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The reduction effect of dietary flavone C- and O-glycosides on the formation of acrylamide and its correlation and prediction with the antioxidant activity of Maillard reaction products

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The effect of dietary flavone C- and O-glycosides on the formation of acrylamide contamination has been investigated in the present work. Flavone glycosides were added in different concentrations, and acrylamide levels were quantified in a potato-based equimolar asparagine-reducing sugar model system via microwave heating. Results indicated a non-linear relationship between addition levels of flavone glycosides and inhibitory rates of acrylamide in the Maillard reaction. The maximum inhibitory rate range (25.3%-63.5%) was observed when the addition levels of all flavone glycosides were 10^{-9} mol l^{-1}. A structure-activity analysis on the ability of flavonoids to reduce the formation of acrylamide revealed that both number and position of the phenol hydroxyl functional groups play an important role in the inhibitory ability of flavonoids. Furthermore, flavone C-glycosides are more effective at inhibiting the formation of acrylamide than flavone O-glycosides despite sharing the same structure aglycone. The rate of inhibition of acrylamide formation correlated well with the change of trolox equivalent antioxidant capacity (ΔTEAC) measured by DPPH (R^2=0.934), ABTS (R^2=0.897) or FRAP (R^2=0.912) assay. Using ΔTEAC as variables, a multiple linear regression (MLR) model could effectively serve as a predictive tool for estimating the reduction of acrylamide by flavone glycosides during microwave heat processing. These observations are important for our understanding and future development of agents which might decrease the formation of this hazardous toxin.

Introduction

Acrylamide is classified as a probable human carcinogen and has widely been found in both Western-style and oriental carbohydrate-rich foods since 2002.[1] Previous studies have shown that acrylamide is generated from the Maillard reaction between asparagine and carbohydrates, which is the source of hundreds of flavour compounds and has been widely applied to the preparation of heat processing foods in food industry.[2,3] Some of critical precursors contributing to the generation of acrylamide include 3-aminopropionamide, decarboxylated Schiff base, decarboxylated Amadori product, acrylic acid and acrolein.[4] Given this toxic nature, it is important to minimize the formation of acrylamide during cooking and food preparation. In a mechanistic view, several studies reported potential strategies for the reduction of acrylamide including the elimination of the key intermediates, formation of other less toxic vinylogous compounds and inhibition of some of the key pathways such as the formation of the Schiff base, Strecker type degradation, *N*-glucoside pathway and β-elimination reaction of the decarboxylated Amadori compounds.[5]

Flavonoids are a large group of plant polyphenolics which contain a benzopyrane-based structure with an attached 2- or 3-phenyl group.[6] Previous study revealed a flavonoid-rich spice mix could effectively reduce acrylamide levels in potato chips.[7] A mechanistic study showed that naringenin (a characteristic compound of flavanones) effectively reduces the formation of acrylamide in the Maillard reaction via directly reacting with its precursors.[8] Flavone glycosides are an important group of flavonoid compounds that include flavone C-glycosides and flavone O-glycosides and occur in significant amounts in fruits and vegetables.[9] Few studies have focused on the role of flavone glycosides in the reduction of acrylamide and related structure-activity elucidations.

Natural antioxidants especially mono- and/or poly-hydroxylated phenolic-rich extracts were widely taken into consideration for the reduction of acrylamide. Previous study found that high addition levels of phenolic compounds were related to low levels of acrylamide formation.[10] We have previously used a natural antioxidant product present in bamboo leaves to inhibit the formation of acrylamide in potato-based foods,[11] fried chicken wings[12] and oriental fried bread sticks,[13] and applied an well-validated ultra-performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS) method for the determination of acrylamide in various food matrixes.[14] Moreover, a close correlation between reduction of acrylamide levels and antioxidant activity of spice extracts was investigated and demonstrated.[15] These original experiments provided insights into the important relationship between the antioxidants present in foods and the formation of acrylamide

during cooking.

Regarding the high correlation with the generation of acrylamide,[16,17] the antioxidant activity of food matrices or model systems could be used to predict the formation and reduction of acrylamide. However, few studies revealed the ability and related mechanism of antioxidants on the reduction of acrylamide and its correlation with antioxidant activities of the Maillard reaction products. Furthermore, the multiple linear regression (MLR) model can be used to predict the overall antioxidant capacity of Maillard reaction products after considering results of multiple antioxidant assays. In this aspect, there is no MLR model available to predict the acrylamide content in Maillard reaction products based on the results of antioxidant capacity measurements.

This study has systematically evaluated the ability of flavone C- and O-glycosides to inhibit the formation of acrylamide in a potato-based Maillard reaction system via microwave heating, and investigated its correlation and prediction with the antioxidant activities of Maillard reaction products.

