Bioactive Compounds and their Antioxidant Capacity in Selected Primitive and Modern Wheat Species

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Abstract: Whole grain foods have been recommended for healthy diets as being recognized sources of dietary fiber and antioxidants. A diverse array of wheat was evaluated in terms of bioactive compounds and antioxidant capacity. The bioactive compounds examined include phenolic acids, carotenoids and tocols which were determined by HPLC. Antioxidant was based on the determination of scavenging capacity of $DPPH^{\cdot}$ and $ABTS^{\cdot+}$ radicals and total phenols assay. Significant differences were observed in total phenols and ferulic acid between wheat species. Wheat species contained four major tocols including 2 tocopherols and 2 tocotrienols with β-tocotrienol being the most abundant tocol. Lutein, the primary carotenoid in wheat, significantly differed among wheat species ranging from 1.0 to 8.1 mg kg^{-1}. Scavenging capacity of $DPPH^{\cdot}$ and $ABTS^{\cdot+}$ radicals significantly varied being 1.97-3.20 and 17.1-24.7 μmole g^{-1}, respectively. The results show the presence of considerable variability among wheat species in antioxidant composition and capacity with certain wheats hold promise for the development of functional foods for health promotion.

Keywords: Modern wheat, primitive wheat, antioxidants, carotenoids, phenolics, tocols, scavenging capacity of DPPH and ABTS.

INTRODUCTION

Wheat has been used as a food since the late Stone Age (ca. 6700 BC) [1]. At present, it is a staple food worldwide. Wheat is rich in the basic nutrients such as energy, protein, vitamins and minerals and also contains rational amounts of bioactive compounds such as dietary fiber and antioxidants. Thus consumption of whole wheat foods such as whole wheat bread or pasta besides other whole grain foods has been recommended for healthy diets. The U.S. Food and Drug Administration (FDA) under the Nutrition and Labeling Education Act (NLEA) has allowed a health claim with regards to the consumption of whole grain foods and reducing the risk of heart disease and certain cancers [2]. Several recent studies have also supported the role of whole grain diets in lowering body mass index and the risk of cardiovascular disease [3], hypertension in women [4] and colorectal cancer [5]. The beneficial health properties of whole grain products have been associated with the presence of higher amounts of dietary fiber and antioxidants and lower calories as compared to their respective refined ones.

A large number of studies have shown that wheat whole grain and wheat bran extracts possess antioxidant properties against oxidation of biologically important molecules such as DNA, proteins and membrane lipids. Wheat bran extracts have been found to inhibit peroxidation of human LDL cholesterol [6], phospholipid liposomes and hydrogen peroxide [7] and to scavenge free radicals of $DPPH^{\cdot}$, $ABTS^{\cdot+}$ and peroxide anion ($O_2^{\cdot-}$) [8]. Durum wheat bran extracts also exhibited stronger antioxidant capacity based on the inhibition of oxidation of soy oil compared to the control sample (oil without additive) and other milling fraction extracts [9]. Nonetheless, wheat antioxidant properties were significantly influenced by wheat genotype and growing location [10] and environmental or growing conditions such as total solar radiation, average daily solar radiation and number of hours exceeding 32°C [11]. Insignificant differences, however, were found between red and white wheat in terms of antioxidant capacity as determined by scavenging DPPH free radical [12].

Wheat contains a diverse array of bioactive compounds that may contribute to its antioxidant capacity. These bioactive components include carotenoids, tocopherols, tocotrienols, phenolic acids, phytic acid, phytosterols and flavonoids. Previous studies have shown that wheat varieties vary in antioxidant properties [6, 13-14] as well as in composition of bioactive compounds such as carotenoids [15-16], tocopherols [8], phenolic acids [17] and anthocyanins [18]. The present study was aimed at exploring differences in antioxidant composition and capacity in selected primitive and modern wheat species. The antioxidant compounds determined in the study include carotenoids, tocopherols, tocotrienols and phenolic acids. The antioxidant properties were based on the determination of scavenging capacity of $DPPH^{\cdot}$ and $ABTS^{\cdot+}$ radicals and content of total phenols. The overall objective was to identify potential wheat cultivars for the development of whole wheat functional foods and/or natural health products.

MATERIALS AND METHODS

Wheat Materials

The wheat species and cultivars used in the present study include two einkorn (*Triticum monococcum* L.) cultivars AC Knowles and PI 418587; emmer (*Triticum turgidum* spp.

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dicoccum) accession Vernal; two Khorasan (*Triticum turgidum* spp. *turanicum*) accession PI211691 CDC Dragon and a commercial sample Kamut; durum (*Triticum turgidum* spp. *durum*) cultivar Kyle; six spelt (*Triticum aestivum* ssp. *spelta*), three winter cultivars Rotkorn, Frankencorn and Heritage, two spring cultivars CDC Nexon and PGR8801 and a commercial organic spelt; six soft wheat (*Triticum aestivum* spp. *aestivum*) cultivars AC Reed, AC Ron, 25W60, Augusta, Freedom and Mendon and four hard wheat (*Triticum aestivum* spp. *aestivum*) cultivars AC Morley, Celtic, Fundulea and Katepwa. The wheat samples were obtained from plots grown at the experimental farms of the University of Saskatchewan, Saskatoon, SK, Canada and the University of Guelph, Guelph, ON, Canada except for einkorn AC Knowles and commercial samples. Einkorn AC Knowles was obtained from the Eastern Cereals and Oilseeds Research Centre, Ottawa, ON, Canada. All the wheat cultivars were conventionally grown using traditional cultivation practice. The commercial organic spelt samples were purchased from the retail market in Guelph, ON, Canada.

