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Inhibitory effect of lotus seedpod oligomeric procyanidins on advanced glycation end product formation in a lactose–lysine model system

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ABSTRACT

Background: Industrial food processing induces protein glycation modifications and toxic advanced glycation end products (AGEs) which affect human health. Therefore, it is of interest to monitor AGEs in food processing. The present study was carried out to investigate the influence of lotus seedpod oligomeric procyanidin (LSOPC) concentrations, solution pH value and metal ions on AGE formation by heat treatment of lactose–lysine model solutions. Nε-(carboxymethyl) lysine (CML), as one of the common AGEs was also determined by HPLC–MS/MS in this experiment.
Results: The results showed that LSOPC can inhibit the formation of AGEs effectively at higher concentrations, lower temperature, and it can reverse the promotion function of metal ions because of its high inhibition activity. Also, LSOPC can inhibit CML formation in the Maillard reaction as well.
Conclusion: These results indicated that LSOPC could be used as functional food ingredients to inhibit AGE formation.

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1. Introduction

Advanced glycation end-products (AGEs) are formed as a result of a non-enzymatic Maillard or 'browning' reaction in which glucose forms adducts with proteins, lipids and nucleic acids [1]. First, carbonyl group forms a reducing sugar and an unprotonated amine group forms a protein producing a nucleophilic addition reaction to form a freely reversible Schiff base. This is subsequently stabilized after rearrangement into Amadori products or Heyns products according to the type of sugar involved (aldoses or ketoses). With additional complex rearrangements such as oxidation, enolization, dehydration, condensation and fragmentation, early glycated products are formed [2]. Subsequent further reactions (cross-linkages and polymerization) lead to the formation of AGEs [3]. AGEs were originally characterized by a yellow-brown fluorescent color and by an ability to form cross-links with and between amino groups, but the term is now used for a broad range of advanced products of the glycation process (also called the "Maillard reaction"). Generally, these compounds can be divided into two types on the basis of chemical structure: one type is the fluorescent properties and crosslinking structure AGEs, such as crossline, 2-(2-furoyl)-4(5)-(2-furanyl)-1H-imidazole (FFI), glyoxal-lysine dimer (GOLD), methyl-glyoxal-lysine dimer (MOLD), fluorolink, pentosidine and vesperlysine, and the other type is the non-fluorescent and non-crosslinking AGEs, such as Nε-(carboxymethyl) lysine (CML), Nε-(carboxyethyl) lysine (CEL), pyrraline and argpyrimidine [4].

Recently, AGEs in vivo have been implicated in the pathogenesis of diabetic complications, including neuropathy, nephropathy, retinopathy, and cataract [5] and other health disorders such as atherosclerosis [6], Alzheimer's disease [7] and chronic kidney disease, as well as other phenotypes related to aging [8]. Other detrimental effects of the glycation process are their contribution to the functional properties of proteins such as their emulsifying, foaming and gelling capacities as well as their solubility [9,10] and the production of toxic and carcinogenic compounds such as the low-molecular weight products, keto-aldehydes, glyoxal, methylglyoxal, 3-deoxyglucosone, heterocyclic amines and acrylamide [11].

The two major sources of human exposure to AGEs are exogenous AGEs found in foods, and endogenous AGEs that are generatiod by abnormal glucose metabolism or as a byproduct of lipid peroxidation. The contribution of dietary AGEs to the total pool of AGEs in the body is likely to be much greater than the contribution from AGEs that are endogenously generated by abnormal glucose metabolism or lipid oxidation [12]. Since dietary AGEs are absorbed as free adducts after

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digestion they are likely to constitute a major source of intracellular and plasma AGEs [13]. Moreover, dietary AGEs are also a major environmental source of proinflammatory AGEs [14]. Industrial processing or cooking of food is rich in AGEs because of the high temperatures that are used in processing, such as deep frying, baking and broiling [15]. Thus, the role of dietary AGEs in human health remains highly controversial and the restriction of food-derived AGEs or the inhibition of absorption of dietary AGEs may be a novel target for therapeutic intervention in the above-mentioned AGE-related disorders.

Lotus seedpod is not an edible part of lotus, which is rich in B-type procyanidins. Lotus seedpod oligomeric procyanidins (LSOPC) (molecular structure; Fig. 1) is a kind of mixture, which doesn't have certain molecular weight. The mean degree of polymerization of LSOPC was 3.21, with 74.2% catechin and 25.8% epicatechin in the terminal units and 26.0%, 43.1%, 30.9% of catechin, epicatechin, epigallocatechin in the extensive units, respectively, which were detected by HPLC/MS. Our laboratory has established the proper extraction technology of LSOPC in recent years [16]. Furthermore, antioxidant properties, metal-chelation and free radical scavenging activity of oligomeric Procyanidins of lotus seedpod (LSOPC) have been extensively identified [17]. In recent years, much attention has been paid to the influence of LSOPC on insulin action and reactive carbonyl species (RCS) scavenging activities, which may provide benefits for diabetic patients [16,17]. Some researchers indicated that LSOPC may play a useful role in the treatment of cognitive impairment caused by Alzheimer's disease and aging due to their excellent performance in scavenging free radicals, antioxidation, anti-lipid peroxidation [18]. Moreover, our previous studies have showed that LSOPC could inhibit AGE formation effectively in simulated physiological environment and the corresponding inhibition mechanisms to scavenging reactive carbonyls by forming adducts with them [19]. However, there are few reports about LSOPC inhibiting AGE formation especially in food system. In this study, a model system was chosen consisting of lactose (as a reducing disaccharide) and lysine (as a very reactive amino acid) to monitor the AGE formation, and observed the effect of different LSOPC concentrations, solution pH values and metal ions on inhibiting AGE formation. This type of modeling system can be a powerful tool to improve our understanding of the evolution of AGEs during food processing with AGE inhibitor.

