

A Rapid Method for Quantifying Total Anthocyanins in Blue Aleurone and Purple Pericarp Wheats

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ABSTRACT

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A simple, rapid method for determining total anthocyanins was developed for use in developing wheat cultivars with dark-blue grains. The method was evaluated as a screening test and for quantification of total anthocyanins in blue and purple wheats and related cereals. Wheat anthocyanins were significantly more extractable in ethanol or methanol than in water at different pH levels. A sample-to-solvent ratio of 1:8 at pH 1 and 25°C was used. Anthocyanin extracts of pigmented wheat and barley grains

exhibited absorbance spectra similar to cyanidin 3-glucoside. The absorbance of anthocyanin extracts of 160 blue wheat experimental lines were significantly correlated with whole-grain Hunterlab color values. Total anthocyanins averaged 157 mg/kg in blue wheat whole meal and 104 mg/kg in purple wheat whole meal, whereas blue wheat bran contained 458 mg/kg as compared with 251 mg/kg in purple wheat bran.

White and red common and amber durum wheats are well known by wheat producers, millers, and bakers, as well as food processors, but blue- and purple-grain wheats are not well known. In the latter wheats, purple pigments are located in the grain pericarp and blue pigments are located in the aleurone layer (Zeven 1991). The division, however, is not entirely clear. Despite early studies on red pigmentation in wheat conducted by Nillson-Ehle (1914), little has been reported on anthocyanins in either blue or purple grains. Information, however, is available on xanthophylls, carotenoids, and flavones—the other common pigments found in wheat. The latter pigments occur in small concentrations in wheat grains and cause detrimental or beneficial effects to end-use products depending on wheat type and product.

With the current trend away from synthetic food dyes, introducing naturally colored grains is of interest to grain producers, processors, and consumers. Purple wheat has been developed and used in New Zealand since 1980 (Bezar 1982). Grains are crushed into relatively large pieces (kibbling process) and added to the exterior of multigrain breads. In addition to wheat, anthocyanins occur in a variety of other pigmented cereal grains such as barley, corn, rye, rice, sorghum, and millet (Mazza and Miniati 1993). Of these, blue corn is used for production of naturally blue tortillas. In addition, an anthocyanin food-coloring agent obtained from purple corn has been patented (Sugiyama Chemical Institute 1977).

To develop wheat cultivars with the dark blue-grain trait for the food industry, a simple, rapid screening method for predicting anthocyanin content is needed. Anthocyanins have been investigated extensively in fruits, vegetables, flowers, and sunflower hulls, but few studies have been conducted on pigmented wheats in terms of anthocyanin quantification and characterization. Dedio et al (1972) reported that the major anthocyanins in the pericarp of purple-grain wheat line UM 606a were cyanidin 3-glucoside and peonidin 3-glucoside, with trace amounts of the corresponding rutinosides, as detected by paper chromatography with rhubarb and plum anthocyanins as standards. No information is available on the concentration of these pigments in blue and purple wheats. The current study was conducted to develop a simple, rapid screening test for pigmented grains based on absorption intensity of anthocyanin. The method was modified based on the procedure of Fuleki and Francis (1968a). The factors that affect anthocyanin extractability in wheat (e.g., solvent type, pH, temperature, and sample-to-solvent ratio) were investigated. The screening method was optimized to quantify

total anthocyanins in blue and purple wheats and other related cereal grains using cyanidin 3-glucoside as a standard. A simple mathematical formula was developed to calculate total anthocyanin content per sample (mg/kg).

MATERIALS AND METHODS

Wheats

Spring-type blue wheat (*Triticum aestivum* L. 'Purendo 38') was grown in standard replicated plots at Saskatoon, SK, Canada, in 1996. The prototype line was selected based on its agronomic performance and quality characteristics. Hard red spring (HRS) wheat cultivars Konini purple (*T. aestivum*) and Katepwa (*T. aestivum*) were grown in the same field trials as blue wheat. A representative sample from each wheat was used in the study. Grain was ground to pass through a 1-mm screen on a cyclone sample mill (Udy, Fort Collins, CO) to produce whole meal. For milling into flour, grain was tempered over an 18-hr period to 14.5% moisture for blue and purple wheats and to 15.5% for HRS wheat. The tempered grain samples were milled on a Quadrumat Jr. flour mill (Brabender Co., South Hackensack, NJ). Milled grain was sifted on a 64-mesh sieve with a Ro-Tap sieve shaker (Tyler Co., Mentor, OH) for 3 min. Flour extraction rates were \approx 71, 73, and 74% for blue, purple, and HRS wheats, respectively. The material retained on the sieve was considered the bran fraction.

