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Comparative Evaluation of the Antioxidant Potential of Infant Cereals Produced from Purple Wheat and Red Rice Grains and LC-MS Analysis of Their Anthocyanins

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Supporting Information

ABSTRACT: Cellular oxidative damage by endogenous and exogenous sources of free radicals and reactive oxygen species is a particular threat in infants. Antioxidant protection is normally achieved through a balance between pro-oxidants and endogenous and/or dietary antioxidants. Comprehensive research is required on optimization to achieve good antioxidant protection through infant foods, in particular, the commercially available infant cereals. This study therefore investigated the properties of whole purple wheat, unpolished red rice, and partially polished red rice before and after processing to produce infant cereals. Total phenolic content (TPC), total anthocyanin content (TAC), oxygen radical absorbance capacity (ORAC), individual anthocyanin components, and cellular antioxidant activity were measured. Home-made and laboratory-made pigmented infant cereals differed in that the latter required longer exposure to higher temperature and enzymatic hydrolysis. Home-made and laboratory-made unpolished red rice infant cereals showed higher total phenolic contents and peroxyl radical scavenging activity than home-made and laboratory-made purple wheat infant cereals; however, the latter had higher TAC. Pigmented infant cereals generally had higher TPC, TAC, and ORAC than the commercial ones ($p < 0.05$). Anthocyanins were identified in whole purple wheat, but they were not detected in unpolished red rice. *C*-Glycosyl apigenin was found in both whole purple wheat and unpolished red rice. Processing significantly decreased anthocyanin and *C*-glycosyl apigenin contents ($p < 0.05$). Purple wheat infant cereals had higher cellular antioxidant activity than unpolished red rice ones ($p < 0.05$). Whole purple wheat infant cereals showed higher antioxidant activity than the commercial infant cereal, suggesting a possibility of improving infant antioxidant status by incorporating this grain in their diet.

KEYWORDS: infant cereals, purple wheat, red rice, antioxidant activity, anthocyanins

INTRODUCTION

Infancy is the key developmental stage in life during which exponential growth and maturation occur. Oxidative stress, which is an imbalance between oxidants and reductants, can occur as a result of exposure either to inadequate endogenous antioxidant systems or via an overload in pro-oxidants. The resultant cellular and molecular damage is linked to a variety of pathologies, and thus proper antioxidant protection is required.[1] Dietary or "natural" antioxidants may be obtained through the diet. In this context, infant cereals, commonly introduced to babies at 4−6 months of age, may have the antioxidant potential to help mitigate oxidative stress. An inverse relationship between intake of whole grains and their products and the risk of chronic diseases has been shown in epidemiological studies.[2,3]

Commercial infant cereals vary widely in composition. They are manufactured according to the dietary requirements of each major developmental stage of the infant. Infant cereals are primarily composed of cereal grains; however, there are additional ingredients such as fruits, prebiotics, added nutrients (iron and vitamins), emulsifiers, whey protein concentrate, honey, plant oils, milk, and beetroot powder. Issues regarding the use of these semisolid foods include stage of introduction and allergic reactions. Processing of infant cereals includes toasting, boiling, drying, and hydrolysis to improve flavor and texture qualities, digestibility (mainly of starch), safety, and shelf life.[4] Pigmented grains, in particular, have been shown to have higher antioxidant potential than the regular nonpigmented cereals.[5−8]

Red rice and purple wheat were selected for the model study of pigmented infant cereals because partially polished red rice infant cereal is a commonly consumed product in Indonesia and purple wheat is grown in Saskatchewan, Canada. Red rice anthocyanins are mainly in the form of cyanidin 3-glucoside, which is also found in purple wheat as the predominant constituent in addition to other anthocyanins.[7] Condensed tannins are also found in purple and red rice.[8] Red rice and purple wheat exhibited higher antioxidant activities in in vitro and in vivo models compared to nonpigmented rice and wheat.[5,6]

The present study aimed to prepare prototype infant cereals using pigmented grains as primary raw ingredients and two types

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Table 1. Samples Including Raw Grains, Infant Cereals Made from Pigmented Grains, and Commercial Infant Cereals Including Their Developmental Stage

	sample name	product name	manufacturer/store/developer	produced in	imported by
	(a) Raw Grains				
a	raw partially polished red rice	Red Raw Rice	M&M Twins Ltd.	Scarborough, ON, Canada	Sri Lanka
b	raw unpolished red rice	Red Cargo Rice	BangSue Chia Meng Rice Mill Co. Ltd.	Bangkok, Thailand	Thailand
c	raw unpolished brown rice short grain, organic		Real Canadian Superstore	Winnipeg, MB, Canada	
d	raw purple wheat	AnthoGrain Wheat	Crop Development Centre, University of Saskatchewan	Saskatoon, SK, Canada	
			provided by InfraReady Products (1998) Ltd.	Saskatoon, SK, Canada	
	(2) Infant Cereals Made from Pigmented Grains				
a	home-made and laboratory-made partially polished red rice				
b	home-made and laboratory-made unpolished red rice				
c	home-made and laboratory-made unpolished brown rice				
d	home-made and laboratory-made whole purple wheat				

	sample name	cereal grain ingredient	sample brand	manufacturer	manufactured in
	(3) Commercial Infant Cereals				
a	Stage 1 (beginner cereals, introduced at 6 months of age)				
i	rice cereal	rice	Beech Nut	Beech-Nut Nutrition Corp.	Canajoharie, NY, USA
ii	rice cereal	rice	Heinz	Heinz Canada	North York, ON, Canada
iii	brown rice cereal	organic brown rice	Organics for Baby	Lucerne Foods	Calgary, AB, Canada
b	Stage 2 (6−8 months)				
i	mixed cereal	oat, wheat, and rice	Heinz	Heinz Canada	North York, ON, Canada
ii	mixed grains cereal	whole wheat, oat, barley, rye, corn, and rice	Nestle	Nestle Canada Inc.	North York, ON, Canada
c	Stage 3 (8−12 months)				
i	wheat and oat cereal with banana and raspberry	wheat and oat	Heinz	Heinz Canada	North York, ON, Canada
ii	rice cereal with apple and pear	organic rice	President's Choice Organics (PC Organics)	Loblaw Inc.	Toronto, ON, Canada
d	Stage 4 (12 months or toddlers)				
i	multigrain cereal with mango, pineapple, and pear	oat, rice, rye and wheat	Heinz	Heinz Canada	North York, ON, Canada
ii	mixed cereals with fruits	wheat, rice, oat, barley, rye, corn, and millet	Milupa, Danone	Van de Water-Raymond Ltd.	Laval, QC, Canada

of processing representing home-made and laboratory-made to simulate household and commercial productions, respectively (see the Supporting Information). Commercial infant cereals were used as controls for comparison with pigmented cereals. Total phenolic content, total anthocyanin content, and total antioxidant activity of the infant cereals were determined. Major antioxidant phytochemicals in the pigmented infant cereals were identified and quantified. Antioxidant properties of selected samples were then assessed using an infant cell culture model.

MATERIALS AND METHODS

Samples. The samples included raw grains, infant cereals made from pigmented grains, and nine commercial samples (Table 1). The commercial samples, selected according to their ingredients and introduction stage, were purchased from major supermarkets in Winnipeg, MB, Canada.

Brown rice was used as a control sample representing nonpigmented grain. The two red rice samples, brown rice, and purple wheat samples were tested as-is (raw grains) and also made into home-made and laboratory-made infant cereals. Pigmented infant cereals including whole purple wheat, unpolished red rice, and partially polished red rice were produced in the present study using two techniques simulating home (HM) and industrial (or laboratory) (LM) methods. The latter involved a long exposure to high heat treatment as well as enzymatic hydrolysis of starch.

