

Reaction mechanisms between acrylamide and mercaptan in high temperature system with different humidity

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The reactions between acrylamide and food aroma mercaptans including 1,2-ethanedithiol, butyl mercaptan, 2-methyl-1-butyl mercaptan and 2-methyl-3-furanthiol under both high temperature and high humidity (HTHH) and high temperature and low humidity (HTLH) were determined in the present study. The results showed that the acrylamide elimination capability of 2-methyl-3-furanthiol was stronger, compared with butyl mercaptan and 2-methyl-1-butyl mercaptan, in the HTHH system, while that was the lowest in the HTLH system. Besides, pH could significantly affect the acrylamide content in HTHH system, but the effect of pH was not so obvious in HTLH system. With the addition of antioxidants, the elimination of acrylamide was decreased significantly. In conclusion, the reaction experienced different mechanisms under the high temperature. The nucleophilic addition and radical addition take the dominant role in HTHH system and HTLH system, respectively.

Key words: food, acrylamide, mercaptans, antioxidants.

INTRODUCTION

The acrylamide is a kind of hazardous substance appearing during the food heat processing in starch-based food [1]. Considering the toxicity damage of acrylamide in liver, neuro and reproduction confirmed by the previous researches, the acrylamide has been denominated as a “potential cancerogen” by the IARC in 1994 [2]. Besides, the acrylamide might react with some food substances due to its active C=C [3, 4].

Mercaptan is a kind of flavor compound widely spread in the plant kingdom, such as the garlic, onion, coffee and food undergoing the heat processing. The mercaptan could attach admirable smell, such as sesame oil, green onion, garlic and barbecue smells, to food with a trace amount. Moreover, over 100 mercaptans are already enrolled as a safe food additives. Recently, the FEMA (Flavour Extract Manufacturers’s Association) has newly published a list of GRAS (Generally Recognized as Safe) additives, which contains lots of mercaptans [5].

Compared with amino and hydroxyl groups, sulphur owns superior alkene nucleophilic addition ability. The “click” reaction between sulphur and alkene has drawn numerous attention [6]. Thus, the

reaction between acrylamide and mercaptan might occur theoretically and some previous study had been done aiming to figure out the reaction kinetics [7, 8]. As far as we know, due to the supreme polarizability of thiol sulfur atoms, both nucleophilic addition and free radical type reaction might be triggered when the sulphur is enrolled with alkene. The complexity of food heat processing procedure results in the complexity of reaction of acrylamide and mercaptan. Therefore, the present study is carried out to illuminate the transformation of acrylamide and mercaptan under different heat process including high temperature and high humidity (HTHH) and high temperature and low humidity (HTLH).

EXPERIMENTAL

Materials and methods

Materials

Acrylamide (>99.9%), butyl mercaptan (≥98%) and DMSO (≥99.9%) were all purchased from Sigma-Aldrich company; [1,2,3-¹³C] Acrylamide isotope internal standard was obtained from the Cambridge Isotope Laboratories Inc; 2-methyl-3-furanthiol (≥98%) and dithioglycol were acquired from Shanghai Aladdin Inc. Ultrapure water was purchased from Watsons. All other reagent were of analytic purity.

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Methods

The establishment of high temperature high humidity model

The HTHH model was established according to Cai[9] with slight modification. The acrylamide (0.1 μmol), different concentration of mercaptan(0~10 μmol) and buffer(0.2 mol/L, 2 mL, sodium citrate pH 3-6, phosphate pH 7-8) were added into 20 mL custom-made stainless steel reaction tube which was lined with teflon. Then, screw the stainless steel cap tightly to ensure the air impermeability and totally mixed on vortex mixture for 5 min. Next, stainless tubes were exposed to the oil bath pan (DF-101S, HengYan, Zhengzhou, China) with temperature ranging from 80~180 °C for 0~30 min. After that, the tubes were cooled immediately in 0 °C water for 20 min. Then 10 μL [1,2,3- ^{13}C] acrylamide isotope internal standard(1mg/mL) was introduced into the tube and the ultrapure water was added to the reaction system to a content volume 10 mL.