Table 1
MS/MS conditions for the compounds studied.

Analyte	Molecular weight	Parent ion (m/z)	Daughter ion (m/z)	tR (min)	DP (V)	CE (eV)	EP (V)	CXP (V)
CML	205	205.2	84.0 130.1	7.20	71 74	31 18	10	12

2. Materials and methods

2.1. Chemical and reagents

Lotus seedpods were obtained from local supermarket (Wu Zhi 2 hao). α-Lactose, L-lysine, phosphate buffer saline (PBS, pH 7.4), D-glucose and FeCl3 were purchased from Sinopharm (Shanghai, China). $FeCl_2 \cdot 4H_2O$, $CuCl_2 \cdot 2H_2O$, $MgCl_2 \cdot 6H_2O$, $ZnCl_2$, $CaCl_2 \cdot 2H_2O$, $SnCl_2 \cdot 2H_2O$ and $AlCl_3 \cdot 6H_2O$ were purchased from Sigma-Aldrich (St. Louis, MO). All other chemicals were of analytical grade.

2.2. Preparation of lotus seedpod oligomeric procyanidins

Fresh lotus seedpod fragments were extracted using 70% ethanol at 60°C for 1.5 h. The crude procyanidin aqueous solution was loaded onto an AB-8 resin (weak polarity macroporous resin, 0.3–1.25 mm particle size, Nankai Hecheng Science & Technology Co., Tianjin, China) column (15 × 3.5 cm, ID), and the fraction eluted by 70% ethanol was collected. The eluent was evaporated, and the procyanidin extract of lotus seedpod was obtained. Subsequently, they were extracted by ethyl acetate to get the oligomeric procyanidins of lotus seedpod (LSOPC), which included catechin monomers, B-type procyanidin dimers, trimers and a few tetramers by LC–MS analysis [20]. The yield of LSOPC was 0.8%. Its purity was 106.22 ± 0.46% compared to that of grape seed procyanidins measured by Butanol-HCl assay [21].

2.3. Preparation of model systems

In order to study the Maillard reaction in real food systems, in particular in milk and milk products, lactose was chosen as the model

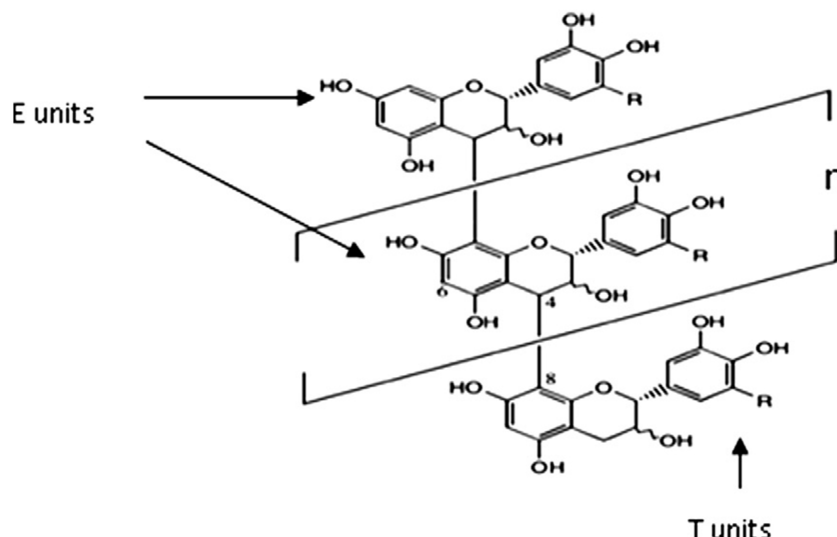

Fig. 1. Scheme of LSOPC: terminal Unit, T-units; extension units, E-units.

