JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Identification and Quantification of Seed Carotenoids in Selected Wheat Species

EL-SAYED M. ABDEL-AAL,*,† J. CHRISTOPHER YOUNG,† IWONA RABALSKI,† PIERRE HUCL,‡ AND JUDITH FREGEAU-REID§

Food Research Program, Agriculture and Agri-Food Canada, 93 Stone Road West, Guelph, Ontario, Canada N1G5C9, Crop Development Centre, University of Saskatchewan, 51 Campus Drive, Saskatoon, Saskatchewan, Canada S7N5A8, and Eastern Cereal and Oilseed Research Centre, Agriculture and Agri-Food Canada, 960 Carling Avenue, Ottawa, Ontario, Canada K1A0C6

Selected primitive and modern wheat species were evaluated on the basis of their carotenoid composition and effects of the genotype and environment on lutein using spectrometry and liquid chromatography. Carotenoids in the wheat extracts were identified and confirmed on the basis of their UV/vis and mass spectra compared with those of authentic standards. The protonated molecule $(M + 1)^+$ at *m*/*z* 569 was the predominant ion for zeaxanthin compared to the fragment ion at *m*/*z* 551 for lutein. A similar carotenoid profile was obtained for the wheat species investigated, but significant differences were observed in the concentration of carotenoids. Einkorn (*Triticum monococcum*) exhibited the highest level of *all-trans*-lutein, averaging 7.41 μg/g with small amounts of *all-trans*-zeaxanthin, *cis*-lutein isomers, and β-carotene. Durum, Kamut, and Khorasan (*Triticum turgidum*) had intermediate levels of lutein (5.41−5.77 μg/g), while common bread or pastry wheat (*Triticum aestivum*) had the lowest content (2.01−2.11 μg/g). Lutein in einkorn appeared to be influenced significantly by environmental growing conditions.

KEYWORDS: Primitive wheat; modern wheat; corn; carotenoids; lutein; LC−UV/vis; LC−MS

INTRODUCTION

Carotenoids are isoprene derivatives found in common fruits, vegetables, and grains which impart yellow or orange color to these agricultural commodities. More than 600 carotenoids have been identified in plants (*1*). Of these only a few carotenoids including α-carotene, β-carotene, lycopene, lutein, zeaxanthin, and β-cryptoxanthin have been found in humans (*2*). Structures of these carotenoids and an example of a *cis*-isomer are presented in **Figure 1**. Lutein and zeaxanthin are non-provitamin A hydroxy-containing carotenoids that play significant roles in promoting the health of eyes and skin and in reducing the risk of age-related macular degeneration (*3*), cataract (*4*), cancer (*5*), and cardiovascular disease (*6*). Clinical studies have shown that lutein and zeaxanthin constitute the macular pigments in the yellow spot or macula lutea of the human retina, providing a protective function, i.e., protect the macula from damage by blue light (*7*), improve visual acuity (*8*), or scavenge harmful reactive oxygen species (*9*). In general, carotenoids are important nutrients for human health and must be provided in the diet.

Fruits and vegetables are generally considered the main dietary source of carotenoids, but certain cereals such as yellow corn, durum wheat, and specialty wheat (e.g., einkorn, Khorasan, and Kamut) have been found to contain relatively high amounts of carotenoids, mainly lutein and zeaxanthin (*10*−*13*). Such grains are staple foods and may offer promising raw ingredients for the development of high lutein functional foods, i.e., bread, cookies, muffins, etc. These foods would be essential for maintaining human health due to the currently low daily intake of lutein (1.5−2 mg) compared to the recommended dose (5−6 mg/day). At present little information has been reported about carotenoids in grains in terms of their composition, effects of the environment and genotype, or stability during storage and processing.

An earlier study on carotenoid composition in two wheat cultivars, Mindum (durum) and Thatcher (common), showed that the main carotenoids are lutein and its esters (*14*). However, both cultivars contained totally different concentrations of these carotenoids; i.e., free lutein amounted to 85% of the total carotenoids in Mindum, whereas lutein esters accounted for 78% in Thatcher wheat. Our previous study showed that lutein is the predominant carotenoid in wheat and accounts for 80−90% of the total carotenoids with small amounts of zeaxanthin, β-carotene, and lutein esters (*12*). In a more recent study, spring wheat flours were richer in carotenoids compared to the flours milled from winter wheats with a few exceptions (*15*). The main carotenoid in spring and winter wheat is lutein, ranging from 71% to 83%, being slightly higher in winter wheat. The present

* To whom correspondence should be addressed. Phone: (519) 780 8031. Fax: (519) 829-2600. E-mail: abdelaale@agr.gc.ca.
† Food Research Program, Agriculture and Agri-Food Canada.
‡ University of Saskatchewan.
§ Eastern Cereal and Oilseed Research Centre, Agriculture and Agri-Food Canada.