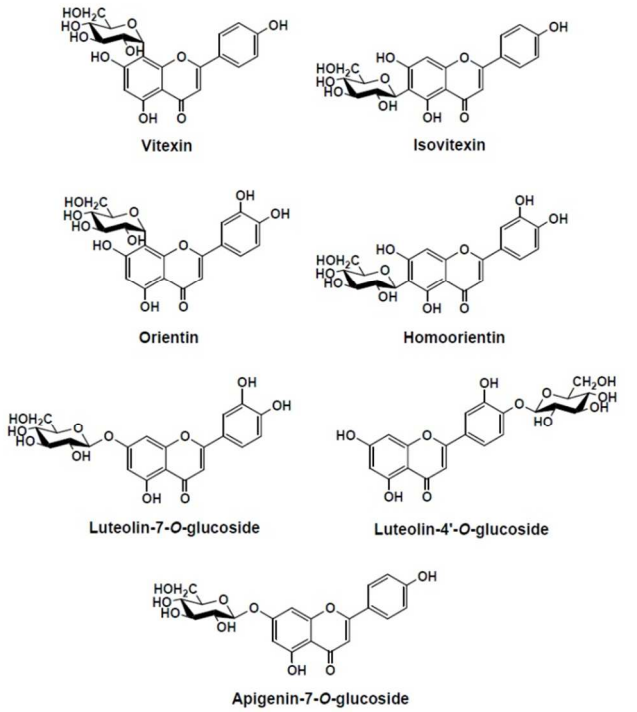

Fig. 1 Chemical structures of flavone glycosides in the present study

Experimental

Materials and chemicals

Homoorientin (luteolin-6-C-glucoside), orientin (luteolin-8-C-glucoside), isovitexin (apigenin-6-C-glucoside) and vitexin (apigenin-8-C-glucoside) were obtained from antioxidant of bamboo leaves by preparative high-performance liquid chromatography (HPLC) according to our previous studies.[18] Apigenin-7-O-glucoside, luteolin-7-O-glucoside and luteolin-4'-O-glucoside were purchased from Extrasynthese Co. (Lyon, France). The chemical structures of the above flavone glycosides were shown in Fig. 1. 2,2-Diphenyl-1-picrylhydrazyl (DPPH), (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (trolox), potassium persulfate and 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ) were purchased from Sigma-Aldrich (St. Louis, MO, USA), whereas 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) was obtained from Genebase Gen-Tech Ltd. (Shanghai, China). Acrylamide, L-asparagine monohydrate and D-(+)-glucose monohydrate were obtained from Sigma-Aldrich, whereas D_3-labelled acrylamide (isotopic purity 99%) was purchased from Cambridge Isotope Laboratories (Andover, MA, USA). Potato powder (Atlantic variety) was obtained from Sanjiang (Group) Potato Products Co., Ltd. (Lintao, China).

Determination of asparagine, glucose, fructose and sucrose in the potato matrix

The contents of asparagine and sugars in the selected potato powder need to be initially analyzed. Considering 90% of all sugars in potatoes consist of glucose, fructose and sucrose, sugar contents were thus determined on these three components.[19] Both asparagine and three sugar contents were quantified by HPLC on a Waters 2695 HPLC chromatograph with a Capcell Pak C_{18} A.Q column (5 μm, 150 mm × 2.0 mm I.D.) protected by a RP_{18} guard column (5 μm, 4.0 mm × 3.0 mm I.D.) (Phenomenex Co., Torrance, CA, USA). The mobile phases were acetonitrile and water (75:25, v/v). The flow rate was 1.0 ml min^{-1}. The injection volume was 30 μl while the column temperature was maintained at 25 °C. Asparagine was monitored at 254 nm with a diode array detector while glucose, fructose and sucrose were monitored with a differential refractive index detector. The external standard method was used for the quantification.

Preparation of potato-based equimolar asparagine-reducing sugar Maillard reaction system

The stock solutions, i.e., asparagine (0.2 mol l^{-1}) and glucose (0.5 mol l^{-1}), were prepared in phosphate buffer (0.1 mol l^{-1}, pH 6.80). The asparagine and reducing sugar (glucose and fructose) concentrations were set to a final equimolar level of 0.14 mol l^{-1} after adding the above stock solutions by considering the original analyzed concentrations of asparagine, glucose and fructose. An aliquot of potato powder (10 g) can be configured into 200 ml potato model system reaction solution.

The use of microwave digestion labstation in the dose-response relationship study

The dose-response relationship in current study was performed in microwave reaction systems using the Ethos D microwave digestion labstation (Milestone Inc., Shelton, CT, USA). In this labstation, there are 9 microwave sample vessels in the carousel, which allows 9 groups of reactions between substrates simultaneously under identical reaction conditions. The ATC-400CE automatic temperature control system (mainly an advanced fiber-optic temperature sensor) and the APC-55 automatic pressure control system within the standard reference vessel allow continuous monitoring and control of internal temperature (±1 °C) and internal vapor pressure (±100 kPa). In addition, a focused and high sensitivity IR sensor is used for monitoring the surface temperature of all sample vessels inside the cavity. The reaction temperature and time and their limits can

be modulated via a digital intelligent control panel connected with all of the above control systems. In the preliminary test of our study, the maximum pressure of the asparagine-sugar reactions was determined as 950 kPa, which was safe enough (maximum safe pressure: 3500 kPa) to perform the reaction using this labstation.

