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Effect of chlorogenic acid on hydroxymethylfurfural in different Maillard reaction systems

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Abstract

Chlorogenic acid significantly increased hydroxymethylfurfural (HMF) formation in systems of glucose reacted with glycine, glutamate, and cysteine at 160°C for 36 min but decreased HMF formation in the glucose/lysine reaction system or glucose alone. Adjusting the pH to 7.0 using phosphate buffer increased HMF formation in the glutamate/glucose system and glucose alone but significantly decreased HMF formation in the three other Maillard reaction systems. HMF was eliminated when it was heated together with all four amino acids. Cysteine and lysine eliminated 100% and 71.5% of the added HMF, respectively. However, the addition of chlorogenic acid hindered the elimination capacity of lysine, glycine, and cysteine for HMF.

Introduction

Hydroxymethylfurfural (HMF) could be used as a marker of quality for a wide range of processed fruits, coffee, honey, and milk, and is also used for monitoring the heating process applied to cereal products (Capuano and Fogliano, 2011). HMF can be formed through three reactions: the decomposition of 3-deoxyosone in the Maillard reaction and the direct dehydration of sugars under acidic conditions (caramelisation) during thermal treatments applied to foods (Kroh, 1994; Ameur *et al.*, 2007; Capuano and Fogliano, 2011), and also, through the condensation of carbonyl compounds (Cammerer *et al.*, 1999), which are produced by enzymatic processes, caramelisation, the Maillard reaction, and lipid oxidation (Arribas-Lorenzo, 2010; Arena *et al*., 2011).

The oral LD_{50} value for HMF in rats is 3.1 g/kg body weight. HMF is cytotoxic and irritating to the eyes, upper respiratory tract, skin, and mucous membranes (Ulbricht *et al*., 1984; Chen *et al.*, 2010). HMF has also been reported to show low carcinogenic activity by inducing and promoting aberrant crypt foci in rat colon and increasing the number of small intestine adenomas and the incidence of hepatocellular adenomas (Archer *et al*., 1992; Boopathy *et al*., 1993; Capuano and Fogliano, 2011). HMF can be converted *in vitro* and *in vivo* by sulfotransferase into sulfoxymethylfurfural (SMF), a compound that has been found to be mutagenic in conventional Ames tests, initiating tumours in mice skin (Lee *et al*., 1995; Surh and Tannenbaum, 1994).

Since HMF shows some toxic effects to humans and is a quality indicator for many foods, more investigations should be conducted to explore its formation mechanism and influencing factors. By comparing the typical products that contain high amounts of HMF, we found that HMF content is positively related to the chlorogenic acid or cichoric acid contents of food. In high-temperature processed foods (Capuano and Fogliano, 2011), the highest HMF content was detected in coffee (400 mg/kg to 4100 mg/kg) and toasted chicory (200 mg/kg to 22500 mg/kg), which contains 5500 mg/kg and 13200 mg/kg chlorogenic acid (Mazzafera, 1999) and cichoric acid (Wills and Stuart, 1999), respectively. In dried fruits, plums contain 10 times more chlorogenic acid than pineapple and produce almost 7 times as much HMF, even though the organic acids of pineapple are double those of plum (Cordenunsi1*et al*., 2010; Murkovic and Pichler, 2006; Nakatani *et al*., 2000). In this research, model Maillard reaction systems were used to investigate whether or not chlorogenic acid increases HMF production.

Materials and Methods

Materials

L-lysine, L-glutamate, and L-cysteine were purchased from Aladdin Reagents Database, Inc. (Shanghai, China). Glycine and glucose were obtained from the Tianjin Damao Chemical Reagent Factory

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(Tianjin, China). HMF (98.5% purity) was purchased from the Sigma-Aldrich Company (St. Louis, MO, USA). Chlorogenic acid (98% purity) was obtained from Huahua Shengde Bio-Tech Co., Ltd. Methanol (HPLC grade) was obtained from Dikma (Dikma Technologies Inc., Lake Forest, CA, USA Distilled water was further purified using a Milli-Q water purification system (Millipore, Bedford, MA, USA).

