotective effect of the extract to its anthocyanin components alone.

Rice Antioxidants May Prevent the Formation of Atherosclerotic Foam Cells and Reduce Platelet Hyperactivity

If inflammation is not restricted, activated macrophages can form complexes with oxidized LDL-cholesterol and produce foam cells, which is the initial event in the formation of atherosclerotic fatty streaks and their progression into atherosclerotic plaques (Qureshi et al., 2002; Xia et al., 2006; Cordero et al., 2010; Yang et al., 2011). Fatty streaks may also contain thrombocytes (aggregated platelets) (Xia et al., 2006; Yang et al., 2011). Most LDL-cholesterol oxidation occurs in the extracellular subendothelial space of arteries; thus, foam cells deposit primarily within arterial walls, causing reduced diameter and decreased blood flow and oxygen supply (Huang et al., 2013). Atherosclerosis is the inflammatory process that leads to the onset of cardiovascular diseases, including peripheral artery disease (obliterative arteriopathy), high blood pressure (hypertension), myocardial infarction (heart attack), cerebrovascular disease (stroke), heart rhythm problems (arrhythmias), and congestive cardiac failure (heart failure) (Xia et al., 2006; Cordero et al., 2010; Siddiqui et al., 2010; Wu et al., 2013a). The cardiovascular risk is dependent on the interactions between foam cells, platelet hyperactivity, and blood coagulation (Cordero et al., 2010; Huang et al., 2013).

Several authors have proposed that the atheroprotective effect of rice antioxidants is due to their inhibition of the generation of oxidized LDL and their promotion of cholesterol efflux from macrophages; effects which ultimately reduce the formation of foam cells (Qureshi et al., 2002; Iqbal et al., 2003; Wilson et al., 2007; Rattanachitthawat et al., 2010; Siddiqui et al., 2010; Young et al., 2012). However, these mechanisms have yet to be proved.

Platelet activation involves a complex signaling cascade that includes many proteins (e.g., thromboxane A2, prostacyclin, calmodulin, P-selectin, and platelet factor 4). Hyperactive platelets rarely cause severe luminal narrowing, but they are easily ruptured, eroded, or calcified, and are prone to clotting (thrombosis formation) (Cordero et al., 2010; Huang et al., 2013). Vulnerable platelets are generally composed of an atrophic fibrous cap of collagen I, proteoglycan, and smooth muscle cells; the thinner the fibrous cap, the greater the risks of platelet rupture (Qureshi et al., 2001; Xia et al., 2006). The anthocyanins-rich extract from rice reduced platelet hyperactivity, hypertriglyceridemia, and body weight in dyslipidemic rats by directly decreasing the levels of thromboxane A2 and the thrombogenic ratio of thromboxane A2 and prostacyclin (Yang et al., 2011). The extract was composed of 43.2% anthocyanins, 21.6% polysaccharides, 16.6% flavonoids, 8.2% unidentified components, and 4.9% proteins (Yang et al., 2011). The chronic diet intake of the anthocyanin-rich extract also inhibited atherosclerotic plaque progression and enhanced plaque stabilization in apolipoprotein-E-deficient mice by increasing the collagen I area and reducing the content of MMP-1 in the lesions of the brachiocephalic arteries. Furthermore, mRNA levels of tissue factor 4 and iNOS in the aortas were highly decreased (Xia et al., 2006). Anthocyanins possess highly antioxidant activities in vitro. Nevertheless, there was no difference in serum antioxidant activity measured by the 2,2-

azinobis(3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging activity (ABTS) assay, among rats treated with anthocyanin-rich extract and control rats (Xia et al., 2006). The daily intake of anthocyanins in human diet has been estimated to be as much as 180ï 250 mg/day in regions where vegetables and fruits, which contain a high amount of these compounds, are widely consumed (Um et al., 2013). It should be pointed out that the latter studies (Xia et al., 2006; Yang et al., 2011) used anthocyanins doses (300ï 500 mg/kg body weight/day) much higher than those generally attainable through rice diet use. The tocotrienol-rich extract from rice also facilitated the maintenance of optimal platelet function in C57BL/6 apolipoprotein-E-deficient (2/2) mice by decreasing the secretion of the tissue factor (Qureshi et al., 2001).

Rice Antioxidants May Regulate the Activity of Hepatic Glucose-Regulating Enzymes and Improve Glucose Intolerance

Blood flow decreases as foam cells are deposited in arteries, triggering an increase in blood glucose levels. Conversely, high blood glucose concentrations can alter the metabolism of arterial-wall cells and result in the formation of foam cells (Guo et al., 2007; Cordero et al., 2010; Pinent et al., 2012; Huang et al., 2013). Blood glucose levels also rise when insufficient insulin is produced by the pancreas (Guo et al., 2007; Pinent et al., 2012). It has been demonstrated using cellular models that ROS have a causal role in insulin resistance (Guo et al., 2007). Insulin regulates glucose homeostasis by suppressing hepatic glucose production and stimulating peripheral glucose uptake by cells (Guo et al., 2007; Pinent et al., 2012). High rates of glucose production (hyperglycemia) and low net synthesis of glycogen (the storage form of glucose)

underlie the symptoms of type-2 diabetes mellitus. Diabetic complications include cataract formation, retinal damage, kidney failure, and nerve damage (Lazarou et al., 2012; Pinent et al., 2012; Zhu et al., 2012; Marrazzo et al., 2013). Glucokinase (hexokinase IV) is considered to be an important regulator of blood glucose levels and is expressed predominantly in the liver and pancreatic ß-cells (Jung et al., 2007; Kim et al., 2010). When blood glucose concentrations exceed the threshold level, glucokinase phosphorylates glucose to glucose-6-phosphate for glycogen synthesis. Phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6pase) on the other hand regulate gluconeogenesis and glucose output in the liver.

Oral administration of a phenolic acid-rich extract from rice (41.65% purity, rich in ferulic and protocatechuic acids) to C57BL/KsJ-*db/db* type 2 diabetic mice improved glucose regulation (decreased blood glucose and increased plasma insulin levels) mainly by restoring hepatic glucokinase and glycogen storage activity. When a commercial ferulic acid extract was used, however, the glucokinase activity remained unchanged, while the glycogen storage activity was restored (Jung et al., 2007). A phytic acid-rich extract from rice (composition not provided) administered as a single dose, also reduced the risk of high-fat-dietï induced hyperglycemia in C57BL/6N mice by elevating hepatic glucokinase activity and reducing PEPCK and G6pase activities. However, no significant difference in the insulin levels among the groups of mice was observed after seven weeks of treatment (Kim et al., 2010).

Blood-glucose-lowering properties of the tocotrienol-rich extract from rice have also been demonstrated in streptozotocin-induced type 1 diabetic rats by Siddiqui et al. (2010). Tocotrienols were found to act as modulators of peroxisome proliferator-activated receptors (PPAR), which are transcriptional factors that regulate the expression of genes involved in

carbohydrate and lipid metabolism (Ohara et al., 2009). Binding of tocotrienols to PPAR enhanced the expression of glucose transporter 4, which in turn promoted insulin-mediated glucose uptake (Siddiqui et al., 2010). The purity of the tocotrienol-rich extract as determined by high-performance liquid chromatography was 29.2% tocotrienols, 39.2% tocopherols, and 31.6% unidentified components. However, considering that this extract has a higher percentage of tocopherols than of tocotrienols, it may not qualify as tocotrienol-rich.

Rice ɔ-oryzanol have been reported to ameliorate nerve function in streptozotocin-induced peripheral diabetic neuropathy model rats. Among the many effects observed (including amelioration of hyperglycemia-induced hyperalgesia, reduction of formalin-induced nociception, and attenuation of oxidative stress), the activity level of Na^+/K^+-ATPase, a biochemical marker associated with the development of diabetic neuropathy, was significantly elevated in the sciatic nerve after eight weeks of treatment (Ghatak and Panchal, 2012).

LESSONS LEARNED FROM IN VITRO AND IN VIVO STUDIES

Taken together, these results show that rice antioxidants exert health effects that are not directly attributable to their antioxidant activities. For example, although anthocyanin-rich extracts from rice increased the plasma total antioxidant capacity, the effect was small and it was nonexistent a few hours after consumption (Xia et al., 2006). The values of lipid parameters on the other hand, tend to be reduced by most antioxidant-rich extracts from rice (Qureshi et al., 2002; Berger et al., 2005), as are biomarkers of systemic inflammation (Min et al., 2010; Shalini et al., 2012). Although many mechanisms are postulated from the different studies, the precise

mechanism in most cases remains unknown. Tocotrienols are well known for their role in inhibiting the activity of the hepatic enzyme HMGCoA-R (Qureshi et al., 2002), whereas tricin exerts its effect via inhibition of COX-2 activity (Shalini et al., 2012). For many other antioxidants, the underlying molecular mechanism remained unclear. Overall, it appears that these mechanisms are additive in producing the desired health effects. In addition, a possibility remains that rice antioxidants trigger various cell responses owing to their redox properties. Therefore, further studies are needed in order to develop a better understanding of whether and how these compounds exert protective effects on metabolic health.

An important finding is that in most cases, antioxidants extracted from rice promote health more effectively than do pure commercial antioxidants (Qureshi et al., 2001; Jung et al., 2007; Nurul-Husna et al., 2010; Canan et al., 2012; Saenjum et al., 2012), although Norazalina et al. (2013) found similar effects with phytic acid. That finding is biologically significant and supports the notion that the food matrix significantly affects the activity of antioxidant compounds; furthermore, it shows that the results from one cereal species cannot be simply extrapolated to other cereals. Although these studies show that rice antioxidants have a promising therapeutic potential compared with commercial antioxidants, the former have a serious limitation: In most studies, the antioxidant-rich extract contains less than 50% of the compound of interest. Very disturbingly, 18 of 32 papers assessed did not provide the composition of their antioxidant-rich extracts or referred to publications which did not.

For example, in studies that used the anthocyanin-rich extract from rice, the total anthocyanin composition was 19% (Um et al., 2013), 41.9% (Hou et al., 2013), and 43.2% (Xia et al., 2006; Yang et al., 2011). Although those studies extrapolated benefits achieved using the

extract to benefits that might be achieved using the antioxidant itself, it cannot be ruled out that other bioactive compounds in the extract contributed partially or equally to the effects observed. Thus, it is impossible to state with any certainty which component or combination of components contributed more. Dose is also a concerned in studies using anthocyanin-rich extracts from rice. Contrary to studies using rice tocotrienols and ɔ-oryzanol which observed effects at both low and high doses of the tested antioxidant, in vivo studies using rice anthocyanins (six of eight studies; Min et al., 2010 and Hou et al., 2013 are the exceptions) and phytic acid (two of three studies, Norazalina et al., 2013 is the exception) did not include a doseï response relationship in their design. In most of these studies, the single-dose of antioxidant tested greatly exceeded that that might be received through dietary exposure (Chen et al., 2006; Xia et al., 2006; Guo et al., 2007; Kim et al., 2010; Yang et al., 2011; Zhu et al., 2011; Young et al., 2012; Um et al., 2013).

In contrast with flavonoids, the correlation between the increased antioxidant activity in vitro and a protective effect has not been positively established for rice phenolic acids using cells lines, or animal models (Jung et al., 2005; Wilson et al., 2007; Ohara et al., 2009; Kim et al., 2012). However, because of the possibility of synergism between these compounds, it is inadequate to draw an affirmative conclusion about the inefficacy of rice phenolic acids. The flavonoids tricin and isovitexin found in high amounts in rice are generally purported to have anticancer properties, and as a result, the health effects of whole grain rice consumption have been widely publicized. However, only a handful of in vitro studies using cell lines have been published on the issue (Lin et al., 2002; Huang et al., 2005; Shalini et al., 2012). The use of cell lines understandably has many limitations (Tirzitis and Bartosz, 2010; Pinent et al., 2012; Fardet

and Chardigny, 2013) and may ultimately make it difficult to support claims related to tricin and isovitexin.

The health effects of rice phytic acid have been established using cell lines (Nurul-Husna et al., 2010; Canan et al., 2012) and rats (Kim et al., 2010; Young et al., 2012; Norazalina et al., 2013). Interestingly, phytic acid extracts of high purity (Ó 98%) are easily obtained with rice. However, the relevance of these findings for humans is not yet clear. Correlations between data from animals and humans with respect to efficacy and safety appear imperfect with regard to phytic acid, as the compound decreases absorption of micronutrients in the gastrointestinal tract, which is a major disadvantage. Thus, further research is required to assess the relative benefits and risks of phytic acid ingestion.

Studies of ɔ-oryzanol have reported a number of therapeutically useful biological activities in cell lines (Kong et al., 2009; Saenjum et al., 2012), adipocytes (Ohara et al., 2009), rodents (Wilson et al., 2007; Kong et al., 2009; Ohara et al., 2009; Oka et al., 2010; Ghatak and Panchal, 2012; Kim et al., 2012), and humans (Berger et al., 2005). However, in most studies, the ɔ-oryzanol rich extract contained less than 10% ɔ-oryzanol (Saenjum et al., 2012). This fact implies that the ɔ-oryzanol contained in the extracts may not be the determining factor that improves health. Other bioactive components in the extracts probably also played important synergistic roles. In humans, there is evidence that gut fermentation causes degradation of ɔ-oryzanol into ferulic acid, which is progressively absorbed (Berger et al., 2005). Therefore, in vitro studies using ɔ-oryzanol in its native form might do little to elucidate its role in health promotion.

The exception appears to be rice tocotrienols; there is strong evidence that consumption of these substances translates into improved health outcomes. The multi-therapeutic properties of rice tocotrienols as hypoglycemic (Siddiqui et al., 2010), hypocholesterolemic (Qureshi et al., 2002), antithrombotic (Qureshi et al., 2001), and antiproliferative (Iqbal et al., 2003; Nakashima et al., 2010) agents in various experimental animal and human models have been reported. Plant phytochemicals are poorly absorbed from both the stomach and small intestine, and plasma concentrations range from nmol to μmol/L (Visioli et al., 2011; Landete, 2013; Lavecchia et al., 2013). Improved bioavailability of rice tocotrienols has been addressed; the high-performance liquid chromatography analyses of serum samples obtained from mice and humans after they consumed the tocotrienol-rich extracts showed 25ï 200% higher tocotrienol levels than those found in the control groups. The levels were estimated to be similar to the threshold concentration that provides effective protection against oxidative stress-induced damages (Qureshi et al., 2001). That finding, together with the high purity of the extracts (i.e., 88.2%), strongly supports the notion that increasing the consumption of rice provides an increase in health effects associated with tocotrienols.

However, how these antioxidants exert their effects may be more complex, and the discussion should not be ascribed to bioavailability alone. Other factors, such as rate of absorption, individual differences in metabolism, and long-term effects, could play roles. Here, some suggestions are offered regarding studies that seek to establish the role of rice antioxidants in disease prevention and mitigation.

1. Current knowledge about the effects of rice antioxidants may be limited because rice is rarely consumed alone and because of the short duration of dietary intervention studies (usually

fewer than eight weeks). It has been argued that because doses used in most dietary intervention studies are far greater than those that could be achieved by consumption of whole-grain rice, it is difficult to judge to what extent these compounds may contribute to the overall health efficacy of consuming rice grains (Visioli et al., 2011; Landete, 2013; Lavecchia et al., 2013). However, it is possible that regular consumption of antioxidants as part of the diet, as it is the case with rice in many countries, could result in the ingestion of significant amounts over time, and hence in a consistently exposure of cells to these plant components. Therefore, long-term studies in large scale-clinical trials are needed to estimate which rice antioxidant is associated with reduced disease risks.

2. This analysis shows that the health benefits of rice cannot be attributed solely to any single compound, as most antioxidant-rich extracts also contain other bioactive compounds, such as unsaturated fatty acids and dietary fibers. Therefore, the synergistic effects of antioxidant compounds are worth investigating in future research. Cycloarternyl *trans*-ferulate, for example, exhibits positive synergism with the drug TRAIL in inhibiting the growth of metastatic and resistant SW620 cells (Kong et al., 2009). Furthermore, low doses of the drug cisplatin in combination with tocotrienol-rich extracts from rice have been reported to eliminate the chemoresistance of H28 cells to cisplatin (Nakashima et al., 2010). Therefore, the use of rice antioxidants as adjuvants is a promising area of research.

3. All of the above-mentioned studies (with the exception of Ohara et al., 2009) found that rice antioxidants combat ROS and exert their health effects in a dose-dependent manner, with the highest activity usually obtained with the highest content of a given compound. This finding suggests that the total plasma antioxidant status may be improved by using specific rice varieties

that deliver the highest possible levels of bioavailable antioxidants (Pinent et al., 2012). Therefore, managing these compounds prior to harvest by understanding their responses to environmental factors and cultural practices is worth investigating (Cohen and Kennedy, 2010).

4. Most rice antioxidants are covalently bound to the indigestible cell wall matrix, which limits their bioavailability because the matrix severely hinders their access to the necessary enzymes in the upper gut (Ragaee et al., 2012; Ktenioudaki et al., 2013; Nayak et al., 2013; Zhao et al., 2013). It has been clearly demonstrated using rice tocotrienols that increased antioxidant bioavailability can be achieved through grain processing. The stabilized rice bran used in the preparation of the tocotrienol-rich extract from rice contained much higher levels of tocotrienols and also contained two new tocotrienols, *d*-didesmethyl tocotrienol and *d*-desmethyl tocotrienol (Qureshi et al., 2001, 2002; Iqbal et al., 2003; Nakashima et al., 2010). The difference in antioxidant bioavailability led to striking changes in clinical and physiological markers associated with reduced risk of diabetes (Siddiqui et al., 2010), atherosclerosis (Qureshi et al., 2001, 2002) and cancer (Iqbal et al., 2003; Nakashima et al., 2010), compared with the tocotrienol-rich extract obtained from non-stabilized rice. This offers proof-of principle for increased bioavailability of antioxidant compounds following processing. It is also necessary to mention that most of the studies cited utilized antioxidant-rich extracts from raw rice. In epidemiological investigations, it would be useful to characterize antioxidant intake from a diet of processed rice.

5. Finally, ROS should not be seen as inherently harmful as they are generated in various life-sustaining responses, such as attacking invading pathogens. Following that line of reasoning, either a deficient or excessive intake of antioxidants could adversely affect the maintenance of an

optimal immune response (Berger et al., 2012). For example, tocotrienols are well known for their role in lowering serum cholesterol levels by inhibiting the activity of the hepatic enzyme HMGCoA-R, while tocopherols induce the activity of this enzyme. Since tocotrienols are converted to tocopherols in vivo, intake of tocotrienols above a certain dose could be counterproductive (Qureshi et al., 2002). Therefore, identifying the factors that affect rice antioxidant content (Table 1) could enable estimation of dietary intake. This knowledge could also improve understandings of how antioxidants can be preserved during processing, and will maximize the chances of obtaining the potential health benefits associated with these compounds.

PRE-HARVEST FACTORS INFLUENCING ANTIOXIDANT COMPOUNDS IN RICE

Influence of Soil Type

A wide diversity of soils is used for rice cultivation, but these soils can roughly be classified into clay loam, silt loam, and sandy loam according to the proportions of sand, silt, and clay (Pelig-Ba, 2009; Butsat and Siriamornpun, 2010; Somsana et al., 2013). Sandy-loam soils are usually coarse-textured, light in color, and contain large particles; these soils remain loose, are very well drained, and do not retain nutrients. Clay loam and silt-loam soils are typically grey and brown, contain fine particles, and retain nutrients, moisture, and organic matter better than sandy soils (Pelig-Ba, 2009).

Differences in soil structure have been reported to affect the content of phenolic compounds in rice, with grains from clay-loam soils having higher concentrations of phenolics

than those from other soils. Butsat and Siriamornpun (2010) analyzed phenolic compounds in KDML 105 rice grown on three different soils (sandy loam, clay loam, and silty clay loam) in Thailand. Phenolic contents of grains from plants grown in sandy-loam soils were lower than those from plants grown in the other soils; the total phenolic content (TPC) was 29ï 54% lower (Butsat and Siriamornpun, 2010). In the study by *[name deleted to maintain the integrity of the review process]*, the TPC and the total flavonoid content (TFC) contents were, respectively, 9ï 30% and 3ï 15% lower in the brown rice, white rice, and bran, when grains from Ariete rice grown on a clay loam soil (15.7% sand, 27.9% silt, 56.4% clay) were compared with those from plants grown on a sandy loam soil (84% sand, 5% silt, 11% clay). In the husk, however, grains from plants grown on the sandy loam soil had more phenolic compounds. However, when seven black rices were planted in a randomized block design in three upland field environments (sandy, sandy loam, and clay loam soils), no difference in the total anthocyanin content (TAC) was observed among the different soil types (Somsana et al., 2013). A number of methods have been developed to determine the antioxidant capacities of chemical compounds and include 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity (DPPH), ferric ion reducing antioxidant power (FRAP), 2,2-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging activity (ABTS), oxygen radical absorbance capacity (ORAC), and ROS-scavenging capacities. Reported losses in DPPH for rice grown in sandy-loam soils ranged from 35ï 45% (Butsat and Siriamornpun, 2010), and from 11ï 41 *[name deleted to maintain the integrity of the review process]*. For the FRAP, losses of 29ï 50% were reported (Butsat and Siriamornpun, 2010). It should be emphasized that DPPH gives information on the antiradical activity of the extract (i.e., the ability of the compounds present in the extract to react with free radicals in a single free

radical reaction). However, in many cases this activity does not correspond to the antioxidant activity (i.e., the ability to inhibit the process of oxidation, which usually involves a set of different reactions) (Tirzitis and Bartosz, 2010). On the other hand, the FRAP measures the ability to reduce iron. Because reduced metals are active propagators of radical chains via hydroperoxide reduction to $RO^{Å}$, FRAP is a reasonable screen for the ability to maintain redox status in cells or tissues. However, the reducing capability based upon the ferric ion is not relevant to antioxidant activity mechanistically since iron is considered too slow a measure of antioxidant potential (Prior et al., 2005). Therefore, the clinical outcomes of these changes in in vitro antioxidant activities due to environmental factors may only be ascertained by in vivo experiments.