Chemicals. Folin−Ciocalteu's phenol reagent, 2,2′-azobis(2-amidinopropane) dihydrochloride (APPH), and ferulic acid standard were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO). Fluorescein

(FL) and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Fisher Acros Organics (Morris Plains, NJ). α-Amylase enzyme extracted from *Bacillus subtilis* was obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO). 2′,7′-Dichlorofluorescin diacetate (DCFH-DA) was purchased from Invitrogen Canada, Inc. (Burlington, ON, Canada). The cyanidin 3-glucoside and peonidin 3-glucoside standards with purities of 99.9 and >97%, respectively, were purchased from Sigma-Aldrich (Oakville, ON, Canada). The apigenin standard was obtained from Chromadex (Irvine, CA) graded Primary Standards (P) for quantitative validation. The adjusted purity was 97.5%.

HPLC grade methanol was used in the extraction. Hydrochloric acid for extraction was purchased from Fisher Acros Organics. MS grade acetonitrile and formic acid were used in the LC-MS analysis. HPLC and MS grade solvents were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO).

Sample Preparation. Raw grains (brown rice, partially polished red rice (red rice 1), unpolished red rice (red rice 2), and purple wheat) were ground to pass through a screen of 0.42 mm prior to production of infant cereals. Water (355.5 mL) was added to 64.5 g of ground grain (±20% solid in water) prior to boiling for 10 min with constatnt stirring. The mixture was then cooled for 30 min. Infant cereals were then freeze-dried (VirTis, Genesis-Pilot Lyophilizer, SP Industries, Warminster, PA). The processing method for laboratory-made pigmented infant cereals was adapted from a patented method.[9] Ground grain samples (50 g) were placed in an air oven and toasted uncovered at 120 °C (DL-110A-2 Stabil-Therm Constant Temperature Cabinet, Blue M Electric Co., Blue Island, IL) for 30 min. The sample was then cooled for 30 min and dispersed in 250 mL of room temperature water (20% solid in water) with constant whisking. Powdered enzyme (0.02 g of α-amylase, *B. subtilis* (~50 units/mg with 1 unit of enzyme releasing 1 μmol of maltose per minute at pH 6.9 at 25 °C) was dispersed in 5 mL of water and added to the grain mixture to allow for enzymatic hydrolysis of starch in a 60 °C water bath for 90 min with stirring every 5 min. The mixture was boiled (90−100 °C) for 2 min with constant stirring. After cooling for 1 h, infant cereals were then lyophilized (VirTis, Genesis-Pilot Lyophilizer, SP Industries).

A sample mill (Black & Decker, Hunt Valley, MD) was used to grind the freeze-dried infant cereals to pass through a screen of 0.42 mm.

Moisture Content Determination. Ground, raw grains and infant cereals were dried overnight at 100 °C in an air-oven following AOAC method 930.15 (1990) for moisture determination. All samples were analyzed in triplicate.

Extraction. The extraction of antioxidant components in raw grains, infant cereals made from pigmented grains, and commercial infant cereals was adapted from methods described earlier.[10] Raw grains and freeze-dried infant cereals (1 g) ground to pass through a screen of 0.42 mm were extracted with 1 M HCl/methanol (v/v, 15:85) (10 mL). The mixture was sonicated (Bransonic 5510OR-DTH, Branson Ultrasonic Corp., Danbury, CT) for 45 min, shaken every 5 min, and centrifuged at 26920*g* (15000 rpm, SSA-34 Rotors, RC5C Sorvall Instruments, Thermo Scientific, Asheville, NC) at ambient temperature for 15 min. The supernatant was collected, and the centrifuge pellet was washed. The supernatants were stored at −20 °C for determination of total phenolic content (TPC), total anthocyanin content (TAC), and oxygen radical absorbance capacity (ORAC). Extraction for LC-MS analysis and cell culture assay followed the same procedure until this point. The supernatant was recentrifuged to remove precipitation. The supernatant was then rotary-evaporated at 35 °C for about an hour (IKA RV10, IKA Works, Inc., Wilmington, NC). Two milliliters of 80% methanol for LC-MS analysis and 2 mL of distilled and deionized water for cell culture assay were added to reconstitute the samples from the rotary evaporator followed by sonication, microcentrifugation at 12000 rpm for 5 min (IEC Micromax Microcentrifuge, Thermo Fisher Scientific Inc., Waltham, MA), and filtering with a 0.45 μm PTFE filter. Samples were then analyzed using LC-MS.

Chemical Model Assays. *Total Phenolic Content Determination.* The TPC of the extracts from infant cereals was measured using a modified method.[11] Folin−Ciocalteu phenol reagent was first diluted 10 times, and 200 μL of extract added to 1.5 mL of the diluted Folin−Ciocalteu phenol reagent. Sodium carbonate solution (60 g/L) (1.5 mL) was then added to the mixture. The reaction was allowed to take place at room temperature for 120 min. The absorbance of the solution was measured at 725 nm against a blank of distilled water (Ultrospec 1100 *pro*, Biochrom Ltd., Cambridge, U.K.). Ferulic acid was used as a standard, and the results are reported as micrograms of ferulic acid equivalents per gram. All analyses were conducted in triplicate.

Total Anthocyanin Content Determination. The TAC of the infant cereals was determined using a modified method.[12] Acidified methanol (1 M HCl/methanol, 15:85, v/v) (24 mL) was added to ground samples (commercial samples were used as-is) (3 g), and the mixture was shaken manually. The mixture was then adjusted to pH 1 using 6 N HCl. The sample was sonicated (Bransonic 5510OR-DTH, Branson Ultrasonic Corp.) for 15 min with shaking every 5 min. The sample was centrifuged at 26920*g* (15000 rpm, SSA-34 Rotors, RC%C Sorvall Instruments, Thermo Scientific, Asheville, NC) at ambient temperature for 15 min. The supernatant collected was made up to 50 mL with acidified methanol. Acidified methanol was used as the blank, and the absorbance was read at 535 nm. The absorbance read at 535 nm showed a linear relationship with concentration producing a standard curve of cyanidin 3-glucoside in acidified ethanol (Ultrospec 1100 *pro*, Biochrom Ltd.). Beer's law was used to calculate the molar absorptivity of cyanidin 3-glucoside. Calculation of total anthocyanin concentration was as

$$C = (A/\varepsilon) \times (\text{vol}/1000) \times \text{MW} \times (1/\text{sample wt}) \times 10^6$$

where C is the concentration of total anthocyanin (μg/g), A is the absorbance reading, ε is the molar absorptivity (cyanidin 3-glucoside = 25965 cm^{-1} M^{-1}), vol is the total volume of anthocyanin extract (50 mL), and MW is the molecular weight of cyanidin 3-glucoside (= 449). The calculation is simplified to $C = A \times 288.21$ μg/g. Analysis was conducted using four replicates.

Oxygen Radical Absorbance Capacity Determination. The ORAC values were obtained using a modified method.[13] The measurement was taken using an FLx800 microplate fluorescence reader (BioTek Instruments, Inc., Winooski, VT) with fluorescence filters at an excitation wavelength of 485/20 nm and an emission wavelength of 528/20 nm. The instrument was monitored by KC4 3.0 software, version 29. A dilution factor of 500 of the supernatant was used for the samples, rutin was used at 10 μM as a control, and Trolox standards were used at 0, 6.25, 12.5, and 50 μM. The remainder of the procedure was according to the method of Li et al.[10] The calculation of antioxidant capacity was based on the method used by Huang et al.[13] A regression equation between the Trolox concentration and the net area under the fluorescence decay curve was constructed. The formula to obtain the area under curve (AUC) was

$$\text{AUC} = 0.5 + \frac{f_1}{f_0} + \ldots + \frac{f_i}{f_0} + \ldots + \frac{f_{49}}{f_0} + 0.5\frac{f_{50}}{f_0}$$

where f_0 = initial fluorescence reading at 0 min and f_i = fluorescence reading at time i min. Net AUC = AUC(blank) − AUC(sample). The final ORAC values were expressed as Trolox equivalent and determined according to the standard curve. All analyses were conducted in triplicate.

