

1-24-2007

High-Amylose Corn Exhibits Better Antioxidant Activity than Typical and Waxy Genotypes

Wende Li
Shanxi University

Chunliang Wei
University of Manitoba

Pamela J. White
Iowa State University, pjwhite@iastate.edu

Trust Beta
University of Manitoba

Follow this and additional works at: http://lib.dr.iastate.edu/fshn_ag_pubs

 Part of the [Food Chemistry Commons](#)

The complete bibliographic information for this item can be found at http://lib.dr.iastate.edu/fshn_ag_pubs/49. For information on how to cite this item, please visit <http://lib.dr.iastate.edu/howtocite.html>.

This Article is brought to you for free and open access by the Food Science and Human Nutrition at Iowa State University Digital Repository. It has been accepted for inclusion in Food Science and Human Nutrition Publications by an authorized administrator of Iowa State University Digital Repository. For more information, please contact digirep@iastate.edu.

High-Amylose Corn Exhibits Better Antioxidant Activity than Typical and Waxy Genotypes

Abstract

The consumption of fruits, vegetables, and whole grains rich in antioxidative phytochemicals is associated with a reduced risk of chronic diseases such as cancer, coronary heart disease, diabetes, Alzheimer's disease, cataract, and aged-related functional decline. For example, phenolic acids are among the main antioxidative phytochemicals in grains that have been shown to be beneficial to human health. Corn (*Zea mays* L.) is a major staple food in several parts of the world; thus, the antioxidant activity of several corn types was evaluated. The 2,2-Diphenyl-1-picrylhydrazyl free radical (DPPH•) scavenging activity, total phenolic content (TPC), antioxidant capacity of lipid-soluble substances (ACL), oxygen radical absorbance capacity (ORAC), and phenolic acid compositions of typical and mutant genotypes (typical-1, waxy, typical-2, and high-amylose) were investigated. The DPPH• scavenging activity at 60 min was 34.39–44.51% in methanol extracts and 60.41–67.26% in HCl/methanol (1/99, v/v) extracts of corn. The DPPH• scavenging activity of alkaline hydrolysates of corn ranged from 48.63 to 64.85%. The TPC ranged from 0.67 to 1.02 g and from 0.91 to 2.15 g of ferulic acid equiv/kg of corn in methanol and HCl/methanol extracts, respectively. The TPC of alkaline hydrolysates ranged from 2.74 to 6.27 g of ferulic acid equiv/kg of corn. The ACL values were 0.41–0.80 and 0.84–1.59 g of Trolox equiv/kg of corn in methanol and HCl/methanol extracts, respectively. The ORAC values were 10.57–12.47 and 18.76–24.92 g of Trolox equiv/kg of corn in methanol and HCl/methanol extracts, respectively. ORAC values of alkaline hydrolysates ranged from 42.85 to 68.31 g of Trolox equiv/kg of corn. The composition of phenolic acids in alkaline hydrolysates of corn was *p*-hydroxybenzoic acid (5.08–10.6 mg/kg), vanillic acid (3.25–14.71 mg/kg), caffeic acid (2.32–25.73 mg/kg), syringic acid (12.37–24.48 mg/kg), *p*-coumaric acid (97.87–211.03 mg/kg), ferulic acid (1552.48–2969.10 mg/kg), and *o*-coumaric acid (126.53–575.87 mg/kg). Levels of DPPH• scavenging activity, TPC, ACL, and ORAC in HCl/methanol extracts were obviously higher than those present in methanol extracts. There was no significant loss of antioxidant capacity when corn was dried at relatively high temperatures (65 and 93 °C) postharvest as compared to drying at ambient temperatures (27 °C). Alkaline hydrolysates showed very high TPC, ACL, and ORAC values when compared to methanol and HCl/methanol extracts. High-amylose corn had a better antioxidant capacity than did typical (nonmutant) corn genotypes.

Keywords

Antioxidant capacity, high-amylose corn, waxy corn, drying temperature

Disciplines

Food Chemistry | Food Science

Comments

Posted with permission from *Journal of Agricultural and Food Chemistry* 55 (2007): 291–298, doi:10.1021/jf0622432. Copyright 2007 American Chemical Society.

High-Amylose Corn Exhibits Better Antioxidant Activity than Typical and Waxy Genotypes

WENDE LI,^{†,‡} CHUNLIANG (VICTOR) WEI,[‡] PAMELA J. WHITE,[§]
 AND TRUST BETA^{*,‡}

Cereal Chemistry & Quality Analysis Laboratory, Faculty of Chemistry & Chemical Engineering, Shanxi University, Taiyuan City 030006, People's Republic of China, Department of Food Science, University of Manitoba, Winnipeg, Manitoba R3T 2N2, Canada, and Department of Food Science and Human Nutrition, Iowa State University, Ames, Iowa 50011

The consumption of fruits, vegetables, and whole grains rich in antioxidative phytochemicals is associated with a reduced risk of chronic diseases such as cancer, coronary heart disease, diabetes, Alzheimer's disease, cataract, and aged-related functional decline. For example, phenolic acids are among the main antioxidative phytochemicals in grains that have been shown to be beneficial to human health. Corn (*Zea mays* L.) is a major staple food in several parts of the world; thus, the antioxidant activity of several corn types was evaluated. The 2,2-Diphenyl-1-picrylhydrazyl free radical (DPPH[•]) scavenging activity, total phenolic content (TPC), antioxidant capacity of lipid-soluble substances (ACL), oxygen radical absorbance capacity (ORAC), and phenolic acid compositions of typical and mutant genotypes (typical-1, waxy, typical-2, and high-amylose) were investigated. The DPPH[•] scavenging activity at 60 min was 34.39–44.51% in methanol extracts and 60.41–67.26% in HCl/methanol (1/99, v/v) extracts of corn. The DPPH[•] scavenging activity of alkaline hydrolysates of corn ranged from 48.63 to 64.85%. The TPC ranged from 0.67 to 1.02 g and from 0.91 to 2.15 g of ferulic acid equiv/kg of corn in methanol and HCl/methanol extracts, respectively. The TPC of alkaline hydrolysates ranged from 2.74 to 6.27 g of ferulic acid equiv/kg of corn. The ACL values were 0.41–0.80 and 0.84–1.59 g of Trolox equiv/kg of corn in methanol and HCl/methanol extracts, respectively. The ORAC values were 10.57–12.47 and 18.76–24.92 g of Trolox equiv/kg of corn in methanol and HCl/methanol extracts, respectively. ORAC values of alkaline hydrolysates ranged from 42.85 to 68.31 g of Trolox equiv/kg of corn. The composition of phenolic acids in alkaline hydrolysates of corn was *p*-hydroxybenzoic acid (5.08–10.6 mg/kg), vanillic acid (3.25–14.71 mg/kg), caffeic acid (2.32–25.73 mg/kg), syringic acid (12.37–24.48 mg/kg), *p*-coumaric acid (97.87–211.03 mg/kg), ferulic acid (1552.48–2969.10 mg/kg), and *o*-coumaric acid (126.53–575.87 mg/kg). Levels of DPPH[•] scavenging activity, TPC, ACL, and ORAC in HCl/methanol extracts were obviously higher than those present in methanol extracts. There was no significant loss of antioxidant capacity when corn was dried at relatively high temperatures (65 and 93 °C) postharvest as compared to drying at ambient temperatures (27 °C). Alkaline hydrolysates showed very high TPC, ACL, and ORAC values when compared to methanol and HCl/methanol extracts. High-amylose corn had a better antioxidant capacity than did typical (nonmutant) corn genotypes.