Rice grown in sandy-loam soils may accumulate higher levels of tocols and ɔ-oryzanol than rice grown in clay soils. Tocopherols were reported to be 46ï 53% higher (Butsat and Siriamornpun, 2010), and 23ï 36% higher *[name deleted to maintain the integrity of the review process]*; and ɔ-oryzanol was reported to be 39ï 55% higher (Butsat and Siriamornpun, 2010) and 47ï 86% higher *[name deleted to maintain the integrity of the review process]* in grains produced in sandy-loam soils.

Rice grown in sandy-loam versus clay and silt-loam soils also contained 6ï 16% more phytic acid (*name deleted to maintain the integrity of the review process*), although other studies showed higher increases (up to 98% in a Ghanaian rice variety; Pelig-Ba, 2009). In the study by Pelig-Ba (2009), however, samples were collected from two different districts of Ghana. The districts differed not only in soil type, but also in altitude and precipitation and temperature patterns. Phosphorus, used in the synthesis of phytic acid, is adsorbed on fine particles (clay and

silt), which renders it less available for utilization by plants (Pelig-Ba, 2009). Higher concentrations of N, K, and Zn in clay-loam soils also limit the availability of phosphorus (Depar et al., 2013). By contrast, sand facilitates infiltration of water and does not impede the movement of free phosphorus, which becomes available for plant uptake (Pelig-Ba, 2009).

Influence of Elevated Carbon Dioxide Concentration ([CO_2])

Atmospheric CO_2 is the substrate for carbon fixation, the main abiotic resource for plants; CO_2 enhances canopy photosynthesis, which increases plant growth (Goufo et al., 2014a). Data from the Intergovernmental Panel on Climate Change indicate that global atmospheric [CO_2] has increased by 30% during the last 100 years, from 290 to 394 µmol/mol, with the largest increase in recent decades (www.ipcc.ch). It is anticipated that [CO_2] will continue to increase and may reach 550 µmol/mol by the middle of the 21st century. There is a general consensus that increased emissions of CO_2 and other greenhouse gases (methane, ozone, nitrous oxide) into the atmosphere is the major cause of observed changes in climate. Among greenhouse gases, the impact of CO_2 is highest because its emissions are much higher than those of the other gases (www.ipcc.ch).

Following exposure to high [CO_2] (550 vs. 375 µmol/mol) in open-top chambers from sowing till harvesting, the contents of phenolic compounds, tocols, and ɔ-oryzanol decreased in all fractions of the rice Ariete, with 3ï 18%, 8ï 14%, and 10ï 15% losses for the TPC, TFC, and DPPH, respectively (Goufo et al., 2014a). The highest reductions were found for gallic acid (33%), caffeic acid (83%), *p*-hydroxybenzoic acid (100%), and chlorogenic acid (100%). For

tocols, decreases of 2ï 69% were observed in the white rice and the brown rice while increases of 25ï 82% were observed in the husk and the bran. For ɔ-oryzanol, decreases of 2ï 35% were reported in all rice fractions (Goufo et al., 2014a). A trend in decreased phytic acid content was observed but was not significant *[name deleted to maintain the integrity of the review process]*.

All the pathways that lead to antioxidant syntheses in rice share precursor and intermediate molecules (Fig. 1). The inositol lipid-independent pathway that leads to the synthesis of phytic acid involves the phosphorylation of *myo*-inositol, which is produced by the reaction between sucrose and galactinol from the Calvin cycle (Frank et al., 2007; Thitisaksakul et al., 2012). Phytic acid can also be produced by sequential phosphorylation of 1D-*myo*-inositol 3-phosphate generated from glucose 6-phosphate during glycolysis (Frank et al., 2007). Sucrose content tends to increase under elevated [CO_2] *[name deleted to maintain the integrity of the review process]*. However, increased sucrose did not translate into increased phytic acid content, probably because sucrose was preferentially utilized for growth of the rapidly developing plant. The biosynthetic of phenolics (phenylpropanoid metabolism) starts with the amino acid L-phenylalanine, which is synthesized from chorismate, the final product in the shikimate pathway (Shih et al., 2008). Phenylalanine content was reported to decrease under elevated [CO_2] *[name deleted to maintain the integrity of the review process]*, which could explain the decreased content of phenolics. In addition, phenylalanine is essential for protein synthesis, and under elevated [CO_2], the protein pathway could be favored (Goufo et al., 2014a). The shikimate pathway produces tyrosine, which is an immediate precursor for tocotrienol biosynthesis (Chaudhary and Khurana, 2009). Chlorophyll *a* from the tetrapyrrole pathway is the substrate for the synthesis of phytol from which tocopherols are formed (Chaudhary and Khurana, 2009).

Both tyrosine and chlorophyll *a* contents are reported to decrease under elevated [CO_2] *[name deleted to maintain the integrity of the review process]*, which might explain the observed reduction in tocol contents. The isoprenoid pathway, although located in the cytoplasm, is involved in delivering ferulate moiety lines for γ-oryzanol synthesis in combination with the phenylpropanoid pathway (Miller and Engel, 2006). Ferulic acid content decreased in all rice fractions under elevated [CO_2] (Goufo et al., 2014a) and this could explain the decreased γ-oryzanol content.

Influence of Elevated Temperature

The rise in [CO_2] and associated greenhouse gases is predicted to cause a 3ï 5 °C increase in global surface temperature during this century (www.ipcc.ch). Although most plants exhibit a temperature threshold appropriate to the environment in which they evolved, rice is currently grown near its optimum temperature in most parts of the world (Britz et al., 2007); therefore, small changes in temperature would affect rice yield and quality.

Elevated temperatures reduce the contents of phenolics and phytic acid in rice, an effect that is more pronounced in white rice than in brown rice. A 17% decrease in the TPC has been reported when grains of ten rices grown in India during the *aman* season (July to November, average temperature of 30 °C) were compared to those of plants grown during the *boro* season (November to February, average temperature of 20 °C) (Dutta et al., 2012); and 29% when grains of two rices grown in Taiwan during the summer (ripening period) were compared with those of plants grown during the winter (Kesarwani et al., 2012). In a field study conducted using

open-top chambers with the daytime temperature maintained at 28 °C in one chamber and 33 °C in the other chamber during the entire growing life of rice, a 3ï 22% and 3ï 21% decrease were recorded for the TPC and the TFC, respectively *[name deleted to maintain the integrity of the review process]*. Chae et al. (2004) subjected three rices grown in pots in phytotrons to temperatures of 18, 21, 24, and 27 °C during the ripening stage; the cyanidin-3-*O*-glucoside content of rice increased by 35ï 136% as temperature increased from 18 to 24 °C, but it decreased by 22ï 83% from 24 to 27 °C. In all those studies (Chae et al., 2004; Dutta et al., 2012; Kesarwani et al., 2012), the TPC and TFC were found to have a strong relationship to antioxidant measures such as DPHH, reducing power, and ROS scavenging activity. In the study by Dutta et al. (2012) for example, grains of plants grown at elevated temperatures displayed the lowest hydroxyl ion scavenging activity. As a reminder, the hydroxyl radical is the most reactive free radical in biological systems and it has been regarded as highly damaging to almost every molecule. However, as in other antioxidant tests, reaction conditions employed for the hydroxyl ion scavenging test are far from physiological. The steady-state concentration of the hydroxyl radical is practically zero, meaning that it is not possible to know whether these changes in in vitro antioxidant activities will be sufficient to offer protection against oxidative stress in vivo (Tirzitis and Bartosz, 2010).

Decreases in phytic acid content (7ï 67%) were observed when the daytime temperature was raised from 28 to 33 °C in open-top chambers *[name deleted to maintain the integrity of the review process]*. Ahn et al. (2010) calculated a 30% decrease in phytic acid when they compared the phytic acid contents of nine rices cultivated in four different locations that varied in average

mean air temperature (16, 18, 20, and 21 °C). A negative relationship also existed between precipitation and phytic acid content (Ahn et al., 2010).

As the temperature increased from 28 to 33 °C in open-top chambers, levels of tocols in rice also increased by 2ï 11% *[name deleted to maintain the integrity of the review process]*. Interestingly, tocol contents increased in the bran, husk, and brown rice under elevated temperature, but decreased by 3ï 42% in the white rice *[name deleted to maintain the integrity of the review process]*. Increases of 5ï 20% were recorded when the temperature was increased by 4.5 °C (Britz et al., 2007). In the latter study, grains from six rices grown to maturity in temperature-gradient greenhouses were analyzed. Temperatures were maintained near ambient (30/23 °C day/night), at one end of the greenhouse and at approximately 4.5 °C (i.e., 35/27 °C day/night) above ambient at the other end. Elevated temperature caused significant increases in Ŭ-tocopherol (five of six varieties) and Ŭ-tocotrienol (four of six varieties), and significant decreases in *γ*-tocopherol (three of six varieties). The following increases have been reported for ɔ-oryzanol: 7ï 10% from 18 to 27 °C (Chae et al., (2004); 33ï 197% from 28 to 33 °C *[name deleted to maintain the integrity of the review process]*; 6ï 106% when the temperature was raised by 4.5 °C (Britz et al., 2007); and 19ï 47% from 23 to 29 °C when three rices grown in pots were exposed to elevated temperature in phytotrons after the flowering stage (Nakano et al., 2013). Tocols accumulate in plant tissues as a defense against biotic and abiotic stresses (Britz et al., 2007); this could explain the observed increases under elevated temperatures. Moderate increases in temperature also resulted in increased ɔ-oryzanol, suggesting that ɔ-oryzanol might also be involved in plant stress responses.

Influence of Organic Farming

Organic agriculture is an ecological production/management system based on minimal use of off-farm inputs, and prohibition of the use of synthetic fertilizers and pesticides (Park et al., 2010; Tuaño et al., 2011; Kesarwani et al., 2012). Organically grown products are developed using cultural methods such as crop rotation; use of cover crops, green manures, and compost; and biological pest control (Sirikul et al., 2009; Cho et al., 2012). ñConventionalò farming refers to systems that rely on external inputs to achieve high yield (Cho et al., 2012). Consumers demand organic products because they are more environmentally benign than foods produced using conventional methods. Some consumers believe that organic foods have superior nutritional content; yet, controversy remains as to the perceived quality advantage of organically grown products.

There is a shortage of information about the effects of organic farming on antioxidant composition of foods, but the available evidence suggests that greater concentrations of antioxidants are found in organic than in conventional rice. Examples include 89% higher TPC (Park et al., 2010), 22% higher TPC (Kesarwani et al., 2012); and 35% higher TPC in rice bran (Sirikul et al., 2009). Furthermore, all three studies found a strong correlation between organic farming and antioxidant activities (DPPH and reducing power). A major limitation of these studies, however, is that no information was available about the growing conditions under which the organic and conventional rice grains were produced. These increases, if true, are consistent with a negative correlation between fertilizer/pesticide application and phenolic compounds (Cohen and Kennedy, 2010). Pressures from pathogens and predators on organic systems might

explain the higher content of phenolics observed in organic rice. However, Tuaño et al. (2011) reported lower TPC (4ï 22%) in organic rice compared with conventional rice. In the study, a split-plot experimental design was used consisting of two main plots (ñwith pesticideò and ñwithout pesticideò) and three subplots (control, organic fertilizer and inorganic fertilizer). The decrease following application of inorganic fertilizers and pesticides was significant while that following application of organic fertilizers and pesticides was not significant (Tuaño et al., 2011).

Tocol increases of 36% in rice bran (Sirikul et al., 2009) and ɔ-oryzanol increases of 9% in brown rice (Cho et al., 2012) have also been noted in organic rice, compared with conventional rice. Whereas Sirikul et al. (2009) simply bought rice samples from farmers; Cho et al. (2012) used fields with organic (green manure) and conventional (pesticides and fertilizers) plots divided by an 8-m-wide road. In contrast, Sirikul et al. (2009) reported 32% lower ɔ-oryzanol content in organic rice bran compared to conventional rice bran. The study by Tuaño et al. (2011) also indicated that ɔ-oryzanol content was decreased (21ï 25%) following application of fertilizers and pesticides. Application of organic fertilizers and pesticides also reduced tocol contents (30ï 83%), but no effect was observed after the use of inorganic fertilizers and pesticides (Tuaño et al., 2011). Because of the low number of available studies, a definitive conclusion about the effects of organic farming on rice tocol and ɔ-oryzanol content cannot be reached.

Phytic acid content was also higher (23%) in organic than in conventional rice (Park et al., 2010), which is consistent with findings of decreased phytic acid synthesis with increasing application of nitrogen (8ï 17%; Ning et al., 2009) and zinc (5ï 48%; Depar et al., 2013).

Influence of Harvest Date

Five stages are distinguishable in the development of the rice grain: (i) anthesis, 0ï 7 days after flowering (DAF), during which at least one grain on the main panicle is elongated to the end of the green husk; (ii) milky stage, 8ï 14 DAF, during which the endosperm begins to form as a milky liquid while the husk is still green; (iii) dough stage, 15ï 21 DAF, characterized by solidification of the milky liquid into a sticky white mass and at least one grain having a yellow husk suitable for dehulling; (iv) yellow-ripe stage, 22ï 28 DAF, when the endosperm becomes hard and opaque and at least one grain on the main panicle has a brown husk; and (v) fully ripe stage, 29ï 35 DAF, when all grains have a brown husk and optimum moisture content for harvesting has been reached (Butsat et al., 2009).

Phenolic acids and flavonoids are abundant in the early stages of grain development but progressively diminish as the grain matures. From milky phase to maturity, TPC losses from 11ï 24% have been reported for the husk (Grains of KDML 105 rice were collected at 7, 14, 21, 28, and 35 DAF, corresponding to the anthesis, milky, dough, yellow-ripe and fully ripe grain stages, respectively; Butsat et al., 2009), from 17ï 68% for the rough rice (grains of two rices were collected at 6ï 9, 12ï 15, and 18 DAF, corresponding to the milky, dough, and yellow-ripe stages, respectively; Lin and Lai, 2011), and from 35ï 82% for the brown rice (grains of three rices were collected at 7, 14, 21, and 42 DAF, corresponding to the milky, dough, yellow-ripe and full ripe stages, respectively; Shao et al., 2014). Although levels of some soluble phenolic acids fluctuate during ripening, similar trends were found for these antioxidants. For example, ferulic acid decreased from 6.42 mg/100 g during the milky stage to 1.81 mg/100 during the fully ripe stage

(Shao et al., 2014). In a black rice variety, however, the TPC increased (from 6ï 37%) as the grain matured (Shao et al., 2014). The TFC was also reported to decrease in the rough rice (by 38ï 75%) from the milky to the fully ripe stage, although in one variety, the TFC increased from anthesis to the dough stage and then decreased at maturity (Lin and Lai, 2011). The decrease in antioxidant contents and activities (Butsat et al., 2009; Lin and Lai, 2011; Shao et al., 2004) during grain formation is probably related to the progressive metabolism of soluble phenolics into oligomers such as lignin. The content of cyanidin-3-*O*-glucoside in two Korean rices was also reported to decrease (43ï 142%) between anthesis and maturity (Kim et al., 2013). In one variety, however, the content of cyanidin-3-*O*-glucoside increased by 290% between 20 days and 30 days after heading and then decreased by 63% until 50 days after heading (Kim et al., 2013). Unfortunately, the authors did not specify the grain development stage corresponding to each sampling. Curiously, the TAC in rice was also reported to increase between the milky and dough stages by 558%; and then to decrease until maturity by 49% (Shao et al., 2014). Over all, the TAC observed during the fully ripe stage was 316% higher than that during the milky stage (Shao et al., 2014).

The degree of maturity also influences tocol and ə-oryzanol contents in rice, although there are inconsistencies among studies. Tocol losses of 8ï 49% in rough rice were reported by Lin and Lai (2011). This was in contrast to a slight increase in eight tocols during the milky phase and a steady increase thereafter (by 51ï 380% at maturity) in two rices in the study by Wang et al. (2013). Lin and Lai (2011) found non-significant differences in ɔ-oryzanol content in developing and mature grains of KFSW rice, and a 13% decrease in ɔ-oryzanol from the milky to the dough stage followed by a 60% increase to the fully ripe stage in TK16 rice. These findings indicate

that the timing of rice harvest influences the content of rice antioxidants. Immature rice grains appear to contain high levels of antioxidants and could be used for the production of nutraceutical foods.

To the authorsô knowledge, no studies have examined the influence of harvest date on phytic acid content in rice.

POST-HARVEST FACTORS INFLUENCING ANTIOXIDANT COMPOUNDS IN RICE

Influence of Parboiling

After harvest, rice is directly dried or is parboiled then dried. There have been few studies on the effect of drying on rice antioxidants. In rice samples dried using a superheated steam fluidized bed dryer at 170 °C for 2.5, 3 and 4 min followed by shade drying at ambient temperature, the TPC and DPPH of one variety were reduced (15ï 17%), while those of the second variety were increased (4ï 24%) (Rumruaytum et al., 2013). Parboiling is an ancient process in Asia, Africa, the Americas, and to a limited extent in Europe, and its function is to improve the milling recovery of rice by hardening the endosperm (Pestana et al., 2009; Walter et al., 2013; Pradeep et al., 2014). Parboiling provides several other advantages including grain sterilization, increased shelf life, and reduced stickiness of cooked rice (Khatoon and Gopalakrishna, 2004; Pascual et al., 2011; Min et al., 2014). Techniques for parboiling rice vary among countries. The traditional process involves soaking rough rice for 4ï 24 h in water at room temperature, followed by boiling or steaming at 100 °C for 30ï 90 min until the starch is

gelatinized and the lemma and palea start to separate (Pradeep et al., 2014). Recent methods adopted in most parboiling studies use warm water below the temperature at which starch gelatinizes (60ï 65 °C) for 3ï 6 h, followed by sterilization by autoclaving (110ï 121 °C) for 5ï 20 min and drying (40ï 60 °C) for 2ï 24 h. The parboiled rice is then cooled and dried to 14% moisture at a maximum temperature of 37 °C before storage or milling (Khatoon and Gopalakrishna, 2004; Pestana et al., 2009; Pascual et al., 2011; Walter et al., 2013).

It is commonly accepted that parboiling results in inward migration of water-soluble vitamins and minerals from the bran to the endosperm (Pestana et al., 2009; Walter et al., 2013). For fat-soluble vitamins, results are contradictory. In the study by Pascual et al. (2011), tocol contents in grains were greatly reduced after parboiling, with losses of 60ï 93% in three rices; whereas in the study by Min et al. (2014), they were greatly increased (8ï 30%) in six rices. Khatoon and Gopalakrishna (2004) also reported total tocol losses of 92ï 100%. However, in their study, two commercial non-parboiled varieties were compared with a commercial parboiled variety. Parboiling also reduced the ɔ-oryzanol content of rice by 20ï 27% in the study by Pascual et al. (2011), but increased it by 9ï 43% in the study by Min et al. (2014). Two recent studies reported 60% (Pestana et al., 2009) and 60ï 66% (Pradeep et al., 2014) higher ɔ-oryzanol content in parboiled than in non-parboiled rice bran, supporting the hypothesis that parboiling could cause ɔ-oryzanol to migrate from the endosperm to the bran. However, a lower content of tocopherols (65ï 70%) was observed in the parboiled rice bran (Pestana et al., 2009).

Parboiling reduced the TPC of brown rice by 33ï 87% but had no such effect in white rice (Walter et al., 2013). In the study by Min et al. (2014), parboiling reduced the TPC, TFC, TAC, and total proanthocyanidins (TPAC) in six rices by 16ï 91%, 57ï 91%, 6ï 37%, and 16ï 57%,

respectively. TPC reductions of 43ï 89% in the bran following parboiling of three rices have also been reported by Pradeep et al. (2014). Hence, cooked parboiled rice might lack the antioxidant properties of freshly cooked rice, as evidenced by significant reduction of DPPH and FRAP values (Walter et al., 2013; Min et al., 2014; Pradeep et al., 2014). This reduction in phenolic content in parboiled rice could be related to leaching of these compounds during the process, as evidenced by the presence of phenolic compounds in parboiling water (Walter et al., 2013). Because sterilization uses temperatures above 100 °C, it is also possible that phenolics may be degraded during parboiling.

