Oryza Vol. 56 No. 3, 2019 (263-284)
DOI https://doi.org/10.35709/ory.2019.56.3.3

Phenolic compounds and antioxidant activities in dehusked and polished pigmented rice varieties

Rajarajeswari Jayaraman[1], Chandrasekhar Yadavalli[1], Vasudeva Singh[2]* and Farhath Khanum[1]

[1]Dept. of Nutrition, Biochemistry & Toxicology, Defence Food Research Laboratory, Mysore, Karnataka, India
[2]Department of Studies in Food Science & Nutrition, Manasa Gangotri, University of Mysore, Mysore, Karnataka, India
**Corresponding author e-mail: singhva2003@gmail.com*

Received : 5 August 2019 Accepted: 20 September 2019 Published : 30 September 2019

ABSTRACT

Present study was conducted to characterise eight red rice varieties in their de-husked and polished form for their phytochemical contents and anti-oxidant activities. Ferulic acid, gallic acid, quercetin, vanillic acid and rutin were identified in their free phenolic extracts. Dehusked rice free phenolics prevented free radical formation, metal chelation ferric to ferrous reduction and also influenced the reducing power. In polished rice, a moderate increase in antioxidant properties was noticed. The methanol extract showed higher phenolic content and anti-oxidant activities compared to extracts made in other solvents. Dehusked form of Kamdhari, Black basumati, Sirsi and Jyothi varieties exhibited higher amount of phytochemical components and antioxidant activities among the varieties. In dehusked form, total carotenoid content was more in Aravadan pillai and GK-4 varieties, whereas ascorbic acid content was high in Karisale, Kamdhari, Black basmati, Sirsi and Kasubai varieties. Thus, the dehusked and polished rice of these varieties with sufficient phenolic contents appear to be more useful as compared to normal rice with respect to their antioxidant properties.

Key words: *Pigmented rice, antioxidants, antioxidant activity, red rice, polished rice*

INTRODUCTION

Rice (*Oryza sativa* L.) is consumed by a large population worldwide and is considered as a basic source of metabolic energy (Monks et al., 2013). Amylose content of the grains is the major concern to select the rice varieties for consumption. Milled rice (polished rice) is most commonly consumed and it is prepared by removing about 7-12% of the bran fraction by mechanical milling. The shelled rice or dehusked rice or brown rice is obtained by removing the inedible cover of rice grain. The level of fibre and fat were reduced during milling process due to removal of seed coat, pericarp and aleurone layer which ultimately affects the sensory properties and storage stability. The pigmented rice varieties which have dark red, dark purple, dark blue, brown red, black purple, or dark red-purple grains have received greater attention as healthier foods (Min et al., 2009). The pigmented rice consumed in China, Japan, and Korea widely was considered as enriched with high quantity of phytochemicals, better in taste and for health improvements (Nakornriab et al., 2008). It is also consumed in different parts of India like Mangalore, Kerala and Odisha and these rice contain plenty of phytochemicals like flavones, tannin, phenolics, sterols, tocols, γ-oryzanols and important nutrients like proteins and essential oils (Parrado et al., 2003). Major functional components of pigmented rice are anthocyanins- a group of red to purple water-soluble flavonoids possessing antioxidant, anti-carcinogenic, anti-allergic, anti-inflammatory and anti-atherosclerosis activities (Hyun and Chung, 2004; Moreno et al., 2005; Zhang et al., 2006b). Carotenoids and ascorbic acid are the essential sources of plants which are mainly known for their antioxidative properties. Rice normally lacks in ascorbic acid and carotenoids.

Oxidative stress is the main reason that results in various chronic diseases which are caused by high levels of reactive oxygen species (ROS) (Goufo et al., 2017). Enzymatic and non-enzymatic antioxidants are also called cellular antioxidants and attenuate the harmful effects of ROS. Nowadays, consumption of natural antioxidants has considerably increased due to their health benefits compared to synthetic antioxidants which have adverse effect on long term intake (Goufo et al., 2014). Pigmented rice varieties are mainly used to strengthen kidney function, treatment of anaemia, promotion of blood circulation, removal of blood stasis, improvement in blood flow and treatment of diabetes and also amelioration of sight (Ma et al., 2000).

Pigmented rice varieties such as black and red rice are generally used as functional food and their extracts are used as food colorants in bread, ice cream, and liquor (Yoshinaga, 1986). They have wide range of bioactive properties such as antioxidant, anti-carcinogenic, anti-atherosclerosis, anti-allergic activities and found useful in amelioration of anemia (Xu and Wang, 1989; Chen et al., 2000; Ichikawa et al., 2001; Ling et al., 2001, Wang et al., 2007). Parboiled pigmented cross linked rice flour was used for the preparation of gluten free functional food for the regulation of glucose homeostasis and prevention of dyslipidaemia which helped in treating complications related to diabetes (Hameeda et al., 2016). Influence of parboiling of red paddy varieties by simple hot water soaking on phytochemical, content and antioxidant properties of rice has been reported (Jayaraman et al., 2019).

Evaluation of antioxidant activities and phenolic compounds in a large varieties of pigmented rice were reported in previous investigations (Nam et al., 2006; Rattanachitthawat et al., 2010; Daiponmak et al., 2014; Sanghamitra et al., 2017). Correlation analysis was performed to quantify the association between antioxidant activities and phenolic contents (Butsat & Siriamornpun, 2010). Esterified and bound phenolic fractions being, cross linked with proteins and carbohydrates are less bioavailable compared to the of free phenolic fractions. (Sakthi kumaran et al., 2015). Antioxidant activities of the rice varieties were evaluated to understand the antioxidant potential in terms of their bioavailable and non-bioavailable fractions. Apart from phenolics and flavonoids of dehusked form of red rice varieties, there have been scanty reports on comparative study on dehusked and polished red rice varieties with respect to their proanthocyanidins, anthocyanins, total ascorbic acid and total carotenoids content. Therefore, the present study was done to determine the variation in the phyto-chemicals and anti-oxidant activities of de-husked, polished forms of eight pigmented rice varieties.

MATERIALS AND METHODS

Collection of pigmented paddy rice varieties

Pigmented paddy rice varieties used for the present work (Jyothi, Aravadan pillai, GK-4, Kamdhari, Black basumati, Kasubai, Karisale and Sirsi) were collected from Vishweshwaraiah Canal Farm, University of Agricultural Sciences, Mandya, Karnataka. These varieties were harvested during December 2013, stored at -20°C till use.

Shelling/de-husking and polishing

The respective paddy rice was brought to room temperature and dehusked in a McGill sheller by adjusting the clearance between the metal roller and rubber containing roller. The shelled rice (100 g) of each variety was subjected to milling/polishing (~15 %) using a Satake type polisher for 2 min, where an emery coated circular disc was used. Collected bran was sieved through #22 mesh sieve and the bran adhering to the milled rice was removed by sieving through the same sieve. The collected polished rice were used for various analysis along with their respective de-husked rice which was red in colour.

Sample preparation

The dehusked and polished rice were grinded in a mixie till it passed completely through #85 mesh sieve and the flours were subjected to defatting. 5 g of each dehusked and polished rice flour were defatted with hexane in a Soxhlet extractor at 40°C for 2 hr and the same process was repeated 2-3 times for the complete extraction of fat. After removing fat, the samples were kept at room temperature for the complete evaporation of hexane and used to analyse various assays.

Extraction of free, esterified and bound phenolics

The free, bound and esterified phenolics were extracted in dehusked and polished rice of paddy rice varieties according to the procedure described by Krygier et al. (1982) and Naczk and Shahidi (1989) with slight

modifications. The defatted samples (2 g) were extracted six times with 40 ml of methanol-acetone-water (7:7:6, v/v/v) mix at room temperature. The mixtures were then centrifuged (5000 g, 15 min), and supernatants were collected and combined. The solvent was evaporated at 30°C under vacuum and reduced to approximately 40 ml. Concentrated supernatants were filtered through Whatman No. 1 filter paper and the collected fractions were labelled as free phenolics. The pellet on the Whatman filter paper was further treated with 30 mL of 4 M NaOH for 4 h at room temperature (21 ± 2°C). The samples were flushed with nitrogen and packaged in airtight glass sample vials. The resultant hydrolysate was acidified to pH 2 using 6 M HCl and extracted six times with diethyl ether. The ether extracts were then combined and evaporated to dryness at 30°C under vacuum. The phenolic acids extracted were those liberated from their esters and labelled as esterified phenolic acids. The leftover meal after extractions was treated with 20 ml of 4 M NaOH for 4 h at room temperature (21 ± 2°C). The samples were flushed with nitrogen and then acidified to pH 2 with 6 M HCl followed by centrifugation (5000 g, 15 min). The mixture was extracted six times with diethyl ether. The ether extracts were combined and evaporated to dryness under vacuum at 30°C. The phenolic acids so extracted were labelled as bound phenolics. Free, esterified, and bound phenolics were dissolved separately in 2 ml of methanol and stored at - 20°C until use.

Total polyphenolic content

The extract of free, bound and esterified phenolics prepared from rice grains was used to determine phenol content by using Folin-Ciocalteau reagent (FC reagent) (Singleton et al., 1999). To different volumes of phenolic extracts, 800 µl of freshly prepared diluted FC reagent and 2 ml of 7.5% Na_2CO_3 were added. The contents were diluted to 7 ml with distilled water and kept in dark for 30 min. The absorbance was read at 760 nm. The content of phenols were expressed in terms of mg ferulic acid equivalents per 100 g of sample.

Total flavonoids content

Total flavonoid content was estimated by the method described by Zhishen et al. (1999). The total flavonoid content of free, bound and esterified phenolics of rice extracts were expressed as mg quercetin equivalents per 100 g of sample.

Total flavonol content

The total flavonol content was estimated as per Yermakov et al. (1987) method with slight modifications. 0.05 ml of bound phenolic extract was taken and volume was made up to 1 ml with methanol. Then 0.5 ml of vanillin (1 % in methanol) and 0.5 ml 25 % H_2SO_4 (in methanol) were added. The tubes were mixed and allowed to react for 15 minutes at room temperature. The absorbance was measured at 500 nm in a UV visible spectrophotometer against blank. For blank, 1.0 ml of methanol was taken and treated in the same way as sample. The results were expressed in terms of mg quercetin equivalents per 100 g of sample.

Tannin content

The bound phenolics of rice extracts were treated with 5 ml of 1 % HCl butanol and boiled in a water bath for 3 h at 100°C for tannin determination. The extract solution (0.4 ml) was mixed with 1 ml of sulphuric acid/ methanol solution and 1 % vanillin in methanol. A control was prepared by adding 100 % methanol instead of vanillin solution. All the sample mixture were incubated for 15 min at 30°C in a water bath and the absorbance was measured at 500 nm. The results were expressed in terms of mg catechin equivalents (CE Eq) per 100 g of sample (Reed et al., 1982).

Determination of anthocyanin content

The analysis method for anthocyanin content was modified from the method used by Hosseinian et al (2008). The bound phenolics of pigmented rice extract (20 µl) was added into 2 mL of potassium chloride buffer (0.03 mol/l, pH 1.0) and 2 mL of sodium acetate buffer (0.4 mol/l, pH 4.5). Each of them was left for 15 min before taking an absorption measurement using spectrophotometer (Libra S22, Biochrom, England) at 550 nm and 700 nm. Distilled water was used as a blank. The anthocyanin concentration (mg/l) of sample was expressed as Cyanidin-3-glucoside equivalents.

Determination of proanthocyanidins

Proanthocyanidins were determined by butanol-HCl assay (Hagerman et al., 2000). In brief, 0.5 ml aliquots of prepared extracts (free and bound) were transferred into test tubes. After addition of 3.0 ml butanol-HCl

reagent (butanol: HCl, 95:5; V/V) and 0.1 ml 2 % ferric reagent (2 % ferric ammonium sulfate in 2M HCl), test tubes were vortexed and put into a boiling water-bath for 60 min. After cooling, absorbance was recorded at 550 nm against blank, containing 0.5 ml of solvent instead of the extract. The proanthocyanidins concentration (mg/l) of sample was expressed as Cyanidin-3-glucoside equivalents.

Estimation of ascorbic acid

Ascorbic acid content in pigmented rice varieties were analysed by the spectrophotometric method described by Roe and Keuther (1943). The concentration of ascorbate in the samples were calculated and expressed in terms of mg ascorbic acid equivalents per 100 g of sample.

Estimation of total carotenoids

Total carotenoids in pigmented rice varieties were estimated by the method described by Zakaria et al. (1979). The amount of total carotenoids in pigmented rice varieties were expressed in terms of mg carotenoids equivalents per 100 g of sample.

$$\text{Total carotenoids (mg/100g)} = \frac{\text{Sample OD x Conc of standard}}{\text{Standard OD x sample weight}} \times 100$$

High-performance liquid chromatography (HPLC) of phenolics and flavonoids

Phenolics and flavonoids of free rice phenolic extracts were analysed by reverse phase HPLC on Shimpak C-18 column (model LC-10A, Shimadzu Corporation, Japan) using a diode array detector operating at 280 nm and 320 nm. A gradient solvent system, consisting of 0.2 % Tri fluro acetic acid (Solvent A) and methanol (Solvent B), at a flow rate of 0.75 ml/min was as follows: 0-30 min, 0-15 % B in A; 30-50 min, 15 % B in A; 50-60 min, 15-25 % B in A; 60-90 min, 15-100 % B in A; 90-100 min, 100-0 % B in A. Standards of caffeic, coumaric, ferulic, gallic, protocatechuic, catechin, rutin, vanillic and trans-cinnamic acids used for identification of polyphenols (Alu'datt et al., 2013).