KEYWORDS: Antioxidant capacity; high-amylose corn; waxy corn, drying temperature

INTRODUCTION

Approximately 35% of deaths due to cancer in the United States are related to diet (1). Therefore, current trends of dietary modifications have shifted to focus on the prevention of diseases (2). Studies on antioxidants have strongly indicated that the

consumption of grains, vegetables, and fruits may prevent many diseases and promote good health (2, 3). Grains are a major source of antioxidants in our daily diets. The main antioxidative components in grains are classified as phytoestrogens, phenolic compounds, and other substances and include anthocyanins, lignans, phytic acid, tannins, sterols, vanillin, ferulic acid (FA), caffeic acid (CA), *p*-hydroxybenzoic acid (*p*-HA), protocatechuic acid, *p*-coumaric acid (*p*-CA), gentisic acid, sinapic acid, isoferulic acid, chlorogenic acid, vanillic acid (VA), *p*-hydroxyphenylacetic acid, and syringic acid (SA) (4). Beneficial effects

* To whom correspondence should be addressed. E-mail: Trust_Beta@UManitoba.CA.

[†] Shanxi University.

[‡] University of Manitoba.

[§] Iowa State University.

of antioxidants on promoting health are believed to be achieved through several possible mechanisms, such as directly reacting with and quenching free radicals, chelating transition metals, reducing peroxides, and stimulating the antioxidative defense enzyme system (5). Corn (*Zea mays* L.) is one of the world's most important grains, with phytochemical substances that display antioxidant activity (6–8). For example, purple-pigmented corn kernels are very rich in anthocyanins with well-established antioxidant and bioactive properties (9). Also, the total antioxidant activity of sweet corn becomes elevated by 44% after thermal processing (6). Corn has a greater total phenolic content (TPC) and total antioxidant activity than do wheat, oats, and rice (7).

Antioxidant properties of grain are affected by many factors, however, such as genotype (10) and growing conditions (11, 12). In this study, typical and mutant corn genotypes with different amylose contents subjected to low, medium, and high drying temperatures (DTs) were used. The objectives of the present study were to provide antioxidant data of typical, waxy, and high-amylose corn and therefore to understand their value as a potential source of natural antioxidants.

MATERIALS AND METHODS

Sample Description. Four corn types [typical-1 (F1-T1), typical-2 (F3-T2), waxy (F2-W), and high-amylose (F4-HA)] grown at the Iowa State University Agricultural Experiment Station plots (Ames, IA) were used. F1-T1 (Pioneer 3335) and waxy corn seeds (Pioneer 33A63) were from the same genetic parents. F3-T2 (B73 × Oh43) and high-amylose (B73ae × Oh43ae) were also from the same genetic parents. Corn was harvested at about 21% moisture by using a combine harvester and shelled in the field. The moisture content of freshly harvested corn was reduced under different DTs of 27, 65, and 93 °C. Ambient air was circulated at about 27 °C to simulate natural drying until the grain moisture content was reduced to about 12%. Drying at medium and high temperatures was conducted by using a grain oven drier at 65 and 93 °C, respectively, until the grain moisture content was reduced to about 10%. The hot grain was rapidly cooled using ambient air prior to storage. Dried corn was stored in plastic containers at 10 °C for up to 12 months prior to determination of their antioxidant properties. The amylose contents of these four typical and mutant corn genotypes were determined by using the Megazyme amylose/amylopectin assay kit (13) and were 20.4% for F1-T1, 20.9% for F3-T2, 1.9% for F2-W, and 42.2% for F4-HA, respectively.

Chemicals. 2,2-Diphenyl-1-picrylhydrazyl free radical (DPPH[•]), 2,2'-azobis(2-methylpropanamide) dihydrochloride (AAPH), and 12 phenolic acid standards were purchased from Sigma-Aldrich (St. Louis, MO). Phenolic acid standards used for high-performance liquid chromatography (HPLC) analysis were as follows: gallic, gentisic, *p*-coumaric, *m*-coumaric, caffeic, sinapic, ferulic, syringic, *o*-coumaric, vanillic, protocatechuic, and *p*-HAs. An assay kit was purchased from Analytik Jena USA, Inc. (The Woodlands, TX) for the determination of antioxidant capacity of lipid-soluble substances (ACL) based on photochemiluminescence. Trolox and fluorescein were purchased from Fisher Acros Organics (New Jersey) for use with the oxygen radical absorbance capacity (ORAC) assay. All other chemicals and solvents were of the highest commercial grade and used without further purification.

Sample Extraction. Corn whole-meal was prepared by grinding grain with a sample mill containing a 1 mm mesh sieve (Krups 50, Germany). Methanol (100%) and HCl (12.1 M)/methanol (1/99, v/v) were used as solvents for extraction of finely ground grain. The extraction procedure involved the addition of 20 mL of solvent to 2.0 g of ground grain each in 50 mL brown bottles and shaking the grain for 16 h at ambient temperature in a rotary shaker (Fermentation Design Inc., Allentown, PA) set at 300 rpm. Material in 50 mL tubes was then centrifuged at 7800g (10000 rpm, SS-34 Rotors, RC5C Sorvall Instruments) at 5 °C for 15 min. The extracts of supernatant fluid were kept at –20 °C in the dark until further analysis for DPPH free radical scavenging activity, TPC, ACL, ORAC, and phenolic acid composition.

DPPH[•] Scavenging Activity. The DPPH methods of Brand-Williams et al. (14) and Yu et al. (10) were modified for this assay. The method involves the reaction of the antioxidants with the stable DPPH[•] in 95% ethanol solution. Briefly, a 60 μM DPPH[•] solution was freshly made in 95% ethanol solution. Grain extracts (200 μL) were reacted with 3.8 mL of the DPPH[•] solution for 60 min. The absorbance at 515 nm was measured against a blank of pure 95% ethanol at *t* = 0, 5, 10, 20, 30, 40, 50, and 60 min. The chemical kinetics of antioxidant activity of grain extracts also were recorded. The antioxidant activity was calculated as follows:

$$\% \text{ DPPH}^{\bullet} \text{ scavenging activity} = (1 - [A_{\text{sample}}/A_{\text{control}=0}]) \times 100$$

DPPH tests were all carried out in duplicate.

Determination of TPC. The TPC of extracts was determined by using modified procedures of the Folin–Ciocalteu method (15–18). An extract (200 μL) was added to 1.9 mL of freshly diluted 10-fold Folin–Ciocalteu reagent (BDH Inc., Toronto, ON). Sodium carbonate solution (1.9 mL) (60 g/L) was then added to the mixture. After 120 min of reaction at ambient temperature, the absorbance of the mixture was measured at 725 nm against a blank of distilled water. FA was used as a standard, and results were expressed as FA equivalents. All analyses were performed in duplicate.

Determination of ACL. The measurement of antioxidant capacity of water- or lipid-soluble substances by means of photosensitized chemiluminescence (PCL) was reported earlier (19–22). A Photochem photochemiluminometer (Analytik Jena AG, Germany) was used to determine ACL. After dilution with methanol, grain extracts (20 μL) were added to components of the ACL kit: 2280 μL of reagent 1 (methanol), 200 μL of reagent 2 (buffer solution), and 25 μL of reagent 3 (photosensitizer). The mixtures were run in the Photochem, and results were obtained from a workstation employing PCL software. ACL was assayed as the area under the curve (AUC) and expressed as μg of Trolox equivalent (TE)/mg of corn. The formula used for calculation was as follows:

$$\text{TE in sample } (\mu\text{g}/\text{mg}) = (\text{quantity} \times \text{dilution factor} \times \text{M} \times \text{volume}) / (\text{pipetted volume} \times \text{sample})$$

where quantity, nmol of TE; M, molar mass (250.3 ng/nmol for Trolox); volume, extraction volume of sample in mL; pipetted volume, μL of volume used for measurement; and sample, grain weight in mg. The tests were carried out in duplicate.