Color Measurement

Color of wheat whole meal, flour, and bran was measured with a Hunterlab color difference meter (Color Quest 45/0, Hunter Associates Laboratory, Reston, VA) as *L*, *a*, and *b* values after standardization with a white color standard. Total color difference (ΔE) was calculated as:

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2}$$

where Δ is the difference between standard and sample readings.

Chemical Analysis

Protein (N \times 5.7) and soluble and insoluble dietary fiber in wheat whole meal, flour, and bran were determined by Approved Methods 46-11A and 32-21, respectively (AACC 1995). Starch was measured as glucose according to the method described by McCleary et al (1997). Carotenoid determination was based on Approved Method 14-50, using water-saturated *n*-butanol as a solvent.

Extractability of Anthocyanin in Blue and Purple Wheats

Extractability of wheat anthocyanins was studied with several types of solvent (water and acidified water, ethanol, and methanol). Acidified solvents consisted of 85 mL of solvent and 15 mL of 0.1, 1.0, or 1.5*N* HCl to provide different levels of acidic pH.

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Ground whole meal (3 g) was weighed in a 50-mL centrifuge tube, and 24 mL of solvent was added. The mixture was shaken for 30 min and centrifuged at $27,200 \times g$ for 15 min. The supernatant or pigment extract was poured into a 50-mL volumetric flask and made up to volume. Absorbance was measured at 535 nm against a reagent (solvent) blank. The pH of each extract also was recorded with a pH meter. To study the effects of pH level (1, 3, 5, and 8), temperature (25, 40, and 60°C), and sample-to-solvent ratio (1:3, 1:5, 1:8, and 1:12), ethanol was used as a solvent, and the pH level of the mixture was adjusted to the desired value with HCl or NaOH solutions of 1–4*N*.

Determination of Total Anthocyanin in Blue and Purple Wheats

On the basis of extractability results, a simple, rapid method for determining total anthocyanin in pigmented wheats was established. A ground wheat sample (3 g) was weighed in a 50-mL centrifuge tube, and 24 mL of acidified ethanol (ethanol and HCl 1.0*N*, 85:15, v/v) was added. The solution was mixed and adjusted to pH 1 with 4*N* HCl. The resulting solution was shaken for 15 min, readjusted to pH 1 if necessary, and the solution was shaken for an additional 15 min. The tube was centrifuged at $27,200 \times g$ for 15 min, and the supernatant was poured into a 50-mL volumetric flask and made up to volume with acidified ethanol. Absorbance was measured at 535 nm against a reagent blank. Cyanidin 3-glucoside or Kuromanin from Extrasynthese (Genay, France) was used as a standard pigment. A series of cyanidin 3-glucoside standard solutions was prepared at 0–0.02 mmol (0–27 µg/3 mL). Absorbance was read at 535 nm against a reagent blank. The concentrations showed a linear relationship against absorbance (Fig. 1) and had regression and determination coefficients of 0.0197 and 0.999, respectively. Total anthocyanin content per sample (mg/kg) was calculated as cyanidin 3-glucoside:

$$C = (A/\epsilon) \times (\text{vol}/1,000) \times \text{MW} \times (1/\text{sample wt}) \times 10^6$$

where *C* is concentration of total anthocyanin (mg/kg), *A* is absorbance reading, ϵ is molar absorptivity (cyanidin 3-glucoside = $25,965 \text{ cm}^{-1} \text{ M}^{-1}$), Vol is total volume of anthocyanin extract, and MW is molecular weight of cyanidin 3-glucoside = 449.