Effect of chlorogenic acid on HMF formation in four Maillard reaction systems

A solution (4 mL) containing 1 mmol amino acid (L-lysine, L-glutamate, L-cysteine, and glycine respectively) and 1 mmol glucose or 1 mmol glucose alone, with or without different amounts of chlorogenic acid (0.002 mmol, 0.02 mmol, and 0.2 mmol), was placed in a sealed stainless test tube, capped, and reacted in an oil bath at 160°C for 36 min. At the end of the reaction, the tubes were cooled immediately in an ice bath. HMF was detected by HPLC.

A contrast experiment was designed to investigate whether or not the pH of the systems attributed to the addition of chlorogenic acid would affect HMF formation. The experimental design was as follows: 0.2 mmol chlorogenic acid, 1 mmol glucose, with or without addition of 1 mmol amino acids (L-lysine, L-glutamate, L-cysteine, and glycine), and 4 mL phosphate buffer (0.2 mol/L, pH 7.0).

Effect of chlorogenic acid, amino acids, and glucose on HMF elimination

An amino acid solution (1 mmol; L-lysine, L-glutamate, L-cysteine, and glycine) or glucose in 3 mL of deionised water was mixed with 1 mL of HMF standard solution at 2000 μg/mL. The mixtures (with or without the addition of 0.02 mmol chlorogenic acid) were placed in a sealed stainless test tube, capped, and reacted in an oil bath at 160°C for 36 min. At the end of reaction, the tubes were cooled down immediately in an ice bath. HMF was detected by HPLC.

HMF analysis

HMF determination was based on the method described by Chen *et al*. (2010) with slight modifications. Samples were filtered using a 0.45 μm membrane. HMF quantification was performed on a Shimadzu LC-20AT system (Shimadzu, Tokyo, Japan) equipped with a diode array detector and LC-solution software. A Zorbax SB-Aq (4.6 mm × 250 mm, 5 μm) column (Agilent Technologies Co., Ltd.) was selected for HMF analysis. The injection volume was 5 L. Elution was carried out at a flow rate of 0.5 mL/min under isocratic conditions at 40°C using 5% of methanol aqueous solution as the mobile phase. HMF was detected at 284 nm and quantified according to the standard curve.

Results and Discussions

Effect of chlorogenic acid on HMF formation

In this research, four kinds of amino acids, namely, neutral (glycine), acid (glutamate), alkaline (lysine) and neutral with SH group (cysteine) were used to establish Maillard reaction systems. Chlorogenic acid (0, 0.002, 0.02, and 0.2 mmol) was added at different amounts to test its effects on HMF formation. Table 1 showed that chlorogenic acid significantly promoted HMF formation in the model systems of glucose reacting with glycine, glutamate, and cysteine. The extent of enhancement increased with the amount of chlorogenic acid added. However, the addition of chlorogenic acid slightly decreased HMF formation in the glucose/lysine reaction system (Table 1). Heating glucose alone produced much less HMF than heating glucose together with amino acids (except for cysteine). Co-heating of glucose with chlorogenic acid produced less HMF than heating glucose alone, and the effect was dependent on the chlorogenic acid concentration (Table 1).

Adjusting the pH to 7.0 using phosphate buffer decreased HMF formation in all of the Maillard reaction systems, except for the glucose/glutamate system to which 0.2 mmol chlorogenic acid had been added (Table 1). As mentioned in the Introduction section, HMF is formed through three pathways. In acidic conditions, the Maillard reaction is inhibited, and HMF formation is decreased through this pathway. Lower pH values increase the formation of a highly reactive fructofuranosyl cation, which can be directly converted into HMF (Capuano and Fogliano, 2011).

However, chlorogenic acid did not promote HMF formation through pH adjustment, as co-heating glucose with chlorogenic acid in this research did not increase but decrease HMF formation (Table 1). Furthermore, addition of chlorogenic acid to the glutamate/glucose reaction system after pH adjustment produced more HMF than in natural pH. These findings exclude the possibility that chlorogenic acid increases HMF formation by decreasing the pH in the reaction system.

The results in this research indicated that chlorogenic acid and amino acids showed synergic effects with HMF formation, the mechanism of which should be further investigated.

