Examination of Conditions Inhibiting the Formation of Acrylamide in the Model System of Fried Potato

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Acrylamide (AAm) is produced in food through the reaction of asparagine and reducing sugar. We examined several methods of reducing the level of AAm using potato tubers. The fried model system that we employed consisted of thin slices that were first treated in water under different conditions before frying. A sufficient amount of water present in the fry material acts as an inhibitor against the formation of AAm and allows only a negligible amount of AAm to form. It was found that given the low content of water, the fry material temperature was sufficiently high to allow a relatively large level of AAm to form. Examination of water treatment prior to frying revealed that higher-temperature treatment water and longer treatment time resulted in the formation of lower levels of AAm. Moreover, removing some of the residual heat had an inhibiting effect on the formation of AAm.

Key words: acrylamide; potato processed food; reducing sugar; amino acid; water treatment

Swedish researchers reported in April 2002 that cooking foods that contain a high content of carbohydrates at high temperatures contributed to the formation of acrylamide (AAm).1,2) Other researchers have reported methods of analyzing the AAm contained in certain foods3,4) and AAm levels in various processed foods have been studied.5,6) It was found that heating the asparagines (Asn) and reducing sugars in food contributes to the formation of AAm,7–9) but details about the mechanism for AAm formation and how such formation can be inhibited have yet to be fully understood. The purpose of our study was to clarify this mechanism of AAm formation and its inhibition using a fried model system based on sliced pieces of potato.

Materials and Methods

Reagents and materials. For all of our studies, we used only guaranteed reagents (provided by Wako Pure Chemical Industries, Osaka, Japan) or equivalent.

Preparation of fried model system. Toyoshiro potato tubers grown in Hokkaido were stored at 5 ºC for five months after harvest. The unpeeled potatoes were washed in tap water and then sliced into pieces 1.42 mm thick with a slicer (manufactured by Urschel Laboratories, Indiana, USA). The slices of potato tuber were trimmed with a pattern cutter to obtain 15 g of potato tuber. These pieces were immersed in distilled water for water treatment lasting 0, 2, 5, 10, and 30 min at 20 ºC, 40 ºC, 60 ºC, and 80 ºC in 1000-mL beakers placed in an water-bath shaker. During immersion, the material was stirred. After water treatment, the slices of potato tuber were wiped off for frying.

Frying.

Frying conditions: different time periods of frying. First, 600 g of palm oil were poured into an iron pan and heated to 180 ºC on an electric stove. Upon reaching said temperature, the heating process was stopped and five slices of fry material were fried in the iron pan for different periods of time (0, 3, 15, 30, 40, 50, 60, 70, 80, and 90 s). After each frying process was completed, the sample was frozen with liquid nitrogen and placed in a freezer set at −27 ºC.

Frying conditions: fixed frying time period. A 17 kg mixture of rice and palm olein (1:1) was placed in a fryer (Eishin Denki, Kanagawa, Japan) and heated to 180 ºC. Ten g of fry material were fried in the fryer for different periods of time (0, 3, 15, 30, 40, 50, 60, 70, 80, and 90 s). After each frying process was completed, the sample was frozen with liquid nitrogen and placed in a freezer set at −27 ºC.

Analysis of AAm. The resulting samples were crushed in a fiber mixer MX-X103 (Matsushita Electric Industrial, Osaka, Japan). Two g of specimen were taken for analysis. AAm was analyzed as the monobromoderivative by gas chromatography–mass spectrometry (GC-
MS) using the method described in the previous paper.3) Analysis of amino acids. Amino acids were analysed according to the standard method.10) Analysis of reducing sugar. The resulting samples were crushed in a mixer. Twenty g of specimen were collected in beakers. Then 100-ml of a 40% solution of ethanol were added to the specimens and homogenized for one min. The homogenized specimens were filtered through 5A filter paper. The resulting filtrates were cleaned through subsequent filtration using the Advantec DISMIC-25cs (Toyo Roshi Kaisha, Tokyo, Japan), and then subjected to HPLC analysis under the following conditions:

- Column: Zorbax carbohydrate analysis, 4.6 mm (ID) × 150 mm (5 μm),
- Mobile phase: 75/25 acetonitrile/water
- Flow rate: 1.4 ml/min.
- Temperature: 30 °C
- Detector: HP 1100 RID, 30 °C
- Sample volume: 10 μl

Measurement of water content. The samples were crushed in a fiber mixer. Ten g of resulting material were flattened on an aluminum plate. These assemblies were dried in a temperature chamber for 5h at 100 °C, and then weighed to determine the residual water content of each specimen.

Measurement of fry material temperature. A long, fine-sheathed thermocouple (T34, φ0.25 straight sheath, Okazaki Manufacturing Company, Hyogo, Japan) was inserted sideways near the center of the specimen slices of potato. A portable temperature recorder (NR-1000, Keyence Corporation, Osaka, Japan) was then employed to collect temperature data at 1-s intervals.

Results and Discussion

Fried model system

Figure 1 shows the frying oil temperature, fry material temperature, residual water content, and amount of AAm formed during the frying process of the fried model system. The measurements showed that when the water content of the fry material was higher than 10%, the fry material maintained a temperature of about 100 °C in frying oil set at a temperature slightly higher than 170 °C. With lower water content, the fry material temperature began to rise sharply until it reach the frying oil temperature. Browning reaction occurred as the water content dropped and the fry material temperature rose. Hardly any AAm formed while a fry material temperature of about 100 °C was maintained due to the high water content. When the water content dropped below a certain level, thus raising the fry material temperature, AAm began to form. The rate of AAm formation steadily increased until the frying test was complete within 90 s. Thus these results suggest that

![Fig. 1. Formation of Acrylamide in Frying Process. Five slices of fry material were washed in tap water for 100 s, and then fried for 90 s in 600 g of palm oil heated at 180 °C.](image-url)
the most important factors in the frying process in terms of AAm formation are the frying oil temperature when the browning reaction begins with lower water content, and the duration of frying.

**Effect of treatment-water temperature**

Figure 2A shows the reducing sugar content when treating the fry material in water for 2 min at 20°C, 40°C, 60°C, and 80°C. In all cases except at a treatment temperature of 40°C, the water treatment reduced the amounts of certain kinds of reducing sugar as compared to the control samples that were not treated in water. In fact, for treatment at 80°C, the amounts of glucose and fructose were 0.9 g/100 g and 0.7 g/100 g (dry weight) respectively, while those of the control samples were 1.4 g/100 g and 1.1 g/100 g (dry weight) respectively. It was conjectured that water treatment of a specimen dissolved some reducing sugar content from the surface of the specimen into the treatment water. The lack of significant difference in reducing sugar content between the specimens and control samples at 40°C was attributed to increased reducing sugar content due to the action of an enzyme known as invertase. Another reason for this difference was the gelatinization and disintegration of potato starch cells during water treatment at 60°C and 80°C. Potato starch is known generally to gelatinize at 60±5°C.

Our measurements indicate that the amounts of free amino acids and Asn tend to drop when the treatment-water temperature rises. Water treatment at 20°C, 40°C, 60°C, and 80°C reduced the total amounts of free amino acids to 3.5, 2.9, 2.5, and 1.1 g/100 g (dry weight) respectively, and reduced Asn to 1.1, 0.9, 0.8, and 0.3 g/100 g (dry weight) respectively (Fig. 2B). The water treatment temperature had no effect on the water content of the water-treated fry materials (data not shown). We measured AAm in fry material treated in water and fried (Fig. 3). The amounts of AAm in the specimens were lower at high water-treatment temperatures than in the control samples, and correlated with the levels of free amino acids and Asn. Water treatment at 80°C contributed to a 72% reduction in AAm. Conversely, water treatment at temperatures exceeding the gelatinization temperature for starch resulted in a significantly different taste and feel of fried samples than those of samples fried after being treated in water at a temperature below the gelatinization temperature. This difference was due to the starch gelatinizing at high temperatures before it was fried. Water treatment thus reduces the components that affect the taste of fried potato, such as reducing sugars and free amino acids. For this reason, the taste and feel of fried products should be considered when the base material is treated in water at high temperatures before frying.