ORAC Assay. The ORAC assay, first developed by Cao et al. (23), was used in this study according to Huang et al. (24) and Dávalos et al. (25) but with some modifications. An FLx800 microplate fluorescence reader (Bio-Tek Instruments, Inc., Winooski, VT) was used with fluorescence filters for an excitation wavelength of 485/20 nm and an emission wavelength of 528/20 nm. The plate reader was controlled by KC4 3.0 software (version 29). Dilution of sample, rutin control, and Trolox standard was done manually. The quantity of 300 μL each of buffer solution (blank) and diluted sample solution, rutin control, and Trolox standard was transferred to a 96-well flat bottom polystyrene microplate (Corning Inc., Corning, NY) by hand according to their designated positions. A full automation of plate-to-plate liquid transfer was programmed by using a Precision 2000 automated microplate pipetting system (Bio-Tek Instruments, Inc.). Specifically, 120 μL of fluorescence working solution was transferred from reagent holder to each well of the second 96-well microplate. Then, 20 μL each of buffer solution (blank), Trolox standard, diluted samples, and 20 μM rutin control from designated wells of the first 96-well microplate was transferred to designated wells of the second 96-well microplate. The latter was quickly covered with an adhesive sealing film, then shaken for 3 min at 37 °C in the incubator and incubated in the preheated (37 °C) FLx800 microplate reader for a total period of 20 min. The second 96-well microplate was transferred back to its original station in the Precision 2000 automated microplate pipetting system, followed by automatically transferring 60 μL of AAPH solution from the reagent holder to designated wells. Thus, the total volume for each well was 200 μL. The second 96-well microplate was quickly covered again with an adhesive sealing film and immediately transferred to the FLx800

Table 1. DPPH[•] Scavenging Activity of Corn Whole-Meal Extracts and Alkaline Hydrolysates (at 60 min)^a

corn genotype	DT (°C)	%		
		EM-DPPH [•] scavenging	EHM-DPPH [•] scavenging	AH-DPPH [•] scavenging
F1-T1	27	35.34 b	61.73 cd	48.63 i
F1-T1	65	35.59 b	60.61 d	59.85 bc
F1-T1	93	35.92 b	61.86 cd	52.49 gh
F2-W	27	42.31 a	63.16 bcd	54.23 fg
F2-W	65	40.44 a	61.61 d	56.62 de
F2-W	93	43.12 a	62.61 bcd	51.31 h
F3-T2	27	34.39 b	61.85 cd	54.83 ef
F3-T2	65	34.84 b	60.46 d	57.87 cd
F3-T2	93	35.71 b	62.27 bcd	55.80 def
F4-HA	27	44.50 a	65.00 abc	60.35 b
F4-HA	65	44.51 a	67.26 a	64.85 a
F4-HA	93	43.81 a	65.45 ab	64.84 a
LSD		4.39	3.37	2.20

^a LSD, least significance difference at $P = 0.05$ level of probability. Mean values for samples having similar letters in the same column are not significantly different. EM, extracts in methanol system; EHM, extracts in HCl/methanol system; AH, alkaline hydrolysates; F1-T1, F3-T2, typical corn; F2-W, waxy corn; F4-HA, high-amylose corn; and DPPH[•], DPPH free radical.

microplate reader, and the fluorescence was measured every minute for 50 min at 37 °C. The peroxy radical was generated by AAPH during measurement, and fluorescein was used as the substrate (26). All of the reaction mixtures were prepared in the measured plate in duplicate, and at least three independent assays were performed for each grain extraction.

Data were processed according to Cao et al. (27) and Huang et al. (24). The final ORAC values were calculated by using a regression equation between the Trolox concentration and the net area under the fluorescence decay curve. The AUC was calculated as follows:

$$\text{AUC} = 0.5 + f_1/f_0 + \dots + f_{49}/f_0 + 0.5(f_{50}/f_0)$$

where f_0 = initial fluorescence reading at 0 min and f_i = fluorescence reading at time i min.

The net AUC was obtained by subtracting the AUC of the blank from that of the sample. ORAC values were expressed as TEs by using the standard curve. Final results were in g of TE/kg of corn.

Determination of Phenolic Acid Composition. Sample preparation and HPLC analysis were according to the methods of Mpofu et al. (18) and Li et al. (28). Briefly, ground corn (2 g) was hydrolyzed by using 4 M NaOH (60 mL) for 4 h under nitrogen at ambient temperature. The hydrolyzed mixture was adjusted to pH 1.5–2.5 by using ice-cold 6 M HCl and then centrifuged at 7800g (10000 rpm, RC5C, Sorvall Instruments, DuPont, Wilmington, DE) at 5 °C for 20 min. The supernatant was extracted three times with ethyl acetate (70 mL), and the combined organic phase was retained. The organic phase was dehydrated by adding 2 g of anhydrous Na₂SO₄. The organic phase was evaporated to dryness at 35 °C by using a rotary vacuum evaporator (RE III Rotavapor, Büchi, Switzerland). The residue was redissolved in 4 mL of 50% methanol and filtered through a 0.45 μm nylon filter. The filtrate (alkaline hydrolysate) was stored in the dark at –20 °C and subsequently analyzed by HPLC to obtain phenolic acid composition of corn samples. Antioxidant properties of alkaline hydrolysates were also evaluated, including DPPH[•] scavenging activity, TPC, and ORAC.

HPLC analysis was performed on a Waters model 2695 chromatograph instrument (Waters, Mississauga, ON, Canada) equipped with a Waters 2996 photodiode array detector. Phenolic acids were separated on a reverse-phase Waters μBondapak RP-C18 column (300 mm × 3.9 mm) with a gradient of solvent A [water containing 1% (v/v) acetic acid] and solvent B (100% methanol) for 33 min at a flow rate of 1.5 mL/min. The solvent gradient was programmed as follows: at 0 min 15% B, 10 min 20% B, 16 min 23% B, 24–28 min 27% B, and 30–33 min 15% B. Phenolic acids in the eluents were monitored at 280

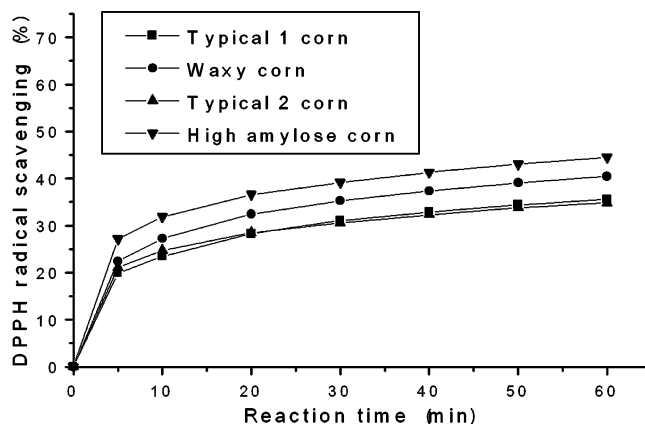


Figure 1. Antioxidant activity kinetics of corn whole-meal methanol extracts using DPPH free radical (DPPH[•]). Note: Corn samples were dried at 65 °C.

nm. Identification of the phenolic acids was accomplished by comparing the retention times of peaks in grain extract to those of phenolic acid standards. The HPLC analyses were carried out in duplicate.

Statistical Analysis. Data were reported as means of measurements and subjected to analysis of variance. Least significant difference (LSD) was calculated by using Fisher's protected LSD test at $P = 0.05$. Quantitative results were expressed on a dry weight basis.