Under test conditions, the equation formula can be simplified to:

$$C = (A/25,965) \times (50/1,000) \times 449 \times (1/3) \times 10^6$$

or

$$C = A \times 288.21 \text{ mg/kg}$$

Beer's law was used to calculate molar absorptivity of cyanidin 3-glucoside, which ranged from 25,591 to 26,559 $\text{cm}^{-1} \text{ M}^{-1}$, with an average of $25,965 \text{ cm}^{-1} \text{ M}^{-1}$, standard deviation of 520, and coefficient of variation of 2%. Mean molar absorptivity was used to calculate the concentration of total anthocyanins in pigmented wheat and barley samples.

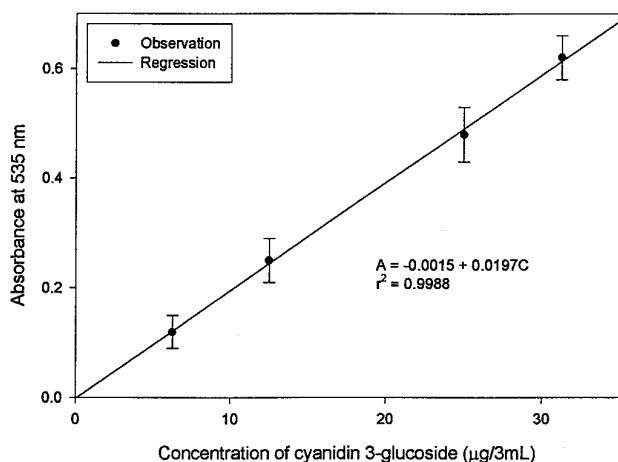


Fig. 1. Standard curve of cyanidin 3-glucoside in acidified ethanol.

Statistical Analysis

Data were analyzed by analysis of variance, using Statistical Software Release 12 (Minitab, State College, PA). The reported data are means of at least three replicate determinations. Duncan's multiple range method was used to identify significant differences among means.

RESULTS AND DISCUSSION

Composition of Pigmented Wheats

The three constituents (starch, protein, and fiber) with the largest impact on end-use characteristics of wheat were determined in blue, purple, and red wheats (Table I). The blue wheat sample contained higher starch and lower protein levels than the HRS wheat sample, while the purple wheat sample was similar to the HRS wheat. Soluble and insoluble dietary fiber content was similar among the three wheat samples. After milling, starch, protein, and soluble and insoluble dietary fiber contents were similar among all wheat flours. Blue wheat bran, however, contained $\approx 3.5\%$ more starch and ≈ 0.5 – 1.5% less protein than purple and red wheats (Table I). Soluble and insoluble dietary fiber was similar for all wheat brans.

TABLE I
Chemical Composition of Blue-, Purple-, and Red-Wheat Grains and Their Milled Products (% db)

Wheat Type and Product	Starch	Protein (N \times 5.7)	Dietary Fiber	
			Soluble	Insoluble
Whole meal				
Blue	66.8 \pm 1.2 ^a	12.2 \pm 0.3	1.9 \pm 0.05	11.9 \pm 0.4
Purple	64.5 \pm 0.9	14.0 \pm 0.2	2.0 \pm 0.05	11.6 \pm 0.6
Red	64.1 \pm 0.7	14.1 \pm 0.2	1.8 \pm 0.02	10.9 \pm 0.4
Flour fraction				
Blue	79.1 \pm 1.2	11.4 \pm 0.3	0.9 \pm 0.02	1.8 \pm 0.1
Purple	78.1 \pm 0.6	13.2 \pm 0.4	1.0 \pm 0.01	2.1 \pm 0.1
Red	77.2 \pm 0.5	13.3 \pm 0.3	1.5 \pm 0.02	2.0 \pm 0.1
Bran fraction				
Blue	24.0 \pm 0.8	15.6 \pm 0.4	2.3 \pm 0.09	40.8 \pm 1.2
Purple	20.3 \pm 0.2	17.2 \pm 0.2	2.3 \pm 0.05	42.2 \pm 2.0
Red	20.5 \pm 0.9	16.1 \pm 0.3	2.4 \pm 0.11	41.0 \pm 0.9

^a Mean \pm standard deviation.

