Phenolic acids inhibit the formation of advanced glycation end products in food simulation systems depending on their reducing powers and structures

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ABSTRACT
The concentration of advanced glycation end products (AGEs) in foods, which are formed by Maillard reaction, has demonstrated as risk factors associated with many chronic diseases. The AGEs inhibitory activities of five common phenolic acids (protocatechuic acid, dihydroferulic acid, p-coumaric acid, p-hydroxybenzoic acid and salicylic acid) with different chemical properties had been investigated in two food simulation systems (glucose-bovine serum albumin (BSA) and oleic acid-BSA). The results substantiated that the AGEs inhibitory abilities of phenolic acids in the oleic acid BSA system were much better than the glucose-BSA system for their strong reducing powers and structures. Among them, dihydrogenferulic acid showed strong inhibition of AGEs formation in oleic acid-BSA system at 0.01 mg/mL compared to nonsignificant AGEs inhibitory effect in oleic acid-BSA system at 10-fold higher concentration (0.1 mg/mL). This study suggests that edible plants rich in phenolic acids may be used as AGEs inhibitor during high-fat cooking.

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Introduction
Advanced glycation end products (AGEs) are a group of heterogeneous compounds formed by Maillard reaction (Hu et al. 2014), and the researches on them were mainly focused on their influence on color and flavor of foods since being discovered in 1912 (Hodge 1953). In 1958, Allen et al reported the first example of a Maillard reaction product (glycated hemoglobin) in vivo (1958). Studies have shown that many chronic diseases, such as diabetes mellitus, cardiovascular disease, Alzheimer and some types of cancers are connected to AGEs levels in vivo (Sasahira et al. 2007; Kang et al. 2010; Luevano-Contreras & Chapman-Novakofski 2010; Vlassara & Striker 2011) by disturbing the structure and function of some major proteins engaging them to crosslink and increase oxidative stress and inflammation (Sparvero et al. 2009; Saito & Marumo 2010; Juranek et al. 2013; Gautieri et al. 2014).

Recent studies have unveiled that restriction in oral intake of potential AGEs sources in mice can reduce oxidative stress, inflammation and serum AGEs levels in healthy individuals or patients, and reinstate AGEs receptor 1 and SIRT1 (nicotinamide adenine dinucleotide – dependent deacetylase sirtuin-1) levels in patients with diabetes mellitus or kidney disease (Vlassara & Striker 2011; Cai et al. 2012; Kellow & Savige 2013; Stirban et al. 2013). Most of the AGEs related complications go on their way through mouth as after oral intake AGEs from foods can be absorbed through the intestine in the form of dipeptide (Geissler et al. 2010). About 50% of ingested Nω-carboxymethyllysine (the marker of AGEs) can be excreted by feces and urine (Delgado-Andrade et al. 2012). Hence, the oral restriction of AGEs from foods can help to prevent or alleviate some chronic diseases such as diabetes mellitus and cardiovascular disease (Vlassara & Striker 2011).

According to previous researches, heating makes foods digestible, delicious and microbiologically safe, but high heating temperature and longtime cooking can significantly increase the AGEs contents in foods (Uribarri et al. 2010). Therefore, it is very meaningful to find a method to minimize AGEs concentrations in foods, while maintaining organoleptic properties and acceptable microbiological safety of foods. Many synthetic compounds and natural products have been
proposed as AGEs inhibitors in vivo, like aminoguanidine (Brownlee et al. 1986), N-phenylthiazolium bromide (Vasan et al. 1996), pyridoxamine (Chen & Francis 2012), polyphenols (Xie & Chen 2013) and plant extracts (Peng et al. 2008; Wu et al. 2013). Although the synthetic compounds, such as pimagedine, demonstrate strong inhibitory abilities on AGEs formation in vivo, they may also result in some side effects. Pimagedine was the first AGEs inhibitor engaged in clinical research and was discontinued due to the side effects (myocardial infarction, congestive heart failure and atrial fibrillation) during phase-III clinical trial in patients with diabetic nephropathy (Bolton & Abdel-Rahman 2002). As compared to synthetic compounds, with a good compatibility with living system natural compounds have considerably less side effects while used as inhibitors. Therefore, natural products may be a good choice to be used for reducing the AGEs contents in foods due to more safety compared with synthetic compounds. Catechin and procyanidins strengthened the above statement as they were proved to be having significant inhibitory abilities against AGEs formation through scavenging dicarbonyl compounds and antioxidant activity (Babu et al. 2008; Wu et al. 2013).

