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Changes in the Main Nutrients, Phytochemicals, and Antioxidant Activity in Yellow Corn Grain during Maturation

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Immature corn grains are becoming increasingly popular as a snack/vegetable, facilitating human consumption in some countries. Of particular interest were their nutritional and functional properties. Therefore, plants at stages M1 (74 DAS), M2 (86 DAS), M3 (98 DAS), and maturity stage (116 DAS), where DAS refers to days after seeding, were investigated for changes of the nutrients, phytochemicals, and antioxidant activity in corn grains during maturation. Results revealed that during maturation of corn grains, the content of reducing sugar and crude protein decreased while starch and total lipids increased ($p < 0.05$). Total carotenoids first decreased, then increased, and then decreased to minimum at maturity stage. Analysis of the main carotenoid compounds determined by HPLC showed that lutein first increased and then decreased ($p < 0.05$), whereas the reverse was found for β-cryptoxanthin. The change in zeaxanthin was consistent with total carotenoids. Total phenolic content decreased; nevertheless, different phenolic fractions varied with various maturation stages. The antioxidant activity determined by DPPH and FRAP assay in total phenolic extracts decreased during maturation, which may explain that antioxidant activity can be attributed to soluble phenolic and total phenolic content.

KEYWORDS: Nutrients; phytochemicals; phenolics; antioxidant activity; maturation; corn (*Zea mays* L.); DPPH; FRAP

INTRODUCTION

Corn (*Zea mays* L.) is a widely consumed cereal that provides food to much of the world's population because of its rich nutrition, such as high contents of starch and resistant starch, well-balanced protein, excellent oil, and several vitamins and minerals (*1*, *2*). Besides, corn contains abundant phytochemicals with bioactivity such as carotenoids (*3*), tocopherols (*4*), phytic acid (*2*), anthocyanins (*5*), and phenolic compounds (*6*), which, following consumption, putatively provide protection from cellular oxidative stress (*2*, *7*), increase antimutagenic activity, and inhibit colorectal carcinogenesis (*2*, *7*, *8*). Now, corn has become an important staple food in the world, especially in Mexico and Central America. Therefore, the research and development of nutrient and functional foods made with corn have become the focus of research in recent years.

Corn products, namely, masa, tortillas, tortilla chips, corn soup, and related products, are mostly obtained from either fresh masa or dry masa flour made from mature grains by milling to remove excess lime and adhered pericarp tissue, which can result in the reduction of nutriment, especially phytochemicals presented mainly in bound form in whole grains (*9*, *10*). This is important because whole grain consumption has been associated with the prevention of cardiovascular disease, type 2 diabetes, and some cancers (*11*). In recent times, immature corn grains have become increasingly popular as a snack/vegetable, facilitating human consumption in some Asian countries, such as China, Japan, and so on, where corn products processed from mature seeds have not become as popular as they are in their countries of origin (*2*). Immature corn grains without any treatment are roasted or boiled with water, and the tender grains are directly popped into mouth, or they can be used for making millet gruel. Nutritionally, they retain the original components, especially some substances enriched in the outer part of the cereal grains and lost more easily during milling, such as vitamin B and minerals (iron, calcium, phosphorus), which is in conformity with the new USDA Dietary Guidelines for Americans that emphasize the need for consumption of whole grains. Therefore, we expect to obtain some information on nutrition characters and functional properties of yellow corn during maturation, which can lay a good foundation for developing whole grain foods and nutraceuticals or food ingredients based on corn. This is critical to enable consumers to gain greater access to the health benefits of corn. However, information on these aspects is very limited in the immature corn grains (*12*−*14*). In the present study, corn ears were picked by hand from plants at four stages, namely, filling stage (M1, 74 days after seeding (DAS)), milk ripe stage (M2, 86 DAS), wax ripe stage (M3, 98 DAS), and maturity stage (116 DAS). The corn grains cut from them were analyzed to investigate changes of main nutrients and phytochemicals in corn

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grains during maturation, as well as to characterize changes in antioxidant activity.

MATERIALS AND METHODS

Corn Materials. Corn cultivar (*Z. mays* L.) Xianyu 335, bred by Pioneer Hi-Bred International, Inc., USA, is the most popular yellow corn cultivar with wide adaptability across different corn-growing regions of China and was used in the study. The cultivar was planted on June 8, 2008, in plots in China Linfen (latitude, 111° 32′ N; longitude, 36° 27′ E; altitude, 450 m). The soil was of the loess type with a loam texture and pH 6.4−7.0. During the growth of the corn, the plots were irrigated twice on June 16 and August 18 and fertilized with carbamide on July 30 so that the cron grew better.