TABLE II
Effect of Solvent Type at Different pH Levels on Extractability of Anthocyanin Pigments from Wheat Grains^a

Solvent Type	Added HCl ^b (N)	pH of Extract	Visible Color	$A_{535\text{nm}}$ ^c	
Blue wheat	None	6.5	Light yellow	0.11d	
	0.1	4.0	Light yellow	0.09d	
	1.0	1.3	Red	0.44bc	
	1.5	1.1	Red	0.46bc	
Ethanol	0.1	2.6	Light pink	0.28cd	
	1.0	1.4	Dark pink	0.92a	
	1.5	1.1	Dark pink	0.91a	
Methanol	0.1	2.4	Pink	0.55b	
	1.0	1.0	Dark pink	0.95a	
	1.5	0.8	Dark pink	0.98a	
Purple wheat	Water	None	6.5	Light yellow	0.07c
		0.1	4.0	Light yellow	0.06c
		1.0	1.4	Light red	0.31b
	Ethanol	1.5	1.2	Light red	0.29b
		0.1	2.8	Red	0.38b
		1.0	1.3	Dark red	0.84a
	Methanol	1.5	1.1	Dark red	0.85a
		0.1	2.5	Dark red	0.36b
		1.0	1.0	Dark red	0.79a
	1.5	0.8	Dark red	0.81a	

^a Sample-to-solvent ratio of 1:8 at 25°C was used in extraction.

^b Ratio of added HCl to solvent was 15:85, v/v.

^c Values followed by different letters indicate significant differences at $P = 0.05$.

Extractability of Anthocyanin Pigments in Wheat

A range of polar solvents with different degrees of acidity from very strong acid to weak acid media was evaluated for the ability to extract anthocyanins from blue and purple wheats (Table II). The pH of extracts varied from 0.8 to 6.5, depending on the concentration of added HCl and solvent type. The pH level had a marked influence on the color of anthocyanin extracts. At lower levels (pH < 2), extracts were red to dark red, while at higher levels (pH > 4), extracts were yellow. Absorbance readings increased with declining pH levels for the three solvents investigated (water, ethanol, and methanol). There were significant differences between the two alcohols and water in the ability to extract anthocyanin from wheat grains. Both acidified alcohols were more efficient than acidified water in extracting wheat anthocyanins. However, there were no significant differences between ethanol and methanol in absorbance readings. Ethanol was chosen for further study because of its lower toxicity relative to methanol.

The pH level had a significant effect on extractability of anthocyanins from blue and purple wheats (Fig. 2). Increasing the level of the extract from pH 1 to pH 5 decreased anthocyanin extractability by $\approx 94\%$ in blue and purple wheats. Extraction of wheat anthocyanins at pH 1 resulted in the highest absorbance readings in both pigmented wheats and stabilized anthocyanin pigments. Fuleki and Francis (1968b) reported that increasing the pH level greatly decreased anthocyanin absorption in the visible region, while absorbance in UV was only slightly affected. Extraction of antho-

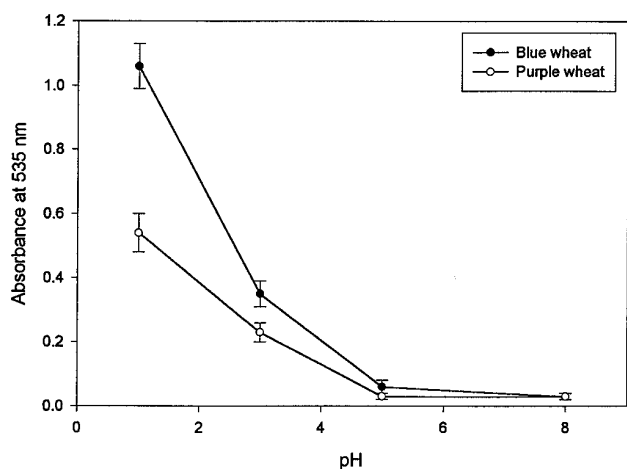


Fig. 2. Effect of pH on extractability of anthocyanins from blue- and purple-grain wheats.

