Antioxidant Activities and Phenolic Compounds of Cornhusk, Corncob and Stigma Maydis

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Palha, espiga e estigma de milho foram extraídos com água, etanol aquoso, metanol aquoso e acetato de etila, respectivamente. O conteúdo fenólico total (TPC), conteúdo flavonóide total (TFC), conteúdo cetoesteróide total (TKC) e atividades antioxidantes [atividade de sequestro de radicais, poder reductor, e poder antioxidante-reductor férrico (FRAP) de 2,2’-difenil-1-picrilhidrazil (DPPH), 2,4,6-tri(2-piridil)-1,3,5-triazina (TPTZ) e 2,2’-azinobis(3-etilbenzotiazolina-6-ácido sulfônico) (ABTS)] dos extratos foram identificados. Os principais componentes antioxidantes foram posteriormente determinados e quantificados por cromatografia líquida de alta eficiência (HPLC). Os resultados revelaram que etanol e metanol aquosos foram mais eficientes na extração de constituintes antioxidantes da espiga, palha e estigma do milho. Oito componentes antioxidantes principais foram detectados como subprodutos e os conteúdos de quatro componentes antioxidantes principais foram determinados. O trabalho presente revelou que a espiga e palha de milho mostram valores de TPC, TFC e TKC altos e similares, e atividade antioxidante con estigma de milho que podem ser usados como potenciais candidatos na prevenção de doenças relacionadas a vários subprodutos oxidantes do metabolismo humano.

Keywords: cornhusk, corncob, stigma maydis, antioxidant activity, phenolics

Introduction

Phenolics are the most important secondary metabolites in cereals, fruits, or other plant products consumed in a normal diet, and plant phenolics might be found in all parts of the plant, such as roots, stems, leaves, bark, fruit, seeds, flowers, pollen, and so on. It is widely known that oxidant by-products of normal metabolism might cause cancer, ageing, cardiovascular disease, immunosystem decline, and brain dysfunction. Researches showed that phenolics had considerable biological activities including antioxidant, antimitrogenic, antitumour, antiatherogenic, and cardioprotective.

Extraction with excellent efficiency was important for the application of natural phenolics in food industry. It is generally known that the yields of phenolics extraction are mainly depended on the polarities of the solvents. Water, ethyl acetate, aqueous solvent of ethanol and methanol are
used for phenolics extraction such as pigmented rice bran extraction, licorice (Glycyrrhiza glabra) extraction, and propolis extraction. Obviously, various solvents should be selected to extract phenolics from different plants.

Cornhusk, corncob, and stigma maydis were by-products of Zea mays L. (Graminaceae). Stigma maydis has been used as herbal medicine for several years in China, and was confirmed to be safe and non-toxic. Stigma maydis is rich in phenolics, flavonoids, ketosteroids, volatile oil, polysaccharide, proteins, steroids, and mineral element, which were known to have significant effect on human health. Previous studies suggested that blue and red pigmented maize could inhibit colorectal carcinogenesis in male rats, possessed antimutagenic, and radical scavenging activities. Furthermore, pharmacological studies of stigma maydis had discovered its remarkable bioactivities including antioxidant, antibacterial, hyperglycemia reduction, anti-depressant, anti-fatigue, and effective diuretic agent. It is interesting that the high scavenging activity of stigma maydis may be due to hydroxyl groups existing in the phenolic compounds that can provide the necessary components as the radical scavenger according to the research previously. Stigma maydis extract can be potentially used as valuable bioactive sources of natural antioxidants. Similarly, the corncob extract exhibit significant antioxidant property according to investigation. Although a few anthocyanins were determined, the exact antioxidant components of corncob remained unknown. For cornhusk, no bioactivity and chemical component has been reported, except for a bioethanol preparation. It is worthy to note that the yields of cornhusk and corncob are higher than stigma maydis in the agricultural production, and most of cornhusk and corncob were thrown away or used as agricultural fertilizer.