Reduction effect of flavone glycosides on the formation of acrylamide

The potato model system consisted of 20% potato matrix, in which the asparagine and reducing sugar concentrations were selectively adapted to a final equimolar level of 0.14 mol l^{-1} for both of components. The remaining 80% consisted of a 0.1 mol/L phosphate buffer (pH 6.8). Then, each flavone glycoside was added into the model system. The concentrations of each flavone glycoside were adapted to a final sequence of 0 (control), 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}, 10^{-9}, 10^{-10} and 10^{-11} mol l^{-1}. The above solutions (10 mL) with addition of flavonoids were added into hermetically closed microwave digestion vessels and microwave-heated for 5 min at 180 °C with a working power of 500 W after a prepared temperature programming of the microwave digestion labstation as follows: room temperature→120 °C (200 W, 5 min); 120 °C→180 °C (500 W, 5 min). Each group of microwave heating experiment was performed in triplicate repeats (n = 3). At the end of heating, the microwave digestion vessels filled with the final reaction products were taken out and immediately cooled in prepared ice water to stop any further reaction. The whole cooling procedure was performed in a special room with stable air temperature (20 °C) adjusted by air-conditioning.

Determination of acrylamide in Maillard reaction products by UHPLC-MS/MS

All of final reaction products in each group were centrifuged at 15,000 rpm for 15 min with a Microfuge 18 centrifuge (Beckman Coulter Inc., Fullerton, CA, USA). The aliquot of the supernatant (0.2 mL) was diluted to 10 mL by the phosphate buffer (pH 6.80). Then, the sample solution was successively pre-treated via the addition of D_3-labelled acrylamide, liquid-liquid extraction with ethyl acetate and clean-up with Oasis HLB solid-phase extraction cartridges (Waters, Milford, MA, USA) according to our previous study.[20] Acrylamide levels in Maillard reaction products in each group were then determined by UHPLC-MS/MS and quantified via the multiple reaction monitoring (MRM) mode. UHPLC detection was performed on an Acquity ultra-high performance liquid chromatography system equipped with the micro vacuum degasser, autosampler and column compartment (Waters, Milford, MA). Tandem mass spectrometry was performed on a Micromass Quattro Ultima Pt mass spectrometer (Micromass Company Inc., Manchester, UK). The instrument was operated using an electrospray source in positive mode (ESI^+). Details about analytical parameters for both UHPLC and MS/MS were optimized and described in our previous work.[14]

Determination of antioxidant properties of Maillard reaction products

The antioxidant activity of the products was simultaneously determined by DPPH, ABTS and ferric reducing ability power (FRAP) assays. The DPPH radicals scavenging activity assay of different products was based on a modified procedure of previous study.[21] The $ABTS^+$ assay was conducted according to previous work with some modifications.[22] On the basis of the procedure described previously,[23] the FRAP assay was modified and improved. Compared to the control groups, results of the change of antioxidant activity of Maillard reaction products via DPPH, ABTS and FRAP assays were all expressed as the change of trolox equivalent antioxidant capacity ($\Delta TEAC_{DPPH}$, $\Delta TEAC_{ABTS}$ and $\Delta TEAC_{FRAP}$) calculated as μmol trolox/mL.

Prediction of Acrylamide Reduction via MLR

Current study investigated the reduction effect of flavone glycosides with eight different addition levels for each on the formation of acrylamide during microwave heat processing. Combined with triplicate test for each experiment, there were 168 groups of experimental data points while each data group included the inhibitory rate of acrylamide and three independent data of ΔTEAC values. Two-thirds of them (112 data points) were used to train the model while the remaining data (56 data points) were used to predict the model. Input data of X_1, X_2, and X_3 represent $\Delta TEAC_{DPPH}$, $\Delta TEAC_{ABTS}$ and $\Delta TEAC_{FRAP}$, respectively, for the target output of inhibitory rate (%) of acrylamide formation affected by flavone glycosides. Using the training data set, the MLR model was established via the linear regression of SPSS (version 16.0) to predict the inhibitory rate of acrylamide. The performance of optimized MLR model was validated using the remaining one-third of totally 168 groups of data points after training. Finally, MLR output values of training and prediction sets were compared with experimental inhibitory rates of acrylamide formation by the effect of flavone glycosides. The performance of MLR models was evaluated by using mean square error (MSE), root mean square error (RMSE), mean absolute error (MAE), mean absolute percentage error (MAPE), correlation coefficient (R^2) and other statistical variables on training and testing set between the predicted values and the experimental values as follows.

$$\mathrm{MSE} = \frac{1}{n}\sum_{i=1}^{n}\left(X_{i0} - X_i\right)^2 \quad (1)$$

$$\mathrm{RMSE} = \sqrt{\frac{1}{n}\sum_{i=1}^{n}\left(X_{i0} - X_i\right)^2} \quad (2)$$

$$\mathrm{MAE} = \frac{1}{n}\sum_{i=1}^{n}\left|X_{i0} - X_i\right| \quad (3)$$

$$\mathrm{MAPE} = \frac{1}{n}\sum_{i=1}^{n}\left|\frac{X_{i0} - X_i}{X_{i0}}\right| \times 100 \quad (4)$$

$$R^2 = \frac{\left[\sum_{i=1}^{n}\left(X_{i0} - \overline{X_0}\right)\left(X_i - \overline{X}\right)\right]^2}{\sum_{i=1}^{n}\left(X_{i0} - \overline{X_0}\right)^2\left(X_i - \overline{X}\right)^2} \quad (5)$$

