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Phytochemical Profiles and Antioxidant Capacity of Pigmented and Non-pigmented Genotypes of Rice (*Oryza sativa* L.)

M.N. Irakli[1]*, V.F. Samanidou[2], D.N. Katsantonis[1], C.G. Biliaderis[3] and I.N. Papadoyannis[2]

[1]Hellenic Agricultural Organization – Demeter, Cereal Institute, 57001, Thessaloniki, Greece
[2]Laboratory of Analytical Chemistry, Department of Chemistry, AUTH, 54124, Thessaloniki, Greece
[3]Laboratory of Food Chemistry and Biochemistry, School of Agriculture,
AUTH, 54124, Thessaloniki, Greece

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Pigmented rice (*Oryza sativa* L.) genotypes become increasingly important in the agro-industry due to their bioavailable compounds that have the ability to inhibit the formation and/or to reduce the effective concentration of reactive cell-damaging free radicals. This study aimed at determining the concentrations of free, and bound phytochemicals and their antioxidant potential (DPPH and ABTS assays) as well as the vitamin E and carotenoids contents of non-pigmented and pigmented rice genotypes. The results confirmed that the content of total phenolics and flavonoids contents, as well as the antioxidant capacity (DPPH and ABTS assays) of pigmented rice was several-fold greater than non-pigmented ones (4, 4, 3 and 5 times, respectively). Compounds in the free fraction of pigmented rice had higher antioxidant capacity relative to those in the bound form, whereas the non-pigmented rice cultivars exhibited the opposite trend. Ferulic acid was the main phenolic acid of all rice genotypes, whereas black rice contained protocatechuic and vanillic acids in higher contents than red rice and non-pigmented rice genotypes. For vitamin E (tocopherols and tocotrienols) and carotenoids (lutein, zeaxanthin and β-carotene) contents, no obvious concentration differences were observed between non-pigmented and pigmented rice, with the black rice exhibiting the highest carotenoid content. Overall, pigmented rice genotypes contain a remarkable amount of bioactive compounds with high antioxidant capacity; therefore, they have great potential as a source of bioactives for developing functional food products with improved health benefits.

Keywords: red rice, black rice, vitamin E, carotenoids, phenolic acids

Abbreviations: PR, pigmented rice genotypes; nPR, non-pigmented rice genotypes; Olympiada, OLY; Alexandros, ALE; Fidgi, FID; Dellrose, DEL; Gladio, GLA; Fragrance, FRA; Dimitra, DIM; Roxani, ROX; Europe, EUR; Axios, AXI; Selenio; SEL; Cigalon, CIG; Escarlate, ESC; Campno, CAM; Black Rice, BLA; Ts, tocopherols; T3s, tocotrienols; PC, phenolic content; FC, flavonoid content; FA, ferulic acid; pCA, p-coumaric acid; GA, gallic acid; SA, sinapic acid; PRCA, protocatechuic acid; 4HBA, 4-hydroxy-benzoic acid; VA, vanillic acid; SRA, syringic acid

*Corresponding author. Phone:+302310471544; E-mail: irakli@cerealinstitute.gr

Introduction

Rice (*Oryza sativa* L.) is a staple food of more than half of the world's population, representing approximately 50% of grain production (Friedman 1996). Although rice is widely consumed as white grain, there are many special cultivars which contain pigments. Their name refers to the kernel which is developed by the deposition of anthocyanins in different layers of the pericarp, seed coat and aleurone (Chaudhary 2003).

Phytochemicals are defined as the bioactive non-nutrient plant compounds found in fruits, vegetables, and whole grains, which are proven clinically and/or epidemiologically to improve human health and well-being (Liu 2004). Cereal grains, especially rice, contain hundreds of phytochemicals, such as tocopherols (Ts), tocotrienols (T3s), γ-oryzanols, various phenolic compounds and carotenoids, which have been reported to possess biological activity (Adom and Liu 2002). Phenolic compounds are among the phytochemicals in cereal grains that are generally considered as natural antioxidants. The major phenolics reported in rice include phenolic acids, mainly ferulic acid (FA) and diferulates, which are present in kernels of nPR (Tian et al. 2004), as well as anthocyanins and proanthocyanidins that characterize PR.