10.1021/jf062764p CCC: $37.00
Published on Web 01/03/2007

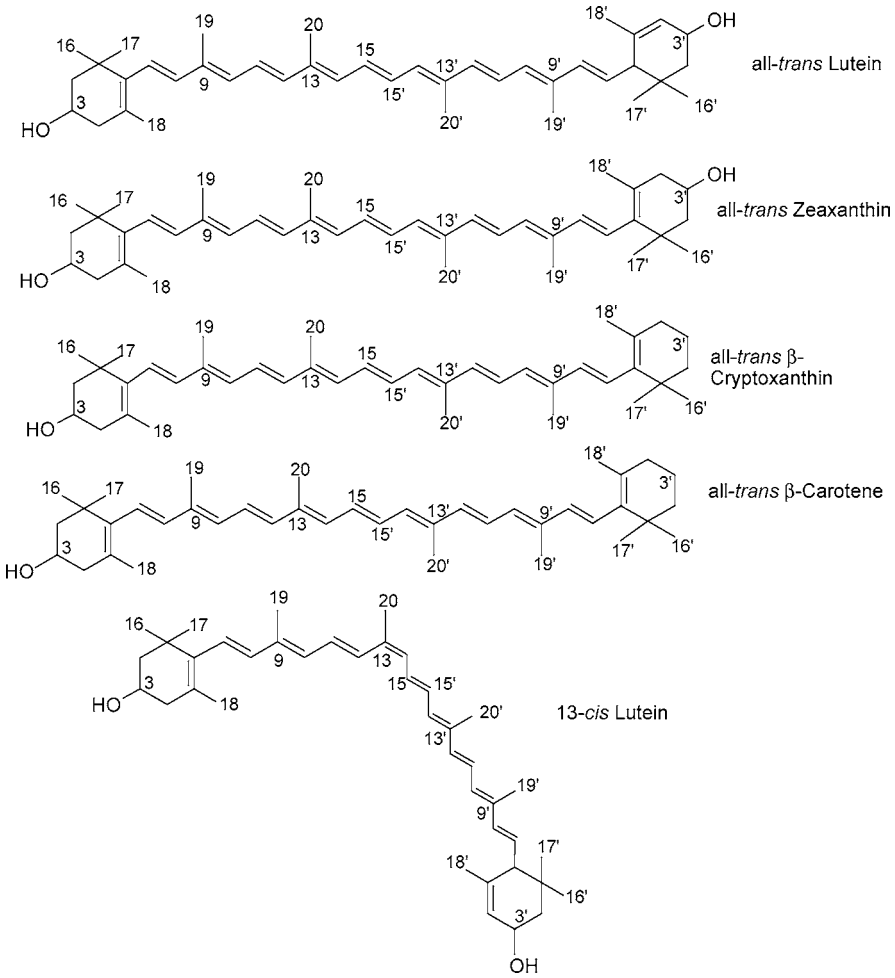

Figure 1. Structure of *all-trans*-lutein, zeaxanthin, β-cryptoxanthin, β-carotene, and 13-*cis*-lutein found in wheat and corn.

study was conducted to investigate the composition of carotenoids, particularly lutein isomers, in diverse wheat species to identify potential species for the development of high-lutein-wheat-based functional foods and to assess the stability of the trait in a number of growing environments for winter- and spring-sown einkorn.

MATERIALS AND METHODS

Wheat Materials. The wheat species used in the present study include two einkorn (*Triticum monococcum* L.) cultivars known to have a high content of lutein, AC Knowles and PI 418587 (*12*), in addition to 24 breeding lines, emmer (*Triticum turgidum* ssp. *dicoccum*) cultivar Vernal and 12 breeding lines, Khorasan (*T. turgidum* ssp. *turanicum*), accession PI211691, a commercial sample of Kamut (*T. turgidum* ssp. *turanicum*), durum (*T. turgidum* ssp. *durum*) cultivar Kyle, four spelts (*Triticum aestivum* ssp. *spelta*), two winter cultivars, Rotkorn and Frankencorn, and two spring cultivars, CDC Nexon and PGR8801, and four common wheats (*T. aestivum* ssp. *aestivum*), two bread or hard wheat cultivars, Katepwa and AC Barrie, and two soft or pastry wheat cultivars, AC Reed and Mendon. The wheat samples were obtained from plots grown at the experimental farms of the University of Saskatchewan, Saskatoon, SK, Canada, except for einkorn AC Knowles, which was obtained from the Eastern Cereals and Oilseeds Research Centre, Ottawa, ON, Canada, and the winter spelts and Mendon wheat that were obtained from the University of Guelph, Guelph, ON, Canada. A commercial yellow corn (*Zea mays*) meal sample was purchased from the retail market in Guelph, ON, Canada, and it was included in the study due to the high content of carotenoids.

AC Knowles einkorn, a cross between a tough glumed *T. monococcum* ssp. *monococcum* M-75-8 and a soft glumed *T. monococcum* ssp. *sinskajae* M-131-8, was collected over 6 years (1996−2001) to study the effects of the year on the lutein content. A second field experiment consisting of a 32-entry test grown as a two-replicate RCBD at four sites in Saskatchewan, Canada (Goodale Farm, Kernen Crop Research Farm, seed farm early seeding date, seed farm late seeding date) was designed to study the effects of the environment on the lutein content. The seed farm early and late seeding dates were 3−4 weeks apart, i.e., early May versus late May or early June, to provide different growing conditions. Three einkorn accessions, PI 418587, TM20450, and TM43450, and a CWRS hexaploid wheat check cultivar, AC Barrie, were entries in the 32-entry experiment. PI 418587 is a selection from *monococcum* subspecies *sinskajae*. The TM accessions were obtained from the Gatersleben Genebank in Germany. The plot size was 4.46 m^2 with five rows planted per plot.

Immediately after harvest, the grains were dried to approximately 10% moisture content and dehulled by passing the hulled grains between a pair of rubber-coated rollers followed by air aspiration. The dehulled (hulls removed by rollers) and hulless (free-threshing) grains were ground on a Cyclone sample mill (Udy Co., Fort Collins, CO) equipped with a 500 μm screen to obtain whole wheat flour. The wheat grains were milled into flour at an extraction rate of approximately 72% with a Brabender Quadrumat Jr. flour mill (Brabender Co., South Hackensack, NJ). The whole grain flours and white flours (72% extraction) were thoroughly mixed to ensure uniformity and kept at 4 °C until extraction and analysis.

Analytical Tests. *Total Yellow Pigment Content*. The total yellow pigment content in the wheat samples was determined using AACC Method 14-50 (*16*) with some modifications. The absorbance was measured at 450 nm (average λ_{max} for carotenoid wheat extracts) instead of 435.8 nm on a spectrophotometer (Varian Inc., Palo Alto, CA), and the total yellow pigment content was calculated on the basis of authentic

lutein (90% purity), which is the main yellow pigment in wheat (*12*), not on the basis of β-carotene as described in the AACC-approved method.

Extraction of Carotenoids. Five solvents (water-saturated 1-butanol, 80% aqueous ethanol, 80% aqueous methanol, methyl *tert*-butyl ether, and tetrahydrofuran, THF) were evaluated for their capacity to extract carotenoids from wheat on the basis of extraction and separation efficiency as determined by reading the absorbance at 450 nm in the colorimetric method and on the basis of the lutein content and shape of the lutein peak in the liquid chromatography (LC) method. In the following experiments, water-saturated 1-butanol was chosen for carotenoid extraction on the basis of the results obtained. An approximately 0.5 g wheat sample was homogenized in 10 mL of solvent for 30 s at 5000 rpm in a PT 10-35 Polytron homogenizer (Kinematica AG, Swizerland), kept for 30 min at room temperature, and homogenized again for 30 s. The mixture was centrifuged at 10000*g* for 5 min, and an aliquot of the supernatant (0.5 mL) was filtered through a 0.45 μm Nylon Acrodisc syringe filter (Pall Gelman Laboratory, Ann Arbor, MI). The first two drops of the filtrate were discarded, and the remainder was collected for LC analyses. All extraction experiments were performed under dim light, and the extraction tubes were wrapped with black paper to avoid sample degradation by photooxidation.