The establishment of high temperature low humidity model

The HTLH model was built following the experiment of Francisco[10] and Cai[9] with minor modification. The acrylamide (0.1 μmol), different concentrations of mercaptan(0~10 μmol) and 300 mg of silica gel were added into 20 mL custom-made stainless steel reaction tube which was lined with teflon. Next, 50 μL buffer(0.2 mol/L, 2 mL, sodium citrate pH 3-6, phosphate pH 7-8) to assure Aw 0.95 and screw the stainless steel cap tightly to ensure the air impermeability and totally mixed on vortex for 5 min. Then, stainless tubes were exposed to the oil bath pan with temperature ranging from 80~180 °C for 0~30 min. After that, the tubes were cooled immediately in 0 °C water for 20 min. Then 10 μL [1,2,3- ^{13}C] acrylamide isotope internal standard(1 mg/mL) was introduced into the tube and the 10 mL ultrapure water was added to the reaction system. Then the system was vortexed for 5 min. After standing, remove the supernatant as sample.

GC-MS determination of acrylamide

The pretreatment and analysis of samples were carried out by Francisco[7] and Zhang[11], respectively, with slight modification. 300 μL 10%(v/v) H_2SO_4 , 400 mL 0.1 mol/L KBrO_3 and 0.5 g KBr was introduced to 2 mL sample in sequence. Then, the system was mixed on the vortex for 3 min and stood for 1 h in 4 °C. After that, 0.1 mL 0.1 mol/L sodium thiosulfate was added to terminate the derivatization reaction. Then, the mixture was introduced to 2 mL ethyl

acetate/hexane (4:1, v:v) for three times and the extract liquor was exposed to anhydrous sodium sulfate for 30 min. Then the pre-dried mixture was totally dried by blowing nitrogen. The dried sample was dissolved in 50 μL ethyl acetate solution, then 10 mL triethylamine was added into the solution, which was then transferred to 100 μL gas sampling bottle.

GC-MS conditions are as follows: Agilent HP 6890 gas chromatography-HP 5975 UPLC-MS-MS (J and W Scientific, Agilent, Santa Clara, CA, USA); HP5-MS polysiloxane capillary column (polysiloxane polymers, 30 m \times \varnothing 0.25 mm, 0.25 μm , J and W Scientific, Agilent, Santa Clara, CA, USA), injection volume: 1 μL splitless. The nitrogen gas flow was 1 mL/min. The temperature of injection port and inspection port were 250 °C and 280 °C, respectively. Mass spectrometer conditions: EI ionization; Ion source temperature was 230 °C and electron energy was 70 eV under the SIM mode. Ion determined: 2-propanamide($[\text{C}_3\text{H}_4\text{NO}]^+$ =70, $[\text{C}_3\text{H}_{479}\text{BrNO}]^+$ =149, $[\text{C}_3\text{H}_{481}\text{BrNO}]^+$ =151, m/z 149 was considered as quantitative ion) and 2-bromine (^{13}C)propionamide($[\text{C}_2\text{H}_38\text{Br}]^+$ = 110, $[\text{C}_3\text{H}_{481}\text{BrNO}]^+$ = 154, m/z 154 was considered as quantitative ion).

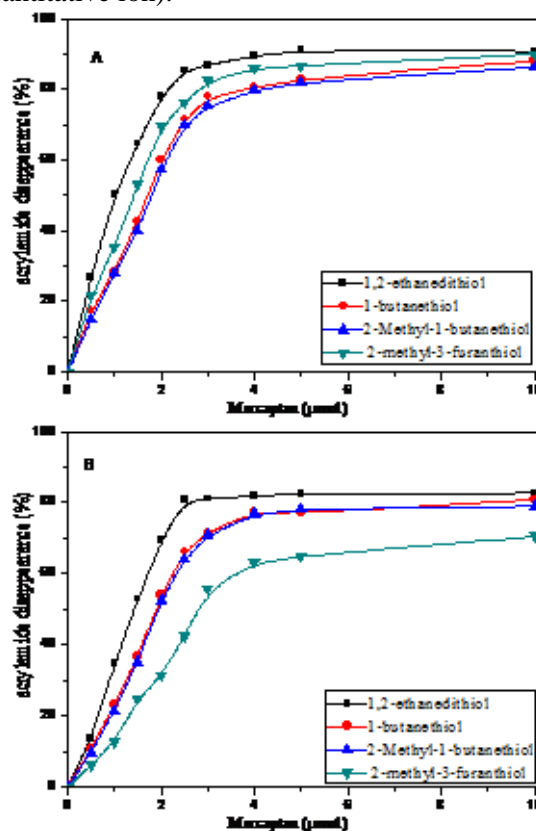


Fig. 1. The different concentrations of mercaptans in acrylamide elimination in (A)HTHH system and (B)HTLH system(acrylamide 0.1 μmol , 160 °C, pH7, reaction time 30 min)