Although PR is currently produced in small amounts, it has the potential to become a valuable raw material for the production of functional foods or natural food colorants. Previous studies have reported the phytochemical content and antioxidant capacity of whole grain white rice (Shen et al. 2009). Other studies also focused on the phytochemicals and antioxidant activity of rice bran (Lai et al. 2009). Compared to white rice, previous studies on PR have mainly dealt with the anthocyanin fraction of the kernel biophenols (Yao et al. 2010). In contrast, other phytochemicals such as vitamin E and carotenoids have not attracted as much attention as other components in rice grains. Therefore, a thorough analysis of the phytochemical profiles and antioxidant capacity of PR is needed. The objectives of the present study were to investigate the distribution of phytochemicals in the free or bound forms of PR and nPR and to evaluate their antioxidant capacities.

Materials and Methods

Chemicals and reagents

Phenolic acids and carotenoids standards, Folin-Ciocalteu reagent, 1,1-diphenyl-2-picrylhydrazyl (DPPH·), 2,2′-azinobis-(3-ethylbenzthiazoline-6-sulfonate) (ABTS) and 6-hydroxyl-2,5,7,8-tetramethychromane-2-carboxylic acid (Trolox) were purchased from Sigma-Aldrich (St Louis, USA). Ts isomers and catechin obtained by Supelco (Bellefonte, USA), whereas T3s isomers by LGC (Wesel, Germany). All other chemicals were of analytical grade.

Rice genotypes, sampling and preparation

The field experiment was carried out at the Experimental Station of the Cereal Institute in Kalochori (Lat: N 40°37'02.69", Long: E 22°49'43.67", 0 m alt.), Thessaloniki, Greece. Twelve nPR (OLY, ALE, FID, DEL, GLA, FRA, DIM, ROX, EUR, AXI, SEL, CIG) and five PR (four red rice: Escarlate, Campno, RRB, RRE and one black rice) were cultivated in experimental plots, using a randomized complete block design with three replications. The soil was fertilized with 150 kg N/ha in three increments, 75 kg P_2O_5/ha and 75 kg K_2O/ha. Each genotype was placed in a plot with plot to plot distance 50 cm. Each plot was consisted by four lines 2 m long. The line to line distance was 25 cm and plant to plant distance was 5 cm. Rice seeds were sown on May 17, transplanted on May 28 and harvested in October. Afterwards, they were air-dried, stored at room temperature for 3 months and were dehusked and grounded in a laboratory mill (Retsch, model ZM 1000, Germany) equipped with a 0.5 mm sieve.

Ts, T3s and carotenoids analysis

Briefly, 0.1 g sample was extracted with 2 mL ethanol and 0.1 mL 80% (w/v) KOH in a water bath at 80 °C for 15 min. After centrifugation, the residue was re-extracted with 2 mL ethanol. The combined extracts were applied to the Oasis cartridges; the retained constituents were eluted with 2 mL dichloromethane, and subsequently evaporated to dryness. Finally, the residue was redissolved in methanol and was analyzed as described by Irakli et al. (2011).

Extraction of free and bound phenolics

Free phenolics were extracted with 70% aqueous methanol by sonication for 15 min, according to Irakli et al. (2012). After centrifugation, the extraction was repeated twice. Supernatants were evaporated, applied to the Oasis cartridge and the adsorbed compounds were eluted with 2 mL 90% (v/v) aqueous methanol. The eluates were evaporated to dryness and the residues were redissolved in mobile phase. The pellets were hydrolyzed with 10 mL of 4 mol/L NaOH for 90 min by sonication. Then, the solution was acidified to pH ~ 2.0, the supernatant was extracted with ethyl acetate and the extracts were evaporated to dryness and redissolved in methanol.

Phenolic acid composition

The composition of phenolic acids of free and bound extracts was performed on an Agilent Technologies 1200 system using the method of Irakli et al. (2012). Samples were separated on a Nucleosil 100 C_{18} column (250 × 4.6 mm, 5 μm), with a mobile phase gradient of solvent A (1% acetic acid, v/v) and solvent B (methanol). The gradient was linear at a flow rate of 1.3 mL/min from 90% to 80% solvent A for 10 min, from 80% to 75% A for the next 10 min, from 75% to 65% A for 10 min and finally from 65% to 35%

A for another 10 min. Phenolic acids were identified by comparing UV spectra and retention times of peaks in rice extracts to those of the standard compounds. Detection and quantification was carried out at 260 nm for VA, 4HBA and PRCA; at 270 nm for GA and SRA; and at 320 nm for FA, SA, and pCA.

Determination of total phenolic content

0.2 mL sample aliquots of free and bound extracts were reacted with 0.8 mL of Folin-Ciocalteu reagent and 2 mL of 7.5% (w/v) sodium carbonate solution and the volume was adjusted to 10 mL with distilled water (Singleton et al. 1999). After 1 h, the absorption at 725 nm was recorded and the results were expressed as mg of gallic acid equivalents (mg GAE) per 100 g sample.