The present study describes the various solvents extraction recovery, total phenolic contents (TPC), total flavonoid contents (TFC), total ketosteroid contents (TKC) and antioxidant activities of cornhusk, corncob, and stigma maydis. Eight phenolic compounds (gallic acid, protocatechuic acid, chlorogenic acid, caffeic acid, felleic acid, rutin, resveratrol, and kaempferol) were detected from above three by-products, and the contents of four main antioxidant components (gallic acid, caffeic acid, felleic acid, and resveratrol) were determined.

**Experimental**

**Chemicals and instruments**

2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) was purchased from Sigma-Aldrich (St. Louis, MO, USA). 2,2′-diphenyl-1-picrylhydrazyl (DPPH) and 2,4,6-tri(2-pyridyl)-1,3,5-triazine (TPTZ) were obtained from J&K Scientific Ltd (Beijing, China). Rutin, kaempferol, and gallic acid were purchased from Aladdin-Reagent (Shanghai, China). Protocatechuic acid, chlorogenic acid, caffeic acid, felleic acid, and resveratrol were obtained from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Folin-Ciocalteu reagent was prepared by our group according the method of GB/T 23527-2009 (CN). Ponasterone A 3-β-D-xylopyranoside (≥ 95%, PA) was separated by our group. Acetonitrile (HPLC grade) was obtained from Fisher Chemicals (New Jersey, USA). The water (resistivity ≥ 18.25 MΩ cm⁻¹) used was purified with a purity water system (Chengdu, China). All other chemicals used were of analytical grade.

A Shimazu UV-VIS 2401 PC spectrometer was used for colorimetric measurements. The Agilent 1200 Series HPLC system consisted of an Agilent G1315D DAD detector and two Agilent G1310A Iso pumps (Agilent Corporation, USA) was used to determine the concentration of phenolics.

**Plant materials**

The cornhusk, corncob, and stigma maydis were collected in Kunming, Yunnan Province, China, in August 2013, and authenticated by Prof Shugang Lu from School of Life Science, Yunnan University. Three voucher specimens (No. DJW-Z01, Z02 and Z03) had been deposited at the Key Laboratory of Medicinal Chemistry for Nature Resource of Yunnan University.

**Extraction**

2 g of sample powders (cornhusk, corncob, and stigma maydis) were soaked in 100 mL of water, 50% (v/v) ethanol, 80% (v/v) ethanol, 50% (v/v) methanol, 80% (v/v) methanol, and ethyl acetate for 24 h, respectively, and extracted with ultrasonic cleaner (whole power altitude model) at 40 °C for three times, 30 min each time, respectively. The extracts were decanted, filtered under vacuum and concentrated in a rotary evaporator. The extracts were made up to 10 mL with methanol to produce stock solution.

**Total phenolic content (TPC)**

Total phenolic content was determined according to the Folin-Ciocalteu method with slight modification.
100 μL of sample was added to 4.5 mL Folin-Ciocalteu reagent which was prediluted ten times with distilled water. After 5 min, a 3.0 mL of Na₂CO₃ (7.5%, m/v) solution was added and the mixture was allowed to stand for 30 min at ambient temperature. Absorbance was measured at 765 nm. A calibration curve was obtained using standard solution of gallic acid (ranging from 0 to 20 μg). The total phenolic content was expressed as mg gallic acid equivalent per 100 g dry weight (mg GAE per 100 g dw).

Total flavonoid content (TFC)

The total flavonoid content was determined by a colorimetric assay described previously with slight modification.³² 1 mL of properly diluted sample mixed with 4.0 mL diluted water was added to 0.3 mL of NaNO₂ (5%, m/v). 5 min later, 0.6 mL of AlCl₃ (10%, m/v) was added. After incubation for 6 min, 4.1 mL of NaOH (1.0 mol L⁻¹) was added to the mixture. The absorbance at 510 nm was measured against a blank solution. A calibration curve was obtained using rutin standard solution (ranging from 0 to 600 μg). The total flavonoid content was expressed as mg rutin equivalent per 100 g dry weight (mg RE per 100 g dw).