RESULTS

Mercaptan contents in reaction

The elimination of acrylamide exhibited a positive correlation with the increasing concentration of mercaptan in both HTHH and HTLH system. The reaction achieved a balance with 5 μmol mercaptan enrolled in the system. As shown in Fig.1, the 1,2-dithioglycol presented the best eliminating acrylamide capability(91%) in HTHH system. The eliminating ability of mercaptan was superior in HTHH system to in HTLH system, which maybe result from the fully reaction between the substrates. Whereas, the reaction was hindered due to the solid substrate adsorption of reactants. Besides, the 2-methyl-3-furanthiol presented the best eliminating ability in HTHH system, while the lowest in HTLH system.

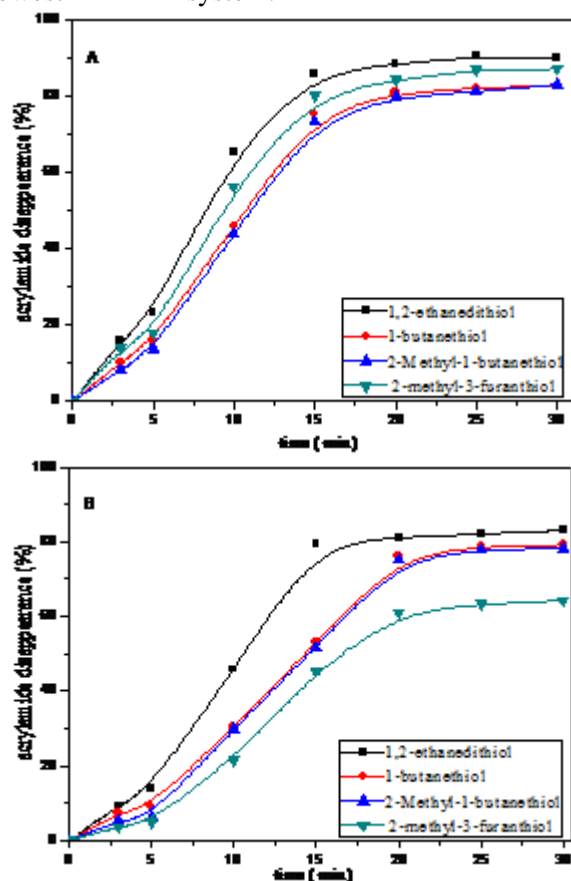


Fig. 2. The different reaction times in the eliminating of acrylamide in (A)HTHH system and (B)HTLH system (acrylamide 0.1 μmol , mercaptan 4 μmol , 160 ° C, pH7)

Reaction time in the acrylamide elimination

As shown in Fig. 2, the elimination of acrylamide was increased with the lengthening of reaction time. The elimination of acrylamide reached the mount (90%) after 25 min reaction. The

acrylamide content decreased with a linear correlation during the first 10 min. The slope of the simulated line could imply the reaction activities between acrylamide and mercaptans. The eliminating ability of 3-methyl-2-furan mercaptan was superior than butyl mercaptan in HTHH system and the result reversed when the reaction was carried out in HTLH system.

Temperature on the eliminating of acrylamide

The eliminating of acrylamide increased with the increasing of heating temperature from 80~160 °C. The elimination achieved a balance in 160 °C. The temperature under 100 °C shows no significant effect in the HTLH system due to the different matrix which hindered the fully contact between the substrates. The temperature over 100 °C shows good inhibition ability on acrylamide.