Determination of total flavonoid content

0.2 mL of free and bound extracts were mixed with 0.15 mL 5% (w/v) sodium nitrite and 0.15 mL 10% (w/v) aluminum chloride hexahydrate, and the mixture was allowed to stand for 5 min, and then 0.5 mL 1 mol/L NaOH was added (Bao et al. 2005). After 30 min, the absorbance at 510 nm was recorded and the results were expressed as mg of catechin equivalents (mg CATE) per 100 g sample.

DPPH radical scavenging activity

The DPPH radical scavenging activity of phenolic extracts was measured as described by Yen and Chen (1995) with some modifications. 0.15 mL extract was reacted with 2.85 mL 0.1 mM methanolic solution of DPPH. After 5 min, the absorbance at 516 nm was recorded. The free radical scavenging activity (%) was calculated by using the following equation:

$$\text{DPPH radical scavenging activity (\%)} = [(A0 - As)/A0] \times 100$$

where A0 is the absorbance of the blank sample and As is the absorbance of the sample at 5 min. Results were expressed as μmol Trolox equivalents (TE) per g sample (μmol TE/g).

ABTS radical scavenging activity

The antioxidant activity was evaluated employing the ABTS assay (Re et al. 1999). Briefly, $ABTS^{\bullet+}$ was obtained by reacting 2 mM ABTS stock solution with 0.73 mM potassium persulfate and the mixture was left to stand in the dark for 12–16 h. The $ABTS^{\bullet+}$ solution was diluted with water to an absorbance at 734 nm of 0.70 ± 0.02. After the addition of 0.1 mL of phenolic extract to 3.9 mL of $ABTS^{\bullet+}$ solution, the absorbance was recorded after 4 min. The $ABTS^{\bullet+}$ scavenging capacity was expressed as previously described for the DPPH assay.

Statistical analysis

Experimental data (all analyses were conducted at least in triplicate) were subjected to one-way analysis of variance using SPSS 19.0 software package. The least significant difference (LSD) at a 5% level of probability was applied to compare the means for identification of any significant difference.

Results

Phenolic (PC) and flavonoid contents (FC)

The free, bound and total PC in whole rice flour are presented in Table 1. The free PC in the nPR and PR varied from 19.8 to 47.3 mg GAE/100 g and 124.4 to 237.8 mg GAE/100 g, respectively. In addition to free PC, rice genotypes contained significant amounts of bound PC ranging from 79.8 to 98.3 mg GAE/100 g and 33.8 to 59.2 mg GAE/100 g for PR and nPR, respectively. Among all, red rice (91.6 mg GAE/100 g), had the highest bound PC, followed by black rice (79.8 mg GAE/100 g) and nPR. In nPR the mean value of the bound PC was approximately 1.5 times higher than the free PC, however, in PR the bound PC was 2 times lower than that of free one. The total PC of the nPR and PR ranged from 60 to 96.4 mg GAE/100 g and 209.6 to 320.9 mg GAE/100 g, respectively. CAM had the highest total PC followed by BLA, but these values were not significantly different.

The free, bound and total FC of the nPR and PR are also presented in Table 1. The free FC of the nPR and PR varied from 26.3 to 43.5 mg CATE/100 g and 104.4 to 145.6 mg CATE/100 g, respectively. The bound FC of the nPR and PR varied from 14.2 to 23.1 mg CATE/100 g and 47.5 to 74.2 mg CATE/100 g, respectively. In all rice genotypes, the mean value of the bound FC was about 2 times lower than the free one. The total FC of the nPR and PR ranged from 43.1 to 59.6 mg CATE/100 g and 169.9 to 219.8 mg CATE/100 g, respectively. ESC had much higher total FC than the other PR whose differences were not significant.

Comparing the total PC of nPR and PR, the average values of free, bound and total PC of PR, at 185.4, 89.2 and 274.6 mg GAE/100 g, respectively, were 6.1, 2.0 and 3.7 times higher, respectively, than those of nPR (30.4, 43.9 and 74.3 mg GAE/100 g, respectively). Comparing the total FC of nPR and PR, the mean values of free, bound and total FC of PR, at 123.6, 66.2 and 189.7 mg CATE/100 g, respectively, were 3.5, 4.0 and 3.7 times higher, respectively, than those of nPR (34.9, 16.5 and 51.3 mg CATE/100 g, respectively).