Corn was picked by hand at different maturation stages M1 (74 DAS), M2 (86 DAS), M3 (98 DAS), and maturity stage (116 DAS). At each stage, the fresh weight of 100 grains was recorded, and moisture percentage of the corn samples was determined using an oven-dry method. Samples were immediately frozen in liquid N_2 to block the enzymatic activities and stored at −40 °C before analysis. The number of replicates was at least three.

Reagents. 2,4,6-Tri(2-pyridyl)-*s*-triazine (TPTZ) and ascorbic acid were purchased from Fluka (Buch, Switzerland). Sodium carbonate, sodium acetate, acetonitrile, and methanol were from Merck (Darmstadt, Germany). 2,2-Diphenyl-1-picrylhydrazyl (DPPH), lutein, zeaxanthin, β-cryptoxanthin, and gallic acid (GA) were from Sigma (St. Louis, MO). All other chemicals and reagents used in the experiments were of analytical grade.

Reducing Sugar and Starch Analysis. Five grams of frozen corn grains was ground and extracted with 100 mL of distilled water for 30 min at 40 °C, and then the homogenates were centrifuged at 4000*g* for 10 min in a centrifuge (Eppendorf 5417R, Germany). The supernatants after centrifugation were analyzed for reducing sugar. Reducing sugar content was determined using the method of Jemai et al. (*15*) with some modifications. A mixture of 1 mL of corn extracts and 2 mL of 3,5-dinitrosalicylic acid (DNS) reagent was homogenized and boiled for 5 min and then cooled immediately using ice cubes. The absorbance of the reaction mixtures was measured using a spectrophotometer (UV2000, Hitachi) at 765 nm wavelength, and the amount of reducing sugar was determined using a DNS standard curve. Starch was determined according to AACC approved method 76-13. All analyses were performed in triplicate.

Total Lipids and Protein Analysis. Total lipids were determined gravimetrically by extraction with acid solvent consisting of 60:40:1 (v/v/v) chloroform, methanol, and hydrochloric acid as described by Marchello et al. (*16*). Corn protein fractions were extracted according to the Osborne procedure with some modifications. Namely, ground samples were sequentially extracted with distilled water (pH 7.0), salt solution (2.0% NaCl), ethanol (75%, v/v), and dilute alkali (0.02 M NaOH) to yield albumin, globulin, prolamin, and glutelin, respectively. Each fraction was extracted at a 1:10 (w/v) solid-to-solvent ratio. Each extraction was carried out at room temperature for 30 min by an ultrasonic homogenizer (Scientz-IID, Ningbo Scientz Biotechnology Co., Ltd., Zhejiang, China) and centrifuged afterward at 4000*g* for 10 min. For each solvent, supernatants were combined, and the protein content was determined by AACC approved method 46-10 (N × 6.25).

Carotenoid Analysis Using Reverse-Phase HPLC. The content of total carotenoids was determined according to AACC method 14-50. Carotenoid analysis was carried out according to the method reported previously (*3*, *17*) with some modifications. Prior to analysis, 1.0 g of frozen corn grain was ground using a mortar and pestle and transferred to a 50 mL screw-capped culture tube, and 6.0 mL of methanol containing 0.1 g of butylated hydroxytoluene/L was added. The test tubes were sealed with screw caps and placed in an 85 °C water bath for 10 min. The test tubes were then removed from the water bath, and 120 μL of 80% KOH was added to each tube. Samples were then vortexed and returned to the water bath for 10 min for saponification to occur. After the samples had been saponified, the test tubes were immediately placed in an ice bath to cool, and then 3 mL of cold deionized water was put into each test tube, followed by 3 mL of hexane. The test tubes were vortexed and immediately centrifuged at 4000*g* for 10 min. The upper organic phase was collected into a 10 mL disposable test tube and was evaporated to dryness with a universal vacuum system. The dried extract was reconstituted in 100 μL of methyl-*tert*-butyl ether followed by 300 μL of methanol. Samples were stored at −20 °C under nitrogen until injection into the HPLC column.

The carotenoid extracts were filtered through a nylon syringe filter (0.45 μm) (Filtrex Technology, Singapore) prior to HPLC analysis and analyzed in a Waters 1525 HPLC chromatograph system (Waters, Milford, MA) equipped with a Waters 2487 diode array detector (DAD) and a C30 carotenoid column (Waters; 5 μm, 4.6 mm × 250 mm) using 100% methanol (containing 1.0 g of ammonium acetate/L) (solvent A) and 100% methyl *tert*-butyl ether (MTBE) (solvent B) as the mobile phase. The following gradient was used: 0−15 min, 100% solvent A; 15−25 min, linear gradient to 10% solvent B; 25−35 min, linear gradient to 30% solvent B; 35−55 min, linear gradient to 50% solvent B. The injection volume was 10 μL, and the flow rate was 1.0 mL/min. Peak area integration was at 453 nm. The extracts and standard compounds were analyzed under the same analysis conditions, and all of the above experiments were replicated three times.

