Reducing Acrylamide in Fried Snack Products by Adding Amino Acids

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ABSTRACT: The aims of this study were to develop commercial methods for reducing the acrylamide content in processed foods and apply them to commercial snacks. The formation of acrylamide in fried foods was found to depend on the composition of raw materials as well as the frying time and temperature. In potato chips, acrylamide was rapidly formed at over 160 °C, with the amount proportional to the heating duration and temperature. Free amino acids were used to reduce acrylamide, with lysine, glycine, and cysteine having the greatest effects in the aqueous system. Lysine and glycine were effective at inhibiting the formation of acrylamide in wheat-flour snacks. In potato snacks, the addition of 0.5% glycine to pallets reduced acrylamide by more than 70%. Soaking potato slices in a 3% solution of either lysine or glycine reduced the formation of acrylamide by more than 80% in potato chips fried for 1.5 min at 185 °C. These results indicate that the addition of certain amino acids by soaking the uncooked products in appropriate solutions is an effective way of reducing acrylamide in processed foods.

Keywords: acrylamide, potato chips, lysine, glycine, cysteine, soaking

Introduction

Acrylamide, classified as a Group 2A carcinogen (that is, probable human carcinogen), has been detected in common foods, such as potato chips, French fries, cookies, cereals, and bread, that are prepared or cooked at a temperature of over 120 °C (IARC 1994; Ono and others 2003; Granda and others 2004). Although there is no clear evidence that acrylamide directly causes cancers in humans (Mucci and others 2003), it is considered an undesirable contaminant in processed foods; therefore, its concentration should be reduced to the lowest technically achievable level. Unfortunately, there is currently insufficient knowledge about the mechanisms of acrylamide formation and the techniques that can reduce or prevent the formation of acrylamide in processed foods.

Potato (*Solanum tuberosum* L.) is cultivated throughout the world and is a staple dietary item in many countries. It can be stored for prolonged periods, is available all year, and is a source of many essential nutrients. Potatoes are always cooked before consumption, traditionally by baking, frying, steaming, or boiling. The food industry also processes potatoes into powder and granules that are used in processed foods, such as snacks and noodles. The volatile compositions of baked potatoes vary quantitatively and qualitatively according to cultivars and/or growing conditions (Kita and others 2004), and the contents of glucose and amino acids can include asparagine (Oruna-Concha and others 2001). These observations indicate that various reactions including sugar degradation and the Maillard reaction can occur that can produce undesirable products such as acrylamide. Asparagine and glucose in the potato tuber are important precursors of acrylamide in fried potato chips (Mottram and others 2002; Stadler and others 2002), and acrylamide has been found to be more abundant in potato chips than in other processed foods (Nemoto and others 2002; Rosén and Hellenä 2002; Takatsuki and others 2003).

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The factors affecting acrylamide formation in processed foods are processing time, temperature, and concentrations of reactants such as asparagine and reducing sugars. Possible approaches for reducing acrylamide in processed foods include (1) removing the reactants, such as asparagine and reducing sugars, (2) interrupting reactions using other reactants, (3) decreasing the processing temperature and time, and (4) removing acrylamide after its formation.

Several studies have investigated decreasing the formation of acrylamide by decreasing acrylamide precursors, asparagine and reducing sugars, by soaking or blanching of potato slices in various solutions (Grob and others 2003; Jung and others 2003). The use of water as a soaking solution has very little or no effects on the acrylamide content (Grob and others 2003; Haase and others 2003). Kita and others (2004) examined the effect of soaking in solutions with different pH values on the formation of acrylamide in sliced potato chips. Soaking or blanching of potato slices in acid solutions was effective, with the greatest reduction in acrylamide (90%) occurring in acetic acid solutions (60 min at 20 °C). However, a sour taste was detected when either citric acid or acetic acid was used. A large decrease in acrylamide content (74%) was also observed after soaking potato slices in a 1% NaOH solution, but the base solution made the appearance, taste, and flavor of the fried crisps unacceptable. This illustrates the importance of selecting a suitable soaking solution for commercial applications.