HPLC-MS/MS Analysis. Cyanidin 3-glucoside, peonidin 3-glucoside, apigenin 6-*C*-glucoside-8-*C*-arabinoside, and cinnamic acid esters were separated, identified, and quantified in infant cereals by using an HPLC (Separation Module, Waters 2695) with a photodiode array detector (Waters 2996) and an autosampler (Waters 717plus). The HPLC was coupled to a quadrupole time-of-flight mass spectrometer

(Q-TOF MS) (Micromass, Waters Corp., Milford, MA). Analysis was performed with a Gemini 5 μ C18 110 Á column (150 mm × 4.60 mm) (Phenomenex, Torrance, CA). A gradient of solvent A (1% formic acid in water) and solvent C (1% formic acid in acetonitrile) was used for 50 min at a flow rate of 0.5 mL/min. The gradient was as follows: at 0 min, 5% C; 5 min, 10% C; 15 min, 15% C; 20 min, 20% C; 30 min, 25% C; 40 min, 40% C; 45−50 min, 10% C. The photodiode array detector was set at 280, 320, and 520 nm. The sample was then introduced into the Q-TOF MS. The Q-TOF MS was calibrated with sodium iodide in the negative mode, and full mass spectra were obtained with a capillary voltage of 1.8 kV and a cone voltage of 45 V. Desolvation gas (N_2) and cone gas (He) were used at flow rates of 900 and 50 L/h, respectively. The temperature settings for the desolvation gas and ion source were 350 and 150 °C, respectively. Identification of *C*-glycosyl flavones was achieved by using tandem mass spectrometric technique with a collision energy of 30 kV, and quantification was done using apigenin standard. The analyses were done in duplicate.

Cell Culture Assay. The present study used a primary human fetal small intestine cell line (FHs 74 Int, CCL-241, American Type Culture Collection (ATCC), Manassas, VA) to further investigate the in vitro intracellular antioxidant effects of cereal grain consumption. FHs 74 Int cells are an epithelial cell line obtained from the small intestine of a human fetus; thus, the genomic profile and antioxidant pathways are arguably a better model for this study than more commonly used adult colonyte cell lines such as Caco-2. The growth medium was 5 mL of Dulbecco's modified Eagle medium with D-glucose content (an energy source) (DMEM, Invitrogen Canada Inc., Burlington, ON, Canada) supplemented with 10% fetal bovine serum, 10 mg/mL penicillin/streptomycin, 200 mM L-glutamine, 1 mg/mL insulin, 20 mg/mL extra cellular growth factor, 2.5 nM sodium pyruvate, and 1 mg/mL human transferrin.

Cell culture medium was changed every 5 days. EDTA (0.02%) and trypsin (0.25%) (1000−2000 *N*-benzoyl-L-arginine ethyl ester (BAEE) units/mg, where 1 BAEE unit produces a ΔA_{253} of 0.001 per minute at pH 7.6 at 25 °C using BAEE as substrate, reaction volume = 3.2 mL, 1 cm light path) were used during the routine passage of the cell lines. Cells were incubated at 37 °C in a 5% CO_2 humidified incubator for optimal growth. EDTA and trypsin solutions were used during routine passage of the cell line to detach and resuspend cells from the cell culture flask surface. EDTA chelates calcium and prevents calcium-dependent adhesion between cells, and prevents clumps of cells in liquid suspension.

Cytotoxicity Assay. Home-made and laboratory-made whole purple wheat and unpolished red rice infant cereals and Stage 3 Heinz Wheat and Oat Cereal with Banana and Raspberry infant cereal (Heinz Canada, North York, ON, Canada) were tested for their cytotoxicity using the MTT cell proliferation assay (ATCC) according to a method by Ellis et al. with some modifications.[14] Infant cereal extracts (500 mg/mL) were further diluted with medium to concentrations of 2 and 0.5 mg/mL. An infant small intestine cell suspension (100 μL) was seeded in a 96-well plate and maintained until confluent (a concentration of 1 × 10^5/mL) for 72 h. The medium in all wells was aspirated out. Then 100 μL of 2 and 0.5 mg/mL sample medium extracts and 100 μL of fresh medium for control wells were added. Blank wells contained medium only. Cells were treated and left in the incubator for 4 h. MTT reagent (10 μL) was added to all wells including control wells (without removing treatment solutions and medium in order not to disrupt the cells) and left in the incubator for 3 h. Dots of purple precipitate were visible under the microscope, and 100 μL of detergent reagent was added to all wells including controls. The plate was swirled gently. The covered plate was then left in the dark overnight at room temperature. The plate cover was removed, and the plate was measured for absorbance at 590 nm using an Opsys MR 96-well plate reader (Dynex Technologies, Chantilly, VA). Cytotoxicity level (percent) was calculated by comparing absorbance to that of controls. Analysis was done in eight replicates.

Dichlorofluoresin Diacetate Assay. The infant cereals were then tested for their effects in cellular oxidative status using the cellular antioxidant activity (CAA) method with some modifications.[15] Sample extracts (500 mg/mL) were further diluted with medium to concentrations of 2 and 0.5 mg/mL. An infant small intestine cell suspension (100 μL) was seeded in a 96-well plate and maintained until confluent (a concentration of 1 × 10^5/mL) for 72 h. The medium in all wells was then aspirated, and 100 μL of 2 and 0.5 mg/mL sample medium extracts and 100 μL of fresh medium for control wells were added. Blank wells contained medium only. The cells were treated and left in the incubator for 1 h. At 30 min of incubation, 100 μL of 10 μM DCFH-DA solution was added to all wells including positive (control with AAPH oxidant added) and negative (control without AAPH oxidant added) controls. The final concentration of the DCFH-DA solution was then 5 μM. After 1 h of incubation, all 200 μL of treatment solutions was removed, and 100 μL of 500 μM AAPH dissolved in Hank's buffered salt solution (HBSS) was added to all wells except negative control wells (100 μL of HBSS was added instead). The plate was then immediately placed into a Fluoroskan Ascent FL 96-well plate reader (ThermoLabsystems, Franklin, MA). Temperature was set at 37 °C, emission wavelength at 527 nm, and excitation wavelength at 485 nm, and measurements were taken every 15 min for 1 h. Analysis was done in eight replicates. The CAA value was calculated by integrating the area under the curve of fluorescence versus time at each concentration (2 and 0.5 mg/mL) of infant cereal sample extracts as

$$\text{CAAvalue} = \{1 - (\int \text{SA} / \int \text{CA})\} \times 100$$

where SA is the area under sample fluorescence versus time curve and CA is the area under positive control fluorescence versus time curve.

Statistical Analysis. All data were converted to dry weight basis and reported as means of duplicate or triplicate analyses. One-way analysis of variance of results was performed using SAS statistical software version 9.2 (SAS Institute Inc., Cary, NC). Significant differences among sample means for raw grains and home-made, laboratory-made, and commercial infant cereals for each analysis were tested using Tukey's test at $p < 0.05$. Pearson correlation coefficients were calculated for TPC and ORAC.