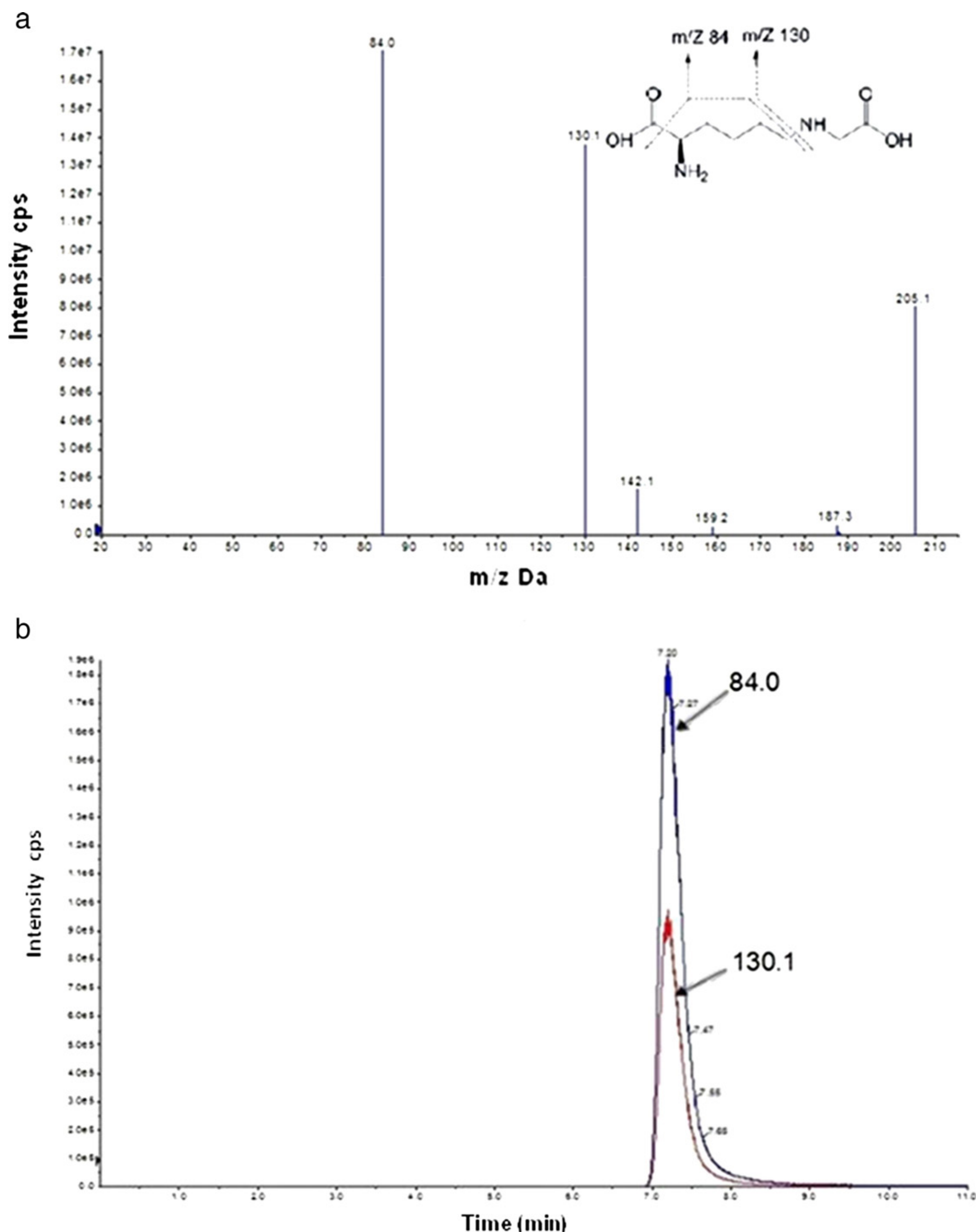

Fig. 2. HPLC–MS/MS response and scheme of mass fragmentation for CML. a—production mass spectra; b—MS chromatogram.

carbohydratio, and lysine was chosen as the model amino acid. The development of the Maillard reaction in food system depends on processing time and temperature, as well as other environment factors. Higher reaction temperature and longer reaction time will both promote the reaction. The formation of AGEs can be used to quantify the intensity of the Maillard reaction. α-Lactose and L-lysine were dissolved in bidistilled water at a concentration of 0.045 mol/L. In order to research the influence of different concentrations of LSOPC on the course of the Maillard reaction, LSOPC was dissolved at different concentrations from 1 mg/mL to 0.02 mg/mL. The reaction solutions were prepared at different pH values ranging from 5 to 8 to determine the influence of pH conditions on AGE formation. As to determine the influence of metal ions on the AGE formation in the process, different concentrations of metal ion salt solutions were prepared: Ca^{2+}, Cu^{2+}, Fe^{2+}, Mg^{2+} and Zn^{2+} were dissolved at the concentrations of 0.1, 1, 10 and 50 ppm and Al^{3+}, Fe^{3+} and Sn^{2+} were dissolved at the concentrations of 0.1, 1 and 10 ppm respectively because of their low solubilities.

2.4. Measuring the development of the Maillard reaction

Test tubes containing 0.8 mg/mL LSOPC and 0.045 mol/L α-lactose/L-lysine model mixtures were screw-sealed and heated in a constant temperature water bath at 80°C and 100°C. The samples were taken after 0.5, 1, 1.5, 2, 2.5, 3 and 3.5 h at 80°C; 4, 6, 8, 10, 15, 20, 30 and 60 min at 100°C to monitor the course of the reaction. After heating, the tubes were immediately cooled under ice bath and stored in a refrigerator (-20°C) to terminate reaction. Then, all

samples quantitatively assessed the formation of fluorescent AGEs using a spectrofluorimeter (Shimadzu RF-5301) at excitation and emission wavelengths of 370 nm and 440 nm, respectively. The same mixtures without LSOPC were as control. The mixtures without heating were as blank. The percent inhibition then calculated as: %inhibition = (Fcontrol - Fsample) / (Fcontrol - Fblank) × 100. Thus, the optimum reaction time at 80 and 100°C were obtained.

Then, the model mixtures contain 0.045 mol/L α-lactose/L-lysine and different concentrations of LSOPC, LSOPC (0.02, 0.05, 0.1, 0.2, 0.5, 0.8 and 1 mg/mL) at 80°C and LSOPC (0.1, 0.2, 0.5, 0.8 and 1 mg/mL) at 100°C. They were all heated for optimum reaction time. The inhibition ratios were obtained by the same way above. IC_{50} values were calculated from inhibition ratios obtained at all tested concentrations.

Subsequently, 0.8 mg/mL LSOPC and 0.045 mol/L α-lactose/L-lysine model mixtures were dissolved in 0.2 M phosphate buffer saline in order to adjust the pH to 5, 5.5, 6, 6.5, 7, 7.5 and 8, respectively. The solutions were all heated for optimum reaction time at 80°C and 100°C. Then, the inhibition ratios of LSOPC at different pH values were obtained by the same way above.

Finally, metal ions were added to a mixed solution of 0.8 mg/mL LSOPC and 0.045 mol/L α-lactose/L-lysine model mixtures. Each mixture was heated at 80°C under pH 5 or 6.5 for optimum reaction time. The inhibition ratios of LSOPC under different metal ion conditions were obtained by the same way above.

2.5. Inhibition effect of LSOPC on CML formation

2.5.1. Sample preparation

The model mixtures containing α-lactose (0.045 mol/L), L-lysine (0.045 mol/L) and LSOPC (0.2, 0.4, 0.6, 0.8 and 1 mg/mL) were heated at 100°C for 30 min. All samples were cooled in ice water to stop any further reaction then continue to purification steps.