Although the authors couldnôt find studies that compared the phytic acid content of parboiled and non-parboiled rice, soaking and steaming result in phytic acid losses (Liang et al., 2008a; Albarracín et al., 2013). Over all, parboiling appears to be the least promising means of retaining antioxidant compounds in rice (as compared to the other processing techniques discussed below).

Influence of Storage

After harvesting and/or parboiling, dried rice maintained at 14% moisture content is commonly stored in jute bags before milling. Changes during storage (ageing) occur in the first three to six months after harvest (Srijesdaruk et al., 2001; Rohrer et al., 2002; Htwe et al., 2010). During that period, constituents in the grain equilibrate to more stable physical forms, which results in a harder grain that can withstand the frictional forces of milling (Lloyd et al., 2000; Kongkiattikajorn et al., 2010; Thanajiruschaya et al., 2010). This is why rice is generally milled

after 3ï 6 months of storage. Rice is stored in its rough form in most parts of the world but storage of brown rice is also widespread (Zhou et al., 2004; Goufo, 2008; Pascual et al., 2011). Brown rice, however, is more sensitive to temperature because of the absence of the insulating enclosing husk. The shelf life of milled rice is usually shortest, followed by brown rice and rough rice (Zhou et al., 2004; Liu et al., 2010; Thanajiruschaya et al., 2010).

During storage, temperature and time are the critical factors that influence the antioxidant composition of rice. With the exception of Zhou et al. (2004) and Pascual et al. (2011), rice was stored in rough form in all the studies mentioned below. Increasing storage time causes losses of antioxidant compounds at temperatures from 4 to 45 °C. Published losses in TPC range from 3% to 69% and can be categorized as follows: 5ï 26% in three rices stored for 6 months, with the lowest losses at 4 °C compared to 37 °C (Zhou et al., 2004); 60ï 69% in KDML 105 rice bran for rice stored for 7 months at 25 and 37 °C (Kongkiattikajorn et al., 2010); 33ï 42% in two rices stored for 1, 2, 3, and 4 months at 20, 30, and 40 °C (Htwe et al., 2010); 58ï 65% in KDML 105 rice stored for 7 months at 25 and 37 °C (Thanajiruschaya et al., 2010); and 3ï 8% in Hom Daeng rice stored for 6 and 12 months at 15 and 28 °C (Tananuwong and Tangsrianugul, 2013). In the latter study, rice grains were vacuum-packed in OPP/AL/LLDPE or Nylon/LLDPE pouches before storage; and reductions were found to be greater in samples stored in Nylon/LLDPE pouches. Htwe et al. (2010) investigated changes in soluble and insoluble phenolics associated with storage and found a gradual decline of insoluble phenolics throughout the storage period. However, soluble phenolics increased after the first month of storage (probably due to the hydrolysis of insoluble phenolics) and then sharply declined after two months. These decreases could be related to enzymatic and/or non-enzymatic oxidation and hydrolysis of antioxidants

during storage. Liu et al. (2010) found that longer storage durations (36 months at room temperature) at ambient temperature corresponded to a 7ï 47% increase in the TPC in three *indica* and three *japonica* rice varieties. However, additional studies using long storage periods would be necessary to confirm that finding. A 49ï 51% decline in the TFC was reported in KDML 105 rice stored for 7 months at 25 and 37 °C (Thanajiruschaya et al., 2010), and a 10ï 36% decline in the TAC was observed in two rices stored for 1, 2, 3, and 4 months at 20, 30, and 40 °C (Htwe et al., 2010). By contrast, Htwe et al. (2010) found a 6ï 15% increase in the DPPH in brown rice after 4 months storage and attributed this to an increase in carotene content during storage. Interestingly, Tananuwong and Tangsrianugul (2013) found that when rice was stored in cooked form, its antioxidant activity was not affected by storage duration or temperature. Other reported reductions in antioxidant activities with storage time include a 4ï 5% decrease in DPPH in *indica* rice stored for 36 months (Liu et al., 2010); a 6ï 9% decrease in reducing power in rice stored for 12 months (Tananuwong and Tangsrianugul, 2013); a 89ï 90% decrease in the inhibition of lipid peroxidation in rice stored for 7 months (Thanajiruschaya et al., 2010); and a 54ï 55% decrease in the ABTS in rice bran stored for 7 months (Kongkiattikajorn et al., 2010). The ABTS assay is widely used in vitro and in vivo to monitor the ability of antioxidant compounds (non-enzymatic antioxidants) in extracts and plasma to interfere with reactions with peroxyl radicals. However, it may not truly reflect in vivo activities, as ABTS is not a physiological free radical (Prior et al., 2005; Tirzitis and Bartosz, 2010). Therefore, it remains unknown whether those decreases translate into physiological effects.

Storage time also has deleterious effects on tocol and ɔ-oryzanol contents in rice. The following losses have been reported for tocols: 38ï 46% in two rices stored for 3 and 6 months at

room temperature (Srijesdaruk et al., 2001); 57ï 71% in three rices (parboiled and non-parboiled) stored at room temperature for 6 months (Pascual et al., 2011); 56ï 79% in rice bran (irradiated and non-irradiated) stored for 1, 3, 7, 24, and 52 weeks at room temperature (Shin and Godber, 1996); and 59ï 78% in rice bran (stabilized and non-stabilized) stored for 7, 35, 105, 165, 210, and 375 days at room temperature (Shin et al., 1997). In the study by Kongkiattikajorn et al. (2010), storage at 25 and 37 °C for 7 months had a non-significant effect on tocopherol contents in KDML 105 rice. ɔ-Oryzanol proved to be more stable than tocols during storage, with losses of 20ï 56% in rice bran stored for 2 and 4 months at 25 and 45 °C (Chitropas et al., 2004); 17ï 20% in three rices (parboiled and non-parboiled) stored for 6 months at room temperature (Pascual et al., 2011); 39ï 62% in rice bran (irradiated and non-irradiated) stored for 1, 3, 7, 24, and 52 weeks at room temperature (Shin and Godber, 1996); and 50ï 62% in rice bran (stabilized and non-stabilized) stored for 7, 35, 105, 165, 210, and 375 days at room temperature (Shin et al., 1997). The retention of ɔ-oryzanol during storage decreased linearly with increasing extrusion temperature during stabilization (Shin et al., 1997). Alpha-tocopherol and Ŭ-tocotrienol were found to be less stable than the other tocols during storage, and ɔ-tocotrienol and ɔ-oryzanol exhibited a steady rate of decomposition during storage (Shin and Godber, 1996; Shin et al., 1997). Azrina et al. (2010) found a steady increase at room temperature (from 71% to 82%) in ɔ-oryzanol content in bran (stabilized and non-stabilized) of seven rices during the first three month of storage, then a progressive decrease (56ï 67%) after 12 months of storage. Nutraceutical contents in the bran of rough-stored rice also increased during the first three months of storage (75ï 138% for tocols, 2ï 28% for ɔ-oryzanol) and then decreased during the

following 4ï 6 months in six U.S. rices (33ï 40% decrease in tocols and 52ï 88% decrease in ɔ-oryzanol) (Rohrer et al., 2002).

Storage at ambient temperature for 1, 6, and 12 months had a non-significant effect on phytic acid content in nine rices (Ahn et al., 2010), probably due to low activity of phytase, the enzyme responsible for the degradation of phytic acid. However, that result is preliminary and thus not conclusive.

Earlier studies suggested that tropical temperatures (18ï 45 °C) affected the antioxidant composition of rice. Chitropas et al. (2004) found an 11ï 19% increase in the DPPH of rice bran when the storage temperature of KDML 105 rice was raised from 25 to 45 °C for 2 and 4 months. Kongkiattikajorn et al. (2010) also found an increase in the TPC (4ï 30%) in KDML 105 rice bran when the storage temperature was raised from 25 to 37 °C for 7 months. However, more recent studies provide evidence that contradict these earlier findings. No influence of temperature (15 and 28 °C) was observed on the TPC, TAC, reducing power, or DPPH in rice stored in different types of pouches for 6 and 12 months (Tananuwong and Tangsrianugul, 2013). Storage temperatures compared by Htwe et al. (2010) (20, 30 and 40 °C for 2 rices stored for 1, 2, 3, and 4 months) and Thanajiruschaya et al. (2010) (25 and 37 °C for KDML 105 rice stored for 7 months) did not affect the TPC, TAC, TFC, or inhibition of lipid peroxidation of rice varieties. Increasing the storage temperature from 21 to 38 °C for 1, 2, 3, 4, 5, and 6 months had no effect on tocotrienol contents in six U.S. rices (Rohrer et al., 2002). Kongkiattikajorn et al. (2010) found no change in the ABTS in bran between samples stored for 7 months at 25 and 37 °C. Although a slight increase (4ï 6%) was found in tocol content, this was not significant (Kongkiattikajorn et al., 2010). Changes in antioxidant contents in rice occur mainly when

storage temperatures below and above 15 °C are compared. For example, the differences in tocol and ɔ-oryzanol contents in bran samples stored at ï 20 and 4 °C for 2 months were not significant (Lloyd et al., 2000). Reported losses resulting from increased temperatures are 6ï 15% for several phenolic acids from 4 to 37 °C in three rices stored for 6 months (Zhou et al., 2004); 18ï 59% for ɔ-oryzanol in the bran from -20 to 45 °C in KDML 105 rice stored for 2 and 4 months (Chitropas et al., 2004); and 20ï 28% for tocols from 7 to 38 °C in six rices stored for 1, 2, 3, 4, 5, and 6 months (Rohrer et al., 2002). The destruction of tocols at high temperatures may be related to hydrolysis of free fatty acids by lipases followed by lipoxygenase oxidation of the free unsaturated fatty acids. In addition, tocols are oxidized directly (Shin et al., 1997). Storage at temperatures below 15 °C could sufficiently retard these changes, as respiration and oxidation rates are lowered by cooling (Lloyd et al., 2000).

Influence of Irradiation

Insects, microorganisms, and rodents cause losses in quantity and quality of rice grains during storage. Of all pest control methods tested (chemical fumigants, vapor heat, irradiation), irradiation has proven to be effective in killing insects and reducing food-borne microbial growth, especially when large quantities of rice require long-term storage (Ramarathnam et al., 1989; Lee et al., 2004; Rastogi, 2012). Irradiation involves placing rough rice in a wooden box or airtight plastic bag and exposing it to carefully controlled amounts of ionizing radiation (far infrared rays, gamma rays, or X rays), which transfers heat evenly to the center of the sample. Irradiation used

for rice treatment is usually limited to a dose of 15 kGy (Ramarathnam et al., 1989; Shin and Godber, 1996; Lee et al., 2004; Jeon et al., 2006; Zhu et al., 2010; Shao et al., 2013).

Research shows that the effects of irradiation on rice phenolics might depend on the dose applied and the form in which the rice is irradiated. Zhu et al. (2010) irradiated three rices (whole grain) with ɔ-rays at room temperature in a ^{60}Co irradiator with a dose rate of 1.0 kGy/h at doses of 2, 4, 6, 8, and 10 kGy; and then stored the irradiated rice in the dark at room temperature for 6 months. They found that irradiation at 2ï 6 kGy significantly decreased the TPC and TAC in a variety (by 9ï 67%), while 8 kGy resulted in a 9ï 11% increase. Similar findings at different doses were found for the two other rices, which suggested that a suitable dose of irradiation could cause an increase in phenolic compounds in whole-grain rice during storage. In a follow-up study by the same authors under the same experimental conditions (Shao et al., 2013), not the brown rice, but the rough rice was irradiated. Irradiation increased the TPC (4ï 11%) and ABTS (6ï 14%) at all doses and in all rices, suggesting that rough-irradiated rice was preferable to whole grain-irradiated rice in improving antioxidant contents in rice. When rice husks were irradiated with an Far Infrared heater (300 W) which emitted radiation within the 2ï 14 µm wavelength range, antioxidant properties increased by 58ï 171% (TPC, Lee et al., 2004); and 35ï 82% (superoxide anion, hydrogen peroxide, hydroxyl radical, and nitric oxide radical scavenging activities, Jeon et al., 2006). The effect of the irradiated rice husk extract on DNA damage induced by H_2O_2 in human lymphocytes was also evaluated (Jeon et al., 2006). Irradiated rice husk extract exhibited a protective effect, as indicated by DNA strand breakage decreasing from 38% to 22% with the irradiated extract and from 49% to 28% with the non-irradiated extract as compared with H_2O_2-treated positive controls. When human lymphocytes were post-incubated

with the rice husk extract for 30 min after exposure to H_2O_2, the protective ability of the rice husks remained unchanged. The ROS-scavenging activities were highly correlated with decreased DNA damage. The authors suggested that the presence of antioxidant compounds could have protected against oxidative DNA damage and contributed to stimulation of DNA repair. The presence of insoluble phenolics in rice probably contributes to the high levels of phenolic compounds observed after irradiation. Irradiation could cleave covalent bonds and liberate phenolic compounds from repeating polymers. However, irradiation may also degrade phenolics; the final TPC appears to depend on the balance between the two processes.

In contrast to phenolics, irradiated rice shows lower contents of tocols and ɔ-oryzanol compared with non-irradiated rice, and the decomposition of individual E vitamers and steryl ferulates increases with increasing irradiation dose. When the bran from two rices was irradiated at doses of 5, 10, and 15 kGy with a dose rate of 0.98 kGy/h using a ^{60}Co irradiator and samples analyzed after 1, 3, 7, 24, and 52 weeks of storage at ambient temperature, reported losses of tocols and ɔ-oryzanol were 46ï 86% and 11ï 22%, respectively (Shin and Godber, 1996). Irradiation of eight rices with and without intact husks with doses of 5, 10 and 15 kGy by a ^{60}Co irradiator (0.68 kGy/h) caused losses of 72ï 78% and 30ï 39% for Ŭ-tocopherol and ɔ-oryzanol, respectively (Ramarathnam et al., 1989). Only trace amounts of Ŭ-tocopherol could be detected in rice at a dose of 15 kGy. Overall, those changes were less severe for rice grains irradiated with intact husks (Ramarathnam et al., 1989). It is also reported that tocotrienols are more sensitive to irradiation than tocopherols (Shin and Godber, 1996). Double bonds between certain carbons atoms in long-chain fatty acids esterified with glycerol are selectively attacked by free radicals produced by irradiation (Shin and Godber, 1996); this should explain why tocotrienols with three

double bonds in their side chains are more susceptible to irradiation than tocopherols without double bonds. ɔ-Oryzanol was found to be more resistant to irradiation than tocols (Ramarathnam et al., 1989; Shin and Godber, 1996). In the case of ɔ-oryzanol, irradiation probably weakens the links between sterols and ferulic esters, as evidenced by a large increase in the amount of free sterol in irradiated rice lipids (Shin and Godber, 1996).

To the authorsô knowledge, no studies have examined the influence of irradiation on phytic acid content in rice.

Influence of Milling

After storage, rice is milled before further processing. The milling process consists of three fundamental operations: (i) removing the husks from rough rice to obtain the brown rice; (ii) subjecting the brown rice to abrasive or friction pressure to remove the bran and produce milled rice; and (iii) separating entire grains from broken grains (Gopala et al., 1984; Sidhu and Bajaj, 1988; Ha et al., 2006; Liang et al., 2008b; Fujita et al., 2010). In most parts of the world, rice is used as white rice after removing the bran (Lloyd et al., 2000; Rohrer and Siebenmorgen, 2004; Finocchiaro et al., 2007; Jang and Xu, 2009; Goufo et al., 2014a). The degree of milling is an important factor in determining the nutritional value and the economic return of rice and is defined as the mass loss due to bran removal (Liang et al., 2008b). Rice antioxidants are mainly concentrated in the bran (Damayanthi, 2001; Ha et al., 2006; Schramm et al., 2007; Wang et al., 2011; Laokuldilok et al., 2013); hence, the degree of milling has a significant effect on their contents. The bran is composed of three layers: the pericarp composed of epicarp, mesocarp, and

cross layer; the seed coat composed of testa and tegmen; and the aleurone layer (Sidhu and Bajaj, 1988; Yđmaz et al., 2013). The germ is enclosed in the seed coat (Yđmaz et al., 2013). Depending on the amount of bran removed, the milling degree can be classified as low (3% of external tissues removed, i.e., 5ï 20 s milling), medium (about 6% of external tissues removed, i.e., 21ï 40 s milling), and high (about 9% of external tissues removed, i.e., 40ï 60 s milling) (Rohrer and Siebenmorgen, 2004; Jang and Xu, 2009; Wang et al., 2013). Several studies have attempted to determine the layer specificity of antioxidant compounds in rice as a means of maximizing the economics of efficiently extracting these compounds.

Phenolic acids and flavonoids might be mostly located in the outer layers of the bran. Laokuldilok et al. (2013) subjected five rices to abrasive milling for 10, 20, and 30 s corresponding, to 5, 7, and 10% milling degree, respectively. The bran collected after 10 s milling contained 9% and 22% more TPC than the bran collected after 20 and 30 s of milling, respectively, indicating that phenolics were mainly located in the medium and outer layers of the bran. In the study by Finocchiaro et al. (2013) using two rices, 3% and 6% milled rices lost 52ï 70% and 51ï 87%, respectively, of the initial ABTS found in the 0% milled rice. However, the inner layer of the bran (9% milling) was not evaluated. In addition, the authors found an even distribution of sinapic, caffeic, ferulic, and coumaric acids in the bran of one variety. In a U.S. purple rice bran, however, the TPC and DPPH in the inner bran layers (collected between 40 and 60 s milling) were 48.91 mg catechin equivalent/100 g and 43.36 mmol trolox equivalent/100 g respectively, whereas they were 11.39 mg catechin equivalent/100 g and 7.82 mmol trolox equivalent/100 g, respectively, in the outer + medium bran layers (collected between 0 and 40 s milling) (Jang and Xu, 2009).

Anthocyanins and proanthocyanidins are clearly concentrated in the outer layers of the bran as demonstrated by several studies. In the study by Finocchiaro et al. (2007) using a red rice, 3% and 6% milled rice grains lost 76% and 86% of the initial proanthocyanidin found in the 0% milled rice, respectively. Matrix-assisted laser desorption/ionization imaging mass spectrometry (MALDI-IMS) is an emerging technology that allows the simultaneous investigation of the content and spatial distribution of biomolecules in plants. Using MALDI-IMS, Yoshimura et al. (2012) confirmed that the major rice anthocyanins (cyanidin-3-*O*-glucoside and peonidin-3-*O*-glucoside) were predominantly localized in the outer layers of the bran, while petunidin-3-*O*-arabinoside and cyanidin-3-*O*-arabinoside contents showed no significant differences across the bran layers. However, another study contradicts these findings, showing levels of anthocyanins in the inner bran layers to be 8 times higher than those in the outer+medium bran layers (Jang and Xu, 2009).

Tocols are also reported to be concentrated in the outer layers of the bran. In two U.S. rices, tocols were found in higher levels in the bran from shorter milling durations (10ï 20 s, corresponding to 3% milling) compared to 30 s (6% milling) (Rohrer and Siebenmorgen, 2004). Schramm et al. (2007) milled two rices at nine time settings from 5 to 45 s in 5-s intervals. Although not statistically significant, the highest concentration of bran tocols was present at the 10-s setting and the second highest concentration was obtained at the 5-s setting. In the study by Laokuldilok et al. (2013), which compared milling degrees of 5%, 7%, and 10%, the Ŭ-tocopherol content of rice bran decreased with longer milling time in three of five varieties studied. In the other two varieties, no consistent trend was observed. Finocchiaro et al. (2007) found that low milling (3%) removed 39ï 83% of tocols while medium milling (6%) removed

71ï 84%, indicating that most tocols were in the outer layers. Ha et al. (2006) observed a total tocol content in brown rice (0% milling) of 37.67 mg/kg rice. The total content of tocols in rice samples with milling degrees of 6%, 8%, and 9% were 21.75, 16.91, and 11.58 mg/kg rice, respectively. In one study, however, tocols were mostly found in the inner layers (Jang and Xu, 2009), while in another study, they were found in the medium layers of the bran (Lloyd et al., 2000). In the latter study, two rices underwent a three-break milling treatment in three successive and separate commercial-sized milling machines, yielding the outer, medium and inner bran layers. The tocol concentration was highest for rice bran taken immediately after milling break 2 (Lloyd et al., 2000). Similar to tocols, ɔ-oryzanol appears to be mostly concentrated in the outer layers of the bran. These results have been confirmed in other studies (Rohrer and Siebenmorgen, 2004; Ha et al., 2006; Finocchiaro et al., 2007; Schramm et al., 2007; Laokuldilok et al., 2013). For example, the content of ə-oryzanol decreased from 329 to 95 mg/kg after 6% milling and to 56 mg/kg after 10% milling in the study by Ha et al. (2006). In the study by Lloyd et al. (2000), the bran collected directly after milling break 1 had the highest ɔ-oryzanol content. In another study, the ɔ-oryzanol content in bran (two varieties) from 7% milling was 48.5ï 98.2 mg/100 g; in the bran from 10% milling, it was 54.8ï 147.7 mg/100 g (Damayanthi, 2001). However, the 7% milling as used by the authors would include both the outer and medium layers. On the other hand, Jang and Xu (2009) found an even distribution of ə-oryzanol in the bran of a purple rice. In their study, however, the outer and medium bran layers were combined for the analyses.