Statistical analysis

All acrylamide levels were expressed as mean ± standard deviation (SD) values in triplicates and corresponding inhibitory rates were also calculated. Data obtained from dose-response study on the correlation between acrylamide levels and addition levels of flavone glycosides were evaluated using Duncan multiple comparison test. Statistical analysis was performed using the Statistical Product and Service Solutions (SPSS) version 16.0 statistical software (SPSS Inc., Beijing, China). Differences were considered significant at $p<0.05$ in all statistical tests.

Results and Discussion

Mimicking of Maillard reaction system via microwave heating

Microwave heating has been recognized as an effective way to the generation of acrylamide in carbohydrate-rich foods compared to various conventional heating.[24] In the present work, considerable levels of acrylamide were generated in Maillard reaction model systems via microwave heating because the microwave heating style in the process of high temperature and short time promotes the kinetics of acrylamide generation. However, few studies focused on the profile of acrylamide generation and elimination via microwave heating. Current study investigated the reduction effect of flavone C- and O-glycosides on the formation of acrylamide under microwave heating mode, which provided pioneer evidence in the control of acrylamide during microwave processing.

Using the mixed standard solution of analytes (10 mg ml^{-1} each), asparagine, glucose, fructose and sucrose were simultaneously determined by HPLC. Results indicated that original contents of the four substrates in potato matrix were 0.37 g kg^{-1}, 0.15 g kg^{-1}, 0.14 g kg^{-1} and 0.61 g kg^{-1}, respectively. Subsequently, the spiking levels of asparagine and glucose standards were calculated based on the purpose of ensuring the occurrence of an equimolar asparagine-reducing sugar Maillard reaction system. Finally, the formula of such a Maillard reaction system was composed of 699.3 ml of asparagine (0.2 mol l^{-1}) and 279.9 ml of glucose (0.5 mol l^{-1}) in phosphate buffer (0.1 mol l^{-1}, pH 6.80). The reaction system was then mixed with potato powder (50 g) and compensated with phosphate buffer solution (pH 6.80) to make the final volume (1 l). The total concentrations of both asparagine and reducing sugars in the final reaction system solution were checked as (0.14 ± 0.01) mol l^{-1} via HPLC after the preparation of the Maillard reaction system. The characteristics of such a potato-based equimolar asparagine-reducing sugar Maillard reaction system include high baseline levels of acrylamide, effective generation of acrylamide, appropriate mimicker of acrylamide formation in characteristic food matrices.

Comparison of dose-response effects of flavone C- and O-glycosides on the reduction of acrylamide

To investigate and compare the effect of selected flavone glycosides on their ability to decrease the formation of acrylamide, the dose-response relationship experiments were performed. Results demonstrated a non-linear concentration-dependent relationship in all of asparagine-reducing sugar-flavonoid potato models and showed the ability of flavone glycosides to inhibit the formation of acrylamide within their addition level ranges of 10^{-11}–10^{-4} mol l^{-1} (Fig. 2). Such non-linear correlation may be ascribed to multiple factors, including the antioxidant activity of food matrices, the antioxidant of Maillard reaction products, and the inherent property of antioxidants, which is so-called "antioxidant paradox".[25] First, Summa et al.[17] demonstrated that the increasing cooking time resulted in increasing acrylamide content, antioxidant activity and colour in a biscuit product. Thus, the acrylamide content may increase significantly with the enhancing level of flavonoids and advance the antioxidant activity of frying foods due to the promotion of Maillard reaction. Second, the acrylamide content may be decreased due to the quinine–amine interaction between antioxidants and the direct precursor of acrylamide. Quinones are formed via the oxidation of the polyphenols, such as flavonoids, and subsequently react with key intermediates including amine groups such as 3-aminopropionamide.[26] The inhibitory rate of acrylamide via the addition of antioxidants may be judged by taking both of factors into consideration and ensuring which factor plays a predominant role during the Maillard reaction. The results indicated from Fig. 2 clearly showed that the increasing content of acrylamide occurs when the addition level of flavonoids increases from 10^{-9} to 10^{-4} mol l^{-1} due to the predominant factor of the promotion of Maillard reaction. Fig. 2 also indicated that the decreasing content of acrylamide is observed when the addition level of flavonoids increases from 10^{-11} to 10^{-9} mol/L due to the predominant factor of the quinine-amine interaction. Such a non-linear relationship between the inhibitory rates of acrylamide via the effect of antioxidant substances and the antioxidant properties in Maillard reaction products has also been previously demonstrated. Li et al.[27] found an antioxidant extract from bamboo leaves effectively reduces the formation of acrylamide in cookies in a non-linear way. Similar phenomenon has been observed when some commercial antioxidants such as BHA, BHT and vitamin C.[28] Previous mechanistic study showed the role of aqueous rosemary extract and catechin compounds in the formation pathway of acrylamide during the Maillard reaction. The dual prooxidant and antioxidant properties of antioxidants occurred during the dose-response relationship study, which depends on the addition level of antioxidants.[29]