Determination of Different Phenolic Fractions. Four different fractions of phenolic compounds were extracted from the corn samples: insoluble cell-wall-bound phenolics (ICP) and free (FP), soluble ester-bound (SEP), and soluble glycoside-bound phenolics (SGP). Extraction was based on a procedure previously described (*6*) with some minor modifications. Five grams of frozen corn grain was ground and extracted with 50 mL of 80% methanol at room temperature and in the absence of light for 20 min, and then the homogenates were centrifuged at 4000*g* for 15 min at 4 °C in a centrifuge. After centrifugation, the combined supernatants were analyzed for free, soluble ester-bound, and soluble glycoside-bound phenolics, and the residues were reserved for determination of insoluble cell-wall-bound phenolics.

The combined supernatants were evaporated under vacuum at 40 °C to about 20 mL. The aqueous suspension was adjusted using 6 N HCl to pH 2.0 and centrifuged. The clear supernatant was extracted five times with ethyl acetate (20 mL each). The ethyl acetate extract was reduced to dryness under vacuum at 40 °C, and the resulting precipitate was resuspended in 10 mL of methanol. This solution was used to determine free phenolics.

The remaining aqueous solution was divided into two parts. The fraction for soluble glycoside-bound phenolic determination was hydrolyzed in 10 mL of 6 N HCl for 1 h at 4 °C, whereas soluble ester-bound phenolics were released by alkaline hydrolysis with 20 mL of 2 N NaOH for 4 h in the dark and under a nitrogen atmosphere. After both digestions, the pH of the solutions was adjusted to 2.0, and the phenolics were extracted five times with ethyl acetate (20 mL each). The ethyl acetate extracts were reduced to dryness in a vacuum system, and the resulting precipitates were resuspended in 10 mL of methanol.

The residues containing insoluble cell-wall-bound phenolics incorporated into the cell wall were hydrolyzed directly with 20 mL of 2 N NaOH under the same conditions as the soluble ester-bound phenolics. After acidification to pH 2.0 using 6 M HCl, supernatants were pooled and then extracted five times with ethyl acetate (20 mL each). The ethyl acetate extracts were reduced to dryness in a vacuum system, and the resulting precipitates were resuspended in 10 mL of methanol. All of the extracts were stored at −40 °C until used.

Phenolic content was determined according to the Folin−Ciocalteu colorimetric method as described by Xu et al. (*18*). Briefly, an aliquot (0.5 mL) of the suitable diluted phenolic extracts, 2.5 mL of deionized water, and 0.5 mL of 1.0 M Folin−Ciocalteu reagent were mixed in 10 mL volumetric flasks and vortexed. After 8 min, 1.5 mL of 7.5% sodium carbonate solution was added and mixed thoroughly. The absorbance of the reaction mixtures was measured using a spectrophotometer (UV2000, Hitachi) at 765 nm wavelength after incubation for 2 h at room temperature. Methanol was used as the blank, and gallic acid (GA) was used for calibration of standard curve (0−500 mg/L). Phenolic content was expressed as milligrams of gallic acid equivalents (GAE) per 100 g on a dry matter basis (DM).

Determination of Antioxidant Activity. DPPH radical scavenging activity of phenolics was assessed by measuring the capacity of bleaching a purple-colored methanol solution of DPPH radicals as described by Xu et al. (*18*). Briefly, 0.5 mL of phenolic extracts was mixed with 4.5 mL of 60 μM DPPH dissolved in methanol. The mixture was shaken vigorously and left to stand for 30 min in the dark, and the absorbance was measured at 517 nm against a methanol blank. The scavenging rate on

Table 1. Physical Parameters of the Corn Grains at Different Maturation Stages [a]

maturation stage[b]	DAS[c]	seed color	100-grain fresh wt (g)	moisture (%)	100-grain dry wt (g)	% dry matter accumulation
M1	74	creamy white	18.46 ± 0.57 c	75.34 ± 0.79 a	4.55 ± 0.09 d	24.66 d
M2	86	creamy yellow	37.08 ± 0.87 b	56.63 ± 1.30 b	16.08 ± 0.76 c	43.37 c
M3	98	yellow	39.07 ± 0.39 a	42.07 ± 1.19 c	22.66 ± 0.62 b	57.93 b
maturity	116	golden yellow	39.98 ± 0.60 a	18.61 ± 0.57 d	31.64 ± 0.46 a	81.39 a

[a] Numbers represent mean values of three independent replicates ± SD. Different letters indicate statistically significant differences between the means ($p < 0.05$) for each physical parameter. [b] M1−M3 refer to the different maturation stages. [c] DAS, days after seeding.

