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Antioxidant-related and kinetic studies on reduction effect of catechins and esterified catechins on acrylamide formation in microwave heating model system

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The reduction effect of catechins and esterified catechins on the kinetic behavior of acrylamide formation and its correlation with the change of antioxidant property of Maillard reaction products in an equimolar asparagine-glucose microwave heating model system was investigated. Results indicated that both catechins and esterified catechins could effectively reduce the formation of acrylamide and achieve a maximum inhibitory rate when their addition levels were 10^{-9} mol L^{-1}. Furthermore, 5'-phenolic hydroxyl and/or 3-gallate groups in the chemical structure of catechins and/or their derivatives play an important role in the reduction of acrylamide formation. Also, the reduction effect of esterified catechins is better than that of catechins. Meanwhile, inhibitory rates of acrylamide affected by either catechins or esterified catechins correlated well (R^2: 0.757-0.940) with the trolox equivalent antioxidant capacity (ΔTEAC) of Maillard reaction products measured by three different antioxidant evaluation methods, i.e. 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) or ferric reducing antioxidant power (FRAP) assay. The kinetic study revealed that the processes of conversion from glucose into fructose, asparagine-fructose reaction and the generation of acrylamide in Maillard reaction systems are significantly suppressed, which may elucidate the action site of catechins and esterified catechins on the reduction of acrylamide during the Maillard reaction.

Introduction

Acrylamide has been classified as Group 2A-"probably carcinogen to humans" by the International Agency for Research on Cancer.[1] Meanwhile, it is also regarded as a kind of neural, genetic and reproductive toxin. In 2002, Swedish scientists found considerable levels of acrylamide contaminant in heat processing carbohydrate-rich foods.[2] Soon after its discovery in foods, mechanistic studies revealed that acrylamide can be generated from the Maillard reaction between the free amino acid asparagine and reducing sugars.[3,4] Furthermore, some critical direct precursors contributing to the formation of acrylamide have also been identified such as 3-aminopropionamide, decarboxylated schiff base, decarboxylated Amadori product, acrylic acid and acrolein.[5]

Investigations on the reduction of acrylamide in different heat processing foods have been highlighted in recent years. Various methods have been used to minimize acrylamide levels. The inhibitory measures can be considered via agronomic and processing strategies.[6] One of representative reduction methods is the control of contents of participating substrates, i.e. reducing sugars and asparagine. Besides, other inhibition approaches include pH modification and the use of cations, hydrogencarbonates and specific amino acids during food processing.[7] Some advanced ways to the reduction of acrylamide such as the addition of asparaginase, irradiation and genetic modification techniques were also used.[8] Recently, natural antioxidants especially mono- and poly-hydroxylated phenolic-rich extracts were used for the reduction of acrylamide. Zhu et al.[9] demonstrated higher addition levels of phenolic compounds were related to lower levels of acrylamide (R=-0.692). Besides, the use of *o*-diphenolic-rich virgin olive oils may be regarded as a reliable strategy to reduce the formation of acrylamide in fried potatoes.[10] However, the application of phenolic-rich virgin olive oils may dually reduce or enhance the generation of acrylamide, which depends on their usage levels.[11] Therefore, precautions and control of addition levels of phenolic-rich olive oils should be especially considered. Alternatively, flavonoid-rich herbal extracts were also demonstrated as phenolic-rich antioxidant additives for the reduction of acrylamide in foods. Our previous study found 74.1% and 76.1% of acrylamide were reduced by the use of antioxidant of bamboo leaves, a flavonoid-rich extract, in potato crisps and French fries, respectively.[12] Mechanistic work showed that naringenin, a characteristic compound of flavanones, could effectively reduce the formation of acrylamide in the Maillard reaction via directly reacting with its precursors.[13] In another mechanistic view, antioxidants could inhibit acrylamide formation by preventing lipid oxidation, thus limiting the accumulation of carbonyls.[14] Besides, antioxidants may also act as active agents in preventing the generation of a "reactive

carbonyl pool", trapping key Maillard reaction intermediates (e.g. Schiff base), participating in the precipitation of asparagine, reacting with acrylamide via Michael addition reactions and eliminating the formation of acrylamide as their oxidised forms.[15]

Catechins were extracted from green tea, which belong to the family of flavanols and their derivatives (mainly esterified catechins). Thus, catchins and their derivatives may have a great potential on reducing acrylamide and exerting their antioxidant properties. The correlation between levels of tea catechins and the reduction of acrylamide during the roasting process has been demonstrated.[16] Nevertheless, few studies have mechanistically investigated the effect of catechins on the reduction of acrylamide and its correlation with antioxidant properties of the Maillard reaction. Besides, a structure-activity analysis of the ability of flavonoids to reduce the formation of acrylamide in our previous study revealed that both the number and position of phenol hydroxyl functional groups play an important role in the inhibition ability of flavonoids,[17] indicating different efficiency of catechins and esterified catechins may contribute to the reduction of acrylamide.

Several different mathematic models have been adopted to better understand the mechanisms about the formation and elimination of acrylamide.[7] Afterwards, multi-reaction response model has been widely used to estimate the kinetic constants, which derived from the dynamic mass balance principle or the network reaction approach. It is possible that the generation and elimination of acrylamide in different stages during the Maillard reaction can be predicted by the knowledge of mechanisms and kinetic models. The reduction of acrylamide via the use of natural antioxidants may affect the generation and elimination of acrylamide on a kinetic basis. Therefore, investigations on the kinetics of acrylamide affected by natural antioxidants are appropriate to the occasion.