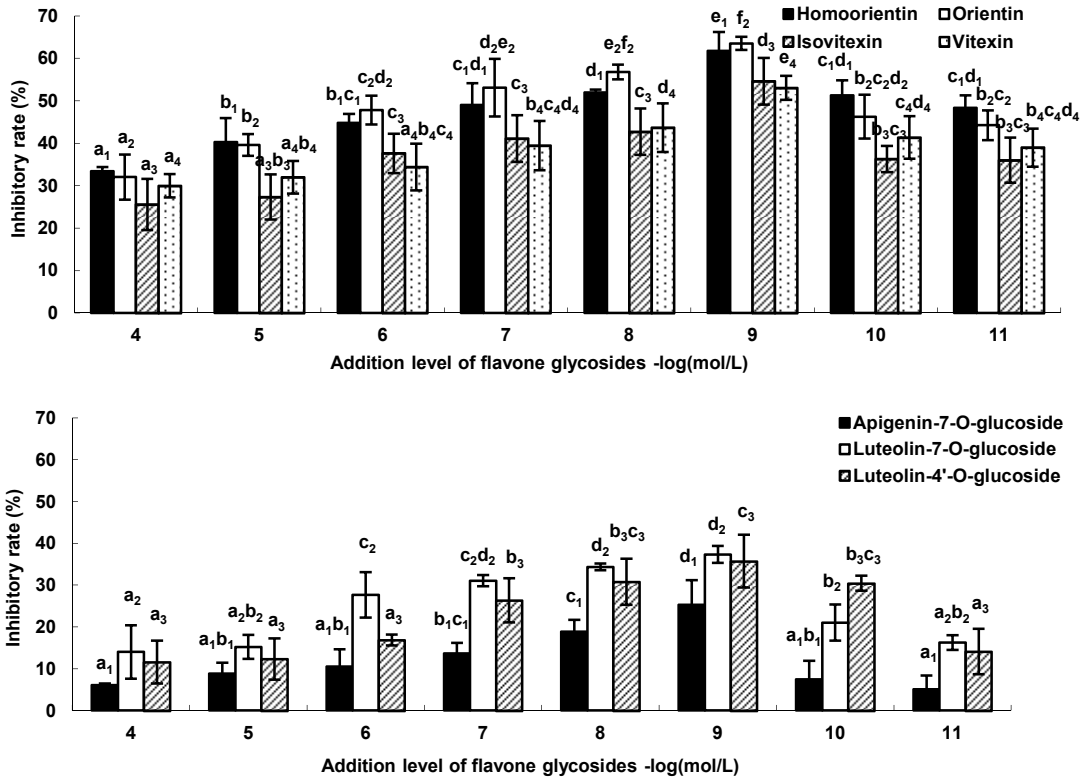

Fig. 2 Dose-response relationship between the inhibitory rate of acrylamide and addition levels of representative flavone glycosides in potato-based microwave heating systems. The bars in all of figures indicate the mean value of inhibitory rate. Data were expressed as mean ± SD (n = 3). (A) reduction of acrylamide by the use of flavone C-glycosides; (B) reduction of acrylamide by the use of flavone O-glycosides. Letters a-f show the statistical results of Duncan multiple comparison test. Different letters shown in different bars present significant difference among each other (p<0.05).

The dose-response relationship study also demonstrated that the maximum inhibitory effect was presented when the addition level of all flavone glycosides was 10^{-9} mol l^{-1}. Following such optimal addition level, (i) the inhibitory effect of four flavone C-glucosides ranged from 53.0% to 63.5%, in which orientin exerted the maximum inhibitory effect (n = 3, Fig. 2A); (ii) the inhibitory effect of three flavone O-glucosides ranged from 25.3% to 37.3%, in which luteolin-7-O-glucoside exerted the maximum inhibitory effect (n = 3, Fig. 2B). The above results allowed us to consider the inhibitory effect of flavone glycosides on a structural basis. Compared to the effect of flavone O-glucosides, the inhibitory effect of flavone C-glucosides was obviously better, indicating the important role of phenol hydroxyl groups rather than alcoholic hydroxyl groups in the chemical structure of flavonoids in the reduction of acrylamide. In detail, flavone C-glycosides are more effective at inhibiting the formation of acrylamide than flavone O-glycosides despite sharing the same structure aglycone (e.g. homoorientin > luteolin-7-O-glucoside). This may be due to the number variation of phenol hydroxyls in flavone glycosides compared to their aglycones. The insertion of C-glucoside does not only maintain the original number of phenol hydroxyls, but also increase the number of alcoholic hydroxyls. However, the insertion of O-glucoside decreases a phenol hydroxyl. Few studies focused on the structure-activity relationship study about the reduction of acrylamide formation by natural product additives. Previous study observed a positive correlation between the carbonyl value of selected additives and acrylamide formation in a model system.[28] Thus, the role of bioactive carbonyl compounds having antioxidant properties on the conversion of asparagine into acrylamide attracted recent concern. Certain phenolic compounds bearing a carbonyl group may compete with the carbonyl group of reducing sugars in Maillard reactions and thus impact the formation and kinetics of acrylamide.[30] However, the correlation between hydroxyl groups of the chemicals and formation of acrylamide in various food matrices has not been demonstrated so far. A polyphenol antioxidant is a type of antioxidant containing a polyphenolic or natural phenol substructure and widely distributed in natural herbs.[31,32] The role of phenol hydroxyl groups of the polyphenol antioxidants in the inhibition of acrylamide generation will be interesting for investigating the reduction mechanism of acrylamide via antioxidant additives. The present study provides pioneer results on the promising effect of phenol hydroxyl group-rich flavonoids on the inhibition of acrylamide, which is important for further elucidation of acrylamide scavenging mechanism via exogenous additives.

