Advance Journal of Food Science and Technology 6(5): 668-679, 2014
DOI:10.19026/ajfst.6.92
ISSN: 2042-4868; e-ISSN: 2042-4876

Submitted: February 11, 2014 Accepted: April 17, 2014 Published: May 10, 2014

Research Article

Antioxidant Properties, Degradation Kinetics and Storage Stability of Drinks Prepared From the Cooking Water of Pigmented Rice

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Abstract: Pigmented rice, consisting of black rice and red rice, is known to contain antioxidant compounds in its bran that tend to leach out into the water during cooking. As the rice is usually cooked in excess water which is discarded after cooking, the purpose of this study is therefore, to evaluate the rice cooking water as an antioxidant drink in terms of its antioxidant properties, storage stability and anthocyanin degradation kinetics. The results showed that the percentages of antioxidant extractability from pigmented rice into the cooking water were 88.42 and 103.26%, respectively for red rice and black rice, respectively. However, red rice drink possessed significantly ($p<0.05$) higher antioxidant activity than black rice drink, except for its total monomeric anthocyanin content. The drinks showed good microbiological stability throughout 12 weeks of storage when kept at 4°C, while those stored at 25°C lasted for 4 weeks. There was a significant decrease of antioxidant content, chroma and pH and increase in L value and hue angle, while less significant changes were observed for total soluble solids and viscosity of the drinks during the storage stability study. The degradation of anthocyanins in both drinks kept at different temperatures followed first-order reaction kinetics. According to the findings of this study, black rice and red rice cooking water have the potential of being new antioxidant drinks.

Keywords: Anthocyanin, antioxidant drink, antioxidant properties, black rice, degradation kinetics, red rice, storage stability

INTRODUCTION

Pigmented rice, or also known as coloured rice, consists of black rice and red rice. Anthocyanins are the pigments responsible for the bran colour of black rice (Escribano-Bailon *et al*., 2004), which has been proven to have up to 96 times more anthocyanin content than pigmented (blue, pink, purple and red) corn, wheat and barley with the values reaching as high as 3276 µg/g (Abdel-Aal *et al*., 2006). Meanwhile, proanthocyanidins are the pigments responsible for red rice colour (Oki *et al*., 2002), although according to several studies (Abdel-Aal *et al*., 2006; Yoshinaga *et al*., 1986; Morimitsu *et al*., 2002), anthocyanins can also be found in red rice in lower concentration up to 35 times less than that of black rice. However, despite its less anthocyanin content, red rice contains higher antioxidant activity compared to black rice (Muntana and Prasong, 2010) due to its proanthocyanidin content (Finocchiaro *et al*., 2007). Previous investigations have shown that anthocyanins and proanthocyanidins contained in black rice and red rice possessed anti-oxidative, anti-inflammatory activities (Hu *et al*., 2003; Xu *et al*., 2001; Ling *et al*., 2001; Xia *et al*., 2003) and anti-cancer properties (Kamei *et al*., 1998; Chen *et al*., 2006; Nam *et al*., 2005) in chemical and biological body systems.

Domestic and commercial methods of rice cooking include steaming, cooking in excess water and cooking in rice cooker or combi oven. Cooking in excess water leads to leaching of water-soluble antioxidants into the cooking water. Anthocyanins in black rice are widely known as water-soluble compounds (Bridle and Timberlake, 1997). A study conducted by Finocchiaro *et al*. (2007) stated that the antioxidant power of the cooking water of dehulled red rice confirmed that based on 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) assay, approximately 57.66% of the total antioxidant content of red rice was lost into the cooking water during boiling. Therefore, antioxidants in the black and red rice cooking water can be preserved by further utilizing it as antioxidant drink.

There has been a number of non-alcoholic cereal drinks being studied and consumed, such as oat milk, rice milk, barley drink and sorghum drink. There are also traditional cereal drinks such as kunu from Nigeria (Ayo, 2004; Gaffa *et al*., 2002) which is made from a mixture of cereals such as millet, acha, corn and

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sorghum; boza from Bulgaria (Todorov and Dicks, 2005; Ivanova *et al*., 2000) which is produced through fermentation of different cereals by lactic acid bacteria and yeast; and chicha morada from Peru (Ramos Escudero *et al*., 2012; Pedreschi and Cisneros-Zevallos, 2007) which is prepared from purple corn immersed in boiling water. However, there are limited studies on the antioxidative effects of these cereal drinks. Studies on antioxidative effects of rice milk (Mitchell and Collins, 1999) and oat milk (Onning *et al*., 1998) showed that consumption of both drinks did not increase total antioxidant capacity of plasma significantly.

Fruits and vegetables have been widely used as raw materials for antioxidant beverages, mostly in the form of juices or drinks. Total phenolic content and total monomeric anthocyanin content of single strength pomegranate juice, which is a known source of anthocyanins, were 3.09 mg gallic acid equivalent (GAE)/mL and 80.2 mg/L, respectively (Cam *et al*., 2009). A study conducted by Konic-Ristic *et al*. (2011) on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity IC_{50} and total monomeric anthocyanin content of different types of single strength berry juices, consisting of red raspberry, black raspberry, blackcurrant, red currant and bilberry, gave results ranging from 9.1×10^{-4} to 3.14×10^{-3} and 300-1800 mg/L, respectively. For vegetables, single strength asparagus juice and broccoli juice were found to possess DPPH radical scavenging activity of 1.2 M trolox equivalent (TE) and 0.4 M TE, total phenolic content of 0.5 mg catechin equivalent (CE)/mL and 0.6 mg CE/mL and total flavonoid content of 0.3 mg rutin equivalent (RE)/mL and 0.1 mg RE/mL, respectively (Sun *et al*., 2007).