The aim of this study was to (i) investigate the optimal addition level of esterified catechins and related mechanisms for reducing the formation of acrylamide compared to the use of catechins; (ii) demonstrate the correlation between antioxidant properties of the Maillard reaction products and the reduction of acrylamide affected by catechins or esterified catechins; and (iii) reveal the action site of catechins on the reduction of acrylamide during the Maillard reaction on a kinetic basis.

Experimental

Materials

The potato powder made of the Atlantis variety was purchased from Sanjiang (Group) Potato Products Co., Ltd. (Lintao, China). Epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC) and epigallocatechin gallate (EGCG) were provided by Tea Research Institute of Chinese Academy of Agricultural Sciences (Hangzhou, China) while their chemical structures were shown in Fig. 1.

Chemicals

Acrylamide, D-(+)-glucose monohydrate, 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-tri(2-pyridyl)-*s*-triazine (TPTZ) and 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (trolox) were purchased from Sigma-Aldrich (St. Louis, MO, USA). 2,2-Azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) was purchased from Shanghai Genebase Technology Co., Ltd. (Shanghai, China). L-asparagine monohydrate was purchased from Biocity Science and Technology Inc (Beijing, China). D_3-acrylamide, $^{13}C_6$-glucose and $^{15}N_2$-asparagine were obtained from Cambridge Isotope Laboratories (Andover, MA, USA). Formic acid (purity≥96%) was supplied by Tedia (Fairfield, OH, USA) while methanol was obtained from Merck (Whitehouse Station, NJ, USA). Ultrapure water was passed through a Milli-Q Gradient A10 system (Millipore, Bedford, MA, USA) throughout the experiments. The Maillard reaction was performed via microwave heating system in a potato matrix.

Establishment of microwave heating system (MHS)

A portion of potato powder (1 g) was added into the centrifuge tube containing the ultrapure water (20 mL). After ultrasonic vibration for 20 min, the sample solution was centrifuged at 15000 r min^{-1} for 15 min. The supernatant was then passed through a 0.22 μm filter and ready for injection. The parameter of HPLC was improved according to previous analytical method.[18] Asparagine was quantified by diode array detector while glucose, fructose and sucrose were monitored by differential refractive detector. HPLC analyses were performed on a 2695 high-performance liquid chromatograph system (Waters, Milford, MA, USA), which includes 2996 diode array detector and 2414 differential refractive detector. Chromatographic separation was achieved via using a Capcell Pak C_{18} A.Q. column (150 mm × 2.0 mm i.d., 5 μm) at a flow rate of 1 mL min^{-1}. The mobile phase using an isocratic gradient elution programme consisted of 75% acetonitrile (v/v). The injection volume was 10 μL. Finally, the concentrations of asparagines and glucose in the potato matrix were measured. Based on the deduction of these two Maillard reaction substrates in the original matrix, the addition levels of asparagine and glucose were calculated in order to ensure the occurrence of an equimolar asparagine-glucose Maillard reaction system. As a result, 699.3 mL of asparagine (0.2 mol L^{-1}) and 279.9 mL of glucose (0.5 mol L^{-1}) were mixed with a portion of potato powder (50 g) and compensated with phosphate buffer solution (pH 6.80) to prepare the Maillard reaction solution (1 L) with an equimolar level of asparagine and glucose (0.14 mol L^{-1}). The microwave heating reaction between asparagine and glucose was performed via an Ethos D microwave digestion labstation from Milestone Inc. (Shelton, CT, USA). The labstation was set up and conducted according to our previous work.[19] In detail, the mixed reaction solutions in sealed digestion vessels were all microwave-heated for 5 min at 180 °C under a working power of 500 W after a prepared temperature programme of the microwave digestion labstation as follows: room temperature→120 °C (200 W, 5 min); 120 °C→180 °C (500 W, 5 min). The fluctuation range of heating temperature in the microwave system was less than ± 1 °C. The present potato-based equimolar asparagine-glucose Maillard reaction system has many advantages, including high baseline levels of acrylamide, effective generation of acrylamide via microwave heating with high reaction temperature and short reaction time, appropriate mimicker of acrylamide production in characteristic food matrices such as potatoes, etc.[17,20] At the end of heating, the microwave digestion vessels filled with the final reaction products were taken out from the labstation and immediately cooled in prepared ice water to stop any further reaction. The whole cooling procedure was performed

in a room with stable air temperature (20 °C) adjusted by air-conditioning. The cooled reaction products were ready for sampling during the pre-treatment of final reaction products.

Reduction effect of catechins and esterified catechins on the formation of acrylamide

Two esterified catechins (i.e. ECG and EGCG) and two non-esterified catechins (i.e. EC and EGC) were investigated for their reduction effects. After dissolving in a small amount of dimethyl sulfoxide (DMSO), they were all diluted to eight different levels, i.e. 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8} and 10^{-9} mol L^{-1} with 0.1 mol L^{-1} phosphate buffer (pH 6.80). Then, different levels of catechins and esterified catechins (100 μL) were added into the prepared potato-based Maillard reaction solution (10 mL). A control group was prepared using the same matrix without any addition of catechin compound. When all the groups were well-prepared, the prepared solutions were reacted at 180 °C for 5 min to mimic the Maillard reaction in the microwave heating system.

Pre-treatment of final products from the Maillard reaction

The internal standard solution of D_3-acrylamide (2 μg mL^{-1}) was prepared in advance. An aliquot of (200 μL) of Maillard reaction products was diluted to 10 mL with phosphate buffer in a colorimetric cylinder. Then, an aliquot of dilution (2 mL) was mixed with the internal standard solution (500 μL). After the solution was well vortex-mixed, liquid-liquid extraction with ethyl acetate and solid-phase extraction (SPE) purification were successively conducted according to our previous work.[21] The elution was finally transferred to an auto-sampler vial for the ultra-high performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS) analysis.