The present study was conducted to determine the effect of the addition of amino acids that might influence the production of acrylamide in aqueous model systems and then to apply this knowledge to the processing of fried model snacks and commercial potato chips. Furthermore, we examined the effects of soaking uncooked potato slices in lysine and glycine solutions on the amount of acrylamide in fried potato chips.

Materials and Methods

Samples

Frozen potatoes were obtained from Taekyung Nong San (Kyonggido, Korea). Samples used in the model system were made at

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the pilot laboratory of Nongshim (Kyonggido, Korea). L-asparagine, L-lysine monohydrochloride, glycine, L-cysteine monohydrochloride, and monosodium L-glutamate were purchased from Joi Science (Seoul, Korea). Because acrylamide is not present in raw potatoes, test samples were made containing wheat flour, sodium chloride, glucose, and asparagine. The test samples were dried before frying to control the reaction conditions. An atmospheric-pressure fryer was purchased from Dae Il (Seoul, Korea).

Chemicals

All reagents were of analytical grade unless otherwise stated. Acrylamide (99.9%) and formic acid were purchased from Sigma Chemical (St. Louis, Mo., U.S.A.). Methanol and acetic acid were purchased from Merck (Darmstadt, Germany). $^{13}C_3$-labeled acrylamide (99.0%) and d_5-3-chloropropanediol (99.4%) were purchased from Cambridge Isotope Laboratory (Andover, Mass., U.S.A.) and used as internal and recovery standards, respectively. High-purity water was obtained from an ultrapure water system (Human Science, Seoul, Korea).

Aqueous model system study

An aqueous model system was used to study the effects of food additives on the reduction in acrylamide. The amino acids used by the additives tested can be described as follows: (1) L-glutamic acid is an acidic and nonessential amino acid, (2) glycine is a neutral and the simplest amino acid, and the only one that is not optically active, (3) L-cysteine is a neutral and sulfur-containing nonessential amino acid, and (4) L-lysine is a basic and essential amino acid in human nutrition. To inhibit the formation of acrylamide in aqueous model systems, each amino acid was added at 0.5% to each aqueous solution containing 50 m*M* glucose and 50 m*M* asparagine. Each solution was heated for 20 min at 150 °C in an oven, and the acrylamide contents in each solution were directly analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS).

Model control snacks

Dough for the model control snacks was processed as follows. Wheat flour (64.5%), sodium chloride (1%), glucose (1%), and asparagine (0.5%) were premixed for 5 min before the addition of water (33%); all ingredients were then mixed for 20 min. The dough was passed through the 1st sheeting roller with a 1-mm gap, and the resulting sheets were passed again through the 2nd sheeting roller also with a 1-mm gap. The sheets then stood for 1 h at room temperature. The semi-dried sheets were passed through a cutting roller to produce hexagonal model snacks, which were dried in a hot-air dryer for 4 h at 70 °C. For model snacks containing amino acids, 0.1% to 3% glycine, cysteine, and lysine were added to the model control formula and samples were made using the same processing conditions. The control and experimental model snacks were fried at 180 ± 3 °C for 0.1, 0.5, 1, or 3 min.

Commercial snacks

Dough for the commercial fried snacks was processed as follows. Wheat flour (38%), frozen potatoes (27%), starch (30%), and other special additives (5%) were premixed for 10 s and then mixed for 10 min while being steamed. The dough was passed through a 1st sheeting roller with a 3-mm gap and then through a 2nd sheeting roller also with a 3-mm gap. The sheets were aged for 24 h at 15 °C and then passed through the cutting roller to produce hexagonal pellets, which were dried in a hot-air dryer for 3 h at 70 °C. Dried pellets in the 1st batch were kept for 2 d at room temperature and then again dried in a hot-air dryer for 4 h at 80 °C. Those in the 2nd batch were fried at 200 ± 3 °C for 25 s. We used different frying temperatures depending on the food materials and these are commercially used in Korea.