A 100 μL volume of incubated solution was reduced overnight at 4°C by sodium borohydride solution (0.2 M, 100 μL). Each sample was centrifuged at 20,000 rpm for 60 min in an ultracentrifuge at -4°C (Sigma, Germany), the isolated solution was eluted on a 6 mL Cleanert C18 cartridge (Agela, China). The Cleanert C18 was washed with 3 mL of methanol–water–formic acid (30:70:0.1, v/v/v). The eluate was dried under vacuum and dissolved in 0.1% aqueous formic acid (1 mL). 10 μL volume of sample was analyzed by HPLC–MS/MS system.

2.5.2. Determination of CML content by HPLC–MS/MS

The HPLC–MS/MS analysis for the determination of CML content was as described by Ahmed and Assar with some modifications [22,23]. The chromatographic system consisted of a HPLC system (Waters, USA) coupled to an AB Sciex API 5000 turbo-ion-spray triple quadrupole tandem mass spectrometer using the electrospray positive ionization (ESI+) method. Separations were conducted on a Symmetry C18 column (4.6 ∗ 250 mm, 5 μm, waters, Ireland), conditioned at 25°C. A 10 μL volume of eluate or CML was injected into the reversed column, and eluted with a mixture of methanol–water–formic acid (30:70:0.1, v/v/v) at a flow rate of 0.2 mL/min. The main operational parameters of the mass spectrometer were summarized as follows: Source/gas collision gas (CAD) at 6 psi, curtain gas (CUR) at 20 psi, ion source gas 1 (GS1) at 60 psi, ion source gas 2 (GS2) at 50 psi, ionspray voltage (IS) at 5500 V, and temperature (TEM) at 600°C.

The precursor ions to the product ions with rich structure features were chosen for MRM detections of CML. Compound quantitative optimization wizard was used to optimize the desolvation potential (DP), entrance potential (EP), collision energies (CEs) and collision cell exit potential (CXP) of these compounds. The respective conditions were summarized in Table 1. Sample peaks corresponding to CML were calculated by using the equation of the standard curve. The equation is y = 0.1249x - 0.7418 (R^2 = 0.9944). The concentration of CML solution was 12.5, 25, 50, 100, 200, and 400 ppb, respectively.

Fig. 2 illustrated the product ion spectra of CML by HPLC–MS/MS. m/z 130 was selected as qualitative ions to as the daughter ion for analyze, and the most abundant fragment was m/z 84 selected for quantification to obtain high sensitivity.

2.6. Statistical analyses and graph drawing

The data of samples was analyzed by SPSS 18.0 (Expressed as mean ± S.D.). IC_{50} was calculated by Probit Regression with SPSS. The graph was drawn by OriginPro 8.0 and the compound structure was drawn by ChemBioDraw 12.0.

3. Results and discussion

3.1. Optimum reaction time

Fig. 3 showed the formation level of AGEs by heating for 0.5–3.5 h at 80°C and heating for 4-60 min at 100°C. AGEs were enhanced greatly during heating. While with 0.8 mg/mL LSOPC added, the formation of AGEs was significantly inhibited. The inhibition ratio reached highest value when heated for 2 h at 80°C (Fig. 3a) and 10 min at 100°C (Fig. 3b) respectively. So we chose 2 h (at 80°C) and 10 min (at 100°C) as the optimum reaction times.

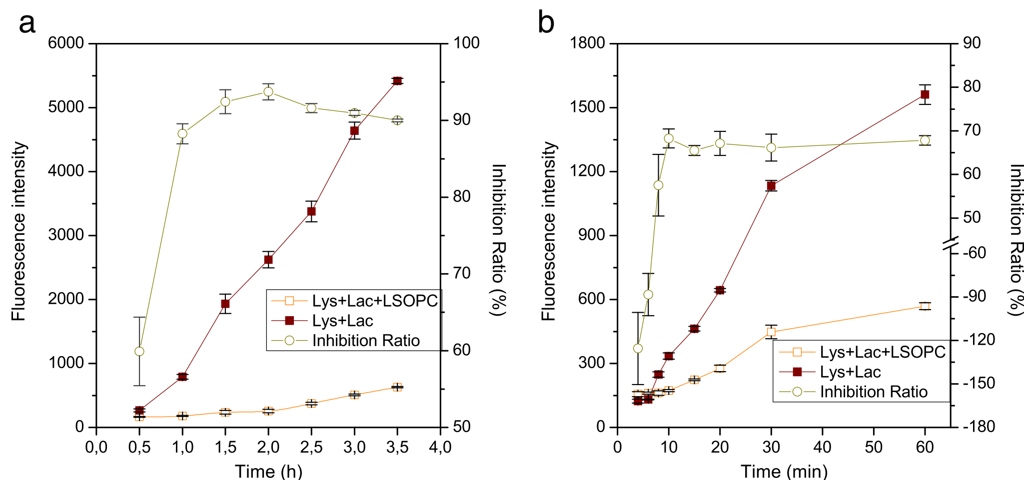

Fig. 3. Inhibition of 0.8 mg/mL LSOPC on non-enzymatic glucosylation of protein in model system of α-lactose and L-lysine during different reaction times: (a) heated at 80°C for 0.5–3.5 h, (b) heated at 100°C for 5–60 min.

