CHAPTER IV
ANTIOXIDANT ACTIVITY AND DNA PROTECTIVE PROPERTIES OF RICE GRASS JUICES

4.1 Introduction

In Thailand, rice is an important economic crop and its grains have been consumed as a major food. Various colored and white rice cultivars have been distributed throughout Thailand. Rice grains are generally consumed as cooked rice. Therefore, a number of studies have investigated the content of nutrients, active compounds, and antioxidants, and biological activities of rice grain extracts of colored and white rice (Lin and Lai 2011). The extracts from colored rice have shown greater antioxidant efficacy than those of white rice. The presence of phenolic compounds and anthocyanins results in the high antioxidant activity of colored rice (Chen et al. 2006; Sadabpod et al. 2010). Numerous food supplements and functional foods have been developed from rice grains and brans of colored rice. However, the chemical constituents in the inedible parts of rice plants including straw (Miyazawa et al. 2008) and seed husk (Butsat et al. 2009) indicate the possibility to use rice plants as ingredients for the development of new food supplements or functional foods.

Rice is a cereal crop belongs to the Poaceae or 'Grass' family. The young plants at the jointing stage, also called grasses of cereal crops are a rich source of phytochemicals and antioxidants (Gruenwald 2009; Kulkarni et al. 2006). Wheatgrass (*Triticum aestivum* L.) is a famous cereal grass. Juice squeezed from wheatgrass has been consumed as a health-promoting food since the 1980s (Falcioni et al. 2002; Wigmore 1985). Over the past two decades, numerous studies have investigated the

active constituents and biological activities of juice squeezed from wheatgrass harvested at the jointing stage. Wheatgrass juice exhibited high antioxidant efficacy (Kulkarni et al. 2006) and immunomodulatory activity (Hemalatha et al. 2012). Furthermore, the antioxidant activity of wheat sprout extracts has also been investigated and wheat sprout extracts have been found to protect DNA from oxidative damage (Falcioni et al. 2002). The antioxidant potential of wheatgrass juice suggests that the juice squeezed from Thai rice grasses harvested at the jointing stage is likely to possess various active compounds that exhibit antioxidant activity. However, knowledge of the antioxidant activity of rice grass juice is limited (Benjawan et al. 2010). Thus, the active compounds and biological activities of Thai rice grass juice are interesting and should be investigated.

Hence, the aim of this study was to determine the total phenolic content, total monomeric anthocyanin content, and antioxidant activity of grass juices from various cultivars of colored and white rice and wheat using 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP), β-carotene bleaching (BCB), and thiobarbituric acid reactive substances (TBARS) assays. Furthermore, the rice grass juices exhibiting strong antioxidant activity and a high level of total phenolic compounds and/or total monomeric anthocyanins were also subjected to DNA nicking assays to evaluate DNA protective properties.

4.2 Materials and methods

4.2.1 Chemicals and spectrophotometric measurement

The chemicals and reagents used in all experiments were of analytical and HPLC grade. The Folin-Ciocalteu reagent; 6-hydroxy-2,5,7,8-tetramethylchroman-2-

carboxylic acid (Trolox); 2,2-diphenyl-1-picrylhydrazyl (DPPH); 2,4,6-tris(2-pyridyl)-*s*-triazine (TPTZ); 2,6-di-*tert*-butyl-4-methylphenol (BHT); 2-thiobarbituric acid (TBA); β-carotene type II; linoleic acid; trichloroacetic acid (TCA); 30% (w/v) hydrogen peroxide; and TWEEN® 40 were purchased from Sigma-Aldrich (Sigma-Aldrich, St. Louis, MO). Absolute ethanol was purchased from Merck Millipore (Merck, Darmstadt, Germany). pBR322 DNA, the VC Lambda/*Hin*dIII marker, and agarose were purchased from Vivantis (Vivantis, Selangor Darul Ehsan, Malaysia). Absorbance measurements to determine the total phenolic content, free radical scavenging activity, and ferric reducing antioxidant power were performed using a SpectraMax M5 Multi-Mode Microplate Reader and SoftMax Pro 5.2 software (Molecular Devices, Sunnyvale, CA). An Evolution 600 UV-Vis Spectrophotometer (Thermo Fisher Scientific, Waltham, MA) was used to determine the anti-lipid peroxidation activity and total monomeric anthocyanin content.

4.2.2 Plant materials and juice preparation

Seeds from colored and white rice and wheat were obtained from the Bureau of Seed Multiplication of the Rice Department of Thailand in Bangkok and the Purple Rice Research Unit, Chiang Mai University, Chiang Mai, Thailand (**Table 4-1**).

Rice and wheat seeds were washed and soaked overnight in tap water. After washing with distilled water, seeds were planted in vermiculite medium in plastic trays and were watered with tap water until the seeds germinated. Rice grass and wheatgrass were grown under fluorescent light (16/8 photoperiod) at 25 ± 2°C and were watered with 2.5 g/l NPK (30-20-10) fertilizer. At the jointing stage immediately prior to the emergence of the second leaf, fresh grasses were rapidly cut above ground, weighed, washed three times with tap water followed by distilled water, dry blotted, and immediately stored at -20°C. Ten grams of fresh grass was cut into small pieces and ground twice with a pestle in a clean mortar containing 5 ml of distilled water. Juices were squeezed through three layers of white cloth and centrifuged at 8000 rpm for 20 min at 4°C. Supernatants were filtered through 0.45-μm syringe filters, lyophilized to a dry powder, and stored at -20°C. Lyophilized powders were reconstituted in distilled water at 20 milligrams of dry extract per milliliter (mg DE/ml), which was diluted to the final concentration required in each assay. Coloration in seed husks, the pericarp, and grasses from colored and white rice was also determined.