Manufacture of potato chips

Outer skin of potato (*Atlantic*) was peeled off and cut to be 1.6 ± 1 mm thick using a turning blade. Residual starches on the surface of sliced potatoes were removed with water, and excess surface water was removed before weighing and frying. The 200 g of sliced potato was presoaked in 5 L of amino acid solutions (0.1% to 3% of lysine, glycine, and cysteine) at 65 ± 5 °C for 1, 3, and 5 min with stirring. They were fried for 10, 15, 20, 25, and 30 min at 120 °C and 140 °C under vacuum, respectively. The control samples were fried for 1.5 min at 185 ± 3 °C under atmospheric pressure. The experiment was conducted 3 times.

Standard solutions

Stock solutions of acrylamide, $^{13}C_3$-labeled acrylamide, and d_5-3-chloropropanediol were prepared in distilled water at concentrations of 1000 μg/mL. The standards were protected from light and stored in a refrigerator at 4 °C.

Internal standard solution. A 1.0-mL aliquot of the $^{13}C_3$-labeled acrylamide stock solution was diluted to 1000 ng/mL, and 2 mL of the resulting solution was added into each sample before extraction.

Recovery standard solution. A working recovery standard (20 μg/mL) was prepared by dilution of 1000 μg/mL standard solution with water. A 2-μL aliquot of this working recovery standard solution was added into 1 mL of each sample and mixed thoroughly before purification with a C_{18} cartridge.

Calibration standard solution. Using a microsyringe, 0.1, 5, 25, 100, 200, 300, 400, and 500 μL of acrylamide stock solution were transferred to a series of 10-mL volumetric flasks, and 20 μL of the internal standard and 20 μL of the recovery standard were added to each flask and then diluted to the correct volume with water. All standard solutions were stored at 4 °C and then stood at room temperature for about 30 min before analysis.

LC-MS/MS analysis

An improved liquid chromatography-tandem mass spectrometry (LC-MS/MS) method was developed with modification of published methods (Nemoto and others 2002; Takatsuki and others 2003) for the determination of acrylamide in processed foods.

Sample analysis was conducted with an HPLC-Sykam S2100 Solvent Delivery System (Sykam, Germany) coupled to MS/MS with an electrospray ionization (ESI) source (Quattro Micro, Manchester, U.K.). The software used to operate the device and perform spectral analysis was MassLynx 4.0. The samples were separated by the Aqua C_{18} HPLC column (2 × 250 mm), packed with 5-μm particles (Phenomonex, Torrance, Calif., U.S.A.), using the mobile phase with aqueous 0.2% acetic acid and 1% methanol at a flow rate of 0.2 mL/min for 14 min. The volume of each sample injected was 20 μL. The electrospray positive ionization source had the following setting: capillary voltage of 4.2 kV, source temperature of 120 °C, desolvation temperature of 240 °C, desolvation gas flow rate of 650 L/h with nitrogen, and an argon gas pressure of 2.5 mbar (used as the collision gas). Acrylamide was determined by multiple reaction monitoring (MRM). MRM was performed by monitoring the 72- to 55-m/z transition for acrylamide, the 75- to 58-m/z transition for $^{13}C_3$-acrylamide, and the 116- to 98-m/z transition for d_5-3-chloropropanediol. For all MRM transitions, the dwell time was 1 s and the inter scan delay time was 0.2 s.

Results and Discussion

Frying time and temperature

Model system studies were performed to elucidate the role of glucose and asparagine in the formation of acrylamide (Figure 1). A 0.1% solution of glucose and asparagine was added to a 250-mL vial with a cap. The samples were heated in an oven at 150 °C for 90 min, and the acrylamide concentration in each solution was directly analyzed by LC-MS/MS. The acrylamide content was found to increase exponentially during this period, up to a maximum of 2750 μg/kg.

