Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 109 (2008) 916–924
FOOD CHEMISTRY
www.elsevier.com/locate/foodchem

Analytical Methods

Measurement of anthocyanins and other phytochemicals in purple wheat

Farah S. Hosseinian [a], Wende Li [a], Trust Beta [a,b,*]

[a] Department of Food Science, University of Manitoba, Winnipeg, Manitoba, Canada R3T 2N2
[b] Richardson Centre for Functional Foods and Nutraceuticals, Smartpark, University of Manitoba, Winnipeg, Manitoba, Canada R3T 2N2

Received 19 November 2007; received in revised form 6 December 2007; accepted 13 December 2007

Abstract

The major anthocyanin composition of normal purple wheat and heat stressed purple wheat were measured using HPLC, LC–MS/MS and the pH differential method. The lignan secoisolariciresinol diglucoside (SDG) and melatonin content were also measured. Total anthocyanin profile of normal purple wheat (491.3 mg/kg) was significantly ($P < 0.05$) lower than that of the heat stressed purple wheat (522.7 mg/kg). Thirteen major anthocyanins were isolated and cyanidin 3-glucoside was the predominant anthocyanin in purple wheat. Using the pH differential method, the total anthocyanin content of normal (500.6 mg/kg) and heat stressed (526.0 mg/kg) purple wheat were similar to those observed using HPLC. The SDG content of normal and heat stressed purple wheat were 770 and 520 μg/kg, while melatonin content was 4 and 2 μg/kg, respectively. The presence of SDG and melatonin in addition to anthocyanins may contribute to the health benefits associated with consumption of coloured cereal grains.

Keywords: Purple wheat; Phytochemicals; Anthocyanins; Secoisolariciresinol diglucoside; SDG; Melatonin; HPLC; LC–MS/MS

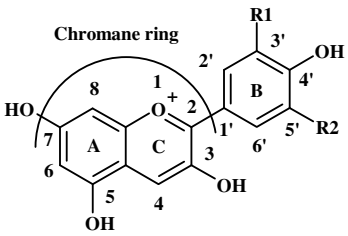
1. Introduction

Anthocyanins have been shown to have some beneficial health effects on oxidative damage, detoxification enzymes, and the immune system (Manach, Mazur, & Scalbert, 2005; Prior & Wu, 2006). They have anti-platelet aggregation and anti-inflammatory properties (Manach et al., 2005). Anthocyanins not only scavenge free radicals, but they also have the ability to bind heavy metals such as iron, zinc, and copper (Li, Shan, Sun, Corke, & Beta, 2005; Prior & Wu, 2006). Also they are an inducer of antioxidant enzymes such as glutathione-*S*-transferase (GST) and superoxide dismutase (SOD) (Duthie et al., 2006; Manach et al., 2005). Anthocyanins appear to have a synergist effect on vitamin C and other flavonoids (Duthie et al., 2006; Manach et al., 2005).

Anthocyanins are the largest group of water soluble natural pigments that give red, violet, and blue colour to many fruits, vegetables, and cereal grains (Choia, Jeonga, & Lee, 2007; Hosseinian & Beta, 2007; Prior & Wu, 2006). Anthocyanins are found in plants in glycosylated forms, generally linked with glucose, galactose, arabinose, rhamnose, xylose, and fructose (Choia et al., 2007; Hosseinian & Beta, 2007; Mazza, Cacace, & Kay, 2004). Cyanidin is the most common anthocyanidin (aglycone) followed by delphinidin, peonidin, pelargonidin, petunidin and malvidin (Oomah & Mazza, 1999). Delphinidin is known to be responsible for the bluish colour, whereas cyanidin and pelargonidin are responsible for purple and red colours in plants (Abdel-Aal el & Hucl, 2003; Lee, Durst, & Wrolstad, 2005; Mazza et al., 2004). The basic structure of anthocyanins is shown in Fig. 1, that includes C-6 (A-ring), C-3 (C-ring), C-6 (B-ring). The A and C rings form the

* Corresponding author. Address: Department of Food Science, University of Manitoba, Winnipeg, Manitoba, Canada R3T 2N2. Tel.: +1 204 474 8214; fax: +1 204 474 7630.
E-mail address: Trust_Beta@umanitoba.ca (T. Beta).

0308-8146/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2007.12.083



Aglycone	R1	R2	colour	λ_{max} (nm)
Cyanidin (Cy)	OH	H	Red	535
Peonidin (Pn)	OCH3	H	Bluish-purple	532
Pelargonidin (Pg)	H	H	Orange-red	520
Malvidin (Mv)	OCH3	OCH3	Purple	542
Delphinidin (Dp)	OH	OH	Purple	546
Petunidin (Pt)	OCH3	OH	Purple	543

Fig. 1. Common anthocyanin structure and corresponding anthocyanidins (aglycones).

chromane ring which is an additional contribution to the aromaticity of the compound (Prior & Wu, 2006).

Secoisolariciresinol diglucoside (SDG) is a lignan (diphenolic) that can be found in oilseeds, cereals grains, fruits, and certain vegetables. SDG (Fig. 2) has been proven to have antioxidant and chemopreventive properties. Flaxseed is the richest source of SDG (7–24 mg/g), which contains ~700–800 times more SDG than any other food (Hosseinian, Muir, Westcott, & Krol, 2007).

Tryptophan serves as the precursor for the synthesis of serotonin (Karasek, 2004). Serotonin is a chemical that helps to keep our moods under control by helping with sleep, calming anxiety, and relieving depression (Karasek, 2004). The mammalian pineal hormone melatonin (*N*-acetyl-5-methoxytryptamine), an aminoindole is produced as a result of serotonin metabolism (5-hydroxytryptamine) (Karasek, 2004). Seratonin and melatonin (Fig. 2) have been shown to have antioxidant, antiaging, and antidepressent properties (Karasek, 2004; Kedziora-Kornatowska et al., 2007). They behave as neuromodulators that play an important role in communication within the nervous system (Karasek, 2004).