Analysis of Carotenoids by LC. The carotenoid extracts were separated and quantified by liquid chromatography with an 1100 series chromatograph (Agilent, Mississauga, ON) equipped with a G1311A quaternary pump, a G1329A temperature-controlled injector, a G1316A temperature-controlled column thermostat, a G1322A degasser, a G1315B photodiode array (PDA) detector, and a ChemStation v.8.04 data acquisition system with the capability of conducting isoabsorbance plot and 3D graphic analyses. The separation was performed on long (25 cm $\times$ 4.6 mm, packing 5 μm) and short (10 cm $\times$ 4.6 mm, packing 3 μm) C30 columns, YMC Carotenoid (Waters, Mississauga, ON). The columns were operated at 35 °C and eluted with a gradient mobile system consisting of (A) methanol/methyl *tert*-butyl ether/nanopure water (81:15:4, v/v/v) and (B) methyl *tert*-butyl ether/methanol (90:10, v/v) at 1 mL/min. The gradient was programmed as follows: 0−9 min, 100−75% A; 9−10 min, 75−0% A; 10−12 min, hold at 0% A; 12−13 min, 0−100% A; 13−15 min, hold at 100% A for the short column. With the long column, a linear gradient from 100% to 0% A within 90 min and back to the original solvent composition (100% A) within 5 min was used. The separated carotenoids were detected and measured at 450 nm, and the identification of the carotenoids was based on the congruence of retention times and UV/vis spectra with those of pure authentic standards.

Four pure authentic carotenoids that are common in grains, *all-trans*-lutein (90% purity) and *all-trans*-β-carotene (95% purity) purchased from Sigma (Sigma-Aldrich Canada Ltd., Oakville, ON) and *all-trans*-zeaxanthin (95% purity) and *all-trans*-β-cryptoxanthin (95% purity) from ChromaDex (ChromaDex Inc., Santa Ana, CA), were used as standards for identification and quantification. Five concentrations in the range of 4−85 ng per injection (20 μL) were prepared for each carotenoid in butanol and used to check the linearity, optimize the analytical method, and generate regression equations for quantification. The regression analysis of the response area and injected amount within the above range showed a linear relationship with a coefficient of determination (R^2) of 0.9997, 0.9993, 0.9990, and 0.9995 for *all-trans*-lutein, *all-trans*-zeaxanthin, *all-trans*-β-cryptoxanthin, and *all-trans*-β-carotene, respectively.

The purity of each compound in the grain extracts was verified on the basis of the spectroscopic properties of each peak using isoabsorbance plot or 3D graphic and peak purity analyses provided with the ChemStation software. Peak purity analysis allows the spectrum of the identified compounds to be identified and confirmed and to determine whether interference occurs.

Confirmation of Carotenoid Identity by Liquid Chromatography−Mass Spectrometry (LC−MS). Confirmation of the identity of each peak was carried out by LC−MS (Thermo Finnigan, San Jose, CA) with a SpectraSystem UV6000LP ultraviolet detector scanning from 190 to 800 nm and an LCQ Deca ion trap mass spectrometer operated in the electrospray positive ionization (ESI +ve) mode scanning from *m*/*z* 50 to *m*/*z* 2000. The LC−MS separation conditions were the same as for the LC−UV/vis analyses.

Machine operating conditions for ESI +ve ionization were as follows: Sheath gas and auxiliary flow rates were set at 91 and 4 (arbitrary units). Voltages on the capillary, tube lens offset, multipole RF amplifier, multipole 1 offset, multipole 2 offset, intermultipole lens, entrance lens, and trap dc offset were set at 35.5, 55.00, 770.00, −4.40, −8.00, −14.00, −58.00, and −10.00 V, respectively. The capillary temperature was set at 350 °C. The source voltage was 5.00 kV. The source current 80.0 μA.

Statistical Analysis. Data obtained from the field experiments were subjected to analysis of variance to study the effects of the genotype and environment on the lutein content, whereas the relationship between the analytical methods was determined by correlation analysis using Minitab software (version 12, Minitab Inc., State College, PA). The data were reported as the means of three replicates ± standard deviation (SD).

Table 1. Efficiency of Carotenoid Extraction from High-Lutein Einkorn Wheat Grains with Five Solvents

solvent	absorbance at 450 nm[a,c]	lutein content[b,c] (μg/g)	lutein peak width (s) and shape
water-saturated butanol	0.2 ± 0.007 a	7.63 ± 0.17 a	36, symmetrical
80% aqueous ethanol	0.23 ± 0.007 a	7.75 ± 0.33 a	36, symmetrical
80% aqueous methanol	0.15 ± 0.004 d	3.04 ± 0.14 b	36, symmetrical
methyl *tert*-butyl ether	0.18 ± 0.004 c	2.81 ± 0.11 c	48, nonsymmetrical
tetrahydrofuran	0.20 ± 0.005 b	2.16 ± 0.10 d	48, nonsymmetrical

[a] Determined by the spectrophotometric method. Figures are means ± SD. [b] Determined by the liquid chromatographic method. Figures are means ± SD. [c] Means within a column followed by the same letter are not significantly different at $p < 0.05$.

RESULTS AND DISCUSSION

Extraction of Carotenoids. Einkorn wheat was used to study the efficiency of five solvents, water-saturated 1-butanol, 80% aqueous ethanol, 80% aqueous methanol, methyl *tert*-butyl ether, and THF, in extracting carotenoids due to its significantly high content of lutein and total carotenoids compared to those of other wheat species (*12*). The efficiency of extraction was assessed on the basis of the measurement of absorbance for einkorn extracts at 450 nm and the separation efficiency of lutein by LC. Water-saturated 1-butanol and 80% aqueous ethanol were more efficient in extracting carotenoids from wheat, giving the highest absorbance reading and lutein concentration (**Table 1**). Water-saturated 1-butanol was used for the determination of total yellow pigment content in durum wheat for quality and screening purposes on the basis of the approved method of the American Association of Cereal Chemists (*16*). In the current study, the concentrations of lutein in water-saturated 1-butanol and 80% aqueous ethanol extracts were approximately 2.5 times higher than those in the other three solvent extracts. Additionally, these two extracts as well as 80% aqueous methanol extract produced a better lutein peak shape in the LC method on the basis of the bandwidth and symmetry of the peak (**Table 1**). The lutein peak had a symmetrical triangle-like shape and lower bandwidth in alcohol extracts compared to the other two solvents, methyl *tert*-butyl ether and THF. The effectiveness of various solvents on the extraction of carotenoids from dry and liquid tomatoes was investigated by Scott (*17*), who found that methanol is the most effective solvent for dried materials followed by ethanol, THF, acetone, and hexane, whereas THF was the best solvent for liquid products. Other studies used water-saturated 1-butanol (*12*), ethanol containing 0.1% butylated hydroxytoluene (*18*), or methanol (*19*) for extracting carotenoids from wheat, corn, and wheat, respectively. In the

