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Formation of Acrylamide in a Processed Food Model System, and Examination of Inhibitory Conditions

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Acrylamide (AAm) is formed from asparagine (Asn) and reducing sugar during cooking of foods at high temperature. We examined the formation of AAm in a model system using a glass fiber filter paper, and looked for suitable conditions for inhibiting AAm formation. In frying, the formation rate was about 10 times that in a moistureless oven. Increase of frying temperature and frying time increased AAm formation when the residual moisture was 5% or less. AAm increased with increasing amount of glucose (Glc) addition up to 1 : 1 with respect to Asn, but then decreased. On the other hand, in the case of fructose, as the amount added was increased, AAm increased accordingly. The AAm formation rate with respect to Asn increased when valine (Val) was co-present in a Glc and Asn reaction system. Cysteine and lysine inhibited the AAm formation rate. Pathways for the formation of AAm are proposed.

Key words: acrylamide; asparagine; processed food; frying; oven; glucose; fructose

Introduction

In April 2002, it was announced by Swedish researchers that acrylamide (AAm), a neurotoxic and probably carcinogenic chemical in humans, is generated by cooking food which contains a large amount of carbohydrates[*3]. AAm is a chemical used for the synthesis of polyacrylamide or other chemical products, and it had not been found in normal foods. Subsequently, a method of analyzing AAm in foods was reported[1),2)], and the AAm content in various processed foods was investigated[3)-5),*4),*5)]. The effect of AAm in foods on human health is unknown and no maximum permitted level for AAm in foods has been set. It was found that AAm was formed by heating asparagine (Asn) present in food together with a reducing sugar[6),7)], but many aspects of this mechanism, including how to inhibit it, are not fully understood. In this study, we examined the formation of AAm in a model system using glass fiber filter paper, and looked for suitable conditions for inhibiting AAm formation.

Materials and Methods

1. *Reagents*

5-(Hydroxy methyl) furfural (HMF) from Kanto Chemicals and 3-deoxyglucosone (3-DG) from Dojin Chemical Laboratory were used for protein saccharification studies. All other reagents were obtained from Wako Pure Chemical Industries. Each solution was prepared by dissolving the agent in 0.02 mol/L phosphate buffer to control the pH.

2. *Preparation of filter paper model*

A processed food model was constructed by placing glass fiber filter papers (GA-200, ϕ25 mm, 0.75 mm, Toyo Filter Paper Co.) on sharp supports (toothpicks stuck into polystyrene foam), each one being supported at several points, so that 400 μL of solution per sheet could be absorbed at the center of the filter paper.

3. *Frying procedure*

Palm oil (600 g) was placed in an iron pan and heated by induction heating. When the target temperature was reached, heating was stopped, and ten sheets of glass fiber filter paper on which solutions had been absorbed, were introduced into the pan and fried. After frying, each filter paper was immediately moved to a stainless steel mesh colander, frozen in a freezer (−27℃), and stored.

4. *Heating in moistureless oven*

Glass fiber filter papers with absorbed solution were frozen in a freezer (−27℃) for 3 hours, and dried with a lyophilizer overnight. The dried sample were heated in a constant temperature vessel for 5 minutes, immediately frozen in the freezer (−27℃), and stored.

[*3] http://www.slv.se./engdefault.asp

[*4] http://www.cfsan.fda.gov/~dms/acrydata.html

[*5] http://www.cfsan.fda.gov/~dms/acrydat2.html

5. AAm measurement

Five filter paper samples were placed in a 500 mL Erlenmeyer flask, and 100 μL of 100 μg/mL acrylamide-1-^{13}C solution was added as an internal standard. Ultrapure water (200 mL) was added, the sample was homogenized in a homogenizer (ULTRA TURRAX®, T25 basic, Ika Labortechnik) for 2 minutes, and measurements were performed according to Nemoto *et al.*[1]

6. Measurement of temperature in frying materials

A long, fine-sheathed thermocouple (T34, ϕ0.25 straight sheath, Okazaki Laboratories) was inserted near the center from the side of a glass fiber filter paper, and the temperature was measured with a mobile-type temperature recorder (NR-1000, Keyence Co., Japan).