Table 4-1 Rice and wheat cultivars used in this study

Type	Scientific name	Cultivar	Code	Source
Colored (C) rice	*Oryza sativa* L.	Kum Doisaket	C-KDS	Purple Rice Research Unit, Chiang Mai University, Chiang Mai
		Kum Ka	C-KK	Bureau of Seed Multiplication, Rice Department of Thailand, Bangkok
		Kum Noi'	C-KN	
		Kum Pe	C-KP	
		Kum Ton Khieaw	C-KTK	
		Niaw Dum Chor Mai Phi	C-NDP	
		Riceberry	C-RB	
White (W) rice	*Oryza sativa* L.	Khai Mod Rin 3	W-KMR3	
		Khao Dawk Mali 105	W-KDML105	
		Khao Gaw Diaw 35	W-KGD35	
		Leb Nok Pattani	W-LNP	
		Pathum Thani 1	W-PTT1	
		Plai Ngahm Prachin Buri	W-PNPB	
		RD6	W-RD6	
Wheat	*Triticum aestivum* L.	Fang 60	WG	

4.2.3 Determination of the radical scavenging activity using the DPPH assay

The radical scavenging activity of rice grass and wheatgrass juices was determined using a DPPH assay according to Brand-Williams et al. (1995) with some modifications. Fifty micromolar ethanolic DPPH radical solution was reacted with 10 - 600 µg DE/ml samples or 0.1 – 5.0 µg/ml Trolox. Distilled water and absolute ethanol were used as blanks for the samples and for Trolox, respectively. After 30 min, the absorbance of DPPH radicals in solution was measured at 517 nm against a blank using a microplate reader. The percentage of the radical scavenging activity of the samples and Trolox was calculated and plotted against different sample or Trolox concentrations to obtain the EC_{50} (mg DE/ml). The EC_{50} represents the amount of sample required to scavenge 50% of the initial DPPH concentration.

4.2.4 Determination of the ferric reducing antioxidant power (FRAP)

The ferric reducing antioxidant power of the samples was evaluated using a modified FRAP assay according to Benzie and Strain (1996). A freshly prepared FRAP working solution (300 mM acetate buffer, pH 3.6, 10 mM TPTZ, and 20 mM $FeCl_3 \cdot 6H_2O$) was allowed to react with 400 µg DE/ml samples or 400 µg/ml Trolox or 5-100 µM $FeSO_4 \cdot 7H_2O$ for 30 min in the dark. The absorbance of the reaction mixtures was measured at 593 nm, and a standard curve for $FeSO_4 \cdot 7H_2O$ was plotted. The FRAP value was calculated from the standard curve and is expressed in molar Fe^{2+} per gram dry extract (M Fe^{2+}/g DE).

4.2.5 β-Carotene bleaching (BCB) assay

The anti-lipid peroxidation activity of rice grass and wheatgrass juices was determined using a modified β-carotene bleaching assay according to Takada et al. (2006). β-carotene/linoleic acid emulsions (2 mg of β-carotene, 80 mg of linoleic acid, and 800 mg of TWEEN® 40) were incubated with 800 µg DE/ml samples or 800 µg/ml BHT at 50°C for 2 h. Aerated distilled water was used as a control. A linoleic acid emulsion without β-carotene was used as a blank. The absorbance of the mixtures was measured at 470 nm in 20-min intervals for 120 min against the blank. The percent inhibition of lipid peroxidation was calculated as follows:

$$\text{inhibition of lipid peroxidation (\%)} = [1 - (\frac{\Delta A_{sample}}{\Delta A_{control}})] \times 100,$$

where ΔA_{sample} = the difference between the absorbance of a sample at t = 0 and t = 120 min; and $\Delta A_{control}$ = the difference between the absorbance of a control at t = 0 and t = 120 min.

4.2.6 Thiobarbituric acid reactive substances (TBARS) assay

The inhibitory effect on lipid peroxidation by rice grass and wheatgrass juices was evaluated by measuring thiobarbituric acid reactive substances (TBARS) according to a method described by Nagababu et al. () with minor modifications. The linoleic acid model system (10 mM linoleic acid, 10 mM TWEEN® 40, and 0.1 M sodium phosphate buffer, pH 7.0) was co-incubated with 800 µg DE/ml samples or 800 µg/ml BHT, 0.4 mM ascorbic acid, and 0.4 mM $FeSO_4 \cdot 7H_2O$ at 45°C for 1 h. Distilled water was used as the control. After incubation, 10 mM BHT, 45% (w/v) TCA, and 1.5%

(w/v) TBA were sequentially added to the reaction mixture, heated to 95°C for 10 min, and then cooled down on ice. Reaction mixtures were centrifuged, and the supernatants were collected. The absorbance was measured at 532 nm against a blank consisting of all of the reagents except ascorbic acid and $FeSO_4 \cdot 7H_2O$. The percentage of lipid peroxidation inhibition was calculated as follows:

$$\text{inhibition of lipid peroxidation (\%)} = [1 - (\frac{A_{532} \text{ of the sample}}{A_{532} \text{ of the control}})] \times 100,$$

where A_{532} = the absorbance of TBARS produced in a reaction mixture at 532 nm.

4.2.7 Total phenolic content (TPC) determination

The total phenolic content in rice grass and wheatgrass juices was determined using the Folin-Ciocalteu method according to Singleton et al. (Singleton et al. 1999) with some modifications. The Folin-Ciocalteu reagent at a concentration of 0.2 N was reacted with 400 µg/ml samples or 1-50 µg of gallic acid and incubated in the dark. After 5 min, 7.5% (w/v) Na_2CO_3 was added to the reaction mixtures, which were maintained in the dark at room temperature for 30 min. The absorbance of the blue solution was measured at 765 nm and plotted against the gallic acid concentrations. The TPC was calculated from a standard curve of gallic acid and is expressed as milligrams of gallic acid equivalents per gram of dry extract (mg GAE/g DE).