Phenolic acids are kinds of aromatic compounds containing a phenolic ring and carboxyl group (C6-C1 skeleton) existing in various plant species and are widely investigated owing to their antioxidant activities and some chronic disease prevention (Boeing et al. 2012; Henning et al. 2013; Zamora-Ros et al. 2013; Ky et al. 2014; Shao et al. 2014). The inhibitory activities of some phenolic acids against AGEs formation in vivo (Lin et al. 2011; Sompong et al. 2015) and in vitro (Bhattacherjee & Datta 2015; Vlassopoulos et al. 2015) have been studied. The results have confirmed that the phenolic acids can significantly inhibit the AGEs formation. But the AGEs inhibitory effect of phenolic acids was rarely studied in food processing systems. Many of the cooked foods are carriers of AGEs and this makes study of AGEs inhibition important in a food system to better understand their behavior. In this study, AGEs inhibitory activities of five common phenolic acids (protocatechuic acid, dihydroferulic acid, p-coumaric acid, p-hydroxybenzoic acid and salicylic acid) with different chemical characteristics were investigated in two food simulation systems, glucose-BSA system (designed to simulate the glycation reaction between reducing sugars and proteins in foodstuff like bread and skim milk) and oleic acid-BSA system (designed to simulate the glycation reaction between fatty acids and proteins in foodstuff like meat and egg).

**Materials and methods**

**Materials**

Protocatechuic acid (chromatographic grade, ≥ 97.0%), p-coumaric acid (chromatographic grade, ≥ 99.0%), p-hydroxybenzoic acid (chromatographic grade, ≥ 99.5%), salicylic acid (chromatographic grade, ≥ 99.0%), tripyridyl triazine (TPTZ), aminoguanidine (AG), glyoxal (GO (40% aqueous solution)), 2,3-dimethylquinoxaline (DQ) and 1,2-phenylenediamine (PD) were purchased from Aladdin Chemical Co. Ltd. (Shanghai, China). Dihydroferulic acid (chromatographic grade, 96.0%) was purchased from Tokyo Chemical Industry Co. Ltd. (Shanghai, China). 2,2′-Azinobis-(3-ethylbenzthiazoline-6-sulfonate) (ABTS) was purchased from Sigma-Aldrich Co. (San Francisco, CA). Bovine serum albumin (BSA, Biological reagent, ≥ 96.0%), D-glucose, oleic acid, diethylenetriamine pentaacetic acid (DTPA) and 2-thiobarbituric acid (TBA) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). All the other chemicals mentioned here or otherwise used were of analytical quality.

**Inhibition of AGEs formation**

The AGEs inhibitory effects of phenolic acids were investigated in two food simulation systems, glucose-BSA system (simulating the reaction between reducing sugars and proteins) and oleic acid-BSA system (simulating the reaction between fatty acids and proteins).

**The procedure of glucose-BSA system**

The procedure was used as described previously (Wu et al. 2013) with minor modifications (the reaction time was optimized in Figure S1A in the supplemental data). Briefly, the reactions were conducted in a volume of 1 mL containing 36 mg/mL D-glucose, 5 mg/mL BSA and different concentrations (1.0, 0.5, 0.1, 0.05, 0.01 mg/mL) of the tested phenolic acids (or AG as positive control). All the materials were dissolved in 0.2 M pH 6.8 phosphate-buffered saline (PBS) and mixed well. The solutions were kept at 60°C for 30h. Then, all treatments were diluted to 4 mL with distilled water and AGEs contents were detected using fluorospectro photometer (F-4600, Hitachi Ltd., Tokyo, Japan) at excitation (EX) and emission (EM) wavelengths of 370 nm and 440 nm, respectively.

**The procedure of oleic acid-BSA system**

The procedure was used as described previously (Wu et al. 2013) with minor modifications (the reaction time was optimized in Figure S1B in the supplemental data)....
data). The procedure was almost as the glucose-BSA system only 36 mg/mL D-glucose was replaced with 2 mg/mL oleic acid and reaction time was increased to 36 h.