Immediately after harvest, the wheat grains were dried to approximately 10% moisture content. For hulled wheat, the grains were dehulled by passing the hulled grains between a pair of rubber-coated rollers followed by air aspiration. The dehulled (hulls removed by rollers) and hull-less (free-threshing) wheat 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 whole grain flours were thoroughly mixed to ensure uniformity and kept at 4°C until extraction and analysis.

Analytical Tests

Extraction of Bioactive Compounds

Extracts for the determination of total phenols and antioxidant activities were prepared as previously described [19] with slight modifications. Whole wheat flours (5 g) were mixed with 50 mL of 80% methanol and the mixture was purged with stream of nitrogen and thoroughly mixed on IKA shaker (IKA – Vibrax – VXR, IKA Works, Brazil) for 30 min. After centrifugation at 10, 000 *g* for 5 min, the extracts were transferred into culture tubes and purged with stream of nitrogen and kept in a refrigerator until analysis.

Carotenoids were extracted with water saturated 1-butanol as described in our previous study [16]. Approximately 0.5 g of wheat sample was homogenized in 10 mL of solvent for 45 sec at 5, 000 rpm in a PT 10-35 Polytron homogenizer (Kinematica AG, Swizerland), kept for 30 min at room temperature and homogenized again for 45 sec. The mixture was centrifuged at 6,000 *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 HPLC analysis.

For the preparation of tocol (tocopherol plus tocotrienol) extracts, 1 g sample was digested with 7.3 mL of 11% KOH in aqueous ethanol (55%) under nitrogen at 80°C for 10 min. The tube was shaken during heating to ensure the mixture remained homogeneous. The mixture was cooled in ice bath and 4 mL of trimethyl pentane (TMP) was added and mixed on a tube shaker or vortex. After centrifugation at 10, 000 *g* for 5 min, the supernatant (TMP extract) was filtered through a 0.45 μm Nylon Acrodisc syringe filter (Pall Gelman Laboratory, Ann Arbor, MI). The extract was evaporated under stream of nitrogen to dryness, and the residue was dissolved in 0.5 mL of the mobile solvent.

Phenolic acid extracts were prepared as outlined in Abdel-Aal and others [17]. The wheat samples were digested with 2M NaOH under nitrogen for 1 h, neutralized with 6M HCL and extracted with diethyl ether/ethyl acetate (1:1, v/v). After centrifugation at 10, 000 *g* for 10 min, the supernatant was transferred into a separatory funnel and the organic layer was collected. The extraction was repeated and the two extracts were pooled and dewatered with anhydrous sodium sulphate. The extract was evaporated at 40°C to dryness and the residue was dissolved in aqueous 95% ethanol prior to analysis.

All extraction experiments were performed under dim light and the extraction tubes were wrapped with black paper to avoid sample degradation by photooxidation.

Total Phenols Content

Total phenols content was based on Folin-Ciocalteu method of Kaluza *et al*. [20] using ferulic acid as a standard. The reaction mixture contained 250 μL of grain extract, 250 μL of diluted Folin-Ciocalteu reagent and 500 μL of saturated sodium carbonate solution. The mixture was brought up to 5 mL with distilled water and the contents were mixed and kept in darkness for 30 min. The mixture was centrifuged at 10, 000 *g* for 5 min and the absorbance was read at 725 nm. The total phenols content was calculated as ferulic acid equivalent using authentic ferulic acid.

Analysis of Carotenoids, Tocols and Phneloic Acids by HPLC

High performance liquid chromatography 1100 Series chromatograph (Agilent, Mississauga, ON) equipped with a G1311A quaternary pump, G1329A temperature controlled injector, G1316A temperature controlled column thermostat, G1322A degasser, G1315B photodiode array detector (PDA) and ChemStation v.8.04 data acquisition system with capability of conducting isoabsorbance plot and 3D graphic analyses was employed for the separation and quantification of carotenoids, tocols and phenolic acids. For carotenoids, the separation was performed on short (10 cm x 4.6 mm, packing 3 μm) C30 column YMC Carotenoid (Waters, Mississauga, ON). The column was operated at 35°C and eluted with a gradient mobile system consisting of: (A) methanol/methyl *tert*-butyl ether/nano pure water (81:15:4, v/v/v) and (B) methyl *tert*-butyl ether/methanol (90:10, v/v) at 1 mL min^{-1}. The gradient was programmed as follows: 0-9 min, 100 to 75% A; 9-10 min, 75-0% A; 10-12 min, hold at 0% A; 12-13 min, 0 to 100% A; and 13-15 min, hold at 100% A. The separated carotenoids were detected and measured at 450 nm and the identity of carotenoids was based on the congruence of retention times and UV/Vis spectra with those of pure authentic standards.

The separation and quantification of tocols were performed on Supelcosil LC-NH_2 (25 cm x 4.6 mm, packing 5 μm) C18 column (Supelco Canada, Oakville, ON). The column was operated at 25°C and eluted with a gradient mobile system consisting of (A) hexane and (B) ethyl acetate at 1 mL min^{-1}. The gradient was programmed as follows: 0-14

min, 85% A; 14-15 min, 85 to 50% A; 15-19 min 50% A; 19-20 min 50 to 85% A and hold at 85% A for 4 min. The separated tocols were detected and measured at 298 nm and the identity was based on the congruence of retention times and UV/Vis spectra with those of pure four authentic standards (α-, β-, γ-, δ-tocopherol).

