NOTE

Einkorn: A Potential Candidate for Developing High Lutein Wheat

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The role of dietary components in promoting good health and reducing the risk of chronic diseases has become well recognized and scientifically accepted. As a result, several health claims for food and food components including cereal-based products are allowed under the United States Food and Drug Administration's nutrition labelling regulations (Anonymous 2001). Besides the health benefits associated with whole grain consumption, cereals also are recognized sources of health-enhancing bioactive components such as dietary fiber, phenolics, tocopherols, and carotenoids. Development of genotypes of cereal grains with high levels of such components should be of interest to cereal producers and processors as well as consumers. Einkorn, a small soft-seeded diploid wheat (*Triticum monococcum* L.), contains elevated levels of protein and yellow pigments (D'Egidio et al 1993; Abdel-Aal et al 1995; Borghi et al 1996; Corbellini et al 1999; Matuz et al 2000). It had been reported also earlier in the past century, that einkorn flour possessed high protein and yellow color (Clerc et al 1918). Einkorn may also hold promise for the production of bakery products rich in carotenoids and proteins (Borghi et al 1996). However, no data are reported on individual carotenoids and xanthophylls in einkorn.

Lutein, a xanthophyll (hydroxylated carotenoid), is the major yellow pigment in wheat (*T. aestivum* L.) (Lepage and Sims 1968). A number of human epidemiological (Seddon et al 1994; Brown et al 1999; Chasan et al 1999) and clinical (Bone et al 2001; Landrum and Bone 2001) studies have shown that lutein intake is associated with reduced incidence of age-related macular degeneration (AMD), the leading cause of irreversible blindness in elderly people, and cataracts. In addition, lutein and zeaxanthin were the only carotenoids found in the macular region of the retina, supporting a protective role for lutein in AMD (Fullmer and Shao 2001). A daily intake of ≈6 mg of lutein might reduce the risk of AMD by ≈57%, relative to an intake of 0.5 mg per day (Seddon et al 1994). In mice, dietary lutein delayed and inhibited mammary tumor growth and enhanced lymphocyte proliferation (Chew et al 1996). In addition, diets rich in carotenoids were associated with a reduced incidence of cancer (Hughes 2001) and better health (Handelman 2001). The current study was undertaken to identify new alternative and functional wheats based on lutein content.

MATERIALS AND METHODS

Selected Canadian-grown alternative wheats, including three genotypes and 12 breeding lines of einkorn, four winter and spring spelts (*T. aestivum* ssp. *spelta*), one cultivar each of emmer (*T. turgidum* spp. *dicoccum*), Kamut (*T. turgidum* spp. *turanicum*), and Khorasan (*T. turgidum* spp. *turanicum*) were used in the present study. One cultivar each of commercially produced hard red spring wheat cv. Katepaw (*T. aestivum* spp. *aestivum*), soft white spring wheat cv. AC Reed, and durum cv. Kyle (*T. turgidum* spp. *durum*) were also included. The einkorn accessions or cultivars, AC Knowles, PI 418587, and TM23 (PI 355523) were spring types. The 12 breeding lines of einkorn are sister lines of AC Knowles and are all from a cross between a tough-glumed *T. monococcum* ssp. *monococcum* M-75-8 (Cereal Research Centre, Winnipeg, MB) and a soft-glumed *T. monococcum* ssp. *sinskajae* M-131-8 (Univ. of California, Riverside, CA). The wheats were ground into whole meal and roller-milled into flour at an extraction rate of ≈72% and into semolina at a yield of ≈55%.