Total ketosteroid content (TKC)

The total ketosteroid content was measured according to the method with minor modification.³³ 100 μL of properly concentrated sample was added to the tube, and the solvent was volatilized at 80 °C. 100 μL vanillin (50 mg mL⁻¹, in CH₃COOH) and 0.4 mL of HClO₄ were added, the mixture was allowed to stand for 15 min at 60 °C. Then, the solution was cooled promptly by ice-water bath and 2.5 mL of glacial acetic acid was added. The absorbance was recorded at 550 nm. A standard curve was prepared by using standard solution of ponasterone A 3-β-D-xylopyranoside (PA) (ranging from 0 to 800 μg). The TKC were expressed as mg PA equivalent per 100 g dry weight (mg PAE per 100 g dw).

Antioxidant activities

DPPH radical-scavenging activity

The DPPH free radical-scavenging activity was performed by the method described previously with slight modification.³⁴ 100 μL of each sample at proper concentration was mixed with 3.9 mL of ethanolic solution containing 0.075 mmol L⁻¹ DPPH. The mixture was shacked vigorously, and then left to stand for 30 min in the dark. The absorbance was measured at 515 nm. The absorbance of the control was obtained by replacing the sample with ethanol. DPPH radical scavenging activity of the sample was calculated as follow:

\[
\text{DPPH radical-scavenging activity (\%) = \left[1 - \frac{\text{absorbance of sample}}{\text{absorbance of control}}\right] \times 100}
\]

A standard curve was obtained using Trolox standard solution at various concentrations (ranging from 0 to 3.00 μmol L⁻¹) in methanol. The antioxidant activities of samples were expressed as μmol Trolox equivalent per 100 g of dry weight (μmol TE per 100 g dw).

ABTS radical-scavenging activity

The ABTS assay was measured using an improved ABTS method described previously.³⁵ The ABTS radical cation (ABTS⁺) solution was prepared by the reaction of 7 mmol L⁻¹ ABTS and 2.5 mmol L⁻¹ potassium persulphate, after incubation at room temperature in the dark for 12-16 h. The ABTS⁺ solution was then diluted with PBS buffer solution (200 mmol L⁻¹, pH 7.4) to obtain an absorbance of 0.70 ± 0.02 at 734 nm. 2 mL of sample was added to 2 mL of ABTS⁺ solution and mixed vigorously. The reaction mixture was allowed to stand at room temperature for 6 min and the absorbance at 734 nm immediately recorded. The ABTS radical scavenging activity of the sample was calculated as follow:

\[
\text{ABTS radical-scavenging activity (\%) = \left[1 - \frac{\text{absorbance of sample}}{\text{absorbance of control}}\right] \times 100}
\]

A standard curve was obtained using Trolox standard solution at various concentrations (ranging from 0 to 3.00 μmol L⁻¹) in methanol. The antioxidant activities of samples were expressed as μmol trolox equivalent per 100 g of dry weight (μmol TE per 100 g dw).

Reducing power

The reducing power was evaluated according to the previous method with minor modification.³⁶ 100 μL of sample at proper concentration was made up to 0.75 mL with phosphate buffer (300 mmol L⁻¹, pH 6.6), and 1.5 mL of 1% (m/v) K₃Fe(CN)₆ was added. The mixture was shaked vigorously and left to stand for 20 min at 50 °C. After the addition of 1.5 mL of 10% (m/v) trichloroacetic acid, the mixture was centrifuged at 3000 rpm for 10 min. A 1.5 mL of supernatant was mixed with distilled water (1.5 mL), and 0.3 mL of 0.1% (m/m) FeCl₃ was added before the absorbance was determined at 700 nm. A standard curve was obtained using trolox standard solution at various concentrations (ranging from 0 to 2.91 μmol L⁻¹)
in methanol. The antioxidant activities of samples were expressed as μmol Trolox equivalents per 100 g of dry weight (μmol TE per 100 g dw).