7. Statistical analysis

Statistical analysis was performed by using Student's *t*-test with the SPSS software system for Windows Ver. 10.0J (SPSS Japan, Tokyo, Japan). In all cases, probability (p) values below 0.05 were considered significant.

Results and Discussion

1. Effect of processing temperature, time and pH

Figure 1 shows photographs and AAm formation rates in glass fiber filter papers prepared by adding 400 μL of an equimolar mixed solution (pH 6.6) of glucose (Glc) and Asn per sheet of glass fiber filter paper (Glc 7.21 mg, Asn 5.28 mg) and frying or heating in a moistureless oven at various temperatures. In solution frying, the formation rate of AAm was about 10 times that in the moistureless oven, which suggests that moisture participates in AAm formation. Likewise, in solution frying, the degree of browning is large, whereas in the moistureless oven, the browning reaction did not progress as much. In the moistureless oven, browning was slight, although AAm formation was observed from 120℃ as in earlier reports[8),9)]. In solution frying, at 100–140℃, the temperature was too low for frying under ordinary pressure, and an analysis was not performed. In solution frying and heating in the moistureless oven, the formation rate fluctuated with maxima at 180℃ and 200℃, respectively. In the higher temperature regions beyond the maxima, a decreasing tendency was seen, probably because the decomposition rate of AAm becomes higher than the formation rate.

Figure 2 shows the effect of pH on the AAm formation rate. In the moistureless oven, a difference of pH did not influence the formation rate of AAm, in contrast with solution frying, in which the formation rate increased at higher pH. The degree of browning was deeper in solution frying than in the moistureless oven, and it increased at higher pH in solution frying. On the other hand, in the moistureless oven, browning was unaffected by pH. It was considered that this was because the ring structure of Glc is opened at higher pH, increasing the reactivity[10)]. On the other hand, in the moistureless oven, Glc was present as a powder, and the ring structure would be favored in the solid state.

2. AAm formation rate in the frying process

Figure 3(A) shows the frying oil temperature, frying material temperature and residual moisture during the frying process. When there is sufficient moisture (5% or more), even if the frying oil temperature is 170℃ or more, the temperature in the frying material stays in