DPPH* radical scavenging activity

The stable 2,2-diphenyl-1-picrylhydrazyl (DPPH+) radical was used to measure the free radical scavenging activity of free and bound rice phenolics extracts and the method followed was described as per Goupy et al. (1999). The reactants consist of 500 µl of diluted sample and 500 µl of freshly prepared DPPH (6 mg/10 ml) incubated in dark for 30 min at ambient temperature and the absorbance was read at 517 nm. The results were expressed in terms of mg catechin equivalents per 100 g of sample.

ABTS* radical scavenging assay

Radical scavenging capacity of the free and bound rice phenolic extracts were evaluated against the ABTS according to the method of Auddy et al. (2003). The results were expressed in terms of trolox equivalents per 100 g of sample.

Hydrogen peroxide (H_2O_2) scavenging activity

The free and bound extract of pigmented rice samples were dissolved in 3, 4 ml of 0.1 M phosphate buffer (pH 7.4) and mixed with 600 µl of a 43 mM solution of hydrogen peroxide. The absorbance value (at 230 nm) of the reaction mixture was recorded from 0 to 40 min and then at every 10 min. For each concentration, a separate blank sample was used for background subtraction (Ruch, Chung, & Klaunig, 1984). The results were expressed in terms of ascorbic acid equivalents per 100 g of sample.

Hydroxyl radical scavenging assay

Hydroxyl radical scavenging activities of free and bound rice extracts were determined according to the method of Halliwell et al. (1987). The results were reported in terms of mg quercetin equivalents per 100 g of sample.

Metal chelating activity (binds Fe^{2+})

Metal chelating activity was measured according to the method of Suter and Richter (2000) with minor modifications. The reaction mixture containing ferrous chloride (200 µM) and potassium ferricyanide (400 µM), with various concentrations of free and bound rice extracts was made to 1 ml with double distilled water and mixed. The reaction mixture was incubated at 20°C for 10 min. Formation of the potassium hexacyanoferrate complex was measured at 700 nm using a UV-Vis spectrophotometer. The assay was carried out at 20°C to prevent Fe^{2+} oxidation. Lower absorbance indicated a higher iron chelating capacity. The results of metal

chelating activity of free and bound rice phenolic extracts were expressed in terms of mg EDTA equivalents per 100 g of sample.

Determination of ferric reducing antioxidant power (FRAP)

The FRAP assay is based on the reduction of the Fe (III)-TPTZ complex to the ferrous form at low pH. This reduction is monitored by measuring the absorption change at 595 nm (Benzie & Strain, 1999). Briefly, 200 µl of free and bound rice extract was mixed with 1.3 ml of the FRAP reagent. The absorbance was measured at 595 nm after incubating for 30 mins at 37°C. FRAP reagent was prepared freshly before the experiment which consists of 0.3 M acetate buffer (pH 3.6), 10 mM TPTZ in 40 mM HCl and 20 mM $FeCl_3$ in a ratio of 10:1:1 (v/v/v). The results were expressed as mg $FeSO_4$ equivalents per 100 g of sample.

Total anti-oxidant activity

Various concentrations of free and bound rice phenolic extracts and 1.23 ml of phospho-molybdenum reagent solution (0.6 M sulphuric acid, 28mM sodium phosphate and 4 mM of ammonium molybdate) were added and incubated at 90°C for 90 min. The absorbance was read at 695 nm and the total antioxidant capacity was expressed as ascorbic acid equivalents per 100 g of sample (Prieto et al., 1999).

Nitric oxide scavenging activity

Sodium nitroprusside (10 mM) in phosphate buffered saline was mixed with different concentrations of rice extract which is dissolved in methanol and incubate at room temperature for 150 min. The same reaction mixture without the methanol extract but the equivalent amount of methanol serves as the control. After the incubation period, 0.5 ml of griess reagent (1% sulfanilamide, 2% H_3PO_4 and 0.1% N-(1- naphthyl) ethylenediamine dihydrochloride was added. The absorbance of the chromophore formed was read at 546 nm. (Sreejayan and Rao, 1997). The results were expressed in terms of mg ferulic acid equivalents per 100 g of sample.

Reducing power activity

The reducing power of free and bound rice phenolic extracts were determined by the method of Yen and Duh (1993) with slight modifications. Different concentrations of rice extracts were mixed with 2.5 ml of phosphate buffer (200 mM, pH 6.6) and 2.5 ml of 1 % potassium ferricyanide. The mixtures were incubated for 20 min at 50ºC. After incubation, 2.5 ml of 10 % trichloroacetic acid were added to the mixtures, followed by centrifugation at 2100 rpm for 10 min. The upper layer (2.5 ml) was mixed with 2.5 ml of distilled water and 0.5 ml of 0.1% ferric chloride and the absorbance of the resultant solution were measured at 700 nm. The results were presented in terms of mg butylated hydroxy toluene equivalents per 100 g of sample.

Antioxidant activity in beta carotene linoleate emulsion system

The antioxidant activity of the free and bound rice phenolic extracts were determined using the thiocyanide method (Kikuzaki & Nakatani, 1993). The antioxidant activity was calculated as percentage of inhibition against blank.

Statistical analysis

Minitab statistical software was used to analyse the data. All experiments were performed in triplicates and the mean values with standard deviations were expressed in the results. Significance level ($p<0.05$) for the obtained results were calculated using Tukey - Kramer multiple comparison test by one-way ANOVA.

RESULTS & DISCUSSION

Phenolics composition

The contents of free, bound and esterified polyphenols are expressed in Table 1. The free phenolic content in the dehusked and polished rice of various varieties (Jyothi, Aravadan pillai, GK-4, Kamdhari, Black basumati, Kasubai, Karisale and Sirsi) varied from 79.40 to 487 and 9.42 to 61.3 mg FAE/100 g respectively. The loss of free polyphenols on polishing among the eight varieties varied from ~48 to ~94 %, with a maximal loss observed in Jyothi variety and minimal loss in Aravadan pillai followed by GK-4, Kasubai, Sirsi, Karisale, Black basumati, Kamdhari varieties. The bound phenolic content of red and polished rice of these varieties ranged from 114.9 to 243.10 and 21.80 to 71.10 mg FAE/100 g respectively. Loss of bound polyphenols upon polishing varied from ~55 to ~91%. Maximal loss observed in Karisale variety and minimal loss in

Table 1. Free, bound and esterified Polyphenolic contents of dehusked and polished rice of various pigmented varieties.

Samples	Free (mg FA Eq:/100g)	Bound (mg FA Eq:/100g)	Esterified (mg FA Eq:/100g)
Jyothi (RR)	168.63 ± 6.9^{e}	143.00 ± 2.6^{c}	98.4 ± 1.80^{a}
Jyothi (PR)	9.42 ± 0.7^{j}	30.10 ± 1.8^{h,i,j}	20.6 ± 1.80^{h}
Aravadan pillai (RR)	79.40 ± 4.1^{f,g}	136.40 ± 8.4^{c,d}	46.4 ± 2.90^{b}
Aravadan pillai (PR)	31.20 ± 2.5^{i,j}	22.00 ± 0.6^{i,j}	15.7 ± 1.00i
GK-4 (RR)	103.60 ± 0.9^{f}	114.90 ± 11.1^{d}	35.1 ± 1.80^{d}
GK-4 (PR)	38.70 ± 0.6^{h,i,j}	44.00 ± 4.2^{g,h,i}	16.2 ± 0.50^{i}
Kamdhari (RR)	487.00 ± 27.8^{a}	180.90 ± 9.4^{b}	40.7 ± 0.50^{c}
Kamdhari (PR)	50.60 ± 2.2^{g,h,i}	71.10 ± 0.7^{e}	15.6 ± 0.05^{i,j}
Black basumati (RR)	381.60 ± 19.0^{b}	243.10 ± 17.2^{a}	26.9 ± 0.80^{f}
Black basumati (PR)	40.00 ± 1.5^{h,i}	69.10 ± 3.8^{e,f}	27.8 ± 1.30^{e,f}
Kasubai (RR)	202.90 ± 10.2^{d}	179.70 ± 7.6^{b}	35.7 ± 3.00^{d}
Kasubai (PR)	61.3 ± 0.7^{g,h}	49.00 ± 4.7^{f,g,h}	24.2 ± 1.40^{f,g}
Karisale (RR)	258.30 ± 9.6^{c}	237.00 ± 4.0^{a}	12.0 ± 2.20^{j}
Karisale (PR)	38.10 ± 1.2^{h,i,j}	21.80 ± 2.4^{j}	27.8 ± 1.20^{e,f}
Sirsi (RR)	369.70 ± 11.4^{b}	202.60 ± 13.7^{b}	22.1 ± 0.04^{g,h}
Sirsi (PR)	59.00 ± 4.0^{g,h,i}	58.60 ± 0.1^{e,f,g}	30.7 ± 1.10^{e}

RR: Dehusked / Red rice, PR: Polished rice; Values are presented in mean ± SD of 3 replicates; Values in columns not sharing the same superscript are significantly different for $P < 0.05$. FAE- Ferulic acid equivalents.

Kamdhari followed by GK-4, Sirsi, Black basumati, Jyothi, Kasubai and Aravadan pillai. Interestingly in four varieties the loss was in the range of 71 to ~ 73%, indicating the degree of adherence by the various bran layers were almost same, in these varieties.

The esterified phenolic content of dehusked and polished pigmented rice varieties ranged from 12.0 to 98.4 and 15.6 to 30.7 mg FAE/100 g respectively. The percentage reduction in esterified polyphenols of polished rice compared with dehusked rice varied from 32 to 79 %. Maximal loss occurred in Jyothi variety and moderate changes were found in Aravadan pillai, GK-4, Kamdhari and Kasubai varieties. While polished rice varieties such as Black basumati, Karisale and Sirsi showed higher esterified polyphenols and this could be because of more esterified phenolic extraction during polishing. Free, bound and esterified phenolics of dehusked rice were found to be more compared to polished rice and this may due to the presence of pigmented rice bran. Jyothi, Kamdhari, Black basumati, Kasubai, Sirsi varieties had a relatively high free phenolic content among the eight varieties of dehusked rice. However, dehusked varieties of Black basumati, Karisale, Sirsi showed reduction in esterified phenolic content compared to polished rice. Phenolic content of pigmented red and black rice was previously reported for dehusked rice and polished rice by Paiva et al. (2014). Hence the content of free, bound and esterified phenolics were not reported in different fractions of pigmented rice varieties. Free polyphenolic contents of dehusked rice varieties (Jyothi, Aravadan pillai, Gk-4, Kamdhari, Black basumati, Kasubai, Karisale and Sirsi) were high compared to bound and esterified polyphenols. The bound phenolics were found to be high in polished rice varieties of Jyothi, GK-4, Kamdhari and Black basumati compared to other varieties. Previous studies reported the phenolic content of Thai red and polished rice varieties which varied between 79.2 to 691.4 mg FAE/100g, 39.2 to 58.9 mg GAE/ 100g. It can be seen that the reported phenolic contents in the present study are in comparison with the previous studies (Ti et al., 2014; Sompong et al., 2011). The variation in the free, bound and esterified phenolics between eight rice varieties mainly due to the growth period, quantity of irrigation of water, growth conditions such as temperature stress (Iqbal et al., 2007).

Total flavonoids content

Flavonoids are ingested by humans and found to have anti-inflammatory, anti-allergic and anti-cancer activities (Crozier et al., 2006). The free and bound flavonoids content in the shelled and milled fractions (Dehusked and polished rice) of eight pigmented rice varieties are listed in Table 2. The free flavonoid content in the

Table 2. Total flavonoids, flavonol, and tannin of dehusked and polished rice of pigmented rice varieties.