For the separation and quantification of phenolic acids, a Supelcosil LC 18 column (25 cm x 4.6 mm, packing 5 μm) (Supelco Canada, Oakville, ON) was employed and operated at 25°C and flow rate of 1 mL min^{-1}. The gradient mobile system consisted of (A) 6% formic acid and (B) acidified acetonitrile and was programmed as follows: 0-35 min, 100 to 82% A; 35-40 min, 82% A; 40-42 min, 82-100% A. The separated phenolic acids were detected at 5 different wavelengths (230, 260, 275, 320 and 330 nm), but only ferulic acid was quantified in this study at 320 nm. The identity of phenolic was confirmed on the basis of the congruence of retention times and UV/Vis spectra with those of pure authentic standards.

Antioxidant Capacity Tests

Radical DPPH Scavenging Capacity

The free radical scavenging capacity of wheat extracts was determined using the stable 2,2-diphenyl-1-picrylhydrazyl radical ($DPPH^{\cdot}$) as outlined by Yu *et al*. [11]. The antioxidant reaction was initiated by transferring 1 mL of wheat extract into a test tube containing 4 mL of 80% methanol and 1 mL (containing 1 mmole) of freshly prepared $DPPH^{\cdot}$ solution. The final concentration of $DPPH^{\cdot}$ in the reaction mixture was 167 μmole. The reaction was monitored by reading absorbance at 517 nm after 10 min. A blank reagent was used to study stability of $DPPH^{\cdot}$ over the test time. The scavenging capacity of grain extracts was calculated as μmole DPPH/g sample.

Radical Cation ABTS Scavenging Capacity

The radical cation (2,2'-azino-di-[3-ethylbenzthiazoline sulphonate] ($ABTS^{\cdot+}$) scavenging capacity was measured using Randox Laboratories assay kit (San Francisco, CA). Trolox (6-hydroxy 2,5,7,8-tetramethylchroman-2-carboxylic acid) provided in the kit was used as an antioxidant standard and for the calculation of scavenging capacity of wheat extract as trolox equivalent. The scavenging capacity of grain extracts was calculated as μmole ABTS/g sample.

Statistical Analysis

The data were subjected to analysis of variance and least significance difference (LSD) multiple comparisons to identify significant differences between wheat species and cultivars using Minitab software (version 12, Minitab inc., State College, PA).

RESULTS AND DISCUSSION

Bioactive Compounds

Wheat grains contain a variety of bioactive substances that could contribute to the health benefits of whole wheat foods such as reducing the risk of coronary heart disease and certain types of cancer. Total phenols content (TPC) and total antioxidant capacity have extensively been used in the literature to evaluate grains in relation to their health benefits or in the identification of potential candidates for the development of grain-based functional foods. In the present study, TPC expressed as ferulic acid equivalent, the predominant phenolic acid in wheat, significantly varied between wheat species and cultivars ranging from 881 μg/g to 2382 μg/g (Table **1**). This demonstrates a broad range of TPC in wheat species, which would offer rich genetic resources for the development of wheat-based functional foods. A High antioxidant Swiss red wheat was found to contain an average of 1800 μg/g TPC which is within the current range [21]. In the latter study the aleurone fraction was found to possess the highest level of TPC being approximately 3000 μg/g compared to the whole grain (1800 μg/g) and bran fraction (2700 μg/g). The study also showed that micronization of aleurone resulted in an increase in TPC level (4040 μg/g) compared to 3000 μg/g TPC in the non-micronized or raw aleurone samples. In another study, TPC in pearled wheat fractions of 8 Canadian cultivars varied significantly among cultivars and pearling fractions with values of 1300-5300 μg/g [12]. Total phenols content was more than 4000 μg/g in the first (5% bran removal) and second (10% barn removal) pearling fractions which was higher than the other three pearling fractions (15, 20 and 25% outer layers removal).

In the present study, wheat samples were categorised into five groups on the basis of number of chromosomes (e.g. 14, 28 and 42) and cultivation era (e.g. primitive versus modern). Significant differences were observed among the five wheat groups (diploid, tetraploid, hexaploid, primitive and modern) (Fig. **1A**). Diploid or *monococcum* wheats, emmer wheat, some spelt cultivars Frankencorn and Rotkorn, organic spelt and soft wheat cultivar Augusta contained significantly higher levels (> 2000 μg/g) of TPC compared to the other wheats examined (Table **1**). Significant differences in TPC among wheat cultivars [10] and growing locations [11] have previously been reported.