Phenolic acids composition

Eight phenolic acids were detected in the free and bound forms in whole rice flour of nPR and PR: GA, PRCA, 4HBA, VA, SRA, pCA, FA and SA. HPLC chromatograms of phenolic acids in nPR and PR are shown in Fig. 1S*. The distribution of phenolic acids in free

*Further details about the Electronic Supplementary Material (ESM) can be found at the end of the article.

Table 1. Phenolic and flavonoid contents of rice genotypes

Geno-type	Phenolic content (mg GAE/100 g)			Flavonoid content (mg CATE/100 g)		
	Free	Bound	Total	Free	Bound	Total
Non-pigmented rice						
OLY	35.9±2.5^{def}	41.8±3.9^{fgh}	77.7±3.6^{efg}	33.6±0.1^{efg}	17.6±0.1^{ef}	51.2±0.2^{efg}
ALE	29.0±1.7^{def}	36.0±2.2^{h}	65.0±1.4^{g}	37.5±0.2^{efg}	14.4±0.1^{f}	51.9±0.2^{efg}
FID	30.7±2.4^{def}	59.2±4.0^{d}	89.9±3.7^{ef}	27.6±0.1^{fg}	23.1±0.2^{d}	50.7±0.3^{efg}
DEL	31.2±4.0^{def}	34.7±5.5^{h}	65.9±3.6^{g}	30.6±0.2^{efg}	20.3±0.2^{de}	50.9±0.3^{efg}
GLA	28.9±1.9^{def}	37.7±2.5^{gh}	66.6±3.6^{g}	42.1±0.8^{e}	15.2±0.1^{f}	57.4±0.8^{ef}
FRA	19.8±1.5^{f}	46.9±2.8^{efg}	66.6±3.7^{g}	43.5±0.6^{e}	16.1±0.1^{ef}	59.6±0.6^{e}
DIM	47.3±0.8^{d}	48.9±3.7^{ef}	96.3±4.5^{e}	26.3±0.3^{g}	18.3±0.2^{def}	44.6±0.2^{fg}
ROX	26.2±2.4^{def}	33.8±1.7^{h}	60.0±0.9^{g}	39.1±0.8^{efg}	14.5±0.1^{f}	53.6±0.8^{efg}
EUR	22.8±0.2^{ef}	38.4±1.4^{gh}	61.2±1.4^{g}	35.3±0.2^{efg}	14.6±0.1^{f}	49.9±0.1^{efg}
AXI	44.4±4.1^{de}	52.0±2.0^{de}	96.4±2.2^{e}	39.8±0.4^{ef}	14.2±0.1^{f}	54.0±0.4^{efg}
SEL	25.6±1.6^{def}	50.6±1.9^{def}	76.2±3.3^{efg}	28.5±0.4^{fg}	14.5±0.1^{f}	43.1±0.4^{g}
CIG	22.9±2.1^{ef}	46.5±3.5^{efg}	69.3±4.9^{fg}	34.3±0.4^{efg}	14.7±0.2^{f}	49.0±0.5^{efg}
Mean	30.4±8.5	43.9±8.0	74.3±13.2	34.9±6.8	16.5±2.9	51.3±6.0
Pigmented rice						
ESC	203.9±13.7^{b}	89.2±5.3^{abc}	293.2±8.4^{b}	145.6±3.5^{a}	74.2±0.4^{a}	219.8±0.7^{a}
CAM	227.3±23.6^{a}	93.6±5.3^{ab}	320.9±18.1^{a}	131.3±3.6^{b}	47.5±0.2^{c}	178.8±0.5^{cd}
RRB	133.5±6.4^{c}	98.3±7.1^{a}	231.8±11.4^{c}	124.6±5.3^{bc}	70.0±0.3^{ab}	194.5±0.5^{b}
REB	124.4±13.5^{c}	85.2±4.8^{bc}	209.6±13.9^{d}	104.4±2.5^{d}	65.5±0.2^{b}	169.9±0.4^{d}
BLA	237.8±5.0^{a}	79.8±4.0^{c}	317.6±7.3^{a}	112.0±5.0^{cd}	73.6±0.4^{a}	185.6±0.5^{bc}
Mean	185.4±53.0	89.2±7.2	274.6±51.0	123.6±15.4	66.2±10.5	189.7±18.3

Values are mean of three replications ± standard deviation; values with the same letter in each column are not significantly different at the 0.05 level.

or bound forms of all rice genotypes is presented in Fig. S2. The total contents of free phenolic acids in nPR and PR ranged from 11.6 to 22.9 and 23.4 to 45.3 μg/g, respectively, while the bound phenolic acids ranged from 195.1 to 417.3 and 364.8 to 673.3 μg/g, respectively.