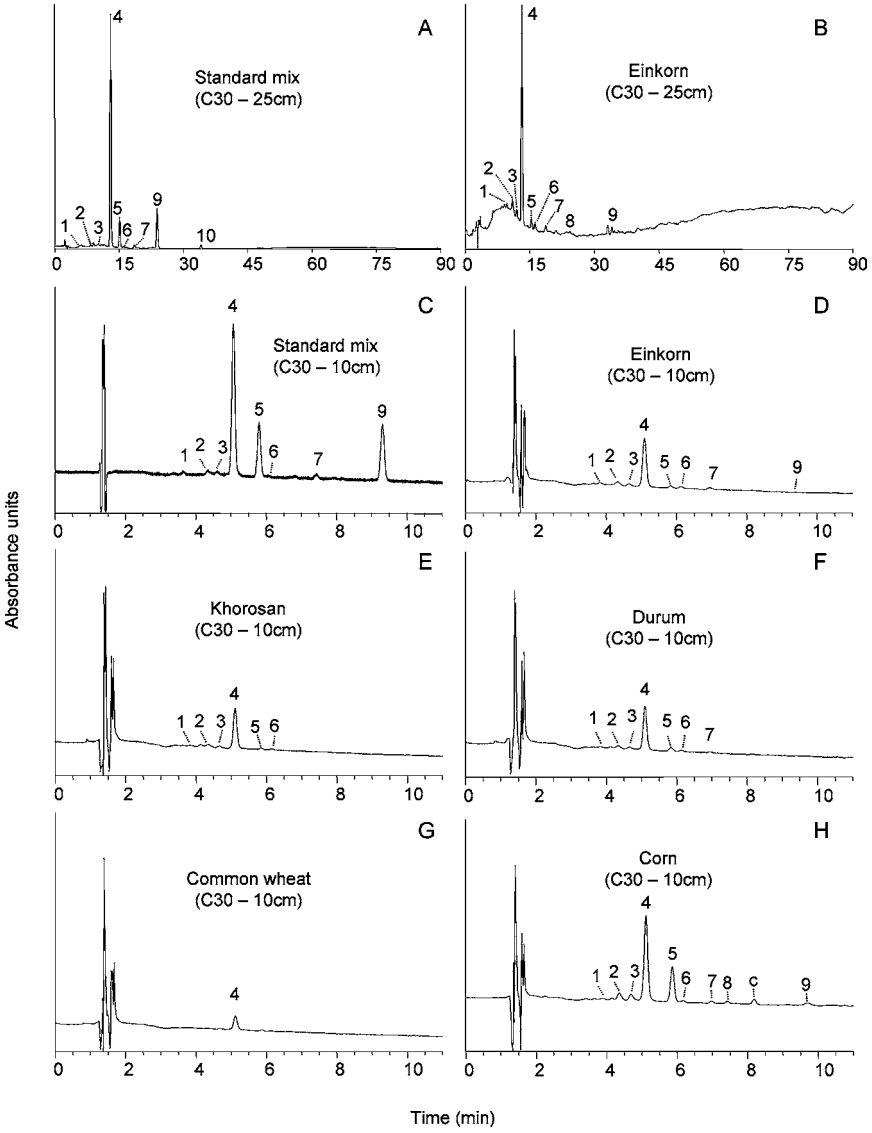

Figure 2. LC–UV/vis chromatograms of carotenoids separated from a standard mixture and einkorn, Khorasan, durum, common wheat, and corn extracts on long and short C30 columns: (1) 15-*cis*-lutein, (2) 13-*cis*-lutein, (3) 13′-*cis*-lutein, (4) *all-trans*-lutein, (5) *all-trans*-zeaxanthin, (6) 9-*cis*-lutein, (7) 9′-*cis*-lutein, (8) 9-*cis*-zeaxanthin, (9) *all-trans*-β-cryptoxanthin (10) *all-trans*-β-carotene.

present study, water-saturated 1-butanol was chosen for further experiments due to its common use in determining the total yellow pigment content for selecting intense amber durum wheat in a breeding program as well as in evaluating the quality of semolina and pasta.

Separation and Quantification of Carotenoids. Initially, separation of carotenoids in the standard mixture and wheat and corn extracts was performed on a long C30 column as described by Sander and others (*20*). This polymeric column is capable of separating hydroxylated and hydrocarbon carotenoids and their geometrical isomers in a single run, and it exhibited superior shape selectivity for the separation of lutein and zeaxanthin isomers compared to a C18 column (*21*). Typical LC chromatograms showing the separation and identity of carotenoids separated from standard mixtures and wheat and corn extracts on long and short C30 columns are presented in **Figure 2**. Separation of the four-carotenoid standard mixture (*all-trans*-lutein, *all-trans*-zeaxanthin, *all-trans*-β-cryptoxanthin, and *all-trans*-β-carotene) showed the presence of contaminant *cis*-isomer carotenoids including 15-*cis*-lutein, 13-*cis*-lutein, 13′-*cis*-lutein, 9-*cis*-lutein, and 9′-*cis*-lutein (**Figure 2A**). This separation was run for 95 min to be able to separate polar and nonpolar carotenoids present in wheat and corn extracts, and later the time was cut to 55 min since no carotenoids were detected after 40 min (**Figure 2B**). Later, the long C30 column was replaced with a short C30 column to reduce the run times further and to save solvents and cost while maintaining the same resolution. Similar quality separation was obtained with the short column for the standard mixture (**Figure 2C**) and einkorn extract (**Figure 2D**) compared to that of the long column (**Figure 2A,B**), but the run time was substantially reduced to only 15 min. The short column was also successfully employed for the separation of carotenoids from Khorasan (**Figure 2E**), durum (**Figure 2F**), common wheat (**Figure 2G**), and corn (**Figure 2H**) extracts.