Samples	Total flavonoids (mg Qu Eq:/100g)			Total flavonol (mg Qu Eq:/100g)	Tannin (mg CAT Eq:/100g)
	Free	Bound	Esterified		
Jyothi (RR)	119.2 ± 5.4^{d,e}	344.2 ± 2.7^{c,d}	79.2 ± 2.1^{a}	1.18 ± 0.08^{f}	1.95 ± 0.07^{d,e}
Jyothi (PR)	80.8 ± 5.4^{e,f}	265.4 ± 10.8^{e,f}	-nd-	0.86 ± 0.02^{g}	-nd-
Aravadan pillai (RR)	44.2 ± 2.7^{f,g}	363.5 ± 13.5^{c}	30.0 ± 1.0^{e}	1.14 ± 0.02^{f}	1.95 ± 0.07^{d,e}
Aravadan pillai (PR)	9.6 ± 2.7^{g}	236.5 ± 13.5^{f}	-nd-	1.10 ± 0.08^{f}	-nd-
GK-4 (RR)	36.5 ± 2.7^{g}	336.5 ± 24.4^{c,d}	60.4 ± 1.6^{c}	0.86 ± 0.02^{g}	3.10 ± 0.10^{a,b}
GK-4 (PR)	5.8 ± 2.7^{g}	244.2 ± 13.5^{f}	-nd-	0.38 ± 0.02^{h}	-nd-
Kamdhari (RR)	446.2 ± 10.8^{a}	430.8 ± 32.6^{b}	36.2 ± 2.1^{d}	2.04 ± 0.05^{d}	3.30 ± 0.10^{a}
Kamdhari (PR)	117.3 ± 2.7^{d,e}	250.0 ± 11.7^{f}	-nd-	1.52 ± 0.05^{e}	2.65 ± 0.07^{b,c}
Black basumati (RR)	376.9 ± 10.9^{b}	500.0 ± 38.0^{a}	21.2 ± 1.6^{f}	3.28 ± 0.01^{b,c}	2.30 ± 0.60^{c,d}
Black basumati (PR)	200.0 ± 0.1^{c}	334.6 ± 12.6^{c,d}	-nd-	0.98 ± 0.02^{f,g}	1.20 ± 0.06^{g}
Kasubai (RR)	361.5 ± 21.7^{b}	423.1 ± 5.4^{b}	22.7 ± 1.6^{f}	3.22 ± 0.08^{c}	3.0 ± 0.15^{a,b}
Kasubai (PR)	336.5 ± 29.9^{b}	253.8 ± 10.8^{f}	-nd-	1.74 ± 0.02^{e}	1.20 ± 0.10^{f,g}
Karisale (RR)	465.4 ± 10.8^{a}	482.7 ± 13.5^{a}	15.8 ± 0.5^{g}	3.50 ± 0.01^{a,b}	2.25 ± 0.07^{c,d}
Karisale (PR)	167.3 ± 2.7^{c}	257.7 ± 10.8^{f}	-nd-	1.60 ± 0.01^{e}	-nd-
Sirsi (RR)	450 ± 21.0^{a}	475.0 ± 19.0^{a}	71.9 ± 2.7^{b}	3.64 ± 0.02^{a}	1.80 ± 0.10^{d,e,f}
Sirsi (PR)	157.7 ± 0.1^{c,d}	307.7 ± 21.7^{d,e}	-nd-	2.10 ± 0.08^{d}	1.45 ± 0.07^{e,f,g}

RR: Dehusked / red rice PR: Polished rice; Values are presented in mean ± SD of 3 replicates; Values in columns not sharing the same superscript are significantly different for $P < 0.05$. Qu E- Quercetin equivalents, CAT E- Catechin equivalents.

dehusked and polished rice of these varieties varied from 36.5 to 465.4 and 5.8 to 336.5 mg QUE/100g respectively. The loss of free flavonoid content on polishing varied from ~7 to 84 %, with a maximal loss observed in Aravadan pillai, GK-4, Kamdhari varieties and minimal loss in Jyothi, Black basumati, Kasubai varieties. Karisale and Sirsi varieties showed an intermediate loss percentage comparatively. The bound flavonoid content of dehusked and polished rice of these varieties ranged from 336.5 to 500 and 236.5 to 334.6 mg QUE/100g respectively. The loss of bound flavonoid content on polishing varied from ~23 to ~47 %, with a minimal loss observed in Jyothi, GK-4 varieties and other varieties showed an intermediate loss percentage comparatively. Whereas, the free flavonoid content of dehusked rice of these varieties were found to be high compared to bound flavonoid content. The varieties such as Kamdhari, Jyothi, Black basumati, Kasubai, Karisale and Sirsi showed highest free flavonoid content than other two rice varieties. In previous studies, free and bound flavonoid content reported in the range of 306.4 to 525.2 mg CE/100g and 97.9 to 198.7 mg CE/100g respectively (Ti et al., 2014). These varietal discrepancies in free and bound flavonoid content of dehusked and polished rice of these varieties is due to the influence of genetic and environmental factors. The esterified flavonoids content of dehusked rice varieties varied from 15.8 to 79.2 mg Qu Eq:/100 g and the same was not detected in polished rice varieties.

Total flavonol content

The total flavonol content in dehusked and polished rice of bound phenolic extracts are presented in Table 2. The flavonol content of dehusked and polished rice of these varieties varied from 0.86 to 3.64 mg QUE/100g and 0.38 to 2.10 mg Quercetin Equivalents (QUE)/100g respectively. The percentage reduction in total flavonol content in polished rice of various varieties compared with dehusked rice ranged from ~4 % to 70 % with a maximal loss in Black basumati variety and minimal loss in Aravadan pillai followed by Kamdhari, Jyothi, Sirsi, Kasubai varieties. Karisale and GK-4 varieties showed an intermediate loss percentage comparatively. The dehusked rice of these varieties such as Kamdhari, Black basumati, Kasubai, Karisale and Sirsi showed higher flavonol content. The flavonol content in rice varieties were not reported in previous studies but the optimal conditions for the extraction of flavonols such as myricetin, quercetin and kaempferol were investigated in plant extracts (Wang & Helliwell, 2001).

Tannin content

In recent years tannins in small quantities are incorporated into foodstuffs like beverages for taste (Parr et al., 2000). Tannins reduce carbohydrate digestibility and bioavailability and therefore considered as anti-nutrient when it's taken in large quantity. In addition to this, tannin has anticancer, cardiovascular, gastro-protective, anti-ulcerogenic and cholesterol lowering properties. It also promotes urinary tract health (Prior et al., 2005). Thus tannins at lower concentrations are beneficial. The tannin content of dehusked and polished rice of pigmented varieties varied between 1.95 to 3.30 mg Catechin Equivalents (CE)/100 g and 1.20 to 2.65 mg CE/100g respectively (Table 2). Dehusked rice of these varieties exhibited highest tannin content than polished rice due to the presence of aluerone, pericarp and seed coat. Different degree of milling resulted in the loss of tannin content in the polished rice except Kamdhari, Black basumati, Kasubai and Sirsi varieties. Tannin content were not detected in polished rice varieties of Jyothi, Aravadan pillai, GK-4 and Karisale. Whereas, the percentage reduction in tannin content of other polished rice is as follows; Kamdhari (19.6%), Black basumati (48 %), Kasubai (60 %) and Sirsi (19 %). The tannin content of pigmented rice varieties in their red and polished form were not reported in previous studies.

Proanthocyanidin content

Proanthocyanidin are flavonoid oligomers widely distributed in plants that are known to have a wide range of benefits for human health (Xie et al., 2006). The free and bound proanthocyanidin content of red and polished rice of pigmented varieties were expressed as cyanidin chloride equivalents are shown in Table 3. The free proanthocyanidin (PA) content in the dehusked and polished rice of these varieties varied from 68.6 to 953.6 and 24.2 to 88.7 mg Cy/100 g respectively. The percentage reduction in free proanthocyanidin content of polished rice of these varieties compared with dehusked rice ranged from 65 % to 94 % with a maximal loss in Jyothi, Kasubai, Sirsi, Black basumati, Karisale, Kamdhari varieties and minimal loss in Aravadan pillai, GK-4 varieties. The bound proanthocyanidin content in the dehusked rice and polished rice of these varieties ranged from 80.7 to 204.4 and 17.4 to 59.1 mg Cy/100 g respectively. The percentage reduction in bound proanthocyanidin content of polished rice of these

Table 3. Total anthocyanins and proanthocyanidins of dehusked and polished rice of pigmented varieties.

Samples	Total proanthocyanidins (mg Cy Eq/100 g)		Total anthocyanin content (mg cy Eq/100g)
	Free	Bound	
Jyothi (RR)	193.6 ± 9.5^{e}	98.1 ± 3.8^{d}	$10.18 \pm 0.1^{d,e}$
Jyothi (PR)	$33.6 \pm 5.7^{g,h}$	17.4 ± 0.9^{i}	4.84 ± 0.2^{h}
Aravadan pillai (RR)	$68.6 \pm 2.8^{f,g}$	$80.7 \pm 4.7^{d,e}$	$12.02 \pm 0.1^{c,d}$
Aravadan pillai (PR)	24.2 ± 0.1^{h}	$28.2 \pm 0.9^{h,i}$	$4.00 \pm 0.1^{h,i}$
GK-4 (RR)	88.7 ± 3.8^{f}	80.7 ± 1.9^{e}	$9.26 \pm 0.3^{e,f}$
GK-4 (PR)	$28.2 \pm 1.9^{g,h}$	$26.9 \pm 0.9^{h,i}$	$2.17 \pm 0.2^{i,j}$
Kamdhari (RR)	953.6 ± 0.9^{a}	139.8 ± 7.6^{c}	19.20 ± 1.0^{a}
Kamdhari (PR)	$60.5 \pm 1.8^{f,g,h}$	$37.6 \pm 1.9^{g,h}$	$5.84 \pm 0.2^{g,h}$
Black basumati (RR)	739.7 ± 4.6^{b}	160.0 ± 3.8^{b}	$13.50 \pm 0.1^{b,c}$
Black basumati (PR)	73.9 ± 0.1^{f}	59.1 ± 1.2^{f}	$3.50 \pm 0.1^{h,i,j}$
Kasubai (RR)	516.4 ± 18.0^{c}	141.2 ± 0.2^{c}	15.20 ± 0.1^{b}
Kasubai (PR)	$68.6 \pm 2.8^{f,g}$	$47.0 \pm 2.8^{f,g}$	1.50 ± 0.1^{j}
Karisale (RR)	347.0 ± 9.5^{d}	204.4 ± 8.5^{a}	$10.50 \pm 0.7^{d,e}$
Karisale (PR)	$32.2 \pm 0.2^{g,h}$	$32.2 \pm 0.1^{h,i}$	$7.51 \pm 0.2^{f,g}$
Sirsi (RR)	685.9 ± 10.4^{b}	$150.6 \pm 0.9^{b,c}$	15.02 ± 0.7^{b}
Sirsi (PR)	87.4 ± 2.8^{f}	59.1 ± 0.1^{f}	$1.66 \pm 0.1^{i,j}$

RR: Dehusked / Red Rice PR: Polished Rice; Values are presented in mean ± SD of 3 replicates; Values in columns not sharing the same superscript are significantly different for $P < 0.05$. Cy Eq: Cyanidin chloride Equivalents

varieties compared with dehusked rice ranged from 63 % to 84 % with a maximal loss in Jyothi, Karisale varieties and minimal loss in other varieties. Proanthocyanidins content of free phenolic extracts was higher than the bound phenolic extracts of dehusked pigmented rice extracts. The free and bound proanthocyanidin content were significantly higher in Kamdhari, Black basumati, Kasubai, Karisale, Jyothi and Sirsi varieties than other varieties. In previous studies, proanthocyanidin content of red rice cultivar reported as 222 mg per 100 g and average value of 11 red rice cultivars were found to be 169 mg /100 g (Min et al., 2012; Gunaratne et al., 2013). Our studies have shown almost 50% of the reported values which may be due to the varietal differences

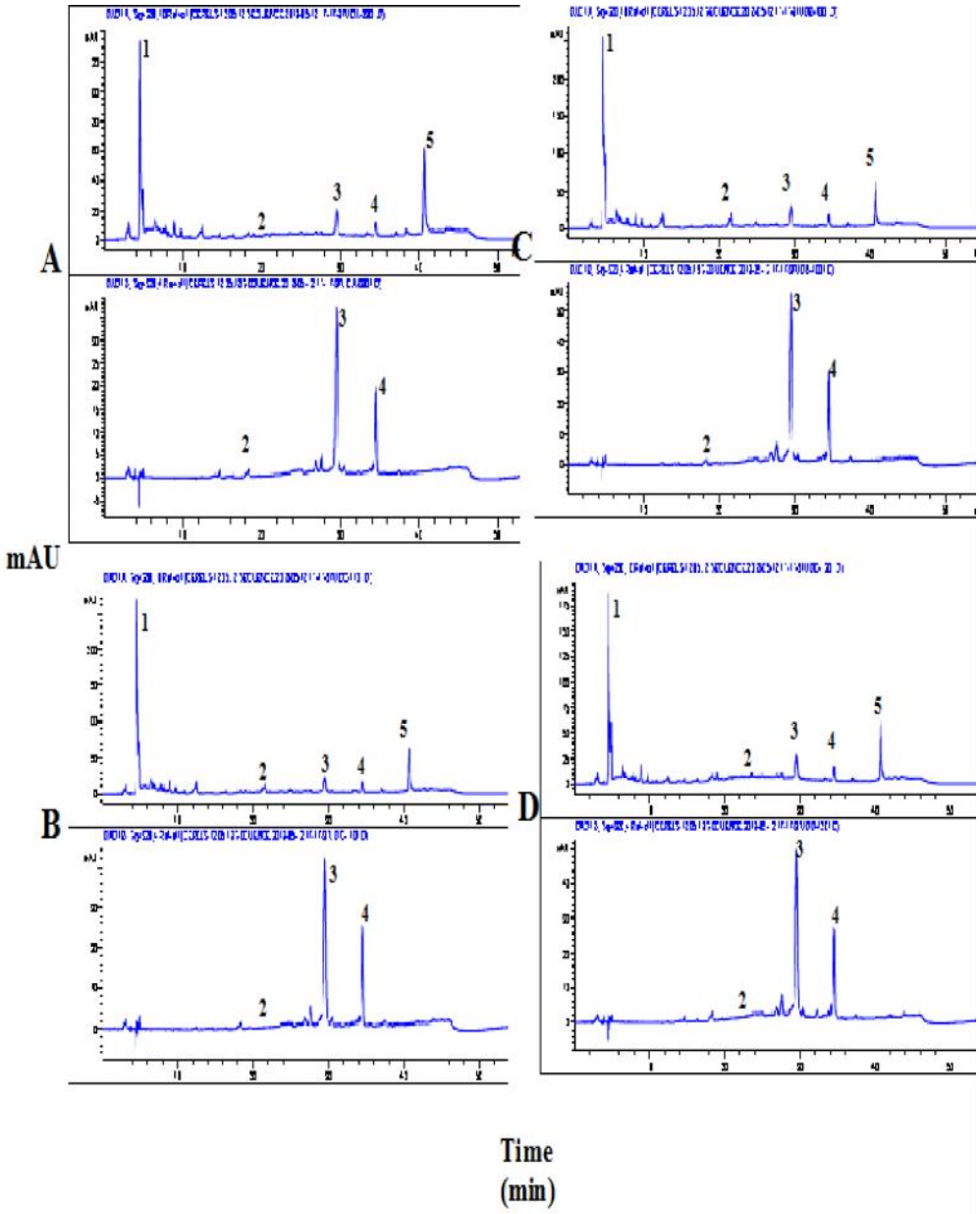

Fig.1. HPLC Chromatogram of phenolic acids and flavonoids. 1. Gallic acid, 2. Vanillic acid, 3.Ferulic acid, 4. Rutin, 5. , 4. Rutin, 5. Quercetin; A- Jyothi, B- Aravadan pillai, C-GK-4, D- Kamdhari.