The free phenolics extract of black and red rice presented 2.18 and 0.98 μg of GA, 13.67 and 11.02 μg of PRCA, 2.37 and 2.03 μg 4HBA, 11.62 and 1.42 μg VA, 1.56 and 1.16 μg SRA, 4.8 and 3.91 μg pCA, 8.34 and 6.44 μg FA, 0.78 and 3.13 μg SA per g sample, while the bound phenolics extract of BLA presented 7.56 and 4.87 μg of GA, 199.67 and 43.29 μg PRCA, 11.72 and 11.66 μg 4HBA, 78.56 and 3.96 μg VA, 8.64 and 6.62 μg SRA, 48.48 and 72.53 μg pCA, 273.02 and 262.74 μg FA, 36.98 and 34.70 μg SA per g of sample, respectively. The free phenolics extract of nPR presented only 1.07 μg of GA, 1.07 μg PRCA, 1.76 μg 4HBA, 1.16 μg VA, 1.14 μg SRA, 3.09 μg pCA, 4.55 μg FA,

Table 2. Tocopherols (Ts), tocotrienols (T3s) and vitamin E (Ts+T3s) contents (μg/g) of rice genotypes

Genotype	Tocopherols				Tocotrienols				Total (Ts+T3s)
	α-T	(β+γ)-T	δ-T	total	α-T3	(β+γ)- T3	δ-T3	total	
Non-pigmented rice									
OLY	3.29^{i}	0.79^{b}	nd	4.08^{g}	1.35^{g}	10.53^{ab}	2.94^{bc}	14.81^{bcd}	18.89^{fg}
ALE	10.03^{cd}	0.16^{fg}	nd	10.19^{c}	4.03^{c}	6.93^{ef}	1.96^{fg}	12.92^{d}	23.11^{de}
FID	7.13^{fg}	0.42^{cde}	nd	7.29^{de}	5.95^{b}	6.52^{ef}	3.55^{a}	16.01^{b}	23.30^{de}
DEL	9.03^{de}	0.16^{fg}	nd	10.15^{c}	3.19^{d}	8.85^{cd}	2.91^{bcd}	14.95^{bcd}	25.10^{cde}
GLA	10.86^{bc}	0.43^{cd}	nd	11.29^{bc}	6.21^{b}	10.96^{a}	1.83^{g}	19.00^{a}	30.30^{b}
FRA	5.21^{h}	0.10^{fg}	nd	5.31^{fg}	2.16^{fg}	2.72^{h}	0.41^{h}	5.29^{f}	10.60^{i}
DIM	7.15^{fg}	0.03^{g}	nd	7.18^{def}	4.50^{c}	7.65^{de}	2.62^{cde}	14.77^{bcd}	21.95^{def}
ROX	14.85^{a}	0.53^{c}	nd	15.38^{a}	4.50^{c}	6.21^{f}	2.28^{efg}	13.00^{d}	28.38^{bc}
EUR	7.90^{ef}	0.04^{g}	nd	7.95^{d}	4.20^{c}	7.14^{ef}	2.28^{efg}	13.62^{cd}	21.57^{ef}
AXI	14.20^{a}	0.20^{efg}	nd	14.40^{a}	7.58^{a}	10.34^{ab}	2.41^{def}	20.32^{a}	34.72^{a}
SEL	9.81^{cd}	0.16^{efg}	nd	10.01^{c}	4.38^{c}	7.56^{de}	2.34^{ef}	14.27^{bcd}	24.29^{de}
CIG	6.24^{fgh}	1.16^{a}	nd	6.40^{def}	2.36^{ef}	2.95^{h}	0.84^{h}	6.15^{f}	12.52^{hi}
mean	8.81	0.35	nd	9.14	4.20	7.36	2.20	13.76	22.90
Pigmented rice									
ESC	14.09^{a}	0.28^{def}	nd	14.37^{a}	6.15^{b}	7.23^{ef}	2.94^{bc}	16.32^{b}	30.69^{b}
CAM	9.90^{cd}	0.15^{fg}	nd	10.05^{c}	5.57^{b}	7.30^{ef}	2.94^{bc}	15.81^{bc}	25.86^{cd}
RRB	11.26^{bc}	0.42^{cde}	nd	11.67^{bc}	6.20^{b}	9.24^{bc}	3.32^{ab}	18.76^{a}	30.43^{b}
REB	5.37^{gh}	0.04^{g}	nd	5.41^{efg}	3.02^{de}	4.62^{g}	2.08^{fg}	9.72^{e}	15.13^{gh}
BLA	12.23^{b}	0.15^{fg}	nd	12.41^{b}	6.29^{b}	9.26^{bc}	3.39^{ab}	18.93^{a}	31.34^{ab}
mean	10.57	0.21	nd	10.78	5.45	7.53	2.93	15.91	26.69

Values with the same letter in each column are not significantly different at the 0.05 level; nd, not detected.