Ferulic acid is the main phenolic acid in wheat accounting for up to 90% of the total phenolic acids in wheat [22]. It exists primarily in a bound form with arabinoxylan polysaccharide in the outer layers of wheat grains. It was found to be a significant variable correlated with resistance to wheat midge [17] and a potent antioxidant in inhibiting lipid peroxidation in microsomal membranes and in protecting murine fibroblasts [23]. Ferulic acid significantly differed among wheat species and within a species (Table **1**). This variability in ferulic acid between wheat cultivars indicates differences in genotype and origin. Since ferulic acid in wheat has been associated with disease resistance and strong antioxidant properties it would be a good indication marker in the development of functional wheat. Ferulic acid concentration ranged broadly (220-574 μg/g) in the wheat species examined due to their environmental and genetic diversity used in the current study. The concentration of ferulic acid in 6 western Canadian wheat genotypes representing three commercial classes with different technological qualities was in the middle of the current range being 371-441 μg/g [24]. This indicates that some of the examined wheat cultivars contain relatively high values of ferulic acid (>500 μg/g) which would enhance their antioxidant properties and health benefits. Other studies also showed that ferulic acid in wheat was significantly influenced by growing conditions and

Table 1. Average Concentration (μg/g) of Bioactive Compounds in Selected Primitive and Modern Wheat Species

Wheat	Cultivar	2n Ch. No.	TPC	FA	α-T	β-T	α-T3	β-T3	Lutein
Einkorn	PI418587	14	2319 ± 85	301 ± 12	6.9 ± 0.17	2.3 ± 0.09	5.2 ± 0.21	20.1 ± 0.89	8.1 ± 0.29
	AC Knowles	14	2355 ± 61	479 ± 17	7.2 ± 0.23	2.0 ± 0.08	7.4 ± 0.30	17.5 ± 0.70	7.4 ± 0.21
Emmer	Vernal	28	2323 ± 72	323 ± 13	7.6 ± 0.19	6.0 ± 0.25	3.1 ± 0.12	23.2 ± 0.91	3.7 ± 0.11
Khorasan	CDC Dragon	28	1851 ± 65	220 ± 9	6.2 ± 0.17	2.6 ± 0.08	2.4 ± 0.07	9.9 ± 0.39	4.8 ± 0.13
	Kamut	28	1917 ± 59	326 ± 15	5.5 ± 0.21	2.4 ± 0.07	2.5 ± 0.08	9.6 ± 0.41	4.7 ± 0.15
Durum	Kyle	28	881 ± 33	416 ± 15	7.5 ± 0.25	2.7 ± 0.07	3.6 ± 0.13	17.2 ± 0.55	4.9 ± 0.17
Spelt	CDC Nixon	42	1207 ± 42	325 ± 11	7.2 ± 0.19	3.0 ± 0.12	3.0 ± 0.14	15.1 ± 0.51	1.0 ± 0.04
	PGR8801	42	1121 ± 38	387 ± 15	7.4 ± 0.21	5.5 ± 0.20	2.6 ± 0.11	13.2 ± 0.43	1.1 ± 0.05
	Frankencorn	42	2317 ± 43	384 ± 16	10.0 ± 0.39	5.5 ± 0.18	2.9 ± 0.13	12.4 ± 0.45	1.5 ± 0.08
	Heritage	42	1799 ± 42	527 ± 23	7.3 ± 0.23	3.1 ± 0.11	2.9 ± 0.12	13.9 ± 0.55	1.3 ± 0.06
	Rotkorn	42	2382 ± 73	465 ± 17	11.3 ± 0.42	6.6 ± 0.29	3.8 ± 0.15	20.0 ± 0.95	2.1 ± 0.10
	Organic	42	2006 ± 75	457 ± 15	7.5 ± 0.22	5.6 ± 0.21	2.8 ± 0.10	13.6 ± 0.52	1.4 ± 0.05
Soft wheat	AC Reed	42	1190 ± 41	441 ± 15	9.8 ± 0.35	4.1 ± 0.15	4.0 ± 0.16	18.8 ± 0.63	2.5 ± 0.09
	AC Ron	42	1880 ± 52	472 ± 18	7.9 ± 0.27	4.1 ± 0.17	4.2 ± 0.13	14.3 ± 0.39	1.9 ± 0.08
	Augusta	42	2190 ± 49	556 ± 25	8.1 ± 0.25	3.8 ± 0.15	3.9 ± 0.13	13.8 ± 0.29	1.7 ± 0.05
	Freedom	42	1719 ± 38	463 ± 17	9.0 ± 0.31	4.1 ± 0.22	3.7 ± 0.15	14.0 ± 0.42	1.3 ± 0.04
	Mendon	42	1575 ± 31	502 ± 21	8.9 ± 0.33	4.3 ± 0.20	3.6 ± 0.14	14.1 ± 0.45	1.3 ± 0.05
	25W60	42	1781 ± 42	491 ± 19	8.1 ± 0.29	3.9 ± 0.14	4.3 ± 0.17	13.9 ± 0.43	1.7 ± 0.06
Hard wheat	AC Morley	42	1918 ± 39	574 ± 27	10.2 ± 0.39	4.5 ± 0.21	3.3 ± 0.13	13.9 ± 0.38	1.3 ± 0.04
	Celtic	42	1802 ± 41	490 ± 20	9.9 ± 0.35	4.7 ± 0.25	3.1 ± 0.11	13.8 ± 0.42	1.1 ± 0.03
	Fundulea	42	2145 ± 45	549 ± 23	9.8 ± 0.31	4.3 ± 0.19	3.5 ± 0.12	14.0 ± 0.47	1.5 ± 0.07
	Katepwa	42	1144 ± 35	413 ± 18	11.9 ± 0.40	4.8 ± 0.13	3.0 ± 0.10	14.1 ± 0.38	1.5 ± 0.05
LSD[a]	-	-	107	36	0.60	0.27	0.28	0.92	0.23

Chr.No.=chromosome number; TPC=total phenols content; FA=ferulic acid; T=tocopherol; T3=tocotrienol.
[a]Least significant difference values at $p < 0.05$.

genotypes [17, 24]. The five wheat groups named diploid, tetraploid, hexaploid, primitive and modern also showed significant variations in ferulic acid (Fig. **1B**).