Compared to fruits and vegetables which are perishable commodities, pigmented rice poses as a good raw material for antioxidant drink since it can be stored for a longer time and requires less maintenance due to its low moisture. The lower price of pigmented rice compared to anthocyanin-rich berries and blackcurrant also suggests that it can be an inexpensive source of anthocyanins for food applications (Jang and Xu, 2009). In addition, in most rice-consuming countries, pigmented rice is easier to obtain than berries or blackcurrant. The anthocyanins in pigmented rice are stable in pH 6.6 (Torskangerpoll and Andersen, 2005), while anthocyanin-based drinks made of fruits and vegetables are mostly low in pH (±3-4) (Konic-Ristic *et al*., 2011; Hernandez *et al*., 1999; West *et al*., 1999; Rodrigo *et al*., 2007). Since the anthocyanins in pigmented rice drink are stable at the pH which is approximately neutral, antioxidant drink from pigmented rice is, therefore, suitable to be consumed by people with gastric problems like Gastroesophageal Reflux Disease (GERD) that are usually sensitive to acidic foods and drinks (Hila and Castell, 2003). With its distinctive but pleasant flavour, comparable antioxidant content, cheaper price and better plant cultivation adaptability than other anthocyanin-containing fruits and vegetables, pigmented rice will make an excellent raw material for antioxidant drinks.

The objectives of this study were to determine the total antioxidant content of black and red rice lost into the cooking water, to convert the rice cooking water into antioxidant drinks, to study storage stability of the drinks for 12 weeks, to study the degradation kinetics of anthocyanins in both drinks and to determine the antioxidant and physical properties of the drinks.

MATERIALS AND METHODS

Materials: Two types of commercial pigmented rice (Thai black and red rice) and commercial fruit drinks (consisting of blackcurrant, apple and orange drinks) which are made from concentrates and not enriched with antioxidants, were purchased from local supermarkets. Blackcurrant drink was chosen since it is the most common anthocyanin-containing fruit drink available in the market, while apple and orange drinks were chosen due to their popularity as the most widely consumed fruit drinks with high antioxidant content contributed by phenolic compounds and vitamin C (Cook *et al*., 1998; Miller and Rice-Evans, 1997).

Reagents and chemicals: Gallic acid, sodium acetate (CH_3COONa), sodium carbonate (Na_2CO_3), sodium nitrate ($NaNO_2$), (+)-catechin and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were from Sigma-Aldrich (St. Louis, MO, USA). Methanol, acetonitrile, aluminium chloride ($AlCl_3$), Folin-Ciocalteu phenol reagent, iron (III) chloride hexahydrate ($FeCl_3.6H_2O$) and hydrochloric acid (HCl) were from Merck (Germany). Ethanol was from HmbG Chemicals (Germany). NaOH was from R&M Chemicals (Essex, UK). Trolox, trifluoroacetic acid (TFA) and 2, 4, 6-tripyridyl-s-triazine (TPTZ) were from Acros Organics (New Jersey, USA). Acetic acid glacial ($C_2H_4O_2$) and potassium chloride (KCl) were from JT Baker (New Jersey, USA). Phosphoric acid was from Fisher Scientific (New Hampshire, USA).

Preparation of methanolic extract from pigmented rice: The methanolic extract was prepared for the calculation of antioxidant extractability by hot water extraction to be performed in section 2.4. The extract was prepared based on the optimized methanolic extraction method recommended by Kasim (2013). The extraction of black rice and red rice was conducted in an ultrasonic cleaning bath (Model B5510 E-DTH, 50 kHz, 240 V, 9.51 L capacity, size: 11.5×9.5×6 inch; Branson Ultrasonics Corporation, Danbury, USA) and the work frequency was fixed at 40 kHz. One gram of each pigmented rice was made up to volume with methanol/water solution (78.49% methanol for red rice and 63.18% methanol for black rice) in a 20 mL volumetric flask and sonicated at a fixed temperature (40.42°C for red rice and 47.16°C for black rice) and time (8.75 min and 11.41 min for red rice and black

rice, respectively). The extract was filtered under vacuum through a Whatman No. 4 filter paper and the filtrate was dried using a rotary evaporator at 40°C.

Preparation of hot water extract from pigmented rice as rice drink: Rice drink was prepared based on the results of our preliminary study on the optimum hot water extraction conditions for maximum antioxidant content of rice drink. The independent variables used for the optimization procedures, based on response surface methodology (RSM) were water/rice ratio (20-30 mL/g), extraction time (30-50 min) and extraction temperature (87°-97°C), with DPPH radical scavenging activity IC_{50} as the dependent variable. DPPH radical scavenging activity IC_{50} was selected as the dependent variable due to its stable results with insignificant difference among repetitions ($p = 0.32$) indiciating good repeatability (Thaipong *et al*., 2006). The percentage of antioxidant extractability (%AE) from pigmented rice into the rice drink was calculated using the following equation:

$$\%AE = \frac{IC50\ hot\ water\ extract}{IC50\ methanolic\ extract} \times \frac{W}{R} ratio \times 100\%$$

In which W/R ratio is water/rice ratio.

Based on the optimum extraction conditions, black rice and distilled water were mixed with a ratio of 20 mL/g and incubated in a Julabo SW23 shaking waterbath (Julabo Labortechnik GmbH, Seelbach, Germany). After 40 min at 95.6°C, the rice drink was centrifuged at 3500 g for 10 min. The same procedure was applied to red rice, with extraction conditions of water/rice ratio of 20 mL/g at 97°C for 30 min. The supernatants were used for analyses.

For the storage stability study, 6% sucrose was added as a sweetener for the drinks. The drinks were then hot filled (85°C) into pre-sterilized opaque High Density Polypropylene (HDPP) bottles (330 mL) and followed by immediate closing using plastic caps. After closing, the bottles were cooled to 30°C. Half of the drinks were kept on shelves exposed to fluorescent lamp light; purposely to imitate market conditions, at room temperature (25±2°C), while the rest were kept at chilled temperature (4°C) for 12 weeks. Stability evaluations were conducted weekly.