RESULTS AND DISCUSSION

DPPH[•] Scavenging Activity, Methanol Extracts. The DPPH[•] scavenging activity of corn whole-meal methanol extracts ranged from 34.39 (F3-T2 at DT 27 °C) to 44.51% (F4-HA at DT 65 °C) (Table 1). For each corn genotype, there was no difference in DPPH[•] scavenging activity among the three DTs of 27, 65, and 93 °C. F2-W (40.44–43.12%) and F4-HA (43.81–44.51%) showed a higher DPPH[•] scavenging activity than did F1-T1 (35.34–35.92%) and F3-T2 (34.39–35.71%). Elsewhere, the DPPH[•] scavenging activity of wheat whole meal ranged from 23.66 (Wu blue-grained wheat) to 33.51% (black-grained wheat) with the same methanol solvent system used in the current work (28). A higher total antioxidant activity of corn than wheat, oats, and rice was reported by Adom and Liu (7). The reaction rate of corn whole-meal extracts (dried at 65 °C) with DPPH[•] was rapid in the first 10 min, but after 10 min, it became progressively slow and stable (Figure 1). High-amylose corn (F4-HA) had the highest DPPH[•] scavenging activity during the reaction process when compared to waxy (F2-W) and typical (F1-T1 and F3-T2) corn. Awika et al. (26) reported that the reaction time with DPPH[•] could be up to 8 h, but after 8 h, the change in antioxidant activity for sorghum extracts was very minimal.

HCl/Methanol Extracts. HCl/methanol extracts had greater DPPH[•] scavenging activity than did corn whole-meal methanol extracts (Table 1). For example, at DT 27 °C, the DPPH[•] scavenging activity of F1-T1, F2-W, F3-T2, and F4-HA extracts in methanol was 35.34, 42.31, 34.39, and 44.50%, respectively, but markedly increased in HCl/methanol up to 61.73, 63.16, 61.85, and 65.00%, respectively. Thus, there were marked effects of the solvent system on estimating antioxidant activity. Antioxidant properties of wheat bran extracts in four solvent systems including 50% acetone (v/v), 70% methanol (v/v), 70% ethanol (v/v), and absolute ethanol were evaluated by Zhou and Yu (5), and extracting solvent significantly altered estimation of the antioxidant properties of wheat bran. In the current study, the DPPH[•] scavenging activity in HCl/methanol ranged from 60.46 (F3-T2) to 67.26% (F4-HA) after drying the samples at

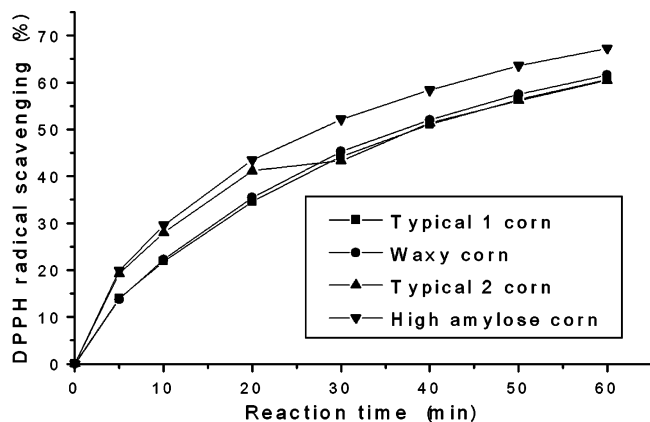


Figure 2. 2. Antioxidant activity kinetics of corn whole-meal HCl/methanol extracts using DPPH free radical (DPPH[•]). Note: Corn samples were dried at 65 °C.

65 °C. For each genotype, differences in DPPH[•] scavenging activity also were not found among the three DTs. Extracting corn with HCl/methanol resulted in an increased DPPH[•] scavenging activity for each genotype; however, there were no differences among F1-T1, F2-W, and F3-T2 extracts. The reaction rate of corn whole-meal extracts in HCl/methanol (Figure 2) was more rapid than in methanol (Figure 1) after 10 min.

Alkaline Hydrolysates. The DPPH[•] scavenging activity of alkaline hydrolysates was evaluated for comparison with corn methanol and HCl/methanol extracts (Table 1). Phenolic acids are the main contributors to DPPH[•] scavenging activity of corn after alkaline hydrolysis to release the bound forms. The DPPH[•] scavenging activity ranged from 48.63 (F1-T1 at DT 27 °C) to 64.85% (F4-HA at DT 65 °C). Differences in DPPH[•] scavenging activity were found among the three drying treatments for F1-T1 and F2-W. The DPPH[•] scavenging activity differed between DT 27 and 65 °C for F3-T2 and among DT 27 and 65 and 93 °C for F4-HA alkaline hydrolysates. Significant differences also were found between the two mutants. High-amylose corn (F4-HA) also differed from the typical corn parent (F3-T2). For each corn genotype treated at the same DT, the DPPH[•] scavenging activity of alkaline hydrolysates was greater than methanol extracts; however, the scavenging activity was more attenuated than for HCl/methanol extracts (Table 1). High-amylose corn (F4-HA) had the highest DPPH[•] scavenging activity at each DT as compared to the other genotypes. Within the first 20 min, reaction rates of alkaline hydrolysates (Figure 3) were faster and their DPPH[•] scavenging activity was higher than the HCl/methanol extracts (Figure 2). Many studies indicate that phenolic substances are largely responsible for antioxidant capacity of cereals (17, 18, 26, 28). The main difference between solvent extracts and alkaline hydrolysates is attributed to the nature of phenolic structures present. Free and bound phenolic compounds are present in grains with levels of the latter being 85% in corn, 75% in oats and wheat, and 62% in rice, respectively (7). The contribution of bound phytochemicals to free radical antioxidant activity of several grains was 90% in wheat, 87% in corn, 71% in rice, and 58% in oats, respectively (7). Free phenolic compounds were the major contributors to the total antioxidant activity in methanol and HCl/methanol extracts, whereas bound phytochemicals were responsible for the free radical scavenging activity of alkaline hydrolysates. The latter are composed mainly of phenolic acids whose presence can easily be detected by using HPLC analysis after alkaline hydrolysis of grains (28).

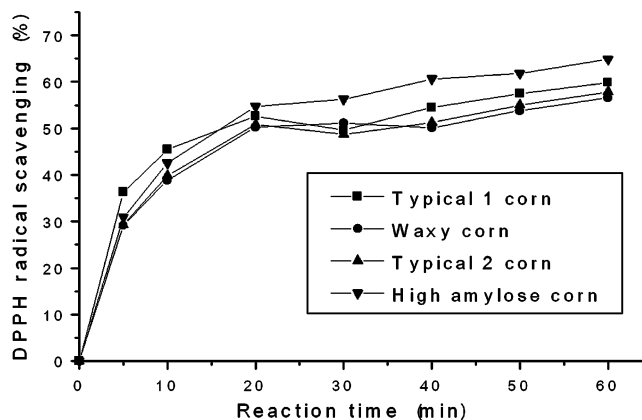


Figure 3. 3. Antioxidant activity kinetics of corn whole-meal alkaline hydrolysates using DPPH free radical (DPPH[•]). Note: Corn samples were dried at 65 °C.