Whole wheat grains contain reasonable amount of total tocopherols and tocotrienols with β-tocotrienol being the primary tocol [25]. In the current study, four tocols including α-tocopherol (α-T), β-tocopherol (β-T), α-tocotrienol (α-T3) and β-tocotrienol (β-T3) were the predominant compounds in all the wheat species examined (Table **1**). The main tocols were β-T3 ranging from 9.6-23.2 μg/g, followed by α-T (5.5-11.9 μg/g), α-T3 (2.5-7.4 μg/g) and finally β-T (2.0-6.6 μg/g). Wheat species (Table **1**) and groups (Fig. **2A-D**) showed significant differences in their contents of the four tocols due to the differences in genotype and origin. The contents of tocols in barley [26] and rice [27] were also found to be influenced by genotype and growing environment. Unlike wheat, γ-T3 and β-T3 were the predominant and smallest tocol in rice, respectively [28].

All-*trans* lutein is the main carotenoid found in wheat species [15]. This polar dihydroxylated carotenoid constituted about 77-83% of the total carotenoids in high lutein wheat species such as einkorn, Khorasan and durum [16]. Common bread and pastry wheat contained lower levels of lutein compared to einkorn, Khorasan or durum since the former wheats have been bred over the years for low yellow pigment content or white endosperm. In the current study, einkorn or *monococcum* wheats contained the highest concentration of lutein (7.4-8.1 μg/g) among all the wheats examined (Table **1**). This is in good agreement with our previous finding [15-16]. Emmer, Khorasan and durum wheats had intermediate levels of lutein (3.7-4.9 μg/g) (Table **1**). Spelt, soft and hard wheats possessed the lowest levels of lutein (1.0-2.5 μg/g) (Table **1**). This indicates that *monococcum* or diploid and tetraploid wheats would be potential candidates for the development of high lutein wheat-based functional foods. The high level of lutein in einkorn, emmer, Khorasan or durum would enhance the daily intake of lutein, but this level still needs to be increased in order to provide the physiological dose, i.e. about 5-6 mg/day. This could be achieved by genetic manipulation and/or carotenoid fortification. In a recent study, einkorn contained carotenoids, mostly lutein, about 2-4 fold higher than non-einkorn wheat with an average of 8.4 μg/g and a maximum of 13.4 μg/g [29]. The significant differences in lutein content among wheat species and cultivars was also supported by grouping the examined wheats based on number of chromosomes and cultivation era (Fig. **1C**).