Determination of DPPH radical scavenging activity: The free radical scavenging activity of samples on DPPH radical was carried out according to the procedure described by Brand-Williams *et al*. (1995). Samples (100 µL) were mixed with 3900 µL aliquots of 80 µM methanolic solution of DPPH. Absorbance of each mixture at 517 nm was measured after 3 h. The percentage of inhibition was calculated using the following equation:

$$\%\ Inhibition = \frac{A0-Ai}{A0} \times 100\%$$

where,

A_0 = The absorbance of the blank (methanolic solution of DPPH)

A_i = The absorbance of each samples

Concentration of the sample which was required to scavenge 50% of the DPPH free radicals (IC_{50}) was estimated using a nonlinear regression algorithm for the preliminary study described in section 2.4, while the results of this study were expressed as µM TE.

Determination of Total Phenolic Content (TPC): Total phenolic content of samples was measured by Folin Ciocalteu reagent assay described by Slinkard and Singleton (1977) and Singleton *et al*. (1999). A 200 µL sample was added to 1.0 mL of Folin-Ciocalteu's reagent in a test cuvette and followed by 0.8 mL of Na_2CO_3 (7.5%). The absorbance of the mixture was measured at 765 nm with a Lambda 25 UV-Vis spectrophotometer (PerkinElmer, CT, USA) after incubation at 30°C for 2 h. Results were expressed as milligrams of GAE per mL of drink or per g of rice sample.

Determination of Total Flavonoid Content (TFC): Determination of total flavonoid content of samples was conducted by using aluminium chloride colorimetric assay as described by Shams-Ardekani *et al*. (2011). One mL of sample or standard solution of catechin (50, 100, 150, 200, 250 and 300 mg/L) was added to a 10 mL volumetric flask containing 4 mL of purified water. 0.3 mL of 5% $NaNO_2$ was then added to the flask and 0.3 mL of $AlCl_3$ (10%) was added after 5 min. After another minute, 2 mL of 1M NaOH was added and the mixture was made up to volume using purified water. The absorbance of the solution was measured at 510 nm using a UV-Vis spectrophotometer versus a reagent blank. Total flavonoid content was expressed as mg CE per one mL of drink or per one gram of rice sample.

Determination of Total Monomeric Anthocyanin Content (TMAC): Total monomeric anthocyanin content of samples was determined using the spectrophotometric method described by Abdel-Aal and Hucl (1999). Twenty four mL of acidified ethanol (ethanol and 1N HCl, 85:15 v/v) was added to 3 g of ground pigmented rice or 3 mL of rice drink/fruit drink sample. The solution was mixed and adjusted to pH 1 using 4N HCl. After being vortexed, the mixture was centrifuged at 3000 g for 10 min. The supernatant was made up to volume in a 50 mL volumetric flask using the previously mentioned acidified ethanol. Total anthocyanins were calculated as mg/kg or mg/L cyanidin-3-glucoside based on the following equation:

$$Total\ anthocyanins = A \times 288.21$$

where,

A = Absorbance at 535 nm

Determination of Ferric Reducing Ability Power (FRAP): Determination of FRAP of samples was conducted following the method established by Benzie and Strain (1996) with slight modifications. FRAP reagent was prepared by mixing 300 mM acetate buffer (pH 3.6) containing 16 mL $C_2H_4O_2$ per litre of the buffer solution, 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) in 40 mM HCl and 20 mM $FeCl_3.6H_2O$ in a ratio of 1:1:10. 2.7 mL of the freshly prepared FRAP reagent was warmed to 37°C and a reagent blank absorbance reading was taken in a UV-Vis spectrophotometer at 593 nm. 150 μL sample and 210 μL water were added into the FRAP reagent and an absorbance reading was taken at 593 nm after 10 min. The results were expressed as μM TE.

Microbiological analyses of pigmented rice drink: The microbiological analyses carried out were total plate count, total coliform count and yeast and mold count based on Vanderzant (1992) recommendations. 0.1 mL of serially diluted samples was spread on each agar plate, consisting of plate count, Eosin Methylene Blue (EMB) and Dichloran Rose Bengal Chloramphenicol (DRBC) agar. Growth was examined after plate count, EMB and DRBC agar plates were incubated at 35°C for 48 h, 35°C for 24 h and 30°C for 5 days, respectively.

Physical analyses of rice drink: An Anton Paar RheolabQC rheometer (Anton Paar GmbH, Germany) and a pH meter (320 Mettler-Toledo Inc., Ohio, USA) were used for measuring the viscosity and pH of the rice drinks. Total soluble solids were analysed using a refractometer (Atago Co. Ltd., Japan). Colour was measured using an Ultra Scan PRO spectrophotometer (Hunter Laboratories Inc., Northern California, USA). A 40 mL of each sample was placed in a 20-mm cell and L (lightness), a (redness) and b (yellowness) values were recorded in total transmittance mode with illuminant D_{65} and 10° observer angle (Giusti and Wrolstad, 2005). The L value represents the lightness of sample, scaled from L = 0 as complete black colour to L = 100 as diffuse white, while the opponent colour axes a+ represents redness, a- represents greenness, b+ is for yellow colours and b-is for blue colours. Lightness (L), Chroma (C) and hue angle (h°) parameters were used because Lab coordinates were unable to express hue and chroma of the drinks directly and difficult to interpret independently (Ramos Escudero *et al*., 2012), therefore C and h° were preferred to be used as indices of the quality of the drinks. C and h° were calculated using the following equations:

$$C = \sqrt{(a^2+b^2)}$$
$$h° = \tan^{-1}(b/a)$$

All analyses were conducted in triplicates.