The effects of environmental stress such as drought, heat, solar radiation, and salt can affect the quality of crops including wheat (Acevedo, Silva, & Silva, 2002; Mpofu, Sapirstein, & Beta, 2006; Oncel, Keles, & Ustun, 2000). Water stress is of common and wide occurrence in nature and is inevitably related to grain yield (Majoul, Bancel, Triboi, Ben Hamida, & Branlard, 2003). Environmental stress likely affects the content of anthocyanin and other phytochemicals in coloured wheats. The major objective of this study was to measure anthocyanin composition of purple wheat using HPLC, UPLC–MS/MS and the pH differential method. The lignan SDG, and melatonin content of these two samples were also measured.

2. Materials and methods

Two purple wheat samples of the same variety but grown in different years were kindly provided from Infra-Ready Products Ltd. (Saskatoon, SK, Canada) and were used in this study. The dry conditions that prevailed in 2005 likely led to a darker appearance to the heat stressed purple wheat compared to the normal purple wheat harvested in 2006. The purple wheat samples were ground using the sample mill (Krups Type 202, Germany) to pass through a 0.5 mm screen. The analyses were conducted on the ground samples. The data were expressed on dry weight basis.

2.1. Chemicals

The solvents: methanol, acetonitrile and formic acid were HPLC grade (Fisher Scientific Co., Ottawa, ON).

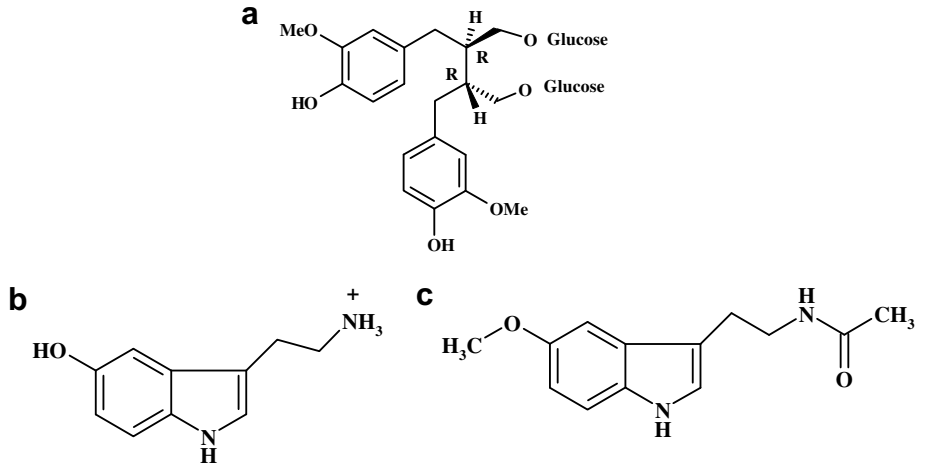


Fig. 2. Structures of (a) SDG ($C_{32}H_{46}O_{16}$, MW = 686.3), (b) serotonin ($C_{10}H_{12}N_2O$, MW = 176.2) and (c) melatonin ($C_{13}H_{16}N_2O_2$, MW = 232.2).

The standards, cyanidin 3-glucoside (Cy-3-glc), cyanidin 3-galactoside (Cy-3-gal), malvidin 3-glucoside (Mv-3-glc), pelargonidin 3-glucoside (Pg-3-glc), peonidin 3-glucoside (Pn-3-glc) and cyanidin chloride (Cy-cl) were purchased from Polyphenols Laboratories AS (Sandnes, Norway). The standards were dissolved in acidified MeOH (1 N HCl, 85:15, v/v) to obtain concentrations of 1 mg/mL each. SDG was purchased from ChromaDex™, Inc. (Santa Ana, CA). Melatonin and serotonin were purchased from Sigma (St. Louis, MO, USA). SDG, melatonin and seratonin standards were dissolved in MeOH to obtain concentrations of 1 mg/mL each.

2.2. Extraction and purification

The extraction and purification of anthocyanins were accomplished according to a modification of the methods reported in the literature (Mazza et al., 2004; Naczk & Shahidi, 2006). Briefly, methanol acidified with HCl (1 N) (ratio 85:15, v/v) was added to ground purple wheat (sample to solvent ratio of 1:8) and the pH adjusted to 1.0. After shaking at 1800 rpm for 45 min, the supernatant was separated from the pellet by centrifuging at 5000*g*. The supernatant was evaporated to dryness at 40 °C and reconstituted in methanol (5 mL). The extraction apparatus included a shaker (G-25, Eberbach Corporation, Ann Arbor, MI, USA), centrifuge (SLA-3000) with Sorvall GS-3 rotor (Sorvall® Instruments, ON, Canada), and rotary evaporator (Yamato RE-51, Cole-Parmer Instrument Company, IL, USA) with a water bath (Thermo-Lift, Fisher Scientific, NJ, USA). To determine recovery of anthocyanins, purple wheat samples were also spiked with standards prior to extraction. The reconstituted extract was filtered through a 0.45 μm syringe filter prior to HPLC analysis.