A specific model system was designed to determine the role of the food matrix in the formation of acrylamide. Changes in acrylamide concentration during the frying of potato chips were monitored (Figure 2). The acrylamide was formed more rapidly at a higher frying temperature (especially above 160 °C), with the amount produced being proportional to the heating duration and temperature. This is consistent with previous suggestions that frying temperatures should be below 175 °C and that the frying time should be as short as necessary to obtain fried products of satisfactory quality (Gertz and Klostermann 2002; Grob and others 2003; Rydberg and others 2003). Mottram and others (2002) found significant amounts of acrylamide when an equimolar mixture of asparagine and glucose was reacted at 185 °C in phosphate buffer in a sealed glass tube. They also observed the temperature dependence of acrylamide formation from 120 to 180 °C. Rydberg and others (2003) reported that the acrylamide content of potato strips in 200 °C. All these results indicate that acrylamide formation is dependent on heating duration and temperature, and the importance of testing real systems so as to reduce acrylamide content in processed foods.

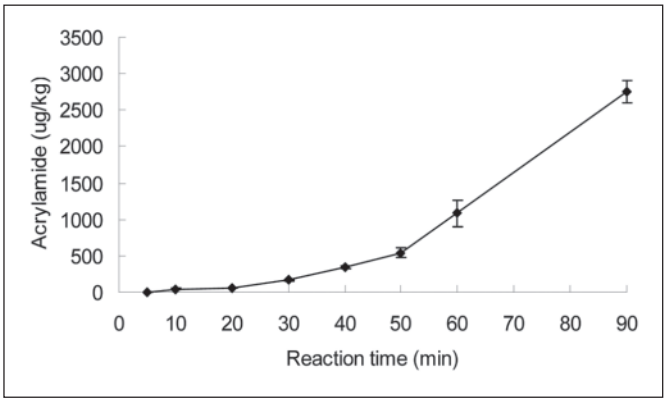

Figure 1—Effect of reaction time on acrylamide formation in a model system at 150 ± 3 °C. A 0.1% solution of glucose and asparagine was added to a 250-mL vial with a cap. The solution was heated at 150 ± 3 °C for 90 min in an oven and analyzed. Data values are mean ± SD (*n* = 3).

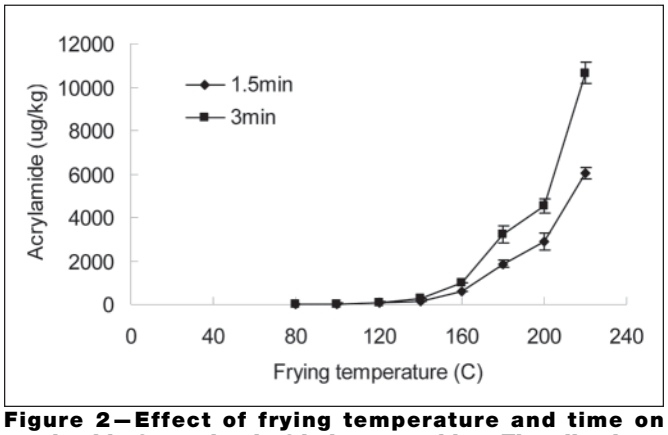

Figure 2—Effect of frying temperature and time on acrylamide formation in fried potato chips. The sliced potatoes were 1.6 ± 0.1 mm thick. Data values are mean ± SD (*n* = 3).

Effects of amino acids in aqueous model systems

An aqueous model system was used to determine the effects of free amino acids on the formation of acrylamide. Glutamic acid, glycine, cysteine, and lysine (each 0.5%) were added into aqueous solutions containing glucose (50 m*M*) and asparagine (50 m*M*) in sealed glass tubes and heated at 150 °C for 20 min. Glycine, L-lysine, and L-cysteine reduced the formation of acrylamide by 95%, 91%, and 87%, respectively (Figure 3), whereas L-glutamic acid inhibited acrylamide formation by less than 20%. This is probably due to the low solubility of the glutamic acid compared with other amino acids in aqueous solution. These results indicate that the addition of certain amino acids to raw materials could inhibit the formation of acrylamide during cooking and/or processing.