Determination of acrylamide in Maillard reaction products

Acrylamide levels of Maillard reaction products in each group were measured by UHPLC-MS/MS with the multiple reaction monitoring (MRM) method. The UHPLC was performed on an Acquity ultra-high performance Liquid chromatograph system equipped with the micro vacuum degasser, auto-sampler and column compartment (Waters, Milford, MA, USA). Chromatographic separation was performed via using a Waters UPLC BEH C_{18} (50 mm × 2.1 mm i.d., 1.7 μm) guarded with a Hypercarb column (10 mm × 2.1 mm i.d.). Details about the UHPLC-MS/MS conditions were conducted by our previous study.[22]

Determination of antioxidant property in Maillard reaction products

The antioxidant property of Maillard reaction products was simultaneously measured by the DPPH, ABTS and ferric reducing antioxidant power (FRAP) assays. (i) *DPPH assay*. The DPPH radicals scavenging activity assay was based on a modified procedure of previous work.[23] In this assay, antioxidants presented in the products reduce the DPPH radicals, which induce a maximal visible absorption at 520 nm. An aliquot of the final products (20 μL) was mixed with 5 mL of the DPPH solution (74 mg L^{-1}). The mixture was then measured at 520 nm by visible spectrophotometer. The trolox standard aqueous solution (concentration range: 0-0.4 mmol L^{-1}) was analyzed under the same conditions, which was used for calibration. The antiradical activity of the products was expressed as the trolox equivalent antioxidant capacity (TEAC), i.e. μmol equivalents of trolox per mL of sample (μmol trolox mL^{-1}). (ii) *ABTS assay*. The ABTS assay was conducted according to previous work with some modifications.[24] In detail, an aliquot of the final reaction solutions (5 μL) was dissolved in 5 mL of the ABTS solution (70 μmol L^{-1}). After well-mixing, the products were also detected by visible spectrophotometer at the maximal wavelength absorption (730 nm). Calibration was performed with a linear range of 0-0.12 mmol L^{-1}. Results were expressed as $TEAC_{ABTS}$ values. (iii) *FRAP assay*. Based on the procedure described previously,[25] the FRAP assay was modified and improved. The FRAP regent consists of TPTZ (10 mmol L^{-1}), $FeCl_3$ (20 mmol L^{-1}) and acetate buffer (0.3 mol L^{-1}) with a ratio of 1:1:10 (pH 3.6). An aliquot of Maillard reaction solutions (6 μL) were allowed to react with 4.5 mL of the above FRAP solution. Then, the absorbance at 595 nm was determined after assay products were well vortex-mixed followed by a 2-h room temperature reaction. The calibration was performed under the same condition as described with a linear range of (0-0.3 mmol L^{-1}). Results were expressed as $TEAC_{FRAP}$ values. If the TEAC values measured by the above three assays were over the linear ranges of their calibration curves, the reaction solutions should be appropriately diluted with water.

Kinetic study on reduction of acrylamide by the use of catechins and esterified catechins in microwave heating system

The kinetic study on the formation and reduction of acrylamide accompanying with the addition of catechins and esterified catechins was also performed in the microwave heating system. The precursor substrate solution contained the equimolar concentration (0.14 mol L^{-1}) of asparagine and glucose. The optimized addition level of catechins and esterified catechins was selected, which presented the maximal reduction effect on acrylamide formation. An aliquot of the optimized level of catechins or esterified catechins (100 μL) was added into the microwave system in the experimental groups while the same volume of phosphate buffer was added into the control group. The mixed reaction solutions in both control and experimental groups in sealed digestion vessels were simultaneously microwave-heated under the selected heating temperature (180 °C) of the labstation temperature programming other than different heating time treatments (0.1, 1, 2, 3, 4, 5, 7.5, 10, 15, 25 and 40 min, totally 11 treatments). The heating treatment of asparagine-glucose-catechin mixture in each test was repeated in triplicates (n = 3). Based on kinetic consideration, all the intermediates were modelled into Schiff base as the proposed central product and the final products could be divided into acrylamide, melanoidins and other final products. The kinetic data analysis and relative rate constant calculation were performed according to previous studies with some modifications.[18] The overall changes in acrylamide concentrations can be formulated by a reaction schedule of 6 consecutive reactions in which k_1, k_2, k_3, k_4, k_5 and k_6 represent the rate constants of asparagine-glucose reaction, conversion from glucose into fructose, asparagine-fructose reaction, acrylamide formation, melanoidins formation and acrylamide degradation, respectively (Fig. 2). The kinetic equations could be written as follows:

$$\frac{dC_{Asn}}{dt} = -k_1 \cdot C_{Asn} - k_3 \cdot C_{Asn} \quad (1)$$

$$\frac{dC_{Glu}}{dt} = -k_1 \cdot C_{Glu} - k_2 \cdot C_{Glu} \quad (2)$$

$$\frac{dC_{Fru}}{dt} = k_2 \cdot C_{Fru} - k_3 \cdot C_{Fru} \quad (3)$$

$$\frac{dC_{Schiff}}{dt} = k_1 \cdot C_{Glu} + k_3 \cdot C_{Fru} - (k_4 + k_5) \cdot C_{Schiff} \quad (4)$$

$$\frac{dC_{AA}}{dt} = k_4 \cdot C_{Schiff} - k_6 \cdot C_{AA} \quad (5)$$

$$\frac{dC_{M}}{dt} = k_5 \cdot C_{Schiff} \quad (6)$$

$$\frac{dC_{D}}{dt} = k_6 \cdot C_{AA} \quad (7)$$

In these kinetic equations, C_{Asn}, C_{Glu}, C_{Fru}, C_{Schiff}, C_{AA}, C_M and C_D represent the concentrations of asparagine, glucose, fructose, Schiff base, acrylamide, melanoidins and degradation products, respectively, while t represents the reaction time. For initial conditions, when the reaction time $t = 0$, $C_{Asn} = C_{Glu} = 0.14$ mol L^{-1} while $C_{Fru} = 0$, $C_{Schiff} = 0$, $C_{AA} = 0$, $C_M = 0$ and $C_D = 0$.