DPPH radicals was calculated according to the following formula: scavenging rate (%) = $[1 - (A_1 - A_s)/A_0] \times 100$, where A_0 is the absorbance of the control solution (0.5 mL of methanol in 4.5 mL of DPPH solution), A_1 is the absorbance in the presence of phenolic extracts in DPPH solution, and A_s, which is used for error correction arising from unequal color of the sample solutions, is the absorbance of the extracts solution without DPPH.

The reducing ability was determined by using the ferric reducing antioxidant power (FRAP) assay described by Xu et al. (*18*). Briefly, the FRAP reagent was freshly prepared from 300 mM sodium acetate buffer (pH 3.6), 10 mM TPTZ solution in 40 mM HCl, and 20 mM $FeCl_3$ solution in proportions of 10:1:1 (v/v), respectively. The FRAP reagent was prepared fresh daily and was warmed to 37 °C in a water bath prior to use. Then 0.1 mL of phenolic extracts was mixed with 1.8 mL of FRAP reagent and 3.1 mL of ultrapure water. The absorption of the reaction mixture was measured at 593 nm after incubation for 30 min at 37 °C. A standard curve was constructed using $FeSO_4$ solution (100−1000 μM). FRAP value was expressed as micromoles of Fe(II) per gram of DM.

Statistical Analysis. All experiments were conducted three times independently, and the experimental data were expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) and Duncan's multiple-range test were carried out to determine significant differences ($p < 0.05$) between the means by SPSS (version 13.0). Correlation coefficient and regression analyses were determined by DPS (version 3.01) and EXCEL program.

RESULTS AND DISCUSSION

Physical Parameters at Different Maturation Stages. The physical characteristics of the corn grains at different maturation stages are shown in **Table 1**. Development of yellow corn grains is characterized by the presence of pericarp color change varying from creamy white to golden yellow, which can be attributed to the increase in carotenoid content (*19*). It is believed that these color stages reflect the changes in carotenoid profiles as well as changes in moisture content, kernel size, and density during maturation of yellow corn. Weight of the 100 grains increased, whereas moisture content decreased sharply from M1 stage to maturity (**Table 1**). For this reason the rate of dry matter accumulation of corn increased significantly ($p < 0.05$) with ripening. These results are consistent with cereal grain ontogenesis, when grain moisture typically ceases to increase during the accumulation of storage materials (such as starch) within seeds (*20*).

Nutritional Profiles at Different Maturation Stages. Nutritional profiles of corn at different maturation stages are shown in **Table 2**. The content of reducing sugar decreased significantly ($p < 0.05$) by 73.63%, from 6.37 g/100 g of DM at M1 stage to 1.68 g/100 g of DM at maturity stage, whereas starch increased significantly ($p < 0.05$) during maturation and increased to 2.1-fold at maturity stage compared with stage M1. Total lipids were not detected until stage M3, and its content was 1.28 g/100 g of DM. Thereafter, total lipids showed a significant increase and increased to 2.17-fold (2.78 ± 0.07 g/100 g of DM) (**Table 2**), which further indicates that oil or fat-soluble substances in corn grains are located in the embryo, which has come into being relatively late (*21*). The content of crude protein decreased

Table 2. Content (Grams per 100 g of DM) of Main Nutrients in Corn Grains at Different Maturation Stages [a]

maturation stage[b]	reducing sugar	starch	crude protein	total lipids
M1	6.37 ± 0.13 a	28.34 ± 0.98 b	13.06 ± 0.30 a	not detected
M2	4.63 ± 0.13 b	30.57 ± 2.58 b	10.19 ± 0.22 b	not detected
M3	3.98 ± 0.09 c	56.14 ± 1.80 a	10.27 ± 0.28 b	1.28 ± 0.05 b
maturity	1.68 ± 0.05 d	59.63 ± 1.94 a	8.77 ± 0.15 c	2.78 ± 0.08 a

[a] Numbers represent mean values of three independent replicates ± SD. Different letters indicate statistically significant differences between the means ($p < 0.05$) for each nutrient. [b] M1−M3 refer to the different maturation stages.