Table 2. TPC of Corn Whole-Meal Extracts and Alkaline Hydrolysates^a

corn genotype	DT (°C)	g/kg		
		EM-equiv of FA	EHM-equiv of FA	AH-equiv of FA
F1-T1	27	0.77 de	1.72 c	2.74 h
F1-T1	65	0.78 de	1.72 c	3.30 g
F1-T1	93	0.84 dc	1.72 c	3.39 gf
F2-W	27	0.95 ba	2.12 ba	4.12 dce
F2-W	65	0.97 ba	2.04 ba	3.93 de
F2-W	93	1.02 a	2.15 a	3.77 fe
F3-T2	27	0.68 f	1.50 d	4.30 cd
F3-T2	65	0.67 f	0.91 e	4.39 c
F3-T2	93	0.73 fe	1.58 d	4.34 c
F4-HA	27	0.91 bc	2.07 ba	6.14 ba
F4-HA	65	0.93 b	2.00 b	5.85 b
F4-HA	93	0.95 ba	1.80 c	6.27 a
LSD		0.08	0.12	0.40

^a LSD, least significance difference at $P = 0.05$ level of probability. Mean values for samples having similar letters in the same column are not significantly different. EM, extracts in methanol system; EHM, extracts in HCl/methanol system; AH, alkaline hydrolysates; equiv, equivalent; F1-T1, F3-T2, typical corn; F2-W, waxy corn; and F4-HA, high amylose corn.

TPC. Methanol Extracts. The TPC ranged from 0.67 (F3-T2 at DT 65 °C) to 1.02 (F2-W at DT 93 °C) g/kg for methanol extracts (Table 2). There was no difference in the TPC among the three DTs for each corn genotype as was observed with DPPH[•] scavenging activity. Differences in TPCs were found between the mutant, F2-W, and its typical parent, F1-T1. The high-amylose mutant, F4-HA, differed also from its typical parent, F3-T2. The TPC of mutants (0.91–1.02 g/kg) was higher than the amount found in typical corn (0.67–0.84 g/kg). Similarly, as compared to methanol extracts of wheat, the TPC of black-grained wheat, Dongjian purple-grained wheat, Dongjian white-grained, and Wu blue-grained wheat whole meals was 1.11, 0.93, 0.82, and 0.71 g/kg, respectively (28). Differences in TPCs among grains indicate that genotypes and varieties are important factors influencing the antioxidant activity of grains.

HCl/Methanol Extracts. The TPC of HCl/methanol extracts ranged from 0.91 (F3-T2 at DT 65 °C) to 2.15 g/kg (F2-W at DT 93 °C) (Table 2). HCl/methanol extracts had greater TPCs than methanol extracts indicating that the presence of acid improved the extractability of phenolic compounds. There were no differences in TPC among the three DTs for F1-T1 and F2-W; however, differences were observed for F3-T2 and F4-HA due to drying treatments. For instance, there were differences in TPC for F3-T2 dried at 27, 65, and 93 °C. The TPC of

Table 3. ACL of Corn Whole-Meal Extracts^a

corn genotype	DT (°C)	g/kg		
		EM-equiv of Trolox	EHM-equiv of Trolox	ACL increase in EHM (%)
F1-T1	27	0.45 fg	0.89 fgh	49.4
F1-T1	65	0.41 h	0.90 fg	54.4
F1-T1	93	0.44 g	0.84 h	47.6
F2-W	27	0.52 e	1.04 de	50.0
F2-W	65	0.47 f	0.86 gh	45.3
F2-W	93	0.58 c	1.00 e	42.0
F3-T2	27	0.60 c	1.10 d	45.5
F3-T2	65	0.55 d	0.92 f	40.2
F3-T2	93	0.53 de	0.99 e	46.5
F4-HA	27	0.70 b	1.27 c	44.9
F4-HA	65	0.80 a	1.59 a	49.7
F4-HA	93	0.70 b	1.34 b	47.8
LSD		0.03	0.06	

^a LSD, least significance difference at $P = 0.05$ level of probability. Mean values for samples having similar letters in the same column are not significantly different. EM, extracts in methanol system; EHM, extracts in HCl/methanol system; equiv, equivalent; F1-T1, F3-T2, typical corn; F2-W, waxy corn; and F4-HA, high amylose corn.

mutants (F2-W and F4-HA) (1.87–2.15 g/kg) was greater than that of typical corn (F1-T1 and F3-T2) (0.91–1.72 g/kg). Differences in TPC also were found between F2-W and F1-T1 and similarly between F4-HA and its typical parent. Estimation of TPC of corn-whole meals was also affected by the solvent system employed as previously reported for wheat bran (5).

Alkaline Hydrolysates. The TPC of alkaline hydrolysates ranged from 2.74 (F1-T1 at DT 27 °C) to 6.27 g/kg (F4-HA at DT 93 °C) (Table 2). The TPC was greater in alkaline hydrolysates than in methanol and HCl/methanol extracts. High-amylose corn (F4-HA) had the highest TPC (5.85–6.27 g/kg) among the genotypes. Alkaline hydrolysates of typical corn (F3-T2 and F1-T1) differed in TPC. For F2-W and F3-T2, there were no differences in TPCs among the three DTs; however, for F1-T1 and F4-HA, differences were found among the drying treatments. For each corn genotype at the same DT, TPCs of alkaline hydrolysates were greater than extracts in methanol and HCl/methanol. Unlike methanol extraction, alkaline hydrolysis enabled release of previously bound phenolic acids. For instance, the TPC of F4-HA at DT 93 °C was 0.95 g/kg in methanol extract, 1.80 g/kg in HCl/methanol extract, and 6.27 g/kg in alkaline hydrolysates.

Although alkaline hydrolysates had greater TPCs than HCl/methanol extracts (Table 2), the DPPH• scavenging activity of hydrolysates was somewhat lower than the HCl/methanol extracts (Table 1). Phenolic acids obtained after alkaline hydrolysis were less effective in scavenging activity than compounds present in HCl/methanol extracts. A better antioxidant activity of bound FA from bran was found in rat plasmas than its pure FA counterpart (29). While a high thermal processing temperature can increase the total antioxidant activity, FA, and TPC by 44, 550, and 54%, respectively, of sweet corn (6), a slight change in the DPPH• scavenging activity and TPC under different drying treatments showed no obvious effect of employing high DT on antioxidant properties of mature corn grains.

ACL Assay. Methanol Extracts. ACL values of methanol extracts ranged from 0.41 (F1-T1 at DT 65 °C) to 0.80 g/kg (F4-HA at DT 65 °C) (Table 3). There were differences in ACL among typical and mutant corn genotypes. Drying treatments also influenced ACL values of corn. For example, ACL values at 65 °C differed significantly from those obtained at 27 and

Table 4. ORAC of Corn Whole-Meal Extracts and Alkaline Hydrolysates^a

corn genotype	DT (°C)	g/kg		
		EM-equiv of Trolox	EHM-equiv of Trolox	AH-equiv of Trolox
F1-T1	27	10.57 c	20.36 cd	43.29 e
F1-T1	65	11.41 bc	20.86 bcd	45.00 de
F1-T1	93	11.25 bc	20.24 cd	46.16 cde
F2-W	27	12.30 ab	20.45 cd	50.68 bc
F2-W	65	13.21 a	24.92 a	48.89 bcd
F2-W	93	12.47 ab	21.92 abc	47.52 bcd
F3-T2	27	11.65 bc	20.23 cd	52.29 b
F3-T2	65	11.15 bc	18.76 d	50.58 bc
F3-T2	93	11.75 bc	19.97 cd	42.85 e
F4-HA	27	12.29 ab	22.86 abc	68.31 a
F4-HA	65	11.50 bc	24.45 ab	63.44 a
F4-HA	93	10.78 c	20.47 cd	66.28 a
LSD		1.46	3.60	5.19

^a LSD, least significance difference at $P = 0.05$ level of probability. Mean values for samples having similar letters in the same column are not significantly different. EM, extracts in methanol system; EHM, extracts in HCl/methanol system; AH, alkaline hydrolysates; equiv, equivalent; F1-T1, F3-T2, typical corn; F2-W, waxy corn; and F4-HA, high amylose corn.