Phytic acid appears to be mainly found in the medium layers of the bran, at the interface of the embryo and the testa. No loss of phytic acid was observed with a 3% milling (6ï 10 s milling); 6% milling (20ï 30 s milling) resulted in a 23% loss of phytic acid, while 9% milling (40ï 60

milling) resulted in a 47% loss for Bijing 37 and Zhongyou 752 rices. For Ganwanxian 30 rice, however, no consistent trend was observed (Liang et al., 2008b). Wang et al. (2011) milled three rices at different time intervals corresponding to 3, 5, 6, 9, 10, 11, 12, 15, 16, 17, 20, and 25% milling. The highest losses were observed after 6% milling for two varieties. For the last variety, similar losses were observed after 6 and 9% milling. Sidhu and Bajaj (1988) found that phytic acid fell to half of its original level after 10% milling (50 s milling); this may be an indication that that phytic acid deposited either in the medium or the outer layer of the bran.

Value-added processing with respect to rice milling has traditionally focused on the whiteness of milled rice, and therefore, the 9% degree of milling has mostly been used (Goufo et al., 2014b). As shown above, this results in a significant loss of antioxidant compounds. The term ñpolished riceò is used to describe milled rice obtained after 3ï6% milling (Rattanachitthawat et al., 2010). Although polished rice has lower market appeal it has a greater antioxidant value, and with the increasing acceptance of functional foods by consumers, its consumption should be encouraged.

Influence of Stabilization

The health benefits associated with rice bran have resulted in the bran being used in many categories of food products (Sharif et al., 2013). However, rice bran is highly susceptible to fat rancidity caused by lipases, which makes it unsuitable for human consumption (Gualberto et al., 1997; Roselina et al., 2011; Thanonkaew et al., 2012; Wataniyakul et al., 2012). In the rice grain, lipases are mainly located in the cross layer and testa, whereas the oil is located in the aleurone

layer and the germ (Yđmaz et al., 2013). During milling, bran lipase and neutral oils (triglycerides) are brought together, resulting in the formation of free fatty acids and glycerol (Ko et al., 2003; Loypimai et al., 2009; Yđmaz et al., 2013). To prevent the formation of these harmful compounds that promote rancidity, endogenous lipases must be inactivated soon after milling. This is achieved by stabilizing the bran through processes including heating with an autoclave (pressured steam-heating), an oven, a steamer, a roaster, an extruder oven, a microwave, or an ohmic heating unit (Gualberto et al., 1997; Shin et al., 1997; Ko et al., 2003; Loypimai et al., 2009; Azrina et al., 2010; Roselina et al., 2011; Thanonkaew et al., 2012; Wataniyakul et al., 2012). These processes use temperatures varying from 105 to 190 °C.

In all stabilization procedures tested, the contents of phenolics increased: 37% for the TPC with autoclave heating (RD-6 rice was steamed at 105 °C for 15 min; Loypimai et al., 2009); 40% for the TPC with microwave heating (150 °C for 3 min with a microwave 800 W power and 2450 MHz frequency; Thanonkaew et al., 2012); 48ï 63% for the TPC with microwave heating (six rices were heated at 110 °C for 8 min with a microwave 67 % power and 1100 W; Roselina et al., 2011); 34ï 55% for the TPC with microwave heating, with a greater increase at 100 °C than at 60 or 80 °C (temperatures of 60, 80, and 100 °C and durations of 5, 10, 20, and 30 min were compared; Wataniyakul et al., 2012); 35% for the TFC with microwave heating (150 °C for 3 min with a microwave 800 W power and 2450 MHz frequency; Thanonkaew et al., 2012). Thanonkaew et al. (2012) also compared how well oven heating (150 °C for 10 min in an oven), roasting (150 °C for 10 min in a cooking pan), and steaming (130 °C for 60 min in a steamer) stabilized the rice bran. All the three methods resulted in an 18ï 38% and 11ï 31% increase in TPC and TFC, respectively. The bran that was stabilized by oven heating contained higher

amounts of phenolics than did the other two types of bran. However, steaming had no effect on the TFC. Rice (RD-6 bran) was stabilized using an ohmic heating unit equipped with titanium electrodes and enclosed on a Teflon tee. An alternative current electricity (50 Hz) with three levels of electrical field strengths (75, 150 and 225 V/cm) was used. The sample was removed from the heating unit after it reached the maximum temperature (60ï 124 °C for 3ï 5 min depending on the sample). Overall, ohmic heating increased the TPC by 81ï 99% (Loypimai et al., 2009). It is likely that stabilization offers a rapid transfer of energy to both the extracting solvent and the rice sample. That energy transfer could cause structural and morphological changes to the grain tissues making them more accessible to the extracting solvent. As a consequence of increased phenolic contents, the stabilized bran extracts exhibited a stronger ability to quench free radicals in vitro (Loypimai et al., 2009; Thanonkaew et al., 2012; Wataniyakul et al., 2012).

Observed effects of stabilization on tocols and ɔ-oryzanol are inconsistent, mainly differing according to the stabilization procedure and the heating temperature and duration. Reductions of ɔ-oryzanol by heating amounted to 21ï 26% at 120 °C (bran of two rices was collected at the outlet of a high temperature steam-injected expander; Lloyd et al., 2000); 9ï 25% at 121 °C with highest losses recorded in the bran stabilized for durations above 3 min (bran from different milling times was autoclaved at 121 °C for 1, 2, 3, 4, and 5 min; Damayanthi, 2011); and 4ï 11% from 110 to 140 °C (bran of three rices was extruded at 110, 120, 130, and 140 ºC with post extrusion holding times of 0, 3, and 6 min; Shin et al., 1997). In the latter study, the ɔ-oryzanol content in stabilized rice bran did not differ for extrusion temperatures between 110 and 120 °C or between 120 and 140 °C. Reductions (16ï 34%) of ɔ-oryzanol in six rices occurred during

microwave (microwave 67 % power, 1100 W) heating at 110 °C for 8 min, with stirring every 3 min (Roselina et al., 2011). No difference was found in ə-oryzanol contents between stabilized and non-stabilized bran immediately after stabilization using microwave and autoclave heating (Azrina et al., 2010). A 43ï 72% increase in ɔ-oryzanol occurred during the first 2 months of storage. After 12 months of storage, however, a 20ï 36% decrease in ɔ-oryzanol was observed (Azrina et al., 2010). Unfortunately, the heating conditions were not provided by the authors. A 4ï 25% decrease in tocol contents occurred due to steam-extrusion heating of rice bran, and the decomposition of tocols increased with increasing temperatures from 100 to 140 °C (Shin et al., 1997). A small decrease in tocols (11ï 17%) was observed by Lloyd et al. (2000) after steam heating at 120 °C, but this effect was not significant. Microwave heating also resulted in reduction of tocols by 10ï 79% as reported by Roselina et al. (2011). An experimental infrared (IR) stabilization system was recently developed (Yđmaz et al., 2013). Rice bran stabilization was achieved using eight different levels of Infrared power (200, 300, 400, 500, 600, 700, 800 and 900 W) for nine different times (1, 2, 3, 4, 5, 6, 7, 8 and 10 min), followed by a 6 months storage period. No significant change in ɔ-oryzanol content but a significant decrease in tocopherol content (12ï 47%) was observed in stabilized rice bran compared with raw bran (Yđmaz et al., 2013).

Conversely, rice bran stabilized with autoclave heating at 160 °C resulted in much higher contents of tocotrienols in addition to the formation of two new tocotrienols (Qureshi et al., 2002). In the study by Ko et al. (2003), tocol contents of microwave-heated bran were 2ï 10% higher than those of non-stabilized bran when the bran was subjected to microwave heating at 2450 MHz for 15ï 120 s. Unfortunately, the heating temperature was not recorded. When the

bran was heated in an electric roaster with constant stirring from 10 to 120 min at 170 °C, 3 to 30 min at 180 °C, and 2 to 20 min at 190 °C, tocol contents of roasted bran were 2ï 13% higher than those of non-stabilized bran. However, longer heating times resulted in a decreased content of tocols (Thanonkaew et al. (2012). Increases of 39% and of 122ï 156% for tocols were reported after autoclave and ohmic heating, respectively (Loypimai et al., 2009). The bran ɔ-oryzanol content was also found to increase following microwave heating (11%) and oven heating, roasting and steaming (6ï 13%) (Thanonkaew et al., 2012). The bran that underwent oven heating had the greatest amount of ɔ-oryzanol, and no difference in ɔ-oryzanol content was observed between bran that was roasted and steamed (Thanonkaew et al., 2012). Increases of 44% and of 54ï 58% for ɔ-oryzanol were reported after autoclave and ohmic heating, respectively (Loypimai et al., 2009). Non-enzymic lipid oxidation is likely to occur during stabilization and could explain the decreased tocol contents observed by some researchers. On the other hand, stabilization can effectively release membrane-bound tocotrienol and tocotrienol-like compounds or can inactivate enzymes involved in the degradation of antioxidants. Therefore, it is recommended that optimum conditions should be established when stabilizing rice bran in order to avoid loss of antioxidants.

Steam-extrusion heating did not affect the phytic acid content of rice in the study by Gualberto et al. (1997). The operation consisted of extruding the bran utilizing screw speeds of 50, 70 and 100% rpm, corresponding to temperatures of 135, 140, and 145 ºC, respectively. The temperature of all barrels, except the feed barrel, were set and controlled at 163 ºC. However, more studies are needed before conclusions can be drawn.

Influence of Soaking

Rice soaking or steeping is applied at both household and industrial scales to soften the grain (Lestienne et al., 2004; Liang et al., 2009b; Albarracín et al., 2013). It is generally the first procedure in processing techniques such as parboiling, fermentation, germination, cooking, and extrusion. After soaking, grains are usually separated from the soaking media by decanting (Liang et al., 2008a; Hiemori et al., 2009; Moongngarm and Khomphiphatkul, 2011; Wu et al., 2013b).

Phytic acid is widely studied in relation to soaking, probably because this compound has two opposing effects on human nutrition. In addition to its antioxidant potential, phytic acid is a strong chelator of nutrients such as Ca, Zn, and Fe, a process that significantly decreases absorption of micronutrients in the gastrointestinal tract (Abulude, 2004; Liang et al., 2009b). Thus, efforts have been made to adjust phytic acid to levels that maintain antioxidant function without substantially changing the bioavailability of minerals. Soaking brown rice in water led to 17% phytic acid removal after 24 h with slow shaking at room temperature (Lestienne et al., 2004); 42ï 59% after 24 h (Liang et al., 2008a); 7% after 48 h at room temperature, with water being changed every 8 h (Moongngarm and Khomphiphatkul, 2011); and 9ï 34% after 24 h (Noreen et al., 2009). In the latter case, however, two of six rices studied showed an increase (7ï 40%) phytic acid content after soaking. A 42ï 59% removal of phytic acid has been reported in brown rice when dry-preheated (30 min at 100 ºC) and wet-preheated (10 min at 115 ºC) grains were soaked at 10 ºC with demineralized water for 24 h, with no difference observed between dry and wet preheated samples (Liang et al., 2008a); and 75ï 100% in white rice when unheated

and preheated (100 ºC for 30 min) grains were soaked in demineralized water and NaAc buffer pH 3.5 at 10 ºC for 24 h, with higher reductions observed with acidic buffer compared with water (Liang et al., 2009b). In the study by Albarracín et al. (2013), brown rice was soaked with 6.6 g/L lactic acid solution for 24, 36 and 48 h, and at 35, 45, and 55 ºC. An 87ï 91% removal of phytic acid was observed, and the amount of phytic acid in the soaking solution increased with increasing temperatures. The most effective treatment was soaking at 45 ºC for 48 h (Albarracín et al., 2013). During soaking, phytic acid is hydrolyzed enzymatically by endogenous phytases or is broken down chemically into other inositol phosphates such as inositol pentaphosphate, inositol tetraphosphate, inositol triphosphate, and possibly inositol di- and monophosphates (Lestienne et al., 2004). Phytic acid may also diffuse into the soaking medium as observed by Liang et al. (2009b) and Albarracín et al. (2013). In the study by Lestienne et al. (2004), however, phytic acid was not detected in the soaking media.

To the authorsô knowledge, information about the effects of soaking on the contents of phenolic acids, tocols, and ɔ-oryzanol in rice have been reported only by Moongngarm and Khomphiphatkul (2011), who found no influence of soaking in water after 48 h at room temperature, but the hydroxyl ion scavenging activity and DPPH increased by 13% and 11%, respectively. For the TAC, both a decrease (19ï 40; Wu et al., 2013b) and no change (Hiemori et al., 2009) after soaking the whole grain of a black rice in water for 1 h at room temperature have been reported.

Influence of Germination

It has been known by indigenous peoples for centuries that to maximize the intake of essential nutrients, rice should be consumed in the form of whole grain (brown rice) (Hübner and Arendt, 2013; Wu et al., 2013a). However, brown rice is not suitable for cooking due to its poor texture, unpleasant odor, and low digestibility (Banchuen et al., 2009; Gujral et al., 2012; Sutharut and Sudarat, 2012; Azmi et al., 2013). Therefore, germination has been used to soften the grain structure of brown rice and to improve its cooking and organoleptic qualities. Germinated rice is produced by first soaking the grain in a solution for up to 24 h at room temperature. Control of microbial contamination is usually achieved by addition of chitosan (Kim and Jang, 2004). After soaking, grains are thoroughly rinsed with water and drained before being spread on trays covered with wet tissue paper. The grains are incubated at room temperature in disinfected dark locations (open or closed) until they begin to sprout (10ï 48 h) (Kim and Jang, 2004; Tian et al., 2004; Sungsopha et al., 2009; Moongngarm and Saetung, 2010; Azeke et al., 2011; Jayadeep and Malleshi, 2011; Gujral et al., 2012).

Germination of brown rice improves the antioxidant potential of rice by increasing the contents of phenolics. The following increases have been reported for the TPC: 57% in two rices after soaking in 0.1% sodium hypochlorite and 0.5% hydrogen peroxide for 30 min and 6 h, respectively, then germination of 18 h (Azmi et al., 2013); 9ï 12% in a rice after soaking in water for 12 h followed by 24 and 48 h germination (Gujral et al., 2012); 34ï 50% in a rice after soaking in water for 21 h (Tian et al., 2004); and 279% in a rice after soaking in 0.1% sodium hypochlorite and 0.5% hydrogen peroxide for 30 min and 6 h, respectively, followed by 18 h germination (Imam et al., 2012). In the study by Banchuen et al. (2009), brown rice was steeped in various solutions (phosphate buffer pH 7, citrate buffer pH 5, citrate buffer pH 3, and distilled

water) at room temperature (30 °C). After 5 h, the soaking solutions were drained off and the grains were wrapped with cheesecloth and left in the dark for 12, 24, 36, and 48 h to germinate; averagely, there was an increase of 43% in ferulic acid content. Sutharut and Sudarat (2012) recently compared germinated rough rice with germinated brown rice. In their study, rough rice and brown rice (three varieties) were soaked in water for 6 and 12 h before further left to germinate for 6, 12, 18, and 24 h. They found that brown rice obtained from germinated rough rice had higher levels of anthocyanins than brown rice obtained from germinated brown rice. Compared to non-germinated rice, germinated brown rice and germinated rough rice showed increased anthocyanin levels of 9ï 35% and 10ï 125%, respectively. Sutharut and Sudarat (2012) suggested that the husk might have prevented anthocyanin loss during germination and proposed that rough rice should be used rather than brown rice in the production of germinated rice. Moongngarm and Saetung (2010) reached a similar conclusion for the TPC. However, germination conditions were not identical in their study: 12 h soaking in water and 24 h germination for the brown rice, 48 h soaking in water and 48 h germination for the rough rice. The following increases have been reported for the TPC after germinating the rough rice: 58% in a rice after soaking in water for 3, 4, 5, and 6 days (Moongngarm and Khomphiphatkul, 2011); 20ï 56% in RD-6 rice after soaking in water for 48 h followed by 48 h germination (Moongngarm and Saetung (2010); and 33ï 67% after soaking rice in water for 3 days, with the soaking water being changed every 24 h (Lee et al., 2007). There is mention of decreased TPC (65ï 82%) and TFC (66ï 71%) following germination of rice (after soaking in water for 12 h) for 1, 2, 3, 4, 5, 6, and 7 days at 25 °C in three different solutions (water, lactic acid and chitosan solubilized in lactic acid and water) (Kim and Jang, 2004); however, it is difficult to reconcile

that decrease with the 9ï 11% increase in the DPPH reported by the same study (Kim and Jang, 2004). Otherwise, all authors have found a strong correlation between phenolic contents and in vitro antioxidant activities in rice after germination (Lee et al., 2007; Sungsopha et al., 2009; Jayadeep and Malleshi, 2011; Moongngarm and Khomphiphatkul, 2011; Gujral et al., 2012; Imam et al., 2012; Sutharut and Sudarat, 2012; Azmi et al., 2013). In the study by Imam et al. (2012), a good relationship was observed between the in vitro antioxidant activity measured by DPPH and ABTS and the ability of methanolic extracts of brown rice and germinated brown rice to improve glycemia in type 2 diabetic rats. Additionally, catalase and superoxide dismutase genes in HEPG2 cells were up-regulated, with germinated brown rice exhibiting higher efficacy than brown rice. In view of the higher antioxidant content (TPC, DPPH, ABTS) of germinated brown rice compared with brown rice, Azmi et al. (2013) studied the potential of the ethyl acetate extract of the two types of rice to prevent apoptosis and modulate processes leading to Alzheimerôs disease. The germinated brown rice extract prevented H_2O_2-induced apoptotic changes in human SH-SY5Y neuronal cells more than brown rice. Furthermore, multiple gene expression analyses showed that the protection of the cells by the extract was linked to their ability to induce transcriptional changes in antioxidant (SOD1, SOD2 and catalase) and apoptotic genes. Sungsopha et al. (2009) also found a strong correlation between in vitro reducing power and ex vivo inhibition of LDL oxidation when comparing brans from germinated and non-germinated rice, but not between DPPH and ex vivo inhibition of LDL oxidation. Compared to DPPH and FRAP, the LDL oxidation assay clearly has relevance to oxidative reactions that might occur in vivo (Prior et al., 2005). However, it is doubted that these effects were due to antioxidant compounds alone as several bioactive compounds such as gamma-aminobutyric acid

and dietary fibers were also increased in the germinated rice. The metabolic activity of the dry rice grain increases as soon as it is hydrated (Wu et al., 2013a). As the grain starts to sprout during germination, several hydrolytic enzymes are activated (Kiing et al., 2009; Gujral et al., 2012). Desirable changes in antioxidants stem primarily from the ability of those enzymes to hydrolyze high-molecular-weight polymers into more simple forms. This is the case for phenolics, which are mostly ether- or ester-linked to cell wall polymers (mainly polysaccharides and lignin) (Goufo et al., 2014b). Tian et al. (2004) isolated two hydroxycinnamate sucrose esters from rice; a 70% decrease in ester contents was observed during germination, whereas free phenolic acid contents increased (Tian et al., 2004).

Germination further increased the total tocol content of brown rice by 46ï 61% in RD-6 rice after soaking in water for 12 h followed by 24 h germination (Moongngarm and Saetung, 2010); 33% in BPT rice after soaking in water for 16 h followed by 5 days germination (Jayadeep and Malleshi, 2011); 47ï 135% in a rice after 3, 4, 5, and 6 days in water (Moongngarm and Khomphiphatkul, 2011); and 125% in a rice bran after soaking in water for 48 h followed by 48h germination (Sungsopha et al., 2009). The following increases in ɔ-oryzanol have been reported: 29% (Sungsopha et al., 2009), 27ï 61% (Moongngarm and Saetung, 2010), and 40% (Moongngarm and Khomphiphatkul, 2011). Some authors, however, did not find any significant difference between the ɔ-oryzanol content of germinated and non-germinated rice (Banchuen et al., 2009; Jayadeep and Malleshi, 2011), whereas decreases of 9ï 69% were found after germination of five Malaysian rices for 24 h in water (Kiing et al., 2009). In the latter study, however, the ɔ-oryzanol content increased by 18ï 28% in three of the eight rices studied (Kiing et al., 2009).

Phytic acid is degraded during germination, with the following decreases reported: 17ï 22% after soaking in water for 12 h followed by 1ï 7 days germination (Kim and Jang, 2004); 27% after 3ï 6 days in water (Moongngarm and Khomphiphatkul, 2011); 13ï 30% after soaking in water for 12 h followed by 24 h germination (Moongngarm and Saetung, 2010); 13ï 40% after 3 days in water (Lee et al., 2007); 4ï 60% after soaking in water for 4ï 24 h followed by 12ï 72 h germination (Liang et al., 2008a); 29% (average) after soaking for 5 h in phosphate buffer pH 7, citrate buffer pH 5, citrate buffer pH 3 or water, followed by 12, 24, 36, and 48 h germination (Banchuen et al., 2009); and 5ï 83% after soaking in 0.5% sodium hypochlorite/0.75% hydrogen peroxide for 5 min. followed by 1ï 10 days germination (Azeke et al., 2011).