Simultaneous determination of acrylamide, asparagine, glucose and fructose by UHPLC-MS/MS during the kinetic study

In the kinetic study, acrylamide, asparagine, glucose and fructose were simultaneously determined by UHPLC-MS/MS according to our previous work.[26] Briefly, chromatographic separation was performed via using a Hypercarb column (100 mm × 2.1 mm i.d., 5 μm, Thermo Electron, San Jose, CA, USA). The mobile phase was formic acid (0.2%, v/v) at a flow rate of 0.2 mL min^{-1}. The chromatographic column was maintained at 40 °C with a run time of 5.5 min per sample. Tandem mass spectrometry was performed on a Quattro Ultima triple-quadrupole mass spectrometer (Micromass Company Inc., Manchester, UK) using the electrospray ionization (ESI) source. The detection of asparagine and $^{15}N_2$-asparagine was operated in negative ion mode due to matrix peak interferences in positive ion mode. All of other analytes including isotope compounds were monitored in positive ion mode. Besides, the melanoidins were measured by a spectrophotometer, which had a maximal visible absorption at 470 nm. The concentration of melanoidins was calculated from the Lambert-Beer equation with an extinction coefficient of 282 L $mol^{-1} \cdot cm^{-1}$ and a 1-cm length of the cuvette.

Statistical analysis and the calculation of kinetic parameters

All acrylamide levels were given as mean ± standard deviation (SD) values in triplicates and corresponding inhibitory rates were subsequently calculated. Compared to the $TEAC_{DPPH}$, $TEAC_{ABTS}$ and $TEAC_{FRAP}$ values in the control groups, three TEAC values of the final reaction products in experimental groups were calculated. The ΔTEAC values were then obtained via observing the difference between TEAC of Maillard reaction products in the treatment groups and the control group. The correlation between ΔTEAC determined by three antioxidant evaluation assays and inhibitory rates of acrylamide affected by catechins and esterified catechins was then investigated. Kinetic studies on the calculation of 6 kinetic parameters were statistically evaluated by the SAS software, version 8.2 (SAS Inst. Inc., Beijing, China). The kinetic parameters of both control and experimental groups were estimated by the Marquardt nonlinear least square regression method. The significance test of kinetic parameters of each experimental group was conducted using the Student's *t*-test method compared to the control group.

Results and Discussion

Comparison study on the reduction effect of catechins and esterified catechins on the formation of acrylamide

To evaluate the thermal stability of catechins/esterified catechins, eight different levels of them were added into current optimized MHS (final concentrations: 10^{-11}-10^{-4} mol L^{-1}) without the presence of asparagine and glucose. Mimicking the microwave heating conditions of dose-response and kinetic studies, the catechin-containing potato-based phosphate buffer solution was thermally processed at 180 °C. The alternation of catechin/esterified catechin levels was analyzed with the increase of microwave heating time by LC-MS/MS according to previous work.[27] Results showed no significant change of all catechins/esterified catechins with the elapse of microwave heating time, indicating their enough thermal stability. A nonlinear and bell-shaped dose-response correlation between acrylamide concentrations in Maillard reaction products and addition levels of catechins/esterified catechins was shown in Fig. 3A. Results indicated that with different concentrations of catechin/esterified catechin treatments, the inhibitory rate of acrylamide affected by EC, ECG, EGC and EGCG ranged 24.4%-50.1%, 50.6%-71.6%, 32.5%-58.4% and 58.7%-91.9%, respectively. The optimal addition levels of either catechins or esterified catechins for suppressing the formation of acrylamide were all 10^{-9} mol L^{-1}. Results of all acrylamide concentrations in the final reaction products in experimental groups were significantly different from the acrylamide concentrations in the control group ($P<0.05$). In detail, negative and positive dose-response relationships of inhibitory rates of acrylamide were observed with the catechin/esterified catechin treatment ranges of 10^{-11}-10^{-9} mol L^{-1} and 10^{-9}-10^{-4} mol L^{-1}, respectively. Such reverse tendency on the reduction of acrylamide may be related to the antioxidant activity of food matrices, the antioxidant property of Maillard reaction products, the inherent property of antioxidants, etc., which is a vivid phenomenon of so-called "antioxidative paradox".[28] First, previous study demonstrated the increase of cooking time leads to the enhancement of acrylamide levels, antioxidant property and colour in a biscuit product.[29] Such observation indicated acrylamide concentrations may increase significantly with the increasing addition level of catechins/esterified catechins and advance the antioxidant property of reaction products due to the promotion of the Maillard reaction. Second, the acrylamide concentrations may be declined due to the quinine-amine interaction between antioxidants and the direct precursor of acrylamide. Quinones are generated via the oxidation of the catechins/esterified catechins, and subsequently react with key

intermediates such as 3-aminopropionamide.[30] The inhibitory rates of acrylamide via the effect of antioxidants may be evaluated by taking both of factors into consideration and ensuring which factor plays a predominant role during the Maillard reaction.[31]