**Ferric reducing-antioxidant power**

The ferric reducing-antioxidant power (FRAP) assay measures the reduction of ferric iron to the ferrous form in the presence of the antioxidant components according to a method with slight modification. The working FRAP reagent was prepared freshly every day by mixing 5.0 mL of TPTZ (10 mmol L\(^{-1}\) in 40 mmol L\(^{-1}\) hydrochloric acid), 5.0 mL of ferric chloride (20 mmol L\(^{-1}\)) and 50 mL of sodium acetate buffer (300 mmol L\(^{-1}\), pH 3.6). The FRAP assay was carried out at 37 °C. 3 mL of the FRAP reagent was mixed with 300 μL of water, and 100 μL of sample was added. The reaction mixture was allowed to stand at 37 °C for 30 min and the absorbance was measured at 595 nm. Standard solutions of ferrous sulfate at various concentrations (ranging from 0 to 60.0 μmol L\(^{-1}\)) were to prepare a standard curve. The antioxidant capacities of the extracts were expressed as μmol ferrous sulfate per 100 g of dry weight (μmol Fe\((II)\) per 100 g dw).

**Phenolics determined by high performance liquid chromatography (HPLC)**

The determination and quantification of phenolic compounds were measured according to a previous method with slight modification. Samples or standards were filtered through a 0.45 μm filter before injection into the HPLC system. HPLC separation, identification and quantification were performed on an Agilent 1200 Series system, equipped with an Agilent G1315D DAD detector, two Agilent G1310A Iso pumps, and Agilent Zorbax SB-C\(_{18}\) (250 × 4.6 mm i.d., 5 μm), and coupled to an Agilent ChemStation (version B.04.02) data-processing station. A gradient elution system consisting of solvent A (water containing 0.2% acetic acid) and B (acetonitrile) was used for the analysis, and the gradient programme was as follows: 0-20 min, 5% solvent B; 20-25 min, 5-10% solvent B; 25-35 min, 10% solvent B; 35-40 min, 10-15% solvent B; 40-50 min, 15% solvent B; 50-60 min, 15-25% solvent B; 60-80 min, 25-35% solvent; 80-90 min, 35% solvent B. The peaks were confirmed by the UV absorptions (280 nm) and the retention times while the flow rate was 1.0 mL min\(^{-1}\), the column temperature was set at 30 °C and the injection volume was 10 μL. Phenolics were identified by using standard addition method, and quantified according to an external standard method. The limit of detection (LOD) and quantification (LOQ) were listed in as follow [phenolic compound, LOD (LOQ)]: gallic acid, 0.00063 μg (0.0025 μg); cafeic acid, 0.00125 μg (0.0025 μg); fumeric acid, 0.00031 μg (0.0025 μg); resveratrol, 0.00031 μg (0.0025 μg); protocatechuic acid, 0.00015 (not quantified, nq); chlorogenic acid, 0.00031 (nq); rutin, 0.00063 (nq); kaempferol, 0.00015 (nq). The linear regression analysis equations and linear ranges are in the Supplementary Information data. Recovery experiments were performed in order to study the accuracy of the method. The recoveries of four phenolic compounds for quantification analysis were range from 95% to 105%.

**Statistical analysis**

The data were presented as mean ± standard deviation (SD) of three determinations for each sample. Statistical analyses were performed using the statistical software (SPSS 19.0, SPSS Inc., USA). A significant difference was evaluated at a level of \(p < 0.05\).

**Results and Discussion**

**Total phenolic content (TPC)**

Phenolics and polyphenolics were main active components in vegetables, fruits, grains and so on. Stigma maydis contains a large amount of phenolics according to the research previously. In this study, the TPC of three by-products including cornhusk, corncob, and stigma maydis extracted with different solvents were determined with Folin-Ciocalteau method.

