Change in Content of Sugars and Free Amino Acids in Potato Tubers under Short-Term Storage at Low Temperature and the Effect on Acrylamide Level after Frying

AkiKO O HARA-TAKADA,1,1 Chie MATSUURA-ENDO,1 Yoshihiro CHUDA,2,3 Hiroshi ONO,3 Hiroshi YADA,3 Mitsuru YOSHIDA,3 Akira KOBAYASHI,1 Shogo TSUDA,1 Shigenobu TAKIGAWA,1 TakahiRO NODA,1 Hiroaki YAMAUCHI,1 and Motoyuki MORI1

1Department of Upland Agriculture, National Agricultural Research Center for Hokkaido Region, Memuro, Hokkaido 082-0071, Japan
2Center for Food Quality, Labeling and Consumer Services, Chuo-ku, Saitama 330-9731, Japan
3National Food Research Institute, Tsukuba, Ibaraki 305-8642, Japan

Received October 29, 2004; Accepted April 19, 2005

Changes in the sugar and amino acid contents of potato tubers during short-term storage and the effect on the acrylamide level in chips after frying were investigated. The acrylamide content in chips began to increase after 3 days of storage at 2°C in response to the increase of glucose and fructose contents in the tubers. There was strong correlation between the reducing sugar content and acrylamide level, \( R^2 = 0.873 \) for fructose and \( R^2 = 0.836 \) for glucose. The sucrose content had less correlation with the acrylamide content because of its decrease after 4 weeks of storage at 2°C, while the reducing sugar in potato tubers and the acrylamide in chips continued to increase. The contents of the four amino acids, i.e., asparatic acid, asparagine, glutamic acid and glutamine, showed no significant correlation with the acrylamide level. These results suggest that the content of reducing sugars in potato tubers determined the degree of acrylamide formation in chips. The chip color, as evaluated by \( L^* \) (lightness), was correlated well with the acrylamide content.

Key words: acrylamide; potato; potato chip; low-temperature storage; reducing sugar

Storing potato tubers at a low temperature is desirable because it inhibits sprouting and reduces the loss due to storage rot.1) The low-temperature storage of potatoes, however, leads to an accumulation of sugars by a process that is called “low-temperature sweetening”.2–4) The processing of potatoes into chips and fries, the accumulated reducing sugars react with free amino acids in the tuber cells, forming unacceptable brown- to black-pigmented products via a non-enzymatic Maillard-type reaction.5) To avoid an increase in the sugar content, potato tubers for chips are generally stored at a temperature around 8 to 12°C.6,7)

It has recently been reported that acrylamide, a compound classified as a probable carcinogen, is present in various foods processed at high temperature.8–10) Acrylamide is formed in the process of Maillard reactions from asparagine in the presence of a carbonyl compound such as reducing sugars.11–14) We have found that chips made from potato tubers stored at 2°C for 2 weeks showed a much darker brown color and higher acrylamide level than those from tubers stored at 20°C.15) A correlation between the reducing sugar (fructose and glucose) level in tubers and the acrylamide level after cooking was clarified.

We have been evaluating the contents of sugars and amino acids in tubers of Japanese cultivars and breeding lines, and their effects on the acrylamide formation during frying with the object of developing new cultivars and storage methods for potatoes to produce chips with a low level of acrylamide and high quality. This study uses Toyoshio, which is a leading cultivar for producing potato chips in Japan, to examine the following points: (1) detailed change patterns of the sugar and amino acid contents in potato tubers during storage for 2 weeks at 2°C; (2) effects of the sugar and amino acid changes on the acrylamide level in chips after frying; (3) difference between early- and regular-harvested tubers in their sugar, amino acid, and acrylamide contents; (4) difference in the sugar, amino acid, and acrylamide contents between regular-harvested tubers stored at 2°C and 18°C for 4 weeks. We will conclude that the content of reducing sugars in raw tubers determines the degree of acrylamide formation in chips.
Materials and Methods

Potatoes (plant material). In this study, the cultivar Toyoshiro, which belongs to the commonly cultivated tetraploid species, Solanum tuberosum ssp. tuberosum, was used. Healthy tubers weighing between 100 g and 130 g were selected and stored under each condition after a 2-week curing treatment. Four replicates consisting of three tubers each were taken on each sampling date. Each tuber was cut in half longitudinally; one half was used for immediate potato chip preparation, and the other half was chopped crosswise into slices (1 × 1 cm, 1 mm thick) and frozen at −40 °C until being used for the extraction of sugars and free amino acids.

Two-week storage at 2°C. Toyoshiro, which had been grown in southern Japan (Kagoshima Prefecture), was used in this short-term storage experiment. Tubers were planted on January 23, 2003 in a field at the Ohsumi branch of Kagoshima Agricultural Experiment Station (Ohsumi, Kagoshima, Japan) and harvested on May 23 (119 days after planting). After curing for 2 weeks at 14°C in the dark, the tubers were stored at 2°C under 80% relative humidity, and samples for analyses were taken on storage days 0, 1, 2, 3, 6, 9, and 14.