It is well-known that the reduction effect of food additives on the formation of acrylamide was closely related to the chemical structure of additives.[32] The carbonyl value, a representative indicator for evaluating the state of lipid oxidation, may contribute to the formation of acrylamide. A positive correlation between the carbonyl value and acrylamide formation was observed in a model system.[33] Further mechanistic study demonstrated that catechins reduce the formation of acrylamide presumably by trapping carbohydrates and/or preventing lipid oxidation.[34] On the other hand, EC, ECG, EGC and EGCG belong to flavanols and their derivatives on a structural basis. The typical differences of chemical structures among different catechins and their derivatives were the functional groups in 3- and 5'-positions of the flavanol skeleton. Results indicated that catechins which contain a phenolic hydroxyl group in 5'-position and/or a gallate group in 3-position of the flavanol skeleton showed a higher inhibitory effect on the acrylamide formation than the catechins which do not contain these functional groups. Among all of catechins, EGCG exerted the strongest inhibitory effect on the formation of acrylamide in all of its addition levels, which may be mainly ascribed to the chemical structure of both 5'-phenolic hydroxyl and 3-gallate. Compared to the effect of catechins EC and EGC, results revealed that the inhibitory effects of esterified catechins (ECG and EGCG) seem much better, indicating the 3-gallate group predominantly contributed to the inhibitory effects of catechins. Besides, the presence of 3-gallate group in the chemical structures of ECG and EGCG introduces 3 additional aromatic phenol hydroxyl functional groups (Fig. 1), which may substantially contribute to the inhibitory effects on the formation of acrylamide.

Correlation between antioxidant properties of Maillard reaction products and acrylamide inhibition

The nonlinear and bell-shaped dose-response relationship between the $TEAC_{DPPH}$/$TEAC_{ABTS}$/$TEAC_{FRAP}$ values and addition levels of catechins/esterified catechins was also observed in Fig. 3B-3D. According to the three assay methods, $\Delta TEAC_{DPPH}$, $\Delta TEAC_{ABTS}$ and $\Delta TEAC_{FRAP}$ values representing the differences between TEAC of Maillard reaction products determined by three methods in catechin treatment groups and control group were calculated accordingly. A linear regression analysis was performed to examine whether ΔTEAC values had a close correlation with acrylamide inhibitory rates (Fig. 4A-4C). The correlation coefficients (R^2) showing the ΔTEAC-inhibitory rate relationship ranged from 0.757 to 0.907, indicating a close linear correlation between ΔTEAC values and acrylamide inhibitory rates among all of investigated experimental data. It can be inferred from the phenomenon of the reaction that acrylamide was effectively suppressed while the color of final reaction products became shallow. The decrease of melanoidins generated from the Maillard reaction may explain the reduction of antioxidant properties of the reaction system. This also means catechins and esterified catechins may affect the process of Maillard reaction, which is needed to be demonstrated by further kinetic studies. Summa et al.[26] investigated the relationship between the antioxidant activity, acrylamide concentration and colour of the self-prepared cookies. However, the direct correlation between the antioxidant property of the reaction products and acrylamide concentration was not concerned. Subsequently, such direct relationship has been systemically investigated in various food matrices with different antioxidant evaluation methods. For example, the linear relationship between acrylamide concentration and total antioxidant capacity (TAC) was found during the frying process (R^2 = 0.8322).[35] Also, acrylamide concentration in biscuits made from two different wheat flour types - wholemeal and white flour type 550 correlated with FRAP and lightness (R^2: 0.47-0.85).[36] These pioneer studies showed the establishment of a model system for simulating the Maillard reaction and the relationship between acrylamide levels and antioxidant properties of reaction products via changing the heating processing conditions, such as baking time, baking temperature, moisture content, etc. Our present study simultaneously demonstrated the reduction of acrylamide by catechins/esterified catechins) and the correlation between acrylamide inhibitory rate and antioxidant property of reaction products. Moreover, high linear correlation coefficients were also observed to quantitatively demonstrate the close relationship. Ciesarová et al.[37] investigated that the effect of other extracts (e.g. pimento, black pepper, marjoram and oregano) on the reduction of acrylamide contents which was in a correlation with antioxidant capacities, in particular with their DPPH-scavenging capacities (R = –0.996). Current study provides a novel experimental system, which comprised of characteristic food matrix and equimolar asparagine-glucose model system. Such design of the experimental system could not only avoid the interference from complex food matrices but also showed the correlation between the reduction of acrylamide formation and correlation with antioxidant properties in mimic food systems.

Kinetic study of acrylamide formation and reduction by optimized addition of catechins

The optimized addition level of both catechins and esterified catechins (10^{-9} mol L^{-1}), which exerted a maximal reduction effect on the acrylamide formation, were used for the kinetic study in the equimolar asparagine-glucose model system. The kinetic model mimicking the Maillard reaction and related kinetic parameters were shown in Fig. 2. A representative UHPLC-MS/MS chromatogram describing the simultaneous determination of asparagine, glucose, fructose and acrylamide in Maillard reaction products using $^{15}N_2$-asparagine, $^{13}C_6$-glucose and D_3-acrylamide as isotope internal standards was shown in Fig. 5. Equations 1 to 7 described the dynamic formation and elimination processes of acrylamide. However, these equations need to be simplified in order to conveniently calculate the kinetic parameters. Based on the kinetic model, calculation results of the kinetic parameters, i.e. rate constants, describing the formation and elimination of acrylamide in the buffered equimolar asparagine-glucose microwave heating system were shown in Table 1. The results indicated that the kinetic parameters k_1, k_5 and k_6, which reflected the rate constants of asparagine-glucose reaction, melanoidins formation and acrylamide degradation, respectively, was not significantly changed (P>0.05). However, parameters k_2, k_3 and k_4, which