Table 1. Effect of chlorogenic acid on the formation of 5-hydroxymethylfurfural in different reaction systems

	HMF (μg/mL)				
System	Blank	0.002 mmol Chl	0.02 mmol Chl	0.2 mmol Chl	0.2 mmol Chl (pH=7)
Glutamate/Glucose	363.9±41.7[b]	461.7±17.0	462.2±2.4	422.0±4.6	648.5±64.0
Lysine/Glucose	1554.7±2.7	1508.7±4.5	1494.0±2.4	1525.9±45.6	798.4±0.2
Glycine/Glucose	179.6±17.1	234.0±3.1	291.8±9.1	742.2±33.8	142.5±0.6
Cysteine/Glucose	2.3±0.0	2.4±0.1	5.7±0.2	9.8±0.1	ND[c]
Glucose	161.6±0.4	155.3±1.9	67.4±0.3	134.8±5.8	143.2±0.2

[a]*Chl, chlorogenic acid;* [b]*Means ± SD(n=3);* [c]*Not detectable*

Effect of chlorogenic acid, glucose, and amino acids on HMF elimination in different reaction systems

Heating HMF with chlorogenic acid only reduced the residual HMF from 199.6 μg/mL to 195.5 μg/mL (Table 2), indicating that chlorogenic acid itself does not eliminate HMF. The carbonyl group of HMF may react with amino acids after its formation. In this research, 0.02 mmol of chlorogenic acid was added to different reaction systems containing HMF to test its effect on HMF elimination. The results in Table 2 showed that HMF remained stable during heating. However, HMF was depleted after the addition of the four amino acids. The most effective amino acids were cysteine and lysine. Cysteine depleted all of the added HMF without the addition of chlorogenic acid, and lysine depleted 71.5% of the added HMF (Table 2), suggesting that the sulfhydryl group in cysteine and ε-NH_2 group in lysine act as active groups to react with HMF.

A small amount of HMF was depleted after addition of 0.02 mmol chlorogenic acid to the HMF mixtures with all of the added amino acids, except for glutamate (Table 2), which further confirmed that chlorogenic acid does not reduce HMF content through elimination of the HMF produced. The chemical properties of chlorogenic acid may contribute to its protective action against HMF elimination. Chlorogenic acid is also quite unstable during heating. In a previous study, for example, no chlorogenic acid was detected in potato products after baking or frying (Mader *et al.*, 2009). When heated, chlorogenic acid could be oxidised to quinones, which then react with amino acids (Kroll *et al*., 2003), thus decreasing the amount of amino acids that can react with HMF.

Heating glucose with the HMF standard solution at 160°C for 36 min produced 83.0 μg/mL of HMF compared with the added amount. However, after addition of chlorogenic acid, HMF formation decreased significantly (Table 2). This finding further proves that chlorogenic acid inhibits the conversion of glucose to HMF. However, the mechanism responsible for such an action should be further investigated.

Table 2. Effect of amino acids on elimination of 5-hydroxymethylfurfural with and without addition of chlorogenic acid

	Reacting without Chl[a]	Reacting with Chl
Composition	Residual HMF (μg/mL)	Residual HMF (μg/mL)
Blank (HMF)	199.6±2.3[b]	195.5±0.4
Glutamate	180.9±1.7	175.2±3.8
Lysine	56.9±2.3	76.2±3.2
Glycine	166.3±9.9	186.7±0.2
Cysteine	ND[c]	8.3±0.0
Glucose	283.0±1.3	236.3±8.3

[a]*Chl, chlorogenic acid;* [b]*Mean ± SD(n=3);* [c]*Not detectable*

Conclusion

Chlorogenic acid significantly increased HMF formation in glucose-amino acid reaction systems but inhibited its formation when co-heated with glucose. Co-heating chlorogenic acid with HMF did not significantly reduce the amount of HMF produced, and addition of chlorogenic acid to the HMF/amino acid co-heating system prevented HMF depletion. Thus, chlorogenic acid influenced HMF formation with different mechanisms in the Maillard reaction and sugar dehydration pathways.

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