2.3. HPLC analysis

The HPLC method for anthocyanin measurements was based on modification of methods reported earlier (Mazza et al., 2004; Naczk & Shahidi, 2006). Analyses were conducted on an HPLC (Waters 2695) system equipped with a photodiode array detector (PDA) (Waters 996), Empower software, and autosampler (Waters 717 plus) (Waters Corp., Milford, MA, USA). The separation of anthocyanins was initially done at three different temperature (23, 35 and 40 °C) on a Luna 3u C18 column (150 × 3 mm i.d., 3 μm) to which a guard column (Phenomenex, Torrance, CA) was attached. An optimum temperature of 35 °C was selected. Mobile phases were A, 4.5% formic acid in double deionized water and B, 100% methanol. The gradient conditions were as follows: solvent B: 0 min, 10%; 30 min, 25%; 40 min, 45%; 42 min, 90%; 45–50 min, 10%. Spectral data (254–600 nm) was collected for all samples. Elution of compounds of interest was monitored at wavelength 520 nm for anthocyanins, at 280 nm and 320 nm for other phenolics. Other chromatographic conditions were as follows: flow rate, 0.4 mL/min; injection volume, 10 μL and run time, 50 min. The separation was performed on a C18 column (150 × 3 mm i.d. 5 μm) (Waters Corp., Milford, MA, USA). Peak identification of each anthocyanin was based on comparison of relative retention time (RT), percentage peak area, and spectral data with those of anthocyanin standards. For SDG, melatonin and serotonin, the mobile phases were A, 0.1% acetic acid in double deionized water and B, 0.1% acetic acid in acetonitrile. The gradient conditions were as follows: solvent B: 0 min, 10%; 5 min, 10%; 10–40 min, 40%; 41–50 min, 10%. Other chromatographic conditions were as follows: flow rate, 0.5 mL/min; injection volume, 10 μL and run time, 30 min. Spectral data was collected at 280 nm and also (254–600 nm) for all samples. Under the current chromatographic conditions, the limit of detection (LOD) and limit of quantification (LOQ) were determined to be 100 ng/mg (S/N > 5) and 200 ng/mg (S/N > 10), respectively.

2.4. Ultra performance liquid chromatography/tandem mass spectrometry (UPLC/MS/MS)

Liquid chromatographic separation was performed on an ACQUITY™ UPLC system (Waters Corp, Milford, MA, USA) with MassLynx™ Software and a PDA detector set at 520 nm for anthocyanins and at 280 nm and 320 nm for other phenolics. The ACQUITY™ UPLC BEH C18 column 1.0 × 100 mm, 1.7 μm was used for separation of anthocyanins (0.2 ml/min). Samples (5 μL) were injected into the liquid chromatograph. Prior to mass spectrometric (MS) analysis, a binary mobile phase consisting of 0.1% formic acid (A) and 100% methanol (B) was used under the following gradient conditions: 0.0 min, 13% B; 8.0 min, 24% B; 22 min, 100% B and at 24.0 min until the end of 26 min run, 13% B, for re-equilibration of the column before the next injection. The eluting stream from the UPLC was introduced into a Waters Quatro Micro™ atmospheric pressure ionization (API) mass spectrometer (MassLynx™ Software, Waters Corp, Milford, MA, USA) equipped with an electrospray ionization (ESI) multi-mode ionization probe (ESI APCI). The MS parameters were optimized using the anthocyanins standards. All spectra were obtained in both positive and negative mode ESI and the scan was set at *m*/*z* 100-1900. MS parameters were as follows: capillary voltage: 3 kV, cone voltage: 30 V, extractor voltage: 3.3 V, source temperature: 100 °C, desolvation temperature: 210 °C, cone gas flow: 50 L/h, desolvation gas flow: 600 L/h. Nitrogen gas was used for desolvation and cone.

2.5. Total anthocyanins measurement using pH differential method

Total anthocyanins were measured according to a modification of the methods described earlier (Fuleki & Francis, 1968); (Lee et al., 2005). Two dilutions of the sample

were prepared, one for pH 1.0 using potassium chloride buffer (0.03 M, 1.9 g KCl into 980 mL distilled water) and the other for pH 4.5 using sodium acetate buffer (0.4 M, 54.4 g $CH_3CO_2Na \cdot 3H_2O$ in 960 mL distilled water). Samples were diluted 10 times to a final volume of 2 mL. The absorbance of each sample was measured at 520 nm against distilled water as blank. The samples had no haze or sediment and thus correction at 700 nm was omitted. The concentration (mg/L) of each anthocyanin was calculated according to the following formula and expressed as Cy-3-glc equivalents:

$$\frac{A \times \mathrm{MW} \times \mathrm{DF} \times 10^3}{\varepsilon \times 1}$$

where A is the absorbance $= (A_{\lambda \text{vis-max}})_{\text{pH 1.0}} - (A_{\lambda \text{vis-max}})_{\text{pH 4.5}}$, MW is the molecular weight (g/mol) = 449.2 g/mol for Cy-3-glc, DF is the dilution factor (0.2 ml sample is diluted to 2 ml, DF = 10), and ε is the extinction coefficient ($L \times \mathrm{cm}^{-1} \times \mathrm{mol}^{-1}$) = 26,900 for Cy-3-glc, where L (pathlength in cm) = 1. For comparison, the same extinction coefficient was used for other standards to calculate the concentration of each anthocyanin and thus results reported is expressed as Cy-3-glc equivalents.

2.6. Statistical analysis

Samples were analysed in triplicate and one way analysis of variance performed using SAS version 9.1. Significant differences were detected at $P < 0.05$.

3. Results and discussion

3.1. Anthocyanins composition using HPLC and UPLC–MS/MS

Interest in the anthocyanin content of coloured cereals such as purple wheat has increased because of their potential as nutraceutical ingredients and functional foods (Choia et al., 2007). Anthocyanins can play a role as antioxidants and provide other health benefits (Manach, Scalbert, Morand, Remesy, & Jimenez, 2004; Prior & Wu, 2006). Anthocyanins are also regarded as safe and effective food colourants (Manach et al., 2004).