represented the rate constants of conversion from glucose into fructose, asparagine-fructose reaction and the formation of acrylamide were much lower than the control group (P<0.05). Therefore, current study showed catechins/esterified catechins selectively affect the kinetic process on the formation and elimination of acrylamide, and significantly reduce the asparagine-fructose reaction pathway, not the asparagine-glucose reaction. Besides, the rate constant of acrylamide formation k_4 calculated from the study in esterified catechins-containing asparagine-glucose MHS was lower than the k_4 calculated from the study when catechins were added into the equimolar asparagine-glucose MHS, which could also elucidate that esterified catechins exert a better reduction effect on acrylamide formation than the reduction effect of catechins. Flavanol-related structures such as catechins and esterified catechins are known to interfere with the Maillard reaction through scavenging of reactive dicarbonyl compounds such as decarboxylated Amadori compound, which was an important intermediate present in the conversion from Schiff base into acrylamide.[38,39] Results of current kinetic investigations regarding the alternation of k_4 via the effect of catechins and esterified catechins were in accordance with the above mechanism. Combined with previous work regarding the correlation between the reduction of acrylamide and aromatic phenol hydroxyls in the chemical structures of catechins and esterified catechins, the finding that esterified catechins exert a better reduction effect on acrylamide formation than the reduction effect of catechins could be demonstrated in both dose-response and kinetic views. However, future mechanistic work should also focus on the effect of catechins and esterified catechins on the prevention of asparagine-fructose reaction but not the original asparagine-glucose reaction.

Conclusions

In the present work, we investigated the effect of catechins and esterified catechins on the formation of acrylamide in a potato-based equimolar asparagine-glucose MHS via the dose-response, antioxidant correlation and kinetic studies. Both catechins and esterified catechins could effectively reduce the generation of acrylamide in the Maillard reaction during microwave heating with the inhibitory rate range of 24.4%-91.9% in a nonlinear bell-shaped dose-response way with the optimized addition levels of 10^{-9} mol L^{-1}, which was ascribed to the so-called "antioxidant paradox" phenomenon. The 5-phenolic hydroxyl and 3'-gallate groups in the chemical structure of catechins/esterified catechins play an important role in the reduction of acrylamide on a structural basis, indicating the reduction effect of esterified catechins on the formation of acrylamide was better than the effect of catechins. Also, the inhibitory rates of acrylamide formation correlated well with the antioxidant properties of Maillard reaction products simultaneously evaluated by three antioxidant capacity assays. Finally, the optimized use of both catechins and esterified catechins could significantly reduce the processes of conversion from glucose into fructose (k_2), asparagine-fructose reaction (k_3) and the formation of acrylamide (k_4) during the Maillard reaction on a kinetic basis. Current study indicated that catechins or catechin-rich extracts (e.g. tea polyphenols) may substantially contribute to the reduction of acrylamide during microwave heat processing.

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Notes and references

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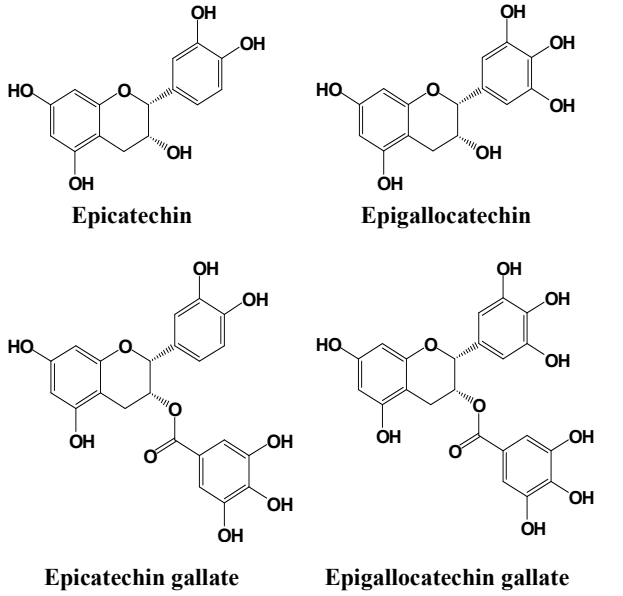


Fig. 1 The chemical structures of catechins and esterified catechins in the present study

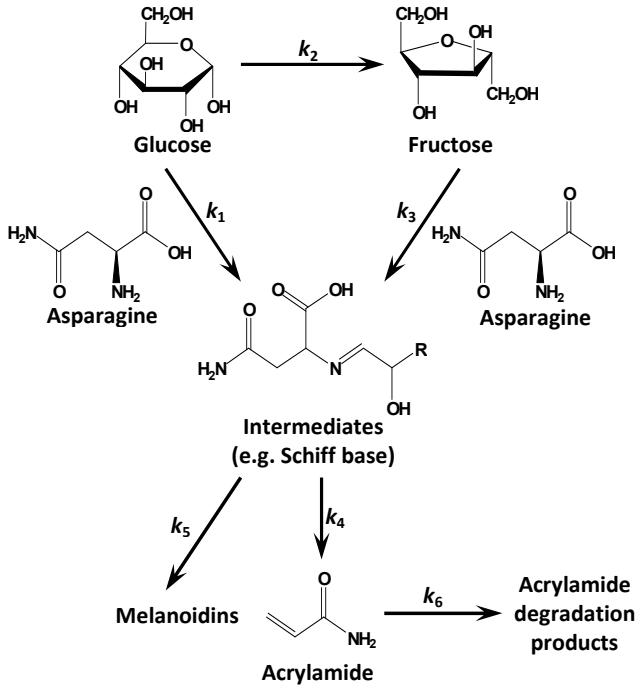


Fig. 2 Kinetic model of acrylamide formation and elimination for mimicking Maillard reactions. k_1-k_6, kinetic parameters.

