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Impact of selected additives on acrylamide formation in asparagine/sugar Maillard model systems

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ABSTRACT

The main purpose of this paper was to study the effect of selected additives, NaCl, $NaHSO_3$, ascorbic acid, cysteine and allicin, on acrylamide (AA) formation in asparagine/fructose (Asn/Fru) and asparagine/glucose (Asn/Glc) by microwave processing. Our results showed that $NaHSO_3$ and cysteine had the best reduction rate on AA formation, but the browning would be influenced by the addition of these additives. Ascorbic acid is another good inhibitor for AA formation both in Asn/Fru and Asn/Glc model systems, but the browning would be inhibited by the high concentration of ascorbic acid. Natural antioxidants are attractive candidates for the development of effective inhibitors for AA formation. In the present study, allicin effectively reduced AA formation and achieved a maximum reduction rate of >50% for the use of allicin at a concentration of 0.0375% in Asn/Fru model system. While in Asn/Glc model system, allicin did not show good reduction rate. Through RSM study, the lowest AA content was 0.181 μg/mL by the addition of allicin in Asn/Fru and 0.029 μg/mL by the addition of ascorbic acid in Asn/Glc model system, respectively. The study could be regarded as a pioneer contribution on AA reduction in Maillard model system by the addition of allicin.

1. Introduction

Acrylamide (AA, $H_2C{=}CH{-}CO{-}NH_2$, CAS No. 79-06-1) is known to be a neurotoxic, genotoxic and carcinogenic compound, which is classified by IARC as a probable human carcinogen (IARC, 1994). Recently, approaches to reduce or mitigate the AA content in food had been vigorously sought. Proposals for lowering AA levels included reducing heating time and temperature, lowering the pH value, as well as using raw materials with low sugar or asparagine content. Many researchers also found that pre-treatments are effective ways to reduce AA content in food. Jung, Choi, and Ju (2003) showed that lowering the pH with citric acid solution before frying could diminish AA formation in French Fries. And other treatments like immersing the samples into NaCl and $CaCl_2$ solutions could effectively reduce AA content in food (Kolek, Šimko, & Šimon, 2006a; Casado, Sánchez, & Montao, 2010).

Natural antioxidants are another important way to reduce AA content in Maillard model system and in food matrix. Zhang and Zhang (2008) found that antioxidant of bamboo leaves (AOB) and extract of green tea (EGT) could effectively reduce AA formation and achieve a maximum reduction rate of 74.4% for the use of AOB and 74.3% for the use of EGT when the addition levels of AOB and EGT were both 10^{-4} mg. Rosemary extraction also played a reduction effect on AA formation up to 25% in commercial potato chips processed with olive oil and rosemary herb (Casado et al., 2010). Similarly, nearly 50% reduction of AA after the addition of a flavonoid spice mix has also been reported by Fernández, Kurppa, and Hyvönen (2003).

Garlic (*Allium sativum*) is an edible plant that has generated significant interest throughout human history as a medicinal panacea. The active component of garlic is thought to be a sulphur-containing compound known as allicin. Allicin (diallylthiosulfinate) is in fresh crushed garlic, representing about 70% of the overall thiosulfinates presented or formed upon crushing the cloves (Han, Lawson, Han, & Han, 1995). Allicin also provides antioxidant protection to the body. The mechanism of the antioxidant active of allicin may be due to scavenge oxygen free radicals (Prasad, Laxdal, Yu, & Raney, 1995; Chung, 2006). Some researches showed that free radicals played a role in the formation of AA by Electron Spin Resonance (ESR) method (Hedegaard, Granby, Frandsen, Thygesen, & Skibsted, 2008). Since allicin has the ability of scavenging oxygen free radicals, would it be possible for allicin to reduce AA formation in Maillard model systems and in food matrix? The purpose of this investigation was to determine the influence of different additives on AA formation in asparagine/fructose (Asn/Fru) and asparagine/glucose (Asn/Glc) model system and mainly discussed the reduction effect of allicin on AA formation, and the optimal conditions of additives applied in Maillard model systems.

2. Materials and methods

2.1. Materials

AA (2-propene amide) (purity>99.8%) and allicin were purchased from Sigma-Aldrich (St. Louis, MO, USA). Asn, Fru, Glc, sodium

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chloride (NaCl), sodium hydrogen sulfite ($NaHSO_3$), ascorbic acid (Vc), and L-cysteine (Cys) were of analytical grade and obtained from Beijing Chemicals Co. (Beijing, China). HPLC methanol was purchased from Merck Ltd. (Beijing, China). HPLC-grade and 0.20 μm filtered water was prepared.

2.2. Establishment of aqueous model systems

The preparation of the model systems was based on the method described by Becalski, Lau, Lewis, and Seaman (2003) and Yuan et al. (2007). A stock 0.1 M Na_2HPO_4 buffer pH 7.5 was prepared. Reaction mixtures were prepared by dissolving solids in phosphate buffer to give a final concentration of 0.25 M Asn and 0.25 M Fru (Asn/Fru model system) and 0.25 M Asn and 0.25 M Glc (Asn/Glc model system).

2.3. Study on the effect of some additives on AA formation in Maillard reaction systems

Sodium chloride (NaCl), sodium hydrogen sulfite ($NaHSO_3$), ascorbic acid, L-cysteine and allicin were used as selected additives in our study. Different concentrations of the additives with 0.2%, 0.5%, 0.8%, 1.2% and 1.5% of NaCl, $NaHSO_3$, ascorbic acid and cysteine were added to Asn/Fru and Asn/Glc model systems according to the references of Kolek et al. (2006a); Kolek, Šimko, and Šimon (2006b), Mestdagh et al. (2008) and Ministry of Health of the People's Republic of China (2007). Different concentrations of allicin with 0.0375%, 0.075%, 0.1875%, 0.375% and 0.5625% was added to Asn/Fru and Asn/Glc model system, respectively according to the reference of Zhang and Zhang (2008) with the use of AOB.