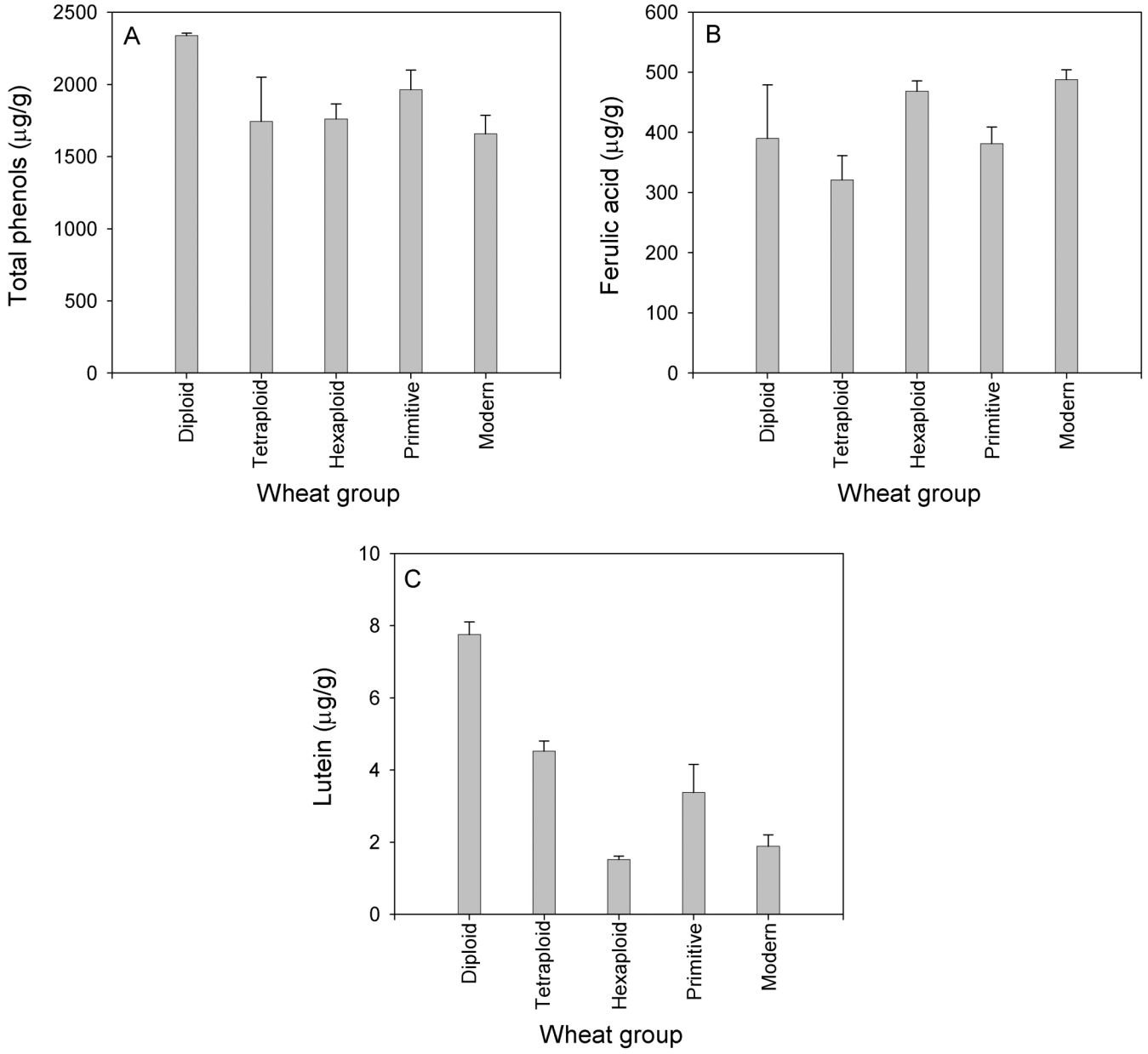

Fig. (1). Differences in total phenols, ferulic acid and lutein among wheat groups (bars represent means and bar error represents standard error of the mean for each group, n=2, 4, 16, 11 and 11 for diploid, tetraploid, hexaploid, primitive and modern wheat, respectively).

Antioxidant Capacity

It has been scientifically acceptable that the reactive oxygen species and free radicals generated during cellular metabolism or peroxidation of lipids and other biological molecules play important roles in the pathogenesis of chronic diseases such as coronary heard disease and cancer. Dietary antioxidants combat reactive oxygen species and free radicals which may help reduce the risk of chronic diseases. In the present study, scavenging capacity of free radicals of DPPH and ABTS were used to measure antioxidant activity of a diverse array of wheat representing different classes and commercial values (Table **2**). Wheat species and cultivars were significantly different in their ability to scavenge DPPH radical. The wheat extracts had scavenging capacity ranging between 1.97 μmole/g and 3.20 μmole/g (Table **2**). In addition, it was noted that diploid, tetraploid and primitive wheats had better DPPH scavenging capacity compared to the modern wheats investigated (Fig. **3A**). However, there were some exceptions in modern wheats. For example, Katepwa hard wheat possessed comparable antioxidant capability to primitive wheats having DPPH scavenging capacity of 3.13 μmole/g (Table **2**).

The antioxidant capacity of the selected wheat species and cultivars was also assessed based on the ability of wheat phenolic extracts to quench ABTS radical cation (Table **2**). Phenolic compounds were found to be active toward scavenging $ABTS^{\cdot+}$ to a different extent depending upon phenolic compound and reaction environment [30]. Wheat extracts contain a large number of phenolic compounds and other antioxidant constituents which may contribute to the scavenging activity of $ABTS^{\cdot+}$. Among the wheat grains examined, spelt cultivar Frankencorn exhibited the greatest scav-