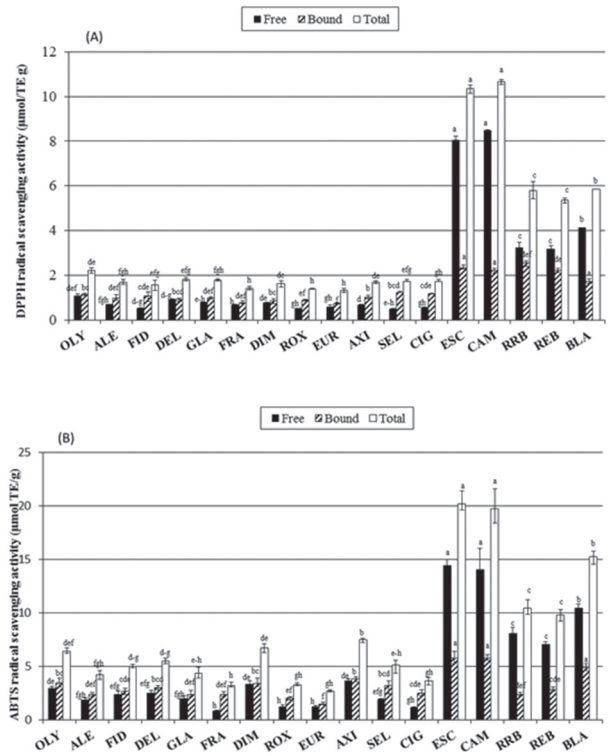

Figure 1. Free radical scavenging of the free and bound phenolic extracts of non-pigmented and pigmented rice genotypes by the DPPH (A) and ABTS (B) assays. Values are mean of three replications ± standard deviation; values with the same letter in the same bar are not significantly different at the 0.05 level

1.61 μg SA per g sample, while the bound phenolics extract of nPR presented 1.71 of GA, 6.73 μg PRCA, 9.20 μg 4HBA, 5.12 μg VA, 4.59 μg SRA, 54.02 μg pCA, 182.73 μg FA, 41.35 μg SA per g sample, respectively.

Ts, T3s and carotenoids contents

The total content of Ts in nPR and PR varied from 4.08 to 15.38 and 5.41 to 14.37 μg/g, respectively (Table 2). The contents of α-T, the most abundant Ts, ranged from 3.29 to 14.85 and 5.37 to 14.09 μg/g for nPR and PR, respectively. The total content of T3s in nPR and PR ranged from 5.29 to 20.32 and 9.72 to 18.93 μg/g, whereas the most abundant isomer was (β + γ) – T3, ranging from 2.72 to 10.96 and 4.62 to 9.26 μg/g, respectively. The vitamin E levels (Ts + T3s) of nPR and PR ranged from 10.60 to 34.72 μg/g and 15.13 to 31.34 μg/g, with a mean value of 22.90 and 26.69 μg/g, respectively.

The contents of carotenoids in rice genotypes were significantly different (Table S1*). Lutein was the primary component of all rice genotypes, ranging from 8.97 to 11.33

μg/100 g in nPR with an average of 10.12 μg/100 g, and 9.37 to 62.21 μg/100 g in PR with an average of 20.74 μg/100 g. Regarding the levels of β-carotene and zeaxanthin ranged from 0.71 to 1.41 and 0.45 to 0.72 μg/100 g in nPR and from 0.77 to 6.90 and 0.60 to 4.02 μg/100 g in PR, respectively. BLA had the highest β-carotene (6.90 μg/100 g) and zeaxanthin content (4.02 μg/100 g). The nPR and red rice exhibited similar values of total carotenoids content ranging from 10.41 to 12.79 μg/g and 10.91 to 12.82 μg/g, respectively, whereas BLA exhibited the highest value with a mean value of 73.13 μg/g.