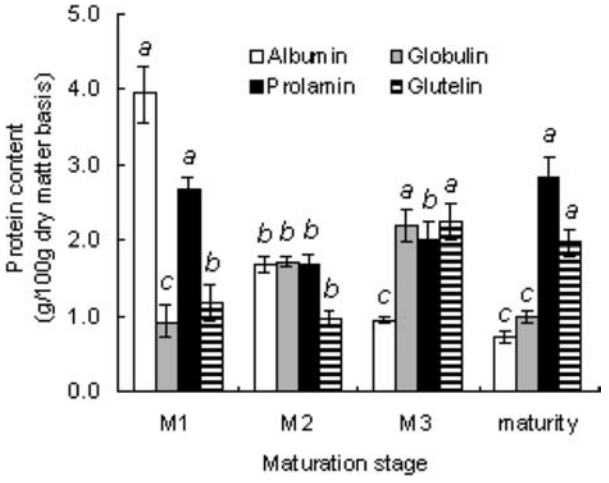

Figure 1. Content (g/100 g of DM) of various protein fractions at different maturation stages of corn grains. Data are expressed as the mean values of three independent replicates ± SD. M1−M3 refer to the different maturation stages. Different letters indicate statistically significant differences between the means ($p < 0.05$) for albumin, globulin, prolamin, and glutelin contents.

significantly ($p < 0.05$) from 13.06 g/100 g of DM at stage M1 to 8.77 g/100 g of DM at maturity stage, but no change was detected between stages M2 and M3. The corn protein fractions also varied significantly ($p < 0.05$) among various maturation stages (**Figure 1**). The content of albumin decreased significantly ($p < 0.05$) by 81.93% from 3.93 g/100 g at M1 stage to 0.71 g/100 g at maturity stage; however, the decrease was not obvious between stages M3 and maturity. Contrary to this, globulin first increased significantly ($p < 0.05$) by 2.35-fold from stages M1 to M3 and then decreased ($p < 0.05$) by 55.25%. The content of prolamin first decreased significantly ($p < 0.05$) by 37.31% between stages M1 and M2 and then increased ($p < 0.05$) to 2.83 g/100 g at maturity stage. Glutelin decreased at first and then increased ($p < 0.05$) to 2.24 g/100 g at stage M3; thereafter, it decreased to 1.98 g/100 g at maturity stage. Landry and Moureaux (*12*) suggested that the quantity of albumin and globulin increases sharply at first and then declines abruptly when the grain ripens; on the contrary, the accumulation of

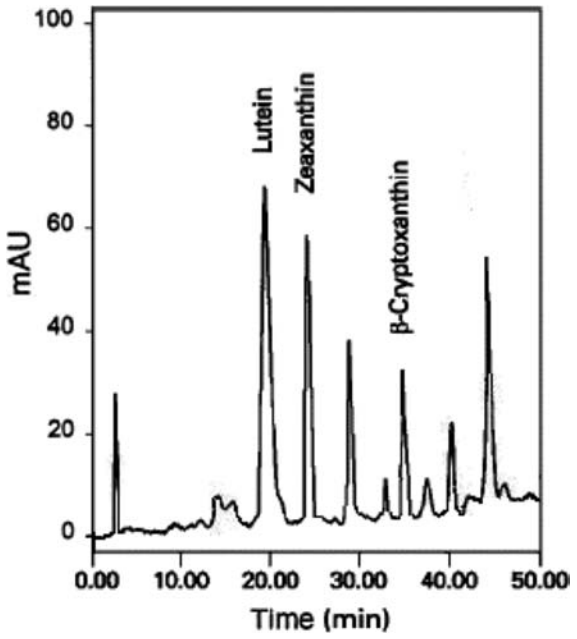

Figure 2. HPLC chromatogram of the carotenoid extracts from the mature corn grains at wavelength 453 nm. The peaks are identified at retention times 19.2, 24.7, and 35.8 min, and correlation coefficient values (R^2) of linear curves were 0.9915, 0.9924, and 0.9855 for lutein, zeaxanthin, and β-cryptoxanthin, respectively. Linearity ranges were all 0.0−10.0 μg/mL.

glutelins is low in immature grain, which was basically the same as the present results on globulin and glutelin. Unexpectedly, this result was quite opposite to what we had studied on albumin, which could be due to an association with corn varieties, sampling time, or different growth conditions. Prolamin and glutelin were the most abundant corn proteins at maturity stage, which was in agreement with previous results (*12*). Besides, we found that the ratio of globulin and albumin in total protein was relatively higher in immature corn grains than in mature grains, which is very important to manufactures of functional foods because of their high bioactivities. Therefore, these changes can produce an effect on nutritive value and processing of corn grains because of differences in biological activity and processing properties of different protein fractions in cereal seed (*22*).