RESULTS AND DISCUSSION

Pigmented Infant Cereal Production. Home-made (HM) infant cereals are often made by mothers keen to ascertain the ingredients fed to their infants. Laboratory-made (LM) formulations were taken to represent industrially manufactured infant cereals. Starch microscopy with bright field showed complete starch gelatinization, which meant the processing methods sufficiently cooked the cereals. Boiling at temperatures of 90−100 °C followed by freeze-drying deactivated the α-amylase.[16]

Total Phenolic Content, Total Anthocyanin Content, and Antioxidant Activity of Raw Grains and Infant Cereals. Table 2 shows the results obtained on TPC, TAC, and antioxidant activity of raw grains and infant cereals. TPC, expressed as micrograms of ferulic acid equivalent per gram of infant cereal, ranged from 532 to 8626 μg/g. Unpolished red rice grain and Beech-Nut Rice Cereal had the highest and lowest TPC, respectively. The average TPC values for raw grains and infant cereals were not significantly different; however, pigmented infant cereals had significantly higher TPC than commercial ones ($p < 0.05$). The highest TPC values were observed in unpolished red rice samples in the order raw grain > HM infant cereal > LM infant cereal. Heinz Stage 3 Wheat and Oat Cereal

Table 2. Total Phenolic Content (TPC), Total Anthocyanin Content (TAC), and Oxygen Radical Absorbance Capacity (ORAC) of Raw Grains and Home-Made (HM) and Laboratory-Made (LM) Pigmented Infant Cereals and Commercial Infant Cereals[a]

no.	sample	TPC (μg ferulic acid equiv/g)[a]	TAC (μg cyanidin 3-glucoside equiv/g)[a]	ORAC (μmol Trolox equiv/g)[a]
1	whole purple wheat grain	4469 ± 34.6 e	251 ± 0.83 a	43.3 ± 2.22 f
2	unpolished brown rice (control)	2079 ± 88.2 jk	nd	31.3 ± 0.44 hi
3	partially polished red rice grain	5232 ± 136 d	15 ± 0.18 h	67.1 ± 2.80 c
4	unpolished red rice grain	8626 ± 207 a	23 ± 0.59 e	87.1 ± 0.42 a
5	HM whole purple wheat	3076 ± 55.1 h	185 ± 0.49 b	48.2 ± 0.21 e
6	HM unpolished brown rice	1712 ± 64.5 l	nd	29.1 ± 0.22 i
7	HM partially polished red rice	3559 ± 62.5 f	9 ± 0.27 j	50.4 ± 2.26 de
8	HM unpolished red rice	6622 ± 145 b	20 ± 0 f	79.0 ± 0.66 b
9	LM whole purple wheat	3390 ± 49.2 fg	95 ± 0.43 c	51.6 ± 0.35 d
10	LM unpolished brown rice	2090 ± 52.7 jk	nd	37.4 ± 0.30 g
11	LM partially polished red rice	3352 ± 92.9 fg	11 ± 0.47 i	52.4 ± 1.13 d
12	LM unpolished red rice	6152 ± 98.7 c	22 ± 0.55 e	79.0 ± 1.07 b
13	brown rice cereal (Organics for Baby)	1972 ± 29.3 l	nd	18.7 ± 0.53 k
14	rice cereal (Beech-Nut)	532 ± 27.5 n	nd	5.9 ± 0.04 m
15	mixed grains cereal (Nestle)	2235 ± 57.7 j	39 ± 0.68 d	12.5 ± 0.17 l
16	rice cereal with fruits (PC Organics)	3265 ± 27.8 gh	10 ± 0.23 i	21.1 ± 0.19 jk
17	mixed cereals with fruits (Milupa)	2242 ± 15.3 j	17 ± 1.22 g	31.9 ± 0.44 hi
18	rice cereal (Heinz)	963 ± 34.1 m	nd	10.1 ± 0.12 l
19	mixed cereal (Heinz)	2060 ± 8.66 jk	4 ± 0.14 k	23.9 ± 1.19 j
20	wheat and oat cereal with fruits (Heinz)	4235 ± 45.8 e	39 ± 0 d	33.8 ± 0.48 h
21	multigrain cereal with fruits (Heinz)	2567 ± 47.3 i	2 ± 0.15 l	19.8 ± 0.49 k

[a] LSD, least significant difference at $p = 0.05$ level of probability; $n = 3$ (TPC), $n = 4$ (TAC), and $n = 3$ (ORAC). Sample means having similar letters are not significantly different. nd, not detected.

with Banana and Raspberry had the highest TPC among the commercial cereals.

TAC, expressed as micrograms of cyanidin 3-glucoside equivalent per gram (of infant cereal), ranged from 2 to 251 μg/g. Whole purple wheat grain had the highest TAC, whereas Heinz Stage 4 Multigrain Cereal with Mango, Pineapple, and Pear had the lowest TAC. Anthocyanins were not detected in brown rice, Organics for Baby Stage 1 Brown Rice Cereal, Beech-Nut Stage 1 Rice Cereal, or Heinz Stage 1 Rice Cereal. The average TAC for pigmented infant cereals was higher but not significantly different from that of commercial ones ($p < 0.05$). The highest TAC was observed in whole purple wheat samples in the order raw grain > HM infant cereal > LM infant cereal. Nestle Stage 2 Mixed Grains Cereal and Heinz Stage 3 Wheat and Oat Cereal with Banana and Raspberry had the highest TAC values among the commercial cereals.

ORAC, expressed as micromoles of Trolox equivalent (TE) per gram of infant cereal, ranged from 5.9 to 87.1 μmol TE/g. Unpolished red rice grain had the highest ORAC value, whereas Beech-Nut Rice Cereal had the lowest. The average ORAC values for infant cereals were significantly higher than those of commercial ones ($p < 0.05$). Heinz Stage 3 Wheat and Oat Cereal with Banana and Raspberry had the highest ORAC value among the commercial samples.

Among the raw grains, red rice showed the highest TPC and ORAC values, whereas brown rice had the lowest values. Pigmented infant cereals showed the same trend in their TPC and ORAC values in the order unpolished red rice > partially polished red rice > whole purple wheat > unpolished brown rice. One study reported TPC of unpolished red rice as 8353 μg FE/g,[17] in close agreement with our findings. TPC (7973 μg FE/g) and ORAC value (209.72 μmol TE/g) of acidified methanol extracts of purple wheat bran were previously reported.[18] The present study found 4469 μg FE/g and 43.3 μmol TE/g, respectively, for whole purple wheat, values that are lower due to dilution by the endosperm material. The TPC and ORAC values had a good correlation (Pearson correlation coefficient = 0.90). To the best of our knowledge, this is the first study comparing the total phenolic content and antioxidant activity of purple wheat versus red rice.

Unpolished red rice had significantly higher TAC than the partially polished one, an indication that anthocyanins are concentrated in the bran layers. TAC values of 93.5 for red rice and 211.9−235 μg cyanidin 3-glucoside equiv/g for blue and purple wheat[7,19] were reported. The present study found TAC values of 23 and 251 μg cyanidin 3-glucoside/g for unpolished red rice and whole purple wheat, respectively. The higher TPC and ORAC values observed in red rice compared to purple wheat are likely due to components other than anthocyanins. Red rice contained condensed tannins as biological antioxidants[8] and tocopherols, tocotrienols, and γ-oryzanol.[20]

Purple wheat bran baked at 177 °C for 20 min had high TPC and ORAC values;[18] however, the present study found decreased TPC and increased ORAC values for laboratory-made infant cereals subjected to toasting at 120 °C for 30 min. The overall TPC, TAC, and ORAC in raw grains and pigmented infant cereals were not significantly different ($p < 0.05$). Thus, high heat treatment and enzymatic hydrolysis did not significantly influence the TPC, TAC, and ORAC.

The average TPC and ORAC values in pigmented infant cereals were higher than those of the commercial ones ($p < 0.05$). However, they were not significantly different in their TAC ($p < 0.05$).