![Figure 1. The total phenolic content of three Zea mays by-products extracted with different solvents. Values were expressed as mean ± SD (n = 3). Abbreviations: W, water; 50%E, 50% (v/v) ethanol; 80%E, 80% (v/v) ethanol; 50%M, 50% (v/v) methanol; 80%M, 80% (v/v) methanol; EA, ethyl acetate.](image-url)
The results (Figure 1) showed that recovery of TPC followed the solvent order of aqueous solvents ≥ water >> ethyl acetate. For all by-products, the highest phenolic content (298.8 ± 11.9 mg GAE per 100 g dw for cornhusk, 283.4 ± 13.2 mg GAE per 100 g dw for corncob, and 399.4 ± 18.9 mg GAE per 100 g dw for stigma maydis) was found in the extract of 80% (v/v) ethanol. The TPC extracted from stigma maydis was slightly higher than other two by-products (cornhusk and corncob). This study revealed that phenolics in *Zea mays* by-products were more extractable by highly polar solvents, which was similar to the description of by-product of eggplant.41

**Total flavonoid content (TFC)**

Flavonoids were one of the main active components in vegetables, fruits, and grains.42 Previous research revealed that free radical was scavenged or radical-reaction would be blocked while flavonoids exiting.43 Flavonoids were also isolated and identified from stigma maydis.44,45 The TFC of the different solvents extracts of three kinds of by-products were determined. The values expressed as mg RE per 100 g dw in Figure 2 decreased in the following order: 80% (v/v) ethanol > other aqueous ethanol ≈ ethyl acetate > water.

The TFC in the extracts of 80% (v/v) ethanol were higher than others, which the contents were 846.8 ± 32.3, 1166.8 ± 45.3, and 956.8 ± 37.8 mg RE per 100 g dw for extracts of cornhusk, corncob, and stigma maydis, respectively. The results showed that 80% (v/v) ethanol was proper solvent for flavonoids extraction from three kinds of *Zea mays* by-products.

**Total ketosteroid content (TKC)**

Ketosteroids, similar to flavonoids, were one of the main antioxidant components. The ketosteroids of *Matteuccia struthiopteris* were determined and obvious DPPH radical-scavenging activity in ketosteroids was found according to previous research.33 Ketosteroids and sterols had been separated and identified from stigma maydis by our group,15 therefore, it’s essential to determinate the TKC of the by-products of *Zea mays*.

![Figure 2](image-url)  
**Figure 2.** The total flavonoid content of three *Zea mays* by-products extracted with different solvents. Values were expressed as mean ± SD (n = 3). Abbreviations are the same as in Figure 1.

![Figure 3](image-url)  
**Figure 3.** The total ketosteroid content of three *Zea mays* by-products extracted with different solvents. Values were expressed as mean ± SD (n = 3). Abbreviations are the same as in Figure 1.

The results of TKC (Figure 3) expressed as mg PAE per 100 g dw followed the solvent order of aqueous solvents > water ≈ ethyl acetate. The TKC of aqueous solvents extracts of cornhusk (range from 4594.2 ± 89.7 to 8233.3 ± 171.7 mg PAE per 100 g dw) and corncob (range from 5823.2 ± 121.2 to 7460.9 ± 213.0 mg PAE per 100 g dw) were slightly higher than TKC of stigma maydis (range from 3568.1 ± 148.4 to 4994.2 ± 194.6 mg PAE per 100 g dw).

**Antioxidant activities**

Oxidation is universally existent and has deleterious effects on both food quality and human health. Previous research declared that oxidative damage can give rise not only to browning, off-flavor, and changing in nutrient value of food, but also to a potential threat to cellular function and formation of compounds which are related to aging acceleration and cardio-vascular disease.46 In this study, the antioxidant activities of three kinds of *Zea mays* by-products were estimated using four methods including DPPH, ABTS, reducing power, and FRAP, respectively.

DPPH is a stable free radical, which has been widely used for studying the free radical-scavenging activities of...
natural antioxidants. The values expressed as μmol TE per 100 g dw in Figure 4 showed that the DPPH radical-scavenging activity followed solvents order of water ≈ aqueous solvents > ethyl acetate.

Previous studies demonstrated that the reducing power of the natural plant extracts might be strongly correlated with their antioxidant activity. It is necessary to discuss the reducing power of a natural plant extract to elucidate the relationship between its antioxidant effect and reducing power. The results in Figure 6 was expressed as μmol TE per 100 g dw, showed that the reducing power followed solvents order of aqueous solvents ≥ water >> ethyl acetate, which was similar to the DPPH radical-scavenging activity.