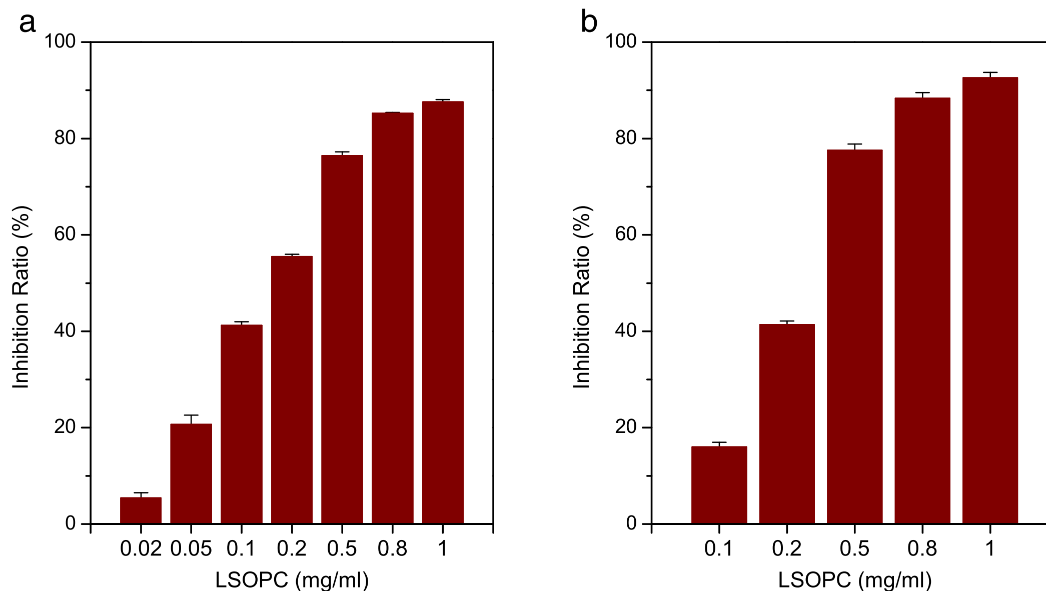

Fig. 4. Inhibition of LSOPC on non enzymatic glucosylation of protein in model system of α-lactose and L-lysine at different concentrations: (a) heated at 80°C for 2 h, (b) heated at 100°C for 10 min.

3.2. Influence of LSOPC concentrations

In order to study the inhibitory effect of LSOPC on non-enzymatic glycation end product formation, different concentrations of LSOPC were added to the lactose–lysine model system. During the reaction period, the generation of AGEs had a negative correlation with the concentrations of LSOPC regardless of the heating temperature: 80°C or 100°C. When heated for 2 h at 80°C, the IC_{50} of LSOPC for AGEs was 0.165 ± 0.019 mg/mL (Fig. 4a) whereas, the IC_{50} of LSOPC was 0.301 ± 0.034 mg/mL when heated for 10 min at 100°C (Fig. 4b). These results showed that the inhibition activity of LSOPC was positively correlated with its concentration and LSOPC had higher inhibition ability at 80°C compared to being heated at 100°C. One possible explanation was that LSOPC had a higher antioxidant activity at a lower temperature [16]. And another reason was that the reaction is fiercer under 100°C to produce a large number of AGEs in a short time, therefore it affected the inhibition activity of LSOPC. Overall, LSOPC had a potential to inhibit the generation of AGEs in the lactose–lysine model system.

3.3. Influence of pH conditions

As we know, pH value varies in real food systems [24]. In order to simulate real food system better, we assessed the inhibition effect of LSOPC on advanced glycation end-product formation in a lactose–lysine model system under different pH values, ranging from 5.0 to 8.0. Fig. 5 showed the formation of AGEs in different pH solutions by heating for 2 h at 80°C and heating for 10 min at 100°C. As the initial pH value was increased, AGEs were increased exponentially. Clearly, the formation of AGEs was to a little extent at low pH level (under pH 6.0). The best pH values for fluorescence AGE formation were maintained in the range from 7.0 to 8.0. The results were in accordance with those of Ai-Nong Yu et al. [25]. With 0.8 mg/mL LSOPC added, the formation of AGEs was significantly inhibited during 7.0 to 8.0. When heated at 80°C for 2 h, inhibition ratio of LSOPC increased from 81.21 ± 1.26% to 89.67 ± 0.60% corresponding to pH 7.0, 7.5, 8.0 (Fig. 5a). Inhibition ratio of LSOPC reached the highest at pH 8.0, being 67.59 ± 1.10% when heated at 100°C for 10 min (Fig. 5b). To conclude, it can be stated that LSOPC had an effective inhibition

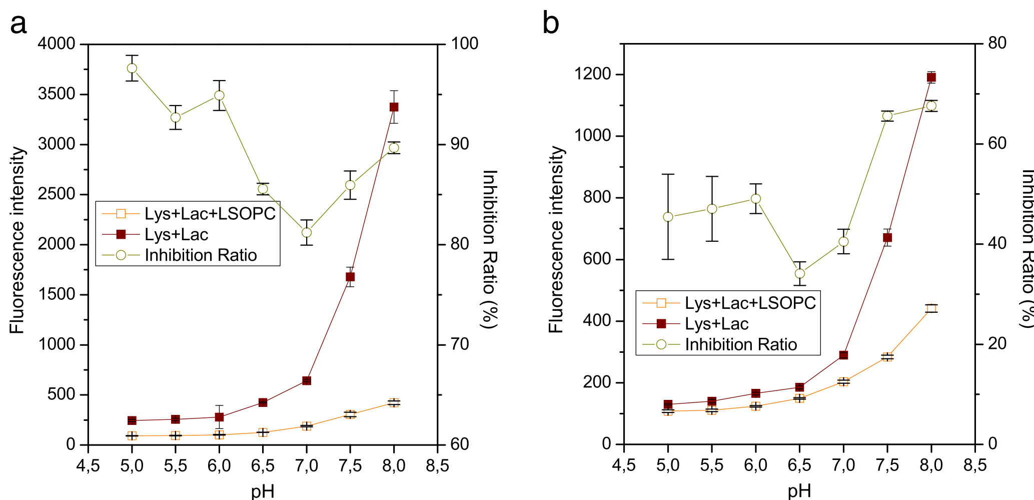

Fig. 5. Inhibition of 0.8 mg/mL LSOPC on non-enzymatic glucosylation of protein in model system of α-lactose and L-lysine at different pH values: (a) heated at 80°C for 2 h, (b) heated at 100°C for 10 min ($n = 3$).