Statistical analysis: Data were reported as means±standard deviations for triplicate determinations of each analysis. Minitab Statistical Software version 14 (State College, Philadelphia, USA) was used to conduct Response Surface Methodology (RSM), one-way ANOVA and Tukey's comparison test purposely for determination of significant differences among values. Statistical significance was defined to be at a level of $p<0.05$.

RESULTS AND DISCUSSION

Percentage of antioxidant extractability into cooking water of pigmented rice: Based on the optimized hot water extraction conditions, both black rice and red rice drinks were produced from the cooking water to have their DPPH radical scavenging activities compared with that of black rice and red rice methanolic extracts which represented 100% extraction, respectively. The percentage of antioxidant extractability (Fig. 1a) from red rice into its rice drink was 88.42%, while that for black rice was 103.26%, although the extraction time taken to obtain maximum antioxidant content for red rice was shorter than that for black rice. This is because under the cooking conditions, anthocyanins, which are the main antioxidant compounds in black rice, are rapidly transformed into protocatechuic acid (Hiemori *et al*., 2009), which is also one type of antioxidant (Masella *et al*., 1999) that probably possessed higher antioxidant activity than anthocyanin. Thus, although anthocyanins are easily destructed by heat (Havlikova and Mikova, 1985), DPPH radical scavenging activity was increased due to the conversion of anthocyanin to protocatechuic acid. This was further shown in the results (Fig. 1b) for total monomer anthocyanin content of both methanolic and hot water extracts of black rice, in which the percentage of anthocyanin extractability into the black rice drink was 88.52%, showing that there was some destruction of anthocyanins due to heat. In a study conducted by Yin *et al*. (2009), it was found that protocatechuic acid possesses anticancer properties modulated via signal transduction pathways and gene expression of enzymes involved in the metastatic cascade of cancer cells. It was also found to be able to protect against oxidative damage induced by *tert*-butylhydroperoxide in rat primary hepatocytes (Tseng *et al*., 1996).

Conforming to the study conducted by Muntana and Prasong (2010) and Sompong *et al*. (2011) which stated that Thai red rice had higher antioxidant content than Thai black rice, the red rice drink showed significantly ($p<0.05$) higher DPPH radical scavenging activity than black rice drink, signifying higher radical scavenging activity although the extraction temperature for red rice was higher than that for black rice. This may indicate that higher temperature is required to

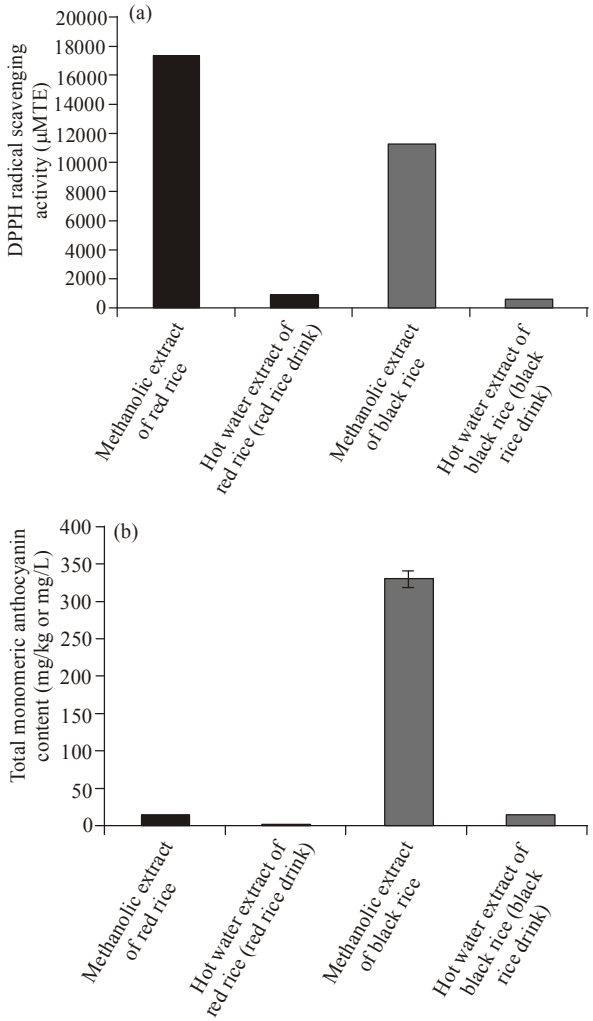

Fig. 1: DPPH radical scavenging activity (a) and total monomeric anthocyanin content (b) of methanolic extracts and hot water extracts of pigmented rice

break the matrix of red rice bran to extract the antioxidants in red rice since proanthocyanidins tend to form strong complexes with insoluble polymeric plant material such as cell wall polysaccharides (Matthews *et al*., 1997; Rohr *et al*., 2000; Scalbert, 1992). Proanthocyanidins are also known as heat-stable antioxidant compounds and are even more heat-stable than ascorbic acid (Satoshi *et al*., 2001). However, in contrast with black rice which had increasing radical scavenging activity after hot water extraction, the percentage of antioxidant extractability for red rice was 88.42%. This might be due to the conversion of proanthocyanidins in red rice into phlobatannins, a water insoluble phenolic substance, after undergoing the heating process (Porter, 1993) which explains the decrease in the percentage of antioxidant extractability into the cooking water.

Antioxidant properties of pigmented rice drinks compared with commercial fruit drinks: The antioxidant properties of pigmented rice and commercial fruit drinks are presented in Table 1. The total phenolic content of the pigmented rice drinks (0.12 to 0.24 mg GAE/mL) was significantly ($p<0.05$) higher than that of the commercial drinks (0.03 to 0.07 mg GAE/mL) used in this study. Red rice drink had significantly ($p<0.05$) higher total phenolic content than black rice drink, which was in compliance with the study conducted by Muntana and Prasong (2010) in which Thai red rice was found to contain higher total phenolic content than Thai black rice.