Table 4. Total carotenoids, total ascorbic acid content of dehusked rice of pigmented varieties.

Samples	Total carotenoids content (mg car Eq:/100g)	Total ascorbic acid content (mg asc Eq:/100g)
Jyothi (RR)	$0.78 \pm 0.06^{d,e}$	5.94 ± 0.1^{f}
Aravadan Pillai (RR)	1.21 ± 0.07^{a}	6.41 ± 0.2^{e}
GK-4 (RR)	1.13 ± 0.04^{a}	6.41 ± 0.2^{e}
Kamdhari (RR)	$0.90 \pm 0.01^{b,c}$	11.38 ± 0.1^{a}
Black basumati (RR)	$0.77 \pm 0.02^{d,e}$	9.50 ± 0.3^{b}
Kasubai (RR)	0.74 ± 0.06^{e}	7.63 ± 0.1^{d}
Karisale (RR)	0.98 ± 0.01^{b}	11.63 ± 0.1^{a}
Sirsi (RR)	$0.87 \pm 0.07^{c,d}$	8.38 ± 0.1^{c}

RR: Dehusked / Red Rice; Values are presented in mean ± SD of 3 replicates; Values in columns not sharing the same superscript are significantly different for $P < 0.05$. Car Eq - Carotene equivalents, Asc Eq- Ascorbic acid equivalents.

Anthocyanin content

Hydrophilic flavonoids are the group of anthocyanins which are responsible for the red colour of pigmented rice. The anthocyanin content of dehusked and polished rice of eight varieties are shown in Table 3. Total anthocyanin content in dehusked rice was significantly higher than polished rice, this may be due to accumulation of pigments in the bran layer of red rice. Total anthocyanin content of dehusked and polished rice of these varieties varied from 9.26 to 15.20 mg Cy/100 g and 1.50 to 7.51 mg Cy/100 g respectively. The percentage reduction in total anthocyanin content of polished rice varieties compared with dehusked rice ranged from 29 % to 90 % with a maximal loss in Sirsi, Kasubai varieties and minimal loss in Jyothi, Aravadan pillai, Kamdhari, Black basumati GK-4 varieties (Table 9). Karisale variety showed an intermediate percentage loss in total anthocyanin content. The dehusked rice of varieties such as Jyothi, Aravadan pillai, GK-4, Kamdhari, Black basumati, Sirsi and Kasubai showed significantly higher anthocyanin content than Karisale variety. The difference in anthocyanin content of dehusked and polished rice of these varieties may be due to the contribution of high phenolic content.

Total carotenoids

Carotenoids are the dietary antioxidants which play important role in photo-protective functions in plants during photosynthesis. Research suggests that the metabolic end product of carotenoids plays an important role in anthocyanin synthesis in many plants (Nagira et al., 2006). The total carotenoid content of pigmented rice flour in their dehusked rice form varied from 0.74 to 1.21 mg carotenoids equivalents per 100 g (Table 4).

Table 5. Radical scavenging activities of dehusked and polished rice of various pigmented varieties.

Samples	DPPH Scavenging activity (mg CAT Eq:/100g)		ABTS Scavenging activity (mg Trolox Eq:/100g)	
	Free	Bound	Free	Bound
Jyothi (RR)	$153.2 \pm 3.6^{b,c}$	309.5 ± 11.1^{a}	173.0 ± 3.7^{d}	-nd-
Jyothi (PR)	14.5 ± 1.3^{i}	97.8 ± 8.8^{c}	5.9 ± 0.4^{g}	-nd-
Aravadan pillai (RR)	120.8 ± 3.7^{d}	302.2 ± 14.1^{a}	163.4 ± 0.75^{d}	-nd-
Aravadan pillai (PR)	14.5 ± 1.3^{i}	98.2 ± 9.1^{c}	5.9 ± 0.4^{g}	-nd-
GK-4 (RR)	82.0 ± 6.2^{e}	312.3 ± 17.6^{a}	177.3 ± 3.7^{d}	-nd-
GK-4 (PR)	34.0 ± 3.2^{h}	99.2 ± 9.7^{c}	$18.3 \pm 0.8^{f,g}$	-nd-
Kamdhari (RR)	$163.9 \pm 3.7^{a,b}$	230.4 ± 3.18^{b}	464.2 ± 27.6^{a}	-nd-
Kamdhari (PR)	$37.1 \pm 0.9^{g,h}$	98.7 ± 8.6^{c}	40.6 ± 1.5^{e}f	-nd-
Black basumati (RR)	$155.5 \pm 3.4^{b,c}$	303.1 ± 9.19^{a}	351.4 ± 6.0^{b}	-nd-
Black basumati (PR)	68.7 ± 5.0^{f}	100.7 ± 10.1^{c}	$42.7 \pm 1.6^{e,f}$	-nd-
Kasubai (RR)	$158.2 \pm 7.2^{a,b,c}$	300.1 ± 10.8^{a}	294.7 ± 10.8^{c}	-nd-
Kasubai (PR)	$39.5 \pm 1.54^{g,h}$	105.3 ± 7.4^{c}	$40.6 \pm 3.1^{e,f}$	-nd-
Karisale (RR)	149.2 ± 2.7^{c}	306.2 ± 10.9^{a}	281.7 ± 7.5^{c}	-nd-
Karisale (PR)	21.9 ± 1.9^{h}	106.3 ± 8.8^{c}	4.9 ± 0.05^{g}	-nd-
Sirsi (RR)	168.4 ± 8.9^{a}	321.9 ± 15.7^{a}	357.5 ± 21.7^{b}	-nd-
Sirsi (PR)	48.1 ± 2.7^{g}	101.5 ± 9.6^{c}	48.9 ± 2.2^{e}	-nd-

RR: Dehusked / Red Rice PR: Polished Rice; Values are presented in mean ± SD of 3 replicates; Values in columns not sharing the same superscript are significantly different for $P < 0.05$. CAT E- Catechin Equivalents.

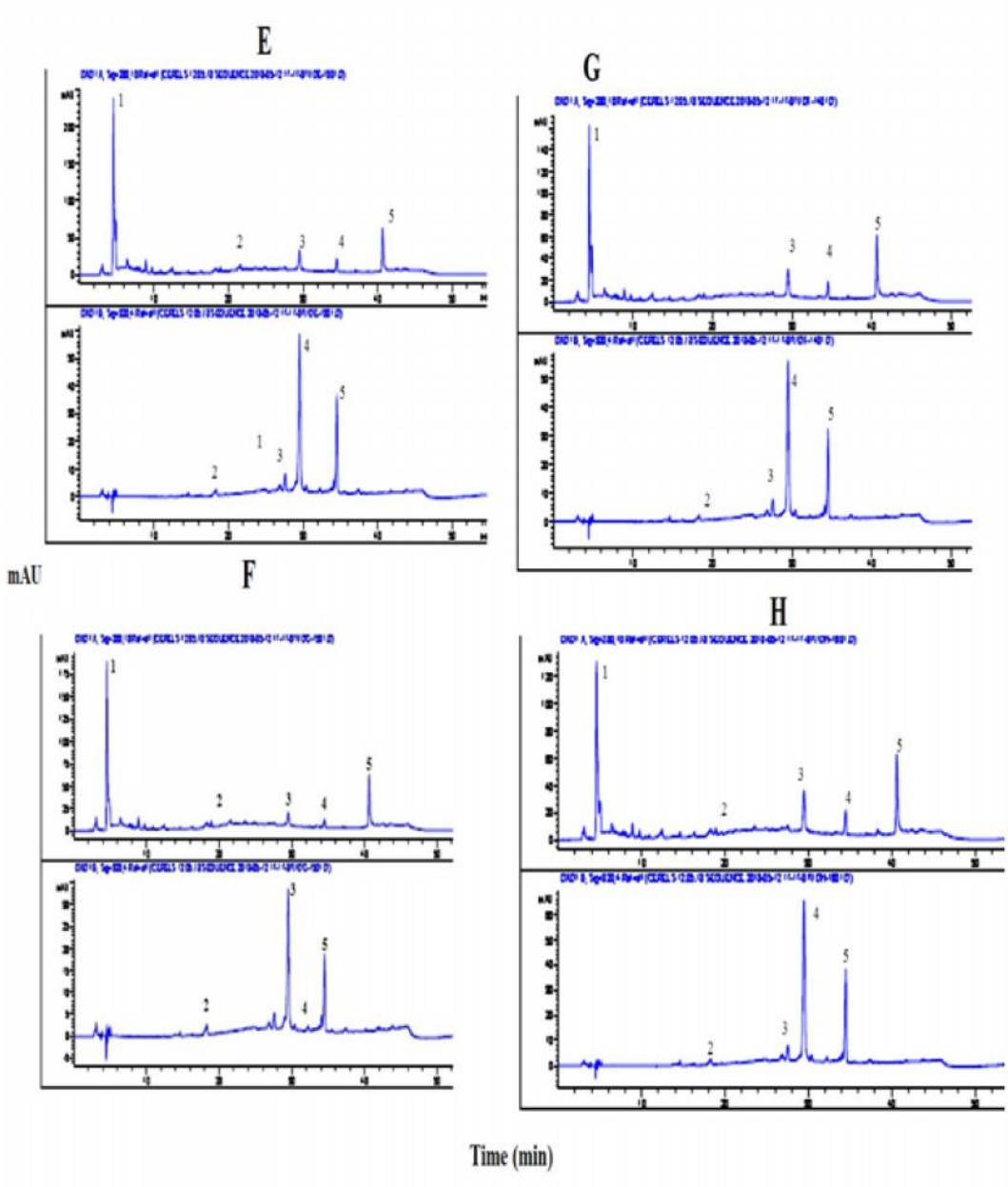

Fig. 2. HPLC Chromatogram of phenolic acids and flavonoids. 1. Gallic acid, 2. Vanillic acid, 3. Ferulic acid, 4. Rutin, 5. , 4. Rutin, 5. Quercetin; E- Black basumati, F- Kasubai, G-Karisale, H- Sirsi.

The carotenoid content was found high in Aravadan pillai and GK-4. While other varieties had lower carotenoid content which may be due to the genetic variability. Previous studies have not reported the content and composition of total carotenoids. Rice grain quality improvement has become very crucial for most breeding programs around the world. Pigmented rice varieties with highest carotenoid content were used for the purpose of bio-fortification to obtain nutrient rich rice grain (Ye et al., 2000).

Ascorbic acid

Ascorbic acid is an essential nutrient required for the human diets. It is mainly found in fruits and vegetables and is predominantly employed in the pharmaceutical

Table 6. Hydrogen peroxide and Hydroxyl radical scavenging activities of dehusked and polished rice of various pigmented varieties.