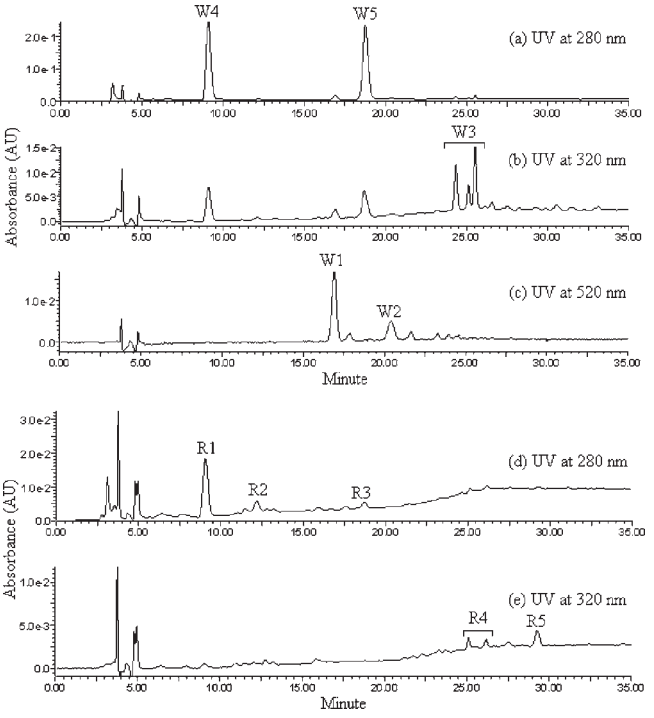

Figure 1. LC chromatograms (0−35 min) of acidified methanol extracts obtained at (a) 280 nm, (b) 320 nm, and (c) 520 nm in raw, whole purple wheat with its five major peaks (W1−W5), and at (d) 280 nm and (e) 320 nm in raw, unpolished red rice with its five major peaks (R1−R5).

Raspberry has a high anthocyanin content,[21] and its presence in Heinz Stage 3 Wheat and Oat Cereal increased the LSD value.

Pigmented infant cereals had significantly higher TPC, TAC, and ORAC than commercial infant cereals. Therefore, they can be considered as potential ingredients for producing infant cereals with higher antioxidant contents. According to the chemical model assays, unpolished red rice seemed to be a good candidate for producing a new infant cereal type because it had the highest TPC and ORAC and medium TAC. Heinz Wheat and Oat Cereal with Banana and Raspberry, a Stage 3 infant cereal, had the highest TPC, TAC, and ORAC among the commercial infant cereals, likely due to the raspberry and black raspberry puree contents. Red rice had significantly higher TPC and ORAC compared to purple wheat, whereas purple wheat had significantly higher TAC than red rice ($p < 0.05$). HM and LM infant cereals did not show significant differences in TPC, TAC, and ORAC ($p < 0.05$). Pigmented infant cereals had significantly higher TPC and ORAC than commercial infant cereals ($p < 0.05$).

HPLC-MS/MS Analysis of Major Components in Acidified Methanol Extracts of Purple Wheat and Red Rice Raw Grains and Infant Cereals. *Identification.* The LC chromatograms of the major peaks found in the acidified methanol extracts of whole purple wheat and unpolished red rice grains obtained at different wavelengths of 280, 320, and 520 nm are shown in Figure 1. There were five major peaks found, W1−W5 for purple wheat and R1−R5 for red rice. The same peaks were not found in the LC chromatogram of red rice sample at the 520 nm wavelength. Tentative MS and MS/MS identifications of these peaks are listed in Table 3 and discussed below.

Peak W1 of m/z 447 found at 16.79 min with the extraction ion chromatogram (EIC) and its MS/MS spectra shown in Figure 3 is identified as cyanidin 3-*O*-glucoside (molecular mass of 449) ($C_{21}H_{21}O_{11}^{+}$) as confirmed using the UV and MS/MS spectra of the kuromanin chloride standard shown in Figure 2. The standard, cyanidin 3-*O*-glucoside chloride ($C_{21}H_{21}ClO_{11}$) (485), had m/z 465 and eluted at 16.79 min. In both MS/MS spectra, the product ion m/z 285 showed the presence of cyanidin aglycone (287) ($C_{15}H_{11}O_6^{+}$). Also observed in the MS/MS spectrum of peak W1 was fragmentation of the hexose molecule (162) ($C_6H_{10}O_5^{-}$). Peak W2 of m/z 461 found at 20.70 min with the EIC and its MS/MS spectrum shown in Figure 4 suggested the presence of peonidin 3-glucoside (463) ($C_{22}H_{23}O_{11}^{+}$). Fragment ion m/z 299 indicated the presence of peonidin aglycone (301) ($C_{16}H_{13}O_6^{+}$) and fragmentation of the hexose molecule (162) ($C_6H_{10}O_5^{-}$). The UV chromatogram and MS/MS spectrum of the peonidin 3-glucoside standard are shown in Figure 3.

Peak W3 and R4 of m/z 563 found at 25.73 and 26.73 min in unpolished red rice and at 25.02, 25.76, and 26.14 min in whole purple wheat, with the EIC and MS/MS spectrum shown in Figure 4, indicated the presence of apigenin 6-*C*-glucoside-8-*C*-arabinoside (564 = 270 + 162 + 132). Fragment ions (A + 83) and (A + 113) at m/z 353 and 383 showed the presence of apigenin aglycone (270) because *C*-glycosyl bonds are rarely completely severed compared with *O*-glycosyl bonds, whereas $[M - H - 18]^{-}$ at m/z 545 suggested the loss of a water molecule.[19,22] The presence of $[M - H - 60]^{-}$, $[M - H - 90]^{-}$, and $[M - H - 120]^{-}$ at m/z 503, 473, and 443, respectively, suggests fragmentation of hexose (162) and pentose molecules (132) because this fragmentation pattern is likely due to cross-ring cleavages in their residues and also because *C*-glycosyl bonds have been found only at 6- and 8-positions.[19,22]

Peak W4 and R1 of m/z 515 found at 9.10 min in purple wheat and at 12.46 min in red rice was identified as dicaffeoyl quinic acid (516 = caffeic acid 180 + caffeic acid 180 + quinic acid 192 − water molecules 36) ($C_{25}H_{24}O_{12}$). The fragment ion $[M - H - 162]^{-}$ at m/z 353 indicated the release of one caffeic acid moiety.[23−25] The fragment ions found at m/z 191 and 179 suggested the presence of quinic acid (192) ($C_7H_{12}O_6$) and caffeic acid (180) ($C_9H_80_4$), respectively.[23,24,28,29] Peak W5 and R3 of m/z 311 found at 18.28 min in purple wheat and at 19.67 min in red rice suggested the presence of monocaffeoyl tartaric acid (312 = caffeic acid 180 + tartaric acid 150 − water 18) ($C_{13}H_{12}O_9$).[26] The fragment ion $[M - H - 162]^{-}$ at m/z 149 showed a caffeic acid (180) ($C_9H_80_4$) moiety and loss of tartaric acid (150) ($C_4H_6O_6$).[24,26] Peak R5 of m/z 311 found at 30.57 min suggested the presence of another isomer of monocaffeoyl tartaric acid. Peak R2 of m/z 333 found at 13.39 min indicated the presence of a vanillic acid dimer, which is dehydrodivanillic acid (334) (2,2′-dihydroxy-3,3′-dimethoxy-5,5′-dicarboxybiphenyl).[27] The fragment ion $[M - H - 162]^{-}$ at m/z 171 showed a vanillic acid moiety (168 + 3) ($C_8H_8O_4$).