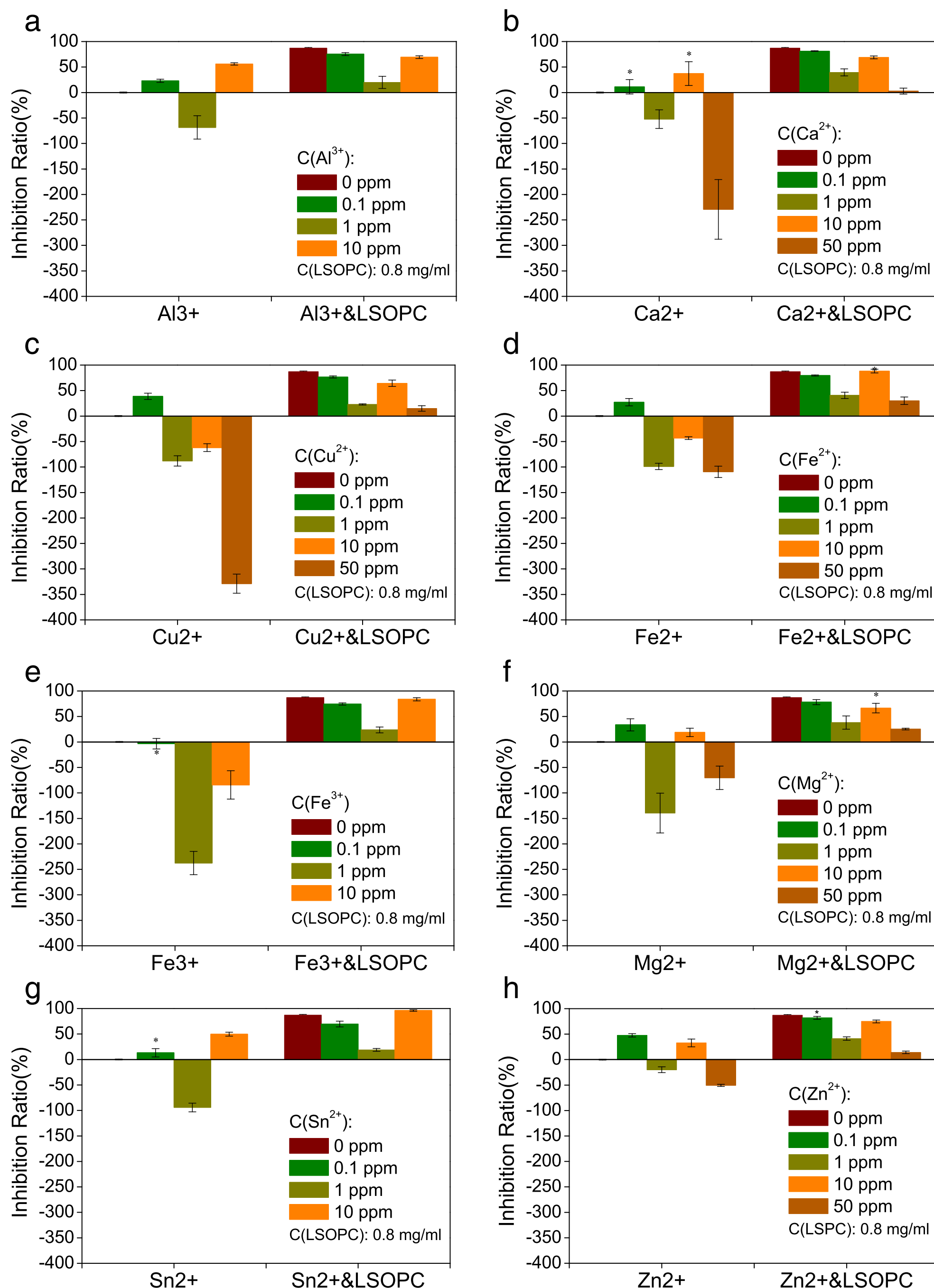

Fig. 6. The interaction of metal irons with LSOPC in model system of α-lactose and L-lysine under pH 5 at 80°C ($n = 3$). Inhibition ratio of all samples except for samples marked with an asterisk were significantly different from that of control ($P < 0.05$): (a) Al^{3+}, (b) Ca^{2+}, (c) Cu^{2+}, (d) Fe^{2+}, (e) Fe^{3+}, (f) Mg^{2+}, (g) Sn^{2+}, (h) Zn^{2+}.

ability when the Maillard reaction happened severely at higher pH. Also, it had a good inhibitory effect at lower pH when there were little AGEs. Meanwhile, LSOPC showed much higher inhibition activity at lower temperature in the same pH environment probably for the reason that LSOPC has a better stability at lower temperature [26].

3.4. Influence of metal ion conditions

The pH value for most of the food is neutral and weak acidic [24]. So we choose pH 5 and 6.5 to do further research. The selection of the metal concentrations was based on values encountered in real food systems, particularly in milk and milk products [27]. The lowest concentrations of the metals applied correspond to their solubilities and the values at which they occur in food. Fig. 6 showed the influence of metal ions on the formation of AGEs and the inhibition effect of metal ions on AGE formation with LSOPC in the lactose–lysine model system when heated at 80°C for 2 h under pH 5.