As previously reported (*12*), *all-trans*-lutein is the main carotenoid found in all wheat extracts. This hydroxy group containing carotenoid constituted about 77−83% of the total carotenoids in the high-lutein wheat species studied (einkorn, Khorasan, and durum) (**Table 2**). *all-trans*-Zeaxanthin was the second major carotenoid and accounted for 9−13% of the total carotenoids. The ratio of lutein to zeaxanthin in two North American bread wheat cultivars that contain low levels of carotenoid was 7.6−11.0 compared to 2.5 for a green harvested

Table 2. Average Concentration (μg/g) of Carotenoids Found in Wheat Species and a Commercial Corn Sample As Determined by Liquid Chromatography

carotenoid	einkorn	Khorasan	durum	common wheat	corn
15-*cis*-lutein (1)[a]	0.31 ± 0.02	nd	nd	nd	0.45 ± 0.02
13-*cis*-lutein (2)	0.37 ± 0.02	0.13 ± 0.01	0.11 ± 0.02	nd	0.11 ± 0.01
13-*cis*-lutein (3)	0.35 ± 0.03	0.13 ± 0.01	0.10 ± 0.01	nd	0.10 ± 0.01
all-trans-lutein (4)	7.41 ± 0.19	5.53 ± 0.11	5.41 ± 0.15	1.89 ± 0.09	21.92 ± 0.66
all-trans-zeaxanthin (5)	0.94 ± 0.05	0.71 ± 0.04	0.49 ± 0.02	tr	10.31 ± 0.29
9-*cis*-lutein (6)	nd	0.06 ± 0.004	0.07 ± 0.004	nd	0.17 ± 0.02
9-*cis*-lutein (7)	0.11 ± 0.01	nd	nd	nd	0.25 ± 0.01
9-*cis*-zeaxanthin (8)	nd	nd	nd	nd	0.54 ± 0.03
all-trans-β-cryptoxanthin (9)	tr	nd	nd	nd	0.95 ± 0.06
all-trans-β-carotene (10)	0.13 ± 0.02	0.09 ± 0.004	0.09 ± 0.005	0.05 ± 0.002	0.31 ± 0.02
total	9.62	6.65	6.27	1.94	35.11

[a] Peak number as in Figure 2. nd = not detected, and tr = trace amount, <0.05 μg/g.

wheat (Freekeh) that exhibited a higher content of carotenoid (*11*). In the present study, this ratio was 7.7−10.8 on the basis of *all-trans* isomers. In general, common bread wheat contained low levels of lutein compared to einkorn, Khorasan, or durum since it has been bred over the years for low yellow pigment content. β-Carotene existed at very low concentration in wheat species, representing only about 1% of the total carotenoids, which suggests that lutein, the main carotenoid in wheat species, should be used in calculating the total yellow pigment content in durum and other wheats and their products, not β-carotene, which is currently used. Several *cis*-isomers were found in wheat grains such as 15-*cis*-lutein, 13-*cis*-lutein, 13′-*cis*-lutein, 9-*cis*-lutein, and 9′-*cis*-lutein (**Figure 2**). These geometrical isomers were relatively high in einkorn, the highest lutein wheat material, compared to Khorasan and durum.

Einkorn, Khorasan, and durum wheats contained higher levels of lutein (5.4−7.4 μg/g) compared to common wheat (1.9 μg/g) (**Table 2**). This indicates that these wheats hold a promise for the development of high-lutein-wheat functional food products. The high level of lutein in einkorn, Khorasan, or durum would enhance the daily intake of lutein, but still needs to be increased to provide the physiological dose. This can be achieved by genetic manipulation or carotenoid fortification. A recent study (*22*) showed that einkorn contains carotenoids, mostly lutein, at an about 2−4 fold higher level than non-einkorn wheat, with an average of 8.4 μg/g and a maximum of 13.4 μg/g. Corn was exceptionally high in lutein at a concentration of 21.9 μg/g (**Table 2**), showing good potential as a blending flour in the development of high-lutein-wheat-based functional foods. In addition to the high content of lutein, corn also had a high concentration of *all-trans*-zeaxanthin (10.3 μg/ g) and small concentrations of *all-trans*-β-cryptoxanthin, 15-*cis*-lutein, 13-*cis*-lutein, 13′-*cis*-lutein, 9-*cis*-lutein, 9′-*cis*-lutein, 9-*cis*-zeaxanthin, and *all-trans*-β-carotene (**Table 2**). The total carotenoid content determined by LC in einkorn, Khorasan, and durum extracts was about 3.2−5.0 times higher than that in common wheat, while that in corn extracts was about 3.6 times higher than the carotenoid levels in einkorn extracts, the highest carotenoid wheat studied.

The identity of carotenoids in wheat and corn extracts was confirmed on the basis of UV/vis and MS properties using authentic carotenoid standards. UV/vis data provide a means of confirming the presence of *cis*-isomers. **Figure 3** shows UV/vis absorption maxima for *all-trans*- and *cis*-lutein. *all-trans*- and *cis*-lutein exhibited three similar absorption peaks, λ_{I}, λ_{II} (λ_{max}), and λ_{III}, at approximately 420, 444, and 475 nm, plus λ_{cis} at 338 nm only in the case of *cis*-isomers. In the absence of standards for *cis*-isomers, these compounds were first characterized by their λ_{max} and the identification of individual *cis*-isomers was based on mass spectra and the relative retention times given by Sander et al. (*20*). The absorption of the *cis*-isomer at 330 nm in combination with mass spectrometric characteristics was previously used to identify *cis*-lutein isomers in wheat (*23*) and marigold flowers (*24*). Wheat and corn extracts contained several *cis*-isomers (**Table 2** and **Figure 2**). Other studies reported 13-, 13′-, 9-, and 9′-*cis*-lutein and 9- and 13-*cis*-zeaxanthin in wheat (*11*, *15*).

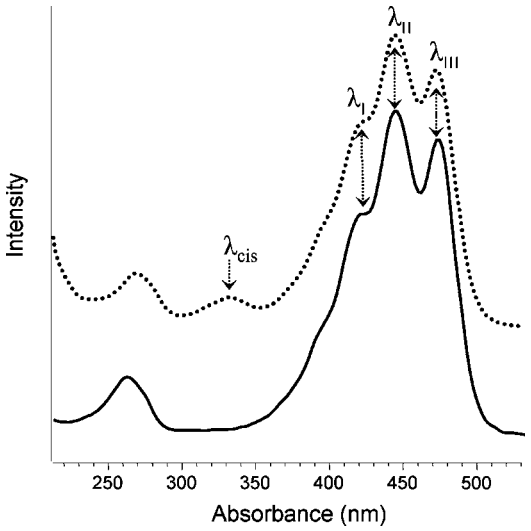

Figure 3. Absorbance spectra of *all-trans*-lutein (solid line) and *cis*-lutein (dotted line).