Influence of Fermentation

Fermented rice has been used in Asian, South American and African cuisine and medicine for centuries (Reddy and Salunkhe, 1980; Shekib, 1988; Cuneo et al., 2000). Beside its direct consumption, fermented rice is also used as a coloring and flavoring agent to preserve and enhance the appearance of foods, mainly fish and meat (Yen et al., 2003; In et al., 2009). The technique of conventional solid-state fermentation (SSF) has been widely applied to produce fermented rice. The traditional SSF technique involves the growth and metabolism of microorganisms on moist solid substrates in the absence of free-flowing water. First, rice is soaked, cooked or steamed in water for 1ï 6 h. After cooling, viscous materials in the rice are washed out with water if necessary. The rice is then sterilized at 121 °C for 15ï 30 min and inoculated with the microorganism. The inoculated rice is incubated at 20ï 35 °C (the conditions

for optimal propagation and mycelium growth) for 1ï 3 weeks. The end product is traditionally sun dried or oven dehydrated at 45ï 65 °C for 6ï 24 h. During fermentation, the culture is generally rotated and agitated under aseptic conditions to supply the microorganisms with oxygen (Yen et al., 2003; Yang et al., 2006; In et al., 2009; Liang et al., 2009a; Kim and Han, 2011; Manosroi et al., 2011; Bao et al., 2013). In modern fermentation, bioreactors are used with low levels of liquid. Generally, rice is covered, sterilized, and placed on trays in the bioreactors. A saline solution is added prior to fermentation. After addition of the microorganisms, the medium is homogenized by adding sterile water to adjust the moisture content to approximately 50%. The trays are then covered with sterile cotton cloth, which enables aeration, and are kept in an incubator at 30 °C for 5ï 10 days (Oliveira et al., 2012).

Several studies have shown that fermentation increases the contents of phenolics in rice. The following increases have been reported for the TPC: 780% in brown rice inoculated with *Pleurotus eryngii* and incubated at 25°C for 40 days (Bao et al., 2013); 32ï 900% in brown rice and white rice inoculated with *Monascus purpureus* and incubated at 25°C for 7 days (Yang et al., 2006); 94% in white rice inoculated with *Phellinus linteus* and incubated at 30 ºC for 14 days (Liang et al., 2009a); 40% in bran inoculated with *Issatchenkia orientalis* at 35 °C for 5 days (Kim and Han, 2011); 14ï 27% in brown rice (five varieties) inoculated with Look Pang powder (containing 3 genus of molds ï *Aspergillus* sp., *Mucor* sp., *Rhizopus* sp. ï 2 species of yeasts ï *Candida krusii*, *Saccharomyces cerevisiae* ï and 1 genus of yeasts ï *Candida* sp. ï) and incubated at 25 °C for 2ï 8 days (Manosroi et al., 2011); 150ï 358% in bran inoculated with *Rhizopus oryzae* in a bioreactor at 25 °C for 1ï 5 days (Oliveira et al., 2012); and 99% in white rice inoculated with *Aspergillus candidus* and incubated at room temperature for 15 days (Yen et

al., 2003). A 130% (Liang et al., 2009a), and a 150% (Manosroi et al., 2011) increase was reported for TFC and TAC, respectively. These numbers clearly show that rice products obtained from fermentation exhibit stronger antioxidant activities than those obtained from germination. The use of enzymes for rice fermentation has also been considered. Sungsopha et al. (2009) described the use of protease and Ŭ-amylase, and Tananuwong and Tangsrianugul (2013) described the use of pepsin, pancreatin, Ŭ-amylase and amyloglucosidase. The greatest increases in TPC were obtained following fermentation with enzymes, with increases as high as 275% in the bran (Sungsopha et al., 2009) and 203% in the brown rice (Tananuwong and Tangsrianugul, 2013). Increases in the contents of phenolics during fermentation can be explained by the ability of microorganisms to degrade lignocellulosic materials due to their highly efficient enzymatic systems. Fungi for example have two types of extracellular enzymatic systems: the hydrolytic system, which produces hydrolases that are responsible for polysaccharide degradation; and a unique oxidative and extracellular ligninolytic system, which degrades and opens phenyl rings (Oliveira et al., 2012). Furthermore, oxidation of phenolics by oxidative enzymes is minimal during fermentation because of the consumption of oxygen by microorganisms (Yen et al., 2003; In et al., 2009; Bao et al., 2013). Fermentation further improves the antioxidant activity of rice (Yen et al., 2003; Yang et al., 2006; Liang et al., 2009a; Sungsopha et al., 2009; Kim and Han, 2011; Manosroi et al., 2011; Oliveira et al., 2012; Bao et al., 2013; Tananuwong and Tangsrianugul, 2013). In their study, Kim and Han (2011) also investigated whether *Issatchenkia orientalis*-fermented rice bran could ameliorate the oxidative stress induced by high glucose and hydrogen peroxide in 3T3-L1 adipocytes. Compared to the non-fermented bran, the fermented bran strongly inhibited ROS generation and TNF-Ŭ expression, and up-regulated the expression

of PPAR-ɔ and adiponectin. However, the mRNA expression of glucose transporter type 4 was not changed by the treatments, indicating that the fermented bran extract could not directly affect glucose transportation. Manosroi et al. (2011) also showed a strong positive relationship between bioactive compound contents (unsaturated fatty acids, TPC, and TAC) and biological activities (tyrosinase, cell proliferation and MMP-2 inhibition activities) of fermented rice. However, the health-improving properties of fermented rice could not have been caused by the release of phenolic acids, but by other compounds released by the fermenting microorganism. For example, *Monascus purpureus* produces Monacolin K, a specific inhibitor of HMGCoA-R in cholesterol biosynthesis (Yang et al., 2006).

Interestingly, fermentation is reported to increase tocols and ɔ-oryzanol contents in bran and brown rice, but to decrease these compounds in white rice. Tocol increases of 100ï 500% have been reported in brown rice inoculated with *Monascus purpureus* and incubated at 25°C for 7 days (Yang et al., 2006). Fermentation with protease and Ŭ-amylase increased the levels of bran ɔ-oryzanol and Ŭ-tocopherol by 23% and 345%, respectively (Sungsopha et al., 2009). In white rice, however, decreases in tocol levels of 100% (Liang et al., 2009a) and 52ï 345% (Yang et al., 2006) have been reported. However, any conclusions should be considered as preliminary because of the small number of studies reporting that behavior.

In all studies published to date, fermentation reduced the phytic acid content of rice, with the highest losses occurring within the first 24 h. Fermentation of brown rice with an acidifying microbota (composition unknown) for 1ï 7 days at 30 °C resulted in a 56ï 96% hydrolysis of phytic acid (Liang et al., 2008a). The following reductions were also observed: 56% in brown rice inoculated with *Leuconostoc mesenteroides* at 30 °C for 18 h (In et al., 2009), 80ï 90% in

brown rice fermented naturally for 48ï 72 h (Marfo et al., 1990), and 26ï 37% in brown rice (alone or added with whey) fermented naturally at 35 °C for 18 h (Sharma and Khetarpaul, 1997). The loss of phytic acid during fermentation might be a result of the activity of phytase that is naturally present in rice, but also may be caused by phytase and phosphatase produced by fermentative microorganisms. Fermentation using an exogenous phytase strain resulted in a 100% loss of phytic acid in white rice, bran, and brown rice after 24ï 172 h at 50 °C (Liang et al., 2009b) and 37% loss in bran after 24 h at 55 °C (Cuneo et al., 2000). Fermentation appears more effective than soaking or germination in reducing phytic acid, since the organic acids produced by microorganisms reduce the pH of the mixture close to the optimum pH for phytase activity (Liang et al., 2009b).

Influence of Boiling and Steaming

In most cases, rice is prepared for consumption by cooking (Abulude, 2004; Noreen et al., 2009; Chamnarnsin and Ahromrit, 2011; Semsang et al., 2012; Min et al., 2014). Rice cooking techniques vary widely and are driven by individual preferences, environmental and economic factors, and cultural traditions. Although this study cannot provide a detailed documentation of all rice cooking techniques, they principally differ according to the following three factors: (i) the heating technique (boiling or steaming); boiling is performed by heating rice in a liquid (usually water); steaming works by keeping rice separated from water but in direct contact with the steam produced by boiling water (Toma and Tabekhia, 1979; Hiemori et al., 2009; Saikia et al., 2012); (ii) the cooking instrument (a conventional electric/gas rice cooker, an electric/gas

pressure cooker, or a stainless pot covered with a lid or left opened); for most rice cookers, the time for which the cooker light remains on is considered the cooking time. When using pots, complete cooking is indicated by full gelatinization of the grains or by loss of opaque, uncooked portions when grains are pressed between glass slides (Finocchiaro et al., 2007; Maisuthisakul and Changchub, 2012; Jantasee et al., 2013); (iii) the rice:water (w/v) ratio (optimum-water-level and in-excess-water-level); the optimum-water-level method allows complete absorption of water by the rice usually using a rice:water ratio Ò 1:4 (w:v) (Marfo et al., 1990; Finocchiaro et al., 2007). The cooking temperature (60ï 100 °C) and duration (10ï 120 min) will vary depending on the method chosen.

Several studies have investigated the effects of thermal processing on the stability of rice antioxidants and have generally found a significant reduction in levels of all phenolic compounds. For the TPC, decreases of 32ï 94% were observed in twenty grains (four rices) soaked in 40 mL distilled water for 30 min at room temperature (27 °C), then cooked by steaming in an autoclave at 100 °C for 10 min; the cooked rice was analyzed after 15, 45, and 90 min with time between analyses having no influence on the reductions (Saikia et al., 2012). In the study by Maisuthisakul and Changchub (2012), brown rice was added with water at a rice:water ratio of 1:2 (two red rices and four non-pigmented rices) and 1:3 (three black rices). Cooking in an electric rice cooker for 1 h resulted into a 21ï 96% decrease of the TPC in eight rices. Finocchiaro et al. (2007) in their study cooked three fractions (0% milled, 3% milled and 6% milled) of two rices at a rice:water ratio of 1:20 (in-excess-water-level) and 1:3.6 (optimum-water-level) for 17ï 40 min depending on the rice variety. The loss of phenolics (44ï 100% for ferulic, sinapic, *p*-coumaric, and caffeic acids) was lower using the optimum-water-level method

compared to the in-excess-water-level method, which could be due to leaching of antioxidants in the liquid used for cooking in the latter method. Other reported TPC losses are as follow: 80ï 90% in grains of Homv Daeng rice cooked in an automatic rice cooker at a rice:water ratio of 1:2 (Tananuwong and Tangsrianugul, 2013); 16ï 57% in grains of three rices cooked in an automatic rice cooker at a rice:water ratio of 1:2 for 40 min (Min et al., 2014); 21ï 72% in grains of 18 rices (parboiled and non-parboiled) cooked at a rice:water ratio of 1:2.5 for 30 min, with losses lower for parboiled rices (Walter et al., 2013); 30ï 47% in grains of 48 rices soaked in water at a rice:water ratio of 1:1.5 and cooked over boiling water (Jantasee et al., 2013); and 12ï 51% in grains of 16 rices cooked for 30 min in a partially covered beaker at a rice:water ratio of 1:4 (Massaretto et al., 2011). For the TFC, losses of 81ï 96% (Saikia et al., 2012) and 38ï 64% (Jantasee et al., 2013) have been reported after cooking. The thermal stability of anthocyanins was assessed in black rice cooked using a conventional rice cooker (90 min), a pressure rice cooker (20 min), or a pot (50 min) (Hiemori et al., 2009) at 1:1.8 grain mass per water volume ratio. The pressure rice cooker resulted in the greatest loss of anthocyanins, followed by the conventional rice cooker and pot. These reductions (65ï 80% for cyanidin-3-*O*-glucoside and peonidin-3-*O*-glucoside) were found to be pH- and temperature dependent, but also to be influenced by process parameters such as glycosidic linkages and food-matrix interactions that occurred during cooking. For example, stability of anthocyanins is reported to increase with the number of methoxyls in the B ring and to decrease as hydroxyls increase (Hiemori et al., 2009). Decreases of 24ï 66% in cyanidin-3-*O*-glucoside and peonidin-3-*O*-glucoside have also been reported after soaking a black rice in water for 1 h, followed by steaming at 100 °C for 20 min (Wu et al., 2013). Reported TAC losses due to cooking are as follows: 46ï 85% (Min et al., 2014),

14ï 51% (Maisuthisakul and Changchub, 2012), and 42ï 66% (Jantasee et al., 2013). In a study by Saikia et al. (2012), anthocyanins were no longer detectable after steaming. For the total TPAC, losses of 64ï 90% (Jantasee et al., 2013) and 60ï 97% (Finocchiaro et al., 2007) have been reported. It has also been noted that for similar cooking times, losses of phenolic compounds tend to be lower for non-pigmented rice than for pigmented rice varieties (Massaretto et al., 2011; Maisuthisakul and Changchub, 2012), probably due to their different soluble/insoluble phenolic ratios. Reductions in phenolics during thermal processing are probably a consequence of degradation of phenolic compounds, depolymerization of higher oligomeric and polymeric structures into dimers and trimers (e.g., proanthocyanidins), polymerization, oxidation, and the formation of Maillard reaction products (which reduce the solubility of phenolic compounds). Although thermal processing can destroy the cell-wall structures and release phenolics from their bound forms, degradation and depolymerization appear to be the dominant reactions at play during cooking. For example, cyanidin-3-*O*-glucoside undergoes deglycosylation during heating and produces cyanidin, which is further degraded into phloroglucinaldehyde and protocatechuic acid (Hiemori et al., 2009; Min et al., 2010). However, Tananuwong and Tangsrianugul (2013) reported a situation in which enhanced extractability of insoluble phenolics overcame the effect of thermal destruction, resulting in greater contents of anthocyanins in the grains after cooking. The in vitro total antioxidant activity of rice evaluated by DPPH, FRAP, and ABTS is also substantially reduced by boiling as a result of reduced phenolics (Finocchiaro et al., 2007; Saikia et al., 2012; Jantasee et al., 2013; Tananuwong and Tangsrianugul, 2013). In the study by Massaretto et al. (2011), the decrease in the TPC after cooking was accompanied by a proportional reduction in the capacity of rice extracts to inhibit the activity of the angiotensin I-

converting enzyme, which is a key component of the rennin-angiotensin system that controls blood pressure (Huang et al., 2013). However, Semsang et al. (2012) did not find any change in the total antioxidant activity (DPPH, FRAP, and ABTS) in rice after cooking, although the TPC decreased.

Contrary to expectation, cooking is reported to increase contents of tocols and ɔ-oryzanol in rice, although there are conflicting reports which suggest differences depending on the rice cultivar. For tocols, increases of 15ï 59% have been reported in brown rice using both ñin-excess-water-levelò and ñoptimum-water-levelò cooking, although tocol content in white rice decreased by 16ï 57% (Finocchiaro et al., 2007). Four Thai pigmented rices were cooked at 65, 75, 85, and 95 °C for 30ï 120 min at a rice:water ratio of 1:5. The ɔ-oryzanol content of cooked rice increased (36ï 88%) as the temperature increased for all cooking durations (Chamnarnsin and Ahromrit, 2011). The increases in ɔ-oryzanol content were tentatively related to the fact that loss of soluble dry matter occurs during cooking, resulting in a higher content of lipophilic compounds per unit dry matter. In addition, tocol molecules that are strongly linked to cellular components can be released during heat treatment (Qureshi et al., 2001). Those studies (Qureshi et al., 2001; Finocchiaro et al., 2007; Chamnarnsin and Ahromrit, 2011) contradict the findings of Pascual et al. (2011) that cooking three rices (non-parboiled and parboiled) for 30 min in a partially covered beaker at a rice:water ratio of 1:4 decreased the contents of tocols and ɔ-oryzanol in brown rice by 71ï 88% and 20ï 33%, respectively. They also contradict the findings of Min et al. (2014), where cooking of six rices (non-parboiled and parboiled) for 40 min in a rice cooker at a rice:water ratio of 1:2 increased the tocol content of one rice, but has no effect on the other five rices. Finocchiaro et al. (2007) also found that ɔ-oryzanol content in white rice and

brown rice of pigmented rice decreased by 8ï 98% after cooking. However, cooking provoked an increase of ɔ-oryzanol concentration in the brown rice of non-pigmented rice.

Boiling reduced the phytic acid content in white rice cooked for 30 min in pots at a rice:water ratio of 1:3 by 31% (Marfo et al., 1990), and that in brown rice of five rices by 10ï 30% (Noreen et al., 2009).

Influence of Baking and Roasting

In most rice consuming-countries in which the Western diet is increasingly popular, maintenance of rice consumption is principally pursued through the development of dried rice products used as breakfast cereals or snacks (Noreen et al., 2009; Gujral et al., 2012). The most popular rice-derived products include puffed/popped rice, rice cakes, crackers, breads, and pasta (Hirawan et al., 2011; Ekasit and Jiraporn, 2013). Baking, roasting, and frying are common industrial processes used to produce these products, as are treatments such as extrusion, pasteurization, and sterilization. Compared with boiling and steaming, which use temperatures below 100 °C, the preparation of these products involves subjecting rice to temperatures of 100ï 300 °C. The preparation of products such as canned rice, rice gruel, and pudding also uses temperatures above 100 °C (Hurrell et al., 2002; Wu et al., 2013b).

As with cooking, heating is a major factor affecting the stability of rice antioxidants during baking and roasting. However, these treatments are found to cause more antioxidant losses than boiling. Upon extrusion at 100 °C using the grit from un-germinated and germinated brown rice, the TPC decreased by 50%. A further decrease of 6ï 15% was observed when the extrusion

temperature was increased from 100 to 120 °C (Gujral et al., 2012). Increasing the temperature from 100 °C (home-made infant cereal) to 120 °C (industrial-made infant cereal) for 30 min during the roasting step in the preparation of infant cereals increased the TPC loss from 18ï 32% to 29ï 36% (Hirawan et al., 2011). ORAC losses also changed from 7ï 22% to 9ï 25% (Hirawan et al., 2011). ORAC measures antioxidant inhibition of peroxyl radicals and thus is more closely related to the physiologically chain breaking antioxidant activity than is DPPH or FRAP (Prior et al., 2005). Home-made infant cereals also had significantly higher cellular antioxidant activities (measured in vitro in the human fetal small intestine cell line FHs 74Int) than industrial-made cereals, suggesting that exposure to higher temperatures during preparation of industrial-made infant cereals might contribute to loss of cellular-effective antioxidants (Hirawan et al., 2011). Large decreases of the TAC (26ï 72) in rice after roasting the cooked black rice at 150 °C for 40 min have also been reported by Wu et al. (2013).

Khao Mao, a popular snack in Thailand, is made from young flattened glutinous rice. To produce Khao Mao, rough rice grains are soaked in water for 6 h, then steamed and roasted or directly roasted at 100 °C for 2 h, then pounded. The flattened grains are separated from broken husk and bran by sieving and winnowing. During the preparation of Khao Mao, rice lost 6ï 56% of its ɚ-oryzanol content (Ekasit and Jiraporn, 2013). When rice bran was heated at 180 °C for 50 h, ɔ-oryzanol was no longer detected; losses of ɚ-oryzanol and its four major components could be described by a first-order kinetics model (Khuwijitjaru et al., 2011).

During the preparation of Kheer, a Pakistani rice-derived product, baking caused a 6ï 33% loss of phytic acid in rice (Noreen et al., 2009); roasting at 120 °C for 30 min during the preparation of infant cereals caused a 7ï 69% loss of phytic acid in brown rice (Frontela et al.,

2008); phytic acid tended to disappear when rice (with and without an Ŭ-amylase pre-treatment) was subjected to extrusion cooking (160 °C) or steam injection/roller-drying (135 °C) for a few minutes during the preparation of cereal-based foods (Hurrell et al., 2002). The temperatures to which rice is subjected during roasting/toasting/frying would not favor endogenous phytase activity, since this enzyme has optimal activity at pH 4-5 and 40-50 °C (Frontela et al., 2008; Noreen et al., 2009); therefore, the observed phytic acid degradation can be attributed to heat only.