Trolox equivalent values were chosen to represent DPPH radical scavenging activity of the drinks for comparison with FRAP values. DPPH radical scavenging activity of pigmented rice drinks (576.06 to 769.22 μM TE) were significantly ($p<0.05$) higher than that of commercial fruit drinks (49.75 to 163.37 μM TE) used in this study. For determination of FRAP, it was found that there was no significant difference between ferric reducing ability of red rice drink (695.97 μM TE) and black rice drink (681.61 μM TE). However, both drinks possessed significantly higher ferric reducing ability than the commercial fruit drinks (38.21 to 83.85 μM TE). These results were in line with the previously reported results for DPPH scavenging activity. Both FRAP and DPPH assays function based on electron-transfer reactions. The difference between the two is that FRAP uses $Fe(III)(TPTZ)_2$ as oxidant under acidic conditions, while DPPH assay is carried out in neutral environment and uses DPPH as an oxidant. This explains why although both assays have the same trolox equivalent units, they gave different values for the same sample. The results showed that black rice drink scavenged $Fe(III)(TPTZ)_2$ better than DPPH compared to other drinks. This was because anthocyanins have higher number of hydroxyl groups than proanthocyanidins in red rice, quercetin in apple and ascorbic acid in orange and those with higher number of hydroxyl groups scavenge Fe(III) $(TPTZ)_2$ faster with more reduction of TEs than those with lower number of hydroxyl groups (Ozgen *et al*., 2006).

Red rice drink had significantly ($p<0.05$) higher flavonoid content than black rice drink with 0.08 mg CE/mL, while black rice drink contained 0.06 mg CE/mL. These values were significantly higher ($p<0.05$) than that in the commercial fruit drinks. The results were in accordance to the findings by Chen *et al*. (2012) in which red rice possessed higher flavonoid content than black rice.

Red rice drink (2.33 mg/L) had up to 6 times lower ($p<0.05$) anthocyanin content than black rice drink (14.53 mg/L). This was in accordance to the study conducted by Abdel-Aal *et al*. (2006) in which anthocyanin content of black rice was 35 times (3276 μg/g) more than that of red rice (93.5 μg/g). Anthocyanin content of both black and red rice drinks

Table 1: Antioxidant properties of pigmented rice drinks and commercial fruit drinks

Samples	Antioxidant Properties				
	DPPH radical scavenging activity (μM TE)	FRAP (μM TE)	Total phenolic content (mg GAE/mL)	Total flavonoid content (mg CE/mL)	Total monomeric anthocyanin content (mg/L)
Black rice drink	576.06±24.40	681.61±20.30	0.12±0.00	0.06±0.00	14.53±0.61
Red rice drink	769.22±23.31	695.97±27.77	0.24±0.00	0.08±0.00	2.33±0.62
Commercial blackcurrant drink	155.01±5.45	83.85±3.49	0.07±0.00	0.01±0.00	1.38±0.23
Commercial apple drink	49.75±2.39	38.21±1.72	0.04±0.00	0.00±0.00	ND
Commercial orange drink	163.37±8.49	83.45±3.21	0.07±0.00	0.02±0.00	ND

[a]: Each value is the average of three independent replicates ± SD; [b]: ND = not detected; [c]: Different letters in the same column indicate significant differences ($p<0.05$)

were significantly ($p<0.05$) higher than that of commercial blackcurrant drink (1.38 mg/L) although blackcurrant is known to be rich in anthocyanins. This could be due to the fact that the results obtained using this method were on cyanidin-3-glucoside basis, while blackcurrant comprises mostly of cyanidin-3-rutinoside (40.15%), delphinidin-3-rutinoside (36.59%) and delphinidin-3-glucoside (14%), with cyanidin-3-glucoside being the minor anthocyanin in its composition (7.08%) (Slimestad and Solheim, 2002). Anthocyanins were not detected in commercial orange and apple drinks.

Microbiological stability of pigmented rice drinks: The microbiological limits for both rice drinks were evaluated according to that of fruit drinks stated by Stannard (1997) for yeast and mold count and that of pasteurized milk by Food Act 1983 Malaysia (MLRB, 2010) for total plate and coliform count because there is no microbiological standard specifically for rice drink to date. Based on the microbiological limits of fruit drinks, the amount of yeast and mold accepted following GMP was <1/100 mL, while total plate count and coliform count according to Food Act 1983 Malaysia (MLRB, 2010) for pasteurized milk were 10^5/mL and absent in 1 g, respectively.

The microbiological analyses for 12 weeks revealed that both rice drinks were able to be kept for 4 weeks at ambient temperature before passing the microbiological limits defined. However, presence of yeast, mold and coliform was not detected throughout the 12 weeks of storage for rice drinks kept at refrigerated temperature (4°C), with total plate count below the microbiological limit.

Antioxidant stability of pigmented rice drinks: Due to change of colour caused by microbial growth which disturbed the readings for antioxidant analyses, antioxidant determination for drinks stored at 25°C was only conducted until week 4 and the results were reported in Fig. 2, together with those kept at 4°C for 12 weeks. Linear regressions and R^2 reported in Table 2 were determined using the following equation:

$$y = mx+c$$

The DPPH radical scavenging activity, total phenolic content and total flavonoid content of black rice drink kept at 4°C were decreased by 27.33, 25.91 and 25.31%, respectively; while that of red rice drink were reduced by 19.94, 12.18 and 13.17%, respectively. For red rice drinks stored at 25°C, the DPPH radical scavenging activity, total phenolic content and total flavonoid content were decreased by 72.91, 32.41 and 43.56%, respectively; while that of black rice drink dropped by 81.46, 50.21 and 66.82%, respectively. Lower values of *m* can be seen in the linear regressions of pigmented rice drinks kept at 4°C, showing more stability of antioxidant compounds due to slower chemical reactions at low temperatures, including degradation of compounds. Stability of antioxidant compounds is greatly affected by external factors such as exposure to light, air and different storage temperatures (2005).