93 °C for F1-T1 and F4-HA. Waxy corn (F2-W) differed in ACL with low, medium, and high DTs. F3-T2 gave higher ACL values at DT 27 °C than at 65 and 93 °C. F4-HA had greater ACL values (0.70–0.80 g/kg) than F1-T1 (0.41–0.45 g/kg), F2-W (0.47–0.58 g/kg), and F3-T2 (0.53–0.60 g/kg).

HCl/Methanol Extracts. ACL values of HCl/methanol extracts ranged from 0.84 (F1-T1 at DT 93 °C) to 1.59 g/kg (F4-HA at DT 65 °C) (Table 3). ACL values were higher by 40.2 (F3-T2 at DT 65 °C) to 54.4% (F1-T1 at DT 65 °C) than levels found in methanol extracts (Table 3). F4-HA differed from the other corn genotypes (F1-T1, F2-W, and F3-T2). Differences in ACL also were found under the three conditions used for drying corn. For example, F3-T2 had higher ACL values at DT 27 °C than at other temperatures, whereas F4-HA had the highest ACL value at 65 °C. F4-HA had greater ACL values (1.27–1.59 g/kg) than did F1-T1 (0.84–0.90 g/kg), F2-W (0.86–1.04 g/kg), and F3-T2 (0.92–1.10 g/kg) HCl/methanol extracts.

The detection principle using Photochem is that continuous irradiation ($h\nu_1$) of an assay mixture containing an antioxidant substance generates free oxygen and other radicals ($S + h\nu_1 \rightarrow R^{\bullet} + \{O_2^{\bullet-}, S^{\bullet-}\}$), which react with and consume antioxidants ($R^{\bullet} + AO \rightarrow \text{products}$), gradually leaving an increasing surplus of radicals available for a retarded rise of the emission $h\nu_2$ ($R^{\bullet} + D \rightarrow h\nu_2$) (30), where S is photosensitizer, R is free radical, AO is antioxidant, and D is free radical detecting substance. The method is simple, rapid, sensitive, and convenient for the assay of antioxidants (30). Luminol plays a double role as a photosensitizer and also an oxygen radical detection reagent (21).

ORAC Assay. Methanol Extracts. ORAC values of methanol extracts ranged from 10.57 (F1-T1 at DT 27 °C) to 13.21 g/kg (F2-W at DT 65 °C) (Table 4). Differences in ORAC values were found between DT 27 and 93 °C only for F4-HA. No major differences in ORAC values were observed when typical and waxy genotypes were dried at low, medium, and high temperatures. However, F2-W (DT 27 °C) differed from F1-T1 (DT 27 °C) and F4-HA (DT 93 °C). ORAC values of waxy corn (DT 65 °C) also differed from the two typical corn mutants (F1-T1 and F3-T2).

HCl/Methanol Extracts. ORAC values of HCl/methanol extracts ranged from 18.76 (F3-T2 at DT 65 °C) to 24.92 g/kg

Table 5. Phenolic Acid Composition in Corn Whole-Meal Alkaline Hydrolysates^a

corn genotype	DT (°C)	mg/kg							
		<i>p</i> -HA	VA	CA	SA	<i>p</i> -CA	<i>m</i> -CA	FA	<i>o</i> -CA
F1-T1	27	10.6 a	7.66 ab	25.73 a	13.55 e	104.03 fg	5.88 a	1596.40 e	387.76 ab
F1-T1	65	5.22 a	ND	9.07 ab	13.14 e	105.56 f	ND	1606.44 e	168.21 b
F1-T1	93	9.21 a	5.24 ab	18.88 ab	12.37 f	97.87 g	ND	1552.48 e	224.73 ab
F2-W	27	ND	7.44 ab	2.53 ab	15.00 d	133.97 e	1.52 bc	1728.51 d	311.06 ab
F2-W	65	ND	14.71 a	6.13 a	14.54 d	140.38 de	ND	1751.81 d	396.44 ab
F2-W	93	6.07 a	6.33 ab	22.22 ab	15.02 d	146.12 d	3.32 ab	1760.28 d	126.53 b
F3-T2	27	5.08 a	3.79 ab	12.97 ab	24.10 a	157.39 c	ND	2110.15 c	272.03 ab
F3-T2	65	ND	9.30 ab	2.70 ab	24.48 a	143.48 d	ND	2134.66 c	377.59 ab
F3-T2	93	ND	4.29 ab	2.32 ab	23.92 a	140.90 de	ND	2107.46 c	381.19 ab
F4-HA	27	7.21 a	3.25 ab	15.64ab	19.93 b	211.03 a	ND	2928.97 ab	320.51 ab
F4-HA	65	6.54 a	3.58 ab	15.23 ab	19.13 c	207.99 ab	ND	2969.10a	316.64 ab
F4-HA	93	ND	8.00 ab	ND	19.12 c	202.64 b	ND	2853.55 b	575.87 a
LSD		12.17	13.06	25.24	0.76	6.95	3.25	77.32	402.57

^a LSD, least significance difference mg/kgce at $P = 0.05$ level of probability. Mean values for samples having similar letters in the same column are not significantly different. F1-T1, F3-T2, typical corn; F2-W, waxy corn; F4-HA, high amylose corn; and ND, not detectable.

Table 6. Intercorrelations (r) among DPPH• Scavenging Activity, TPC, ACL, ORAC, and FA ($n = 12$)^a

	methanol extracts				HCl/methanol extracts				alkaline hydrolysates		
	DPPH•	TPC	ACL	ORAC	DPPH•	TPC	ACL	ORAC	DPPH•	TPC	ORAC
TPC	0.88				0.51				0.82		
ACL	0.68	0.31			0.94	0.30			ND	ND	
ORAC	0.39	0.51	-0.01		0.50	0.68	0.40		0.76	0.94	
FA	0.50	0.12	0.93	-0.026	0.81	-0.06	0.91	0.21	0.81	0.96	0.89

^a ND, not determined; and n , number of samples. All correlations were done at the same level of probability. Bold indicates significance at the $P = 0.05$ level of probability.

(F2-W at DT 65 °C) (Table 4), an increase almost double the levels found in methanol extracts. For each corn genotype under the three different drying conditions, differences in ORAC values were found between DT 27 and 65 °C for F2-W and between DT 65 and 93 °C for F4-HA. Drying F1-T1, F3-T2, and F4-HA at 93 °C resulted in similar ORAC values that differed from those of F2-W (DT 65 °C). F1-T1, F3-T2, and F2-W (DT 27 °C) had similar ORAC values that differed from F4-HA (DT 65 °C). Corn mutants exhibited high ORAC values (24.92 and 24.45 g/kg) for F2-W and F4-HA, respectively, dried at 65 °C postharvest.

Alkaline Hydrolysates. ORAC values of alkaline hydrolysates ranged from 42.85 (F3-T2 at DT 93 °C) to 68.31 g/kg (F4-HA at DT 27 °C) (Table 4). The ORAC levels were significantly enhanced at each DT for each corn genotype as compared to methanol and HCl/methanol extracts. For each corn genotype under the three different drying conditions, similar ORAC values were obtained with one exception. Drying F3-T2 at 93 °C resulted in ORAC values that were slightly lower than levels obtained after treatment at low and medium temperatures. Differences in ORAC values were also found among corn genotypes. ORAC values of high-amylose corn, F4-HA (63.44–68.31 g/kg), were greater than F1-T1 (43.29–46.16 g/kg), F2-W (47.52–50.68 g/kg), and F3-T2 (42.85–52.29 g/kg). High ORAC values of alkaline hydrolysates indicated that released phenolic acids had a better antioxidant capacity for oxygen radicals than did phenolic compounds from methanol extracts.