CONCLUSIONS

Environmental factors may produce either beneficial or deleterious effects on the antioxidant composition of rice. Similarly, rice processing can increase the content of some antioxidants and decrease that of others. Negative effects occur primarily by oxidation of antioxidants, activation of enzymes that degrade antioxidants, or leaching of these compounds from the grain. In most cases, increased antioxidant contents reported during processing resulted from the conversion of insoluble/bound antioxidants into soluble antioxidants (Ragaee et al., 2012; Rastogi, 2012; Ktenioudaki et al., 2013; Nayak et al., 2013; Zhao et al., 2013). There is strong evidence that stabilization increases the bioavailability of rice tocotrienols in humans (Qureshi et al., 2002). Little however, is known about the other rice antioxidants. Bio-processing techniques such as fermentation and germination also have the potential to increase the bio-accessibility of antioxidant compounds bound to the matrix (Gupta and Abu-Ghannam, 2012; Hübner and Arendt, 2013; Kanmani et al., 2013). Whether these techniques can influence the

bioavailability and metabolic fate of rice antioxidants compounds in vivo remains to be determined. Insoluble antioxidants generally reach the colon in an undigested form, where they are modified by microflora to yield different beneficial compounds that may be progressively absorbed into the circulatory system (Gualberto et al., 1997; Wilson et al., 2007; Vitaglione et al., 2008). Insoluble antioxidants also survive gastrointestinal digestion and act extracellularly to prevent colorectal cancer (Weed, 2013; Visioli et al., 2011). Therefore, most processed rice products lack the colon-improving properties of unprocessed rice. Reported increases in antioxidant contents also reflect the loss of dry matter (mainly carbohydrates) during processing. There is a large amount of data on the antioxidant composition of various rice cultivars grown worldwide. Because it is not possible to obtain cultivar-specific processing data for all varieties, the changes presented in this review can guide nutritionists and epidemiologists in estimating dietary intake. Fortification of nutrients by processing to address particular deficiencies has recently emerged as a field of study; hence, this review should be useful to researchers working to enhance the nutraceutical value of rice. Environmental manipulation, in conjunction with genetic selection based on the information presented here, could be used by growers to breed and produce grains with a better ratio of antioxidant compounds. This review covered only publications that contained at least one of the following keywords in their titles: rice or cereal + phenolic, flavonoid, anthocyanin, tocopherol, tocotrienol, ɔ-oryzanol, phytic acid, or antioxidant (144 papers). More papers are probably available, but it is unlikely that their results would contradict the conclusions presented in this study.

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REFERENCES

Abulude, F. O. (2004). Effect of processing on nutritional composition, phytate and functional properties of rice (*Oryza sativa* L.) flour. *Niger. Food J.* **22**: 97ï 104.

Ahn, D. J., Won, J. G., Rico, C. M., and Lee, S. C. (2010). Influence of variety, location, growing year, and storage on the total phosphorus, phytate-phosphorus, and phytate-phosphorus to total phosphorus ratio in rice. *J. Agric. Food Chem.* **58**: 3008ï 3011.

Albarracín, M., González, R. J., and Drago, S. R. (2013). Effect of soaking process on nutrient bio-accessibility and phytic acid content of brown rice cultivar. *LWT* ï *Food Sci. Technol.* **53**: 76ï 80.

Azeke, M. A., Egielewa, S. J., Eigbogbo, M. U., and Ihimire, I. G. (2011). Effect of germination on the phytase activity, phytate and total phosphorus contents of rice (*Oryza sativa*), maize (*Zea mays*), millet (*Panicum miliaceum*), sorghum (*Sorghum bicolor*) and wheat (*Triticum aestivum*). *J. Food Sci. Technol.* **48**: 724ï 729.

Azmi, N. H., Norsharina, I., Mustapha, U. M., and Maznah, I. (2013). Ethyl acetate extract of germinated brown rice attenuates hydrogen peroxide-induced oxidative stress in human SH-SY5Y neuroblastoma cells: role of anti-apoptotic, pro-survival and antioxidant genes. *BMC Complement. Altern. Med.* **13**: 177.

Azrina, A., Maznah, I., and Azizah, A. H. (2010). Extraction and determination of oryzanol in rice bran of mixed herbarium UKMB, AZ 6807: MR 185, AZ 6808: MR 211, AZ6809: MR 29. *ASEAN Food J.* **15**: 89ï 96.

Banchuen, J., Thammarutwasik, P., Ooraikul, B., Wuttijumnong, P., and Sirivongpaisal, P. (2009). Effect of germinating processes on bioactive component of Sangyod Muang Phatthalung Rice. *Thai J. Agric. Sci.* **42**: 191ï 199.

Bao, L., Li, Y., Wang, Q., Han, J., Yang, X., Li, H., Wang, S., Wen, H., Li, S., and Liu, H. (2013). Nutritive and bioactive components in rice fermented with the edible mushroom *Pleurotus eryngii*. *Fungal Biol.* **4**: 96ï 102.

Berger, A., Rein, D., Schäfer, A., Monnard, I., Gremaud, G., Lambelet, P., and Bertoli, C. (2005). Similar cholesterol-lowering properties of rice bran oil, with varied ɔ-oryzanol, in mildly hypercholesterolemic men. *Eur. J. Nutr.* **44**: 163ï 173.

Berger, R. G., Lunkenbein, S., Ströhle, A., and Hahn, A. (2012). Antioxidants in food: mere myth or magic medicine? *Crit. Rev. Food Sci. Nutr.* **52**: 162ï 171.

Britz, S. J., Prasad, P. V. V., Moreau, R. A Jr., Allen, L. H., Kremer, D. F., and Boote, K. J. (2007). Influence of growth temperature on the amounts of tocopherols, tocotrienols, and ɔ-oryzanol in brown rice. *J. Agric. Food Chem.* **55**: 7559ï 7565.

Butsat, S., and Siriamornpun, S. (2010). Antioxidant capacities and phenolic compounds of the husk, bran and endosperm of Thai rice. *Food Chem.* **119:** 606ï 613.

Butsat, S., Weerapreeyakul, N., and Siriamornpun, S. (2009). Changes in phenolic acids and antioxidant activity in Thai rice husk at five growth stages during grain development. *J. Agric. Food Chem.* **57**: 4566ï 4571.

Canan, C., Delaroza, F., Casagrande, R., Baracat, M. M., Shimokomaki, M., and Ida, E. I. (2012). Antioxidant capacity of phytic acid purified from rice bran. *Acta Sci. Technol.* **34**: 457ï 463.

Chae, J. C., Lee, D. J., Jun, D. K., Ryu, S. N., and Shin, J. C. (2004). Changes of anthocyanin pigment cyanidin-3-glucoside, oryzanol content and antioxidant activity as affected by ripening temperature in rice varieties. **In**: Proceedings of the 4th International Crop Science Congress. Brisbane, Australia.

Chamnarnsin, P., and Ahromrit, A. (2011). Effect of heating on gamma-oryzanol content and degree of gelatinization of Thai colored rice. **In**: Proceedings of the 12th ASEAN Food Conference, 16 18 June 2011. BITEC Bangna, Bangkok, Thailand.

Chaudhary, N., and Khurana, P. (2009). Vitamin E biosynthesis genes in rice: Molecular characterization, expression profiling and comparative phylogenetic analysis. *Plant Sci.* **177**: 479ï 491.

Chen, G., Wang, H., Zhang, X., and Yang, S-T. (2013). Nutraceuticals and functional foods in the management of hyperlipidemia. *Crit. Rev. Food Sci. Nutr.* **DOI:** 10.1080/10408398.2011.629354.

Chen, P. N., Kuo, 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**: 218ï 229.

Chitropas, P., Priprem, A., Siri, B., Khamlert, C., and Sripanidkulchai, B. (2004). Factors affecting antioxidation and gamma-oryzanol in developed Hom Dok Mali 105 rice bran tablets. *Khon Kaen Univ. Res. J.* **9**: 59ï 67.

Cho, J. Y., Lee, H. J., Kim, G. A., Kim, G. D., Lee, Y. S., Shin, S. C., Park, K. Y., and Moon, J. A. (2012). Quantitative analyses of individual ɔ-Oryzanol (Steryl Ferulates) in conventional and organic brown rice (*Oryza sativ*a L.). *J. Cereal Sci.* **55**: 337ï 343.

Cohen, S. D., and Kennedy, J. A. (2010). Plant metabolism and the environment: implications for managing phenolics. *Crit. Rev. Food Sci. Nutr.* **50**: 620ï 643.

Cordero, Z., Drogan, D., Weikert, C., and Boeing, H. (2010). Vitamin E and risk of cardiovascular diseases: a review of epidemiologic and clinical trial studies. *Crit. Rev. Food Sci. Nutr.* **50**: 420ï 440.

Cuneo, F., Amaya-Farfan, J., and Carraro, F. (2000). Phytate distribution in stabilized rice bran treated with exogenous phytase. *Ciência Tecnol. Alime.* **20**: 94ï 98.

Damayanthi, E. (2001). Rice bran stabilization and ɔ-oryzanol content of two local paddy varieties "IR 64" and "Cisadane Muncul". *J. Teknol. Ind. Pangan* **12**: 72ï 76.

Deng, G. F., Xu, X. R., Zhang, Y., Li, D., Gan, R. Y., and Li, H. B. (2013). Phenolic compounds and bioactivities of pigmented rice. *Crit. Rev. Food Sci. Nutr.* **53**: 296ï 306.

Depar, N., Rajpar, I., Sial, N. B., and Keerio, M. I. (2013). Grain phytic acid accumulation of domestic and exotic rice genotypes in zinc-deficient soil. *J. Basic Appl. Sci.* **9**: 26ï 30.

Dutta, A. K., Gope, P. S., Banik, S., Makhnoon, S., Siddiquee, M. A., and Kabir, Y. (2012). Antioxidant properties of ten high yielding rice varieties of Bangladesh. *Asian Pac. J. Trop. Biomed.* **2:** S99ï S103.

Ekasit, O., and Jiraporn, B. (2013). Some physical characteristics and bioactive compounds of young flattened rice (Khao-Mao). *Int. Food Res. J.* **20**: 1323ï 1328.

Fardet, A., and Chardigny, J-M. (2013). Plant-based foods as a source of lipotropes for human nutrition: a survey of in vivo studies. *Crit. Rev. Food Sci. Nutr.* **53**: 535ï 590.

Finocchiaro, F., Ferrari, B., Gianinetti, A., Dall'asta, C., Galaverna, G., Scazzina, F., and Pellegrini, N. (2007). Characterization of antioxidant compounds of red and white rice and

changes in total antioxidant capacity during processing. *Mol. Nutr. Food Res.* **51**: 1006ï 1019.

Frank, T., Meuleye, B. S., Miller, A., Shu, Q. Y., and Engel, K. H. (2007). Metabolite profiling of two low phytic acid (lpa) rice mutants. *J. Agric. Food Chem*. **55**: 11011ï 11019.

Frontela, C., Garcia-Alonso, F. J., Ros, G., and Martēnez, C. (2008). Phytic acid and inositol phosphates in raw flours and infant cereals: The effect of processing. *J. Food Comp. Anal.* **21**: 343ï 350.

Fujita, A., Fujitake, H., Kawakami, K., and Nomura, M. (2010). Antioxidant activity of colored rice bran obtained at different milling yields. *J. Oleo Sci.* **59**: 563ï 568.

Ghatak, S. B., and Panchal, S. J. (2012). Protective effect of oryzanol isolated from crude rice bran oil in experimental model of diabetic neuropathy. *Braz. J. Pharmacog.* **22**: 1092ï 1103.

González, R., Ballester, I., López-Posadas, R., Suárez, M. D., Zarzuelo, A., Martínez-Augustin, O., and Sánchez de Medina, F. (2011). Effects of flavonoids and other polyphenols on inflammation. *Crit. Rev. Food Sci. Nutr.* **51**: 331ï 362.

Gopala, K. A. G., Prabhakar, J. V., and Sen, D. P. (1984). Effect of degree of milling on tocopherol content of rice bran. *J. Food Sci. Technol.* **21**: 222ï 224.

Goufo, P. (2008). Evaluating the constraints and opportunities for sustainable rice production in Cameroon. *Res. J. Agric. Biol. Sci.* **4:** 734ï 756.

Goufo, P., Pereira, J., Figueiredo, N., Oliveira M. B. P. P., Carranca, C., Rosa, E. A. S., and Trindade, H. (2014a). Effect of elevated carbon dioxide (CO_2) on phenolic acids, flavonoids, tocopherols, tocotrienols, ɔ-oryzanol and antioxidant capacities of rice (*Oryza sativa* L.). *J. Cereal Sci.* **59:** 15ï 24.

Goufo, P., and Trindade, H. (2014b). Rice antioxidants: phenolic acids, flavonoids, anthocyanins, proanthocyanidins, tocopherols, tocotrienols, ɔ-oryzanol, and phytic acid. *Food Sci. Nutr.* **DOI**: 10.1002/fsn3.86.

Gualberto, D. G., Bergman, C. J., Kazemzadeh, M., and Weber, C. W. (1997). Effect of extrusion processing on the soluble and insoluble fiber, and phytic acid contents of cereal brans. *Plant Foods Hum. Nutr.* **51**: 187ï 198.

Gujral, H. S., Sharma, P., Kumar, A., and Singh, B. (2012). Total phenolic content and antioxidant activity of extruded brown rice. *Int. J. Food Prop.* **DOI**: 10.1080/10942912.2010.483617.

Guo, H., Ling, W., Wang, Q., Liu, C., Hu, Y., Xia, M., Feng, X., and Xia, X. (2007). Effect of anthocyanin-rich extract from black rice (*Oryza sativa* L. indica) on hyperlipidemia and insulin resistance in fructose-fed rats. *Plant Foods Hum. Nutr.* **62**: 1ï 6.

Gupta, S., and Abu-Ghannam, S. (2012). Probiotic fermentation of plant based products: possibilities and opportunities. *Crit. Rev. Food Sci. Nutr.* **52**:183ï 199.

Ha, T. Y., Ko, S-N., Lee, S-M., Kim, H-R., Chung, S-Y., Kim, S-R., Yoon, H-H., and Kim, I-H. (2006). Changes in nutraceutical lipid components of rice at different degrees of milling. *Eur. J. Lip. Sci. Technol.* **108**: 175ï 181.

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**: 1908ï 1914.

Hirawan, R., Diehl-Jones, W., and Beta, T. (2011). Comparative evaluation of the antioxidant potential of infant cereals produced from purple wheat and red rice grains and LC-MS analysis of their anthocyanins. *J. Agric. Food Chem.* **59**: 12330ï 12341.

Hou, F., Zhang, R., Zhang, M., Su, D., Wei, Z., Deng, Y., Zhang, Y., Chi, J., and Tang, X. (2013). Hepatoprotective and antioxidant activity of anthocyanins in black rice bran on carbon tetrachloride-induced liver injury in mice. *J. Funct. Foods* **DOI:** 10.1016/j.jff.2013.07.015.

Htwe, N. N., Srilaong, V., Tanprasert, K., Uthairatanakij, A., Photchanachai, S., and Kanlayanarat, S. (2010). Effects of storage time and temperature on radical scavenging activities and bioactive compounds in colored rice varieties. *J. Food Agric. Environ.* **8**: 26ï 31.

Huang, S. T., Chen, C. T., Chieng, K. T., Huang, S. H., Chiang, B. H., Wang, L. F., Kuo, H. S., and Lin, C. M. (2005). Inhibitory effects of a rice hull constituent on tumor necrosis factor Ǔ prostaglandin E2, and cyclooxygenase-2 production in lipopolysaccharide-activated mouse macrophages. *Ann. N. Y. Acad. Sci.* **1042**: 387ï 395.

Huang, W-Y., Davidge, S. T., and Wu, J. (2013). Bioactive natural constituents from food sourcesð potential use in hypertension prevention and treatment. *Crit. Rev. Food Sci. Nutr.* **53**: 615ï 630.

Hübner, F., and Arendt, E. K. (2013). Germination of cereal grains as a way to improve the nutritional value: A review. *Crit. Rev. Food Sci. Nutr.* **53**: 853ï 861**.**

Hurrell, R. F., Reddy, M. B., Burris, J., and Cook, J. D. (2002). Phytate degradation determines the effect of industrial processing and home cooking on iron absorption from cereal-based foods. *Br. J. Nutr.* **88**: 117ï 123.

Imam, M. U., Musa, S. N. A., Azmi, N. H., and Ismail, M. (2012). Effects of white rice, brown rice and germinated brown rice on antioxidant status of type 2 diabetic rats. *Int. J. Mol. Sci.* **13**: 12952ï 12969.

In, M. J., Choi, S. Y., Kim, H. R., Park, D. B., Oh, N. S., and Kim, D. C. (2009). Acid production and phytate degradation using a *Leuconostoc mesenteroides* KC51 Strain in saccharified-rice suspension. *J. Appl. Biol. Chem.* **52**: 33ï 37.

Iqbal, J., Minhajuddin, M., and Beg, Z. H. (2003). Suppression of 7,12-dimethylbenz[alpha]anthracene-induced carcinogenesis and hypercholesterolaemia in rats by tocotrienol-rich fraction isolated from rice bran oil. *Eur. J. Cancer Prev.* **12**: 447ï 453.

Jang, S., and Xu, Z. (2009). Lipophilic and hydrophilic antioxidants and their antioxidant activities in purple rice bran. *J. Agric. Food Chem.* **57**: 858ï 862.

Jantasee, A., Thumanu, K., Muangsan, N., Leeanansaksiri, W., and Maensiri, D. (2013). Fourier transform infrared spectroscopy for antioxidant capacity determination in colored glutinous rice. *Food Anal. Methods* **DOI:** 10.1007/s12161-013-9637-1.

Jayadeep, A., and Malleshi, N. G. (2011). Nutrients, composition of tocotrienols, tocopherols, and ɔ-oryzanol, and antioxidant activity in brown rice before and after biotransformation. *CyTA – J. Food* **9**: 82ï 87.

Jeon, K. I., Park, E., Park, H. R., Jeon, Y. J., Cha, S. H., and Lee, S. C. (2006). Antioxidant activity of far-infrared radiated rice hull extracts on reactive oxygen species scavenging and oxidative DNA damage in human lymphocytes. *J. Med. Food* **9**: 42ï 48.

Juliano, C., Cossu, M., Alamanni, M. C., and Piu, L. (2005). Antioxidant activity of gamma-oryzanol: Mechanism of action and its effect on oxidative stability of pharmaceutical oils. *Int. J. Pharm.* **299**: 146ï 154.

Jung, E. H., Kim, S. R., Hwang, I. K., and Ha, T. Y. (2007). Hypoglycemic effects of a phenolic acid fraction of rice bran and ferulic acid in C57BL/KsJ-db/db Mice. *J. Agric. Food Chem.* **55**: 9800ï 9804.

Kanmani, P., R. Kumar, R. S., Yuvaraj, N., Paari, K. A., Pattukumar, V., and Arul, V. (2013). Probiotics and its functionally valuable products - a review. *Crit. Rev. Food Sci. Nutr.* **53**: 641ï 658.

Kesarwani, A., Chiang, P. Y., Chen, S. S., and Su, P. C. (2012) Antioxidant activity and total phenolic content of organically and conventionally grown rice cultivars under varying seasons. *J. Food Biochem.* **DOI**: 10.1111/j.1745-4514.2012.00661.x.

Khatoon, S., and Gopalakrishna, A. G. (2004). Fat-soluble nutraceuticals and fatty acid composition of selected Indian rice varieties. *J. Am. Oil Chem. Soc.* **81**: 939ï 943.

Khuwijitjaru, P., Yuenyong, T., Pongsawatmanit, R., and Adachi, S. (2011). Effects of ferric chloride on thermal degradation of ɔ-oryzanol and oxidation of rice bran oil. *Eur. J. Lip. Sci. Technol.* **113**: 652ï 657.

Kiing, I., Yiu, P., Rajan, A., and Wong, S. (2009). Effect of germination on ɔ-oryzanol content of selected sarawak rice cultivars. *Am. J. Appl. Sci.* **6**: 1658ï 1661.

Kim, D., and Han, G. D. (2011). Ameliorating effects of fermented rice bran extract on oxidative stress induced by high glucose and hydrogen peroxide in 3T3-L1 adipocytes. *Plant Foods Hum. Nutr.* **66**: 285ï 290.

Kim, K. S., and Jang, H. D. (2004). Effects of chitosan and lactic acid on enzymatic activities and bioactive compounds during germination of black rice. *J. Food Sci. Nutr.* **9**: 199ï 205.

Kim, S. K., Shin, J-H., Kang, D-K., Kim, S-Y., and Park, S-Y. (2013). Changes of anthocyanidin content and brown rice yield rice varieties among different transplanting and harvesting times. *J. Crop Sci. Biotech.* **58**: 28ï 35.

Kim, S. M., Rico, C. W., Lee, S. C., and Kang, M. Y. (2010). Modulatory effect of rice bran and phytic acid on glucose metabolism in high fat-fed C57BL/6N mice. *J. Clin. Biochem. Nutr.* **47**: 12ï 17.

Kim, S. P., Kang, M. Y., Nam, S. Y., and Friedman, M. (2012). Dietary rice bran component ɔ-oryzanol inhibits tumor growth in tumor-bearing mice. *Mol. Nutr. Food Res.* **56**: 935ï 944.

Ko, S. N., Kim, C. J., Kim, C. T., Kim, H., Chung, S. H., Lee, S. M., Yoon, H. H., and Kim, I. H. (2003). Changes of vitamin E content in rice bran with different heat treatment. *Eur. J. Lip. Sci. Technol.* **105***:* 225ï 228.

Kong C. K. L., Lam, W. S., Chiu, L. C. M., Ooi, V. E. C., Sun, S. S. M., and Wong, Y-S. (2009). A rice bran polyphenol, cycloartenyl ferulate, elicits apoptosis in human colorectal adenocarcinoma SW480 and sensitizes metastatic SW620 cells to TRAIL-induced apoptosis. *Biochem. Pharmacol.* **77**: 1487ï 1496.