Relationship between reduction of acrylamide by flavone glycosides and antioxidant property of Maillard reaction products

Considering the decrease of acrylamide contents is greatly related

to the antioxidant ability of both flavone glycosides and reaction systems, the antioxidant property of Maillard reaction products (mainly melanoidins) was investigated. Details about the antioxidant ability of final reaction products simultaneously determined by the DPPH, ABTS and FRAP assays were shown in Table 1-3. The promising correlations between the inhibitory rates of acrylamide and the antioxidant activity of Maillard reaction products with and without addition of flavone glycosides were observed ($R^2_{\text{Acrylamide-DPPH}}$ = 0.934, $R^2_{\text{Acrylamide-ABTS}}$ = 0.897 and $R^2_{\text{Acrylamide-FRAP}}$ = 0.912). Results indicated that the addition of flavone glycosides does not only inhibit the formation of acrylamide, but also reduce the overall extent of Maillard reaction. Previous work demonstrated that antioxidant compounds and acrylamide formed in the similar stages of the Maillard reaction and at similar rates,[33] which was in agreement with our current correlation study that the antioxidant properties of the Maillard reaction products reduced when the acrylamide levels decreased. It can be inferred from the phenomenon of the reaction that acrylamide was effectively inhibited while the colour of final reaction products became shallow. The amount of melanoidins generated in Maillard reaction decreased may explain the reduction of antioxidant properties of the reaction system. This also means flavone glycosides may reduce the generation of acrylamide and thus affect the process of Maillard reaction. However, the pathway and mechanism of reduction effect need to be demonstrated by further kinetic studies.

Table 1 The antioxidant property of final Maillard reaction products simultaneously determined by the DPPH assay [a]

Addition (mol l^{-1})	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-10}	10^{-11}
Flavonoid	ΔTEAC (μmol trolox ml^{-1}) [b]							
Luteolin-7-O-Glu	1.92	2.34	2.78	3.39	3.63	4.30	2.58	2.07
Luteolin-4'-O-Glu	1.35	1.61	2.25	3.29	3.70	3.85	3.02	2.13
Apigenin-7-O-Glu	1.68	1.79	1.92	2.22	2.78	3.63	2.20	1.78
Homoorientin	4.07	4.57	4.72	5.25	5.28	6.80	5.49	5.19
Orientin	3.37	4.37	5.49	5.67	6.10	6.31	5.13	4.43
Isovitexin	2.42	3.21	4.36	4.51	4.66	6.07	4.80	4.56
Vitexin	2.75	3.07	3.22	4.44	4.82	5.67	3.83	3.74

[a] Data were expressed as the mean value from triplicate test (n = 3).
[b] ΔTEAC, the difference of trolox equivalent antioxidant capacity between the control group and experimental group. μmol trolox ml^{-1}, μmol equivalents of trolox per ml of sample. Glu, glucoside.

Previous study demonstrated that favourable structural requirements for effective radical scavenging or the antioxidant potential of flavonoids follow the famous Bors' three criteria:[34] (i) The o-dihydroxyl (3',4'-diOH, i.e. catechol) structure in the B ring confers high stability to the flavonoid phenoxyl radicals via hydrogen bonding or by expanded electron delocalization; (ii) The C2-C3 double bond (in conjugation with the 4-oxo group) determines the co-planarity of the heteroring and participates in

Table 2 The antioxidant property of final Maillard reaction products simultaneously determined by the ABTS assay [a]

Addition (mol l^{-1})	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-10}	10^{-11}
Flavonoid	ΔTEAC (μmol trolox ml^{-1}) [b]							
Luteolin-7-O-Glu	1.59	1.51	3.40	3.48	4.33	4.34	2.54	2.01
Luteolin-4'-O-Glu	1.62	1.96	2.60	2.77	4.86	4.97	3.00	2.69
Apigenin-7-O-Glu	1.36	1.91	2.12	2.45	4.72	6.65	4.34	3.73
Homoorientin	4.09	4.87	4.94	5.75	6.22	6.53	6.00	5.24
Orientin	3.56	3.85	4.53	4.77	5.90	6.37	5.37	3.68
Isovitexin	2.04	2.93	3.51	3.90	4.09	4.96	3.73	3.15
Vitexin	3.08	3.53	3.95	4.26	5.19	5.35	4.51	3.50

[a] Data were expressed as the mean value from triplicate test (n = 3).
[b] ΔTEAC, the difference of trolox equivalent antioxidant capacity between the control group and experimental group. μmol trolox ml^{-1}, μmol equivalents of trolox per ml of sample. Glu, glucoside.