HPLC Analysis of Carotenoids at Different Maturation Stages. The HPLC chromatogram of carotenoid extracts from mature corn grains is shown in **Figure 2**. Identification of the main carotenoids was performed by comparison to the retention time and UV spectra of authentic standards from Sigma, and then the quantitative data were calculated from their linear calibration curves under analysis conditions. Results for the main carotenoids were expressed as micrograms per gram on a dry matter basis. It can be seen from the figure that the main carotenoid compounds such as lutein, zeaxanthin, and β-cryptoxanthin were detected, and some peaks have not been identified because of shortage of standards. The RP-HPLC quantitative analytical results of main carotenoid compounds and total carotenoids extracted from corn at different stages are shown in **Table 3**. Total carotenoids first decreased, then increased, and then decreased to the lowest level at maturity stage, which is different from the corn grain color, possibly because of the differences in content of moisture and other mediums. The main carotenoid compounds varied among various maturation stages. The content of lutein increased significantly ($p < 0.05$) by 31.73% from stages M1 to M3 and then decreased by 5.69% at maturity stage. The content of zeaxanthin did not change much from stages M1 to M3 and then decreased significantly ($p < 0.05$) by 44.21% between stages M3 and maturity. The decline in content of lutein and zeaxanthin may result from their conversion to metabolites because zeaxanthin is an important precursor for abscisic acid (*23*) and lutein is a precursor for apocarotenoids and some aroma compounds (*24*). The content of β-cryptoxanthin increased significantly ($p < 0.05$) by 39.02% at maturity stage compared with stage M1, whereas no obvious changes in β-cryptoxanthin was detected from stages M1 to M3, indicating that the anabolism of β-cryptoxanthin takes place during the later stage of maturation. Although some authers (*25*, *26*) previously reported that lutein and zeaxanthin were major carotenoid species in mature corn grains, whether they are major carotenoids in immature corn grains or not needs to be further studied.

Table 3. Main Carotenoids Content (Micrograms per Gram of DM) in Corn Grains at Different Maturation Stages [a]

maturation stage[b]	lutein	zeaxanthin	β-cryptoxanthin	total carotenoids
M1	8.52 ± 0.13 c	6.65 ± 0.08 a	0.41 ± 0.05 ab	26.89 ± 0.53 b
M2	12.12 ± 0.12 ab	5.49 ± 0.05 b	0.30 ± 0.05 b	23.43 ± 0.23 c
M3	12.48 ± 0.19 a	6.65 ± 0.09 a	0.38 ± 0.10 b	28.76 ± 0.42 a
maturity	11.77 ± 0.17 b	3.71 ± 0.09 c	0.57 ± 0.05 a	22.78 ± 0.22 c

[a] Numbers represent mean values of three independent replicates ± SD. [b] M1-M3 refer to the different maturation stages. Different letters indicate statistically significant differences between the means ($p < 0.05$) for lutein, zeaxanthin, and β-carotene contents.

Phenolic Profiles at Different Maturation Stages. The levels of four different fractions of phenolic compounds (free, soluble ester-bound, soluble glycoside-bound, and insoluble cell-wall-bound phenolics) and total phenolics varied to different degrees between various maturation stages (**Table 4**). Free phenolic content first decreased and then increased ($p < 0.05$) 58.82% at M3 stage compared with M2; thereafter, it decreased significantly by 77.80% and was minimum at maturity stage. The contents of soluble glycoside-bound and ester-bound phenolics decreased continuously by 71.57 and 59.67% by the end of maturity, respectively. However, soluble ester-bound phenolics did not change much between stages M2 and maturity. Insoluble cell-wall-bound phenolic content first increased significantly ($p < 0.05$) by 15.12% at stage M2 and then decreased continuously by 26.70% from stages M2 to maturity. The content of insoluble cell-wall-bound phenolics is far higher than that of others at the same stages, and it was the predominant form of phenolics, which is in agreement with previous results (*9*, *18*) that the majority of phenolic compounds of cereals are bound or attached through a covalent association with cell wall polysaccharides. Changes of the total phenolic content had a more similar trend to the soluble glycoside-bound phenolics (**Table 4**), but the variation range of the total phenolic content was different from that of the soluble glycoside-bound phenolic content, and its content decreased ($P < 0.05$) by 34.60 from stages M1 to maturity.