4.2.8 Total monomeric anthocyanin content (TMAC) determination

The total monomeric anthocyanin content in rice grass and wheatgrass juices was determined using the pH differential method as described by Lee et al. (Lee et al. 2005). Samples were diluted 1:8 with 25 mM KCl buffer, pH 1.0, and 400 mM sodium acetate buffer, pH 4.5. The absorbance of samples in different pH value systems was measured at 520 and 700 nm. The TMAC is expressed as milligrams of cyanidin-3-glucoside equivalents per gram of dry extract (mg C3GE/g DE) and was calculated as follows:

$$\text{TMAC (mg C3GE/g DE)} = \left(\frac{A \times MW \times DF \times 1000}{\varepsilon \times l \times g} \right),$$

where $A = (A_{520\ nm} - A_{700\ nm})_{pH\ 1.0} - (A_{520\ nm} - A_{700\ nm})_{pH\ 4.5}$; MW = the molecular weight of C3GE, 449.2 g/mol; DF = a dilution factor; ε = the molar extinction coefficient for C3GE, 26900 l/mol·cm; l = the path length, 1 cm; 1000 = a conversion factor; and g = gram of dry extract.

4.2.9 Assessment of the protection against oxidative DNA damage

The DNA protective properties of rice grass and wheatgrass juices were assessed by subjecting supercoiled pBR322 DNA to the Fenton reaction according to the method described by Falcioni et al. (2002) with some modifications. Two hundred nanograms of pBR322 DNA was incubated with samples at 1, 10 and 100 µg DE/ml at room temperature for 10 min. Subsequently, 100 µM $FeSO_4 \cdot 7H_2O$ and 80 mM H_2O_2 were sequentially added to the mixture. The final volume of the mixture was brought to 20 µl with 5 mM sodium phosphate buffer, pH 7.4, and the mixture

was incubated at 37°C for 1 h. The reaction was terminated by adding 6× electrophoresis loading buffer. Treated pBR322 DNA was separated in a 1% (w/v) agarose gel and stained with ethidium bromide. pBR322 DNA was visualized and photographed under UV light using a Gel Doc™ XR+ system (Bio-Rad, Hercules, CA). The relative intensity of the SC pBR322 DNA following exposure to the Fenton reaction was quantified and calculated as a percentage using Image Lab™ software (Bio-Rad, Hercules, CA). Distilled water and 0.1 μM Trolox were used as a control and a positive control, respectively.

4.2.10 Statistical analysis

All analyses were performed in triplicate (n = 9) and the results are reported as the means ± SD. The data were analyzed using Tukey's honestly significant difference (HSD) test in one-way analysis of variance (ANOVA) using SPSS software version 16.0 (SPSS Inc., Chicago, IL). Significant differences were considered at $p < 0.05$. Correlation analysis between assays was performed using Pearson's correlation coefficient (R).

4.3 Results

4.3.1 Coloration in Colored and White Rice

Seven cultivars from both colored and white rice and one wheat cultivar were examined in this study (**Table 4-1**). Color differences were observed in the seed husk, pericarp, and rice grass (**Figure 4-1**). The color of the white rice seed husks was light to dark yellow, whereas the seed husks of the colored rice ranged from light yellow to dark brown (**Figure 4-1a**). Only the colored rice cultivars possessed colored pericarps ranging from red/reddish-brown to dark brown or reddish-purple to dark purple (**Figure 4-1a**). Rice grass pigmentation was observed in six colored rice cultivars: C-KDS, C-KK, C-KN, C-KP, C-KTK, and C-RB. Pigments accumulated in the coleoptiles and leaves of colored grass, and their color varied from reddish-purple to dark purple. Interestingly, grasses of the colored rice cultivar C-NDP were green, which is similar to that of the white rice cultivars and wheat (**Figure 4-1b**). The color of the fresh juice and dry extract of the rice grass and wheatgrass ranged from green to dark purple depending on the pigmentation of the grass (**Figures 4-1c and 4-1d**).

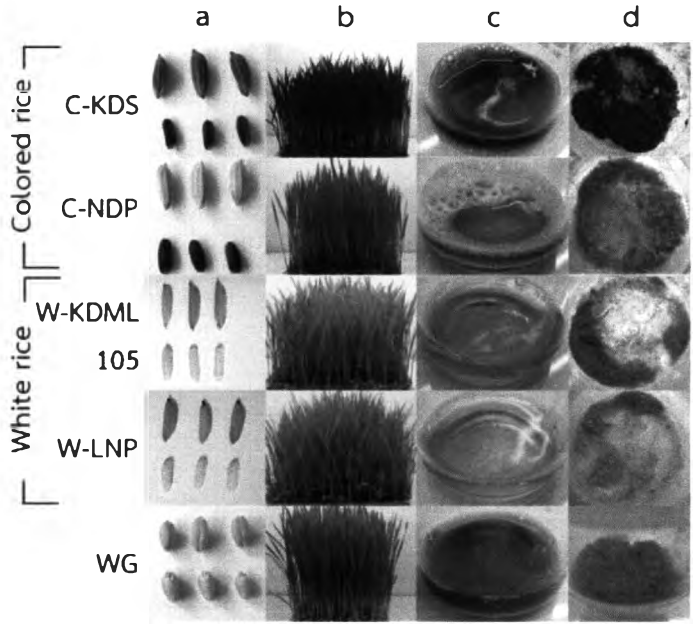

Figure 4-1 Color differences of (**a**) the seed husk and pericarp, (**b**) grasses, (**c**) fresh grass juice, and (**d**) the dry extract of the colored rice cultivars Kum Doisaket (C-KDS) and Niaw Dum Chor Mai Phi (C-NDP), white rice cultivars Khao Dawk Mali 105 (W-KDML105) and Leb Nok Pattani (W-LNP), and wheat (WG).