The anthocyanin content of black and red rice drinks kept at 4°C was decreased by 55.45 and 37.33%, respectively, while for those kept at 25°C, the anthocyanin content declined by 54.98 and 41.11%, respectively. As shown in Fig. 2d, anthocyanin content of both rice drinks dropped drastically at week 2 before decreasing gradually over the next weeks, while DPPH radical scavenging activity, total phenolic content and total flavonoid content did not. This might be due to the occurrence of Maillard reactions in which the sugar present in the drinks were degraded to furfural-type compounds that might increase the antioxidant content of the drinks but accelerate degradation of anthocyanins (Cevallos-Casals and Cisneros-Zevallos, 2004). It can also be seen that although red rice drink contained much lower anthocyanins, the compounds were retained better than those in black rice drink over time, which was illustrated by a less declined slope and lower value of *m*.

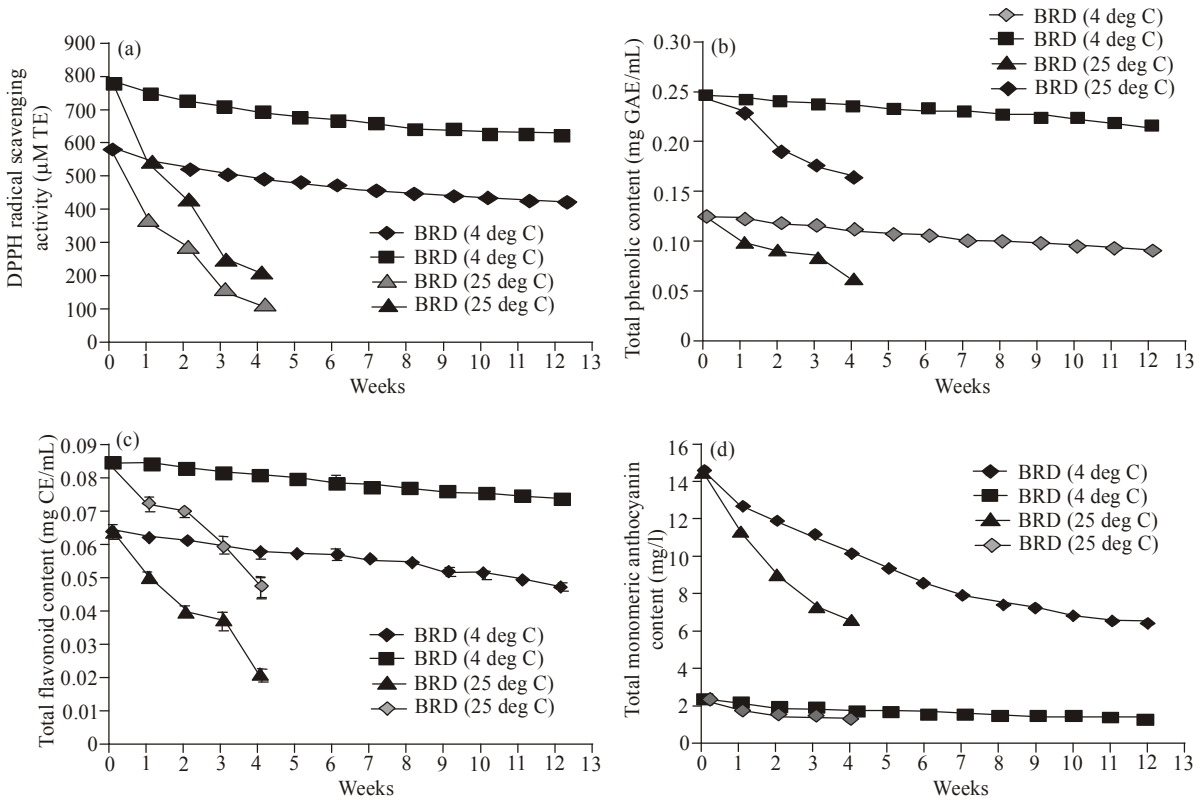

Fig. 2: DPPH radical scavenging activity, (a) total phenolic content, (b) total flavonoid content, (c) total monomeric anthocyanin content and (d) of Black Rice Drink (BRD) and Red Rice Drink (RRD) as function of storage time and temperature

Table 2: Linear regression and R^2 of pigmented rice drinks at different storage temperatures

		Black rice drink		Red rice drink	
Storage temperature	Antioxidant properties	Linear regression	R^2	Linear regression	R^2
4°C	DPPH radical scavenging activity	y = 547.78-11.936x	0.94	y = 756.62-12.137x	0.94
	TPC	y = 0.1215-0.0027x	0.98	y = 0.2457-0.0023x	0.99
	TFC	y = 0.0636-0.0013x	0.98	y = 0.0851-0.0009x	0.99
	TMAC	y = 13.108-0.6418x	0.93	y = 2.4155-0.0718x	0.92
25°C	DPPH radical scavenging activity	y = 639.37-113.28x	0.95	y = 857.9-140.41x	0.95
	TPC	y = 0.1333-0.014x	0.96	y = 0.2627-0.0212x	0.95
	TFC	y = 0.0719-0.0098x	0.96	y = 0.0919-0.0085x	0.97
	TMAC	y = 13.689-1.9867x	0.94	y = 2.2188-0.2342x	0.92

Physical stability of pigmented rice drinks: Red rice and black rice drinks had comparable pH, viscosity and total soluble solids (Fig. 3a toc). The neutral pH of the rice drinks was similar to that previously reported by Torskangerpoll and Andersen (2005). Their very low viscosities showed that centrifugation removed majority of the starch which leached together with the antioxidant compounds during cooking, leaving only the water soluble compounds in the drink. By removing the starch in the cooking water, sedimentation in the rice drink could be prevented. During storage, there was no significant ($p>0.05$) changes to pigmented rice drinks kept at both temperatures in terms of total soluble solids. The viscosity of the drinks stored at 4°C fluctuated until week 6 then remained stagnant throughout the rest of the 12 weeks of storage, while that of those kept at 25°C increased throughout the 4 weeks of storage. In terms of pH, there was no significant ($p>0.05$) changes to the drinks kept at 4°C, while that of those kept at 25°C decreased significantly ($p<0.05$) due to microbial growth.