**Glyoxal scavenging**

The glyoxal scavenging method was adopted from the literature (Peng et al. 2008) with minor modifications. Briefly, GO (5 mM), DQ (5 mM), PD (20 mM), the tested phenolic acids (5 mM) and AG (as control, 5 mM) were prepared in PBS (50 mM, pH 6.8). All the solutions were prepared just before the experiment. 0.3 mL of GO solution and 0.3 mL of the tested phenolic acids were mixed well and kept at 37°C for 1 h. AG and PBS were used as positive and negative control, respectively. Then, the mixtures were cooled in 0°C ice-cold water for 5 min. Subsequently, 0.15 mL of PD (derivatization agent) and 0.15 mL of DQ (internal standard) were added into the cool mixtures and kept at 37°C for 30 min for the completion of derivatization reaction between GO and PD. High-performance liquid chromatography (HPLC) analysis of GO derivatization product and PD was performed on Waters liquid chromatogragh 2695 with diode array detector (Waters Co., Ltd., MA, USA) and Spursil C18 - EP column (5 μ, 250 × 4.6 mm, Dikma technology Co., Ltd., Beijin, China). The flow rate was 1 mL/min and the injected volume was 15 μL. The mobile phases were composed of deionized water with 0.1% formic acid (solvent A) and pure methanol (solvent B). The linear gradient for elution was: 0–4 min, 5–50% B; 4–16 min, 50–50% B; 16–18 min, 50–90% B; 18–19 min, 90–90% B; 19–20 min, 90–5% B; 20–25 min, 5% B. The total running time was 25 min and chromatograms were recorded at 315 nm. The amount of unreacted GO in the samples were calculated based on the ratios of peak area of quinoxaline and DQ. Percentage decrease in GO was calculated using the following equation.

\[
\text{GO decrease percentage} = \frac{(\text{amounts of GO in negative control} - \text{amounts of GO in sample})}{\text{amounts of GO in negative control}} \times 100\%.
\]

**Reducing power of phenolic acids**

**Potassium ferricyanide method**

The method taken from the literature (Yen & Hsieh 1995) was used with some modifications. Briefly, 0.2 mL of appropriately diluted phenolic acids in distilled water were mixed well with 1 mL of 0.5% potassium ferricyanide (dissolved in 0.1 M PBS, pH 6.6) and kept at 50°C for 20 min. Then, 0.5 mL of 10% trichloroacetic acid aqueous solution was added and centrifuged for 10 min at 1000 × g. Subsequently, 0.5 mL supernatant was taken and mixed well with 0.5 mL of 0.1% ferric trichloride aqueous solution and 2 mL of distilled water. The OD values were measured by spectrophotometer (UV-1750 UV-Vis spectrophotometer, Shimadzu Instruments Co., Ltd., Kyoto, Japan) at 700 nm. Vitamin C (VC) was used as the standard and the reducing powers of phenolic acids were expressed as VC (g)/phenolic acid (g).

**Folin–phenol method**

The method given by Greco et al. (2013) was used with minor changes. Briefly, 1 mL of appropriately diluted phenolic acids in distilled water was mixed well with 0.8 mL of folin–phenol reagent and 10 mL of 10% sodium carbonate aqueous solution, and kept at 25°C for 2 h. Then, the OD values were measured by spectrophotometer at 765 nm. VC was used as the standard and the reducing powers of phenolic acids were expressed as VC (g)/phenolic acid (g).

**Antiradical activity of phenolic acids**

**Scavenging ABTS radical (ABTS\(^+\))**

The previously devised method was used with some modifications (Szwajgier 2009). Briefly, 0.2 mL of appropriately diluted phenolic acids in distilled water was mixed well with 2.8 mL of ABTS reagent (0.7 mM ABTS and 0.25 mM potassium persulfate dispersed in 95% ethanol) and kept at 25°C for 10 min. The OD values were measured by spectrophotometer at 735 nm. VC was used as the standard and the antiradical activities of phenolic acids were expressed as VC (g)/phenolic acid (g).