Samples	H_2O_2 Scavenging activity (mg Asc Eq:/100g)		Hydroxyl scavenging activity (mg Quercetin Eq:/100g)	
	Free	Bound	Free	Bound
Jyothi (RR)	$62.1 \pm 3.7^{f,g,h}$	68.6 ± 0.1^{a}	37.2 ± 1.3^{a}	$27.0 \pm 0.2^{d,e,f}$
Jyothi (PR)	$65.3 \pm 2.1^{f,g}$	41.1 ± 2.9^{g}	27.5 ± 0.1^{g}	28.1 ± 0.03^{d}
Aravadan pillai (RR)	$58.6 \pm 1.2^{h,i}$	$36.7 \pm 0.2^{h,i}$	$36.1 \pm 0.7^{a,b}$	$27.7 \pm 0.4^{d,e}$
Aravadan pillai (PR)	78.2 ± 1.2^{c}	$42.6 \pm 1.4^{e,f,g}$	24.0 ± 0.6^{i}	29.2 ± 0.03^{c}
GK-4 (RR)	47.2 ± 2.4^{j}	$39.3 \pm 3.7^{g,h}$	34.8 ± 0.1^{b}	$26.9 \pm 0.06^{e,f}$
GK-4 (PR)	$90.9 \pm 2.4^{a,b}$	33.6 ± 1.0^{i}	29.1 ± 0.4^{f}	$30.9 \pm 0.7^{a,b}$
Kamdhari (RR)	$93.3 \pm 5.8^{a,b}$	$45.9 \pm 2.4^{e,f}$	31.9 ± 1.1^{c}	$27.5 \pm 0.1^{d,e,f}$
Kamdhari (PR)	$67.8 \pm 0.1^{e,f}$	$41.9 \pm 2.5^{f,g}$	35.1 ± 0.1^{b}	31.4 ± 0.2^{a}
Black basumati (RR)	95.5 ± 0.3^{a}	51.6 ± 2.4^{d}	$31.7 \pm 0.06^{c,d}$	$25.8 \pm 0.1^{g,h}$
Black basumati (PR)	54.2 ± 0.2^{i}	60.1 ± 1.4^{b}	29.5 ± 0.1^{f}	$26.5 \pm 0.5^{f,g}$
Kasubai (RR)	80.4 ± 1.2^{c}	$53.8 \pm 0.6^{c,d}$	$31.8 \pm 0.5^{c,d}$	24.7 ± 0.06^{h}
Kasubai (PR)	$72.1 \pm 1.2^{d,e}$	46.2 ± 1.4^{e}	$30.3 \pm 0.2^{e,f}$	$30.0 \pm 1.4^{b,c}$
Karisale (RR)	$90.3 \pm 0.3^{a,b}$	$55.5 \pm 1.2^{c,d}$	$31.1 \pm 0.4^{c,d,e}$	25.0 ± 0.2^{h}
Karisale (PR)	$61.4 \pm 2.1^{g,h}$	$35.4 \pm 0.7^{h,i}$	25.7 ± 0.7^{h}	31.5 ± 0.7^{a}
Sirsi (RR)	89.4 ± 2.1^{b}	56.0 ± 2.4^{c}	$30.4 \pm 0.6^{d,e,f}$	25.2 ± 0.03^{h}
Sirsi (PR)	$77.8 \pm 6.1^{c,d}$	34.9 ± 0.1^{i}	$31.4 \pm 0.9^{c,d,e}$	31.7 ± 0.1^{a}

RR: Dehusked / Red Rice PR: Polished Rice; Values are presented in mean ± SD of 3 replicates; Values in columns not sharing the same superscript are significantly different for $P < 0.05$. Asc - Ascorbic acid Equivalents, Qu Eq- Quercetin Equivalents.

Table 7. Metal chelating, ferric reducing power and total antioxidant capacity of dehusked and polished rice of various pigmented varieties.

Samples	Metal chelating activity (mg EDTA Eq:/100g)		Ferric reducing power (mg $FeSO_4$ Eq:/100g)		Total antioxidant capacity (mg asc Eq:/100g)	
	Free	Bound	Free	Bound	Free	Bound
Jyothi (RR)	117.5 ± 0.9^{a}	239.5 ± 2.5^{a}	$15.2 \pm 0.9^{h,i}$	$6.8 \pm 0.1^{f,g}$	166.4 ± 0.3^{d}	91.3 ± 3.5^{i}
Jyothi (PR)	8.4 ± 0.4^{g}	$32.8 \pm 3.0^{g,h}$	$21.8 \pm 0.07^{c,d}$	$7.0 \pm 0.2^{e,f,g}$	58.2 ± 3.8^{h}	$116.1 \pm 0.7^{f,g}$
Aravadan pillai (RR)	84.9 ± 5.8^{b}	140.2 ± 10.0^{c}	12.8 ± 0.3^{i}	$6.6 \pm 0.1^{g,h}$	164.6 ± 3.2^{d}	$93.1 \pm 6.7^{h,i}$
Aravadan pillai (PR)	$13.3 \pm 1.1^{f,g}$	$52.7 \pm 2.6^{e,f}$	$19.4 \pm 1.2^{d,e,f}$	$7.1 \pm 0.4^{e,f,g}$	$85.9 \pm 3.2^{f,g}$	75.2 ± 0.9^{j}
GK-4 (RR)	82.7 ± 3.2^{b}	128.3 ± 6.8^{c}	12.8 ± 0.3^{i}	5.7 ± 0.4^{h}	138.7 ± 1.2^{e}	$90.8 \pm 0.3^{i,j}$
GK-4 (PR)	$13.0 \pm 0.1^{f,g}$	$33.4 \pm 5.9^{g,h}$	$17.9 \pm 1.2^{e,f,g}$	$6.5 \pm 0.3^{g,h}$	91.6 ± 2.4^{f}	$103.7 \pm 0.4^{g,h,i}$
Kamdhari (RR)	45.8 ± 3.0^{d}	90.3 ± 1.6^{d}	28.9 ± 0.9^{b}	8.5 ± 0.6^{c}	341.9 ± 22.9^{a}	174.6 ± 6.7^{d}
Kamdhari (PR)	$12.2 \pm 0.1^{f,g}$	$44.2 \pm 0.2^{f,g}$	$18.2 \pm 1.6^{e,f,g}$	$7.8 \pm 0.07^{c,d,e}$	$83.5 \pm 4.8^{f,g}$	$115.6 \pm 6.7^{f,g}$
Black basumati (RR)	82.4 ± 7.2^{b}	173.4 ± 8.4^{b}	23.9 ± 1.6^{c}	$7.6 \pm 0.2^{d,e,f}$	318.5 ± 12.1^{a}	279.3 ± 17.0^{a}
Black basumati (PR)	8.1 ± 0.4^{g}	24.7 ± 0.1^{h}	$18.5 \pm 0.4^{e,f,g}$	$8.3 \pm 0.2^{c,d}$	$82.9 \pm 5.7^{f,g,h}$	142.3 ± 0.7^{e}
Kasubai (RR)	57.6 ± 0.8^{c}	63.0 ± 1.6^{e}	$16.3 \pm 0.9^{g,h}$	8.5 ± 0.3^{c}	215.0 ± 5.4^{c}	250.6 ± 6.5^{b}
Kasubai (PR)	$11.3 \pm 0.3^{f,g}$	$52.7 \pm 2.0^{e,f}$	$20.2 \pm 1.0^{d,e}$	$8.3 \pm 0.3^{c,d}$	$82.3 \pm 0.4^{f,g,h}$	$116.3 \pm 3.4^{f,g}$
Karisale (RR)	115.3 ± 2.1^{a}	186.3 ± 2.1^{b}	41.6 ± 2.5^{a}	17.6 ± 0.2^{a}	$157.4 \pm 0.2^{d,e}$	252.3 ± 13.8^{b}
Karisale (PR)	9.7 ± 0.06^{g}	$44.4 \pm 0.1^{f,g}$	29.0 ± 1.69^{b}	$6.6 \pm 0.4^{g,h}$	$61.6 \pm 0.4^{g,h}$	$108.7 \pm 0.2^{f,g,h}$
Sirsi (RR)	31.4 ± 0.3^{e}	93.3 ± 5.1^{d}	28.6 ± 1.3^{b}	17.0 ± 0.9^{a}	267.4 ± 8.8^{b}	207.2 ± 5.2^{c}
Sirsi (PR)	15.8 ± 1.1^{f}	$35.4 \pm 0.9^{f,g,h}$	$17.2 \pm 0.1^{f,g,h}$	9.5 ± 0.2^{b}	89.9 ± 1.2^{f}	120.3 ± 7.6^{f}

RR: Dehusked / Red Rice PR: Polished Rice; Values are presented in mean ± SD of 3 replicates; Values in columns not sharing the same superscript are significantly different for $P < 0.05$. Asc - Ascorbic acid Equivalents, Qu Eq- Quercetin Equivalents.

and cosmetic industry due to its antioxidant properties. In the earlier studies, the content of ascorbic acid has not been reported in pigmented rice varieties. Rice is reported to contain no vitamin C, vitamin A, beta-carotene, or lutein+zeazanthin, and is notably low in fiber. An attempt was made to understand the availability of ascorbic acid in pigmented rice varieties and the results are listed in Table 4. The ascorbic acid content in dehusked rice varieties varied from 5.94 to 11.63 mg Asc E/100 g. Kasubai, Karisale and Kamdhari

Table 8. Nitric oxide, Reducing power and Beta Carotene Linoleate System antioxidant activity of dehusked and polished rice of pigmented varieties.

Samples	Nitric oxide scavenging activity (mg FA Eq:/100g)		Reducing power activity (mg BHT Eq:/100g)		Beta carotene linoleate emulsion system (% Inhibition)	
	Free	Bound	Free	Bound	Free	Bound
Jyothi (RR)	$52.3 \pm 2.6^{a,b}$	$15.4 \pm 0.7^{d,e,f}$	3.63 ± 0.3^{d}	$4.33 \pm 0.1^{d,e}$	$59 \pm 2.4^{a,b}$	78 ± 0.9^{a}
Jyothi (PR)	$16.3 \pm 0.4^{d,e}$	$12.5 \pm 0.4^{e,f,g}$	2.98 ± 0.1^{d}	3.87 ± 0.1^{g}	$32 \pm 1.2^{e,f}$	$69 \pm 1.6^{a,b}$
Aravadan pillai (RR)	41.2 ± 3.5^{b}	$11.3 \pm 0.1^{e,f,g}$	1.18 ± 0.01^{f}	$4.44 \pm 0.1^{e,f}$	61 ± 1.4^{a}	$60 \pm 0.6^{b,c,d,e,f}$
Aravadan pillai (PR)	$14.5 \pm 1.1^{d,e}$	$12.6 \pm 0.09^{e,f,g}$	3.40 ± 0.1^{d}	$4.40 \pm 0.1^{f,g}$	28 ± 1.6^{f}	$66 \pm 0.2^{b,c}$
GK-4 (RR)	41.7 ± 2.8^{b}	$14.7 \pm 1.0^{d,e}$	$1.87 \pm 0.1^{e,f}$	$4.20 \pm 0.01^{e,f}$	$52 \pm 3.2^{b,c}$	$50 \pm 1.2^{g,h,i}$
GK-4 (PR)	10.9 ± 0.02^{e}	$23.5 \pm 0.4^{a,b}$	$2.64 \pm 0.1^{d,e}$	3.23 ± 0.2^{g}	$37 \pm 1.6^{d,e,f}$	$55 \pm 0.1^{d,e,f,g}$
Kamdhari (RR)	54.4 ± 4.2^{a}	$22.4 \pm 2.0^{a,b}$	9.62 ± 0.05^{a}	$5.13 \pm 0.02^{c,d}$	64 ± 4.8^{a}	$53 \pm 1.2^{e,f,g,h}$
Kamdhari (PR)	$17.9 \pm 0.07^{d,e}$	$11.7 \pm 0.01^{e,f,g}$	3.13 ± 0.1^{d}	3.90 ± 0.1^{a}	$45 \pm 0.5^{c,d}$	$57 \pm 1.3^{c,d,e,f,g}$
Black basumati (RR)	56.0 ± 3.8^{a}	$12.7 \pm 0.6^{e,f,g}$	$7.76 \pm 0.1^{a,b}$	$5.99 \pm 0.05^{b,c}$	65 ± 2.4^{a}	$44 \pm 0.1^{h,i,j}$
Black basumati (PR)	$20.0 \pm 0.6^{c,d,e}$	$9.6 \pm 0.1^{f,g}$	$2.77 \pm 0.1^{d,e}$	2.82 ± 0.1^{g}	$44 \pm 0.2^{c,d}$	$60 \pm 0.5^{b,c,d,e,f}$
Kasubai (RR)	57.0 ± 2.4^{a}	10.2 ± 0.6^{g}	5.28 ± 0.01^{c}	$6.12 \pm 0.1^{b,c}$	$40 \pm 0.02^{d,e}$	$53 \pm 1.2^{f,g,h}$
Kasubai (PR)	25.8 ± 1.2^{c}	24.5 ± 0.6^{a}	$2.77 \pm 0.1^{d,e}$	4.13 ± 0.3^{g}	$37 \pm 0.02^{d,e,f}$	$64 \pm 1.3^{b,c,d}$
Karisale (RR)	58.3 ± 0.6^{a}	$9.9 \pm 0.1^{f,g}$	3.79 ± 0.02^{d}	$6.47 \pm 0.08^{b,c}$	$44 \pm 0.8^{c,d}$	$42 \pm 1.1^{i,j}$
Karisale (PR)	$24.1 \pm 0.9^{c,d}$	$17.5 \pm 0.5^{c,d}$	$2.60 \pm 0.2^{d,e,f}$	3.98 ± 0.1^{g}	$31 \pm 1.6^{e,f}$	$64 \pm 0.1^{b,c,d}$
Sirsi (RR)	$59.2 \pm 0.6^{d,e}$	$8.7 \pm 0.3^{f,g}$	$6.66 \pm 0.1^{b,c}$	7.48 ± 0.1^{b}	$59 \pm 2.4^{a,b}$	40 ± 0.8^{j}
Sirsi (PR)	25.9 ± 0.6^{c}	$18.9 \pm 0.8^{b,c}$	$2.80 \pm 0.1^{d,e}$	4.65 ± 0.1^{g}	$33 \pm 0.01^{e,f}$	$63 \pm 0.9^{b,c,d,e}$

RR: Dehusked / Red Rice PR: Polished Rice; Values are presented in mean ± SD of 3 replicates; Values in columns not sharing the same superscript are significantly different for $P < 0.05$. FA E- Ferulic acid Equivalents, BHT Eq- Butylated hydroxy toluene Equivalents.

varieties showed highest ascorbic acid content than other varieties.