Table 3 The antioxidant property of final Maillard reaction products simultaneously determined by the FRAP assay [a]

Addition (mol l^{-1})	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-10}	10^{-11}
Flavonoid	ΔTEAC (μmol trolox ml^{-1}) [b]							
Luteolin-7-O-Glu	0.96	1.19	2.45	3.08	3.90	4.02	2.09	1.67
Luteolin-4'-O-Glu	2.25	2.43	3.04	3.17	4.45	4.74	4.20	2.00
Apigenin-7-O-Glu	0.67	0.81	1.42	1.54	1.61	3.34	1.11	1.33
Homoorientin	4.21	4.96	5.19	5.58	6.40	7.33	6.92	5.00
Orientin	3.07	3.72	4.27	5.46	5.97	7.06	4.74	4.70
Isovitexin	2.30	2.73	4.81	5.31	5.60	6.72	5.34	5.06
Vitexin	2.70	3.44	5.06	5.86	5.92	7.00	3.87	3.22

[a] Data were expressed as the mean value from triplicate test (n = 3).
[b] ΔTEAC, the difference of trolox equivalent antioxidant capacity between the control group and experimental group. μmol trolox ml^{-1}, μmol equivalents of trolox per ml of sample. Glu, glucoside.

radical stabilization via electron delocalization over all three ring systems; (iii) Both 3- and 5-hydroxyl groups are in favour of the maximal radical scavenging capacity and the strongest radical absorption. Compared to results of reduction effect of different flavone glycosides on the formation of acrylamide performed in the present study, the above criteria were in accordance with the conclusion that both the number and position of the phenol hydroxyl functional groups play an important role in the ability of flavone glycosides to inhibit the formation of acrylamide *in vitro*. The flavone glycosides which possess one of the above chemical structures exerted stronger reduction effect on the acrylamide

generation than their counterparts which have not these functional groups. For instance, the reduction effect of homoorientin and orientin which contain the *o*-dihydroxyl (3',4'-diOH) structure in the B ring was better than the effect of isovitexin and vitexin when their addition level was all 10^{-9} mol l^{-1}.

Current study demonstrated that flavone glycosides have promising capacity for the reduction of acrylamide. Four flavone C-glucosides, i.e. homoorientin, orientin, isovitexin and vitexin, are characteristic flavonoid representatives in antioxidant of bamboo leaves (AOB), which could effectively inhibit the formation of acrylamide in various foods in our previous work.[11-13] Besides, AOB has been authorized as an official antioxidant additive in food via China National Standard Committee. Thus, dual capacity of both antioxidant and inhibition of acrylamide of AOB and its characteristic compounds flavone C-glycosides have bright potential in food applications to reduce acrylamide.

Prediction of acrylamide reduction using ΔTEAC variables via MLR

The MLR method was used to predict the inhibitory rates of acrylamide using the antioxidant activity of Maillard reaction products including $\Delta TEAC_{DPPH}$, $\Delta TEAC_{ABTS}$ and $\Delta TEAC_{FRAP}$ values. The regression equation was shown as follows:

$$Y = 8.531X_1 - 0.763X_2 + 2.160X_3 - 4.346 \qquad (6)$$

X_1, X_2 and X_3 represented $\Delta TEAC_{DPPH}$, $\Delta TEAC_{ABTS}$ and $\Delta TEAC_{FRAP}$, respectively, for the output of inhibitory rates (Y) of acrylamide reduced by flavone glycosides. Using the SPSS (version 16.0) regression function, a MLR model for the prediction of inhibitory rates of acrylamide was established while the model performance for the training and testing data set were presented in Fig. 3A and 3B. Results indicated that the correlation coefficient (R^2) related to training data set was 0.946 while that related to testing data set was 0.903. Statistical results from Table 4 indicated that current MLR model could effectively serve as a predictive tool for estimating the reduction of acrylamide by flavone glycosides during microwave heat processing using ΔTEAC values as variables. Recently, several studies contributed to dose-dependent correlation between the reduction of acrylamide via the addition of antioxidants and the antioxidant capacity of various food matrix systems. For example, FRAP and ABTS values in biscuit food matrixes were correlated well on a low level, whereas acrylamide content of biscuits was correlated with FRAP and lightness.[35] Overall, different kinds of antioxidants with different structures or functional groups could react with acrylamide precursors, with intermediates of the reaction or with acrylamide itself and lead to dose-dependent reduction effects.[16] However, few studies focused on the establishment and optimization of predictive models for the estimation of the reduction of acrylamide based on antioxidant capacities of Maillard reaction products from various food matrixes. Compared to the analysis of acrylamide levels, the measurement of ΔTEAC values refers to simple instrumentation, popularized operation and rapid spectrometric analysis. Current predictive model study provides an easy-to-use approach to the estimation of inhibitory rate of acrylamide.