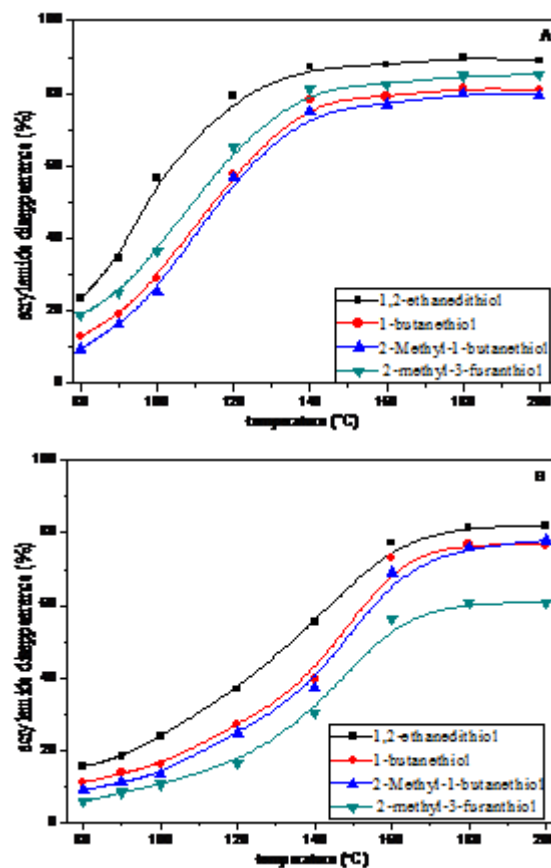


Fig. 3. Different temperatures in eliminating of acrylamide in (A)HTHH system and (B)HTLH system (acrylamide 0.1 μmol , mercaptan 4 μmol , pH7, reaction time 30 min)

pH in eliminating of acrylamide

The effect of pH in elimination reaction of acrylamide varies in different system. Considering the HTHH system, low pH under value pH 6 resulted in the low elimination ability, while under higher pH the eliminations were promoted

dramatically. There was a trace amount of acrylamide left with pH 8. However, compared with HTHH system, the elimination was less sensitive to the pH in HTLH system in which the pH shows less effective in removing the acrylamide(Fig. 4).

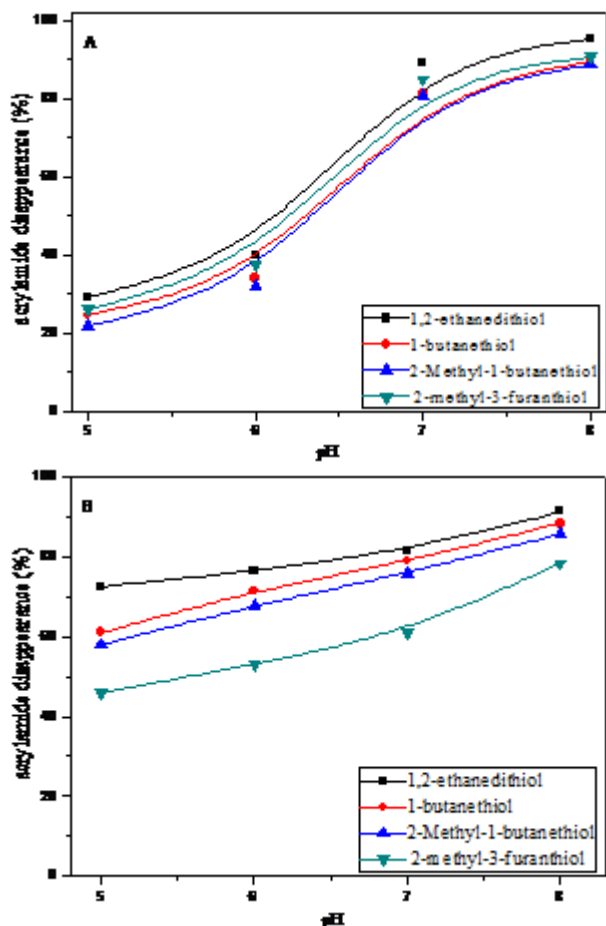


Fig. 4. Different pH in eliminating of acrylamide in (A)HTHH system and (B)HTLH system (acrylamide 0.1 μ mol, mercaptan 4 μ mol, 160 °C, reaction time 30 min)

Oxygen and antioxidants in elimination of acrylamide

Exposed to different antioxidants including Vc and gallic acid, the elimination capabilities of different mercaptans are presented in Fig. 5. The air and nitrogen atmosphere showed no significant effect in HTHH system and when the antioxidants were applied the acrylamide slightly reduced. Whereas, a nitrogen atmosphere showed its negative effect in HTLH system acrylamide elimination. Furthermore, addition of Vc and gallic acid both turned out better elimination and less acrylamide remaining.