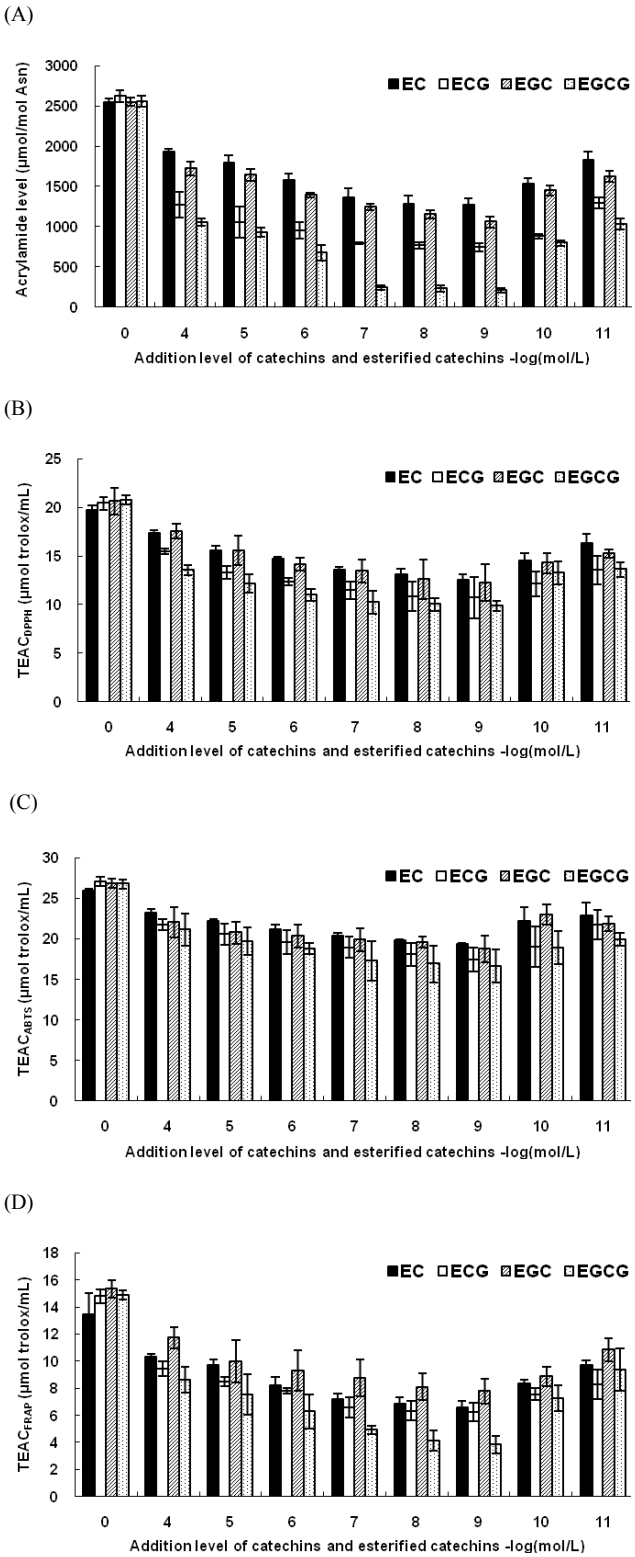
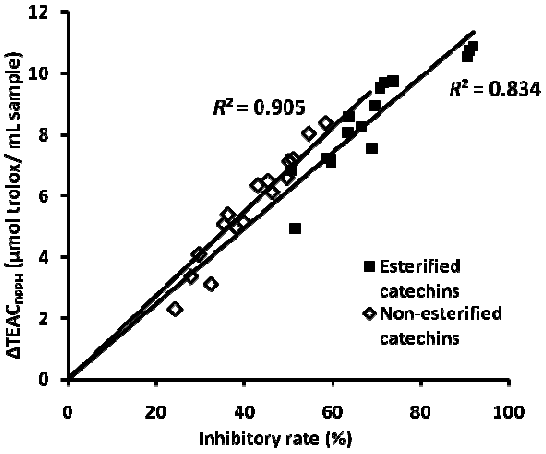


Fig. 3 The bell-shaped correlations between different treatment levels of non-esterified/esterified catechins and (A) acrylamide concentrations; (B) $TEAC_{DPPH}$ values; (C) $TEAC_{ABTS}$ values; or (D) $TEAC_{FRAP}$ values in Maillard reaction products via microwave heating. Data were expressed as mean ± SD (n = 3).

(A)



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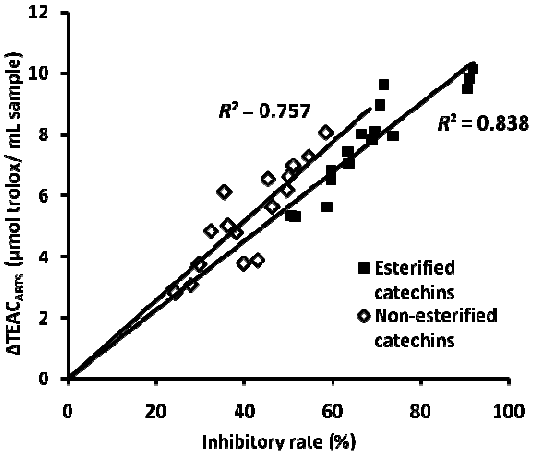