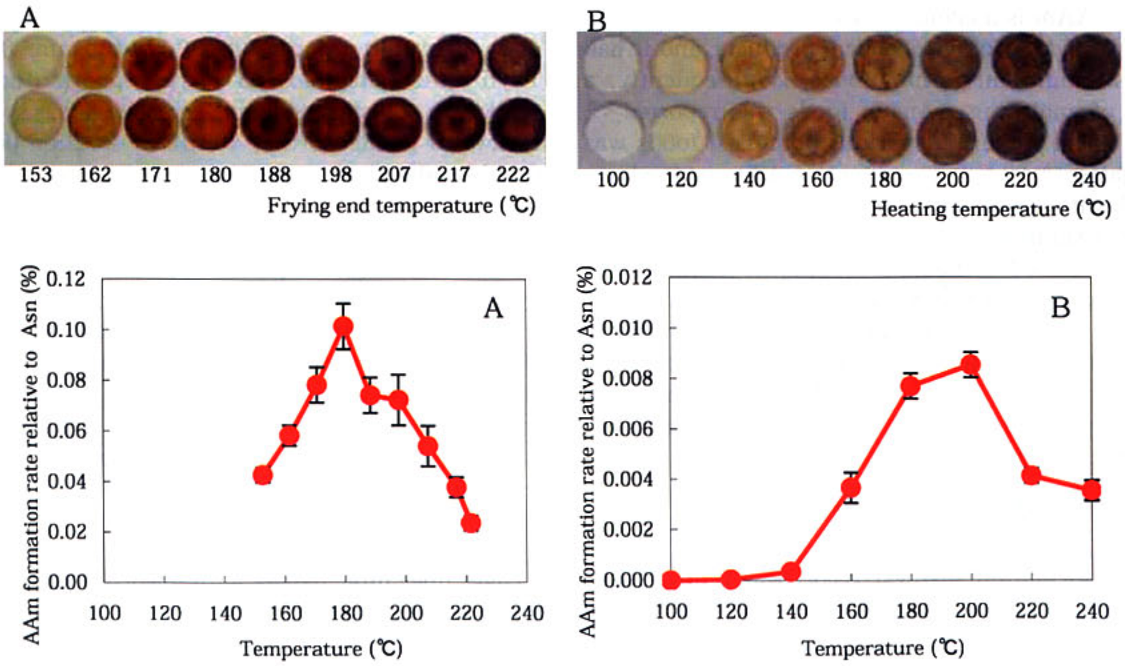

Fig. 1. AAm formation rate variations due to heating
A: Solution frying (frying time, 1 min), B: Moistureless oven (heating time, 5 min)
A 400 μL aliquot of solution (pH 6.6, Glc 40 μmol, Asn 40 μmol) was added per one sheet of glass fiber filter paper. Each value represents mean ± S.D. of triplicate determinations.