DPPH and ABTS radical scavenging capacities

DPPH and ABTS scavenging capacities varied significantly among free and bound fractions, indicating the same trend in both assays (Fig. 1). The free DPPH radical scavenging capacities of nPR and PR ranged from 0.49 to 1.07 and 3.17 to 8.47 μmol TE/g, respectively, whereas the corresponding values for bound extracts ranged from 0.73 to 1.23 and 1.72 to 2.55 μmol TE/g, respectively. The total DPPH radical scavenging capacities of nPR and PR ranged from 1.30 to 2.21 and 5.37 to 10.67 μmol TE/g, respectively. The average of free, bound and total DPPH radical scavenging capacities of the PR, at 5.40, 2.20 and 7.60 μmol TE/g, respectively, were 7.9, 2.3 and 4.6 times higher than those of nPR (0.68, 0.98 and 1.66 μmol TE/g, respectively).

The free ABTS radical scavenging capacities of nPR and PR ranged from 0.83 to 3.66 and 7.01 to 14.43 μmol TE/g, respectively, whereas the corresponding values for bound extracts ranged from 1.47 to 3.80 and 2.79 to 5.73 μmol TE/g, respectively. The total ABTS radical scavenging capacities of nPR and PR ranged from 2.70 to 7.46 and 9.77 to 20.17 μmol TE/g, respectively. The average of free, bound and total ABTS radical scavenging capacities of the PR, at 10.79, 4.29 and 15.07 μmol TE/g, respectively, were 5.2, 1.6 and 3.1 times higher than those of nPR (2.07, 2.72 and 4.79 μmol TE/g respectively).

Discussion

Phenolic profiles of nPR and PR genotypes

The present study demonstrates that mean free PC did not differ significantly ($p > 0.05$) between PR, but free PC in rice is mostly influenced by color of the pericarp. These findings are in accordance with those of Sompong et al. (2011), who found that PC of PR varied significantly between the varieties, but no significant difference was shown between the two colors. Although several studies reported a greater extent of bound phenolics than free phenolics in rice (Adom and Liu, 2002), this was not observed in the present study in the situation of PR. However, the data are in agreement with those reported by De Mira et al. (2009) and Min et al. (2011), who also determined higher concentrations of free phenolics in PR than bound phenolics. This may be due to the fact that PR is rich in anthocyanins that are not esterified to other cellular components (Finochiaro et al. 2007). De Mira et al. (2009) indicated that even after repeated extractions for bound phenolics,

the color of PR extracts remained pink, indicating that it was difficult to completely extract these compounds.

This study also indicated that PR contained around 4 times higher total PC and FC than that of nPR, implying that the amount of phenolics is largely influenced by the pericarp pigments (Goffman and Bergman 2004). The total PC and FC of the nPR and PR in this study was similar to those reported by Saikia et al. (2012), whereas Shen et al. (2009) reported higher values. The possible reasons of these differences could be the different rice genotypes used, the growth conditions and the extraction method applied.

Consistent with other studies (Tian et al., 2004), nPR were noted to be rich in FA and pCA. PRCA following by FA were the predominant species in red rice in the free form, whereas in BLA, PRCA and VA were predominant. Similar results were reported by Sompong et al. (2011), whereas Niu et al. (2013) reported no values for PRCA in 22 red rice samples. GA, 4HBA, SRA and SA were minor constituents in all rice genotypes.

The most abundant bound phenolic acid found in nPR and red rice was FA followed by pCA and SA which was in accordance with previous studies (Tian et al. 2004; Sompong et al. 2011). However, in the BLA the bound phenolic acids followed the order: FA > PRCA > VA > pCA. Interestingly, PRCA was determined in high amounts in the bound form of BLA, although it was reported in low percentages in some red rice varieties (Sompong et al., 2011).

Tocols and carotenoids concentrations

Among the vitamin E components, $(\beta + \gamma) - T3$ was the highest vitamin E isomer present in all rice genotypes, while $(\beta + \gamma) - T$, $\delta - T3$, were present in trace amounts, whereas $\delta - T$ was not detected. The vitamin E content of the nPR was similar to that of the PR. These findings are consistent with those reported by Huang and Ng (2011) even though the levels of tocols vary depending on the genotype and the environmental conditions. Meanwhile, no clear trend in the contents of Ts and T3 was observed between nPR and PR, which is consistent with previous findings (Finochiaro et al. 2007; Min et al. 2011). Some of the PR analyzed in this study had a lower content of total vitamin E than the nPR, indicating that the color of rice does not relate to the total tocols content (Min et al. 2011). Huang et al. (2011) indicated that lipophilic compounds are more concentrated in regions closer to the bran fraction than to the endosperm, especially the germ part, whereas pigments appear to be mainly associated with the bran layer of rice (Min et al. 2011).