HPLC of phenolics and flavonoids

Twelve phenolic compounds were detected in dehusked rice of eight pigmented rice varieties (Jyothi, Aravadan pillai, GK-4, Kamdhari, Black basumati, Kasubai, Karisale and Sirsi). The chromatogram of these varieties are shown in Fig. 1 & Fig. 2. Standard phenolic compounds used for the HPLC detection were gallic acid, chlorogenic acid, vanillic acid, ferulic acid, coumaric acid, ellagic acid, epicatechin, catechin, rutin, myricetin, quercetin and kemphferol. As shown in the chromatogram, phenolic compounds were identified at 280 nm and 320 nm. Among the standards used, five phenolic compounds that were observed in eight dehusked rice varieties were gallic acid, vanillic acid, ferulic acid, rutin and myricetin. The changes in phenolic compounds identification may be due to storage conditions which ultimately leads to destruction of phenolics. The maximum peak absorbance was

Table 9. Percentage reduction in phenolic compounds of pigmented rice varieties after polishing.

Samples	FP	BP	EP	FF	BF	TF	PAF	PAB	TAC
Jyothi	94	79	79	32	23	27	83	82	52
Aravadan pillai	60	84	66	78	35	4	65	65	67
GK-4	63	62	54	84	27	56	68	67	77
Kamdhari	90	61	62	74	42	25	94	73	70
Black basumati	90	72	3	47	33	70	90	63	74
Kasubai	70	73	32	7	40	46	87	67	90
Karisale	85	91	57	64	47	54	91	84	29
Sirsi	84	71	39	65	35	42	88	61	89

FP- Free polyphenols, BP- Bound polyphenols, EP- Esterified polyphenols, FF- Free flavonoids, BF- Bound flavonoids, TF- Total flavonol, FPA- Free proanthocyanidin, BPA- Bound proanthocyanidin, TAC- Total anthocyanin content.

Table 10. Percentage reduction in antioxidant properties of pigmented rice varieties after polishing.

Samples	DPPH		ABTS		H_2O_2		MC		FRAP		TAC		NO		RP		BCLS	
	F	B	F	B	F	B	F	B	F	B	F	B	F	B	F	B	F	B
Jyothi	91	68	97	-	5	40	93	86	-	-	65	-	69	19	18	11	46	12
Aravadan pillai	88	68	96	-	33	16	84	62	-	-	48	19	65	-	-	1	54	-
GK-4	59	68	90	-	93	15	84	74	-	--	34	-	74	-	-	23	29	-
Kamdhari	77	57	91	-	27	9	73	51	37	8	76	34	67	48	67	24	30	-
Black basumati	56	67	88	-	43	16	90	85	22	-	74	49	64	24	64	53	32	-
Kasubai	75	65	86	-	10	14	80	16	-	2	62	54	55	-	48	33	8	-
Karisale	85	65	98	-	32	36	92	76	30	63	61	57	59	-	31	38	30	-
Sirsi	71	69	86	-	13	38	50	62	40	44	66	42	56	-	58	38	44	-

H_2O_2- Hydrogen peroxide, MC- Metal chelating activity, FRAP- Ferric reducing power, TAC- Total antioxidant capacity, NO- Nitric oxide, RP- Reducing power, BCLS- Beta carotene linoeate system.

observed at a wavelength of 280 nm than 320 nm. Ferulic acid was found to be highest in all the dehusked rice varieties than other phenolics present in these varieties. It is also reported that ferulic acid was the highest phenolic compound in HPLC chromatogram of five Indica and Thai dehusked rice varieties (Ti et al., 2014; Sompong et al., 2011). The phenolics compounds were not detected in the chromatogram of polished rice varieties which may be due to high degree of polishing.

Antioxidant activity

Radical scavenging activities of red and polished rice of pigmented varieties

Reports on contribution of free, bound phenolic compounds of dehusked and polished rice of pigmented varieties to antioxidant activity are scanty. DPPH and radical cation ABTS+ scavenging activities of free phenolic extracts of de-husked and polished rice are presented in Table 5. DPPH scavenging activity in free phenolic extracts of dehusked and polished rice of these varieties varied from 82 to 168.4 and 14.5 to 68.7 mg Catechin Eq:/100 g respectively. The percentage changes in DPPH scavenging activity of free phenolic polished rice extracts compared with dehusked rice free phenolic extract ranged from 56 % to 91 % with a maximal loss in Sirsi, Kasubai, Kamdhari, Karisale, Aravadan pillai, Jyothi and minimal loss in Black basumati and GK-4 varieties (Table 10). The bound phenolic extracts of dehusked and polished pigmented rice extracts varied from 230.4 to 321.9 and 97.8 to 106.3 mg CE/100 g respectively. The percentage changes in DPPH scavenging activity of polished rice (bound phenolics) compared with dehusked rice ranged from 57 % to 68 % with a minimal loss in Kamdhari (57 %), Kasubai (64 %), Karisale (65 %), Black basumati (67 %), Aravadan pillai (68 %), GK-4 (68 %) and Jyothi (68 %) varieties. DPPH activity of bound phenolic extracts was found to be higher than free phenolic extracts of dehusked rice varieties and this may be due to the leaching of ester linked phenolics during shelling. ABTS scavenging activity in free phenolic extracts of dehusked and polished rice of these varieties varied from 163.4 to 464.2 and 4.9 to 48.9 mg Trolox Eq:/100 g respectively. The percentage decrease in ABTS scavenging activity of polished rice (free phenolics) compared with dehusked rice ranged from 86 % to 96 % with a maximal loss in all the varieties. The maximum loss of ABTS scavenging activity in the polished rice could be due to the lower concentration of free phenolics as compared to the dehusked rice varieties (as shown in Table 1). The ABTS activity was absent for the bound phenolic rice extracts and this may be due to the saturation of phenolic compounds with ABTS solution.

The hydrogen peroxide scavenging activity in free phenolic dehusked and polished rice extracts ranged between 47.2 to 95.5 and 54.2 to 90.9 mg ascorbic acid equivalents (AAE) per 100 g of sample respectively (Table 6). The percentage reduction in hydrogen peroxide scavenging activity of polished rice of these varieties (free phenolics) compared with dehusked rice varied from 10 % to 43 % with a moderate loss of scavenging activity observed in Kasubai (10 %), Sirsi (13 %), Kamdhari (27 %), and Karisale (32 %), Black basumati (43 %) varieties. However, higher hydrogen peroxide scavenging activity was observed

in the polished rice of GK-4 (93 %), Aravadan pillai (33 %) and Jyothi (5 %) varieties than the dehusked rice of these three varieties. The hydrogen peroxide scavenging activity in bound phenolic of dehusked and polished rice extracts ranged between 36.7 to 68.6 and 33.6 to 60.1 mg AAE per 100 g of sample respectively. The percentage changes in hydrogen peroxide scavenging activity of polished rice of these varieties (bound phenolics) compared with dehusked rice varied from 8.7 % to 40 % with a moderate loss in Kamdhari (8.7 %), Kasubai (14 %), GK-4 (15 %), Karisale (36 %), Sirsi (38 %) and Jyothi (40 %) varieties. Moreover, bound phenolic extracts of Aravadan pillai and Black basumati varieties showed 16 % increase in hydrogen peroxide scavenging activity.

Superoxide and hydrogen peroxide form the hydroxyl radical in the presence of metal ions such as copper/iron which reacts with proteins, lipids, polypeptides and DNA mainly thymine and guanosine. Reaction of hydroxyl radical with aromatic compounds forms hydroxycyclo hexadienyl radical by adding double bond which undergoes further formation of oxygen, peroxyl radical which ultimately decompose phenoxyl type radicals by elimination of water (Lee et al., 2002). Several bioactive components were reported to control the activity of hydroxyl radicals. The hydroxyl radical scavenging activity in free and bound phenolics of dehusked and polished rice of these varieties were estimated and the results were reported in terms of quercetin equivalents (Table 6). The hydroxyl radical scavenging activity in free phenolic dehusked and polished rice extracts ranged between 30.4 to 37.2 and 25.7 to 35 mg quercetin equivalents per 100 g of sample respectively. The percentage reduction in hydroxyl radical scavenging activity of free phenolic extracts of polished rice of these varieties compared with dehusked rice of these varieties ranged from 5 % to 34 % with a minimal loss in Kasubai (5 %), Black basumati (7 %), GK-4 (16 %), Karisale (18 %) and Jyothi (26 %), Aravadan pillai (34 %) varieties. Whereas, in Kamdhari and Sirsi, hydroxyl radical scavenging activity of free phenolic extracts of polished rice showed an increase. The scavenging activity of free phenolic extracts from dehusked rice reduced significantly compared to free phenolics from polished rice of these varieties. The hydroxyl radical scavenging activity in bound phenolic of dehusked and polished rice extracts ranged between 24.7 to 27.7 and 26.5 to 31.7 mg quercetin equivalents per 100 g of sample respectively. The percentage

Table 11a. Correlations of free phenolics to its antioxidant properties.

	Free	TAC	FRAP	MC	ABTS	DPPH	H_2O_2	OH	RP
TAC	0.959								
	0.000								
FRAP	0.496	0.268							
	0.051	0.315							
MC	0.451	0.487	0.113						
	0.080	0.056	0.678						
ABTS	0.963	0.961	0.384	0.577					
	0.000	0.000	0.142	0.019					
DPPH	0.849	0.873	0.263	0.732	0.926				
	0.000	0.000	0.325	0.001	0.000				
H2O2	0.645	0.570	0.549	0.036	0.528	0.386			
	0.007	0.021	0.028	0.895	0.035	0.139			
OH	0.234	0.327	-0.354	0.640	0.368	0.529	-0.222		
	0.384	0.217	0.178	0.008	0.161	0.035	0.410		
RP	0.913	0.873	0.440	0.139	0.816	0.640	0.712	-0.009	
	0.000	0.000	0.088	0.607	0.000	0.008	0.002	0.973	
NO	0.835	0.833	0.339	0.764	0.916	0.960	0.345	0.478	0.603
	0.000	0.000	0.198	0.001	0.000	0.000	0.190	0.061	0.013
BCLS	0.733	0.810	0.019	0.630	0.773	0.806	0.151	0.687	0.528
	0.001	0.000	0.944	0.009	0.000	0.000	0.577	0.003	0.035

TAC- Total antioxidant capacity, FRAP-Ferric reducing power, MC- Metal chelating, H_2O_2- Hydrogen peroxide, OH- Hydroxyl, RP- Reducing power, NO- Nitric oxide, BCLS- Beta carotene linoeate system.

Table 11b. Correlations of bound phenolics to its antioxidant properties.

	Bound	TAC	FRAP	MC	ABTS	DPPH	H_2O_2	OH	RP
TAC	0.811								
	0.000								
FRAP	0.585	0.601							
	0.017	0.014							
MC	0.704	0.285	0.252						
	0.002	0.284	0.346						
ABTS	0.865	0.511	0.404	0.804					
	0.000	0.043	0.121	0.000					
DPPH	0.544	0.443	0.451	0.532	0.494				
	0.029	0.086	0.080	0.034	0.052				
H2O2	-0.795	-0.669	-0.528	-0.549	-0.811	-0.701			
	0.000	0.005	0.035	0.028	0.000	0.002			
OH	0.805	0.764	0.734	0.427	0.673	0.360	-0.624		
	0.000	0.001	0.001	0.099	0.004	0.171	0.010		
RP	-0.372	-0.355	-0.409	-0.225	-0.408	-0.420	0.597	-0.381	
	0.156	0.177	0.115	0.401	0.117	0.106	0.015	0.146	
NO	-0.683	-0.726	-0.635	-0.146	-0.491	-0.055	0.492	-0.667	0.285
	0.004	0.001	0.008	0.590	0.054	0.839	0.053	0.005	0.284

TAC- Total antioxidant capacity, FRAP-Ferric reducing power, MC- Metal chelating, H_2O_2- Hydrogen peroxide, OH- Hydroxyl, RP- Reducing power, NO- Nitric oxide.

increase in bound phenolic of polished rice extracts compared with dehusked rice varied as follows; Karisale (26 %), Sirsi (25.7 %), Kasubai (21 %), GK-4 (14.8 %), Kamdhari (14 %), Aravadan pillai (5.4 %), Jyothi (4 %), Black basumati (2.7 %). Whereas, bound phenolic extracts of dehusked rice varieties showed lower scavenging activity of hydroxyl radical and this may be due to the presence of high amount of phenolic compounds which leads to immediate saturation of reaction mixture.

