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Cysteine alone or in combination with glycine simultaneously reduced the contents of acrylamide and hydroxymethylfurfural

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ABSTRACT

Inhibition of acrylamide formation in food has been extensively reported, but some mitigation methods result in a concomitant increase in hydroxymethylfurfural (HMF), a food contaminant mainly produced through Maillard reaction. Mitigation strategies to reduce HMF are not yet available. This study showed that cysteine alone or in combination with glycine could simultaneously and significantly reduce the content of acrylamide and HMF in asparagine/glucose model as well as in biscuits. Mixing 0.36 g/100 g of cysteine and 0.2 g/100 g (w/w) of glycine into the dough reduced 97.8% and 93.2% of acrylamide and HMF, respectively, in biscuits. Cysteine reduced HMF content possibly by reacting with formed HMF through Michael adduction and Maillard reaction.

1. Introduction

Acrylamide and hydroxymethylfurfural (HMF) are two food contaminants produced during high-temperature food processing.

Acrylamide is mainly formed from the reaction between asparagines and carbonyl compounds (reducing sugars). This reaction firstly formed a Schiff base and then decarboxylate to form an azomethine ylide. Afterward, acrylamide may be formed directly from azomethine ylide, through β-elimination of the decarboxylated Amadori compound, or through deamination of 3-aminopropionamide (Jin, Wu, & Zhang, 2013). Acrylamide induces tumours in several organs in mice and rats and has been designated by the International Agency for Research on Cancer as a "probable human carcinogen" (Capuano & Fogliano, 2011). It is rapidly absorbed from the gastrointestinal tract and can be metabolised to glycidamide, a compound more reactive with DNA and proteins than acrylamide (Pedreschi, Mariotti, & Granby, 2014). Acrylamide is a neurotoxin that inhibits kinesin-based fast axonal transport and decreases neurotransmitter levels, thereby inhibiting neurotransmission (Erkekoglu & Baydar, 2014). The neurotoxic effects of acrylamide can be observed at low dose with long exposures (Erkekoglu & Baydar, 2014), suggesting that dietary acrylamide is harmful to humans, especially children.

The presence of acrylamide in food remains a health risk. According to WHO, the mean margin of exposure (MOE) value based on the carcinogenic effect of acrylamide in mammary glands is 300–310 (Pedreschi et al., 2014), which is lower than 10,000, a criterion regarded as low health concern. Moreover, the detected concentrations of acrylamide and glycidamide haemoglobin adducts in Canadian teenagers indicate the need to reduce acrylamide exposure in the population (Brisson et al., 2014).

5-Hydroxymethylfurfural (HMF), a heterocyclic compound, is a thermal process contaminant in food. It is formed after 1,2-enolization, dehydration and cyclisation reactions from hexose sugars and Amadori product degradation during Maillard reaction, or from direct dehydration of sugars under acidic conditions (Capuano & Fogliano, 2011; Goncuoglu & Gokmen, 2013). HMF content ranges from 1.9 mg/kg to 20 mg/kg in baking products to several grams/kg in coffee, toasted chicory and dried fruits (Capuano & Fogliano, 2011; Goncuoglu & Gokmen, 2013; Petisca, Henriques, Perez-Palacios, Pinho, & Ferreira, 2014).

HMF content in food is related to the heat load applied during processing, which is a common index to evaluate thermal

Abbreviations: Cys, cysteine; Gly, glycine; HMF, hydroxymethylfurfural; LC-MS, liquid chromatography–mass spectroscopy; ORP, oxidation–reduction potential; PBS, phosphate buffer solution; SMF, 5-sulfooxymethylfurfural; SULTs, sulfotransferases.

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damage, ageing, and sensory properties in food products (Anese, Bot, & Suman, 2014; Goncuoglu & Gokmen, 2013; Petisca et al., 2014).

HMF causes death in honey bees (Zirbes et al., 2013), induces genotoxic and mutagenic effects in bacterial and human cells (Svendsen et al., 2012), and promotes colon cancer in rats (Svendsen et al., 2012), although conflicting views exist with regard to the effect of this substance on human health (Abraham et al., 2011; Severin, Dumont, Jondeau-Cabaton, Graillot, & Chagnon, 2010).

HMF initiates colonic aberrant crypt foci in rats and skin papillomas and hepatocellular adenomas in mice. HMF is inactive in *in vitro* genotoxicity tests using standard activating systems but is activated to a mutagen, 5-sulfooxymethylfurfural (SMF), by sulfotransferases (SULTs) (Monien, Engst, Barknowitz, Seidel, & Glattt, 2012; Monien, Frank, Seidel, & Glatt, 2009; Monien & Glatt, 2012). Monien et al. (2009) intravenously injected HMF (793 μmol/kg) into mice and detected a maximum SMF plasma level 2.5 min after HMF administration; 452–551 ppm of the initial HMF dose was converted to SMF and reached the circulation. In contrast to rodents, which mainly express sulfotransferases in liver, humans express sulfotransferases in many tissues, including the intestine, implying that humans could be more susceptible to HMF than conventional rodent models (Svendsen et al., 2012). Moreover, HMF can be converted to acrylamide and furan during Maillard reaction, (Cai et al., 2014; Gökmen, Kocadagli, Göncüoglu, & Mogol, 2012; Mesias-Garcia, Guerra-Hernandez, & Garcia-Villanova, 2010) which are classified as "possibly carcinogenic to humans".