**Scavenging hydroxyl radical (-OH)**

The method from the previous research was used with minor changes (Chun 2010). Briefly, 0.5 mL of appropriately diluted phenolic acids in distilled water was mixed well with 0.5 mL of 0.75 mM o-phenanthroline (dispersed in absolute ethanol), 1 mL of 0.2 M PBS (pH 7.4), 0.5 mL of 0.75 mM ferrous sulfate aqueous solution and 0.5 mL of 0.015% hydrogen peroxide aqueous solution, and kept at 37°C for 1 h. The OD values were measured by spectrophotometer at 536 nm. VC was used as the standard and the antiradical activities of phenolic acids were expressed as VC (g)/phenolic acid (g).
**Fe²⁺ chelating power of phenolic acids**

The Fe²⁺ chelating powers of phenolic acids were tested using the published method with some modifications (Fu et al. 2011). Briefly, 1 mM TPTZ (dispersed in 4 mM hydrochloric acid and 0.2 mM sodium acetate buffer (pH 3.6)), 0.2 mM ferrous sulfate (dispersed in 0.2 mM sodium acetate buffer, pH 3.6), 1 mM EDTA aqueous solution and appropriate concentration of phenolic acids aqueous solution were freshly prepared before measuring. All of these solutions were subjected to sonication for 20 min in an ultrasonic bath (KQ2200DE, Kunshan ultrasonic instrument Co., Ltd. Jiangsu, China). Then, 0.2 mL of phenolic acids (sample) and EDTA (positive control) was mixed well with 0.8 mL ferrous sulfate separately and kept at 30°C. After 24 h, the mixture was centrifuged for 10 min at 2000 × g. Subsequently, 0.4 mL of supernatant was taken and mixed well with 2 mL of TPTZ. The OD values were measured by spectrophotometer at 593 nm. EDTA was used as the standard and the ferrous ion chelating powers of phenolic acids was expressed as EDTA (g)/phenolic acid (g).

**Inhibition of D-glucose autoxidation**

Oxidation of glucose was measured by detecting the degree of oxidative polymerization by fluorescence intensity of oxidation products (the reaction time and detection wavelength were optimized in Figure S2 in the supplemental data) and detecting the content of dicarbonyl compounds using thiobarbituric acid (TBA) method (Gutteridge 1981).

**Analyses of oxidation products**

All the reaction mixtures were conducted in 1 mL volume containing 200 mg/mL D-glucose and 1 mg/mL phenolic acids. All these solutions were dissolved in 0.2 M PBS (pH 6.8) and kept at 60°C for 30 h. All treatments were diluted to 4 mL with distilled water to detect the oxidation products using fluorospectro photometer (EX = 370 nm, EM = 445 nm).

**Analyses of dicarbonyl compounds**

After the reaction of “Analyses of oxidation products,” 0.1 mL of these mixtures were taken and mixed well with 1 mL of TBA (dispersed in 50% acetic acid aqueous solution) and 0.9 mL of distilled water and kept at 100°C for 1 h. All treatments were diluted to 4 mL with distilled water to detect the contents of dicarbonyl compounds using a fluorospectro photometer (EX = 515 nm, EM = 550 nm).

**Inhibition of oleic acid autoxidation**

Oxidation of oleic acid was measured by detecting the content of dicarbonyl compounds using TBA method (Gutteridge 1981). Method followed in “Analyzes of dicarbonyl compounds” was used in which 200 mg/mL D-glucose was replaced with 2 mg/mL oleic acid.

**Inhibition of Amadori compound autoxidation**

Oxidation of Amadori compound was tested using a method from the literature with minor modifications (Fujiwara et al. 2011; Wu et al. 2013). Firstly, Amadori compound was prepared under antioxidative conditions for inhibiting the formation of AGEs as described in the literature (Fujiwara et al. 2011). Briefly, 50 mg/mL BSA was incubated with 300 mg/mL D-glucose in 10 mL of 0.2 M PBS (pH 6.8) in the presence of 1 mM DTPA at 37°C for 7 days followed by dialysis against 0.2 M PBS (pH 6.8) to remove the D-glucose. Then, 5 mg/mL Amadori compound was allowed to reacted with 1 mg/mL phenolic acids in 1 mL of 0.2 M PBS (pH 6.8) at 60°C for 30 h. All treatments were diluted to 4 mL with distilled water to detect AGEs contents using fluorospectro photometer (EX = 370 nm, EM = 440 nm).

**Ionization form detection of protocatechuic acid**

The ionization form of protocatechuic acid was detected by fluorescence method according to the published assay with minor modifications (Yinghua 2006). Briefly, protocatechuic acid (0.01 mg/mL) was prepared in 20 mM PBS (pH 6.8), then the fluorescence of protocatechuic acid at different pH (pH 1.6–11.8, adjusted by hydrochloric acid and sodium hydroxide) was tested using fluorospectro photometer (EX = 287 nm, EM = 336 nm). The ionization form was displayed through the change of the fluorescence.