The ORAC assay measures the relative potencies of antioxidants in preventing protein from free radical attacks (5). The observed changes of ORAC values in methanol and HCl/methanol solvents for the same corn genotype at the same DT suggested that the extracting solvent system may alter the overall estimation of ORAC value of a selected grain sample. High ORAC values indicated that the HCl/methanol system is a

suitable solvent for extracting antioxidants from corn whole-meal. It is important to understand that a selected solvent system, such as change in solvent polarity, affects efficiency of extracting antioxidant compounds and estimation for antioxidant capacity. The ORAC value of extracts in a 50% acetone system was 3–20-fold greater than that in ethanol system (31).

Phenolic Acid Composition. Eight types of phenolic acids in F1-T1 (DT 27 °C) and F2-W (DT 93 °C) and seven types of phenolic acids in F3-T2 (DT 27 °C) and F4-HA (DT 27 and 65 °C) were detected (Table 5). The phenolic acids included *p*-HA, VA, CA, SA, *p*-CA, *m*-coumaric acid (*m*-CA), FA, and *o*-coumaric acid (*o*-CA). However, *p*-HA in F2-W (DT 27 and 65 °C), F3-T2 (DT 65 and 93 °C), and F4-HA (DT 93 °C), VA in F1-T1 (DT 65 °C), CA in F4-HA (DT 93 °C), and *m*-CA in F1-T1 (DT 65 and 93 °C), F2-W (DT 65 °C), F3-T2, and F4-HA were not detectable probably due to low levels and/or loss during the alkaline hydrolysis process. FA was the predominant phenolic acid present in all four types of corn. Other major phenolic acids were *o*-CA (126.53–575.87 mg/kg) and *p*-CA (97.87–211.03 mg/kg) present in corn. Corn genotypes had different FA contents. Mutants had greater FA levels than their typical parents. DT generally did not affect the phenolic acid composition of corn genotypes. Differences in *p*-CA were found between the F4-HA and the other corn genotypes. For *o*-CA, a high LSD value (402.57) indicated its poor stability during preparation by alkaline hydrolysis. The FA concentration decreased in the order F4-HA (2853.55–2969.10 mg/kg) > F3-T2 (2107.46–2134.66 mg/kg) > F2-W (1728.51–1760.28 mg/kg) > F1-T1 (1552.48–1606.44 mg/kg). The F4-HA also had the greatest *p*-CA (202.64–211.03 mg/kg) among corn genotypes. In a previous study, the highest FA level among five wheat bran samples was 2119.4 mg/kg (28), which further confirms that corn contains greater levels of FA than wheat.

Many different phenolic acids contribute to the total phenolic acid composition of corn. Standard phenolic acids decreased in antioxidant activity in the following order: protocatechuic acid > chlorogenic acid > CA > *p*-HA > gentisic acid > FA > VA > SA > *p*-CA (32). Although FA is the predominant phenolic acid in many grains, free phenolic acids, soluble phenolic esters, and insoluble-bound phenolic acids still exist in grains prior to alkaline hydrolysis of grain (33). FA derivatives and free FA in white corn were 2360 and 2484 mg/kg, respectively (34). Protocatechuic acid derivatives and *p*-CA derivatives also were found in white corn (34). FA derivatives such as 5-5', 8-*O*-4', and 8-5' dehydrodiferulic acids were reported to account for 2.5% (w/w) of corn bran, whereas its 8-8' dimer was present only in minor amounts (35). Free, soluble-conjugated, and bound FAs were present in the ratio 0.1:1:100 (7). Alkaline hydrolysis releases soluble-conjugated and bound phenolic compounds in corn as free phenolic acids.

Intercorrelations. The correlations (*r*) between TPC and DPPH• scavenging activity were 0.88, 0.51, and 0.82 in methanol, HCl/methanol extracts, and alkaline hydrolysates, respectively (Table 6). A significant correlation was also found between total phenolics and antioxidant activity of other plant products (16, 17, 36, 37). In another report, no correlation was observed between total phenolics and DPPH• scavenging capacities (10). A high correlation (*r* = 0.94) was found between ACL and DPPH• scavenging activity for HCl/methanol extracts (Table 6). A good correlation (*r* = 0.94) was also found between ORAC values and TPC for alkaline hydrolysates (Table 6). A greater correlation (*r*² = 0.97) was found between DPPH• and ORAC values in sorghum products (26). High correlations (*r*) were found between FA and ACL values (0.91 and 0.93) or DPPH• scavenging activity (0.81) for solvent extracts or TPC (0.96) or ORAC values (0.89) for alkaline hydrolysates, respectively (Table 6). A good correlation (*r* = 0.84) between TPC and FA was also found in wheat genotypes (18). It is apparent that the solvent system had an effect on the correlation between some parameters because of differences in extractability for different phenolic compounds by different solvent systems.

In conclusion, this study reported the antioxidant properties of corn whole-meals in two extracting solvent systems and their alkaline hydrolysates as well as the possible effects of DT on the antioxidant properties of corn. Levels of DPPH• scavenging activity, TPC, ACL, and ORAC in HCl/methanol extracts were higher than those in methanol extracts. The choice of solvent for extracting corn samples affected estimation of the antioxidant properties; thus, detailed characterization of the nature of compounds present in the extracts should be attempted. The antioxidant activity was not attenuated under conditions of medium and high DTs (65 and 93 °C, respectively) as compared to natural drying at an ambient temperature of 27 °C. Hence, proper application of high processing temperature may increase or maintain the antioxidant capacity of cereal and food products. Differences in antioxidant properties were found among corn genotypes. Alkaline hydrolysates of corn whole-meals obviously showed very high antioxidant capacity when compared to extracts in methanol and HCl/methanol system, respectively, except for DPPH• scavenging activity in HCl/methanol system. Overall, high-amylose (F4-HA) corn had the best antioxidant capacity among corn genotypes. The high antioxidant capacity of F4-HA corn may be associated with its high amylose content, but follow-up work is needed. The results suggested that high-amylose corn may have potential for utilization as a novel cereal, rich in natural antioxidants.

ACKNOWLEDGMENT

We thank Wan Yuin (Alison) Ser for technical laboratory assistance and Dr. Koushik Seetharaman for assistance with corn drying and storage.