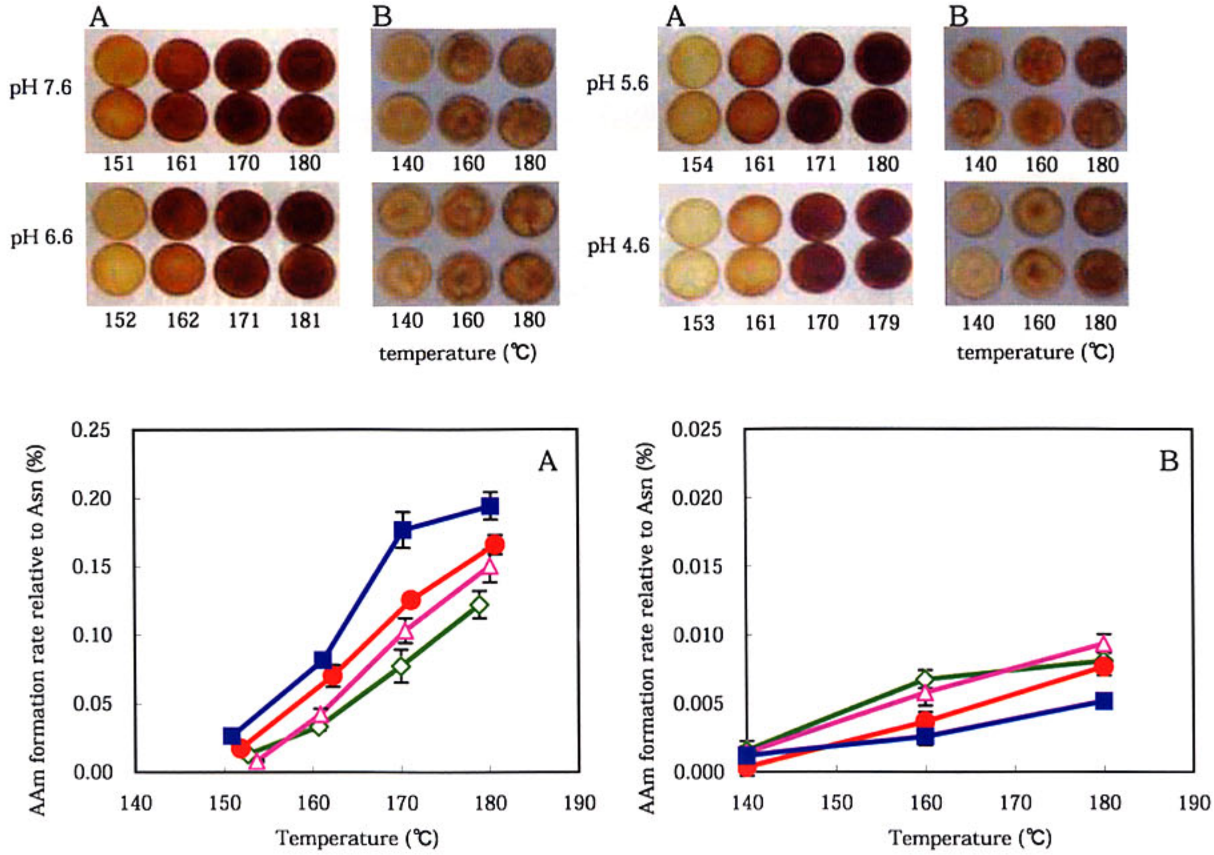

Fig. 2. AAm formation rate variations due to pH
A: Solution frying (frying time, 1 min), B: Moistureless oven (heating time, 5 min)
A 400 μL aliquot of solution (Glc 40 μmol, Asn 40 μmol) was added per one sheet of glass fiber filter paper.
Each value represents mean±S.D. of triplicate determinations.
—■—, pH 7.6; —●—, pH 6.6; —△—, pH 5.6; —◇—, pH 4.6

the region of approx. 100℃. But when the moisture level decreases, the temperature in the frying material rapidly rises and reaches the frying oil temperature. It was also found that the browning reaction progressed further as the moisture level decreased and the material temperature increased. Figure 3(B) shows the AAm formation rate variation. When the moisture level decreased and the frying material temperature increased, AAm formation also increased considerably.

3. Effect of carbonyl compounds

We considered that AAm might be formed *via* the Maillard reaction, and the reaction of Glc, fructose (Fru) and reducing sugar decomposition products[11, 12] [glyoxal (Glyo), methylglyoxal (MeGlyo), HMF, 3-DG] with Asn, during food processing (Fig. 4). A filter paper was impregnated with an equimolar mixed solution (pH 6.6) of Asn and a carbonyl compound, and heated, and the AAm formation rate was measured. In the moistureless oven, although AAm formation was seen with all carbonyl compounds, the formation rate was very low as compared with that in the case of reducing sugar. On the other hand, in solution frying, the formation rate with DG was almost equivalent to that with Glc, while that with Glyo was about 30% as much, and that with HMF was about 3%. With MeGlyo, no AAm was formed. This may be because the low molecular weight compounds (Glyo, MeGlyo, HMF) have low boiling points, and during frying at 180℃, would rapidly evaporate. However, Fru shows about twice the formation rate of Glc, which suggests that it contributes more to AAm formation than does Glc.

4. Comparative effects of Glc and Fru on AAm formation

Figure 5 compares the reactions of Glc or Fru with Asn. The amount of Asn was fixed, and the amount (moles) of reducing sugar was gradually increased to 7 times that of Asn. In solution frying, AAm increased as Glc was increased up to 1 : 1, but it then decreased. It was also found that with Glc, the degree of browning decreased at 4 times or more the amount of Asn. On the other hand, with Fru, if the added amount was increased, AAm increased accordingly. The degree of browning also increased with the increase of Fru. This suggests that Asn and Fru are the key precursors of AAm in solution frying. In a moistureless oven system, a large difference between Glc and Fru was not seen. When Glc and Fru were increased to the same amount as that of Asn, the formation rate increased, but if they were increased further, AAm formation decreased. As in solution frying, with Glc, browning decreased at a 4-fold excess or more, but for Fru, no decrease was seen.