**Fig. 2.** Effect of Water-Treatment Temperature on Fry Material Composition. Slices of fry material were treated in water for 2 min at different temperatures. The amounts of reducing sugar (A) and amino acids (B) in the slices were then measured.
Effect of water-treatment period

Figure 4A shows the reducing sugar content in fried samples treated in water for 2, 5, 10, and 30 min at 60 °C. Figure 4B shows the content of free amino acids. It was observed that the amounts of reducing sugar and free amino acids were reduced after longer water treatment as compared to those of the control samples not treated in water. In water treatment lasting 2, 5, and 10 min, the amounts of glucose were reduced to 1.3, 0.8, and 0.6 g/100 g (dry weight) respectively, and the amounts of fructose were reduced to 1.0, 0.7, and 0.4 g/100 g (dry weight) respectively. Similarly, in these cases, the total amounts of amino acids were reduced to 3.6, 2.9, and 1.2 g/100 g (dry weight) respectively, and the total amounts of Asn were reduced to 1.1, 1.0 and 0.4 g/100 g (dry weight) respectively. Water treatment lasting 30 min reduced the reducing sugar and the total amount of amino acids in the fry material to almost nothing. The water-treatment temperatures had no effect on the water content of the water-treated fry materials (data not shown here). Measurements of AAm in the fry material fried after being treated in water (Fig. 5) showed that water treatment lasting 2–10 min had the effect of reducing AAm by 50%. Thus, 30 min of water treatment achieved a near total (99%) reduction of AAm without any browning reaction. However, it should be noted that water treatment prior to frying can greatly affect the taste and feel of fried products. Our next challenge is to consider the sensory aspects of the water-treatment process.

Effect of cooling after frying on reducing AAm

Fry material was treated in water for 2 min at 20 °C, and then fried for 2 min at 180 °C. After frying, the specimens were frozen (quenched) in liquid nitrogen to examine any AAm-inhibiting effect of quenching on frying (Fig. 6). This experiment showed that quenching had an AAm-inhibiting effect of 14.3% as compared to storage at room temperature. This result suggests that removing some residual heat after frying can help limit the formation of AAm.

Conclusion

Our examinations of possible AAm-inhibiting conditions in processed food based on potato tubers and using the fry model system consisting of slices of potato tuber yielded the following findings: (1) When the residual water content of the fry material became sufficiently low (about 10%) during the frying process, acrylamide (AAm) began to form. (2) The effect of inhibiting AAm formation became more pronounced at higher water treatment temperatures for slices of potato tuber and during longer water treatment. And (3) The effect of inhibiting AAm formation became more pronounced when the fry material was quenched (frozen).

Water treatment prior to frying is technically and economically applicable because it is the general way in potato chips manufacturing. Therefore, the data of this paper carry practical significance.
Fig. 4. Effect of Water-Treatment Period on Potato Tuber Composition.
Slices of fry material were subjected to water treatment at 60 °C for different periods of time. The amounts of reducing sugar (A) and amino acids (B) in the slices were then measured.

Fig. 5. Effect of Water Treatment Period on Acrylamide Formation in Fried Potato Tubers.
A large volume of fry material was treated in water at 60 °C for different periods of time. Specifically, 10-g lots of water-treated fry material were fried for 90 s in a 17-kg mixture of palm olein and rice (1:1) at a constant temperature of 180 °C. The amounts of acrylamide were then measured. Each value represents the mean ± S.D. of triplicate determinations.
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References