Several studies have confirmed that asparagine, a major amino acid in potato, rice, and cereals, is a central factor for acrylamide formation, especially in the presence of reducing sugars such as glucose, whereas cysteine, glutamine, arginine, and aspartic acid produced only trace quantities of acrylamide (Gertz and Klostermann 2002; Mottram and others 2002). Other factors will also influence the formation of acrylamide, such as temperature, moisture content, pH, and the relative proportion of the constituent amino acids. The addition of food additives to optimally decrease acrylamide in heated and/or fried foods and foodstuffs could lead to significant reductions in acrylamide levels. The commercially available additives that are usable for controlling acrylamide reduction are amino acids, citric acid, antioxidants, and emulsifiers. The 1st consideration is whether these are suitable as foods and have an acceptable processing quality. For example, fried potato chips containing citric acid are unsuitable due to the resulting poor quality and taste.

Effects of amino acids in the fried model snack and commercial snacks

The reduction rates of acrylamide in fried model snacks depended on frying time and additive concentration. The effects of lysine

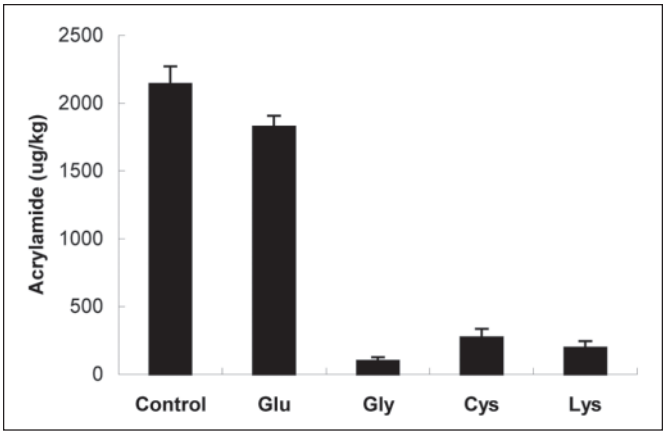

Figure 3—Effects of amino acids on reduction of acrylamide in aqueous model systems. Amino acids were added at 0.5% to each aqueous solution containing 50 m*M* glucose and 50 m*M* asparagine that were heated in sealed glass tubes at 150 °C for 20 min. Cys = cysteine; Glu = glutamic acid; Gly = glycine; Lys = lysine.

content (0.1% to 3.0%) and frying time (0.5 to 10 min) on the reduction rates of acrylamide in fried model snacks are shown in Figure 4a when the model samples were fried for 0.5 min, lysine treatment reduced the acrylamide by up to 70% compared with control, but this reduction decreased rapidly with increasing frying time. In particular, when the model samples including 0.1% lysine were fried for 3 min, the reduction in acrylamide was only 3%. The reduction in acrylamide increased in proportion to the concentration of lysine in the fried model snacks at 1.5 and 3 min. The effects of glycine contents and frying times on the reduction rates of acrylamide in the fried model snacks are shown in Figure 4b. When the model samples were fried for 0.5 min, the presence of 3% glycine reduced the acrylamide by up to 60%, in proportion to increasing frying time. The reduction in acrylamide increased in proportion to the concentration of glycine in the fried model snacks. The effects of cysteine content and frying time on the reduction rates of acrylamide in fried model snacks are shown in Figure 4c, which indicates that cysteine had the smallest effect among the amino acids tested. Moreover, reduction in acrylamide did not depend on the cysteine content and frying time. The solubility of cystein in aqueous solution is extremely low compared with lysine and glycine. This made low effectiveness of cysteine in the reduction of acrylamide. In summary, the effects of lysine and glycine were greater than that of cysteine on reducing acrylamide in fried model snacks.