An advantage of the present study was that the quantification of the anthocyanins was performed by HPLC using anthocyanin references (Fig. 3). The quantification is therefore based on different references and not by one single reference such as cyanidin-3-*O*-glucoside. However, some peaks were still unlabeled due to unavailability of all standards. We selected nine major anthocyanin standards to measure key anthocyanins in the purple wheat. Fewer numbers of standards were reported in previous studies (Kay, Mazza, Holub, & Wang, 2004; Naczk & Shahidi, 2006); however, a reasonable number of references were selected for this study to identify individual anthocyanins present in purple wheat samples. Some peaks were not identified using HPLC due to lack of corresponding anthocyanin standards. The non-identified peaks were considered as anthocyanins using mass spectrometry and their UV absorption at 520 nm. Major anthocyanins in purple wheat and their m/z values are shown in Table 1. The total anthocyanins reported include both identified and non-identified HPLC peaks (Table 2).

Whereas organic solvents such as methanol and acetone are usually used for extraction of anthocyanins, the acidified MeOH showed the highest extraction efficiency (70–100%) (Kay et al., 2004; Mazza et al., 2004; Naczk & Shahidi, 2006). To determine recovery of anthocyanins, purple wheat samples were also spiked with standards prior to extraction. The extraction efficiency ranged from 80 to 100% for the anthocyanins, which is within the range previously reported (Kay et al., 2004; Naczk & Shahidi, 2006). In the calculation of the final results, these recoveries were not taken into account since the identities of some peaks were unknown. Although measuring the recovery% is useful to validate the method for extraction, most studies use only statistical analysis to show the reproducibility of the extraction method (Seeram & Nair, 2002). In this study, the coefficient of variation (CV) for the repeatability of the HPLC injections was less than 10% (Table 2).

Dp-3-glc showed the shortest RT (13.7 min) while Cy-cl showed the longest RT (33.1 min) (Fig. 3). The UV profile at 520 nm was used to distinguish anthocyanins from other phenolics (280 nm). All mass spectra data indicated anthocyanins were present as their glycoside forms (Table 1). No aglycones were observed in the wheat samples. Thirteen major anthocyanins were isolated from these samples and Cy-3-glc was the predominant anthocyanin in both samples (Table 2 and Fig. 3). Total anthocyanin profile of heat stressed purple wheat (522.7 mg/kg) were significantly ($P < 0.05$) higher than the normal purple wheat (491.3 mg/kg) (Table 2). Thus a ~7% increase in the anthocyanin content of heat stressed purple wheat was observed in comparison with the normal purple wheat sample. There were also some unidentified peaks (~29–44%) observed for both normal and heat stressed purple wheat that showed UV absorption at 520 nm (Table 2). Recently, a study on purple corncob anthocyanins showed that the anthocyanins could be acylated accounting for 35.6–54.0% of total anthocyanins (Jing, Noriega, Schwartz, & Mónica Giusti, in press). Thus the unknown anthocyanins in the purple wheat samples could be due to the presence of acylated anthocyanins (Fig. 3). Furthermore, it was reported the environmental factors such as growing location affected anthocyanin levels and antioxidant activities (Mpofu et al., 2006; Jing et al., 2007) of cereals. The present study suggests that the environmental conditions likely affected the anthocyanin levels of the normal and heat stressed purple wheat.

The most abundant anthocyanins in coloured cereal grains are reported to be Cy-3-glc followed by Pg-3-glc, Pn-3-glc (Naczk & Shahidi, 2006; Prior & Wu, 2006). In the present study, Cy-3-glc was also the most abundant anthocyanin in purple wheat; however, this was followed