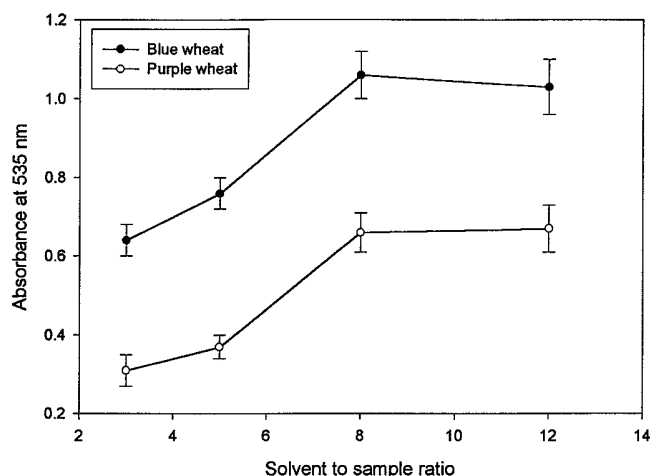


Fig. 3. Effect of solvent-to-sample ratio on extractability of anthocyanins from blue- and purple-grain wheats.

cyanins from blue and purple wheats at different solvent-to-sample ratios also was studied (Fig. 3). Increasing the ratio from 3:1 to 8:1 increased the efficiency of anthocyanin extraction. However, there was no marked difference in anthocyanin extraction between the 8:1 and 12:1 ratios. Nonsignificant increases in anthocyanin extraction were observed when temperature was increased from 25 to 60°C (Fig. 4). The absorbance reading increased by $\approx 15\%$ as temperature increased from 25 to 60°C.

In the original procedure for determining total anthocyanin in berries (Fuleki and Francis 1968a), blended and macerated berries were stored at 4°C overnight to improve extraction efficiency. Fresh fibrous materials such as cranberries require overnight submersion in extraction solvent to allow anthocyanin to diffuse through the cell membranes (Fuleki and Francis 1968a). However, when macerated, ground wheat or bran was stored overnight at 4°C after shaking for 30 min, there were no significant differences between stored and nonstored samples in anthocyanin extraction. For example, absorbance readings were 0.46 (stored) versus 0.44 (nonstored) for whole meal, and 0.49 versus 0.45 for wheat bran. After extraction and centrifugation, anthocyanin extracts were kept in the dark for up to 4 hr, and absorbance was read at 1-hr intervals to study the time required to attain equilibrium between different forms of anthocyanin. There were no significant differences in absorbance reading at 0 hr and after standing for 4 hr for either whole meal or bran. Absorbance readings were 0.45 at 0 hr versus 0.46 after 4 hr of standing for whole meal extracts and 0.49 versus 0.50 for bran extracts (diluted twice). The results indicate that anthocyanin-pigmented wheat extracts require a very short time to reach equilibrium, which may reflect lesser forms of anthocyanins present in pigmented wheats as compared with fruit. In our study, a very short (or no) equilibrium time was used before measuring absorbance. Based on the results obtained, and to simplify the method, anthocyanins were extracted for 30 min with continuous agitation, and absorbance was read after centrifugation.

The stability of blue and purple wheat anthocyanin extracts under cold storage (4°C) conditions was studied up to eight weeks (Fig. 5). Absorbance of anthocyanin extracts was measured on a weekly basis. After one week, extracts became cloudy, presumably partially because of precipitation of soluble proteins. Extracts were centrifuged, and absorbance was measured. There was no significant reduction in absorbance over the test period, but there was a slight decline during the first two weeks, particularly for blue wheat extracts. The pigment extracts from blue wheat whole meal and bran, purple wheat whole meal and bran, and blue barley whole meal had absorbance spectra similar to that of cyanidin 3-glucoside (Fig. 6), the major anthocyanin in purple wheat (Dedio et al 1972). This finding confirmed that the extracted pigments had spectral characteristics