As shown in Fig. 6a, the addition of 1 ppm Al^{3+} promoted the formation of AGEs, whereas the addition of 0.1 and 10 ppm Al^{3+} suppressed the AGE formation. 10 ppm Al^{3+} had the highest inhibition ratio, up to 56.08 ± 2.30%. After 0.8 mg/mL LSOPC added, the inhibition ratio of the model solution containing Al^{3+} at concentrations of 0.1 and 10 ppm were increased to 75.46 ± 2.76% and 56.08 ± 2.30% respectively. And 1 ppm Al^{3+} became to have an inhibition effect in the presence of LSOPC.

The addition of Ca^{2+}, Mg^{2+} and Zn^{2+} in the concentrations of 1 ppm and 50 ppm, increased the formation of AGEs in the lactose–lysine model system, especially for 50 ppm Ca^{2+}. However, the formation of AGEs could be suppressed in addition of 0.1 and 10 ppm Ca^{2+}, Mg^{2+} or Zn^{2+}, and 10 ppm Ca^{2+}, 0.1 ppm Mg^{2+} and 0.1 ppm Zn^{2+} had the highest inhibition ratios, being 37.13 ± 33.37%, 33.60 ± 16.95% and 47.65 ± 3.32%. After 0.8 mg/mL LSOPC was added, all concentrations of metal ions inhibited the formation of AGEs, the highest inhibition ratio of Ca^{2+}, Mg^{2+} and Zn^{2+} all appeared at the concentration of 0.1 ppm, being 69.00 ± 2.92%, 78.17 ± 5.05% and 81.93 ± 2.96% (Fig. 6b, f, h).

Fig. 6c, d show the influence of Cu^{2+} and Fe^{2+} on the formation of AGEs and the inhibition effect of LSOPC on AGE formation with metal ions in the model system. The addition of 0.1 ppm Cu^{2+} or Fe^{2+} suppressed the formation of AGEs, while for the higher concentrations (1, 10, 50 ppm) the formation of AGEs was promoted, especially for 50 ppm Cu^{2+}. After 0.8 mg/mL LSOPC was added, they all inhibited the formation of AGEs including the higher concentrations.

As shown in Fig. 6e, Fe^{3+} promoted the formation of AGEs at all concentrations (0.1, 1 and 10 ppm), especially for 1 ppm. However, all concentrations of Fe^{3+} inhibited the formation of AGEs in the presence of LSOPC. 10 ppm Fe^{3+} had the highest inhibition ratio, up to 74.43 ± 2.13%.

Fig. 6g shows the influences of Sn^{2+} on the formation of AGEs with and without 0.8 mg/mL LSOPC. They were similar to Al^{3+},