LITERATURE CITED

- (1) Doll, R.; Peto, R. The causes of cancer: quantitative estimates of avoidable risks of cancer in the United States today. *J. Natl. Cancer Inst.* **1981**, *66*, 1197–1265.
- (2) Willet, W. C. Diet and health: What should we eat. *Science* **1994**, *254*, 532–537.
- (3) Temple, N. J. Antioxidants and disease: More questions than answers. *Nutr. Res.* **2000**, *20*, 449–459.
- (4) Martínez-Tomé, M.; Murcia, M. A.; Frega, N.; Ruggieri, S.; Jiménez, A. M.; Roses, F.; Parras, P. Evaluation of antioxidant capacity of cereal brans. *J. Agric. Food Chem.* **2004**, *52*, 4690–4699.
- (5) Zhou, K.; Yu, L. Effects of extraction solvent on wheat bran antioxidant activity estimation. *Lebensm.-Wiss. Technol.* **2004**, *37*, 717–721.
- (6) Dewanto, V.; Wu, X.; Liu, R. H. Processed sweet corn has higher antioxidant activity. *J. Agric. Food Chem.* **2002**, *50*, 4959–4964.
- (7) Adom, K. K.; Liu, R. H. Antioxidant activity of grains. *J. Agric. Food Chem.* **2002**, *50*, 6182–6187.
- (8) Zhao, Z.; Egashira, Y.; Sanada, H. Phenolic antioxidant richly contained in corn bran are slightly bioavailable in rats. *J. Agric. Food Chem.* **2005**, *53*, 5030–5035.
- (9) Tsuda, T.; Horio, F.; Uchida, K.; Aoki, H.; Osawa, T. Dietary cyanidin 3-*O*- β -D-glucose-rich purple corn color prevents obesity and ameliorates hyperglycemia in mice. *J. Nutr.* **2003**, *133* (7), 2125–2130.
- (10) Yu, L.; Haley, S.; Perret, J.; Harris, M.; Wilson, J.; Qian, M. Free radical scavenging properties of wheat extracts. *J. Agric. Food Chem.* **2002**, *50*, 1619–1624.
- (11) Yu, L.; Zhou, K. Antioxidant properties of bran extracts from 'Platte' wheat grown at different locations. *Food Chem.* **2004**, *90*, 311–316.
- (12) 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.
- (13) Gibson, T. S.; McCleary, B. V. A procedure to measure amylose in cereal starches and flours with concanavalin A. *J. Cereal Sci.* **1997**, *25*, 111–119.
- (14) Brand-Williams, W.; Cuvelier, M. E.; Berset, C. Use of a free radical method to evaluate antioxidant activity. *Lebensm.-Wiss. Technol.* **1995**, *28*, 25–30.
- (15) Singleton, V. L.; Rossi, J. A., Jr. Colorimetry of total phenolics with phosmolybdic-phosphotungstic acid reagents. *Am. J. Enol. Vitic.* **1965**, *16*, 144–158.
- (16) Gao, L.; Wang, S.; Oomah, B. D.; Mazza, G. Wheat quality: Antioxidant activity of wheat millstreams. In *Wheat Quality Elucidation*; Ng, P., Wrigley, C. W., Eds.; AACC International: St. Paul, MN, 2002; pp 219–233.
- (17) Beta, T.; Nam, S.; Dexter, J. E.; Sapirstein, H. D. Phenolic content and antioxidant activity of pearled wheat and roller mill fractions. *Cereal Chem.* **2005**, *82* (4), 390–393.
- (18) Mpofu, A.; Sapirstein, H. D.; Beta, T. Genotype and environmental variation in phenolic content, phenolic acid composition, and antioxidant activity of hard spring wheat. *J. Agric. Food Chem.* **2006**, *54*, 1265–1270.
- (19) Popov, I.; Lewin, G. Photochemiluminescent detection of antiradical activity. VI. Antioxidant characteristics of human blood plasma, low density lipoprotein, serum albumin and amino acids during *in vitro* oxidation. *Luminescence* **1999**, *14*, 169–174.
- (20) Popov, I. N.; Lewin, G. Photochemiluminescent detection of antiradical activity; IV: Testing of lipid-soluble antioxidants. *J. Biochem. Biophys. Methods* **1996**, *31*, 1–8.

- (21) Popov, I. N.; Lewin, G. Antioxidative homeostasis: characterization by means of chemiluminescent technique. In *Methods Enzymology*; Packer, L., Glazer, A. N., Eds.; Academic Press: New York, 1999; Vol. 300, pp 437–456.
- (22) Popov, I. N.; Lewin, G. Photochemiluminescent detection of antiradical activity. VII. Comparison with a modified method of thermo-initiated free radical generation with chemiluminescent detection. *Luminescence* **2005**, *20*, 321–325.
- (23) Cao, G. H.; Alessio, H. M.; Cutler, R. G. Oxygen-radical absorbance capacity assay for antioxidants. *Free Radical Biol. Med.* **1993**, *14*, 303–311.
- (24) Huang, D.; Ou, B.; Hampsch-Woodill, M.; Flanagan, J. A.; Prior, R. L. High-throughput assay of oxygen absorbance capacity (ORAC) using a multichannel liquid handling system coupled with a microplate fluorescence reader in 96-well format. *J. Agric. Food Chem.* **2002**, *50*, 4437–4444.
- (25) Dávalos, A.; Gómez-Cordovés, C.; Bartolomé, B. Extending applicability of oxygen radical absorbance capacity (ORAC-fluorescein) assay. *J. Agric. Food Chem.* **2004**, *52*, 48–54.
- (26) Awika, J. M.; Rooney, L. W.; Wu, X.; Prior, R. L.; Cisneros-Zevallos, L. Screening methods to measure antioxidant activity of sorghum (*Sorghum bicolor*) and sorghum products. *J. Agric. Food Chem.* **2003**, *51*, 6657–6662.
- (27) Cao, G. H.; Prior, R. L. Measurement of oxygen radical absorbance capacity in biological samples. Oxidants and antioxidants. *Methods Enzymol.* **1999**, *299*, 50–62.
- (28) Li, W.; Shan, F.; Sun, S.; Corke, H.; Beta, T. Free radical scavenging properties and phenolic content of Chinese black-grained wheat. *J. Agric. Food Chem.* **2005**, *53*, 8533–8536.
- (29) Rondini, L.; Peyrat-Maillard, M.-N.; Marsset-Baglieri, A.; Fromentin, G.; Durand, P.; Tomé, D.; Prost, M.; Berset, C. Bound ferulic acid from bran is more bioavailable than the free copound in rat. *J. Agric. Food Chem.* **2004**, *52*, 4338–4343.
- (30) Popov, I. N.; Lewin, G. Photochemiluminescent detection of antiradical activity: II. Testing of nonenzymic water-soluble antioxidants. *Free Radical Biol. Med.* **1994**, *17*, 267–271.
- (31) Zhou, K.; Laux, J. J.; Yu, L. Comparison of Swiss red wheat grain and fractions for their antioxidant properties. *J. Agric. Food Chem.* **2004**, *52*, 1118–1123.
- (32) Onyeneho, S. N.; Hettiarachchy, N. S. Antioxidant activity of durum wheat bran. *J. Agric. Food Chem.* **1992**, *40*, 1496–1500.
- (33) Krygier, K.; Sosulski, F.; Hogge, L. Free, esterified, and insoluble-bound phenolic acids. I. Extraction and purification procedure. *J. Agric. Food Chem.* **1982**, *30*, 330–334.
- (34) Pozo-Insfran, D. D.; Brenes, C. H.; Saldivar, S. O. S.; Talcott, S. T. Polyphenolic and antioxidant content of white and blue corn (*Zea mays* L.) products. *Food Res. Int.* **2006**, *39*, 696–703.
- (35) Saulnier, L.; Thibault, J. F. Ferulic acid and diferulic acids as components of sugar-beet pectins and corn bran heteroxylans. *J. Sci. Food Agric.* **1999**, *79*, 396–402.
- (36) Veliogu, Y. S.; Mazza, G.; Gao, L.; Oomah, B. D. Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. *J. Agric. Food Chem.* **1998**, *46*, 4113–4117.
- (37) Zielinski, H.; Kozłowska, H. Antioxidant activity and total phenolics in selected cereal grains and their morphological fractions. *J. Agric. Food Chem.* **2000**, *48*, 2008–2016.

Received for review August 3, 2006. Revised manuscript received November 2, 2006. Accepted November 16, 2006. Financial assistance was obtained from the Natural Sciences Engineering Research Council of Canada, Canada Foundation for Innovation, and from the National Research Initiative of the U.S. Department of Agriculture Cooperative State Research, Education and Extension Service, Grant 2001-35503-10030.

JF0622432