4.3.2 DPPH Radical scavenging activity and ferric reducing ability

The radical scavenging activity and ferric reducing ability of grass juices were evaluated using DPPH and FRAP assays, respectively. The DPPH radical scavenging activity of the samples is expressed as the EC_{50} value (**Table 4-2**). Rice grass and wheatgrass juices at concentrations ranging from 10-600 µg DE/ml exhibited DPPH radical scavenging activity in a dose-dependent manner (**Figure 4-2**). Generally, the colored juices of most of the colored rice cultivars exhibited strong DPPH radical scavenging activity. The C-KDS cultivar exhibited the greatest radical scavenging activity with an EC_{50} of 0.11 mg DE/ml ($p < 0.05$). The colored rice cultivars C-KDS, C-KK, C-KN, C-KP, C-KTK, and C-RB exhibited a significantly greater DPPH radical

scavenging activity than white rice cultivars and wheat ($p < 0.05$). However, the green juice of the colored rice cultivar C-NDP exhibited a lower radical scavenging activity than wheat. Colored and white rice grass juices could reduce Fe^{3+} to Fe^{2+}. FRAP values indicated that the colored rice exhibited a higher Fe^{3+} reducing capacity than white rice (**Table 4-2**). The colored rice cultivar C-KDS exhibited the highest FRAP value of 1.79 M Fe^{2+}/g DE. In contrast, the white rice cultivar W-PNPB exhibited the lowest FRAP value of 0.44 M Fe^{2+}/g DE.

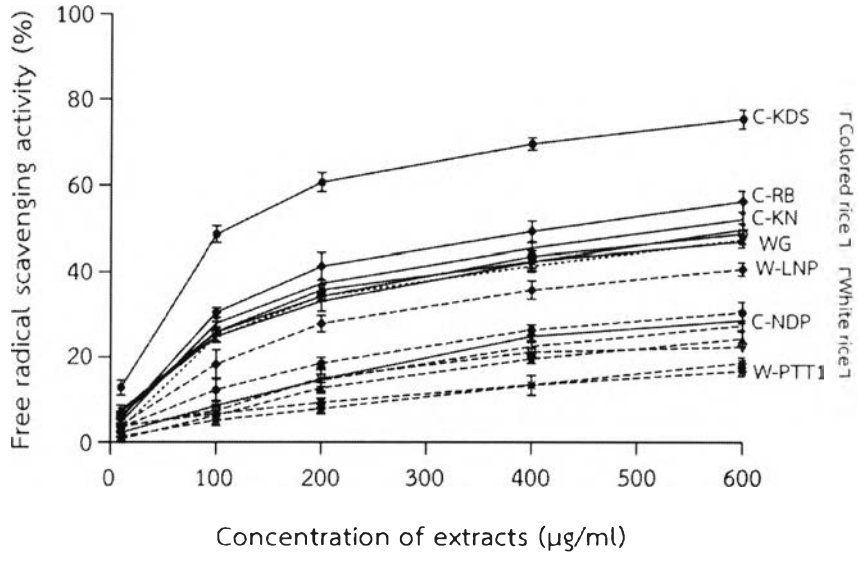

Figure 4-2 DPPH radical scavenging activity (%) of grass juices from colored rice cultivars (solid line), white rice cultivars (dashed line), and wheat (dotted line) at concentrations ranging from 10-600 µg/ml.

4.3.3 Inhibition of lipid peroxidation

The anti-lipid peroxidation activity of rice grass and wheatgrass juices was evaluated using BCB and TBARS assays. Grass juices from colored rice, white rice and wheat at a concentration of 800 µg DE/ml were subjected to the linoleic acid emulsion system to determine the percent inhibition of β-carotene bleaching and TBARS formation (**Table 4-2**). Colored juices from colored rice cultivars exhibited a strong inhibitory effect on lipid peroxidation. The C-KDS cultivar exhibited the greatest anti-lipid peroxidation activity among the examined cultivars ($p < 0.05$) with 93.80% β-carotene bleaching and 94.98% TBARS formation compared with that of the control. However, the green juice from the colored rice cultivar C-NDP exhibited moderate anti-lipid peroxidation activity in BCB and TBARS assays. The anti-lipid peroxidation activity of white rice cultivars was low to moderate. The W-PTT1 cultivar exhibited the lowest inhibition of the lipid peroxidation, with 8.70 and 12.51% β-carotene bleaching and TBARS formation, respectively.

Table 4-2 DPPH radical scavenging activity, ferric reducing capacity (FRAP values), and anti-lipid peroxidation activity in the β-carotene/ linoleic acid system (β-carotene bleaching assay, BCB) and the ascorbate-Fe^{2+} system (thiobarbituric acid reactive substances assay, TBARS) of rice grass and wheatgrass juices*