Kongkiattikajorn, J., Tosingharach, V., Mongkol, S., and Rattanachaisit, P. (2010). Analysis of tocopherol content and antioxidant properties of rice bran and correlation to cereal color during storage. **In**: Proceedings of 48th Kasetsart University Annual Conference, Kasetsart University.

Ktenioudaki, A., Alvarez-Jubete, L., and Gallagher, E. (2013). A review of the process-induced changes in the phytochemical content of cereal grains: The breadmaking process. *Crit. Rev. Food Sci. Nutr.* **DOI**: 10.1080/10408398.2012.667848.

Landete, J. M. (2013). Dietary intake of natural antioxidants vitamins and polyphenols. *Crit. Rev. Food Sci. Nutr.* **53**: 706ï 721.

Laokuldilok, T., Surawang, S., and Klinhom, J. (2013). Influence of milling time on the nutritional composition and antioxidant content of Thai rice bran. *Food Appl. Biosci. J.* **13**: 112ï 130.

Lavecchia, T., Rea, G., Antonacci, A., and Giardi, M. T. (2013). Healthy and adverse effects of plant-derived functional metabolites: the need of revealing their content and bioactivity in a complex food matrix. *Crit. Rev. Food Sci. Nutr.* **53**: 198ï 213.

Lazarou, C., Panagiotakos, D., and Matalas, A-L. (2012). The role of diet in prevention and management of type 2 diabetes: implications for public health. *Crit. Rev. Food Sci. Nutr.* **52**: 382ï 389.

Lee, S. C., Kim, J. H., Jeong, S. M., Ha, J. U., Nam, K. C., and Ahn, D. U. (2004). Antioxidant activity of organic solvent extracts from far infrared-treated rice hulls. *Food Sci. Biotechnol.* **13**: 172ï 175.

Lee, Y. R., Woo, K. S., Kim, K. J., and Jeong, H. S. (2007). Antioxidant activities of ethanol extracts from germinated specialty rough rice. *Food Sci. Biotechnol.* **16**: 765ï 770.

Lestienne, I., Icard, V. C., Mouquet, C., Picq, C., and Trèche, S. (2004). Effects of soaking whole cereal and legume seeds on iron, zinc and phytate contents. *Food Chem.* **89**: 421ï 425.

Liang, C. H., Syu, J. L., and Mau, J. L. (2009a). Antioxidant properties of solid-state fermented adlay and rice by *Phellinus linteus. Food Chem.* **116**: 841ï 845.

Liang, J. F., Han, B. Z., Nout, M. J. R., and Hamer, R. J. (2008a). Effects of soaking, germination and fermentation on phytic acid, total and *in vitro* soluble zinc in brown rice. *Food Chem.* **110***:* 821ï 828.

Liang, J. F., Han, B. Z., Nout, M. J. R., and Hamer, R. J. (2009b). Effect of soaking and phytase treatment on phytic acid, calcium, iron and zinc in rice fractions. *Food Chem.* **115**: 789ï 794.

Liang, J. F., Lia, Z. G., Tsuji, K. C., Nakano, K., Nout, M. J. R., and Hamer, R. J. (2008b). Milling characteristics and distribution of phytic acid and zinc in long-,medium- and short-grain rice. *J. Cereal Sci.* **48**: 83ï 91.

Lin, C. M., Chen, C. T., Lee, H. H., and Lin, J. K. (2002). Prevention of cellular ROS damage by isovitexin and related flavonoids. *Planta Medica* **68**: 363ï 365.

Lin, P. Y., and Lai, H. M. (2001). Bioactive compounds in rice during grain development. *Food Chem.* **127**: 86ï 93.

Liu, X., Li, X., and Lei, G. (2010). Storage effects on total phenolics, antioxidant capacity in Indica-Japonica genotype rice grain. **In**: 4th International Conference on Bioinformatics and Biomedical Engineering (iCBBE), Chengdu, China.

Lloyd, B. J., Siebenmorgen, T. J., and Beers, K. W. (2010). Effects of commercial processing on antioxidants in rice bran. *Cereal Chem.* **77**: 551ï 555.

Loypimai, P., Moonggarm, A., and Chottanom, P. (2009). Effects of ohmic heating on lipase activity, bioactive compounds and antioxidant activity of rice bran. *Austral. J. Basic Appl. Sci.* **3**: 3642ï 3652.

Maisuthisakul, P., and Changchub, C. (2012). Effect of cooking on total phenolic and anthocyanin contents of 9 genotypes from Thai rice grains. *Agric. Sci. J.* **43**: 669ï 672.

Manosroi, A., Ruksiriwanich, W., Kietthanakorn, B. O., Manosroi, W., and Manosroi, J. (2011). Relationship between biological activities and bioactive compounds in the fermented rice sap. *Food Res. Int.* **44**: 2757ï 2765.

Marfo, E. K., Simpson, B. K., Idow, J. S., and Oke, O. L. (1990). Effect of local food processing on phytate levels in cassava, cocoyam, yam, maize, sorghum, rice, cowpea, and soybean. *J. Agric. Food Chem.* **38**: 1580ï 1585.

Marrazzo , G., Barbagallo, I., Galvano, F., Malaguarnera, M., Gazzolo, D., Frigiola, A., DôOrazio, N., and Volti, L. (2013). Role of dietary and endogenous antioxidants in diabetes. *Crit. Rev. Food Sci. Nutr.* **DOI**: 10.1080/10408398.2011.644874.

Massaretto, I. L., Alves, M. F. M., de Mira, N. V. M., Carmona, A. K., and Marquez, U. M. L. (2011). Phenolic compounds in raw and cooked rice (*Oryza sativa* L.) and their inhibitory effect on the activity of angiotensin I-converting enzyme. *J. Cereal Sci.* **54**: 236ï 240.

Miller, A., and Engel, K. H. (2006). Content of *γ*-oryzanol and composition of steryl ferulates in brown rice (*Oryza sativa* L.) of European origin. *J. Agric. Food Che*m. **54**: 8127ï 8133.

Min, B., McClung, A., and Chen, M. H. (2014). Effects of hydrothermal processes on antioxidants in brown, purple and red bran whole grain rice (*Oryza sativa* L.). *Food Chem*. **DOI**: 10.1016/j.foodchem.2014.02.164

Min, S. W., Ryu, S. N., and Kim, D. H. (2010). Anti-inflammatory effects of black rice, cyanidin-3-*O*-*β*-D-glycoside, and its metabolites, cyanidin and protocatechuic acid. *Int. Immunopharmacol.* **10**: 959ï 966.

Moongngarm, A., and Khomphiphatkul, E. (2011). Germination time-dependence of bioactive compounds and antioxidant activity in germinated rough rice (*Oryza sativa* L.). *Am. J. Appl. Sci.* **8**: 15ï 25.

Moongngarm, A., and Saetung, N. (2010). Comparison of chemical compositions and bioactive compounds of germinated rough rice and brown rice. *Food Chem.* **122**: 782ï 788.

Nakano, H., Ono, H., Iwasawa, N., Takai, T., Arai-Sanoh, Y., and Kondo, M. (2013). Isolation and identification of phenolic compounds accumulated in brown rice grains ripened under high air temperature. *J. Agric. Food Chem.* **DOI**: 10.1021/jf403416e.

Nakashima, K., Virgona, N., Miyazawa, M., Watanabe, T., and Yano, T. (2010). The Tocotrienol-rich fraction from rice bran enhances cisplatin-induced cytotoxicity in human mesothelioma H28 cells. *Phytother. Res.* **24**: 1317ï 1321.

Nayak, B., Liu, R. H., and Tang, J. (2013). Effect of processing on phenolic antioxidants of fruits, vegetables and grains - a review. *Crit. Rev. Food Sci. Nutr.* **DOI**: 10.1080/10408398.2011.654142.

Ning, H., Liu, Z., Wang, Q., Lin, Z., Chen, S., Li, G., Wang, S., and Ding, Y. (2009). Effect of nitrogen fertilizer application on grain phytic acid and protein concentrations in *japonica* rice and its variations with genotypes. *J. Cereal Sci.* **50**: 49ï 55.

Norazalina, S., Norhaizan, M. E., and Hairuszah, I. (2013). Suppression of ß-catenin and cyclooxygenase-2 expression and cell proliferation in azoxymethane-induced colonic cancer in rats by rice bran phytic acid (PA). *Asian Pac. J. Cancer Prev.* **14**: 3093ï 3099.

Noreen, N., Shah, H., Anjum, F., Masood, T., and Faisal, S. (2009). Variation in mineral composition and phytic acid content in different rice varieties during home traditional cooking processes. *Pak. J. Life Soc. Sci.* **7**: 11ï 15.

Nurul-Husna, S., Norhaizan, M. E., Hairuszah, I., Abdah, M. A., Norazalina, S., and Norsharina, I. (2010). Rice bran phytic acid (IP6) induces growth inhibition, cell cycle arrest and apoptosis on human colorectal adenocarcinoma cells. *J. Med. Plants Res.* **4**: 2283ï 2289.

Ohara, K., Uchida, A., Nagasaka, R., Ushio, H., and Ohshima, T. (2009). The effects of rice hydroxycinnamic acid derivatives on adiponectin secretion. *Phytomedicine* **16**: 130ï 137.

Oka, T., Fujimoto, M., Nagasaka, R., Ushio, H., Hori, M., and Ozaki, H. (2010). Cycloartenylferulate, a component of rice bran oil-derived ɔ-oryzanol, attenuates mast cell degranulation. *Phytomedicine* **17**: 152ï 156.

Oliveira, M. S., Cipolatti, E. P., Furlong, E. B., and Soares, L. D. S. (2012). Phenolic compounds and antioxidant activity in fermented rice (*Oryza sativ*a) bran. *Ciência Tecnol. Alime.* **32**: 531ï 537.

Park, J. H., Nam, S. H., Kim, Y. O., Kwon, O. D., and An, K.N. (2010). Comparison of quality, physiochemical and functional property between organic and conventional rice. *J. Kor. Soc. Food Sci. Nutr.* **39**: 725ï 730.

Pascual, C. C. I., Massaretto, I. L., Kawassaki, F., Barros, R. M. C., Noldin, J. A., and Marquez, U. M. L. (2011). Effects of parboiling, storage and cooking on the levels of tocopherols, tocotrienols and ɔ-oryzanol in brown rice (*Oryza sativa* L.). *Food Res. Int.* **50**: 676ï 681.

Pelig-Ba, K. B. (2009). Assessment of Phytic Acid Levels in Some Local Cereal Grains in Two Districts in the Upper East Region of Ghana. *Pak. J. Nutr.* **8**: 1540ï 1547.

Pestana V. R., Zambiazi, R. C., Mendonca, C. R. B., Bruscatto, M. H., and Ramis-Ramos, G. (2009). The influence of industrial processing on the physico-chemical characteristics and lipid and antioxidant contents of rice bran. *Grasas Aceites* **60**: 184ï 193.

Pinent, M., Cedó, L., Montagut, G., Blay, M., and Ardévol, A. (2012). Procyanidins improve some disrupted glucose homoeostatic situations: an analysis of doses and treatments according to different animal models. *Crit. Rev. Food Sci. Nutr.* 52: 569ï 584.

Pradeep, P. M., Jayadeep, A., Guha., M., and Singh, V. (2014). Hydrothermal and biotechnological treatments on nutraceutical content and antioxidant activity of rice bran. *J. Cereal Sci*. **DOI**: 10.1016/j.jcs.2014.01.025

Prior, R. L., Wu, X., and Schaich, K. (2005). Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplement. *J. Agric. Food Chem.* 53: 4290ī 4302.

Qureshi, A. A., Salser, W. A., Parmar, R., and Emeson, E. E. (2001). Novel tocotrienols of rice bran inhibit atherosclerotic lesions in C57BL/6 ApoE-deficient mice. *J. Nutr.* **131:** 2606ï 2618.

Qureshi, A. A., Sami, S. A., Salser, W. A., and Khan, F. A. (2002). Dose-dependent suppression of serum cholesterol by tocotrienol-rich fraction (TRF25) of rice bran in hypercholesterolemic humans. *Atherosclerosis* **161**: 199ï 207.

Ragaee, S., Seetharaman, K., and Abdel-Aal, E. M. (2012). Impact of milling and thermal processing on phenolic compounds in cereal grains. *Crit. Rev. Food Sci. Nutr.* **DOI**: 10.1080/10408398.2011.610906.

Ramarathnam, N., Osawa, T., Namiki, M., and Kawakishi, S. (1989). Studies on changes in fatty acid composition and content of endogenous antioxidants during ɔ irradiation of rice seeds. *J. Am. Oil Chem. Soc.* **66**: 105ï 108.

Rastogi, N. K. (2012). Recent trends and developments in infrared heating in food processing. *Crit. Rev. Food Sci. Nutr.* **51**: 737ï 760.

Rattanachitthawat, S., Suwannalert, P., Riengrojpitak, S., Chaiyasut, C., and Pantuwatana, S. (2010). Phenolic content and antioxidant activities in red unpolished Thai rice prevents oxidative stress in rats. *J. Med. Plants Res.* **4**: 796ï 801.

Reddy, N. R., and Salunkhe, D. K. (1980). Effects of fermentation on phytate phosphorus and mineral content in black gram, rice, and black gram and rice blends. *J. Food Sci.* **45**: 1708ï 1712.

Rohrer, C. A., and Siebenmorgen, T. J. (2004). Nutraceutical concentrations within the bran of various rice kernel thickness fractions. *Biosyst. Eng.* **88**: 453ï 460.

Rohrer, C. A., Siebenmorgen, T. J., and Howell, T. A. (2002). Effects of storage conditions on nutraceutical levels in rough rice. *Univ. Ark. Rice Res. Stu.* **504**: 404ï 409.

Roselina, K., Adilah, M. N. N., Hasanah, M. G., Noranizan, M. A., and Mei-Leng, L. (2011). Effects of microwave treatment on the bioactive components of rice chips from various rice mills in Selangor. **In**: Universiti Malaysia Terengganu International Annual Symposium Souvenir, pp. 229ï 234. LS1, Malaysia.

Rumruaytum, P., Borompichaichartkul, C.,and Kongpensook, V. (2013). Effect of drying involving fluidisation in superheated steam on physicochemical and antioxidant properties of Thai native rice cultivars. *J. Food Eng.* **DOI**: 10.1016/j.jfoodeng.2013.08.025.

Russel, R. M. (2010). Integration of epidemiologic and other types of data into dietary reference intake development. *Crit. Rev. Food Sci. Nutr.* **50**: 33ï 34.

Saenjum, C., Chaiyasut, C., Chansakaow, S., Suttajit, M., and Sirithunyalug, B. (2012) Antioxidant and anti-inflammatory activities of gamma-oryzanol rich extracts from Thai purple rice bran. *J. Med. Plants Res.* **6**: 1070ï 1077.

Saikia, S., Dutta, H., Saikia, D., and Mahanta, C. L. (2012). Quality characterisation and estimation of phytochemicals content and antioxidant capacity of aromatic pigmented and non-pigmented rice varieties. *Food Res. Int.* **46**: 334ï 340.

Sangkitikomol, W., Tencomnao1, T., and Rocejanasaroj, A. (2010). Antioxidant effects of anthocyanins-rich extract from black sticky rice on human erythrocytes and mononuclear leukocytes. *Afr. J. Biotechnol.* **9**: 8222ï 8229.

Schramm, R., Abadie, A., Hua, N., Xu, Z., and Lima, M. (2007). Fractionation of the rice bran layer and quantification of vitamin E, oryzanol, protein, and rice bran saccharide. *J. Biol. Eng.* **DOI**: 10.1186/1754-1611-1-9.

Semsang, N., Kawaree, R., Cutler, R. W., Chundet, R., Yu, L. D., and Anuntalabhochai, S. (2012). Improved antioxidant activity of BKOS Thai jasmine rice. *Nat. Prod. Res.* **26**: 1145ï 1151.

Shalini, V., Bhaskar, S., Kumar, K. S., Mohanlal, S., Jayalekshmy, A., and Helen, A. (2012). Molecular mechanisms of anti-inflammatory action of the flavonoid, tricin from Njavara rice (*Oryza sativa* L.) in human peripheral blood mononuclear cells: Possible role in the inflammatory signalling. *Int. Immunopharmacol.* **14**: 32ï 38.

Shao, Y., Tang, F., Xu, F., Wang, Y., and Bao, J. (2013). Effects of ɔ-irradiation on phenolics content, antioxidant activity and physicochemical properties of whole grain rice. *Radiat. Phys. Chem.* **85**: 227ï 233.

Shao, Y., Xu, F., Sun, X., Bao, J., and Beta, T. (2014). Phenolic acids, anthocyanins, and antioxidant capacity in rice (*Oryza sativa* L.) grains at four stages of development after flowering. *Food Chem.* **143**: 90ï 96.

Sharif, M. K., Butt, M. S., Anjum, F. M., and Khan, S. H. (2013). Rice bran: a novel functional ingredient. *Crit. Rev. Food Sci. Nutr.* **DOI**: 10.1080/10408398.2011.608586.

Sharma, A., and Khetarpaul, N. (1997). Effect of fermentation on phytic acid content and *in vitro* digestibility of starch and protein of rice-blackgram dhal-whey blends. *J. Food Sci. Technol.* **34**: 20ï 23.

Shekib, L. A. E. (1988). Evaluation of protein quality, methionine and lysine availability, and phytic acid in natural fermented lentils, rice and their blend. *Alex. J. Agric. Res.* **33**: 135ï 144.

Shih, C. H., Chu, H., Tang, L. K., Sakamoto, W., Maekawa, M., Chu, I. K., Wang, M., and Lo. C. (2008). Functional characterization of key structural genes in rice flavonoid biosynthesis. *Planta* **228**: 1043ï 1054.

Shin, T. S., and Godber, J. S. (1996). Changes of endogenous antioxidants and fatty acid composition in irradiated rice bran during storage. *J. Agric. Food Chem.* **44**: 567ï 573.

Shin, T. S., 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. *J. Food Sci.* **62**: 704ï 728.

Siddiqui, S., Rashid Khan, M., and Siddiqui, W. A. (2010). Comparative hypoglycemic and nephroprotective effects of tocotrienol rich fraction (TRF) from palm oil and rice bran oil against hyperglycemia induced nephropathy in type 1 diabetic rats. *Chem. Biol. Interact.* **188**: 651ï 658.

Sidhu, J. S., and Bajaj, M. (1988). Extended milling of Indian rice. IV. Effect on phytic acid content. *Chem. Mikrobiol. Technol. Leben.* **10**: 172ï 175.

Sirikul, A., Moongngarm, A., and Khaengkhan, P. (2009). Comparison of proximate composition, bioactive compounds and antioxidant activity of rice bran and defatted rice bran from organic rice and conventional rice. *Asian J. Food Ag-Ind.* 2: 731ï 743.

Somsana, P., Wattana, P., Suriharn, B., and Sanitchon, J. (2013). Stability and genotype by environment interactions for grain anthocyanin content of Thai black glutinous upland rice (o*ryza sativa*). *SABRAO J. Breeding Genet.* 45: 523ï 532.

Srijesdaruk, V., Nantachai, K., and Suwannarong, S. (2001). Studies on efficiency of rice bran (*Oryza sativa*) tocopherol extracts as antioxidant. *Khon Kaen Univ. Res. J.* **6**: 34ï 44.

Sungsopha, J., Moongngarm, A., and Kanesakoo, R. (2009). Application of germination and enzymatic treatment to improve the concentration of bioactive compounds and antioxidant activity of rice bran. *Aust. J. Basic Appl. Sci.* **3**: 3653ï 3661.

Sutharut, J., and Sudarat, J. (2012). Total anthocyanin content and antioxidant activity of germinated coloured rice. *Int. Food Res. J.* **19**: 215ï 221.

Tananuwong, K., and Tangsrianugul, N. (2013). Effects of storage conditions and cooking on colour and antioxidant activities of organic pigmented rice. *Int. J. Food Sci. Technol.* **48**: 67ï 73.

Thanajiruschaya, P., Doksaku, W., Rattanachaisit, P., and Kongkiattikajorn, J. (2010). Effect of storage time and temperature on antioxidant components and properties of milled rice. *Khon Kaen Univ. Res. J.* **15**: 843ï 851.

Thanonkaew, A., Wongyai, S., McClements, D. J., and Decker, E. A. (2012). Effect of stabilization of rice bran by domestic heating on mechanical extraction yield, quality, and antioxidant properties of cold-pressed rice bran oil (*Oryza saltiva* L.). *LWT - Food Sci. Technol.* **48**: 231ï 236.

Thitisaksakul, M., Jiménez, R. C., Arias, M. C., and Beckles, D. M. (2012). Effects of environmental factors on cereal starch biosynthesis and composition. *J. Cereal Sci.* **56**: 167ï 180.

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: 4808ï 4813.

Tirzitis, G., and Bartosz, G. (2010). Determination of antiradical and antioxidant activity: basic principles and new insights. *Acta Biochim. Pol.* 57: 139ï 142.

Toma, R. B., and Tabekhia, M. M. (1979). Changes in mineral elements and phytic acid contents during cooking of three California rice varieties. *J. Food Sci.* **44**: 619ï 621.