Numerous studies focus on HMF content and toxicology as well as the influence of composition and process variables on HMF formation, but mitigation strategies specifically addressed to reduce HMF content in foods are not yet available (Capuano & Fogliano, 2011). Preventive strategies are based on changes in formulation, reduction of thermal impact, and removal of HMF formed in the product by vacuum treatments (Anese & Suman, 2013). However, HMF cannot be removed by vacuum treatments in biscuits (Anese et al., 2014).

A number of food additives, such as $CaCl_2$, cysteine, and glycine, have been proven to effectively inhibit acrylamide formation. Mixing these inhibitors with raw materials or immersing of fresh food in solutions containing inhibitors does not significantly affect the processing technology, making this method practical in the food industry (Friedman & Levin, 2008; Ou et al., 2008; Pedreschi et al., 2014). In our previous study, cysteine and glycine significantly influence HMF formation during Maillard reaction; reaction of glucose with cysteine and glycine produces less HMF than reaction with lysine and glutamate (Jiang et al., 2013).

Whether adding these additives alone or in combination can simultaneously inhibit the formation of acrylamide and HMF is unknown. Therefore, this study we used $CaCl_2$, cysteine and glycine alone or their mixtures to investigate their inhibition effect on acrylamide and HMF formation.

2. Materials and methods

2.1. Chemicals

Cysteine, glycine, asparagine, glucose, 2-mercaptoethanol, ethylamine, and calcium chloride were purchased from Aladdin Reagents Database Inc. (Shanghai, China). HMF and acrylamide standard (>99.8%) were obtained from Sigma–Aldrich Company (St. Louis, MO, USA). High-performance liquid chromatography (HPLC)-grade methanol and polyphenol oxidase (845 U/mg) were obtained from J. T. Baker (USA) and Worthington Biochemical Corporation (Lakewood, NJ, USA), respectively.

2.2. Preparation of cysteine, glycine and/or $CaCl_2$ containing asparagine/glucose model reaction systems

An equimolar asparagine/glucose Maillard reaction system was used to investigate the effects of additives on the formation of acrylamide and HMF. Each 20-ml stainless steel test tube contained 4 mL of 0.1 mol/L phosphate buffer solution (pH = 5.7 and 7.0) with 1 mmol asparagine and 1 mmol glucose, as well as different concentrations of cysteine, glycine and $CaCl_2$ alone (0.05, 0.1, 0.25, 0.375 and 0.5 mol/L), As described in our previous research (Cai et al., 2014), the test tubes were capped with Teflon pad-filled stainless steel cap and the mixtures were heated at 160 °C for 15 min in an oil bath installed with a magnetic stirrer (DF-101S, Yuhua Instrument Co. Ltd., Gongyi, Henan Province, China). After cooling in an ice bath, the reaction mixtures were decanted into 14-ml centrifuge tubes and deionized water was added to make a total volume of 10 mL in each tube. The mixtures were then centrifuged at 3000 g for 20 min on an Allegra 21 R centrifuge (Beckman Coulter Inc., Miami, USA). The concentrations of acrylamide and HMF in the supernatant were then determined.

2.3. Preparation of biscuits added with additive mixtures

Biscuits were prepared according to the recipe of Van Der Fels-Klerx et al. (2014) with slight modification. The dough contained 80.0 g wheat flour (passing through a 0.074 mm-sieve), refined palm oil (20.0 g), sucrose (35.0 g), $NaHCO_3$ (0.8 g), water (16.0 g), NH_4HCO_3 (0.4 g) and NaCl (1.0 g). Oil, sucrose, $NaHCO_3$, NH_4HCO_3, NaCl and the additives were initially mixed in water and then mixed with flour to prepare the dough. Based on the optimal concentration obtained from the results of the asparagine/glucose model reaction system, six kinds of additive mixtures (on the basis of dough; their concentrations in added water were similar to that in the model reaction system) were tested: A. 0.08 g/100 g $CaCl_2$ + 0.4 g/100 g cysteine; B. 0.06 g/100 g $CaCl_2$ + 0.65 g/100 g cysteine; C. 0.16 g/100 g $CaCl_2$ + 0.54 g/100 g cysteine; D. 0.18 g/100 g cysteine +0.3 g/100 g glycine; E. 0.36 g/100 g cysteine +0.2 g/100 g glycine; and F. 0.06 g/100 g $CaCl_2$ + 0.29 g/100 g cysteine +0.2 g/100 g glycine The dough (3 × 5 × 0.3 cm) were baked in a convention oven at 190 °C for 15 min, then cooled. 5.0 g of biscuits was ground and ultrasound extracted three times using 20 mL of 800 mL/L methanol according to Michalak, Gujska, and Kuncewicz (2013). Methanol and water in the extracts were removed by a rotary evaporator (RE-52AAA, Shanghai JiaPeng Technology co., LTD, Shanghai, China) under vacuum at 60 °C, the residues were dissolved in 2.0 mL deionized water for the determination of acrylamide and HMF.

2.4. Effect of amino acids, 2-mercaptoethanol and ethylamine on HMF elimination

The mixture (4 mL) in the test tubes, containing 0.1 mmol of HMF and 1.0 mmol of amino acids (Cys or Gly) or 2-mercaptoethanol (SH containing compounds) or ethylamine (NH_2 containing compounds), was allowed to react in an oil bath at 160 °C for 15 min. The residual HMF was detected by HPLC.

2.5. Acrylamide and HMF analysis

Acrylamide and HMF were determined by LC–MS and HPLC, respectively, as we previously described (Cai et al., 2014).