Carotenoids were extracted from wheat materials with water saturated 1-butanol, centrifuged at 1,000 × *g* for 10 min and filtered through Whatman filter paper #41 followed by a 0.45 μm filter. Purified extracts were separated and quantified by HPLC (Thermo Finnigan, Riviera Beach, FL) equipped with quaternary gradient pump (Spectra System P 4000), autosampler (20 μL) (Spectra System AS 3000), and data acquisition system (Xcalibur). A reversed-phase C18 column (250 × 4.6 mm, 5 μm particle size, WAT 054275, Waters, Mississauga, ON) was used and operated at room temperature. The column was eluted at 1 mL/min with a mobile phase gradient of acetonitrile-methanol-methylene chloride changing from 60:30:10 (v/v) to 50:10:40 over 20 min and held for 9 min before being returned to the original composition within 1 min. The separated compounds were subsequently detected and identified with a photodiode array ultraviolet-visible detector (Spectra System UV 6000 LP) and mass spectrometric detector (LCQDeca). Lutein and β-carotene from Sigma (Oakville, ON) were used for calibration and quantification. The concentrations of lutein and β-carotene in standard solutions were in the range of 0.0–3.6 μg/mL and 0.0–0.2 μg/mL, respectively. These ranges were selected to roughly imitate lutein and β-carotene levels in wheat extracts and to be within the linear range of the calibration curves. The coefficient of determination for both compounds was similar ($r^2 = 0.99$). The identity of carotenoids was confirmed on the basis of chromatographic retention times and by coelution with added standards. Identity was further confirmed by congruence of UV and atmospheric pressure chemical ionization mass spectra. Lutein and β-carotene from standards and wheat extracts were characterized by triple-absorption maxima at 422, 447, and 475 nm and at 429, 457, and 484 nm, respectively. Each wheat sample was extracted twice and each extract was analyzed in duplicate. The results are presented as means of four replicates.

RESULTS AND DISCUSSION

In this study, samples of einkorn contained the highest level of lutein among the selected wheat species (Table I). But one of the einkorn samples, TM23, did not contain a high level of lutein. The flour fraction of einkorn samples with high lutein content (PI 418587 and AC Knowles) had higher levels of lutein compared with the bran fraction. The same trend was found in wheats exhibiting intermediate lutein contents (5.4–6.1 mg/kg), such as Khorasan, Kamut, and durum. Einkorn flour fractions obtained from PI 418587 and AC Knowles contained about sixfold more lutein than hard red spring (HRS) wheat, averaging 8.6 mg/kg compared with 1.5 mg/kg. The average lutein content in einkorn whole meals was 8.1 mg/kg for the 12 breeding lines (Table II).

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TABLE I
Lutein Content (mg/kg, db) of Alternative Wheats Grown in Canada

Wheat	Cultivar/Accession	2n Chromosome Number	Product			
			Whole Meal	Flour	Bran	Semolina
Spring Einkorn	TM23	14	1.78	1.61	2.16	. . .
Spring Einkorn	PI 418587	14	8.23	9.05	7.01	. . .
Spring Einkorn	AC Knowles	14	7.29	8.24	5.93	. . .
Spring Emmer	Vernal	28	3.68	2.71	4.19	2.72
Spring Khorasan	PI 211691	28	5.37	6.69	5.22	6.09
Kamut	Commercial	28	6.09	6.67	5.51	6.57
Durum	Kyle	28	5.93	7.42	5.67	6.65
Spring spelt	CDC Bavaria	42	1.03	0.73	1.92	. . .
Spring spelt	PGR8801	42	1.31	0.68	2.41	. . .
Winter spelt	Frankencorn	42	1.61	1.55	2.19	. . .
Winter spelt	Rotkorn	42	2.58	2.18	3.23	. . .
Soft wheat	AC Reed	42	2.39	3.49	3.06	. . .
HRS wheat	Katepwa	42	2.09	1.54	3.22	. . .
LSD ($P < 0.05$)			0.21	0.23	0.15	0.35

TABLE II
Lutein Content in Whole Meals of Selected Einkorn Breeding Lines

Accession Number	Lutein Content[a] (mg/kg, db)
33-56-2	
Line 1	8.14 ± 0.03
Line 2	8.11 ± 0.09
Line 3	7.81 ± 0.07
Line 4	8.27 ± 0.03
Line 5	8.18 ± 0.03
Line 6	7.88 ± 0.08
33-129-3	
Line 1	8.00 ± 0.02
Line 2	8.14 ± 0.07
Line 3	8.05 ± 0.02
Line 4	7.70 ± 0.02
Line 5	8.30 ± 0.10
Line 6	8.69 ± 0.02
Overall mean	8.10
Standard deviation	0.26

[a] Mean ± standard deviation, $n = 4$.