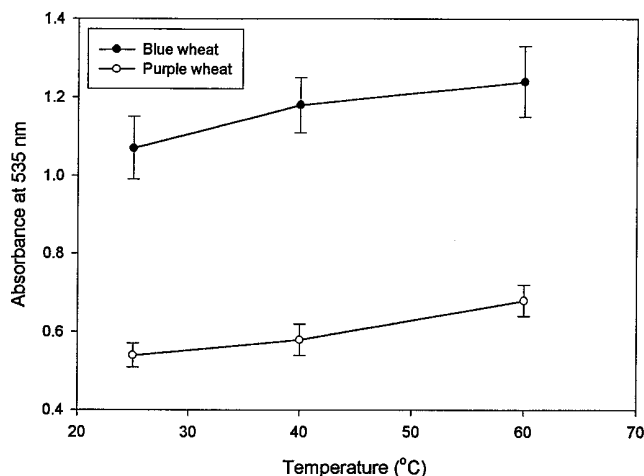


Fig. 4. Effect of temperature on extractability of anthocyanins from blue- and purple-grain wheats, using acidified ethanol solvent.

similar to cyanidin 3-glucoside. Maximum absorbance for cyanidin 3-glucoside and anthocyanin extracts was 535–540 nm.

Based on anthocyanin extractability results, a simplified method was developed for screening pigmented wheats and other related cereals and for quantification of total anthocyanins in these grains. Both screening and quantification methods were based on extraction of anthocyanins with acidified ethanol (85 mL of 95% ethanol + 15 mL of 1N HCl). The extract was made up to volume, and absorbance was measured at 535 nm against a reagent blank.

Screening of Pigmented Wheats

The modified method was evaluated as a screening test in a breeding program aimed at developing anthocyanin-pigmented wheat cultivars. The absorbance of anthocyanin extracts for 160 experimental lines was measured and correlated with grain color values determined with a Hunterlab colorimeter (Table III). Colorimeter scores were significantly correlated with absorbance readings, showing the relevance of both methods in screening wheats based on pigment content. Absorbance readings were negatively correlated with *L*, *a*, and *b* color values but were strongest with *b* ($r = -0.80$) (negative *b* values indicate degree of blue). There were also large variations in *L*, *a*, and *b* values and absorbance readings among experimental wheat lines, indicating an opportunity to select for darker blue wheat. The distribution of anthocyanin content in the experimental lines is shown in Fig. 7. Anthocyanin was normally distributed, 34.6–507.2 mg/kg of wheat sample, with a mean value of 183.0 mg/kg and standard error of 7.6.

The screening method had some advantages over the Hunterlab colorimetric method, including the ability to provide an accurate and absolute value of anthocyanin content in wheat lines and the relatively small amount of sample required (3 g) as compared with the Hunterlab method (35–70 g). Furthermore, sample weight could be reduced to ≈ 200 mg when a total volume of 3 mL was used instead of 50 mL.

Color and Total Anthocyanins in Pigmented Wheat Products

Blue wheat experimental line Purendo 38, purple wheat Konini, and common wheat Katepwa whole meals, flours, and brans were compared in terms of Hunterlab color values and anthocyanin and carotenoid concentrations (Table IV). Blue and purple wheat grains tended to have lower *L*, *a*, and *b* values than red wheat grains. Red wheat grains were less dark than anthocyanin-pigmented wheat, as indicated by higher *L* values and lower ΔE values, but they exhibited greater degrees of red and yellow. The relatively high *b* values in blue wheat samples could reflect the infiltration of blue pigments with other pigments in grains. Purendo 38 has a light red pericarp. Blue, purple, and red wheat flour colors were similar, as indicated by Hunterlab values, with a slight increase in white in red wheat

flour. Blue and purple wheat brans tended to be more similar in *L*, *a*, and *b* color values and differed from red wheat bran. Again, red wheat bran color was lighter than anthocyanin-pigmented wheat brans.