Table 1 lists the effects of lysine and glycine on reducing acrylamide in commercial snacks. Glycine at 0.1% and 0.5% reduced the acrylamide concentration by 43% and 69%, respectively. Rydberg and others (2003) determined the effects of various amino acids on the formation of acrylamide in homogenized potatoes heated at 180 °C for 25 min. The addition of glycine, alanine, lysine, glutamine, and glutamic acid at 35 mmol/kg reduced acrylamide levels by 42% to 70% compared with control. This was probably due to the competitive consumption of acrylamide precursors and/or increased elimination of acrylamide by nucleophilic components in the amino acids. Addition of free amino acids or a protein-rich food component strongly reduced the acrylamide content, probably by promoting competing reactions and/or covalently binding acrylamide formed.

Effects of presoaking

In the present study, the asparagine and reducing sugars were removed from potato slices by dipping them into glycine and lysine solutions. The resulting inhibition of acrylamide formation in potato chips resulted from the loss of substances required for the nonenzymatic browning reaction and a competition reaction between them and asparagine in the potato slices. The effects of dipping potato slices in lysine solutions on the reduction in acrylamide in fried potato chips are shown in Figure 5a. When the potato slic-

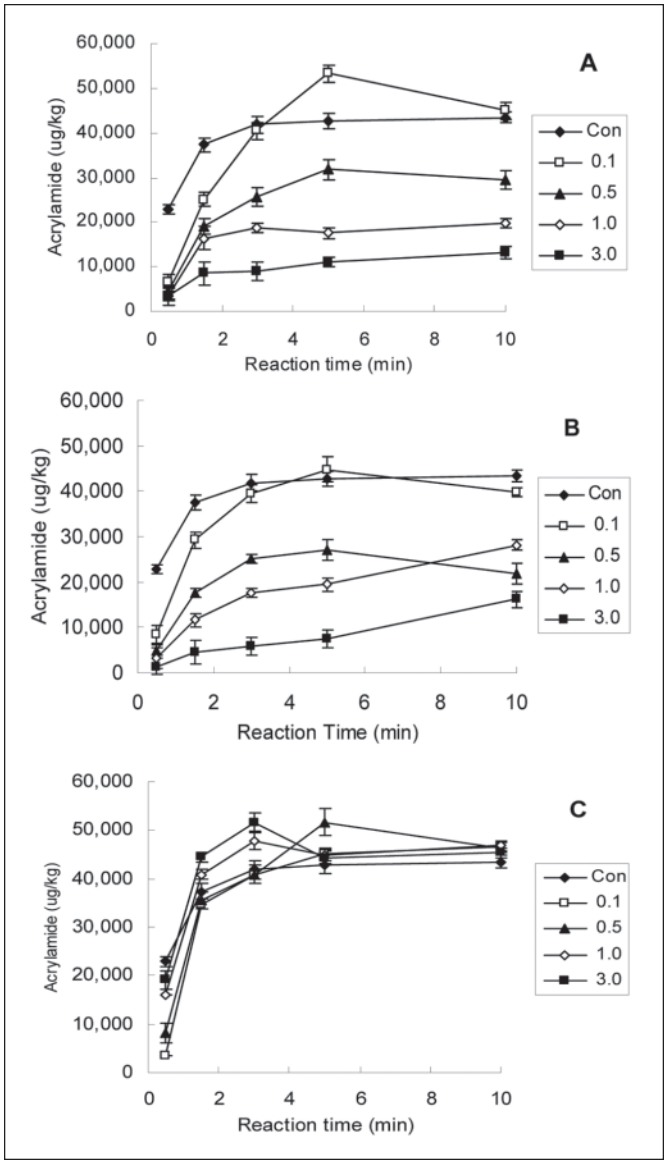

Figure 4—Effects of lysine (A), glycine (B), and cysteine (C) on acrylamide in model samples fried at 180 °C. Data values are mean ± SD (*n* = 3).