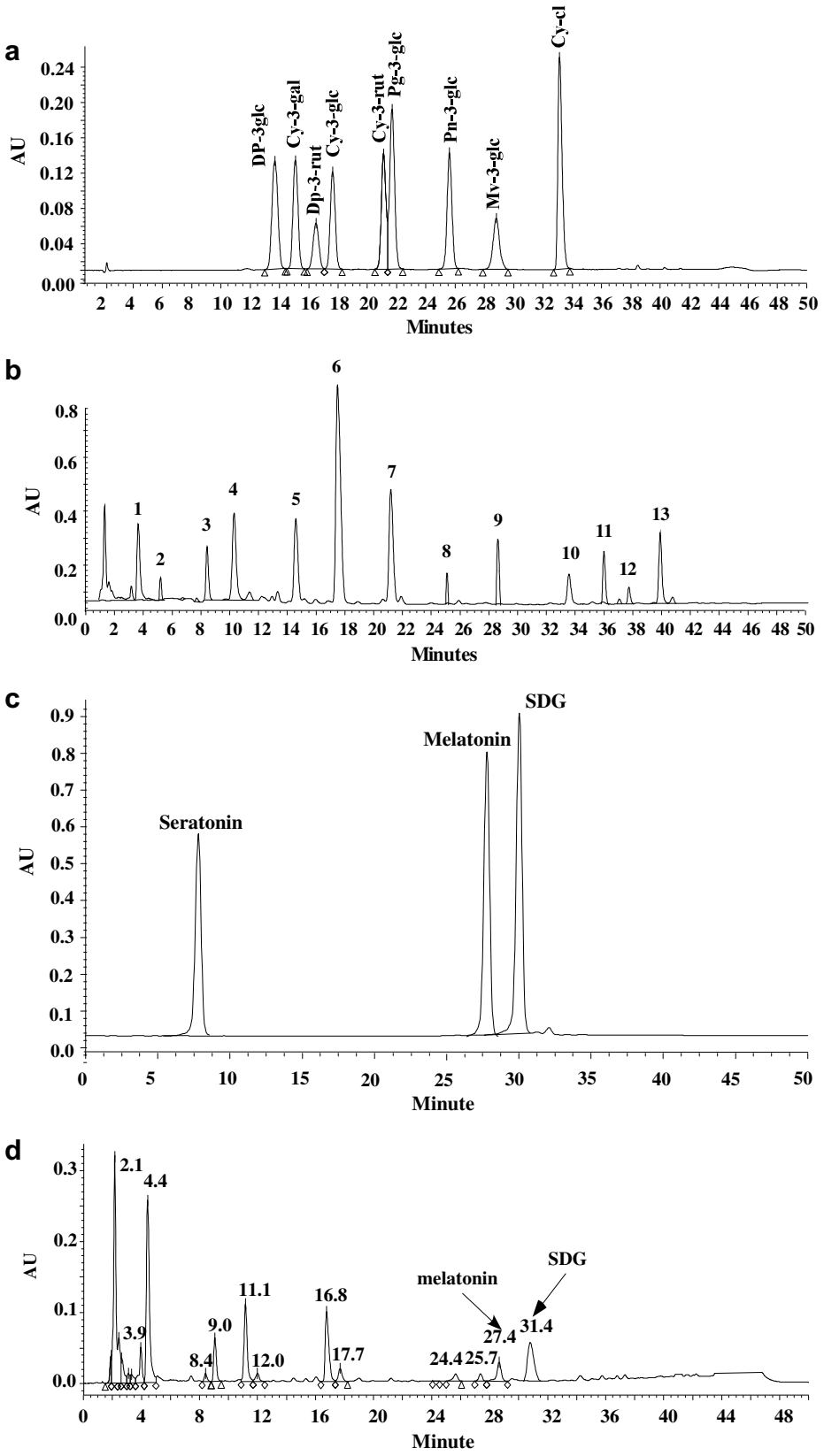


Fig. 3. HPLC chromatograms (520 and 280 nm) for (a) anthocyanin standards, (b) anthocyanin distribution in purple wheat, (c) serotonin, melatonin and SDG standards, and (d) melatonin and SDG in purple wheat.

by Cy-3-gal and Mv-3-glc. Some grains such as blue and purple corn are reported to contain more complex anthocyanins in comparison to red rice and black rice (Choia et al., 2007; Naczk & Shahidi, 2006). Anthocyanins are relatively

Table 1
Mass spectra (m/z) of anthocyanin, secoisolariciresinol (SDG), melatonin and seratonin standards used for identification of phytochemicals in purple wheat

Phytochemical	RT (min)	Major ions (m/z)
Anthocyanin[a]		$[M+H]^+$
Dp-3-gal	3.8	465, 303
Dp-3-ara	5.2	435, 303
Cy-3-ara	8.3	419, 287
Pt-3-glc	10.2	479, 317
Cy-3-gal	15.1	449, 287
Cy-3-glc	17.6	449, 287
Pg-3-glc	21.6	433, 271
Pn-3-glc	25.6	463, 301
Mv-3-glc	28.8	493, 331
Cy-cl	33.1	323, 287
Pg-3-gal	35.9	433, 287
Pg-3-ara	37.8	403, 271
Pn-3-ara	39.9	449, 301
Lignan		$[M-H]^-$
SDG	31.4	685, 361
Other		$[M-H]^-$
Melatonin	27.4	231, 175
Seratonin	7.4	175

[a] Delphinidin 3-galactoside (Dp-3-gal), Delphinidin 3-arabinoside (Dp-3-ara), Cyanidin 3-arabinose (Cy-3-ara), Petunidin 3-galactoside (Pt-3-glc), cyanidin 3-galactoside (Cy-3-gal), Cyanidin 3-glucoside (Cy-3-glc), pelargonidin 3-glucoside (Pg-3-glc), peonidin 3-glucoside (Pn-3-glc), malvidin 3-glucoside (Mv-3-glc), cyanidin chloride (Cy-cl), pelargonidin 3-galactoside (Pg-3-gal), pelargonidin 3-arabinoside (Pg-3-ara) and peonidin 3-arabinoside (Pn-3-ara).

unstable and often undergo degradation during processing and storage (Jing et al., 2007). The total anthocyanin content in plants is also affected by factors such as genetics, light, temperature, and agronomic conditions (Majoul et al., 2003; Jing et al., 2007). Environmental stresses such as drought, heat, salt and UV can adversely alter the nutritional and functionality of crops (Jing et al., 2007). Stress due to drought can cause rupturing of the cell thereby damaging the cell membrane (Majoul et al., 2003). This can cause lipid peroxidation as well as osmolyte leakage. Heat and UV exposure may be associated with free radical formation and singlet oxygen production (Keles & Öncel, 2004). Elevated levels of free radicals and singlet oxygen may increase the oxidative stress in wheat. As a result, specific activities of antioxidant enzymes can increase to protect the plant against environmental stress (Keles & Öncel, 2004). The findings that the anthocyanin content increased due to temperature stress in heat stressed purple wheat strongly suggests the need for future studies investigating the environmental and physiological factors that may affect phenolic composition of purple wheat.

The efficiency of anthocyanin separation was accomplished by raising the column temperature up to 35 °C as previously reported (Hosseinian & Beta, 2007). At present, the most satisfactory method for mixture analysis is the multistep method of separation, isolation, and quantification by HPLC with peak identification by mass spectrometry (MS) (Mazza et al., 2004). Thus HPLC in combination with MS (discussed below) provided elaborate techniques for analysis of anthocyanins in purple wheat samples.