LC−MS was also employed to identify or confirm the identity of the separated carotenoids in wheat and corn extracts. Lutein appears as a protonated $(M + 1)^+$ ion at *m*/*z* 569 under ESI +ve ionization conditions. The molecule then sequentially loses two molecules of water to yield ions at *m*/*z* 551 and 533. These ions along with their relative abundance pattern were used to confirm the identity of lutein in the grain extracts. Although lutein and zeaxanthin have similar molecular weight and structure (**Figure 1**), they exhibited different mass spectra under ESI +ve ionization conditions. The predominant ion for zeaxanthin was the protonated molecular $(M + 1)^+$ ion at *m*/*z* 569 compared to the fragment ion at *m*/*z* 551 in the case of lutein. The different fragmentation patterns for lutein and zeaxanthin could be attributed to the subtle differences in their chemical properties; i.e., in zeaxanthin the two ionone rings are β types, whereas lutein has a β-ionone ring and an ϵ-ionone ring. The ϵ-ionone ring has a C4′−C5′ double bond adjacent to the 3′-hydroxyl group (**Figure 1**), which could encourage more

Table 3. Range and Concentration of Total Yellow Pigments Calculated as Lutein Equivalents and Lutein Determined by Liquid Chromatography in Selected Wheat Species (μg/g)

wheat species	total yellow pigment content range	total yellow pigment content mean[b]	CV (%)	lutein content range	lutein content mean[b]	CV (%)
einkorn (26)[a]	8.56–13.79	10.21 a	12.0	6.37-8.46	7.41 a	14.9
Khorasan (1)	7.66–8.51	8.15 b	3.7	5.58-6.07	5.53 b	3.9
Kamut (1)	7.71–8.52	8.11 b	3.2	5.55-5.97	5.77b	3.3
durum (1)	7.31–7.88	7.61 b	2.5	5.23-5.60	5.41b	3.1
emmer (13)	3.91–6.67	5.76 c	15.9	3.21-4.69	3.97 c	17.3
hard wheat (2)	2.77–3.15	3.01 de	6.1	1.94-2.27	2.11 d	6.5
soft wheat (2)	2.75–3.07	2.89 e	5.8	1.93-2.25	2.01 d	6.7
spelt (4)	3.55–5.21	4.01 d	18.2	0.93-2.01	1.47 d	23.1

[a] Number of cultivars/breeding lines analyzed. [b] Means within a column followed by the same letter are not significantly different at $p < 0.05$.

loss of water molecules under ESI +ve ionization conditions. For β-cryptoxanthin and β-carotene the protonated molecular $(M + 1)^+$ ions at m/z 553 and 537 were the major fragment ions, respectively.

Total Yellow Pigment versus Lutein Content. The total yellow pigment (TYP) content is a common test used in screening durum in breeding programs and in determining the quality of semolina and pasta products. The present study and previous investigations (*12*, *15*, *25*) have shown that lutein is the main carotenoid in wheat, and thus, the relationship between TYP and lutein content may warrant investigation. In fact, several studies have recently investigated the relationship between color and carotenoids in wheat and triticale (*26*) and durum (*27*, *28*). The range and concentration of TYP and lutein in different wheat species is presented in **Table 3**, while the relationship between these two parameters is presented in **Figure 4**. The TYP mean values ranged from 2.9 to 10.2 μg/g compared with 1.5−7.4 μg/g for lutein and showed genetic diversity among wheat species in their content of carotenoids. Additionally, significant variability within wheat species was observed as indicated by the high coefficient of variability values in particular for those species with a large number of samples being analyzed. There were significant positive associations between TYP and lutein content (**Figure 4A**) or TYP and the summation of individual or total carotenoids (**Figure 4B**) with a correlation coefficient (r) of 0.9442 and 0.9886, respectively ($n = 25$). This demonstrates that the colorimetric method or TYP content would be a good predictor of the lutein or total carotenoid content in wheat. In the current colorimetric method two main changes were made: the absorbance was read at 450 nm, and the TYP was calculated as lutein equivalents instead of reading at 438.5 nm and measuring total yellow pigments as β-carotene as in the AACC-approved method. These modifications reflect lutein as the main carotenoid in wheat, which resulted in more accurate determination for the total yellow pigment content. The slope of the relationship between TYP and lutein or TYP and total carotenoids by LC was 1.2260 and 1.1971, respectively (**Figure 4**). This shows that the colorimetric method overestimated the LC method by about 23% for lutein and 20% for total carotenoids, perhaps due to the contribution of other pigments that may exist in the wheat extracts. Several studies showed the positive relationship between color and carotenoids in wheat and triticale (*25*) and durum (*26*, *27*).

The wheat samples were milled into flour and bran fractions to study the distribution of lutein in the kernel of different species. Lutein was found to be higher in the flour fractions compared to the bran fractions except for common wheat, a

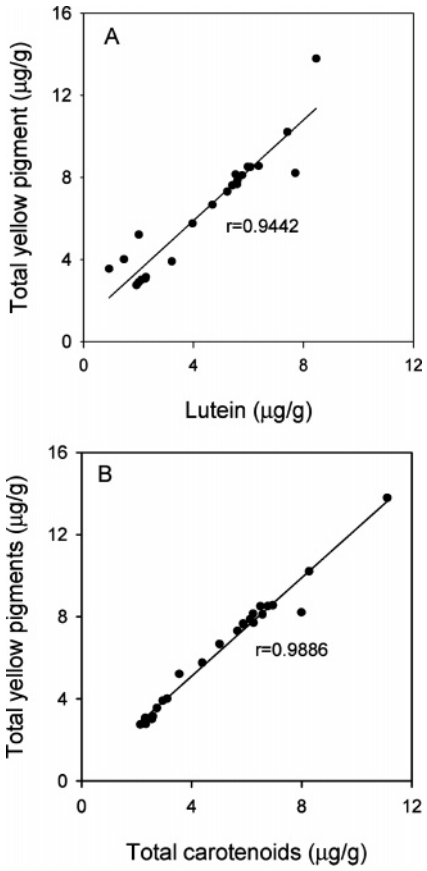

Figure 4. Correlation between the total yellow pigment content determined by spectrophotometry and lutein (**A**) or the total carotenoid content determined by liquid chromatography (**B**).

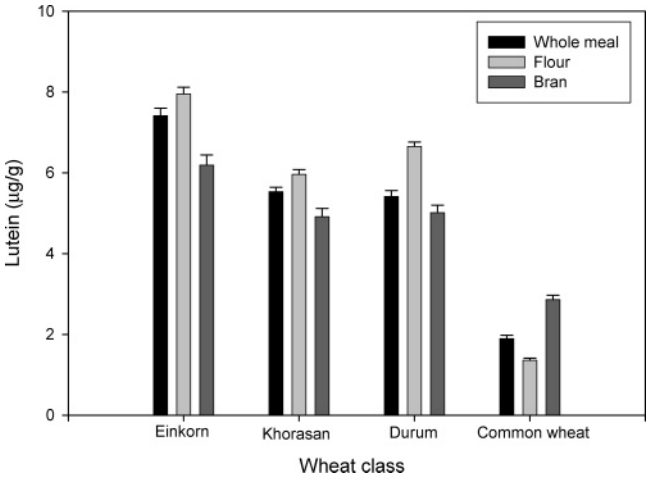

Figure 5. Distribution of lutein in wheat kernels of different species.

low-lutein wheat class (**Figure 5**). It seems that lutein is mainly concentrated in the endosperm in high-lutein wheats such as einkorn, Khorasan, and durum. Several reports have shown that these wheat species produce yellow flours (*29*, *30*). In light of the roles of lutein and zeaxanthin in the health of eyes and skin, such yellow flours may hold potential as natural functional food ingredients.