Quantification. Dicaffeoylquinic acid, dehydrodivanillic acid, and monocaffeoyltartaric acid were not quantified due to unavailability of commercial standards. Anthocyanins were quantified at 520 nm, whereas apigenin *C*-glycosides (as apigenin equivalent) were calculated at 320 nm wavelength. Results are shown in Table 4. For purple wheat samples, cyanidin 3-glucoside and peonidin 3-glucoside ranged from 4.50 to 25.50 μg/g and from 1.34 to 1.95 μg/g, respectively. Apigenin 6-*C*-glucoside-8-*C*-arabinoside ranged from 17.4 to 21.9 μg/g and from 3.16 to 11.4 μg/g in purple wheat and red rice, respectively. Cyanidin 3-glucoside content was significantly higher in purple wheat grains compared to infant cereals. Purple wheat grain also

Table 3. MS/MS Identification of Five Major Peaks in Acidified Methanol Extracts of Raw Whole Purple Wheat and Unpolished Red Rice

no.	retention time (min)	MS, $[M-H]^-$	MS/MS fragment ions		tentative identification except for compounds indicated with*
			Whole Purple Wheat		
W1	16.79	447	$[M-H-162]^-$	285	cyanidin 3-glucoside*
W2	20.70	461	$[M-H-162]^-$	299	peonidin 3-glucoside*
W3	25.02	563	$[M-H-18]^-$	545	apigenin 6-*C*-glucoside-8-*C*-arabinoside
	25.76		$[M-H-60]^-$	503	
	26.14		$[M-H-90]^-$	473	
			$[M-H-120]^-$	443	
			(A + 113)	383	
			(A + 83)	353	dicaffeoylquinic acid
W4	9.10	515	$[M-H-162]^-$	353	
				317	
				191	
				179	
				111	
W5	18.28	311	$[M-H-162]^-$	149	monocaffeoyltartaric acid
			Unpolished Red Rice		
R1	12.46	515	$[M-H-162]^-$	353	dicaffeoylquinic acid
				317	
				191	
				179	
R2	13.39	334	$[M-H-162]^-$	171	dehydrodivanillic acid
R3	19.67	311	$[M-H-162]^-$	149	monocaffeoyltartaric acid (isomer 1)
R4	25.73	563	$[M-H-18]^-$	545	apigenin 6-*C*-glucoside-8-*C*-arabinoside
	26.73		$[M-H-60]^-$	503	
			$[M-H-90]^-$	473	
			$[M-H-120]^-$	443	
			(A + 113)	383	
			(A + 83)	353	
R5	30.57	311	$[M-H-162]^-$	149	monocaffeoyltartaric acid (isomer 2)

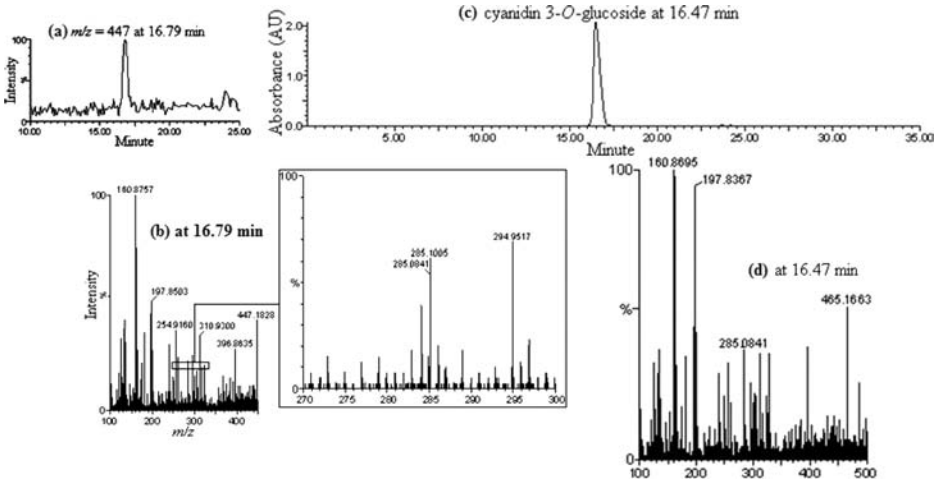

Figure 2. Extracted ion chromatogram (EIC) of peak W1 with m/z 447 (a) with its MS/MS spectrum (b) in whole purple wheat; LC chromatogram of cyanidin 3-*O*-glucoside standard showing peak at 16.47 min and 520 nm (c) and MS/MS spectrum of the standard peak with m/z 465 (d).

had significantly higher levels of peonidin 3-glucoside and apigenin 6-*C*-glucoside-8-*C*-arabinoside than HM infant cereal ($p < 0.05$); however, the two compounds were not found in LM infant cereal. The average apigenin *C*-glycoside content was

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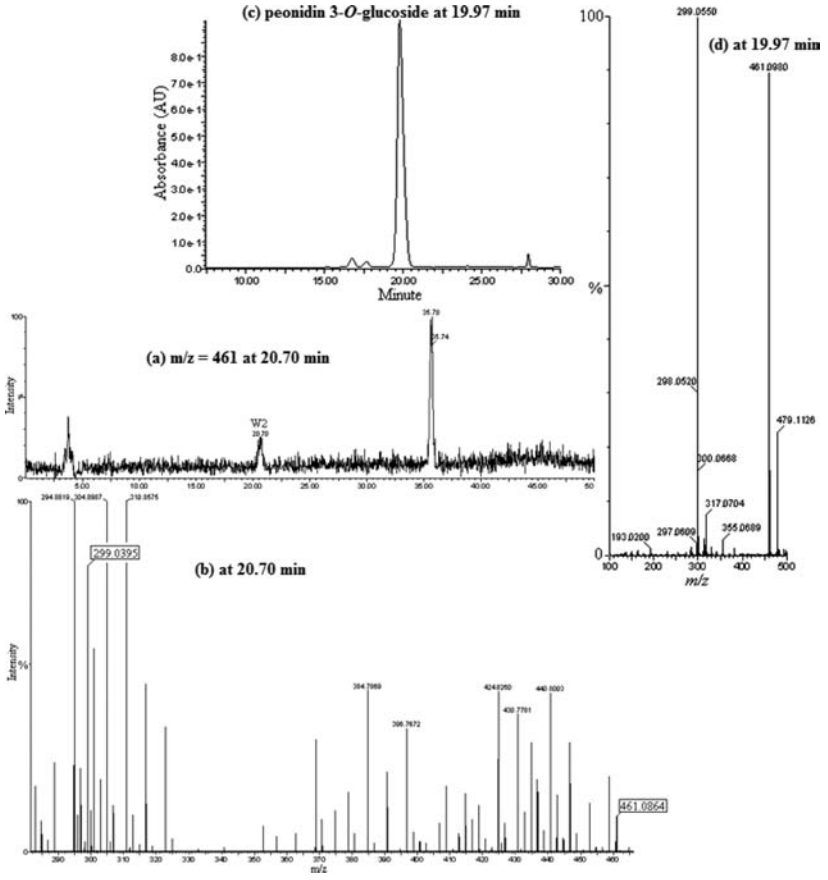

Figure 3. Extracted ion chromatogram (EIC) of peak W2 with m/z 461 (a) and its MS/MS spectrum (b) in whole purple wheat grain; LC chromatogram of peonidin 3-*O*-glucoside standard peak at 19.97 min at 520 nm (c) and MS/MS spectrum of the standard peak with m/z 461 (d).

significantly higher in purple wheat (19.5 μg/g) compared to red rice (7.43 μg/g). HM infant cereals had significantly higher cyanidin 3-glucoside, peonidin 3-glucoside, and apigenin 6-*C*-glucoside-8-*C*-arabinoside contents than laboratory-made ones ($p < 0.05$).

Cyanidin 3-glucoside and peonidin 3-glucoside were found in Charcoal purple wheat, whereas red, yellow, and white wheat grains did not contain any anthocyanins.[19] In another study, cyanidin 3-glucoside and peonidin 3-glucoside were also found in purple wheat and red rice.[7] Cyanidin 3-glucoside contents of 14.0, 20.3, and 4.0 μg/g in red rice and blue and purple wheat, respectively, were previously reported.[7] Whereas no anthocyanins were detected in unpolished red rice grain, the TAC of purple wheat in the present study and that of blue wheat in a previous study were comparable.[7]

Apigenin 6-*C*-glucoside-8-*C*-arabinoside was found in regular and whole-wheat samples as well as in other cereal grains including Charcoal purple wheat[19] and common bread wheat.[30] This is the first time that this compound has been quantified in purple wheat and red rice grains and infant cereals. Apigenin 6-*C*-glucoside-8-*C*-arabinoside and its isomer apigenin 6-*C*-arabinoside-8-*C*-glucoside were identified and quantified as the major yellow color components with concentrations of 8.7 and 10.1 mg/g naringin equivalent in an Australian common bread wheat germ.[30] Apigenin 6-*C*-glucoside-8-*C*-arabinoside was likely not destroyed during processing including high-temperature treatments because whole purple wheat showed similar contents with its infant cereals. Approximately 3 times higher contents of apigenin 6-*C*-glucoside-8-*C*-arabinoside were found in purple wheat compared with red rice. As far as we know, apigenin 6-*C*-glucoside-8-*C*-arabinoside has not been previously reported in red rice grain.