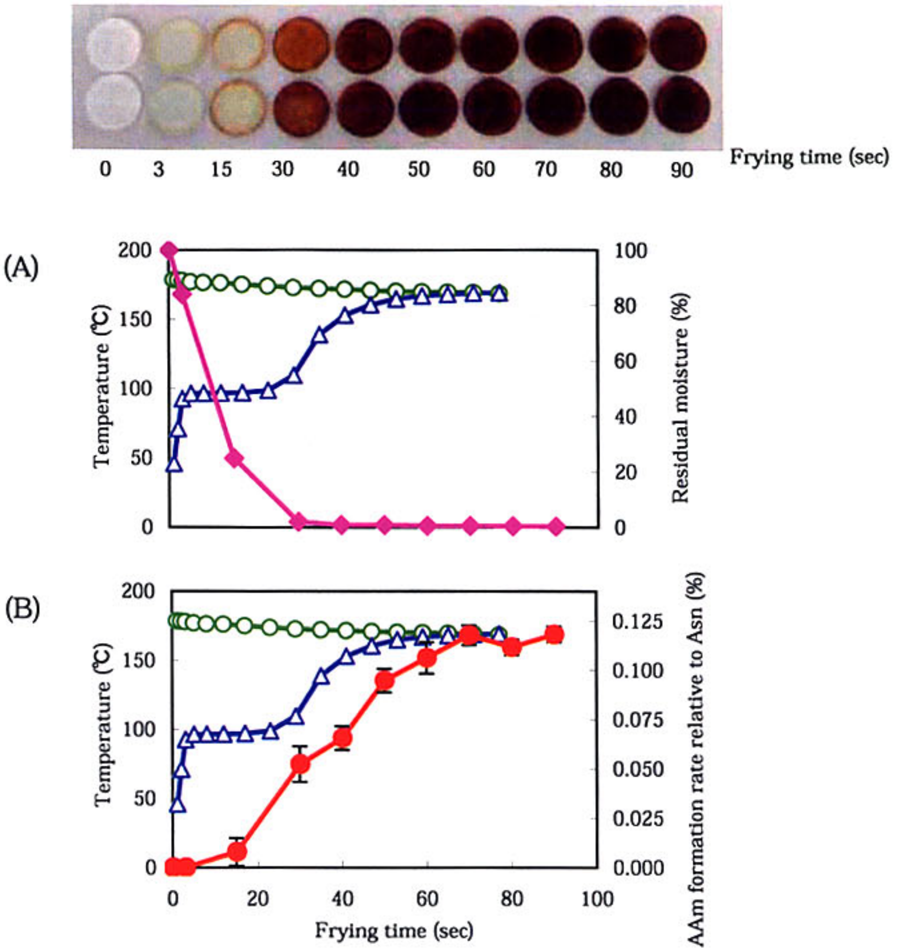

Fig. 3. AAm formation due to frying process

Frying temperature: 180℃

A 400 μL aliquot of solution (pH 6.6, Glc 40 μmol, Asn 40 μmol) was added per one sheet of glass fiber filter paper. Each value represents mean±S.D. of triplicate determinations.

—○—, Oil temperature (℃); —◆—, Residual moisture (%); —△—, Frying material temperature (℃); —●—, AAm formation rate relative to Asn (%)