The identity of each anthocyanin compound in samples and standards was confirmed by the using an ACQUITY™ UPLC–MS/MS equipped with an electrospray ionization source. The mass spectrum of each individual anthocyanin standard was recorded in the positive ESI mode. The mass spectra fragmentation of the anthocyanin present in purple wheat samples showed the same fragmentation patterns as those found in standards and thus supported the identification of each compound.

The major mass fragmentation ions of anthocyanins present in purple wheat samples were associated with their corresponding aglycones (Table 1). Cy-3-glc or Cy-3-gal had m/z 449 while the cyanidin (aglycone) part of the molecule had m/z 287, suggesting the loss of a glucose or galactose (m/z 162) from the above anthocyanins (Wu & Prior, 2005). Since MS cannot make a distinction between these two sugars the RT of each standard observed from HPLC was used for their identification (Fig. 3). Pn-3-glc showed m/z 463 while its fragmented ion had a value of m/z 301 (Table 1). The ion at m/z 301 corresponded to methylation of cyanidin ($287 + 14 = 301$) in Pn-3-glc (Abdel-Aal el, Young, & Rabalski, 2006; Wu & Prior, 2005).

3.2. Anthocyanin composition using pH differential method

Anthocyanin pigments undergo reversible structural transformations with a change in pH manifested by strikingly different absorbance spectra (Fuleki & Francis, 1968; Lee et al., 2005). The oxonium (orange-purple) form predominates at pH 1.0 and the hemiketal (colorless) form at pH 4.5. The pH-differential method is based on this reaction, and permits accurate and rapid measurements for the total amount of anthocyanins, even in the presence of polymerized degraded pigments and other interfering compounds (Fuleki & Francis, 1968; Lee et al., 2005). Since Cy-3-glc was the most abundant anthocyanin in two purple wheat samples, the extinction coefficient of Cy-3-glc standard was used to measure the total anthocyanins (Cy-3-glc equivalent). Levels of individual anthocyanins were significantly different ($P < 0.05$) in the two samples except for Pg-3-glc (Table 2). The total anthocyanin content of heat stressed purple wheat (526.0 mg/kg) was significantly ($P < 0.05$) higher than that of normal purple wheat (500.6 mg/kg). The total anthocyanin contents observed from HPLC (522.7 and 491.3 mg/kg, respectively) were not significantly different ($P > 0.05$) from the results observed using pH differential method (Table 2). The similarity observed in the results of the purple wheat samples indicates their precision using the two different methods.

3.3. SDG and melatonin

HPLC chromatograms of serotonin, melatonin and SDG standards are illustrated in Fig. 3 and their mass spectra (m/z)

Table 2
Anthocyanin composition (mg/kg) of normal purple wheat versus heat stressed purple wheat using HPLC method and pH differential method

Compound	Anthocyanin[a]	Normal purple wheat HPLC method[b]	Normal purple wheat pH differential method	Heat stressed purple wheat HPLC method	Heat stressed purple wheat pH differential method
1	Dp-3-gal	38.3a ± 0.6	36.4b ± 0.3	37.7a ± 0.4	39.4b ± 0.1
2	Dp-3-ara	16.7a ± 0.4	15.1a ± 0.9	16.0a ± 0.8	15.3a ± 0.5
3	Cy-3-ara	25.1a ± 0.1	23.2a ± 0.3	26.5a ± 0.8	25.5a ± 0.6
4	Pt-3-glc	40.4a ± 0.3	38.3b ± 0.6	40.7a ± 0.3	40.1a ± 0.3
5	Cy-3-gal	72.0a ± 0.10	76.1a ± 0.7	66.0b ± 0.3	71.3a ± 0.1
6	Cy-3-glc	103.0b ± 0.4	109.2a ± 0.1	104.9b ± 1.4	110.8a ± 0.4
7	Pg-3-glc	28.8a ± 0.0	29.3a ± 0.0	27.3a ± 0.0	32.2a ± 0.1
8	Pn-3-glc	2.7a ± 0.1	3.3a ± 0.4	3.5b ± 0.0	6.0a ± 0.2
9	Mv-3-glc	51.6b ± 0.7	60.3a ± 0.3	85.7a ± 0.2	90.4a ± 0.6
10	Cy-cl	5.2a ± 0.1	6.8b ± 0.2	9.8a ± 0.0	9.2a ± 0.1
11	Pg-3-gal	26.1a ± 0.5	22.1b ± 0.7	25.6a ± 0.2	21.5b ± 0.1
12	Pg-3-ara	9.3a ± 0.3	11.3b ± 0.3	10.3a ± 0.6	10.2a ± 0.5
13	Pn-3-ara	28.4a ± 0.4	29.3a ± 0.8	27.7a ± 0.8	26.1a ± 0.2
	Total known	447.6a ± 1.0	460.7a ± 0.9	481.7a ± 0.3	499.1a ± 1.2
	Total unknown	43.5a ± 0.5	39.9a ± 2.7	41.0a ± 0.1	29.1b ± 1.8
	Total, all	491.1a ± 1.4	500.6a ± 3.5	522.7a ± 1.9	526.0a ± 3.0
	LSD	1.6	0.1	0.9	1.1
	CV%	0.30	0.05	0.05	0.05

$P < 0.05$ using Fisher's least significance difference (LSD).

[a] Delphinidin 3-galactoside (Dp-3-gal), Delphinidin 3-arabinoside (Dp-3-ara), Cyanidin 3-arabinose (Cy-3-ara), Petunidin 3-galactoside (Pt-3-glc), cyanidin 3-galactoside (Cy-3-gal), Cyanidin 3-glucoside (Cy-3-glc), pelargonidin 3-glucoside (Pg-3-glc), peonidin 3-glucoside (Pn-3-glc), malvidin 3-glucoside (Mv-3-glc), cyanidin chloride (Cy-cl), pelargonidin 3-galactoside (Pg-3-gal), pelargonidin 3-arabinoside (Pg-3-ara) and peonidin 3-arabinoside (Pn-3-ara).