Phenolics are an ecologically significant class of secondary metabolites expressed in all higher plants and are influenced by different metabolic pathways (*27*). The key enzyme for the phenolic metabolism is phenylalanine ammonia-lyase (PAL), and its activity was found to vary greatly with the stage of plant development (*27*). Some authors (*28*) have reported that stress conditions increase either PAL synthesis or activity in plants; other authors (*29*) have found that some stress treatments delay the increase in wound-induced PAL activity. Besides, changes of different phenolics during grain development were related with plant species and varieties (*27*−*30*). In view of this, the reason for complex changes in the content of different phenolics in corn during maturation requires further investigation.

Table 4. Phenolic Content (Milligrams of GAE per 100 g of DM) in Corn Grains at Different Maturation Stages [a]

maturation stage[b]	FP	SGP	SEP	ICP	total phenolics
M1	33.01 ± 0.28 a	57.50 ± 2.56 a	63.18 ± 5.11 a	276.19 ± 20.20 b	429.89 ± 29.12 a
M2	17.77 ± 0.12 c	27.81 ± 1.41 b	39.29 ± 7.38 b	317.94 ± 6.99 a	403.74 ± 13.72 ab
M3	27.79 ± 0.19 b	22.94 ± 0.85 c	28.05 ± 3.78 b	286.74 ± 6.72 ab	365.48 ± 15.02 b
maturity	6.17 ± 0.03 d	16.35 ± 0.91 d	25.47 ± 4.42 b	233.15 ± 10.33 c	281.14 ± 9.28 c

[a] Numbers represent mean values of three independent replicates ± SD. Different letters indicate statistically significant differences between the means ($p < 0.05$) for free, glycoside-bound, soluble ester-bound, insoluble cell-wall-bound, and total phenolic contents. Abbreviations of different phenolics: FP, free phenolics; SGP, soluble glycoside-bound phenolics; SEP, soluble ester-bound phenolics; ICP, insoluble cell-wall-bound phenolics. [b] M1-M3 refer to the different maturation stages.

Table 5. DPPH Radical Scavenging Activity and FRAP from Corn Grain Extracts at Different Maturation Stages [a]

maturation stage[b]	FP	SGP	SEP	ICP	total phenolics
			DPPH (% Reduction)		
M1	92.27 ± 2.90 a	95.29 ± 1.96 a	95.89 ± 3.67 a	94.15 ± 3.12 a	97.32 ± 1.90 a
M2	56.93 ± 1.29 b	94.99 ± 4.64 a	90.61 ± 4.09 a	93.04 ± 1.92 a	95.61 ± 2.15 a
M3	94.23 ± 2.85 a	93.24 ± 1.69 a	54.64 ± 2.63 b	91.31 ± 3.23 a	94.58 ± 1.14 a
maturity	31.00 ± 0.36 c	90.22 ± 2.57 a	20.96 ± 2.03 c	82.96 ± 1.83 b	92.96 ± 2.16 a
			FRAP Value (μmol of Fe(II)/g of DM)		
M1	3.49 ± 0.16 a	1.70 ± 0.25 a	1.87 ± 0.13 a	26.44 ± 1.83 a	37.27 ± 0.77 a
M2	1.89 ± 0.12 b	0.71 ± 0.07 b	1.34 ± 0.07 b	28.64 ± 3.32 a	30.69 ± 1.50 b
M3	1.81 ± 0.14 b	0.73 ± 0.19 b	0.81 ± 0.12 c	26.70 ± 1.11 a	30.45 ± 1.83 b
maturity	0.71 ± 0.05 c	0.49 ± 0.05 b	0.74 ± 0.06 c	20.51 ± 0.73 b	21.89 ± 0.19 c

[a] Numbers represent mean values of three independent replicates ± SD. Different letters indicate statistically significant differences between the antioxidant activity means ($p < 0.05$) for free, glycoside-bound, soluble ester-bound, insoluble cell-wall-bound, and total phenolic extracts. Abbreviations of different phenolics: FP, free phenolics; SGP, soluble glycoside-bound phenolics; SEP, soluble ester-bound phenolics; ICP, insoluble cell-wall-bound phenolics. [b] M1−M3 refer to the different maturation stages.