Samples	DPPH assay	FRAP values	Inhibition of lipid peroxidation (%)‡	
	EC_{50}† (mg/ml)	M Fe^{2+}/g DE	BCB	TBARS
Colored rice				
C-KDS	0.11 ± 0.03^{a}	1.79 ± 0.03^{a}	93.80 ± 1.24^{a}	94.98 ± 0.27^{a}
C-KK	0.64 ± 0.02^{c}	1.13 ± 0.03^{c,d}	91.55 ± 1.73^{a,b}	92.68 ± 0.45^{b}
C-KN	0.54 ± 0.07^{b,c}	0.61 ± 0.02^{f}	91.69 ± 0.74^{a,b}	93.21 ± 0.90^{a,b}
C-KP	0.61 ± 0.05^{c}	1.18 ± 0.04^{c,d}	90.97 ± 0.41^{b}	92.61 ± 0.71^{b}
C-KTK	0.67 ± 0.03^{c}	0.73 ± 0.03^{e}	90.28 ± 1.03^{b}	91.77 ± 0.63^{b}
C-NDP	1.02 ± 0.02^{e}	0.61 ± 0.02^{f}	64.53 ± 1.10^{e}	67.25 ± 1.24^{d}
C-RB	0.43 ± 0.06^{b}	1.06 ± 0.03^{d}	81.33 ± 1.70^{c}	83.51 ± 0.72^{c}
White rice				
W-KMR3	1.02 ± 0.01^{e}	0.50 ± 0.02^{g}	65.08 ± 1.02^{e}	68.44 ± 0.92^{d}
W-KDML105	11.36 ± 0.07^{f}	0.47 ± 0.07^{g,h}	41.17 ± 0.56^{n}	46.60 ± 0.70^{f,g}
W-KGD35	0.98 ± 0.04^{e}	0.54 ± 0.01^{g}	56.40 ± 0.67^{f}	57.86 ± 1.18^{e}
W-LNP	1.34 ± 0.08^{f}	1.31 ± 0.04^{o}	56.49 ± 0.96^{f}	59.62 ± 1.37^{e}
W-PNPB	1.90 ± 0.05^{g}	0.44 ± 0.03^{n}	40.12 ± 1.87^{n}	41.69 ± 1.66^{g}
W-PTT1	1.94 ± 0.06^{g}	0.48 ± 0.03^{g,h}	8.70 ± 1.25^{i}	12.51 ± 0.43^{n}
W-RD6	1.24 ± 0.03^{f}	0.53 ± 0.01^{g}	40.34 ± 0.35^{n}	44.99 ± 0.94^{g}
Wheatgrass	0.81 ± 0.02^{d}	1.08 ± 0.02^{d}	47.80 ± 0.83^{g}	49.39 ± 0.17^{f}
Standard	Trolox		BHT	
	2.43 ± 0.05 μg/ml	769.63 ± 11.97¶	83.32 ± 1.12	86.72 ± 0.13

*Values are expressed as the mean of triplicates ± SD. † EC_{50} represents the effective concentration of the samples or Trolox that can scavenge 50% of the initial DPPH concentration. ‡ The concentration of samples and standard used in the BCB and TBARS assays was 800 μg/ml. ¶ The value is expressed in M Fe^{2+}/g Trolox. Different letters within the same column indicate a significant difference at $p < 0.05$ by Tukey's HSD test.

4.3.4 Total phenolic and total monomeric anthocyanin content

Samples were subjected to a modified Folin-Ciocalteu method for TPC determination (**Figure 4-3**). The TPC of the colored rice cultivars ranged from 1.90 to 4.33 mg GAE/g DE. The C-KDS cultivar exhibited the highest TPC ($p < 0.05$). The TPC of white rice cultivars varied in the range 1.50 - 2.14 mg GAE/g DE, whereas the TPC of wheat was 2.91 mg GAE/g DE. The TPC of C-KDS grass juice was 48 and 100% higher than that of wheat and white rice, respectively. The colored grass juices, particularly C-KDS, exhibited more effective antioxidant activity than the green grass juices.

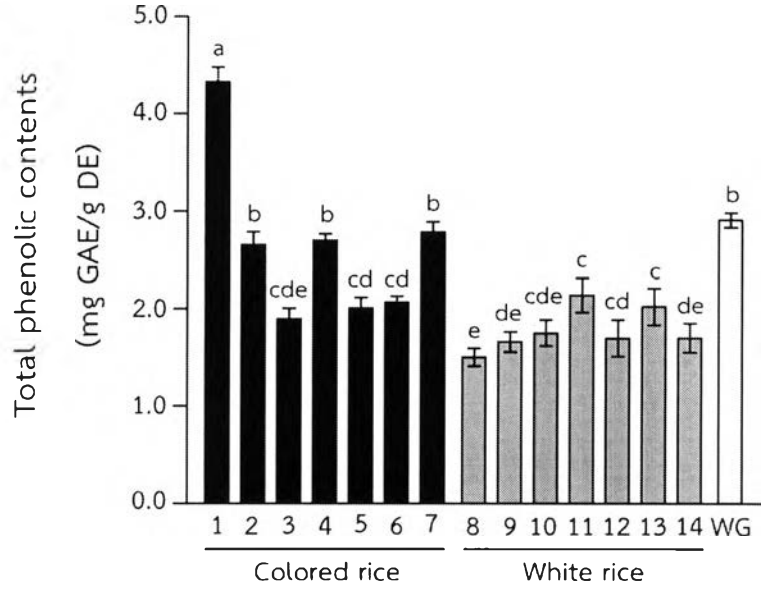

Figure 4-3 Total phenolic content of grass juice from colored rice (1: C-KDS; 2: C-KK; 3: C-KN; 4: C-KP; 5: C-KTK; 6: C-NDP; and 7: C-RB), white rice (8: W-KMR3; 9: W-KDML105; 10: W-KGD35; 11: W-LNP; 12: W-PTT1; 13: W-PNPB; and 14: W-RD6) and wheat (WG). The values are expressed as milligrams of gallic acid equivalents per gram of dry extract (mg GAE/g DE). Different letters above the bars indicate a significant difference at $p < 0.05$.

To determine whether the antioxidant activity of colored rice cultivars was influenced by the presence of anthocyanins, the pH differential method was performed. Monomeric anthocyanins were detected in only the colored grass juices of the colored rice cultivars C-KDS, C-KK, C-KN, C-KP, C-KTK, and C-RB (**Figure 4-4**). The C-KDS cultivar exhibited the highest TMAC at 4.42 mg C3GE/g DE. The TMAC in grass juice from the C-KDS cultivar was 2- and 15-fold greater than that from the C-KK and C-KN cultivars, respectively.