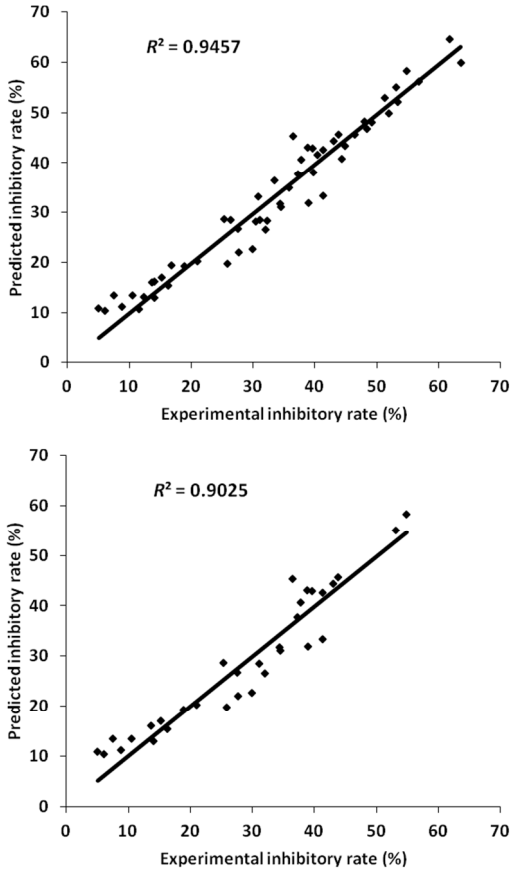

Fig. 3 Predictive performance of inhibitory rates of acrylamide by the MLR model. The regression and fitting performance were investigated using the SPSS system. Correlation between experimental and predictive inhibitory rate of acrylamide (%) for (A) training data set and (B) testing data set.

Table 4 Statistical variables of training and testing data of inhibitory rate (%) in the MLR model

Statistical parameter	Training data set		Testing data set	
	Experimental	Predicted	Experimental	Predicted
	(i) Statistical analysis			
Mean	33.13	33.13	28.49	28.70
Standard error	2.04	1.98	2.43	2.40
Standard deviation	15.24	14.84	13.74	13.58
Median	34.41	32.48	28.49	28.70
Variance	232.31	220.24	188.83	184.49
CV	0.46	0.45	0.48	0.47
Kurtosis	-0.83	-1.02	-0.88	-0.80
Skewness	-0.11	0.12	-0.11	0.43
	(ii) Model performance			
MSE	10.56		14.68	
RMSE	2.77		4.12	
MAE	2.20		3.67	
MAPE	16.99		20.04	

Conclusions

The microwave digestion labstation combined with UHPLC-MS/MS was regarded as a robust tool for mimicking the formation and reduction of acrylamide during Maillard reaction and the quantification of acrylamide in final products. Using the potato-based equimolar asparagine-reducing sugar Maillard reaction and microwave heating systems, the present study describes how flavone C-glycosides and O-glycosides are likely to inhibit the formation of acrylamide and find their dose-response and structure-activity relationships for the decrease of acrylamide contents. Also, the correlation of their reduction effect on acrylamide generation with the antioxidant properties of Maillard reaction products was investigated. The maximum inhibitory rates of flavone glycosides ranging from 25.3% to 63.5% were observed when the addition levels of different flavonoids were all 10^{-9} mol/L. Both the number and position of the phenol hydroxyl functional groups play an important role in the ability of flavone glycosides to inhibit the formation of acrylamide. The 3',4'(ortho)-dihydroxyls in B cycle of the flavonoid molecular greatly contributes to the inhibition of acrylamide. Flavone C-glucosides are more effective at inhibiting the formation of acrylamide than flavone O-glucosides despite sharing the same structure aglycone. The quinine-amine interaction between antioxidants and the direct precursor of acrylamide combined with the effect of flavone glycosides on the promotion of Maillard reaction may explain the mechanism on their abilities to inhibit the formation of acrylamide. Besides, this study revealed a significant linear relationship between inhibitory rates of acrylamide affected by flavone glycosides and antioxidant properties of reaction products. Using ΔTEAC values as variables, a MLR model could effectively serve as a predictive tool for estimating the reduction of acrylamide by flavone glycosides during microwave heat processing. The antioxidant-related study also indicated that the addition of flavone glycosides does not only inhibit the formation of acrylamide, but also reduce the overall extent of Maillard reaction. Further studies will focus on the reduction mechanism of flavonoids on the formation of acrylamide on a structural basis using the quantitative structure activity relationship (QSAR) method and quantitatively investigate the contribution of phenol hydroxyls to the reduction of acrylamide in the Maillard reaction.

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Notes and references

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