**Statistical analyses and graph drawing**

All analyses were run in triplicate and averaged. The data of samples (expressed as mean ± S.D.), Spearman correlation analysis and independent samples t-test were analyzed by SPSS 18 (NY, USA). The graph was drawn by OriginPro 9.0 (Northampton, USA). The compound structure was drawn by ChemBioDraw 12.0 (MA, USA) and the charge distribution of phenolic acids was calculated by the extended Hückle method with ChemBio3D 12.0. (MA, USA).
Results and discussion

**AGEs inhibition by phenolic acids in food simulation systems**

In order to study the inhibitory effects of phenolic acids (protocatechuic acid, dihydroferulic acid, p-coumaric acid, p-hydroxybenzoic acid and salicylic acid) on the formation of AGEs, two food simulation systems; glucose-BSA system (Figure S1A) and oleic acid-BSA system (Figure S1B), were established. In glucose-BSA system (Figure 1A), all the tested phenolic acids except p-coumaric acid had a certain degree of AGEs inhibitory abilities (protocatechuic acid ≈ salicylic acid > dihydroferulic acid > p-hydroxybenzoic acid), but the inhibitory effects of phenolic acids were less than that of positive control (aminoguanidine) at
the same concentration ($p < 0.01$). Surprisingly, $p$-coumaric acid promoted AGEs formation in samples when certain concentration (0.01–1 mg/mL) was used. In oleic acid-BSA system (Figure 1B), all of the tested phenolic acids inhibited AGEs formation. Their inhibitory effects, except $p$-hydroxybenzoic acid and salicylic acid (dihydroferulic acid > $p$-coumaric acid /C25 protocatechuic acid), were better than that of positive control at the same concentration, especially dihydrogenferulic acid which strongly inhibited AGEs formation in the tested concentration (0.01–1 mg/mL).

**Glyoxal scavenging of phenolic acids**

Dicarbonyl compound like glyoxal is an important precursor of AGEs that is formed by the oxidation of reducing sugar, unsaturated fatty acid and Amadori compound (Nguyen et al. 2013). Therefore, the glyoxal scavenging abilities of phenolic acids were investigated, but the results (Figure 2) portended that all of the tested phenolic acids did not have significant ability to scavenge glyoxal ($p > 0.05$). The previous researches showed that catechin, a good dicarbonyl scavenger, could combine with the carbonyl compounds by electrophilic substitution reaction (Wu et al. 2013; Gu et al. 2014). So, the charge distribution (Figure 3) of the tested phenolic acids and catechin was compared and the results showed that the electron-withdrawing property from carboxyl group (COO$^-$ at pH 6.8) of the phenolic acids greatly reduced the electron cloud density on the benzene ring, which may be the reason for no glyoxal scavenging activity of these phenolic acids.

**Antioxidant activity assay of the phenolic acids**

The antioxidant activities of the phenolic acids (protocatechuic acid, dihydroferulic acid, $p$-coumaric acid, $p$-hydroxybenzoic acid and salicylic acid) were measured by their reducing powers, antiradical activities and ferrous ion-chelating powers (Table 1). The results affirmed that all the tested phenolic acids had a certain reducing power, ABTS$^+$•-scavenging activity and •OH-promoting abilities in PBS at pH 6.8 (protocatechuic acid > dihydroferulic acid > $p$-coumaric acid > $p$-hydroxybenzoic acid > salicylic acid). Among them, protocatechuic acid and dihydroferulic acid had higher reducing powers, ABTS$^+$• scavenging activities and •OH promoting abilities than the others. In ferrous ion-chelating test, protocatechuic acid had better chelating power than the other four phenolic acids (Table 1).

**Antioxidation abilities of phenolic acids during the AGEs formation**

The autoxidation of reducing sugar, unsaturated fatty acid and Amadori compound is the necessary pathway of AGEs formation (Nguyen et al. 2013). The inhibition effects of phenolic acids on the autoxidation of glucose, oleic acid and Amadori compound (glycated BSA) were also investigated in this study (Figure 4). Figure 4(A) and (B) indicated that all of the phenolic acids inhibited the autoxidation of glucose (oxidative polymerization) and Amadori compound except $p$-coumaric acid, which was similar to the inhibitory effect on the formation of AGEs in glucose-BSA system. Furthermore, all of the phenolic acids except $p$-coumaric acid had significant promoting effects on the production of carbonyl compounds in the autoxidation of glucose (Figure 4C), which presumptively be the result of phenolic acids inhibiting the polymerization of autoxidation products (Figure 3A). In the experiment of oleic acid autoxidation (Figure 4D), $p$-coumaric acid strongly inhibited the generation of carbonyl compounds. Surprisingly, salicylic acid greatly promoted the generation of carbonyl compounds during the autoxidation of oleic acid.