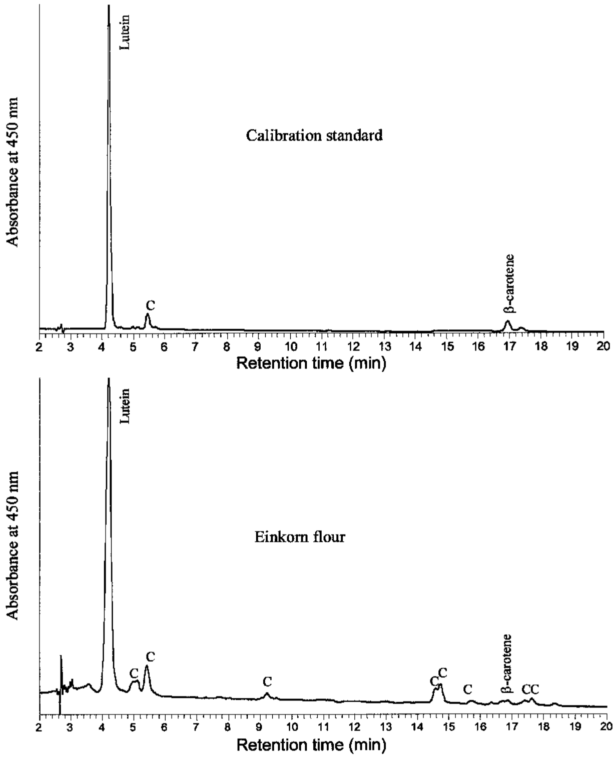

Fig. 1. HPLC chromatograms of lutein and β-carotene in standard and einkorn flour. C = unknown compounds presumed to be carotenoids.

Durum cv. Kyle, a white-seeded tetraploid wheat bred for a high level of yellow pigment was relatively high in lutein, ≈80% of that in einkorn. This would make durum another choice for developing high-lutein wheat. β-Carotene was found at very low levels in all wheats (0.02–0.12 mg/kg in the whole meals). Several unknown compounds, presumed to be carotenoids, also were observed in einkorn on the basis of the characteristic triple-absorption maxima of carotenoids (Fig. 1). Further investigations are underway to identify these compounds and generally to provide more detailed information on carotenoids in wheat.

The results obtained demonstrate that einkorn wheat accessions contain higher levels of lutein and may hold potential as high-lutein functional wheats. The examination of a larger number of monococcum accessions is required to verify our findings. Several studies have shown that einkorn wheats possess high levels of carotenoids or yellow pigments (D'Egidio et al 1993; Abdel-Aal et al 1995; Borghi et al 1996; Corbellini et al 1999). The tetraploid wheats examined (Khorasan, Kamut, and durum) had intermediate lutein content, which make them another potential source of high-lutein wheat. Emmer wheat cv. Vernal was the exception. Lutein intake in the United States ranges from 1.5 to 2 mg/day, which is below the recommended daily intake (Fullmer and Shao 2001). The level is also declining due to a decrease in the consumption of dark green vegetables, the major dietary sources of lutein. As a staple food, high-lutein wheat would enhance the daily intake of lutein by ≈120% on the basis of the recommended daily consumption of whole grain products (i.e., six servings provide 2.1 mg of lutein based on 86% dry matter). This considers lutein alone. The total carotenoid contribution would be higher (e.g., contribution of lutein, zeaxanthin, and other carotenoid isomers). It is not known what level of lutein or total carotenoid would be required for a significant reduction in risk, but clearly the presently identified levels could significantly raise dietary intake. Also, new genotypes with increased lutein levels could be developed. The effect of processing and storage on lutein also needs to be considered. When lutein was added to ready-to-eat cereal (0.5 mg/30 g serving) and cereal bar (1.25 mg/50 g serving) products, it remained stable during processing and storage for six and 12 weeks (Fullmer and Shao 2001).

Because the principal drawback to developing einkorn for commercial use is the low grain yield (Borghi et al 1996; Corbellini et al 1999), the development of high-yield genotypes needs to be emphasized, in addition to the high lutein content. The higher grain yield of durum could make it another choice for developing high-lutein wheat for particular end-uses. Screening a large number of einkorn and durum types for lutein will assist in evaluating this potential. The presence of adhering glumes in einkorn might help sustain grains during development and storage against invasive insects and microorganisms, an important feature in sustainable and organic agriculture. Because the glumes should be removed before processing, specific dehullers are now being developed for dehulling spelt and einkorn. This would also aid in developing the hulled wheat market. We believe that this information should be of interest to breeding programs aimed at developing alternative and functional wheats.

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