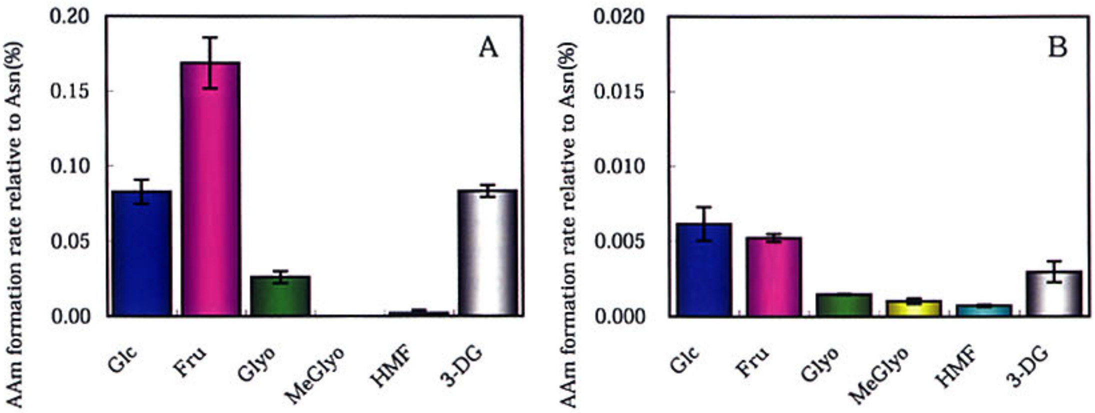

Fig. 4. Effect of carbonyl compounds on AAm formation

A: Solution frying (frying time, 1 min; temperature, 180℃), B: Moistureless oven (heating time, 5 min; temperature, 160℃)

A 400 μL aliquot of solution (pH 6.6, carbonyl compounds 40 μmol, Asn 40 μmol) was added per one sheet of glass fiber filter paper. Each value represents mean±S.D. of triplicate determinations.

5. Effect of free amino acids

To establish how free amino acids affect AAm formation, various amino acids were added in equimolar proportions to an equimolar Glc/Asn system, and their effect was evaluated (Fig. 6). Cysteine (Cys) and lysine (Lys) (especially Cys) inhibited AAm formation in both

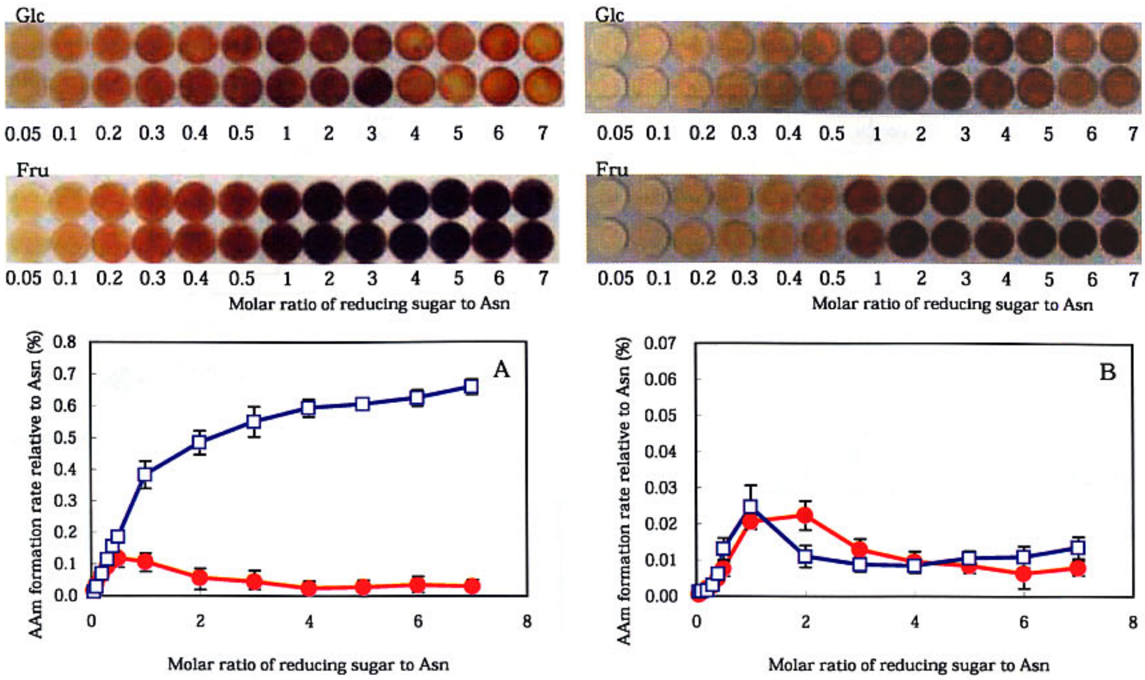

Fig. 5. Comparison of AAm formation rates due to Glc and Fru

A: Solution frying (frying time, 1 min; temperature, 180℃), B: Moistureless oven (heating time, 5 min; temperature, 160℃)

A 400 μL aliquot of solution (pH 6.6, reducing sugar 0–280 μmol, Asn 40 μmol) was added per one sheet of glass fiber filter.