DPPH Radicals Scavenging Activity and FRAP of Phenolic Extracts from Corn at Different Maturation Stages. The antioxidant activity is influenced by many factors, and there are more commonly used methods that each have their advantages and disadvantages for measuring antioxidant activity, which cannot be fully described with one single method (*31*). In this assay, the antioxidant activity of the corn grains is measured by DPPH and FRAP assays, and the results are shown in **Table 5**. DPPH radical scavenging activity from total phenolic extracts had no obvious change during maturation of corn, which could be because different total phenolic extracts have had all of the DPPH radicals scavenged though difference in phenolic content. DPPH radical scavenging activity from four different phenolic fractions varied with various reproductive stages, and the maximum values were 94.23% at M3, 95.29% at M1, 95.89% at M1, and 94.15% at M1 for free, soluble glycoside-bound, soluble ester-bound, and insoluble cell-wall-bound phenolics, respectively. Unexpectedly, soluble glycoside-bound phenolic extracts exhibited a higher scavenging activity than the others, although its content was not very high at each reproductive stage, which may imply that soluble glycoside-bound phenolic extracts might contain some highly reactive antioxidative substances. In the FRAP assay, the antioxidant activity of total phenolic extracts decreased significantly ($p < 0.05$) by 41.27%, from 37.27 μmol of Fe(II)/g of DM at M1 stage to 21.89 μmol of Fe(II)/g of DM at maturity stage,; there was no obvious change between stages M2 and M3. The antioxidant activity determined by FRAP assay from four different phenolic fractions also showed changes with different degrees during maturation. Among phenolic fraction extracts, the antioxidant activity of insoluble cell-wall-bound phenolics was the highest at each maturation stage.

To further investigate the influence of different phenolics on the antioxidant activity of phenolic extracts, the correlation between the antioxidant activity and phenolic contents was established, and correlation coefficients (R) are shown in **Table 6**. Free phenolic content associated ($p < 0.05$) significantly with

Table 6. Correlation Analysis of Phenolics and Antioxidant Activity

	phenolic fraction extracts		total phenolic extracts	
phenolic fraction[a]	DPPH	FRAP	DPPH	FRAP
FP	0.9767 *[b]	0.8880[c]	0.8185	0.9303
SGP	0.7317	0.9922 **	0.9290	0.8884
SEP	0.8125	0.9836 *	0.9372	0.8629
ICP	0.8256	0.9683 *	0.5610	0.5761
soluble phenolics			0.9576 *	0.9420
total phenolics			0. 9621 *	0.9587 *

[a] Abbreviations of different phenolics (FP, free phenolics; SGP, soluble glycoside-bound phenolics; SEP, soluble ester-bound phenolics; ICP, insoluble cell-wall-bound phenolics). [b] Significantly different: **, $p < 0.01$; *, $p < 0.05$. [c] Correlation coefficient R.

DPPH radical scavenging activity of free phenolic extracts and other phenolic fractions associated ($p < 0.05$) with FRAP of their own phenolic extracts. Four phenolic fractions were slightly correlated to the antioxidant activity of total phenolic extracts. Interestingly, soluble phenolics content, that is, the sum of free, glycoside-bound, and soluble ester-bound phenolics, correlated ($p < 0.05$) significantly with the DPPH radical scavenging activity of total phenolic extracts. Total phenolic content correlated ($p < 0.05$) significantly with the DPPH radical scavenging and ferric reducing antioxidant power of total phenolic extracts, which are supported by the previous study (*15*, *18*). From the regression analysis it can be concluded that changes of antioxidant activity of total phenolic extracts from corn during maturation can be attributed to changes of soluble phenolic and total phenolic contents.

In conclusion, the main nutrients, phytochemicals, antioxidant activity, and some physical parameters in yellow corn were found to vary greatly with the stage of corn maturation. Data from the present study indicate that immature seeds possess high concentrations of reducing sugars and low concentrations of starches and total lipids, and they have high contents of protein with

biological activity (albumin and globulin) and higher antioxidant activity. Consequently, immature corn grain can be used as a new raw material for the development of whole grain foods and nutritional intervention suitable for human consumption. Further studies need to be performed on the processing properties of protein and starch in immature corn grain and the effects of processing conditions on nutrients and phytochemicals with biological activity.

ABBREVIATIONS USED

GA, gallic acid; DPPH, 2,2-diphenyl-1-picrylhydrazyl; BHT, 2,6-di-*tert*-butyl-4- hydroxytoluene; GAE, gallic acid equivalents; FP, free phenolics; SGP, soluble glycoside-bound phenolics; SEP, soluble ester-bound phenolics; ICP, insoluble cell-wall-bound phenolics; TPTZ, 2,4,6-tri(2-pyridyl)-*s*-triazine; PAL, phenylalanine ammonia-lyase; DM, dry matter weight of grains; HPLC, high-performance liquid chromatography; FRAP, ferric reducing antioxidant power; DAS, days after seeding.

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