The modified procedure was used to determine total anthocyanins in pigmented wheat and barley and milled products (Table IV). Total anthocyanins averaged 157 mg/kg in blue wheat whole meal and 104 mg/kg in purple wheat whole meal. These concentrations were high when compared with other wheat pigments. Also, two samples of blue aleurone barley contained high concentrations of anthocyanin pigments (174 and 291 mg/kg). After milling, anthocyanins were localized primarily to the bran fraction. Blue wheat bran contained 458 mg/kg of anthocyanins as compared with 251 mg/kg in purple wheat bran. The flour fractions, however, had very low concentrations of anthocyanins, particularly in purple wheat. The lower concentration of anthocyanins in purple wheat flour than in blue wheat flour might be expected because the anthocyanins are located in the pericarp of purple wheat and aleurone layer of blue wheat. Red wheat and its milled products contained lower levels of anthocyanin pigments than the counterpart products of blue and purple wheats. Carotenoids or yellow pigments also were measured in blue- and purple-wheat products. In wheat grains, the pigments were present in relatively lower concentrations than anthocyanins or reddish pigments (Table IV). Purple wheat contained higher levels of carotenoids than blue and red

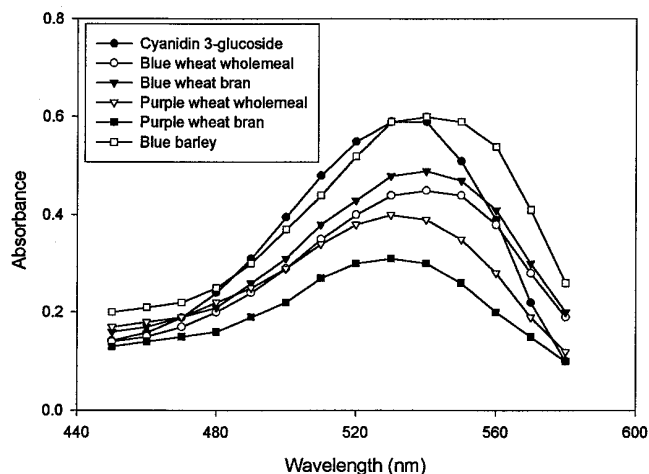


Fig. 6. Absorbance spectra of cyanidin 3-glucoside and anthocyanin extracts from blue and purple wheat whole meal and brans and blue barley.

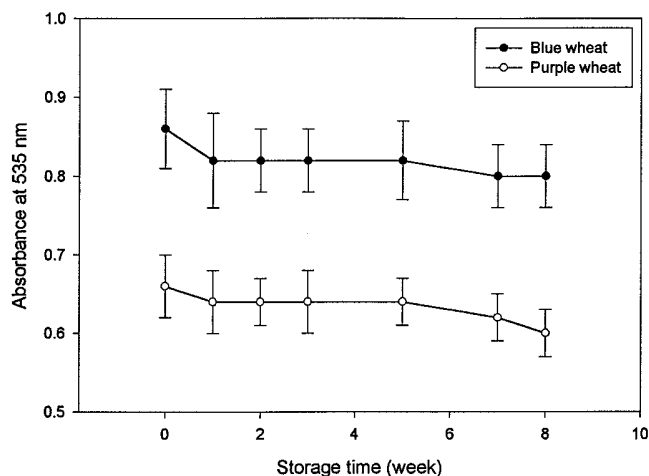


Fig. 5. Stability of blue and purple wheat anthocyanin extracts stored at 4°C.

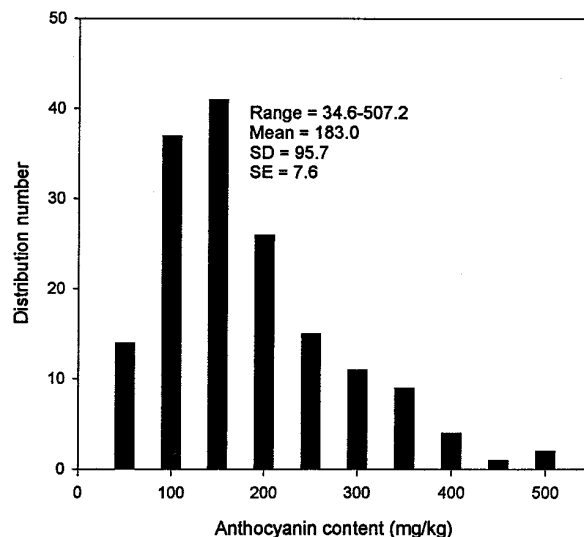


Fig. 7. Distribution of anthocyanin concentration in pigmented wheat experimental lines ($n = 160$).