—●—, Glc; —□—, Fru

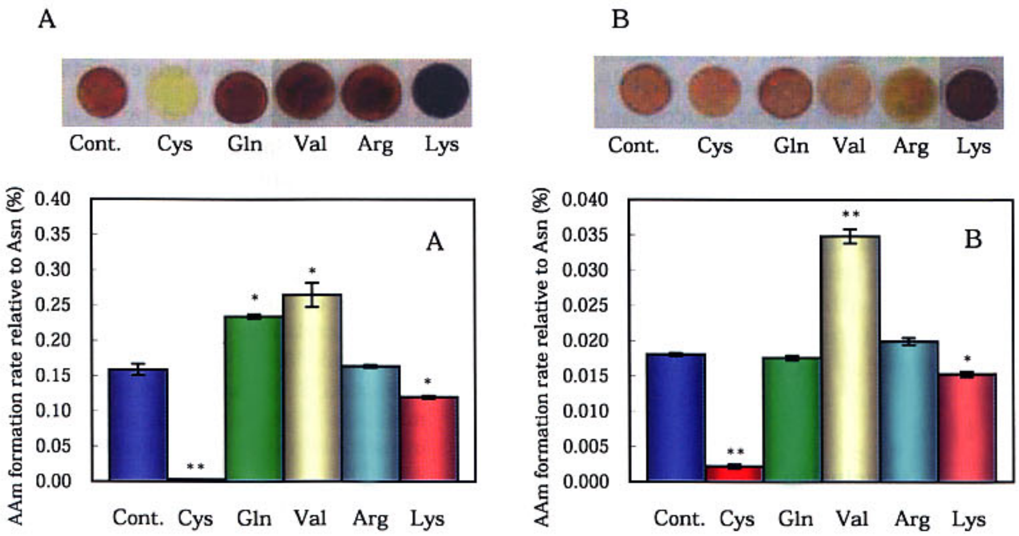

Fig. 6. Effect of various amino acids on AAm formation

A: Solution frying (frying time, 1 min; temperature, 180℃), B: Moistureless oven (heating time, 5 min; temperature, 160℃)

A 400 μL aliquot of solution (pH 6.6, Glc 40 μmol, Asn 20 μmol, amino acids 20 μmol) was added per one sheet of glass fiber filter paper. Each value represents mean±S.D. of triplicate determinations. *Significantly different from the corresponding control group (* $p < 0.05$; ** $p < 0.01$)

solution frying and the moistureless oven. In solution frying, Cys also inhibited browning. On the other hand, valine (Val) and glutamine (Gln) promoted AAm formation in solution frying, and Val also promoted it in the moistureless oven. Arginine (Arg) had little influence on AAm formation.

6. Mechanism of AAm formation in processed foods

Based on the above results and previous findings[6, 7, 9, 10] a mechanism for AAm formation was proposed (Fig. 7). In one pathway, Asn and a reducing sugar react to form AAm via a glycoside (Pathway 1). In another pathway, a free amino acid, such as Val, first reacts with a reducing sugar to form a dicarbonyl com-