**Influence of ionization form of protocatechuic acid on its •OH-scavenging ability**

Antioxidation test (Table 1) demonstrated that protocatechuic acid strongly promoted the generation of •OH. In order to clarify the reason, the •OH-scavenging ability of protocatechuic acid in different ionization forms was detected. The results (Figure 5) unveiled...
that protocatechuic acid did not have significant influence on the generation of •OH in H$_2$L form when pH was less than 3.5. With the rise in pH (4.5–6.5), protocatechuic acid showed great promoting effect on the generation of •OH in H$_2$L- form. With pH higher than 6.5, the promoting effect was significantly reduced when the H$_2$L- form turned to HL$^2_-$. So the •OH promoting ability of protocatechuic acid was due to increase in its H-donating ability through the pathway described in Figure 6 and this phenomenon was supported by the mechanism in catechin found in literature (Furukawa 2003).

Correlation analyses between the antioxidant activities of phenolic acids (analyzed by SPSS 18, Table 2) indicated that the reducing power had positive correlation with the ability of scavenging ABTS$^+$•. But the ability of scavenging •OH showed obvious negative correlation with the reducing power and the ability of scavenging ABTS$^+$•. The antioxidant activities of phenolic acids on oleic acid (Figure 4D) were inconsistent with their reducing power and the ability of scavenging ABTS$^+$• (Table 2), as $p$-coumaric acid was stronger than protocatechuic acid, which is probably due to the powerful promoting •OH ability of protocatechuic acid. Similarly, the promoting effect of $p$-coumaric acid on the autoxidation of glucose (Figure 4A) and Amadori compound (Figure 4B) was probably the result of combined effect of its reducing power and promoting •OH ability. The correlation index between "radical-scavenging activity (•OH)" and "inhibition of oleic acid oxidation (carbonyl)" was −0.90 ($p < 0.01$), while the correlation index between "radical-scavenging activity (•OH)" and "inhibition of Amadori compound (carbonyl)" was 0.60 ($p < 0.05$), which indicates that the influence of •OH on the autoxidation of Amadori compound was more than that on oleic acid.

**Figure 3.** Electron cloud density of phenolic acids.

**Table 1.** Antioxidant ability of phenolic acids.

|                     | Reducing power (potassium ferricyanide method, g VC/g) | Reducing power (folin–phenol method, g VC/g) | Radical-scavenging activity (ABTS$^+$, g VC/g) | Radical-scavenging activity (• OH, g VC/g) | Transition metal ions chelating ability (Fe$^{2+}$, g EDTA/g) |
|---------------------|-------------------------------------------------------|---------------------------------------------|---------------------------------------------|-------------------------------------------|-------------------------------------------------|
| Protocatechuic acid | 1.9824 ± 0.0067                                        | 1.4609 ± 0.0469                              | 1.1522 ± 0.0098                             | −0.3144 ± 0.0178                           | 0.2111 ± 0.0144                                 |
| Dihydrogenferulic acid | 1.1401 ± 0.0067                                        | 1.1177 ± 0.0070                              | 1.2277 ± 0.0208                             | −0.0236 ± 0.0025                           | 0.0005 ± 0.0006                                 |
| $p$-Coumaric acid   | 0.1618 ± 0.0017                                        | 0.7115 ± 0.0047                              | 0.7994 ± 0.0049                             | −0.0239 ± 0.0030                           | 0.0080 ± 0.0011                                 |
| $p$-Hydroxybenzoic acid | −0.0006 ± 0.0011                                       | 0.5789 ± 0.0094                              | 0.0416 ± 0.0031                             | −0.0032 ± 0.0001                           | −0.0002 ± 0.0017                                |
| Salicylic acid      | 0.0005 ± 0.0010                                        | 0.3253 ± 0.0070                              | 0.0059 ± 0.0001                             | −0.0003 ± 0.0007                           | 0.0073 ± 0.0017                                 |