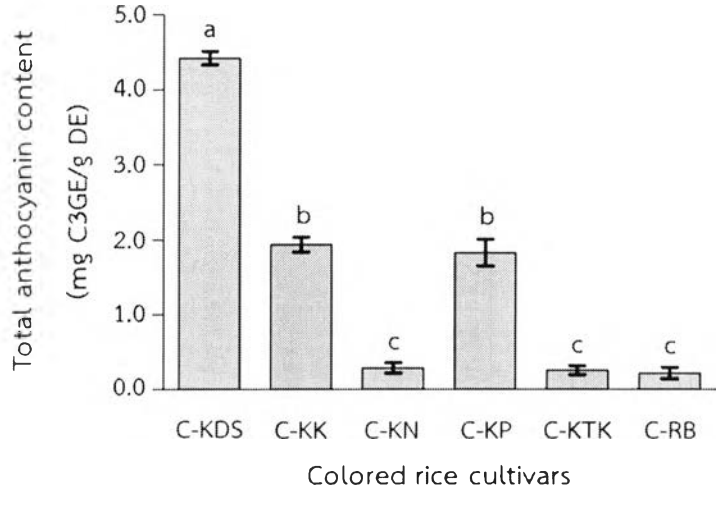

Figure 4-4 Total monomeric anthocyanin content of colored rice grass juices. The values are expressed as milligrams of cyanidin-3-glucoside equivalents per gram of dry extract (mg C3GE/g DE). Different letters above the bars indicate a significant difference at $p < 0.05$.

4.3.5 Correlation analysis of the antioxidant activity, TPC, and TMAC

To determine the relationship between the different antioxidant activity assays, TPC, and TMAC of rice grass juices, Pearson correlation analysis was performed. Significant correlations were found among the assays with $p < 0.05$ (**Table 4-3**). The TPC and TMAC were associated with antioxidant activity. Furthermore, the TMAC exhibited greater correlation with the anti-lipid peroxidation activity than the TPC.

Table 4-3 Correlation coefficients (*R*) between antioxidant activity assays, total phenolic content, and total monomeric anthocyanin content

	Correlation coefficients (*R*)				
	DPPH[a]	FRAP[b]	BCB[c]	TBARS[d]	TPC[e]
FRAP	0.843*				
BCB	0.786*	0.584*			
TBARS	0.773*	0.546*	0.993*		
TPC	0.784*	0.880*	0.491*	0.458	
TMAC[f]	0.694*	0.792*	0.533*	0.503*	0.845*

Values with an asterisk are significant at $p < 0.05$.

[a]DPPH radical scavenging activity; [b]ferric reducing antioxidant power; [c]β-carotene bleaching assay; [d]thiobarbituric acid reactive substances assay; [e]total phenolic content; and [f]total monomeric anthocyanin content.

4.3.6 Inhibition of pBR322 DNA strand breakage induced by the Fenton reaction

To determine the DNA protective effects of rice grass juice, the colored rice cultivars C-KDS and C-KN, which exhibited high antioxidant activity, were selected and individually co-incubated with supercoiled pBR322 DNA. After the co-incubation, the mixtures were subjected to the Fenton reaction (Fe^{2+}/H_2O_2). The ability of grass juices from the rice cultivars C-KDS and C-KN to prevent oxidative damage of supercoiled pBR322 DNA was determined and compared to that of wheat (**Figure 4-5**). After subjecting pBR322 DNA to the Fenton reaction, three pBR322 DNA bands were detected under UV light. The upper band represents a nicked circular (NC) form, which is followed by linear and supercoiled (SC) pBR322 DNA (**Figure 4-5a**). Most of the pBR322 DNA in the control reaction (SC lane) was in a supercoiled form. In the presence of ferrous ion (Fe^{2+} lane) or hydrogen peroxide alone (H_2O_2 lane), pBR322 DNA was partially converted to the NC form. However, the SC DNA was completely converts to the NC and linear forms when incubated with both ferrous ion and hydrogen peroxide (Fe^{2+}/H_2O_2 lane). The positive control (0.1 μM Trolox) demonstrated a potent DNA protective effect. The relative intensity of the SC DNA was 85.07% compared with that of the control (**Figure 4-5b**). The colored rice cultivar C-KDS demonstrated a dose-dependent of DNA protective effect. The relative intensity of the SC DNA was significantly increased from 31.5 to 37.47% upon co-incubation of pBR322 DNA and C-KDS grass juice at 1 and 100 μg/ml, respectively (**Figures 4-5a and 4-5b**). Juice from the C-KN cultivar at concentrations of 1 and 10 μg/ml exhibited DNA protective effects, whereas 100 μg/ml C-KN grass juice enhanced the oxidative DNA damage. This pro-oxidative activity of the C-KN cultivar

reduced the relative intensity of the SC DNA from 27.57 to 11.87% after co-incubation with 10 and 100 µg/ml C-KN grass juice, respectively. A DNA protective effect was also observed upon co-incubation of pBR322 DNA and 1 µg/ml wheatgrass juice, which exhibited a relative intensity for SC DNA of 21.80% (**Figure 4-5b**). However, wheatgrass juice at higher concentrations promoted oxidative damage of pBR322 DNA. The greatest pro-oxidant activity was observed for 100 µg/ml wheatgrass juice, which resulted in completely fragmented DNA (**Figure 4-5a**).