The mercaptans which own outstanding reducibility could be oversensitive to the oxygen exposed to alkali. In the present study, the LC-MS was applied to determine the transformation of

mercaptans. The result confirmed the formation of disulphide, which could not be considered as an effective factor related to the elimination reaction because no significant influence between air and nitrogen atmosphere was observed in HTHH system.

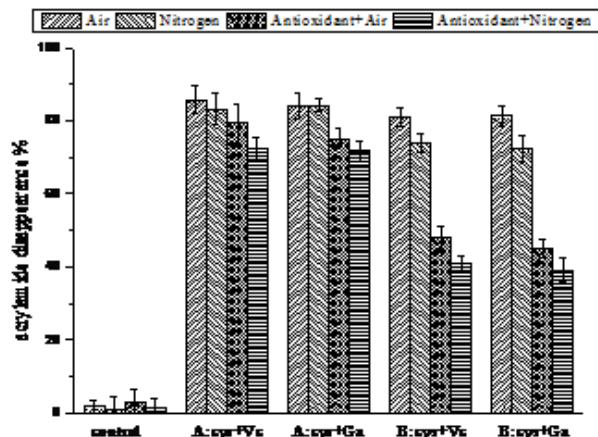


Fig. 5. Oxygen and antioxidants in the eliminating of acrylamide in (A)HTHH system and (B)HTLH system (acrylamide 0.1 μ mol, mercaptan 4 μ mol, antioxidants 2 μ mol, 160 °C, reaction time 30 min)

DISCUSSION

Up to now, there is no significant evidence about acrylamide could lead to cancer[12], while its wide spread in foods, especially fried food which contains high acrylamide, has aroused the public concern and numerous researches focused on its carcinogenic potency. The acrylamide could react with some nucleophiles. The mercaptan is a kind of nucleophilic aromas which usually coexist with acrylamide in food[7]. In the present study, the mercaptans, temperature, time, pH, oxygen and antioxidants in the elimination of acrylamide were measured. 3-methyl-2-furan mercaptan shows superior eliminating ability than butyl mercaptan 2-methyl-1-butyl mercaptan in HTHH system, whereas in the HTLH 3-methyl-2-furan mercaptan turned out the lowest, which implied the different mechanism took place between acrylamide and mercaptans. In the HTHH system, the nucleophilic addition might take place and the sulfur might loss the hydrogen and turn into sulfion. Compared with sulfion of the butyl mercaptan, that of the 3-methyl-2-furan mercaptan is more steady, which could lead to higher eliminating activity. However, it is hard for mercaptans to loss the hydrogen in HTLH system. Thus, the radical addition is the dominant reaction in HTLH, which could explain the better activity of butyl mercaptan due to its less steady radical.

The pH assay shows similar results. The increase in pH could favor the formation of sulfur anion which could improve its nucleophilic ability, thus the elimination was promoted in HTHH system. Whereas in HTLH system, the elimination has not been improved, which could also hint the radical reaction occurred.

In conclusion, the reaction between acrylamide and mercaptans follows different mechanisms. The nucleophilic addition and radical addition take the dominant role in HTHH system and HTLH system, respectively.

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РЕАКЦИОННИ МЕХАНИЗМИ МЕЖДУ АКРИЛАМИД И МЕРКАПТАН ПРИ ВИСОКОТЕМПЕРАТУРНА СИСТЕМА С РАЗЛИЧНА ВЛАЖНОСТ

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(Резюме)

В настоящото изследване са изследвани реакциите между акриламид и хранителни ароматни меркаптани, включващи 1,2-енандитиол, бутил меркаптан, 2-метил-1-бутил меркаптан и 2-метил-3-фурантиол при висока температура и висока влажност (HTHH) и при висока температура и ниска влажност (HTLH). Резултатите показват, че способността за елиминиране на акриламид на 2-метил-3-фурантиол е по-силна в сравнение с тази на бутил меркаптана и 2-метил-1-бутил меркаптана в HTHH системата, докато в системата HTLH е най-ниска. Освен това, рН може значително да повлияе на съдържанието на акриламид в HTHH системата, но ефекта на рН не е толкова очевиден в системата HTLH. С добавянето на антиоксиданти елиминирането на акриламида е значително намалено. В заключение, реакцията се характеризира с различни механизми при висока температура. Нуклеофилното присъединяване и радикалното присъединяване заемат доминиращата роля съответно в HTHH и HTLH системите.