TABLE III
Statistical Estimates and Correlation Coefficients of Total Anthocyanins^a vs. Hunterlab Color Values (*L*, *a*, *b*)^b
for Pigmented Wheat Experimental Lines (*n* = 160)

Estimate	<i>L</i>	<i>a</i>	<i>b</i>	<i>A</i> _{535nm}
Range	20.2–32.3	3.0–5.7	3.6–9.4	0.12–1.76
Mean	26.8	4.3	6.1	0.64
SE ^c	0.14	0.04	0.08	0.03
Correlation coefficient				
Absorbance	–0.62** ^d	–0.55**	–0.80**	...

^a Absorbance reading at 535 nm.

^b *L* = 100 (white) to 0 (black); *a* = + (red), – (green); *b* = + (yellow), – (blue).

^c Standard error.

^d ** = significant at *P* ≤ 0.01.

TABLE IV
Hunterlab Color Values (*L*, *a*, *b*)^a and Pigment Contents of Blue-, Purple-, and Red-Wheat Grains and Their Milled Products (% db)

Wheat Type and Product	<i>L</i>	<i>a</i>	<i>b</i>	Δ <i>E</i> ^b	Anthocyanins (mg/kg)	Carotenoids (mg/kg)
Whole meal						
Blue	74.7 ± 2.1 ^c	–0.9 ± 0.07	4.1 ± 0.2	18.6 ± 0.8	155.6 ± 4.1	5.2 ± 0.3
Purple	76.3 ± 2.5	1.6 ± 0.11	7.0 ± 0.2	17.9 ± 0.9	103.8 ± 1.3	7.2 ± 0.2
Red	80.3 ± 2.0	2.2 ± 0.09	10.6 ± 0.2	16.1 ± 0.8	5.2 ± 0.2	4.6 ± 0.2
Flour fraction						
Blue	87.7 ± 1.9	–1.1 ± 0.05	4.9 ± 0.1	6.5 ± 0.2	22.5 ± 0.8	2.4 ± 0.1
Purple	89.6 ± 1.6	–0.1 ± 0.01	9.5 ± 0.2	8.9 ± 0.4	5.2 ± 0.1	4.6 ± 0.2
Red	88.8 ± 0.9	0.3 ± 0.01	10.4 ± 0.2	10.1 ± 0.4	1.7 ± 0.1	2.9 ± 0.1
Bran fraction						
Blue	54.2 ± 2.5	0.2 ± 0.01	6.1 ± 0.2	39.2 ± 1.6	458.3 ± 11.3	11.0 ± 0.4
Purple	54.4 ± 2.1	3.2 ± 0.11	8.1 ± 0.3	39.5 ± 1.5	250.7 ± 6.3	13.6 ± 0.3
Red	61.4 ± 1.6	5.5 ± 0.12	14.3 ± 0.3	34.8 ± 0.7	10.4 ± 0.4	9.9 ± 0.3

^a *L* = 100 (white) to 0 (black); *a* = + (red), – (green); *b* = + (yellow), – (blue).

^b Total color difference.

^c Mean ± standard deviation.

wheat grains. This finding also was observed with purple-wheat milled products. Durum semolina, used as a control check for carotenoid measurement, contained the highest concentration of carotenoids (6.3 mg/kg) among wheat flours.

CONCLUSIONS

A dark-blue grain spring wheat with moderate protein content currently is being developed for use as a naturally colored food ingredient in cereal-based products. Because blue pigments are located in the outer layers of the grain, as indicated by a high concentration of anthocyanins in the bran fraction, blue wheat could be used in whole meal, crushed grain, or bran products. Anthocyanins in blue and purple wheats were more extractable in alcohol than in water at different pH levels. The pigment extracts were stable when stored at 4°C for up to eight weeks, with the exception of the appearance of cloudiness after the first week.

We developed a simple, rapid method for screening anthocyanin-pigmented wheats and other related cereals and quantifying total anthocyanins in these grains. Anthocyanins occurred at relatively high concentrations compared with other wheat pigments. We currently are developing an HPLC technique to determine the concentration of individual anthocyanins in blue and purple wheats that will provide a better understanding of the stability of these pigments.

ACKNOWLEDGMENTS

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