[b] Different letters in the rows are used to show significant differences among anthocyanins (mean ± stdev) in HPLC method and pH differential method for each sample.

are shown in Table 1. The mass spectra of SDG showed *m/z* 685.6 $[M-H]^-$ and thus MW = 686.6 (Table 1). The *m/z* 708 $[M-H]^-$ corresponded to (SDG + Na^+). The fragmented ion with *m/z* 361 $[M-H]^-$ corresponded to the aglycone SECO (Hosseinian et al., 2007). Melatonin showed *m/z* 231.1 $[M-H]^-$ and thus MW = 232.1, and the fragmented ion with *m/z* 175.1 $[M-H]^-$ corresponded to serotonin molecule with MW = 176.1 (Table 1).

HPLC chromatograms for purple wheat are shown in Fig. 3. The SDG content of the normal purple wheat (770 μg/kg) was significantly ($P < 0.05$) higher than that of heat stressed purple wheat (520 μg/kg) (Table 3), indicating that environmental conditions likely affected the levels of phytochemicals in coloured wheats. The SDG content of purple wheat was within the range reported previously for total lignans 70–7640 μg/kg (Milder, Arts, van de Putte, Venema, & Hollman, 2005) and 80–2990 μg/kg (Smeds et al., 2007) in the whole grain. There is scanty literature on the content in various grains. In wheat, lignans are found mainly in the bran layer. SDG, the major lignan in wheat bran is present in amounts ranging between 421 and 821 (μg/kg) (Qu, Madl, Takemoto, Baybutt, & Wang, 2005). Other peaks at 280 nm most likely corresponded to other phenolics (e.g. phenolic acids) since they are commonly detected at 280 nm.

Table 3
SDG and melatonin content (μg/kg) of normal purple wheat and heat stressed purple wheat

Compound	New purple wheat[a]	Old purple wheat
SDG	770.0a ± 0.1	520.0b ± 0.1
Melatonin	4.0a ± 0.1	2.0b ± 0.3

[a] Different letters in same row are used to show significant differences in SDG and melatonin contents (±standard deviation, triplicate determinations) in two purple wheat samples at $P < 0.05$.

The levels of serotonin and melatonin in purple wheat have not been previously reported. The melatonin content of normal purple wheat (4 μg/kg) was significantly higher ($P > 0.05$) than that of heat stressed purple wheat (2 μg/kg) (Table 3) and no serotonin was observed in these samples. This suggests that serotonin was probably converted to melatonin or the amount was below the detection limit of the HPLC (0.1 μg/mg). Corn, rice, barley grains, and ginger showed melatonin concentrations of 187.8, 149.8, 87.3, 142.3 ng/100 g, respectively (Badria, 2002). The presence of melatonin in cereal grains is important, given that melatonin has been linked to mood, happiness, and brain neuromodulation (Ferrari, 2004). Cereal grains are important daily source of diet and adding colored cereals to the diet may provide additional health benefits with the consumptions. Future studies will help to understand the mechanism of action of phytochemicals in colored cereals.

4. Conclusion

Qualitative and quantitative anthocyanin compositions of colored cereals such as purple wheat are important factors in determining the functionality and health benefits of foods enriched with anthocyanins. Cy-3-glc was the predominant anthocyanin in the normal and heat stressed purple wheat. The heat stressed purple wheat showed higher anthocyanin content in comparison to normal purple wheat. Measurement of total anthocyanin contents, using HPLC and the pH differential methods showed similar results and hence reproducibility of the methods. The presence of SDG in purple wheat may contribute to antioxidant activity. Furthermore, the presence of melatonin in purple wheat samples suggests that cereal grains are potential sources of phytochemicals that may contribute additive health benefits. Future research needs to be conducted in order to determine the heath benefits of such bioactive components *in vitro* and *in vivo*.

Acknowledgements

We are grateful for the financial support provided by the Canada Foundation for Innovation (CFI New Opportunities Fund), and Natural Sciences and Engineering Research Council of Canada (NSERC Discovery Grant and Canada Research Chairs Program). The technical assistance provided by Wan Yuin Ser and Max Abou is greatly appreciated.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.foodchem.2007.12.083.

References

Abdel-Aal el, S. M., & Hucl, P. (2003). Composition and stability of anthocyanins in blue-grained wheat. *Journal of Agricultural and Food Chemistry, 51*(8), 2174–2180.

Abdel-Aal el, S. M., Young, J. C., & Rabalski, I. (2006). Anthocyanin composition in black, blue, pink, purple, and red cereal grains. *Journal of Agricultural and Food Chemistry, 54*(13), 4696–4704.

Acevedo, E., Silva, P., & Silva, H. (2002). Wheat growth and physiology. http://www.fao.org/DOCREP/006/Y4011E/y4011e06.htm.

Badria, F. A. (2002). Melatonin, serotonin, and tryptamine in some Egyptian food and medicinal plants. *Journal of Medicinal Food, 5*(3), 153–157.

Choia, Y., Jeonga, H., & Lee, J. (2007). Antioxidant activity of methanolic extracts from some grains consumed in Korea. *Food Chemistry, 103*, 130–138.

Duthie, S. J., Jenkinson, A. M., Crozier, A., Mullen, W., Pirie, L., Kyle, J., et al. (2006). The effects of cranberry juice consumption on antioxidant status and biomarkers relating to heart disease and cancer in healthy human volunteers. *European Journal of Clinical Nutrition, 45*(2), 113–122.