The carotenoid contents indicated distinct differences depending upon grain color. A comparison with the literature related to HPLC analysis of carotenoids is very difficult due to the few data available obtained by different methods. Our data are in a quite agreement with those of Lamberts and Delcour (2008) who reported similar values for the carotenoids levels of nPR. Other authors (Kim et al. 2010) reported different levels for the PR depending on the carotenoid variability discussed above. Among the nPR and red rice genotypes, no significant differences were observed, which agrees with the findings reported by Kim et al. (2010). Similarly to our results, in another study, screening of black,

red, and colorless rice cultivars for β-carotene indicated that black cultivars had higher β-carotene levels than the other two groups (Frei and Becker 2005). To the best of our knowledge, earlier literature data has not quantitatively compared the content of total carotenoids of durum wheat and black rice. However, Lamberts and Delcour (2008) reported that lutein levels in durum semolina samples were 40 times higher (mean value of 4.2 μg/g) than those of brown rice, while Kim et al. (2010) reported that the corresponding highest average content in black cultivars was 6.4 μg/g, suggesting that black rice could be an alternative source for carotenoids.

Antioxidant activity

Phenolic compounds contribute to antioxidant activity but reports examining free and bound antioxidant activity in red and black rice are limited. The results in our present research have shown that antioxidant activities of free phenolic extracts of PR genotypes were significantly higher than those of bound phenolic extracts, in accordance with the results of Paiva et al. (2014), although they reported DPPH and ABTS scavenging capacities higher than those obtained in the present study. However, the antioxidant capacities of bound extracts from nPR as measured by the two methods were higher than those of their free fractions, which agree with the findings of Adom and Liu (2002). This could be attributable to the higher PC in the bound extracts compared to the free ones. The total DPPH and ABTS radical scavenging capacities were approximately 3 to 5 times higher than those of nPR; this can be attributed to the higher concentration of PC and FC (Laokuldilok et al. 2011). Although the free phenolics extract of red rice genotypes presented lower total PC than the free phenolic extract of BLA, it presented higher antioxidant capacity by both DPPH and ABTS assays, maybe due to the high presence of proanthocyanidins in the outer layers of the red rice (Saikia et al. 2012).

Floegel et al. (2011) compared the ABTS and DPPH scavenging capacities of fruits, vegetables and beverages, reporting a higher ABTS value than DPPH. The higher DPPH than ABTS results for free phenolics extracts reported in the present study could be attributed to the presence of hydrophobic compounds, such as vitamin E, that would be more available for DPPH scavenging capacity, since DPPH is more applicable to hydrophobic systems.

Nowadays, more rice varieties have been developed as healthy foods and gained increasing popularity with consumers. The present study has shown that PR possess relatively higher antioxidant potential than nPR, because of the presence of higher amounts of phenolic compounds rather than lipophilic components. Regardless of the phenolic acid composition, FA is the major phenolic acid in all rice genotypes and is mainly present in the bound form, whereas, PR contained PRCA in higher contents than nPR. This gives opportunities for scientists to breed new PR varieties with higher nutritional values, which the food industry can use to develop new products that will compete in today's expanding functional food markets.

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Electronic Supplementary Material (ESM)

Electronic Supplementary Material (ESM) associated with this article can be found at the website of CRC at http://www.akademiai.com/content/120427/

Electronic Supplementary *Table S1*. Carotenoids contents of rice genotypes

Electronic Supplementary *Figure S1*. Typical chromatograms at 260, 270 and 320nm of (A) standard phenolic acids, (B) free phenolic acids of non-pigmented rice, (C) free phenolic acids of red rice, (D) free phenolic acids of black rice. Key to peak identification: 1, gallic acid; 2, internal standard; 3, protocatechuic acid; 4, 4-hydroxybenzoic acid; 5, vanillic acid; 6, caffeic acid; 7, syringic acid; 8, *p*-coumaric acid; 9, ferulic acid; 10, sinapic acid

Electronic Supplementary *Figure S2.* Distribution of (a) free and (b) bound phenolic acids in non-pigmented and pigmented rice genotypes. GA, gallic acid; PRCA, protocatechuic acid; 4HBA, 4-hydroxybenzoic acid; VA, vanillic acid; SRA, syringic acid; pCA, *p*-coumaric acid; FA, ferulic acid; SA, sinapic acid