The ABTS radical-scavenging activities assay has always been used as a method for total antioxidant activity. And the ABTS radical formed from ABTS-e to ABTS•+ reacts quickly with the electron/hydrogen donors to form color-less ABTS. The values of ABTS radical-scavenging activity in Figure 5 were expressed as μmol TE per 100 g dw, and decreased in the following order: aqueous solvents ≥ water >> ethyl acetate. The values in the extract of 80% (v/v) ethanol were the highest, 252.9 ± 11.6, 263.0 ± 13.5, and 244.1 ± 10.2 μmol TE per 100 g dw were for extracts of cornhusk, corncob, and stigma maydis, respectively. The consequence was in good agreement with that of TPC.

The ferric reducing-antioxidant power (FRAP) is often used as an indicator of phenolic antioxidant activity as important as reducing power. The antioxidant potential of sample was estimated by their abilities which is to reduce Fe(III)-TPTZ to Fe(II)-TPTZ. In this study, FRAP was measured using a standard of FeSO₄ and the results in Figure 7 were expressed as μmol
Fe(III) per 100 g dw. It showed that the antioxidant activities were followed the solvents order of 80% (v/v) ethanol > others aqueous solvents > water and ethyl acetate. That is to say, 80% (v/v) ethanol was a proper solvent for extraction of three kinds of Zea mays by-products, which agreed with the results previously.50

Correlation between antioxidant components and antioxidant activities

Correlation analysis between antioxidants components and antioxidant activities of three by-products extracted with different solvents was performed, and the results in Table 1 suggested that positive correlations were found between TPC and values of all methods of antioxidant activities including DPPH radical-scavenging activity ($r = 0.709$), ABTS radical-scavenging activity ($r = 0.871$), reducing power ($r = 0.935$), and FRAP ($r = 0.477$). However, there was no obvious correlation between TFC and antioxidant activities except the FRAP ($r = 0.680$). The TKC was also slightly correlated with antioxidant activities except DPPH radical-scavenging activities. These data implied that phenolics played an important role in the antioxidant activities of three Zea mays by-products, which agreed with the conclusion described previously.51

Phenolics of three by-products analyzed by HPLC

To clarify the phenolics performed positive antioxidant activities in different solvents extracts of three by-products, eight phenolic components including gallic acid (1) protocatechuic acid (2) chlorogenic acid (3) cafeic acid (4) femlic acid (5) rutin (6) resveratrol (7) kaempferol (8) in Figure 8 were determined by HPLC using standard additions method, and the contents of phenolics were showed in Table 2.

Table 1. Correlations between antioxidant activities and antioxidant components

<table>
<thead>
<tr>
<th>Correlation coefficients</th>
<th>TPC</th>
<th>TFC</th>
<th>TKC</th>
<th>DPPH</th>
<th>ABTS</th>
<th>FRAP</th>
<th>Reducing power</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPC</td>
<td>1</td>
<td>0.385</td>
<td>0.576</td>
<td>0.709</td>
<td>0.871</td>
<td>0.477</td>
<td>0.935</td>
</tr>
<tr>
<td>TFC</td>
<td>–</td>
<td>1</td>
<td>0.247</td>
<td>0.093</td>
<td>0.313</td>
<td>0.680</td>
<td>0.351</td>
</tr>
<tr>
<td>TKC</td>
<td>–</td>
<td>–</td>
<td>1</td>
<td>0.076</td>
<td>0.794</td>
<td>0.372</td>
<td>0.587</td>
</tr>
</tbody>
</table>

*Correlation coefficients were calculated by one-way linear analysis.

Figure 8. Results of identification of phenolics from standards (a) and 80% (v/v) ethanol extracts of cornhusk (b); corncob (c); and stigma maydis (d) by HPLC.
The contents of phenolics followed the solvents order of aqueous solvents > water >> ethyl acetate, especially the content of resveratrol (7), which was in good accordance with TPC determined by Folin-Ciocalteau method. It suggested that aqueous solvent was the proper solvent for phenolics extraction from Zea mays by-products.