Nitric oxide scavenging activity in free and bound phenolic extracts of dehusked and polished rice varieties were reported in terms of ferulic acid equivalents per 100 g of sample (Table 8). The nitric oxide radical scavenging activity in free phenolic dehusked and polished rice extracts ranged between 41.2 to 59 and 10.9 to 25.9 mg ferulic acid equivalents per 100 g of sample respectively. The percentage reduction in free phenolics of various polished rice varieties compared with dehusked rice ranged from 55 % to 74 % with a maximal loss in Aravadan pillai (65 %), Black basumati (64 %), Kamdhari (67 %), Jyothi (69 %) and minimal loss in Kasubai (55 %), Sirsi (56 %) and Karisale (59 %) varieties. The nitric oxide radical scavenging activity in bound phenolic dehusked and polished rice extracts ranged between 8.7 to 22.4 and 9.6 to 24.5 mg FAE equivalents per 100 g of sample respectively. The percentage increase of nitric oxide scavenging activity in polished rice varieties compared with dehusked rice varied as follows; Sirsi (1.17 fold), Kasubai (1.4 fold), Karisale (77 %), GK-4 (60 %), Aravadan pillai (12 %). However, Jyothi, Kamdhari and black basumati varieties showed lower nitric oxide scavenging activity. The activity of nitric oxide radical was found to be high in dehusked rice varieties such as Black basumati, Karisale, Aravadan pillai, GK-4 and Sirsi. However, lower activity was observed in Jyothi, Kasubai, Kamdhari varieties. The higher activity of nitric oxide scavenging was observed in free phenolic extracts compared to bound phenolic extracts of red rice.

Metal chelating activity (binds Fe^{2+})

Ferric ion chelation is the important antioxidative effects for the retardation of metal-catalysed oxidation. The metal chelating activity in free and bound phenolic extracts of red and polished rice were reported in terms of EDTA equivalents (Table 7). The metal chelating activity in free phenolic extracts of dehusked and polished rice of these varieties varied from 31.4 to 117.5 and 8.1 to 15.8 mg EDTA Eq:/100 g respectively. The percentage changes in metal chelating activity of free phenolic polished rice extracts compared with dehusked

rice ranged from 50 % to 93 % with a maximal loss in Kamdhari, Kasubai, Aravadan pillai, GK-4, Black basumati and Karisale, Jyothi varieties and moderate loss observed in Sirsi variety. The metal chelating activity in bound phenolic extracts of dehusked and polished rice varied from 63 to 239.5 and 24.7 to 52.7 mg EDTA Eq:/100 g respectively. The percentage reduction in metal chelating activity of bound phenolic polished rice compared with dehusked rice ranged from 16 % to 86 % with a maximal loss in GK-4, Karisale, Black basumati, Jyothi varieties and minimal loss in Kamdhari, Aravadan pillai, Sirsi varieties. Kasubai variety showed an intermediate percentage loss comparatively. The metal chelating activity of bound phenolic of dehusked rice extracts were found to be higher than free phenolics. Moreover, free and bound phenolic extracts of de-husked rice grains of Jyothi showed higher chelating activity compared to others.

Total antioxidant capacity (TAC)

The total antioxidant capacity of free and bound phenolic rice extracts were reported in terms of ascorbic acid equivalents (Table 7). Total antioxidant activity in free phenolics of rice exists from 138.7 to 341.9 (Red) and 58.2 to 91.6 (Polished) mg ascorbic acid equivalents per 100 g of sample. The percentage reduction in the activity of free phenolic polished rice compared with dehusked rice varied as follows; GK-4 (34 %), Aravadan pillai (48 %), Karisale (61 %), Kasubai (62 %), Jyothi (65 %), Sirsi (66 %), Black basumati (74 %) and Kamdhari (76 %). Total antioxidant activity in bound phenolics of rice exists from 90.8 to 279.3 (red) and 75.2 to 142.3 (polished) mg ascorbic acid equivalents per 100 g of sample respectively. The percentage reduction in the activity of bound phenolic polished rice compared with dehusked rice varied as follows; Aravadan pillai (19 %), Jyothi (27 %) , Kamdhari (33.7 %), Sirsi (41.9 %), Black basumati (49 %), Kasubai (53.5 %), Karisale (56.9 %). However, the total antioxidant activity of GK-4 variety exhibited 14 % increase and this may be due to the presence of phenolic compounds in the aleurone layer. Whereas, lower activity were observed in bound phenolics of dehusked rice varieties; Jyothi & GK-4. The higher activity was found in dehusked rice varieties such as Kamdhari, Black basumati and Sirsi this is in correlation with the phenolic content of those varieties.

Determination of ferric reducing antioxidant power (FRAP)

The Fe^{3+} to Fe^{2+} reduction in de-husked and polished rice extracts of various rice varieties was investigated by using the potassium ferricyanide method and the results were reported in Table 7. The ferric reducing power in free phenolics of rice extracts varied from 12.8 to 41.6 (Red) and 17.2 to 29 (Polished) mg ferrous sulphate equivalents per 100 g of sample respectively. The percentage reduction in the activity of polished rice compared with dehusked rice varied as follows; Black basumati (23 %), Kasubai (24 %), Karisale (30 %), Kamdhari (37 %), and Sirsi (40 %). However, Aravadan pillai, Jyothi and GK-4 varieties resulted 52 %, 43 % and 40 % increase in ferric reducing power. The ferric reducing power in bound phenolics of rice extracts varied from 5.7 to 17 (Red) and 6.5 to 9.5 (Polished) mg ferrous sulphate equivalents per 100 g of sample respectively. The percentage reduction in the activity of polished rice compared with dehusked rice varied as follows: Kasubai (2.4 %), Kamdhari (8 %), Sirsi (41 %) and Karisale (63 %). While, GK-4 (14 %), Black basumati (9 %), Aravadan pillai (8 %) and Jyothi (3 %) varieties were resulted higher ferric reducing power. Whereas, higher activity were found in free and bound phenolics of dehusked rice except Jyothi, Aravadan pillai, GK-4 and Kasubai varieties.

Reducing power activity

Reducing agents are electron donor compounds which can reduce intermediates of the lipid peroxidation which are oxidized; therefore, they may be considered as primary or secondary antioxidants (Zhao et al., 2008). The iron reducing power in free phenolics of dehusked and polished rice extracts ranged from 1.18 to 9.62 and 2.60 to 3.40 mg BHT equivalents per 100 g of sample respectively (Table 8). The percentage reduction in reducing power of free phenolic polished rice extracts compared with dehusked rice ranged from 18 % to 67 % with the minimal loss in Jyothi, Karisale, Kasubai, Sirsi, Black basumati, Kamdhari varieties. Aravadan pillai (1.8 fold) and GK-4 (41 %) varieties resulted higher reducing power activity. The iron reducing power in bound phenolics of dehusked and polished rice extracts ranged from 4.20 to 7.48 and 2.82 to 4.65 mg BHT equivalents per 100 g of sample respectively. The percentage reduction in reducing power of bound

phenolic polished rice extracts compared with dehusked rice ranged from 0.9 % to 53 % with the minimal loss in Aravadan pillai, Jyothi, GK-4, Kamdhari, Kasubai, Sirsi and Karisale, Black basumati varieties. Whereas, the activity of reducing power were found to be higher in free and bound phenolics of dehusked rice varieties such as Kamdhari, Sirsi, Black basumati and Kasubai.

Antioxidant activity in beta carotene linoleate emulsion system

β-carotene is used as a coloring agent in beverages. Its discoloration markedly reduces the quality of those products. The aqueous phase of the emulsion is diluted in the lipid phase by polar antioxidants which are less effective in protecting linoleic acid. Rapid decolorization of β-carotene occurs with the absence of an antioxidant. The extent of β-carotene bleaching by neutralizing linoleate free radicals and other radicals formed in the system is hindered by different antioxidants (Siramon and Ohtani, 2007). The antioxidant activity in beta carotene linoeate emulsion system for the dehusked, polished rice extracts was calculated and the results are reported in terms of percentage inhibition (Table 8). The percentage inhibition in free phenolics of dehusked and polished rice extracts ranged from 40 % to 65 % and 28 % to 45 % respectively. The percentage reduction in the inhibition of polished rice compared with the dehusked rice varied as follows which is shown in the increasing order; Kasubai (8 %), GK-4 (29 %), Karisale (30 %), Kamdhari (30 %), Black basumati (32 %), Sirsi (44 %), Jyothi (46 %) and Aravadan pillai (54 %). The percentage inhibition in bound phenolics of dehusked and polished rice extracts ranged from 40 % to 78 % and 55 % to 69 % respectively. The bound phenolic extracts of polished rice showed higher activity than the dehusked rice varieties. The percentage increase in the inhibition of polished rice compared with dehusked rice varied as follows; Kamdhari (8 %), Aravadan pillai (10 %), GK-4 (10 %), Kasubai (21 %), Black basumati (36 %), Karisale (52 %), Sirsi (58 %). Jyothi (12 %) variety exhibited lower inhibition in antioxidant activity. Lower antioxidant activity were observed in the bound phenolics of dehusked rice varieties except Jyothi.

Correlation between phenolic compounds and antioxidant properties

Further, the free phenolic fraction of the rice varieties exhibited significant positive correlation to the total antioxidant capacity, ABTS, DPPH radical scavenging activity, hydrogen peroxide radical scavenging activity, reducing power activity, nitric oxide radical scavenging activity and beta carotene linoeate antioxidant activity with $r^2 = 0.959, 0.963, 0.849, 0.645, 0.913, 0.835, 0.733$ and $p<0.01$ (Table 11a & 11b). Lower or negative correlation in the assays involving bound phenolic fractions and flavonoids suggested the importance of free phenolic acids for their antioxidant capacity among the pigmented rice varieties.

CONCLUSION

Our results highlight the amount of antioxidant components present in the pigmented (Red) rice varieties. Red rice varieties due to their inherent phenolics may have application as natural antioxidants. In this study, flavonol, tannin, total carotenoids and ascorbic acid have been reported. This study also reports the ability of red rice phenolics to combat metal ions, hydroxyl radical, nitric oxide, and exhibit ABTS radical scavenging activity, total antioxidant activity and antioxidant activity in beta carotene linoeate system identified in the study, have been reported. Some of the varieties in their de-husked form showed higher anti-oxidant properties. However, the components of antioxidants were drastically reduced in polished rice of the varieties with moderate increase in antioxidant properties. Bound phenolic extracts of polished rice showed maximum retention in all the antioxidant components and its properties compared to other extracts. An increase were found in ferric reducing power of Jyothi, Aravadan pillai, GK-4 and kasubai varieties. Hydroxyl radical scavenging activity of polished red rice varieties were found to be higher than dehusked red rice varieties. Among the polished rice varieties, Aravadan pillai and GK-4 observed to have minimal loss in the antioxidant contents. Hence, usage of red rice either in de-husked or in polished form may be an attractive possibility to develop antioxidant rich health products of rice like noodles, pastas and diabetic foods etc.

ACKNOWLEDGEMENTS

For the present study, Director, DRDO-Defence Food Research Laboratory, Mysore Chairperson, Department of Food Science and Nutrition, Mysore University, Mysore supported and authors thank them

profoundly. Mrs. Rajarajeswari Jayaraman wishes to gratefully acknowledge the financial support from Indian Council of Medical Research, New Delhi, in carrying out this investigation by sanction of Emeritus Medical Scientist position to Dr. Vasudeva Singh.

REFERENCES

Alu'datt MH, Rababah T, Ereifej K and Alli I (2013). Distribution, antioxidant and characterization of phenolic compounds in soybeans, flaxseed and olives. Food Chem. 139: 93-99. https://doi.org/10.1016/j.foodchem.2012.12.061

Auddy B, Ferreira M, Blasina F, Lafon L, Arredondo F, Dajas F, Tripathi PC, Seal T and Mukherjee B (2003). Screening of antioxidant activity of three Indian medicinal plants traditionally used for the management of neurodegenerative diseases. J. Ethnopharmacol. 84: 131-138 https://doi.org/10.1016/S0378-8741(02)00322-7

Benzie IFF and Strain JJ (1999). Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. Methods Enzymol. 299: 15-27 https://doi.org/10.1016/S0076-6879 (99) 99005-5

Butsat S and Siriamornpun S (2010). Antioxidant capacities and phenolic compounds of the husk, bran and endosperm of Thai rice. Food Chem. 119: 606-13 https://doi.org/10.1016/j.foodchem.2009.07.001

Chen Q, Ling WH, Ma J and Mei J (2000). Effects of black and dehusked rice on the formation of aortic plaques and blood lipids in rabbits. J. Hyg. Res. 29:170-172. https://europepmc.org/abstract/med/12725067

Crozier A, Ashihara H and Clifford MN (2006). Plant Secondary Metabolites and the Human Diet. Blackwell Publishing. Oxford. England. Google books

Daiponmak W, Senakun C and Siriamornpun S (2014). Anti-glycation capacity and antioxidant activities of different pigmented Thai rice. International J. Food Sci. Tech. 49: 1805-1810 https://doi.org/10.1111/ijfs.12487

Goufo P and Trindade H (2017). Factors influencing antioxidant compounds in rice. Crit. Rev. Food Sci. Nutr. 57: 893-922. https://doi.org/10.1080/10408398.2014.922046

Goufo P and Trindade H (2014). Rice antioxidants: Phenolic acids, flavonoids, anthocyanins, proanthocyanidins, tocopherols, tocotrienols, γ-oryzanol, and phytic acid. Food Sci. Nutr. 2: 75-104. https://doi.org/10.1002/fsn3.86