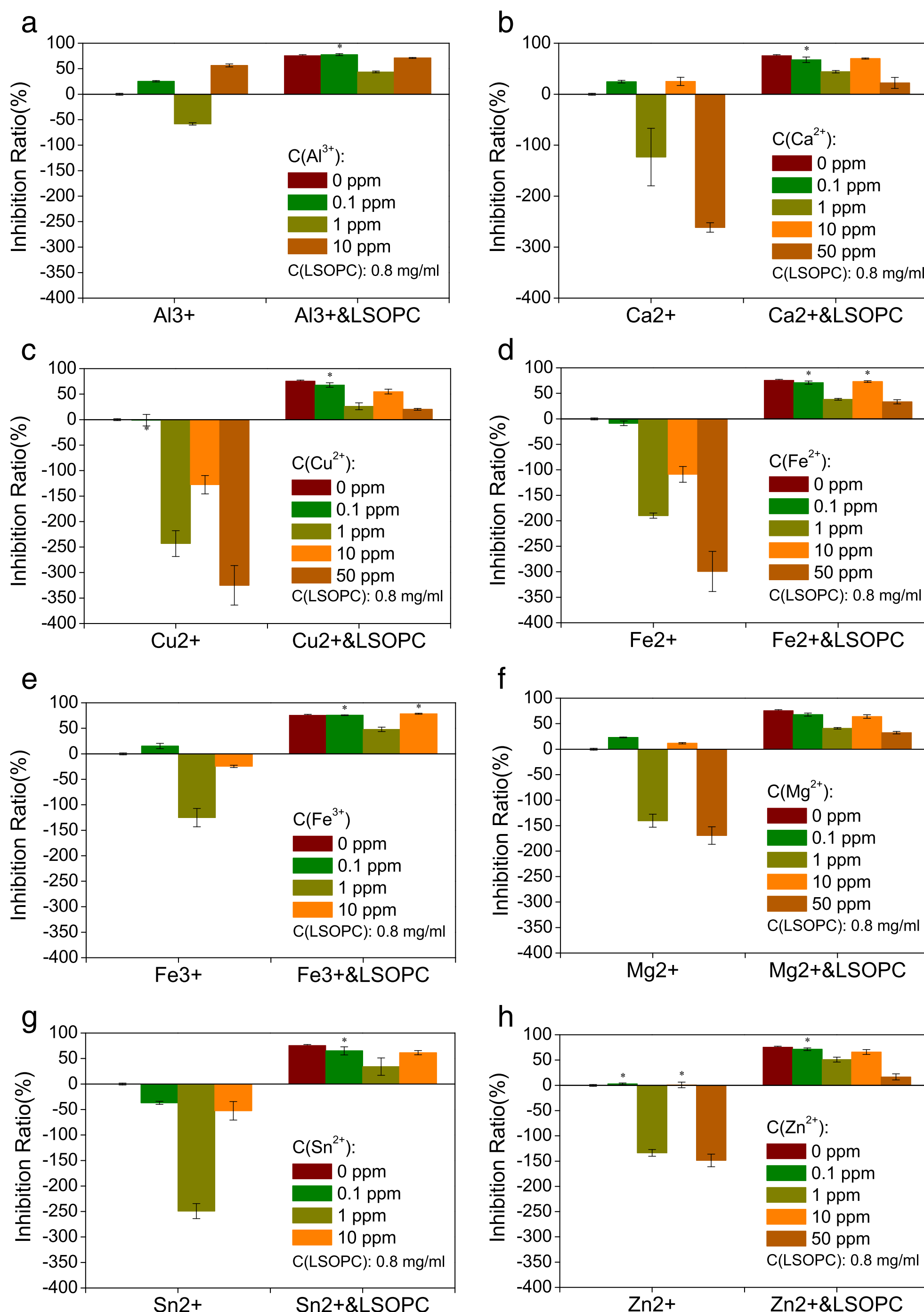

Fig. 7. The interaction of metal irons with LSOPC in model system of α-lactose and L-lysine under pH 6.5 at 80°C ($n = 3$). Inhibition ratio of all samples except for samples marked with an asterisk were significantly different from that of control ($P < 0.05$): (a) Al^{3+}, (b) Ca^{2+}, (c) Cu^{2+}, (d) Fe^{2+}, (e) Fe^{3+}, (f) Mg^{2+}, (g) Sn^{2+}, (h) Zn^{2+}.

only 1 ppm Sn^{2+} promoted the formation of AGEs in the absence of LSOPC. Sn^{2+} at all concentrations (0.1, 1 and 10 ppm) inhibited the formation of AGEs in the presence of LSOPC, the inhibition ratios were 69.58 ± 5.60%, 18.80 ± 2.88% and 96.18 ± 1.50% (Fig. 6g).

Fig. 7 shows the influences of metal ions on the formation of AGEs with and without LSOPC when heated at 80°C for 2 h under pH 6.5. The inhibition effects of most metal ions did not change compared to that heated at pH 5.0. However, there were still some differences. For Cu^{2+} and Fe^{2+}, they had higher promotion effects at higher concentrations (1, 10, 50 ppm) when heated at pH 6.5. By contrast, Fe^{3+} has weaker promotion effects. When solution was heated at pH 5.0, 0.1 and 10 ppm Sn^{2+} inhibited the formation of AGEs in the absence of LSOPC, but 1 ppm Sn^{2+} had an opposite effect. In contrast, Sn^{2+} at all concentrations (0.1, 1 and 10 ppm) promoted the formation of AGEs when heated at pH 6.5. After 0.8 mg/mL LSOPC was added, they had the same influences on the formation of AGEs. When heated at pH 5.0, Zn^{2+} at the concentration of 0.1 and 10 ppm had higher inhibition effect than that heated at pH 6.5, but lower promotion effect. Their inhibition effects were similar in the presence of LSOPC.

The effect of metal ions on AGEs formation was found to depend on the type of amino acid and heating time, as well as on the type of metal ion. It is known that a transition metal ion catalyzes the Maillard reaction by the oxidative pathway [28,29] and the Maillard reaction was suppressed by the coagulation of melanoidin in the presence of various metal ions. According to Fallico and Ames [30], there was respectively only a small effect of ion on the model systems. However, in this study, almost all metal ions inhibited the formation of AGEs at lower concentrations and had promotion effects at higher concentrations. Cu^{2+} and Ca^{2+} ions at higher concentration (50 ppm) enhanced AGE formation the most. However, they had inhibition effects at all concentrations after LSOPC added. The possible reason was that LSOPC has a significant inhibition effect because of their antioxidant properties and carbonyl scavenging capacity [19], and it can reverse the promotion function of metal ions. As figures shown, the inhibition ratio of LSOPC decreased in the presence of metal ions, indicating that LSOPC-metal ion chelates had a lower inhibition effect than LSOPC. pH was found to have few contributory effect to the formation of AGEs, except for Sn^{2+} and Zn^{2+}.

3.5. Effect of LSOPC on CML formation

The effect of LSOPC on CML formation was shown in Fig. 8. In α-lactose/L-lysine system, CML generation had a negative correlation with the concentrations of LSOPC. 0.2, 0.4, 0.6, 0.8 and 1 mg/mL LSOPC inhibition rates were 26.725 ± 1.84%, 28.241 ± 1.67%, 30.141 ± 1.43%, 32.846 ± 2.2% and 38.132 ± 2.86%, respectively. These results showed that LSOPC had a significant inhibition activity on the formation of CML compared to other flavonoids such as rutin and quercetin [31].

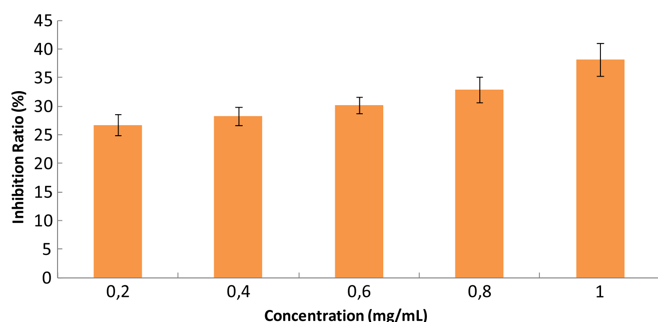

Fig. 8. Inhibition of CML formation by LSOPC in lactose–lysine model systems. Data points are the mean of triplicate measurements ± SD.

4. Conclusions

The presence of AGEs in food products raises concern since only one-third of absorbed dietary AGEs are excreted, while the rest is presumably incorporated into body tissues and is responsible for food and age-related diseases [32,33]. So it is clear that a better understanding and monitoring of AGE formation during food processing is required. To conclude, it can be stated that LSOPC can inhibit the formation of AGEs effectively at high concentrations under temperature 100°C, and it can reverse the promotion function of metal ions because of its high inhibition activity. In addition, LSOPC can inhibit CML formation in Maillard reaction. Thus, LSOPC could be helpful to prevent AGE formation in model system and with the potential to be used as functional food ingredients.

Conflict of interest

The authors declare that they have no conflict of interest.

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