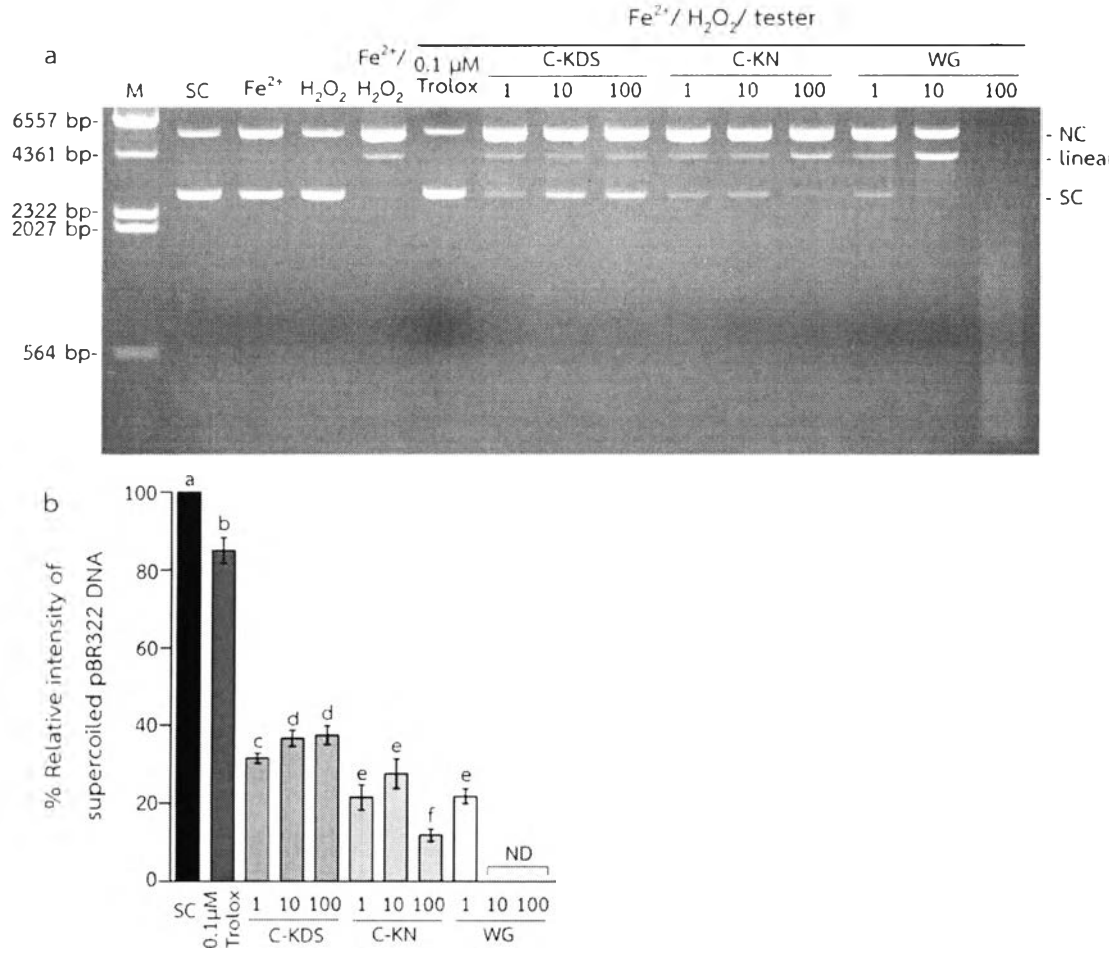

Figure 4-5 DNA protective effects of grass juice from the colored rice cultivars Kum Doisaket (C-KDS) and Kum Noi (C-KN), and wheat (WG). (**a**) Agarose gel electrophoresis of pBR322 DNA following exposure to the Fenton reaction. M: the VC Lambda/*Hind* III marker; SC: supercoiled pBR322 DNA; NC: nicked pBR322 DNA; Fe^{2+}: DNA + Fe^{2+}; H_2O_2: DNA + H_2O_2; Fe^{2+}/H_2O_2: DNA + Fe^{2+} + H_2O_2 and Fe^{2+}/H_2O_2/tester: DNA + Fe^{2+} + H_2O_2 + 0.1 μM Trolox or 1, 10 and 100 μg/ml of C-KDS, C-KN or WG grass juices. (**b**) The relative intensity of supercoiled pBR322 DNA subjected to the Fenton reaction in the presence of 0.1 μM Trolox or 1, 10 and 100 μg/ml of the C-KDS, C-KN, or WG grass juices. The relative intensity of the control (SC) is set at 100%. Different letters above the bars indicate a significant difference at $p < 0.05$.

4.4 Discussion

The antioxidant activity, total phenolic content, and total monomeric anthocyanin content of juices from wheatgrass and colored and white rice grasses were investigated in this study. Grasses from 14 Thai colored and white rice cultivars and wheat were grown under controlled conditions and harvested at their jointing stage at 15 and 7 days for rice and wheat, respectively. Rice grass and wheatgrass were squeezed to obtain juice, which was lyophilized to a dry powder. Juice preparations were processed without high-temperature treatment to preserve thermally sensitive antioxidants, and solvents were not utilized to eliminate solvent effects (Calzuola et al. 2004; Dudonne et al. 2009). Natural antioxidants in crude plant extracts possess multifunctional activities; thus, a single antioxidant activity assay is insufficient to predict and measure the antioxidant efficacy of natural antioxidants (Antolovich et al. 2002; Dudonne et al. 2009; Frankel and Finley 2008; Prior and Cao 1999). The utilization of assays that measure electron/radical scavenging activity in combination with anti-lipid peroxidation assays is recommended for the determination of natural antioxidant potential (Moon and Shibamoto 2009). Therefore, four different antioxidant assays, i.e., DPPH, FRAP, BCB, and TBARS assays, were used to determine the antioxidant efficacy of wheatgrass and colored and white rice grass juices in this study. Rice grass and wheatgrass juices exhibited antioxidant activity in different assays (**Table 4-2**). Positive results in all of the antioxidant activity assays used in this study indicate that the various antioxidants presented in rice grass and wheatgrass juices include a DPPH radical scavenger, a metal ion chelator, and a lipid peroxidation inhibitor. Interestingly, colored grasses from most of the colored rice cultivars exhibit a high level of anthocyanins in the

coleoptiles and leaves (**Figure 4-1**). Colored grass juices demonstrated more effective antioxidant activity than green juices. Our results are consistent with the previous study of antioxidant efficacy of rice bran in which colored rice bran exhibited stronger antioxidant activity than white rice bran (Laokuldilok et al. 2010). Notably, the most colored rice cultivar Kum Doisaket significantly exhibited the highest antioxidant efficacy in all assays ($p < 0.05$).