Effect of the Harvest Year, Environment, and Growing Conditions on the Lutein Content. Several studies have shown that environmental and growing conditions significantly affect bioactive components such as phenolic acids (*31*), total phenolic content (*32*), and antioxidant activity (*33*, *34*) in wheat. Little or no data have been published with regard to the effects of

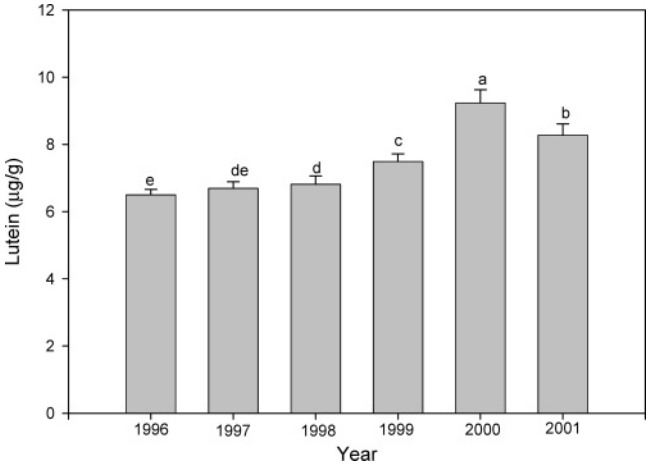

Figure 6. Effect of the harvest year on the lutein content of einkorn cultivar AC Knowles. Columns having the same letter are not significantly different at $p < 0.05$.

Table 4. Average Lutein Content As Influenced by the Wheat Cultivar and Location

variable	lutein content[a] (mg/g)
cultivar	
wheat AC Barrie	1.18 ± 0.11 c
einkorn PI 418587	4.69 ± 0.90 b
einkorn TM20450	6.32 ± 0.97 a
einkorn TM43450	6.41 ± 0.55 a
location	
Goodale	5.51 ± 2.89 a
Kernen	4.32 ± 2.30 b
seed farm, early	4.63 ± 2.51 b
seed farm, late	4.16 ± 2.14 b

[a] Within cultivar or location, means within a column followed by the same letter are not significantly different at $p < 0.05$.

genetics and environmental conditions on lutein in wheat. The effect of the year on the lutein content in einkorn cultivar AC Knowles over a 6-year period is presented in **Figure 6**. In general, the lutein content varied significantly over the years, ranging from 6.5 μg/g (1996) to 9.2 μg/g (2000); in 2000, the lutein content was exceptionally high. During the examined period, the months of May, June, and July were warmer and drier except for in 2000, when they were cooler and wetter. These findings suggest that it will be difficult to control the carotenoid content as environmental conditions will vary from year to year during grain development, and thus, choosing high-lutein wheat materials will be critical to minimize losses in lutein.

In another experiment, three einkorn accessions selected for a high content of lutein and a common wheat check grown in four environments in 2003 were used to investigate the influence of the growing site on the lutein content. Significant differences in the lutein content were observed among wheat accessions and sites (**Table 4**). As expected, the common wheat check contained the lowest level of lutein, whereas einkorn accessions had higher levels of lutein. Among the three einkorn wheats there were significant differences at all four sites. These field experiments demonstrate that the lutein content is influenced by the growing conditions. Further research is needed to measure genotype × environment interaction and to determine optimal agronomic practices to maximize the concentration of lutein and other carotenoids in wheat.

In conclusion, high-lutein wheat species were found to exhibit simple carotenoid profiles consisting mainly of *all-trans*-lutein and *all-trans*-zeaxanthin with small concentrations of other carotenoids and isomers, but exhibited significant differences in lutein content. Lutein in einkorn wheats appeared to be influenced significantly by the environmental growing conditions, which suggests more research is needed to determine optimal agronomic practices to maximize the concentration of lutein and to study the effects of genotype × environment interaction on lutein and other bioactive constituents in wheat.

all-trans-Lutein was found at considerable levels in *monococcum* and *turgidum* wheats, whereas corn contained substantial levels of *all-trans*-lutein and zeaxanthin. Since lutein and zeaxanthin have been reported to play significant roles in promoting the health of eyes and skin and in reducing the risk of several chronic diseases, the identified high-lutein wheat and corn materials could be used to enhance the daily intake of lutein as staple foods. In this respect, the stability and efficacy of these carotenoids in the end products are being investigated.

ACKNOWLEDGMENT

The excellent technical assistance provided by C. Perron and H. Braitenbach is greatly appreciated.

LITERATURE CITED

(1) Kahklon, T. S.; Keagy, P. M. Benefits and sources of functional foods. *Cereal Foods World* **2003**, *48*, 112−115.
(2) Khachik, F.; Englert, G.; Daitch, C. E.; Beecher, G. R.; Tonucci, L. H.; Lusby, W. R. Isolation and structural elucidation of the geometrical isomers of lutein and zeaxanthin in extracts from human plasma. *J. Chromatogr., A* **1992**, *582*, 153−159.
(3) Bone, R. A.; Landrum, J. T.; Mayne, S. T.; Gomez, C. M.; Tibor, S. E.; Twaroska, E. E. Macular pigment in donor eyes with and without AMD: A case-control study. *Invest. Ophthalmol. Visual Sci.* **2001**, *42*, 235−240.
(4) Olmedilla, B.; Granado, F.; Blanco, I.; Vaquero, M.; Cajigal, C. Lutein in patients with cataracts and age-related macular degeneration: A long-term supplementation study. *J. Sci. Food Agric.* **2001**, *81*, 904−909.
(5) Michaud, D. S.; Feskanich, D.; Rimm, E. B.; Colditz, G. A.; Speizer, F. E.; Willett, W. C.; Giovannucci, E. Intake of specific carotenoids and risk of lung cancer in 2 prospective US cohorts. *Am. J. Clin. Nutr.* **2000**, *72*, 990−997.
(6) Osganian, S. K.; Stampfer, M. J.; Rimm, E.; Spiegelman, D.; Manson, J. E.; Willett, W. C. Dietary carotenoids and risk of coronary disease in women. *Am. J. Clin. Nutr.* **2003**, *77*, 1390−1399.
(7) Sperling, H. G.; Johnson, C.; Harwerth, R. S. Differential spectral damage to primate cones. *Vision Res.* **1980**, *20*, 1117.
(8) Olmedilla, B.; Granado, F.; Blanco, I.; Vaquero, M. Lutein, but not α-tocopherol, supplementation improves visual function in patients with age-related cataracts: A 2-y double-blind placebo-controlled pilot study. *Nutrition* **2003**, *19*, 21−24.
(9) Handelman, G. J. The evolving role of carotenoids in human biochemistry. *Nutrition* **2001**, *17*, 818−822.
(10) Frégeau-Reid, J.; Abdel-Aal, E.-S. M. Einkorn: A potential functional wheat and genetic resource. In *Specialty grains for food and feed*; Abdel-Aal, E.-S. M., Wood, P. J., Eds.; American Association of Cereal Chemists, Inc.: St. Paul, MN, 2005; pp 37−61.
(11) Humphries, J. M.; Khachik, F. Distribution of lutein, zeaxanthin and related geometrical isomers in fruit, vegetables wheat and pasta products. *J. Agric. Food Chem.* **2003**, *51*, 1322−1327.
(12) Abdel-Aal, E.-S. M.; Young, J. C.; Wood, P. J.; Rabalski, I.; Hucl, P.; Frégeau-Reid, J. Einkorn: A potential candidate for developing high lutein wheat. *Cereal Chem.* **2002**, *79*, 455−457.
(13) Moros, E. E.; Darnoko, D.; Cheryan, M.; Perkins, E. G.; Jerrell, J. Analysis of xanthophylls in corn by HPLC. *J. Agric. Food Chem.* **2002**, *50*, 5787−5790.