The colour of black rice drink differed significantly ($p<0.05$) from that of red rice drink (Fig. 3d to f). Black rice drink showed significantly lower L value than red rice drink, which indicated that red rice drink was brighter in colour. Red rice drink also had a significantly ($p<0.05$) higher hue angle, showing a colour more towards red, while black rice drink leaned more towards a purple colour. During their respective

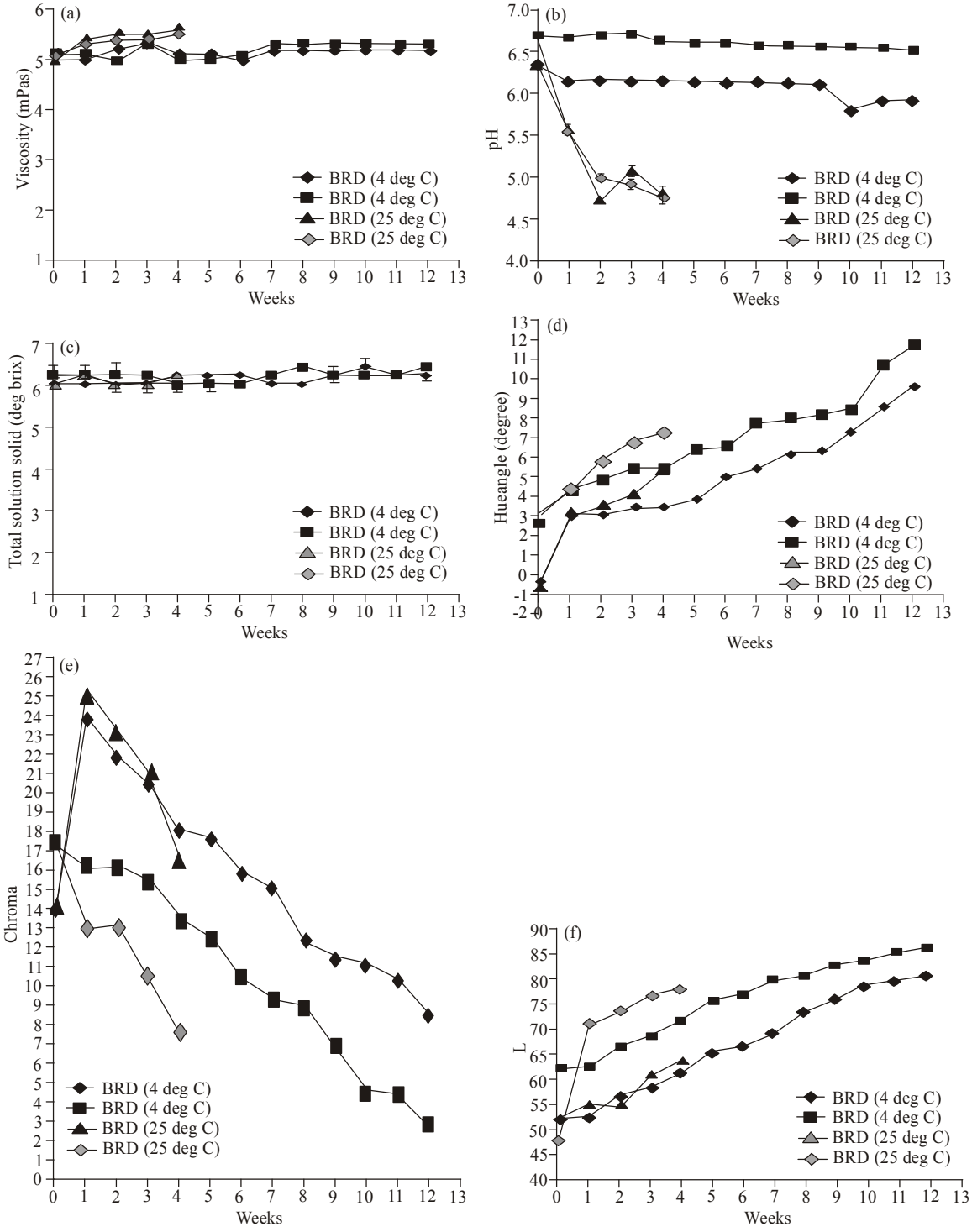

Fig. 3: Viscosity, (a) pH, (b) total soluble solids, (c) hue, (d) chroma, (e) L value and (f) of black rice drink (BRD) and red rice drink (RRD) as function of storage time and temperature

storage time, drinks kept at 4°C and 25°C showed significantly ($p<0.05$) increasing L values as well as hue angles, while their chroma values decreased significantly ($p<0.05$), indicating formation of colourless chalcones due to anthocyanin degradation (Malien-Aubert *et al*., 2001).