The decreases in cyanidin 3-glucoside content were 5 and 82% during the processing of whole purple wheat to HM and LM infant cereals, respectively, whereas decreases of 31 and 100% were found in peonidin 3-glucoside content. Similarly, decreases of 12 and 21% were found in the apigenin 6-*C*-glucoside-8-*C*-arabinoside content. In red rice, the apigenin 6-*C*-glucoside-8-*C*-arabinoside content decreased by 32 and 72%. Processing did not affect TPC, TAC, and ORAC significantly; however, individual content of cyanidin 3-glucoside, peonidin 3-glucoside, or apigenin 6-*C*-glucoside-8-*C*-arabinoside was affected significantly. Higher and longer exposure temperature treatment in the processing of LM infant cereal likely contributed to the higher losses of cyanidin 3-glucoside and apigenin 6-*C*-glucoside-8-*C*-arabinoside and complete loss of peonidin 3-glucoside contents compared with the home-made ones. Cooking using a pressure cooker, rice cooker, and gas range was found to thermally degrade cyanidin 3-glucoside; however, as a result of the same thermal treatment, protocatTheechuic acid was produced.[31] The level of this particular phenolic acid was found to be 2.7−3.4 times higher after cooking.[31] A study found that the consumption of 0.3 g of red rice powder/g of rabbit body weight in one diet that lasted for 10 weeks resulted in inhibition against atherosclerotic plaque formation and increased antioxidant status,[32] whereas another study reported that the consumption of 0.5 g of cooked red rice or 0.3 g of uncooked red rice/g of rat body weight

in one diet maintained for 6 days protected against Fenton reaction-based renal lipid peroxidation.[6] A normal person of 60 kg would eat 1 cup of rice at approximately 185 g of red rice in one Asian-style diet, and this would equal 0.003 g of red rice/g of body weight. To the best of our knowledge, there has not been a study on the effects of consumption of red rice on human health. Pro-oxidant factors were used in animal diets and thus required higher levels of consumption of red rice than in a normal diet. Incorporation of red rice bran powder in a diet may be useful to concentrate the above identified phytochemicals.

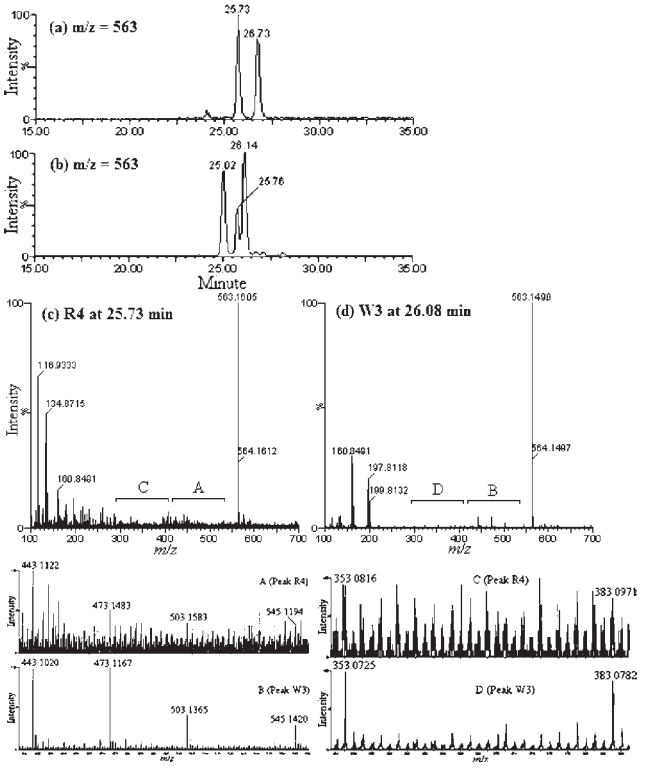

Figure 4. EIC of peaks R4 (a) and W3 (b) with m/z 563 and their retention times in unpolished red rice and whole purple wheat, respectively; MS/MS spectra of peak R4 (c) and W3 (d) with m/z 563 at their retention times in unpolished red rice and whole purple wheat, respectively (top). The bottom figures are highlights of areas in the spectra indicated above (A–D) showing fragment ions of peaks R4 and W3.

Cell Culture Assay of Home-Made and Laboratory-Made Whole Purple Wheat, Unpolished Red Rice, and Commercial Infant Cereal. *Cytotoxicity Assay.* The MTT cell proliferation assay (ATCC) was used to evaluate the cytotoxicity of HM, LM, whole purple wheat, unpolished red rice, and commercial Heinz Stage 3 infant cereals. The cytotoxicity assay was performed to confirm that the sample treatments of pigmented cereal grains had minimal negative effect on the viability of the infant small intestine cells. Sample treatments were compared with the cytotoxicity of commercial samples, and the cytotoxicity levels of commercial samples were used as a threshold as these commercial samples are consumed regularly by the general public. There was no nutrient impact described by the cytotoxicity assay; however, the DCFH-DA assay further explored the antioxidant activity of the sample treatments. Figure 5 shows the cytotoxicity of the sample treatments with different concentrations (2 and 0.5 mg/mL) represented by cell viability compared to the untreated control (control having 100% viability). The cell viability of sample-treated cells ranged from 71 to 91%, and the

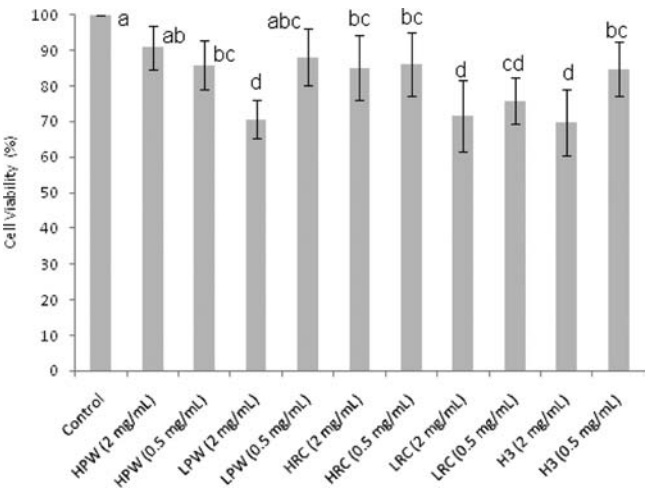

Figure 5. Cytotoxicity of home-made (HPW) and laboratory-made whole purple wheat (LPW), home-made (HRC) and laboratory-made unpolished red rice (LRC) infant cereals, and commercial sample of Heinz Stage 3 (Wheat and Oat Cereal with Banana Raspberry) at concentrations of 2 and 0.5 mg/mL in confluent FHs 74 Int (normal fetal small intestine cell line). Control was untreated cells at 100% cell viability. Data represent the mean ± SD, n = 8. Different letters (a–d) indicate significant differences.