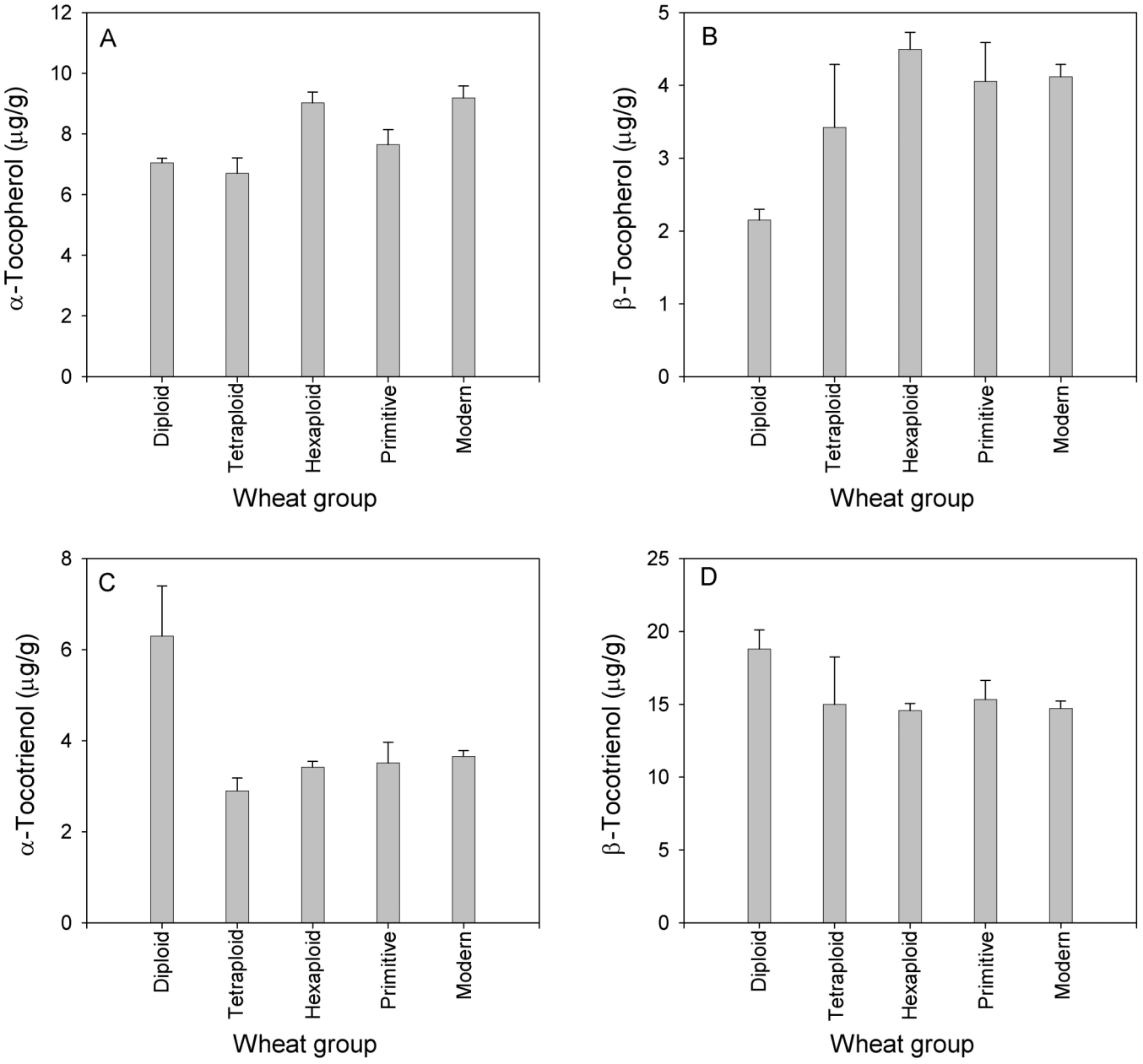

Fig. (2). Differences in tocoppherols and tocotrienols among wheat groups (bars represent means and bar error represents standard error of the mean for each group, n=2, 4, 16, 11 and 11 for diploid, tetraploid, hexaploid, primitive and modern wheat, respectively).

enging capacity toward $ABTS^{\cdot+}$ (Table **2**). Other wheat cultivars such as Kamut, spelt cultivar Rotkorn and hard wheat cultivars celtic and Katepwa had comparable scavenging capacity to that of spelt cultivar Frankencorn.

When the wheat cultivars grouped into diploid, tetraploid, hexpploid, primitive and modern categories, slight differences in $ABTS^{\cdot+}$ scavenging capacity were observed among the five groups (Fig. **3B**). This trend was different from that found in the case of DPPH radical. Both DPPH and ABTS tests are electron transfer based reactions in which they involve one redox reaction with the oxidant as an indicator of the reaction endpoint [31]. In the DPPH test, the colored stable DPPH radical is reduced in the presence of an antioxidant into a non-radical DPPH-H. The ABTS test is based on the formation of $ABTS^{\cdot+}$ by reacting ABTS with metmyoglobin and H_2O_2 to form a relatively stable blue-green colored compound which is reduced in the presence of an antioxidant. In wheat a large number of bioactive compounds exist which may possess scavenging activity against $DPPH^{\cdot}$ or $ABTS^{\cdot+}$. However, capacity of scavenging activity against $DPPH^{\cdot}$ or $ABTS^{\cdot+}$ by wheat antioxidants would vary depending upon concentration of individual bioactive compounds in wheat extracts and their synergic effects.

Eight Canadian wheat cultivars and their pearling fractions were reported to be different in their DPPH scavenging capacity [12]. The study indicated that pearling is an effective milling technique to obtain wheat bran fractions enriched in phenolics and antioxidants, thereby maximizing

Table 2. Average Scavenging Capacity (μmole/g) of DPPH and ABTS Radicals in Selected Primitive and Modern Wheat Species

Wheat	Cultivar	2n Ch. No.	DPPH	ABTS
Einkorn	PI418587	14	3.06 ± 0.12	18.4 ± 0.81
	AC Knowles	14	3.18 ± 0.13	21.1 ± 0.89
Emmer	Vernal	28	3.20 ± 0.15	21.3 ± 0.93
Khorasan	CDC Dragon	28	3.01 ± 0.14	16.7 ± 0.67
	Kamut	28	3.17 ± 0.11	22.4 ± 0.99
Durum	Kyle	28	3.09 ± 0.09	19.0 ± 0.77
Spelt	CDC Nixon	42	3.12 ± 0.11	17.1 ± 0.63
	PGR8801	42	3.03 ± 0.13	17.3 ± 0.67
	Frankencorn	42	3.16 ± 0.14	24.7 ± 1.01
	Heritage	42	3.14 ± 0.09	18.9 ± 0.83
	Rotkorn	42	3.12 ± 0.08	21.8 ± 1.05
	Organic	42	3.17 ± 0.13	19.6 ± 0.89
Soft wheat	AC Reed	42	2.21 ± 0.09	20.7 ± 0.92
	AC Ron	42	2.67 ± 0.12	19.5 ± 0.93
	Augusta	42	2.38 ± 0.07	17.3 ± 0.79
	Freedom	42	2.53 ± 0.10	20.2 ± 0.88
	Mendon	42	2.05 ± 0.11	19.5 ± 0.94
	25W60	42	1.97 ± 0.09	19.2 ± 0.87
Hard wheat	AC Morley	42	2.43 ± 0.11	20.1 ± 0.89
	Celtic	42	2.40 ± 0.09	23.8 ± 0.95
	Fundulea	42	2.63 ± 0.12	19.9 ± 0.82
	Katepwa	42	3.13 ± 0.10	22.5 ± 0.85
LSD[a]	-	-	0.22	1.86

DPPH=2,2-diphenyl-1-picrylhydrazyl radical; ABTS=2,2'-azino-di-[3-ethylbenzthiazoline sulphonate; Chr.No.=chromosome number.
[a]Least significant difference values at $p < 0.05$.