Phenolic compounds are water-soluble antioxidants that are commonly found in fruits, vegetables, and plant extracts. Hydroxyl groups and their resonance stabilization effects on the phenol rings of phenolic compounds are responsible for plant antioxidant activity (Dai and Mumper 2010; Pasko et al. 2009; Rice-Evans et al. 1996; Vinson et al. 2001). A positive correlation between antioxidant activity and the phenolic content of crude extracts has been demonstrated among different plant parts and species such as rice bran (Laokuldilok et al. 2010), wild Indian black plums (Banerjee et al. 2005), barley grass (PaulíčkoVá et al. 2007), and common edible fruits (Garcia-Alonso et al. 2004). In this study, Pearson correlation coefficients (R) indicated a relationship between the antioxidant activity, TPC, and TMAC of rice grass and wheatgrass juices (**Table 4-3**). The R values indicated that the TPC and TMAC were involved in the DPPH radical scavenging activity, ferric reducing ability, and anti-lipid peroxidation activity of rice grass and wheatgrass juices. The TMAC in colored rice grass juices was more associated with inhibitory effects on lipid peroxidation. The effective radical scavenging activity, ferric reducing ability, and anti-lipid peroxidation activity of rice grass and wheatgrass juices may result from a synergistic effect between phenolic compounds and other non-phenolic antioxidants. This synergistic effect in crude plant mixtures has also been reported by Vinson et al. who

demonstrated that crude extracts from commonly consumed fruits exhibited higher antioxidant activity than most of pure phenolic compounds and vitamin antioxidants (Vinson et al. 2001).

Reactive oxygen species (ROS) such as hydroperoxyl, superoxide, and hydroxyl radicals play an important role in oxidative DNA damage. This deleterious effect causes dysfunction in biological processes in the human body leading to age-related and chronic diseases (Finkel and Holbrook 2000; Mayne 2003). Plant extracts that can scavenge ROS may prevent oxidative DNA damage (Kapiszewska et al. 2005). Therefore, the colored rice cultivars Kum Doisaket and Kum Noi, which exhibited high antioxidant activity, were selected and analyzed to assess their DNA protective properties. Rice grass juices from the Kum Doisaket and Kum Noi cultivars and wheatgrass juice were individually co-incubated with supercoiled pBR322 DNA that was further subjected to the Fenton reaction (Fe^{2+}/H_2O_2), and their DNA protective effects were assessed. Our results indicated that rice grass and wheatgrass juices exhibit partial DNA protection (**Figure 4-5**). Only the Kum Doisaket cultivar, which contained the highest level of total monomeric anthocyanins, demonstrated a dose-dependent DNA protective effect. This result suggested that the anthocyanins in colored rice grass juice may be responsible for these DNA protective effects. Anthocyanins can protect DNA from hydroxyl radicals generated in the Fenton reaction by forming an anthocyanin-DNA copigmentation complex (Sarma and Sharma 1999). The dose-dependent DNA protective effect of rice grass juice from the Kum Doisaket cultivar is consistent with the DNA protective pattern of wheat sprout extract demonstrated by Falcioni et al. (2002). Rice grass juice from the Kum Noi cultivar also prevented hydroxyl radical-induced oxidative damage of pBR322 DNA.

However, the DNA protective effect of the Kum Noi cultivar was lower than that of the Kum Doisaket cultivar. The low level of total monomeric anthocyanins in the grass juice from the Kum Noi cultivar may affect its DNA protective properties. Unexpectedly, grass juices of the Kum Noi cultivar and wheat at high concentrations promoted pBR322 DNA oxidative damage. This pro-oxidant activity may result from phenolic compounds in these grass juices. Plant extracts that contain a high level of phenolic compounds, may exhibit pro-oxidant activity in the presence of redox-active metals such as iron and copper. The redox cycling of phenolic compounds is catalyzed by redox-active metals and produces phenoxyl radicals, ROS, and other organic radicals that can attack DNA and other biomolecules in biological systems (Sakihama et al. 2002). A high total phenolic content in wheatgrass juice may contribute to its strong pro-oxidant activity, which is consistent with polyphenol-rich extracts from Mediterranean spices (Joubert et al. 2005) and rooibos tea (Kapiszewska et al. 2005).

4.5 Conclusion

This study is the first to report the antioxidant activity and the total phenolic and total monomeric anthocyanin content of juices squeezed from grasses harvested at the jointing stage from various Thai rice cultivars that included colored and white rice. The findings of our study suggest that colored rice grass juices that contain a high level of total monomeric anthocyanins exhibit higher antioxidant activity than white rice and wheat. Anthocyanins present in colored rice grass juices may be responsible for their high antioxidant efficacy. Notably, the colored rice cultivar Kum Doisaket exhibited effective antioxidant activity and DNA protective properties and

contains the highest level of anthocyanins. Thus, colored rice cultivars may be used as primary ingredients in food supplements or functional foods. These results are useful for the development of new functional foods from rice grass cocktails. Additional studies are necessary to isolate and characterize the bioactive compounds present in rice grass juice. Bioassay-guided fractionation and the elucidation of the mechanisms underlying the bioactivities of rice grass juice may aid in the development of value-added products from rice. Furthermore, this information of antioxidant activity and active compounds in Thai rice grass juices could be used for the recruitment and selection of rice cultivars for crop improvement programs.