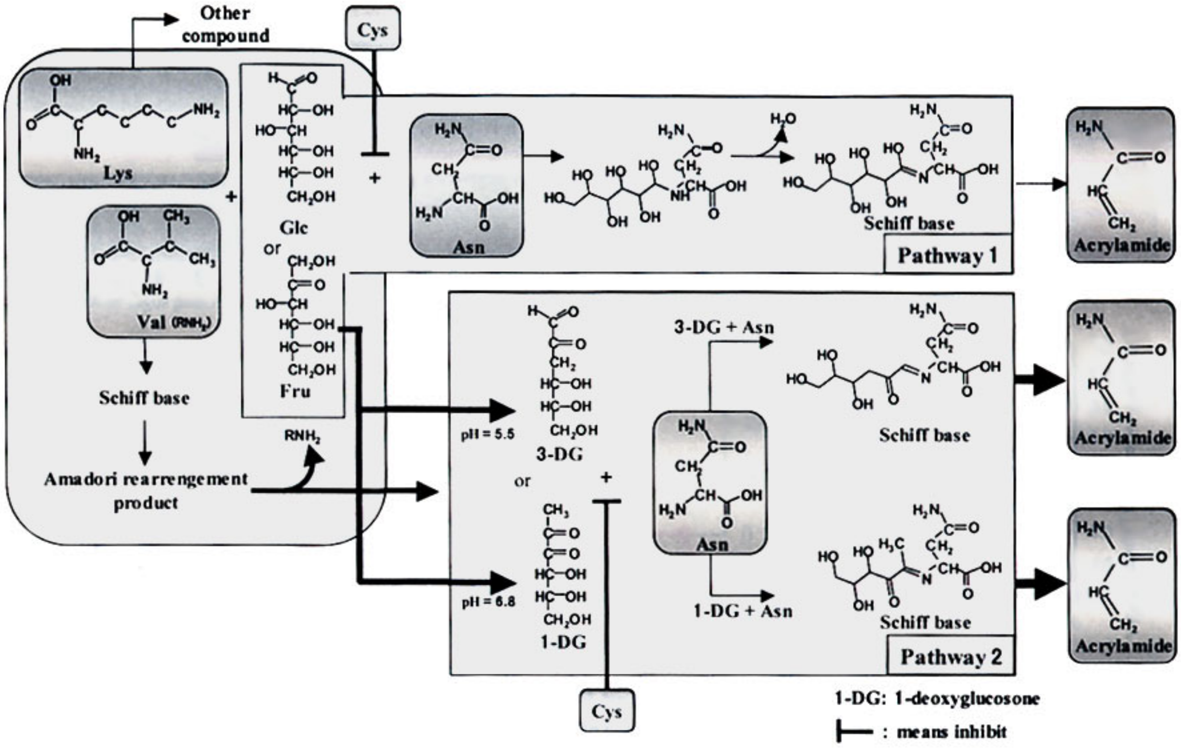

Fig. 7. Possible pathways of AAm formation in processed foods

pound (3-DG), or Fru alone is converted to a dicarbonyl compound, and these dicarbonyl compounds then react with Asn (Pathway 2). In a system where Glc is present in a large amount relative to Asn (Fig. 5), Pathway 1 will be favored. On the other hand, Fru is readily converted to highly reactive 1-deoxyglucosone (1-DG) or 3-DG[11, 12], so pathway 2 will be favored in the presence of large amounts of Fru. When Val and Asn are both present, the reducing sugar first reacts with Val to form a dicarbonyl compound, which reacts with Asn to give AAm. On the other hand, when Lys and Asn are both present, Lys reacts with reducing sugar, but little dicarbonyl compound is formed, so AAm formation is decreased. The thiol residue in Cys reacts with a carbonyl residue[13, 14], and this may account for the inhibitory effect of Cys.

Conclusion

AAm formation was investigated in a model system consisting of a glass fiber filter paper impregnated with a free amino acid and a carbonyl compound such as a reducing sugar.

1) The AAm formation rate was maximum at 180℃ in solution frying and at 200℃ in the moistureless oven.
2) The AAm formation rate was reduced at lower pH of the frying material.
3) Increase of frying temperature and frying time increased AAm formation when the residual sample moisture fell to 5% or less.
4) Fru effectively formed AAm.
5) Free amino acids co-present with Asn affect the AAm formation rate, and Cys has an inhibitory effect.
6) We propose possible pathways for the formation of AAm.

Acknowledgements

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