Replication level: Three. The concentrations for tested phenolic acids were 0.05, 0.5, 5 and 50 mg/mL. The results were determined by the linear range of standard control (VC or EDTA).
Studies have indicated that the formation of AGEs can be inhibited by scavenging reactive carbonyls, free radical, chelating transition metal ions and reducing action (Fujiwara et al. 2011; Wu et al. 2011). However, phenolic acids could not scavenge reactive carbonyls because of the electron-withdrawing property from carboxyl group (COO\(^{-}\) at pH 6.8), which is probably the reason of the low AGEs inhibitory activity of phenolic acids in glucose-BSA system (Figure 1A). The AGEs inhibitory effect of phenolic acids in oleic acid-BSA system (Figure 1B) was much better than it in glucose-BSA system (Figure 1A), which is due to the combined effect of their strong antioxidation on oleic acid (Figure 4D) and weak antioxidation on glucose (Figures 4A and C) and Amadori compound (Figure 4B). In oleic acid-BSA system (Figure 1B), the AGEs inhibitory effects of protocatechuic acid and p-coumaric acid were having negative correlation with their concentrations, which probably were related to their powerful promoting •OH abilities and relatively weak promoting •OH ability (Table 1). As expected, the AGEs inhibitory effects of p-hydroxybenzoic acid and salicylic acid (Figure 1B), which could hardly promote the generation of •OH, were having positive correlation with their concentration. In glucose-BSA system (Figure 1A), the AGEs promoting effect of p-coumaric acid might be the result of its weak reducing power and slight promoting •OH ability (Table 1).

**Correlation between AGEs inhibitory activities and the structures of phenolic acids**

Literature showed that the antioxidant activities of phenolic acids were determined by the number and position of the hydroxyl groups bound to the aromatic ring and the type of substituent (Gulcin 2012). The catechol group of protocatechuic acid gave it great reducing power and scavenging ABTS\(^{+}\)• ability (Table 1) and subsequently a certain AGEs inhibitory ability (Figure 1). The increase in generation of •OH from catechol group also made its AGEs inhibitory ability weaker than dihydrogenferulic acid in oleic acid-BSA system (Figure 1B). The good AGEs inhibitory ability would be related to their antioxidant activity.
Figure 5. Influence of ionization form of protocatechuic acid on its scavenging •OH ability.

Figure 6. Proposed pathway of protocatechuic acid promoting •OH.
of salicylic acid in glucose-BSA system (Figure 1A) was possibly the result of the Fe$^{2+}$ chelating power (Table 1) from its ortho substitution of hydroxyl group with carboxyl group.

**Conclusion**

Phenolic acids are kinds of natural compound with some biological activity and widely exist in various plant species (Ky et al. 2014; Shao et al. 2014). This study confirmed that phenolic acids were effective in inhibiting the formation of AGEs by their reducing power in food simulation systems, especially in oleic acid-BSA system. The previous research revealed that fat-rich meats were the main source of dietary AGEs (Uribarri et al. 2010). These means that foods from plant sources (rich in phenolic acids) could be used as AGEs inhibitors in processing of high-fat foods. In this study, 60°C was selected as the reaction temperature but not 95°C or higher, because the high temperature will coagulate BSA solution resulting in the inhomogeneity of reaction, poor reproducibility of experiments and undetectability of AGEs in current methods. In consideration of most foodstuffs are solid, the glycation systems are liquid (beneficial to the homogeneity of glycation reaction), and the reaction rate in liquid state is higher than in solid state. So, the concentration of BSA was lower than common foodstuff. The AGEs inhibitory effects of phenolic acids might be a little higher than it in real food processing and the AGEs inhibitory effects of some foods from plant sources in cooking need further research. In addition, mg/mL was preferred concentration unit of phenolic acids instead of molarity which may affect the comparison between different phenolic acids as the difference on molar basis was giving a poor picture of comparison. (The phelolic acids carrying the molar masses of 154 g/mol (protocatechuic acid), 196 g/mol (dihydro ferulic acid), 164 g/mol (p-coumaric acid) and 138 g/mol (p-hydroxybenzoic acid and salicylic acid) possessed highest test concentration of 6.5 mmol/L, 5.1 mmol/L, 6.1 mmol/L and 7.2 mmol/L, respectively). But it did not affect the results as all the statistical analyzes were based on a same unit.

**Disclosure statement**

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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