Trigueros, L., Peña, S., Ugidos, A. V., Sayas-Barberá, E., Pérez-Álvarez, J. A., and Sendra, E. (2013). Food ingredients as anti-obesity agents: a review. *Crit. Rev. Food Sci. Nutr.* **53**: 929ï 942.

Tuaño, A. P. P., Xu, Z., Castillo, M. B., Mamaril, C. P., Manaois, R. V., Romero, M. V., and Juliano, B. O. (2011). Content of tocols, ɔ-oryzanol and total phenolics and grain quality of brown rice and milled rice applied with pesticides and organic and inorganic nitrogen fertilizer. *Philip. Agric. Sci.* **94**: 211ï 216.

Um, M. Y., Ahn, J., and Ha, T. Y. (2013). Hypolipidaemic effects of cyanidin 3-glucoside rich extract from black rice through regulating hepatic lipogenic enzyme activities. *J. Sci. Food Agric.* **93**: 3126ï 3128.

Visioli, F., De La Lastra, C., Andres-Lacueva, C., Aviram, M., Calhau, C., Cassano, C., DôArchivio, M., Faria, A., Favé, G., Fogliano, V., Llorach, R., Vitaglione, P., Zoratti, M., and Edeas, M. (2011). Polyphenols and human health: a prospectus. *Crit. Rev. Food Sci. Nutr.* **51**: 524ï 546.

Vitaglione, P., Napolitano, A., and Fogliano, V. (2008). Cereal dietary fibre: a natural functional ingredient to deliver phenolic compounds into the gut. *Trends Food Sci. Technol.* **19**: 451ï 463.

Walter, M., Marchesan, E., Massoni, P. F. S., da Silva, L. P., Sartori, G. M. S., and Ferreira, R. B. (2013). Antioxidant properties of rice grains with light brown, red and black pericarp colors and the effect of processing. *Food Res. Int.* **50**: 698ï 703.

Wang, K. M., Wu, J. G., Li, G., Zhang, D. P., Yang, Z. W., and Shi, C. H. (2011). Distribution of phytic acid and mineral elements in three indica rice (*Oryza sativa* L.) cultivars. *J. Cereal Sci.* **54**: 116ï 121.

Wang, X., Song, Y-E., and Li, J-Y. (2013). High expression of tocochromanol biosynthesis genes increases the vitamin E level in a new line of giant embryo rice. *J. Agric. Food Chem.* **61**: 5860ī 5869.

Wataniyakul, P., Pavasant, P., Goto, M., and Shotipruk, A. (2012). Microwave pretreatment of defatted rice bran for enhanced recovery of total phenolic compounds extracted by subcritical water. *Bioresour. Technol.* **124**: 18ï 22.

Weed, D. L. (2013). The quality of nutrition and cancer reviews: a systematic assessment. *Crit. Rev. Food Sci. Nutr.* **53**: 276–286.

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**: 105ï 112.

Wu, F., Yang, N., Toure, A., Jin, Z., and Xu, X. (2013a). Germinated brown rice and its role in human health. *Crit. Rev. Food Sci. Nutr.* **53**: 451ï 463.

Wu, L., Zhai, M., Yao, Y., Dong, C., Shuang, S., and Ren, G. (2013b). Changes in nutritional constituents, anthocyanins, and volatile compounds during the processing of black rice tea. *Food Sci. Biotechnol.* **22**: 917ï 923.

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 Eï deficient mice. *J. Nutr.* **136**: 2220ï 2225.

Xiao, J., and Kai, G. (2012). A review of dietary polyphenol-plasma protein interactions: characterization, influence on the bioactivity, and structure-affinity relationship. *Crit. Rev. Food Sci. Nutr.* **52**: 85ï 101.

Yang, J. H., Tseng, Y. H., Lee, Y. L., and Mau, J. L. (2006). Antioxidant properties of methanolic extracts from monascal rice. *LWT – Food Sci. Technol.* **39**: 740ï 747.

Yang, Y., Andrews, M. C., Hu, Y., Wang, D., Qin, Y., Zhu, Y., Ni, H., and Ling, W. (2011). Anthocyanin extract from black rice significantly ameliorates platelet hyperactivity and hypertriglyceridemia in dyslipidemic rats induced by high fat diets. *J. Agric. Food Chem.* **59**: 6759ï 6764.

Yen, G. C., Chang, Y. C., and Su, S. W. (2003). Antioxidant activity and active compounds of rice koji fermented with *Aspergillus candidus*. *Food Chem.* **83**: 49ï 54.

Yđmaz, N., Tuncel, N. B., and Kocabēyđk, H. (2013). Infrared stabilization of rice bran and its effects on *γ*-oryzanol content, tocopherols and fatty acid composition. *J. Sci. Food Agric.* **DOI**: 10.1002/jsfa.6459.

Yoshimura, Y., Zaima, N., Moriyama, T., and Kawamura, Y. (2011). Different localization patterns of anthocyanin species in the pericarp of black rice revealed by imaging mass spectrometry. *PLoS ONE* **7**: e31285.

Young, K. M. I., Kim, S. M., Rico, C. W., and Lee, S. C. (2012). Hypolipidemic and antioxidative effects of rice bran and phytic acid in high fat-fed mice. *Food Sci. Biotechnol.* **21**: 123ï 128.

Zhao, S., Baik, O-D, Choi, Y. J., Kim, S-M. (2013). Pretreatments for the efficient extraction of bioactive compounds from plant based biomaterials. *Crit. Rev. Food Sci. Nutr.* **DOI**: 10.1080/10408398.2011.632698.

Zhou, Z., Robards, K., Helliwell, S., and Blanchard, C. (2004). The distribution of phenolic acids in rice. *Food Chem.* **87**: 401ï 406.

Zhu, F., Cai, Y. Z., Bao, J., and Corke, H. (2010). Effect of ɔ-irradiation on phenolic compounds in rice grain. *Food Chem.* **120**: 74ï 77.

Zhu, W., Jia, Q., Wang, Y., Zhang, Y., and Xia, M. (2012). The anthocyanin cyanidin-3-O-ɓ-glucoside, a flavonoid, increases hepatic glutathione synthesis and protects hepatocytes against reactive oxygen species during hyperglycemia: Involvement of a cAMP-PKA-dependent signaling pathway. *Free Rad. Biol. Med.* 52: 314ï 327.

Table 1. Factors influencing antioxidant compounds in Rice

	Phenolic acids and flavonoids	Anthocyanins and proanthocyanidins	Tocopherols and tocotrienols	λ-oryzanol	Phytic acid
Sandy vs. Clay soils	**Decrease** (Butsat and Siriamornpun, 2010; Anonymous, 2014)	**No effect** (Somsana et al., 2013)	**Increase** [a] (Butsat and Siriamornpun, 2010; Anonymous, 2014)	**Increase** (Butsat and Siriamornpun, 2010; Anonymous, 2014)	**Increase** (Pelig-Ba, 2009; Anonymous, 2014)
Elevated carbon dioxide	**Decrease** (Goufo et al., 2014a)	NOT AVAILABLE	**Decrease** [a] (Goufo et al., 2014a)	**Decrease** (Goufo et al., 2014a)	**No effect** (Goufo et al., 2014a)
Elevated temperature	**Decrease** (Dutta et al., 2012; Kesarwani et al., 2012; Anonymous, 2014)	**Increase** (Chae et al., 2004)	**Increase** (Britz et al., 2007; Anonymous, 2014)	**Increase** (Chae et al., 2004; Britz et al., 2007; Nakano et al., 2013; Anonymous, 2014)	**Decrease** (Ahn et al., 2010; Anonymous, 2014)
Organic vs. Conventional farming	**Increase** [b] (Sirikul et al., 2009; Park et al., 2010; Kesarwani et al., 2012)	NOT AVAILABLE	**Inconsistent** (Tuaño et al., 2011) = decrease (Sirikul et al., 2009) = increase	**Inconsistent** (Sirikul et al., 2009; Tuaño et al., 2011) = decrease (Cho et al., 2012) = increase	**Increase** (Park et al., 2010)
Harvest date	**Decrease** [c] (Butsat et al., 2009; Lin and Lai, 2011; Shao et al., 2014)	**Inconsistent** [d] (Kim et al., 2013) = decrease (Shao et al., 2014) = increase	**Inconsistent** (Lin and Lai, 2011) = decrease (Wang et al., 2013) = increase)	**Inconsistent** (Lin and Lai, 2011) KFSW rice = no effect TK16 rice = increase	NOT AVAILABLE

Drying	**Inconsistent** (Rumruaytum et al., 2013) SYP rice = decrease Nauykaur rice = increase	NOT AVAILABLE	NOT AVAILABLE	NOT AVAILABLE	NOT AVAILABLE
Parboiling	**Decrease** (Walter et al., 2013; Min et al., 2014; Pradeep et al., 2014)	**Decrease** (Min et al., 2014)	**Inconsistent** (Khatoon and Gopalakrishna, 2004; Pascual et al., 2011) = decrease (Min et al., 2014) = increase	**Inconsistent** (Pascual et al., 2011) = decrease (Khatoon and Gopalakrishna, 2004) = no effect (Min et al., 2014) = increase	NOT AVAILABLE
Storage time	**Decrease** [e] (Zhou et al., 2004; Htwe et al., 2010; Kongkiattikajorn et al., 2010; Thanajiruschaya et al., 2010; Tananuwong and Tangsrianugul, 2013)	**No effect** (Htwe et al., 2010; Tananuwong and Tangsrianugul, 2013)	**Decrease** [f] (Shin and Godber, 1996; Shin et al., 1997; Srijesdaruk et al., 2001; Rohrer et al., 2002; Pascual et al., 2011)	**Decrease** (Shin and Godber, 1996; Shin et al., 1997; Rohrer et al., 2002; Chitropas et al., 2004; Azrina et al., 2010; Pascual et al., 2011)	**No effect** (Ahn et al., 2010)
Storage temperature	**No effect** [g,h] (Htwe et al., 2010; Thanajiruschaya et al., 2010; Tananuwong and	**No effect** (Htwe et al., 2010; Tananuwong and Tangsrianugul, 2013)	**No effect** (Rohrer et al., 2002; Kongkiattikajorn et al., 2010)	**No effect** (Chitropas et al., 2004)	NOT AVAILABLE

	Tangsrianugul , 2013).				
Irradiation	**Increase** (Lee et al., 2004; Jeon et al., 2006; Zhu et al., 2010; Shao et al., 2013)	**Increase** [i] (Zhu et al., 2010)	**Decrease** (Ramarathna m et al., 1989; Shin and Godber, 1996)	**Decrease** (Ramarathna m et al., 1989; Shin and Godber, 1996)	NOT AVAILABLE
Milling degree	**Outer layer of the bran** [j] (Finocchiaro et al., 2007; Laokuldilok et al., 2013)	**Outer layer of the bran** (Finocchiaro et al., 2007; Yoshimura et al., 2012)	**Outer layer of the bran** [k] (Rohrer and Siebenmorgen , 2004; Ha et al., 2006; Finocchiaro et al., 2007; Schramm et al., 2007; Laokuldilok et al., 2013)	**Outer layer of the bran** (Lloyd et al., 2000; Damayanthi, 2001; Rohrer and Siebenmorgen , 2004; Ha et al., 2006; Finocchiaro et al., 2007; Schramm et al., 2007; Laokuldilok et al., 2013)	**Medium layer of the bran** (Sidhu and Bajaj, 1988; Liang et al., 2008b; Wang et al., 2011)
Stabilizati on	**Increase** (Loypimai et al., 2009; Roselina et al., 2011; Thanonkaew et al., 2012; Wataniyakul et al., 2012)	NOT AVAILABLE	**Inconsistent** (Shin et al., 1997; Roselina et al., 2011; Yilmaz et al., 2013) = decrease (Lloyd et al., 2000) = no effect (Qureshi et al., 2002; Ko et al., 2003; Loypimai et al., 2009;	**Inconsistent** (Shin et al., 1997; Damayanthi, 2001; Lloyd et al., 2000; Azrina et al., 2010; Roselina et al., 2011) = decrease (Azrina et al., 2010; Yilmaz et al., 2013) = no effect (Loypimai et	**No effect** (Gualberto et al.,1997)

			Thanonkaew et al., 2012) = increase	al., 2009; Azrina et al., 2010; Thanonkaew et al., 2012) = increase	
Soaking	**No effect** (Moongngarm and Khomphiphatkul, 2011)	**Inconsistent** (Hiemori et al., 2009) = no effect (Wu et al., 2013) = decrease	**No effect** (Moongngarm and Khomphiphatkul, 2011)	**No effect** (Moongngarm and Khomphiphatkul, 2011)	**Decrease** [l] (Lestienne et al., 2004; Liang et al., 2008a; Liang et al., 2009b; Noreen et al., 2009; Moongngarm and Khomphiphatkul, 2011; Albarracín et al., 2013)
Germination	**Increase** [m] (Tian et al., 2004; Lee et al., 2007; Banchuen et al., 2009; Moongngarm and Saetung, 2010; Moongngarm and Khomphiphatkul, 2011; Gujral et al., 2012; Imam et al., 2012; Azmi et al., 2013)	**Increase** (Sutharut and Sudarat, 2012)	**Increase** (Sungsopha et al., 2009; Moongngarm and Saetung, 2010; Jayadeep and Malleshi, 2011; Moongngarm and Khomphiphatkul, 2011)	**Increase** [n] (Sungsopha et al., 2009; Moongngarm and Saetung, 2010; Moongngarm and Khomphiphatkul, 2011) = increase (Banchuen et al., 2009; Jayadeep and Malleshi, 2011) = no effect	**Decrease** (Kim and Jang, 2004; Banchuen et al., 2009; Lee et al., 2007; Liang et al. 2008a; Moongngarm and Saetung, 2010; Azeke et al. 2011; Moongngarm and Khomphiphatkul, 2011)
Fermentation	**Increase** (Yen et al., 2003; Yang et al., 2006;	**Increase** (Manosroi et al., 2011)	**Inconsistent** (Yang et al., 2006; Liang et al., 2009a)	**Increase** (Sungsopha et al., 2009)	**Decrease** (Marfo et al., 1990; Sharma and

	Liang et al., 2009a; Sungsopha et al., 2009; Kim and Han, 2011; Manosroi et al., 2011; Oliveira et al., 2012; Bao et al., 2013; Tananuwong and Tangsrianugul, 2013)		= decrease in white rice (Yang et al., 2006 ; Sungsopha et al., 2009) = increase in brown rice and bran		Khetarpaul, 1997; Cuneo et al., 2000; Liang et al., 2008a; In et al., 2009; Liang et al., 2009b)
Boiling and steaming	**Decrease** (Finocchiaro et al., 2007; Massaretto et al., 2011; Maisuthisakul and Changchub, 2012; Saikia et al., 2012; Jantasee et al., 2013; Tananuwong and Tangsrianugul, 2013; Walter et al., 2013; Min et al., 2014)	**Decrease** [o] (Finocchiaro et al., 2007; Hiemori et al., 2009; Maisuthisakul and Changchub, 2012; Saikia et al., 2012; Jantasee et al., 2013; Wu et al., 2013; Min et al., 2014)	**Inconsistent** (Pascual et al., 2011) = decrease (Finocchiaro et al., 2007) = increase (Min et al., 2014) = no effect	**Inconsistent** (Finocchiaro et al., 2007; Pascual et al., 2011) = decrease Chamnarnsin and Ahromrit (2011) = increase (Min et al., 2014) = no effect	**Decrease** (Marfo et al., 1990; Noreen et al., 2009)
Baking and roasting	**Decrease** (Hirawan et al., 2011; Gujral et al., 2012)	**Decrease** (Wu et al., 2013)	NOT AVAILABLE	**Decrease** (Khuwijitjaru et al., 2011; Ekasit and Jiraporn, 2013)	**Decrease** (Hurrell et al., 2002; Frontela et al., 2008; Noreen et al., 2009)

[a] Anonymous, (2014): the increase was in the bran, brown rice and husk while a decrease was observed in the white rice

[b] Tuaño et al. (2011) reported lower phenolics in organic rice compared with conventional rice

[c] Shao et al. (2014) studied the effect of harvest date on three rices. In two rices, phenolics decreased with increased harvesting time while in one rice, phenolics increased

[d] In the study by Kim et al. (2013), one of three varieties showed increased phenolics with increased harvesting times

[e] Liu et al. (2010) found that longer storage durations (36 months) corresponded to a 7ï 47% increase in the TPC in six rices

[f] No effect of storage time was observed on tocopherols by Kongkiattikajorn et al. (2010)

[g] The comparison is made with storage temperatures between 20 and 45 °C

[h] Kongkiattikajorn et al. (2010) found an increase in phenolic contents in rice bran when the storage temperature was raised from 25 to 37 °C for 7 months

[i] The effects of irradiation on rice phenolics depended on the dose applied (Zhu et al., 2010)

[j] The study by Jang and Xu (2009) contradicted all other studies, showing higher levels of phenolics, anthocyanins, tocols and ə-oryzanol in the inner layer of the bran

[k] According to Lloyd et al. (2000), tocols are predominantly found in the medium layer of the bran

[l] In the study by Noreen et al. (2009), soaking reduced the content of phytic acid in four varieties and increased it in two varieties

[m] Germination decreased the contents of phenolics in one study (Kim and Jang, 2004); however, it is difficult to reconcile that decrease with the increase in the DPPH

[n] Decreases of ɔ-oryzanol were found after germination of five Malaysian rices, whereas increases were found in three rices (Kiing et al., 2009)

[o] Tananuwong and Tangsrianugul (2013) reported a situation in which cooking resulted in greater contents of anthocyanins in the grains

Figure caption

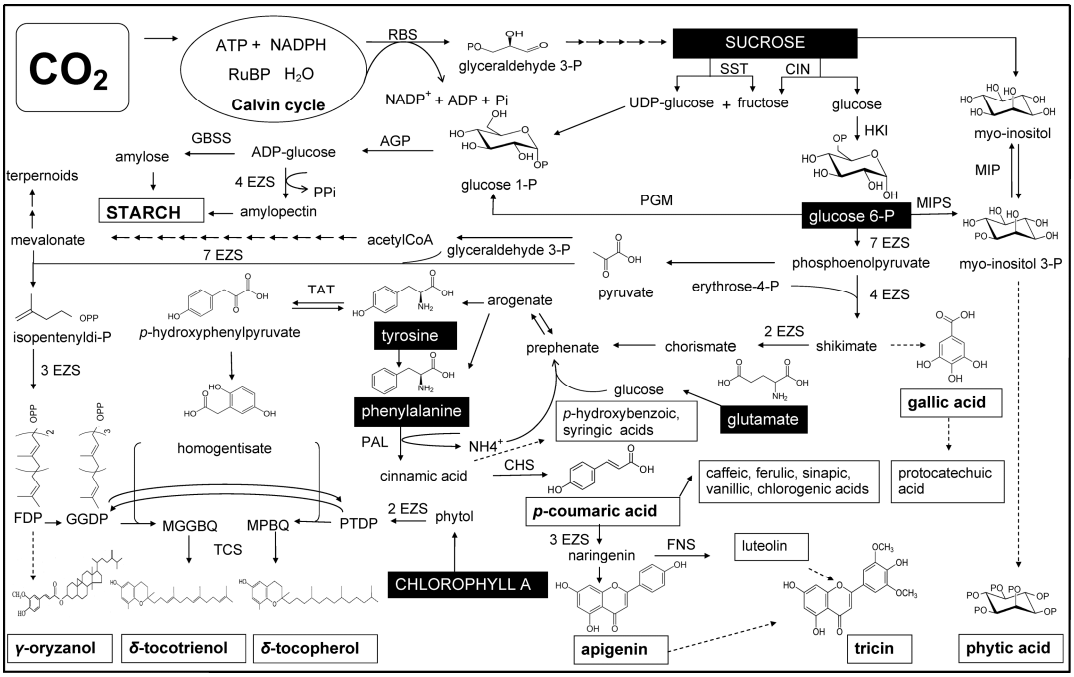

Figure 1. Schematic of rice metabolism affected by increased atmospheric CO_2 concentration. Pathway sketched are as proposed by Thitisaksakul et al. (2012) (starch), Shi et al. (2008) (phenolic acids and flavonoids), Chaudhary and Khurana (2009) (tocopherols and tocotrienols), Frank et al. (2007) (phytic acid), and Miller and Engel (2006) (*γ*-oryzanol). The dotted lines indicate not fully elucidated pathways. Abbreviations: EZS: enzymes or steps, SST: sucrose synthase, CIN: cell wall invertase, PGM: phosphoglucoisomerase, AGP: ADP-glucose pyrophosphorylase, GBSS: granule bound starch synthase, RBS: rubisco, MIP: *myo*-inositol monophosphatase, MIPS: 1D-*myo*-inositol 3-phosphate synthase, CHS: cinnamate 4-hydroxylase, FNS: flavone synthase, TCS: tocopherol cyclase, TAT: tyrosine aminotransferase, HKI: hexokinase, PAL: phenylalanine ammonia lyase, PTDP: phytyl diphosphate, MPBQ: 2-methyl 6-phytyl 1,4-benzoquinone, MGGBQ: 2-methyl 6-geranylgeranylbenzoquinone, GGDP: geranylgeranyl diphosphate, FDP: farnesyldiphosphate.