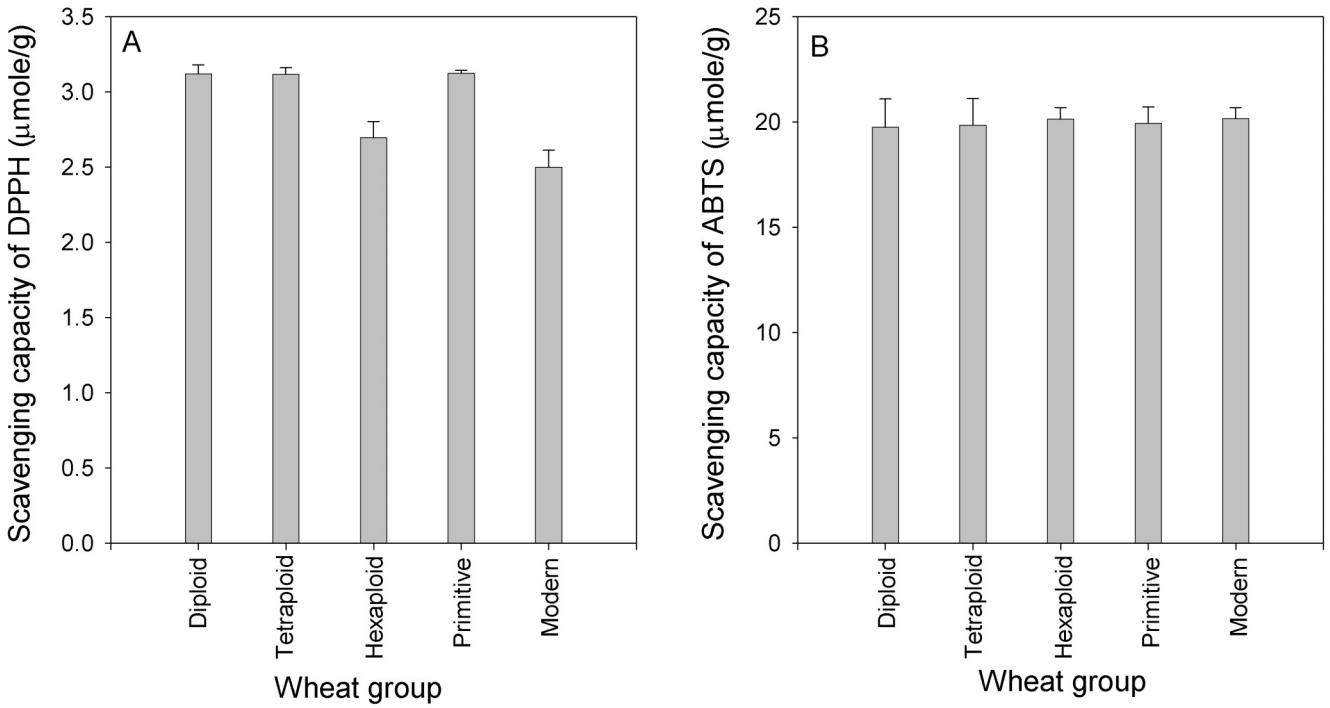

Fig. (3). Differences in scavenging capacities of DPPH and ABTS among wheat groups (bars represent means and bar error represents standard error of the mean for each group, n=2, 4, 16, 11 and 11 for diploid, tetraploid, hexaploid, primitive and modern wheat, respectively).

health benefits of wheat. The current study showed that certain wheats such as emmer (3.20 μmole/g), einkorn (3.06-3.20 μmole/g), Khorasan (3.01-3.17 μmole/g), spelt (3.03-3.17 μmole/g) and hard wheat cultivar Katepwa (3.13 μmole/g) would be potential high antioxidant candidates based on their DPPH scavenging capacity values. In another study, micronization, a post-harvest treatment, has been found to boost antioxidant activity of wheat aleurone fraction obtained from a Swiss red wheat [21]. The results of the present study demonstrate that certain wheat samples contain relatively higher antioxidant capacity than others. The antioxidant properties in these promising wheat species would be even enhanced further by employing the proper pre-processing technologies such as pearling and/or micronization in the development of high antioxidant wheat-based food ingredients.

CONCLUSIONS

Wheat contains a variety of bioactive compounds and antioxidants including dietary fiber, tocopherols, tocotrienols, phenolic acids, phytic acid, phytosterols, flavonoids, etc. These bioactive compounds play significant roles in human health. The wheat species and cultivars examined showed significant differences in their contents of TPC, ferulic acid, tocols and lutein, as well as in their free radical scavenging capacities. Certain wheat species have been found to possess relatively high levels of bioactive compounds and antioxidant activity. Examples of these wheats include einkorn, the earliest cultivated wheat by mankind, emmer, spelt and certain modern wheats. Einkorn would hold promise for the development of high antioxidant/lutein functional foods. In addition, some spelt cultivars such as Frankencorn and Rotkorn and hard wheat cultivar Katepawa which exhibited high scavenging activities toward DPPH and ABTS radicals would be potent antioxidant natural ingredients.

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