**Conclusions**

In present study, the solvent extraction, phenolics, and antioxidant activities of three Zea mays by-products were investigated. The results revealed that phenolics in Zea mays by-products were more extractable by 80% (v/v) ethanol and eight phenolics of gallic acid, protocatechuic acid, chlorogenic acid, cafeic acid, femlic acid, rutin, resveratrol, and kaempferol played important roles for strong antioxidant activities of these by-products. Furthermore, cornhusk, and corncob possessed same high TPC, TFC, and TKC as stigma maydis and exhibited strong antioxidant activities. Therefore, cornhusk and corncob could be used as potential antioxidant candidates for further development like stigma maydis.

**Supplementary Information**

Supplementary data are available free of charge at http://jbcs.sbq.org.br as PDF file.

**Acknowledgements**

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**Table 2.** The contents of phenolic compounds of three Zea mays by-products determined by HPLC.

<table>
<thead>
<tr>
<th>Sample</th>
<th>W</th>
<th>50%E</th>
<th>80%E</th>
<th>50%M</th>
<th>80%M</th>
<th>EA</th>
<th>W</th>
<th>50%E</th>
<th>80%E</th>
<th>50%M</th>
<th>80%M</th>
<th>EA</th>
</tr>
</thead>
<tbody>
<tr>
<td>gallic acid</td>
<td>1.46 ± 0.04</td>
<td>1.91 ± 0.06</td>
<td>1.95 ± 0.07</td>
<td>2.44 ± 0.07</td>
<td>2.69 ± 0.07</td>
<td>0.46 ± 0.07</td>
<td>8.58 ± 0.56</td>
<td>1.60 ± 0.05</td>
<td>1.84 ± 0.05</td>
<td>4.50 ± 0.14</td>
<td>2.77 ± 0.14</td>
<td>13.47 ± 0.14</td>
</tr>
<tr>
<td>cafeic acid</td>
<td>1.74 ± 0.05</td>
<td>2.26 ± 0.07</td>
<td>2.26 ± 0.07</td>
<td>1.34 ± 0.08</td>
<td>2.72 ± 0.08</td>
<td>0.41 ± 0.08</td>
<td>1.09 ± 0.03</td>
<td>2.06 ± 0.09</td>
<td>2.99 ± 0.11</td>
<td>3.72 ± 0.11</td>
<td>3.40 ± 0.11</td>
<td>5.11 ± 0.11</td>
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<tr>
<td>femlic acid</td>
<td>2.22 ± 0.01</td>
<td>0.96 ± 0.03</td>
<td>1.47 ± 0.03</td>
<td>1.18 ± 0.03</td>
<td>0.77 ± 0.03</td>
<td>0.25 ± 0.03</td>
<td>1.25 ± 0.06</td>
<td>1.52 ± 0.08</td>
<td>1.49 ± 0.08</td>
<td>1.24 ± 0.08</td>
<td>1.72 ± 0.08</td>
<td>0.10 ± 0.03</td>
</tr>
<tr>
<td>resveratrol</td>
<td>0.89 ± 0.04</td>
<td>3.56 ± 0.05</td>
<td>8.82 ± 0.05</td>
<td>5.58 ± 0.05</td>
<td>4.13 ± 0.05</td>
<td>0.55 ± 0.05</td>
<td>0.94 ± 0.05</td>
<td>8.73 ± 0.06</td>
<td>11.01 ± 0.06</td>
<td>10.57 ± 0.06</td>
<td>11.28 ± 0.06</td>
<td>0.49 ± 0.03</td>
</tr>
</tbody>
</table>

Values were mean ± SD (n = 3). The contents were expressed as mg per 100 g dw; aW, 50%E, 80%E, 50%M, 80%M, and EA were expressed as extracts extracted with water, 50% (v/v) ethanol, 80% (v/v) ethanol, 50% (v/v) methanol, 80% (v/v) methanol and ethyl acetate, respectively; the nd means not determined under the LODs.

**References**