Ferrari, C. K. (2004). Functional foods, herbs and nutraceuticals: Towards biochemical mechanisms of healthy aging. *Biogerontology, 5*(5), 275–289.

Fuleki, T., & Francis, F. J. (1968). Determination of total anthocyanin and degradation index for cranberry juice. *Food Science, 33*(1), 78–83.

Hosseinian, F. S., & Beta, T. (2007). Saskatoon and wild blueberries have higher anthocyanin contents than other Manitoba Berries. *Journal of Agricultural and Food Chemistry, 55*(26), 10832–10838.

Hosseinian, F. S., Muir, A. D., Westcott, N. D., & Krol, E. S. (2007). AAPH-mediated antioxidant reactions of secoisolariciresinol and SDG. *Organic & Biomolecular Chemistry, 5*(4), 644–654.

Jing, P., Noriega, V., Schwartz, S. J., & Mónica Giusti, M. (2007). Effects of growing conditions on purple corncob (*Zea mays* L.) anthocyanins. *Journal of Agricultural and Food Chemistry*, doi: 10.1021/jf070755q.

Karasek, M. (2004). Melatonin, human aging, and age-related diseases. *Experimental Gerontology, 39*(11–12), 1723–1729.

Kay, C. D., Mazza, G., Holub, B. J., & Wang, J. (2004). Anthocyanin metabolites in human urine and serum. *British Journal of Nutrition, 91*(6), 933–942.

Kedziora-Kornatowska, K., Szewczyk-Golec, K., Czuczejko, J., van Marke de Lumen, K., Pawluk, H., Motyl, J., et al. (2007). Effect of melatonin on the oxidative stress in erythrocytes of healthy young and elderly subjects. *Journal of Pineal Research, 42*(2), 153–158.

Keles, Y., & Öncel, I. (2004). Growth and solute composition in two wheat species experiencing combined influence of stress conditions. *Russian Journal of Plant Physiology, 51*(2), 203–209.

Lee, J., Durst, R. W., & Wrolstad, R. E. (2005). Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines by the pH differential method: Collaborative study. *Journal Association of Official Analytical Chemists International, 88*(5), 1269–1278.

Li, W., Shan, F., Sun, S., Corke, H., & Beta, T. (2005). Free radical scavenging properties and phenolic content of Chinese black-grained wheat. *Journal of Agricultural and Food Chemistry, 53*(22), 8533–8536.

Majoul, T., Bancel, E., Triboi, E., Ben Hamida, J., & Branlard, G. (2003). Proteomic analysis of the effect of heat stress on hexaploid wheat grain: Characterization of heat-responsive proteins from total endosperm. *Prometoics, 3*(2), 175–183.

Manach, C., Mazur, A., & Scalbert, A. (2005). Polyphenols and prevention of cardiovascular diseases. *Current Opinion in Lipidology, 16*(1), 77–84.

Manach, C., Scalbert, A., Morand, C., Remesy, C., & Jimenez, L. (2004). Polyphenols: Food sources and bioavailability. *American Journal of Clinical Nutrition, 79*(5), 727–747.

Mazza, G., Cacace, J. E., & Kay, C. D. (2004). Methods of analysis for anthocyanins in plants and biological fluids. *Journal Association of Official Analytical Chemists International, 87*(1), 129–145.

Milder, I. E., Arts, I. C., van de Putte, B., Venema, D. P., & Hollman, P. C. (2005). Lignan contents of Dutch plant foods: A database including lariciresinol, pinoresinol, secoisolariciresinol and matairesinol. *British Journal of Nutrition, 93*(3), 393–402.

Mpofu, A., Sapirstein, H. D., & Beta, T. (2006). Genotype and environmental variation in phenolic content, phenolic acid composition, and antioxidant activity of hard spring wheat. *Journal of Agricultural and Food Chemistry, 54*(4), 1265–1270.

Naczk, M., & Shahidi, F. (2006). Phenolics in cereals, fruits and vegetables: Occurrence, extraction and analysis. *Journal of Pharmaceutical and Biomedical Analysis, 41*(5), 1523–1542.

Oncel, I., Keles, Y., & Ustun, A. S. (2000). Interactive effects of temperature and heavy metal stress on the growth and some biochemical compounds in wheat seedlings. *Environmental Pollution, 107*(3), 315–320.

Oomah, B. D., & Mazza, G. (1999). Health benefits of pytochemicals from selected Canadian crops. *Trends in Food Science and Technology, 10*, 193–198.

Prior, R. L., & Wu, X. (2006). Anthocyanins: Structural characteristics that result in unique metabolic patterns and biological activities. *Free Radical Research, 40*(10), 1014–1028.

Qu, H., Madl, R. L., Takemoto, D. J., Baybutt, R. C., & Wang, W. (2005). Lignans are involved in the antitumor activity of wheat bran in colon cancer SW480 cells. *Journal of Nutrition, 135*(3), 598–602.

Seeram, N. P., & Nair, M. G. (2002). Inhibition of lipid peroxidation and structure-activity-related studies of the dietary constituents anthocyanins, anthocyanidins, and catechins. *Journal of Agricultural and Food Chemistry, 50*(19), 5308–5312.

Smeds, A. I., Eklund, P. C., Sjoholm, R. E., Willfor, S. M., Nishibe, S., Deyama, T., et al. (2007). Quantification of a broad spectrum of lignans in cereals, oilseeds, and nuts. *Journal of Agricultural and Food Chemistry, 55*(4), 1337–1346.

Wu, X., & Prior, R. L. (2005). Identification and characterization of anthocyanins by high-performance liquid chromatography–electrospray ionization–tandem mass spectrometry in common foods in the United States: Vegetables, nuts, and grains. *Journal of Agricultural and Food Chemistry, 53*(8), 3101–3113.