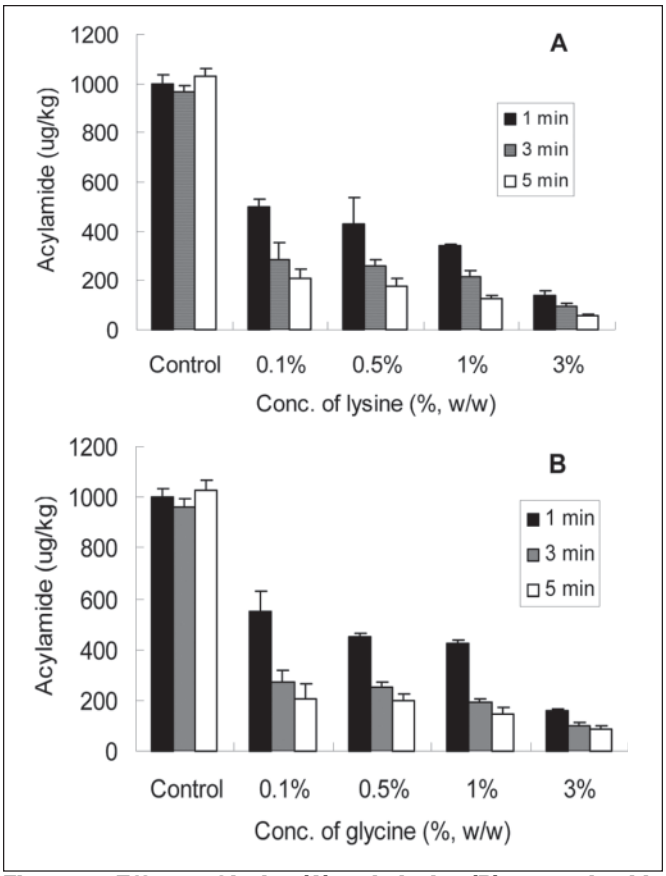

Figure 5—Effects of lysine (A) and glycine (B) on acrylamide in fried potato chips. Data values are mean ± SD (*n* = 3).

Table 1—Effects of lysine, glycine, and cysteine solutions on acrylamide in commercial snacks

Treatment	Acrylamide (μg/kg)	Reduction (%)
Control	1154 ± 79	
Lysine 0.1%	657 ± 4	43 ± 4
0.5%	353 ± 10	69 ± 2
Glycine 0.1%	795 ± 14	31 ± 4
0.5%	543 ± 9	53 ± 3
Cysteine 0.1%	935 ± 6	19 ± 5
0.5%	1008 ± 39	13 ± 8

Data values are mean ± SD (n = 3).

es were dipped for 1, 3, and 5 min in a 0.1% lysine solution, the reductions in acrylamide were 45%, 72%, and 79%, respectively; for a 0.5% lysine solution they were 55%, 74%, and 80%; and for a 1% lysine solution they were 58%, 81%, and 86%, respectively. When the concentration of the lysine solution was 3%, the reductions in acrylamide were 84% to 92%.

The effects of soaking potato slices in glycine solutions on reducing acrylamide in fried potato chips are shown in Figure 5b. When the potato slices were soaked for 1, 3, and 5 min in a 0.1% glycine solution, the reductions in acrylamide were 50%, 71%, and 79%, respectively; and for a 0.5% glycine solution they were 57%, 74%, and 82%, respectively. Dipping the potato slices in 1% and 3% glycine solutions reduced acrylamide formation by up to 88% and 94%, respectively. These results indicate that dipping potato slices into glycine can significantly reduce the acrylamide contents in potato chips without requiring the use of a vacuum fryer and hence this method could be incorporated into a general manufacturing process (Granda and others 2004).

Conclusions

The acrylamide content in potato chips increases as frying temperature and duration increase, especially at above 150 °C. In an aqueous model system, the presence of glycine, cysteine, and lysine in wheat-flour dough had the largest effects on the reduction in acrylamide, with lysine and glycine successfully preventing the formation of acrylamide in the fried product. Dipping potato slices in 3% lysine or 3% glycine for 1 min reduced the acrylamide in potato chips by 80%. These results could be applied to reducing acrylamide levels in commercial snacks and potato chips.

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