Goupy P, Hugues M, Boivin P and Amiot MJ (1999). Antioxidant composition and activity of barley (*Hordeum vulgare*) and malt extracts and of isolated phenolic compounds. J. Sci. Food Agric. 79(12): 1625-1634 https://doi.org/10.1002/(SICI)1097-0010(199909)79:12<1625::AID-JSFA411>3.0.CO;2-8

Gunaratne A, Wu K, Li D, Bentota A, Corke H and Cai YZ (2013). Antioxidant activity and nutritional quality of traditional red-grained rice varieties containing proanthocyanidins. Food Chem. 138: 1153-1161 https://doi.org/10.1016/j.foodchem.2012.11.129

Hagerman A, Harvey-Mueller I and Makkar HPS (2000b). Quantification of tannins in tree foliage-a laboratory manual. FAO/ IAEA, Vienna pp. 4-7 https://books.google.co.in/books

Halliwell B, Gutteridge JMC and Aruoma OI (1987). The deoxyribose method: a simple "test tube" assay for determination of rate constants for reactions of hydroxyl radicals, Anal Biochem. 165: 215-219 https://doi.org/10.1016/0003-2697(87)90222-3

Hameeda BNI, Pradeep SR, Singh V, Srinivasan K and Jayadeep A (2016). Beneficial influence of phosphorylated parboiled red dehusked rice (*Oryza sativa* L.) in streptozotocin-induced diabetic rats. Starch/Stärke. 68: 568-580 https://doi.org/10.1002/star.201500289

Hosseinian FS, Li W and Beta T (2008). Measurement of anthocyanins and other phytochemicals in purple wheat. Food Chem. 109 (4): 916-924

Hyun JW and Chung HS (2004). Cyanidin and malvidin from *Oryza sativa* cv. Heungjinjubyeo mediate cytotoxicity against human monocytic leukemia cells by arrest of G(2)/Mphase and induction of apoptosis. J. Agric. Food Chem. 52: 2213-2217 https://doi.org/10.1016/j.foodchem.2007.12.083

Ichikawa H, Ichiyanagi T, Xu B, Yoshii Y, Nakajima M and Konishi T (2001). Antioxidant activity of anthocyanin extract from purple black rice. J. Med. Food. 4: 211-218 https://doi.org/10.1089/10966200152744481

Iqbal S, Bhanger MI and Anwar F (2007). Antioxidant properties and components of bran extracts from selected wheat varieties commercially available in Pakistan. LWT- Food Sci Tech. 40: 361-367 https://

doi.org/10.1016/j.lwt.2005.10.001

Jayaraman R, Uluvar H, Khanum F and Singh V (2019). Influence of parboiling of red paddy varieties by simple hot soaking on physical, nutrient, phytochemical, antioxidant properties of their dehusked rice and their mineral, starch, and antioxidant's bioaccessibility studies. J. Food Biochem. e12839. doi:10.1111/jfbc.12839

Kikuzaki H and Nakatani N (1993). Antioxidant effects of some ginger constituents. J. Food Sci. 58: 1407-1410 https://doi.org/10.1111/j.1365-2621.1993.tb06194.x

Kim JK, Lee SY, Chu SM, Lim SH, Suh SC, Lee YT et al., (2010). Variation and correlation analysis of flavonoids and carotenoids in Korean pigmented rice (*Oryza sativa* L) cultivars. J. Agric. Food Chem. 58: 12801-12809 https://doi.org/10.1021/jf103277g

Krygier K, Sosulski F and Hodge L (1982). Free, esterified, and insoluble bound phenolic acids. 1. Extraction and purification procedure. J. Agric. Food Chem. 30: 330-334 https://doi.org/10.1021/jf00110a028

Lee JC, Kim HR, Kim J and Jang YS (2002). Antioxidant activity. of ethanol extract of the stem of Opuntia ficus-*indica* var. Saboten. J. Agric. Food Chem. 50: 6490-6496 https://doi.org/10.1021/jf020388c

Ling WH, Cheng QX, Ma J and Wang T (2001). Red or black rice decrease atherosclerotic plaque and increase antioxidant status in rabbits. J. Nutr. 131:1421-1426 https://doi.org/10.1093/jn/131.5.1421

Ma J, Chen WX and Ling WH (2000). The health efficacy of de-husked rice and black rice. J. Food Sci. 21: 139-140

Min B, Chen MH and Green BW (2009). Antioxidant activities of purple rice bran extract and its effect on the quality of low-NaCl, phosphate free patties made from channel catfish (*Ictalurus punctatus*) belly flap meat. J. Food Sci. 74: 268-277 https://doi.org/10.1111/j.1750-3841.2009.01108.x

Min B, Gu L, Mcclung AM, Bergman CJ and Chen M (2012). Free and bound total phenolic concentrations, antioxidant capacities, and profiles of proanthocyanidins and anthocyanins in whole grain rice (*Oryza sativa* L.) of different bran colours. Food Chem. 133: 715-722 https://doi.org/10.1016/j.foodchem.2012.01.079

Monks JF, Vanier NL, Casaril J, Berto RM, Oliveira M, de Gomes CB, Carvalho MP, de Dias ARG and Elias MC (2013). Effects of milling on proximate composition, folic acid, fatty acids and technological properties of rice. J. Food. Compost. Anal. 30: 73-79 https://doi.org/10.1016/j.jfca.2013.01.009

Moreno YS, Sanchez GS, Hernandez DR and Lobato NR (2005). Characterization of anthocyanin extracts from maize kernels. J. Chromatogr. Sci. 43: 483-487 https://doi.org/10.1093/chromsci/43.9.483

Naczk M and Shahidi F (1989). The effect of methanol-ammonia-water treatment on the content of phenolic acids of canola. Food Chem. 31: 159-164 https://doi.org/10.1016/0308-8146(89)90026-5

Nakornriab M, Sriseadka T and Wongpornchai S (2008). Quantification of carotenoid and flavonoid components in brans of some Thai black rice cultivars using supercritical fluid extraction and high-performance liquid chromatography-mass spectrometry. J. Food Lipids 15: 488-503 https://doi.org/10.1111/j.1745-4522.2008.00135.x

Nam SH, Choi SP, Kang MY, Koh HJ, Kozukue N and Friedman M (2006). Antioxidative activities of bran extracts from twenty one pigmented rice cultivars. Food Chem. 94: 613-620 https://doi.org/10.1016/j.foodchem.2004.12.010

Nagira Y, Ikegami K, Koshiba T and Ozeki Y (2006). Effect of ABA upon anthocyanin synthesis in regenerated torenia shoots. J. Plant Res. 119: 137-144 DOI:10.1007/s10265-005-0256-9

Paiva FF, Vanier NL, Berrios JD, Pan J, de Almeida Villanova F, Takeoka G and Elias MC (2014). Physicochemical and nutritional properties of pigmented rice subjected to different degrees of milling. J. Food Compost. Anal. 35(1): 10-17 https://doi.org/10.1016/j.jfca.2014.05.003

Parr AJ and Bolwell GP (2000). Phenols in the plant and in man. The potential for possible nutritional enhancement of the diet by modifying the phenols content or profile. J. Sci. Food Agri. 80: 985-1012 https://doi.org/10.1002/(SICI)1097-0010(20000515)80:7<985::AID-JSFA572>3.0.CO;2-

Parrado J, Miramontes E, Jover M, Marquez JC, Angeles Mejias M, Collantes De Teran L, Absi E and Bautista J (2003). Prevention of brain protein and lipid oxidation elicited by a water-soluble oryzanol enzymatic extract derived from rice bran. E. J. Nutri. 42: 307-314 https://doi.org/10.1007/s00394-003-0424-4

Prieto P, Pineda M and Aguilar M (1999). Spectrophotometric

quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Anal. Biochem. 269: 337-341 https://doi.org/10.1006/abio.1999.4019

Prior RL and Liwei G (2005). Occurrence and biological significance of proanthocyanidins in the American diet. Phytochem. 66: 2264-2280

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: 796-801 https://doi.org/10.1016/j.phytochem.2005.03.025

Reed JD, McDowell RE, Van Soest PJ and Horvath PJ (1982). Condensed tannins: A factor limiting the use of cassava forage. J. Sci. Food Agric. 33: 213-20 https://doi.org/10.1002/jsfa.274033030

Roe JH and Kuether GA (1943). The determination of ascorbic acid in whole blood and urine through 2,4-di nitro phenyl hydrazine. J. Biol. Chem. 47: 399-410 http://www.jbc.org/content/147/2/399.short

Ruch RJ, Chung SU and Klaunig JE (1984). Spin trapping of superoxide and hydroxyl radicals. Methods Enzymol. 105: 198-209 https://doi.org/10.1016/S0076-6879(84)05026-6

Sakthikumaran P and Vijayalakshmi G (2015). Therapeutical properties of ferulic acid and bioavailability enhancement through feruloyl esterase. J. Func. Foods 17: 657-666 http://dx.doi.org/10.1016/j.jff.2015.06.013

Sanghamitra P, Bagchi TB, Sah RP, Sharma SG, Sarkar S and Basak N (2017). Characterization of red and purple-pericarp rice (*Oryza sativa* L.) based on physico-chemical and antioxidative properties of grains. Oryza 54(1): 57-64

Singleton VL, Orthofer R and Lamuela-Raventós RM (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. Methods Enzymol. 299: 152-178 https://doi.org/10.1016/S0076-6879(99)99017-1

Siramon P and Ohtani Y (2007). Antioxidative and antiradical activities of Eucalyptus camaldulensis leaf oils from Thailand. J. Wood Sci. 53: 498-504 https://scialert.net/abstract/?doi=pjbs.2009.1564.1570

Sompong R, SiebenhandlEhn S, Berghofer E and Schoenlechner R (2011). Extrusion cooking properties of white and colour dehusked rice varieties with different amylose content. StarchStärke, 63(2): 55-63.

Sreejayan N and Rao MNA (1997). Nitric oxide scavenging by curcuminoids. J. Pharm. Pharmacol. 49: 105-107 https://doi.org/10.1111/j.2042-7158.1997.tb06761

Suter M and Richter C (2000). Anti and pro-oxidative properties of PADMA 28, a Tibetan herbal formulation. Redox Report, 5, 17-22. https://doi.org/10.1179/rer.2000.5.1.17

Ti H, Zhang R, Zhang M, Wei Z, Chi J, Deng Y and Zhang Y (2015). Effect of extrusion on phytochemical profiles in milled fractions of black rice. Food Chem. 178: 186-194 https://doi.org/10.1016/j.foodchem.2015.01.087

Wang H and Helliwell K (2001). "Determination of flavonols in green and black tea leaves and green tea infusions by high performance liquid chromatography," Food Res. Int. 34: 223-227 https://doi.org/10.1016/S0963-9969(00)00156-3

Wang Q, Han P, Zhang M, Xia M, Zhu HL, Ma J, Hou MJ, Tang Z and Ling WH (2007). Supplementation of black rice pigment fraction improves antioxidant and anti-inflammatory status in patients with coronary heart disease. Asia Pac. J. Clin. Nutr. 16: 295-301 DOI 10.6133/apjcn.2007.16.s1.56

Xu F and Wang EM (1989). The effects of pigmented rice on hemoglobin regeneration in anemic rats. Acta Nutr. Sin. 11: 120-125 http://wprim.whocc.org.cn/admin/article/articleDetail?WPRIMID=550046&articleId=550046

Ye X, Al-Babili S, Klo¨ ti A, Zhang J, Lucca P, Beyer P and Potrykus I (2000). Engineering the provitamin A (β-carotene) biosynthetic pathway into (carotenoid-free) rice endosperm. Sci. 287: 303-305 https://science.sciencemag.org/content/287/5451/303

Yen GC and Duh PD (1993). Anti-oxidative properties of methanolic extracts from peanut hulls. J. Am. Oil Chem. Soc. 70: 383-386 https://doi.org/10.1007/BF02552711

Yermakov AI, Arasimov VV and Yarosh NP (1987). Methods of Biochemical Analysis of Plants, John Wiley and Sons, New York. https://www.cabdirect.org/cabdirect/abstract/19801688892

Yoshinaga K (1986). Liquor with pigments of red rice. J. Brew. Soc. Jpn. 81: 337-342

Zakaria M, Simpson KL, Red PR and Krstulovic A (1979). Use of reversed phase HPLC analysis for the

determination of provitamin A carotenes in tomatoes. J. Chromatogr. 176: 109-17 https://doi.org/10.1016/S0021-9673(00)92091-0

Zhang MW, Guo BJ, Zhang RF, Chi JW, Wei ZC, Xu ZH and Zhang Y (2006b). Separation, purification and identification of antioxidant compositions in black rice. Sci. Agric. Sin. 39: 153-160 https://doi.org/10.1016/S1671-2927(06)60073-4

Zhao H, Fan W, Dong J, Lu J, Chen J, Shan L et al., (2008). Evaluation of antioxidant activities and total phenolic contents of typical malting barley varieties. Food Chem. 107: 296-304. https://doi.org/10.1016/j.foodchem.2007.08.018

Zhishen J, Mengcheng T and Jianming W (1999). Research on antioxidant activity of flavonoids from natural materials. Food Chem. 64: 555-559