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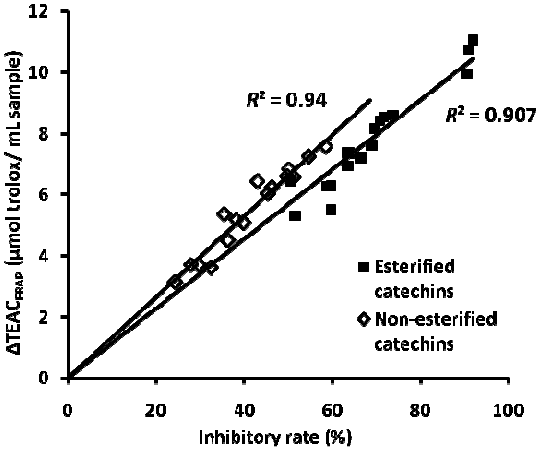

Fig. 4 Correlation between the effect of catechins/esterified catechins on the reduction of acrylamide and ΔTEAC of Maillard reaction products. (A) $TEAC_{DPPH}$-inhibitory rate relationship; (B) $TEAC_{ABTS}$-inhibitory rate relationship; (C) $TEAC_{FRAP}$-inhibitory rate relationship. Catechins, EC and EGC; Esterified catechins, ECG and EGCG.

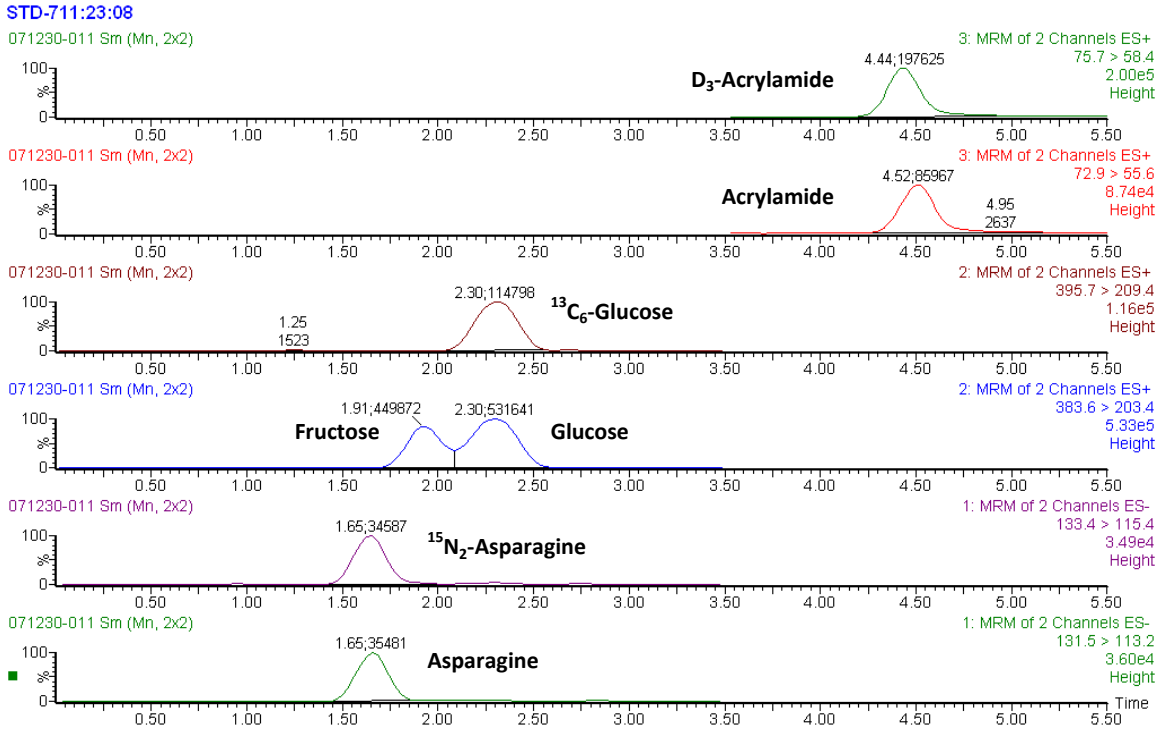

Fig. 5 A representative UHPLC-MS/MS chromatogram describing the simultaneous determination of asparagine, glucose, fructose and acrylamide in Maillard reaction products using $^{15}N_2$-asparagine, $^{13}C_6$-glucose and D_3-acrylamide as isotope internal standards.

Table 1 Parameter estimation of kinetic models via the effect of catechins on the reduction of acrylamide (n = 3)

Catechins/esterified catechins	Parameters of kinetic models [a]					
	k_1 (min^{-1})	k_2 (min^{-1})	k_3 (min^{-1})	k_4 (min^{-1})	k_5 (min^{-1})	k_6 (min^{-1})
Control group	0.143 ± 0.026	0.636 ± 0.009	0.718 ± 0.005	5.093 ± 0.447	4.121 ± 0.397	0.099 ± 0.009
EC	0.135 ± 0.043	0.577 ± 0.065*	0.591 ± 0.069*	3.033 ± 0.344**	4.167 ± 0.398	0.112 ± 0.036
ECG	0.105 ± 0.019	0.524 ± 0.019**	0.548 ± 0.018**	2.780 ± 0.541**	4.308 ± 0.223	0.101 ± 0.029
EGC	0.133 ± 0.037	0.529 ± 0.021**	0.558 ± 0.020**	2.888 ± 0.391**	4.115 ± 0.661	0.099 ± 0.014
EGCG	0.094 ± 0.015	0.523 ± 0.025*	0.537 ± 0.012**	2.134 ± 0.515**	4.050 ± 0.411	0.083 ± 0.012

[a] The significant difference was evaluated by Student's t-test. * P<0.05, ** P<0.01.