Degradation kinetics of anthocyanins in pigmented rice drinks during storage: Degradation kinetics of anthocyanins in pigmented rice drinks were studied at two different storage temperatures; 4°C and 25°C. The degradation kinetics was calculated using the standard equations for zero and first-order reactions below. After

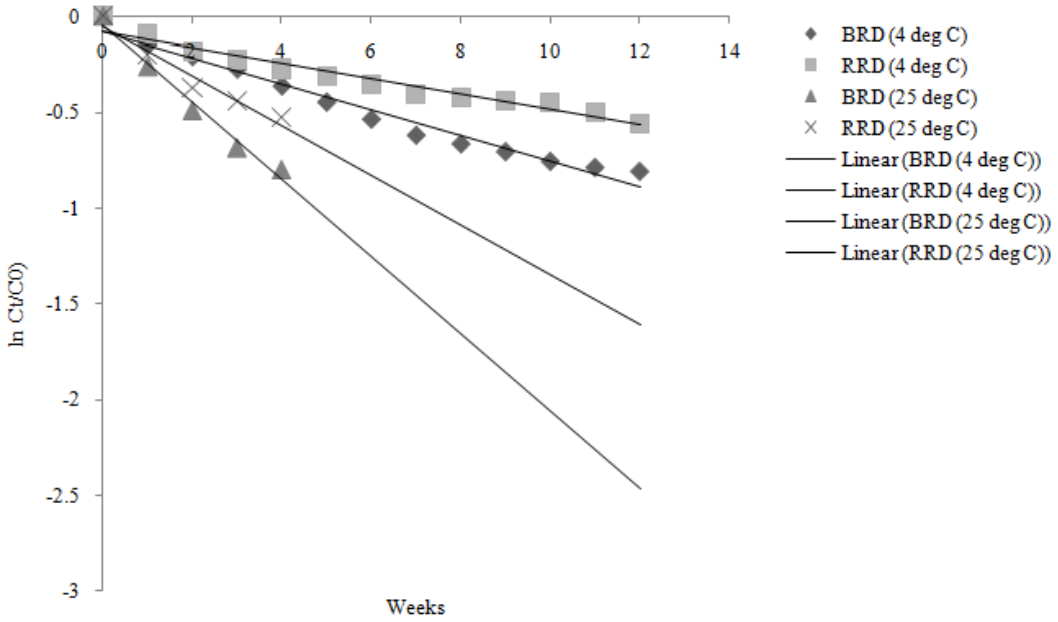

Fig. 4: Effect of storage time on anthocyanin content of Black Rice Drink (BRD) and Red Rice Drink (RRD) at different storage temperatures

fitting the results to both equations, degradation rate constant (k) was determined:

$$C_t = -kt+C_0$$
$$\ln [C_t] = -kt+\ln [C_0]$$

Due to higher R^2, the results were more fitted to the first-order reaction, which was in compliance with the study conducted by Hou *et al*. (2013) on anthocyanin content of black rice. However, to date, there is no study yet on degradation kinetics of anthocyanins in red rice. Using the k determined, the half-life ($t_{1/2}$) was also calculated by the following equation:

$$t_{1/2} = -\ln 0.5/k$$

where,
C_0 = The initial anthocyanin content
C_t = The monomeric anthocyanin content after t weeks
$t_{1/2}$ = The half-life
k = The first-order degradation rate constant (weeks^{-1}) (Fig. 4 and Table 3).

The results for both black rice and red rice drink showed that k values increased with increasing temperature, showing less thermal stability of anthocyanins at higher storage temperature. This might be caused by factors such as Maillard reaction, since as the temperature increases, Maillard reaction also increases, causing copigmentation, which sustains anthocyanin stability, to become less stable (Gradinaru *et al*., 2003).

Black rice drink was shown to have less thermal stability (6.79×10^{-2}/weeks and 20.21×10^{-2}/weeks for storage at 4°C and 25°C, respectively) than red rice drink (4.09×10^{-2}/weeks and 13.05×10^{-2}/weeks for storage at 4°C and 25°C, respectively). This was further proven with up to 3 times less $t_{1/2}$ for the drinks stored at 25°C, with black rice drink having less $t_{1/2}$ (10.21 weeks and 3.43 weeks for storage at 4°C and 25°C, respectively) than red rice drink (16.95 weeks and 5.31 weeks for storage at 4°C and 25°C, respectively). Stability of anthocyanins in red rice drinks might be caused by copigmentation of anthocyanins with other components which were not present in black rice. Proanthocyanidins could be a possible group of compounds since proanthocyanidins are known to be suitable for copigmentation with anthocyanins that may enhance coloration (Macheix *et al*., 1990).

Table 3: Effect of storage on the k and $t_{1/2}$ values of anthocyanin degradation in pigmented rice drinks

	Black rice drink		Red rice drink	
Attributes	4°C	25°C	4°C	25°C
k (weeks^{-1}) $\times10^{-2}$	6.79	20.21	4.09	13.05
$t_{1/2}$ (weeks)	10.21	3.43	16.95	5.31
R^2	0.97	0.98	0.95	0.95

CONCLUSION

The water obtained from cooking black and red rice in excess water has high potential to be developed into antioxidant drinks since antioxidant compounds present in the rice bran tend to leach out into the cooking water. The percentages of antioxidant extractability from red rice and black rice were 88.42 and 103.26%, respectively. It was found that red rice drink had significantly higher ($p<0.05$) antioxidant power than black rice drink and both rice drinks

possessed significantly higher ($p<0.05$) antioxidant power compared to different commercial fruit drinks evaluated in this study, indicating that hot water extraction showed great capability in extracting antioxidants from pigmented rice. The anthocyanins in both drinks followed first-order reaction kinetic during storage. The variations in terms of antioxidant content and physical properties were observed more at storage temperature of 25°C than that of those kept at 4°C. The drinks were able to be kept for 4 weeks at room temperature, while those kept at 4°C remained microbiologically stable throughout 12 weeks of storage. Therefore, it is suggested for the drinks to be kept at refrigerated temperature, or addition of permitted preservatives may be required for the drinks to be able to be kept at room temperature for longer periods.

ACKNOWLEDGMENT

Authors thank Padiberas Nasional Berhad (BERNAS) for the financial support of this research.

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