Table 4. HPLC Quantification of Major Peaks in Acidified Methanol Extracts of Whole Purple Wheat Grain and Unpolished Red Rice Uncooked Grains and Home-Made (HM) and Laboratory-Made (LM) Infant Cereals

		concentration (μg/g)		
no.	sample	cyanidin 3-glucoside	peonidin 3-glucoside	apigenin 6-*C*-glucoside-8-*C*-arabinoside[a]
		Whole Purple Wheat		
1	uncooked grain	25.5 ± 0.74 a	1.95 ± 0.04 a	21.9 ± 0.07 a
2	HM infant cereal	18.6 ± 0.29 b	1.34 ± 0.09 b	19.2 ± 0.28 b
3	LM infant cereal	4.50 ± 0.00 c		17.4 ± 0.04 c
		Unpolished Red Rice		
4	uncooked grain			11.4 ± 0.27 a
5	HM infant cereal			7.74 ± 0.46 b
6	LM infant cereal			3.16 ± 0.09 c

[a] Concentration of apigenin 6-*C*-glucoside-8-*C*-arabinoside as μg/g apigenin equivalent.

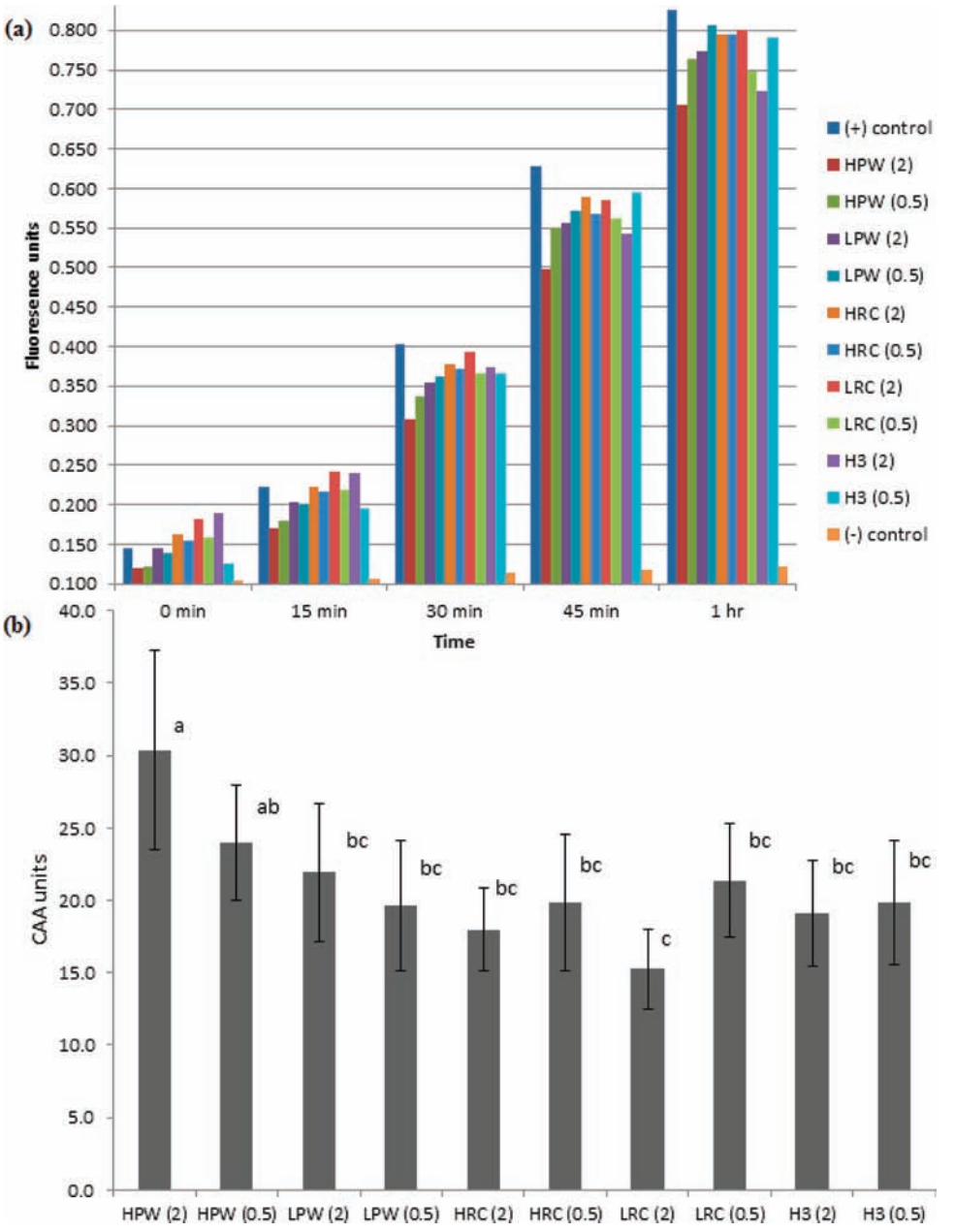

Figure 6. (a) AAPH-induced free radical oxidation of DCFH to DCF represented in fluorescence units by (+) control over time. (−) Control shows cell conditions without addition of AAPH over time. Treatment using home-made (HPW) and laboratory-made whole purple wheat (LPW), home-made (HRC) and laboratory-made unpolished red rice (LRC), and commercial Heinz Stage 3 (H3) infant cereals at two different concentrations, 2 (2) and 0.5 mg/mL (0.5), shows fluorescence intensity values between the control values. (b) CAA values of HPW, LPW, HRC, LRC, and H3 infant cereals at different concentrations, 2 (2) and 0.5 mg/mL (0.5) (mean ± SD, $n = 8$). Different letters indicate significant differences among raw grains and infant cereals ($p < 0.05$).

viability of those treated with commercial samples was 70% (2 mg/mL) and 85% (0.5 mg/mL). Because the HM and LM infant cereals behaved similarly or better than the commercial samples, pigmented infant cereals were considered to pose no toxicity against the fetal small intestine cell lines ($p < 0.05$).

Dichlorofluorescin Diacetate Assay. The reaction of the sample-treated, normal fetal small intestine cell lines against AAPH-induced free radical oxidation is shown in Figure 6. AAPH induced the oxidation of the dye DCFH to DCF. The fluorescence intensity of DCF was measured to represent the rate of oxidation. Positive (+) controls showed the fluorescence intensity of the AAPH oxidation over time. Negative (−) controls were cells to which AAPH was not added. They were used to illustrate conditions devoid of any oxidation inducer. Treatments using home-made whole purple wheat (HPW), laboratory-made whole purple wheat (LPW), home-made unpolished red rice (HRC), laboratory-made unpolished red rice (LRC), and commercial Heinz Stage 3 (H3) infant cereals at two different concentrations, 2 (2) and 0.5 mg/mL (0.5), showed inhibition of oxidation as their fluorescence intensities were higher than the negative control; however, they were lower than the positive control over time.

CAA was then calculated on the basis of the fluorescence intensity of infant cereals and AAPH-treated cells (Figure 6). The CAA ranged from 15.3 to 30.4 CAA units ($p < 0.05$). HM whole purple wheat infant cereal at 2 and 0.5 mg/mL showed the highest antioxidant activity, whereas LM unpolished red rice cereal gave the lowest CAA at 2 mg/mL. Other pigmented infant cereals at 0.5 mg/mL and commercial Heinz Stage 3 at both concentrations did not differ significantly in their CAA. Thus, HM purple wheat had significantly higher CAA than LM red rice and commercial infant cereals ($p < 0.05$). Longer exposure to higher temperature for LM and commercial infant cereals might contribute to loss of cellular-effective antioxidants. On the basis of the current studies using in vitro techniques, homemade whole-grain pigmented infant cereals had higher antioxidant activities compared to commercial infant cereals.

■ ASSOCIATED CONTENT

Supporting Information. Home and industrial simulations of infant cereal production were applied to pigmented grains to obtain purple wheat products with higher antioxidant activity than the commercial infant cereals. This material is available free of charge via the Internet at http://pubs.acs.org.

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