(14) Lepage, M.; Sims, R. P. Carotenoids of wheat flour: Their identification and composition. *Cereal Chem.* **1968**, *45*, 600−604.
(15) Konopka, I.; Czaplicki, S.; Rotkiewicz, D. Differences in content and composition of free lipids and carotenoids in flour of spring and winter wheat cultivated in Poland. *Food Chem.* **2006**, *95*, 290−300.
(16) American Association of Cereal Chemists. Method 15-50. *Approved Methods of the AACC*, 10th ed.; St. Paul, MN, 2000.
(17) Scott, K. J. Observations on some of the problems associated with the analysis of carotenoids in foods by HPLC. *Food Chem.* **1992**, *45*, 357−364.
(18) Kurilich, A. C.; Juvik, J. A. Simultaneous quantification of carotenoids and tocopherols in corn kernel extracts by HPLC. *J. Liq. Chromatogr. Relat. Technol.* **1999**, *22*, 2925−2934.
(19) Zandomeneghi, M.; Festa, C.; Carbanaro, L. Front-surface absorbance spectra of wheat flour: Determination of carotenoids. *J. Agric. Food Chem.* **2000**, *48*, 2216−2221.
(20) Sander, L. C.; Sharpless, K. E.; Pursch, M. C30 stationary phases for the analysis of food by liquid chromatography. *J. Chromatogr., A* **2000**, *880*, 189−202.
(21) Dachtler, M.; Kohler, K.; Albert, K. Reversed-phase high-performance liquid chromatographic identification of lutein and zeaxanthin stereoisomers in bovine retina using a c30 bonded phase. *J. Chromatogr., B* **1998**, *720*, 211−216.
(22) Hidalgo, A.; Brandolini, A.; Pompei, C.; Piscozzi, R. Carotenoids and tocols of einkorn wheat (*Triticum monococcum ssp. monococcum* L.). *J. Cereal Sci.* **2006**, *44*, 182−193.
(23) Abdel-Aal, E.-S. M.; Akhtar, M. H. Recent advances in the analyses of carotenoids and their role in human health. *Curr. Pharm. Anal.* **2006**, *2*, 195−204.
(24) Tsao, R.; Yang, R.; Young, J. C.; Zhu, H.; Manolis, T. Separation of geometric isomers of native lutein diesters in marigold (*Tagetes erecta* L.) by high-performance liquid chromatography-mass spectrometry. *J. Chromatogr., A* **2004**, *1045*, 65−70.
(25) Panfili, G.; Fratianni, A.; Irano, M. Improved normal-phase high-performance liquid chromatography procedure for the determination of carotenoids in cereals. *J. Agric. Food Chem.* **2004**, *52*, 6373−6377.
(26) Humphries, J. M.; Graham, R. D.; Mares, D. J. Application of reflectance colour measurement to the estimation of carotene and lutein content in wheat and triticale. *J. Cereal Sci.* **2004**, *40*, 151−159.
(27) Fratianni, A.; Irano, M.; Panfili, G.; Acquistucci, R. Estimation of color of durum wheat. Comparison of WSB, HPLC and reflectance colorimeter. *J. Agric. Food Chem.* **2005**, *53*, 2373−2378.
(28) Hentsche, V.; Kranl, K.; Hollmann, J.; Lindhauer, M. G.; Böhm, V.; Bitsch, R. Spectrophotometric determination of yellow pigment content and evaluation of carotenoids by high-performance liquid chromatography in durum wheat grain. *J. Agric. Food Chem.* **2002**, *50*, 6663−6668.
(29) Abdel-Aal, E.-S. M.; Hucl, P.; Sosulski, F. W.; Bhirud, P. R. Kernel, milling and baking properties of spring-type spelt and einkorn wheats. *J. Cereal Sci.* **1997**, *26*, 363−370.
(30) D'Egidio, M. G.; Nardi, S.; Vallege, V. Grain, flour and dough characteristics of selected strains of diploid wheat, *Triticum monococcum* L. *Cereal Chem.* **1993**, *70*, 298−303.
(31) Abdel-Aal, E.-S. M.; Hucl, P.; Sosulski, F. W.; Graf, R.; Gillott, C.; Pietrzak, L. Screening spring wheat for midge resistance in relation to ferulic acid content. *J. Agric. Food Chem.* **2001**, *49*, 3559−3566.
(32) Yu, L.; Zhou, K. Antioxidant properties of bran extracts from "Platte" wheat grown at different locations. *Food Chem.* **2004**, *90*, 311−316.
(33) Yu, L.; Haley, S.; Perret, J.; Harris, M. Comparison of wheat flours grown at different locations for their antioxidant properties. *Food Chem.* **2004**, *86*, 11−16.
(34) Yu, L.; Perret, J.; Harris, M.; Wilson, J.; Haley, S. Antioxidant properties of bran extracts from "Akron" wheat grown at different locations. *J. Agric. Food Chem.* **2003**, *51*, 1566−1570.

Received for review September 26, 2006. Revised manuscript received November 17, 2006. Accepted November 17, 2006. This is Scientific Publication S284 of the Food Research Program, Agriculture and Agri-Food Canada, Guelph, ON, Canada. The research conducted at the University of Saskatchewan was funded by a grant from the Saskatchewan Agriculture Development Fund.

JF062764P