2.4. Study on the reduction of AA formation in Asn/Fru and Asn/Glc model system by selected additives via response surface methodology (RSM)

The formation of AA influenced by selected additives was performed by RSM method according to Bråthen, Kita, Knutsen, and Wicklund (2005) with some improvements. The independent variables were microwave power (x_1), initial pH value (x_2), heating time (x_3) and selected additives content (x_4), and their interactions between each other were also taken into consideration. Corresponding coefficients of both variables and interaction variables were established by Design-Expert software 7.0 while the data and their response surface graphs were also drawn by Design-Expert software 7.0. Response surface equations could be calculated of the type:

$$y = \beta_0 + \sum_{i=1}^{4} \beta_i x_i + \sum_{i=1}^{4} \beta_{ii} x_i^2 + \sum_{i<j} \beta_{ij} x_i x_j$$

where y is AA content, β_0 is a constant, β_i, β_{ii}, and β_{ij} is the linear, quadratic and cross product coefficients, respectively.

2.5. Model systems treated with microwaving

All microwave treatments were carried out in a MAS-II microwave oven of a frequency of 2455 MHz with maximum power of 800 W (dimensions of the microwave oven: length of 35 cm, width of 35 cm and height of 25 cm, Shanghai Xinyi microwave instrument Co., Shanghai, China), which was equipped with a condenser. Twenty milliliters of Asn/Fru or Asn/Glc model system mixtures with different contents of additives were added into glass flasks (50 mL). The flasks were placed in the middle of the bottom and connected with the condenser. With a short time period for microwaving, less than about 1 min, the mixtures began to boil. As for the single factors of added additives on AA formation, the model systems were treated for 20 min at 700 W. As for the RSM test, the model systems were treated for 5 to 30 min at 500 to 900 W with initial pH values from 5.0 to 9.0 (Table 1). All treatments were performed for three times. After the treatment, the samples were immediately withdrawn and cooled in ice water to stop any further reaction for AA analysis.

2.6. AA analysis

The HPLC analysis for the determination of AA content was described by Rosen, Gökmen, and Knol (Rosen & Hellenas, 2002; Gökmen, Şenyuva, Acar, & Sarıoğlu, 2005; Knol et al., 2005). This method has been confirmed by HPLC-MS/MS in this experiment. The analyses were performed using a HPLC system (Shimadzu Co., Japan), which equipped with a C_{18} guard column (4.0 × 2.0 mm i.d., Shanghai ANPEL Scientific Instrument Co., Ltd., Shanghai, China) connected to the analytical column of a reversed Venusil MP-C18 (4.6 × 250 mm, 5 μm, Agela Co., Beijing). Twenty microliters of solution was injected onto the column, and eluted isocratically at 30 °C with water containing 2% methanol at a flow rate of 0.7 mL/min. AA was measured by its absorbance at 205 nm with a UV detector and quantified by the external standard procedure with a calibration curve. The established calibration curve was made from a series of standard solutions of AA dissolved in distilled water at the concentration levels of 0.05, 0.1, 0.2, 0.8, 1.0, 2.0, and 5.0 μg/mL. The curve showed excellent linearity correlation ($R^2 > 0.9998$).

2.7. Statistical analysis

Statistical analysis was performed by using Student's *t*-test with SPSS 15.0 software. Analysis of variance (ANOVA) was tested on a significance level of $p = 0.05$.

3. Results and discussion

3.1. Effect of selected additives on AA formation in Asn/Fru and Asn/Glc model system

The effects of these additives on AA formation in Asn/Fru and Asn/Glc model systems were shown in Figs. 1–5. When equimolar amounts of Asn and Fru were pyrolyzed without the addition of NaCl, a maximum of 1.587 μg/mL of AA was formed at 700 W for 20 min in Asn/Fru model system. After the addition of NaCl solution with different concentrations of 0.2%, 0.5%, 0.8%, 1.2% and 1.5%, the reduction rates were only 3.02%, 4.73%, 9.45%, 12.41%, and 14.78%, respectively. In Asn/Glc model system, 0.857 μg/mL of AA was formed without the addition of NaCl, and the reduction rates were 1.98%, 3.97%, 9.51%, 11.09%, and 24.68%, respectively, with the addition of NaCl solution at the mentioned concentration. In the study of Gökmen and Şenyuva (2007), however, increasing the amount of Na^+ ion decreased AA formation by 59%. Further increase in the amounts of Na^+ ion did not bring any improvement for the reduction, but increased the amount of AA formed. Gökmen and Şenyuva (2007) showed that the presence of cation in the reaction mixture not only influenced the type and concentration of characteristic reaction products during heating, but also influenced the rate of decomposition of reaction precursors significantly. In our study, at lower concentrations of 0.2%, 0.5%, 0.8% and 1.2%, NaCl showed a similar reduction rate to AA formation in both systems, while at a concentration of 1.5%, NaCl showed an effective reduction rate in Asn/Glc model system than that Asn/Fru model system. Maybe due to the effect of cation on different rate of decomposition of Fru and Glc.

$NaHSO_3$ is a food additive used to prevent oxidation and kill bacteria. The effect of $NaHSO_3$ on AA formation was shown in Fig. 2. The reduction rate reached a 98.11% after added 0.2% of $NaHSO_3$ solution in Asn/Fru model system. After addition of the 0.8% $NaHSO_3$ solution in Asn/Fru model system, the AA formation was completely