Four-week storage at 2°C and 18°C. Toyoshiro, which had been grown in northern Japan (Hokkaido Prefecture), was used in this experiment. Tubers were planted on April 28, 2003 in a field of the Department of Upland Agriculture, National Agricultural Research Center for Hokkaido Region (Memuro, Hokkaido, Japan) and harvested on September 11, 2003 (regular harvesting, 136 days after planting). After curing for 2 weeks at 18°C in the dark, the regular-harvested tubers were stored at 2°C or 18°C under 80% relative humidity, and samples for analyses were taken on storage days 0, 14, and 28.

Preparation of potato chips and chip color determination. From one half of the tubers, 5 slices of 1.3 mm thickness (ca. 20 g each) were prepared. Fifteen slices from 3 tubers of a replicate were washed in water for 100 seconds and then fried at 180°C in 9-liter of cottonseed oil for 90 seconds according to the standard method. For the experiment of two-week storage at 2°C, 6 chips with representative color were selected from each sample, and the L* (lightness) of the central area of each chip was measured by a colorimeter with an aperture of 10 mm in diameter (NR-3000, Nippon Denshoku, Tokyo, Japan). For the other experiments, all 15 chips were crushed into small pieces (ca. 3 × 3 mm), and the color of the pieces was measured in triplicate by a colorimeter with an aperture of 50 mm in diameter (CR-410, Konica Minolta, Tokyo, Japan).

Analysis of acrylamide in the chips. After the color determination, the concentration of acrylamide in the chips was analyzed by gas chromatography–mass spectrometry (GC–MS) after bromination as previously described.10 The chips (5 g) were homogenized in 200 ml of water with internal standard acrylamide-d3 (Polymer Source, Quebec City, Canada) and then centrifuged. The supernatant (2 ml) was applied to a mixed-mode SPE cartridge (Isolute Multimode (500 mg), International Sorbent Technology, Mid Gla
gen, UK) and eluted with water. The SPE eluate was brominated with KBr-HBr-bromine water, extracted with ethyl acetate, and injected into a GC–MS instrument (GCMS-QP2010, Shimadzu, Kyoto, Japan) with a CP-Sil 24 CB Lowbleed/MS column (equivalent to OV-17, 0.32 mm i.d. × 30 m, 0.5-μm film thickness, Varian, Walnut Creek, CA, U.S.A.). Fragment ion peaks of 2,3-dibromopropionamide derived from acrylamide at m/z 150 and 152, and those from acrylamide-d3 at m/z 153 and 155 were monitored by selected ion monitoring (SIM) for quantification.

Analysis of sugars in the tubers. The extraction and determination of sugars were carried out as previously described.16 Frozen tuber slices (ca. 10 g fresh weight) were homogenized in 80% (v/v) ethanol, and the sugars in the homogenate were extracted at 80 °C for one hour. After centrifugation, the supernatant was dried under vacuum, dissolved in distilled water, and passed through a membrane filter (0.2-μm Omnifore, Millipore, Tokyo, Japan). The concentrations of fructose, glucose, and sucrose in the filtrate were determined by HPLC (SC-8020, Tosoh, Tokyo, Japan) with a TSKgel Amide-80 column (4.6 × 250 mm, Tosoh).

Analysis of free amino acids in the tubers. Frozen tuber slices (ca. 10 g fresh weight) were homogenized with an Ultra-Turrax instrument (T25, Ika Works, Selangor, Malaysia) for 1 min in 40 ml of 80% (v/v) ethanol, and the amino acids in the homogenate were extracted at room temperature for one hour. The extract was passed through 2 layers of filter paper (No. 2, Advantec, Tokyo, Japan), and the resulting filtrate was dried in a vacuum evaporator. The dried sample was dissolved in 10 ml of a 0.25 mol/l lithium citrate buffer (pH 2.2, Wako, Osaka, Japan) and passed through a Sep-Pak C18 cartridge (Waters, Massachusetts, U.S.A.) and a 0.45-μm olefine-polymer filter (Kurabo, Osaka, Japan). The concentrations of aspartic acid, asparagine, glutamic acid, and glutamine in the filtrate were determined by HPLC (LC-10A amino acid analysis system, Shimadzu, Kyoto, Japan) with a Shim-pack Amino-Li column (6 × 100 mm, Shimadzu). Quantification was performed by using an external standard solution (type H amino acid standard solution, Wako, Osaka, Japan) with authentic asparagine and glutamine.
Results and Discussion

The major sugars in potato tubers are fructose, glucose, and sucrose. Asparagine is the most abundant of the free amino acids but glutamine, glutamic acid, aspartic acid, threonine, arginine, and valine are also major ones in potatoes. These components were used to perform model studies for acrylamide formation, which gave the result that a substantial amount of acrylamide was formed from asparagine in the presence of reducing sugars. Acrylamide was also formed from glutamine and aspartic acid with the reducing sugars, although the amount was much less than that from asparagine. We therefore focused on changes in the contents of the two major reducing sugars, fructose and glucose, together with sucrose, and of the four free amino acids, aspartic acid, asparagine, glutamic acid and glutamine, in the potato tubers during storage.

Tuber components during 2 weeks of storage at 2 °C and acrylamide in chips

Figure 1 shows changes in the contents of the four free amino acids and three sugars in raw tubers during 2 weeks of storage at 2 °C and of acrylamide in the chips made from them. The contents of amino acids were almost constant throughout the storage period (Fig. 1A). However, the sucrose content increased markedly after a 3-day lag period and reached 8.1 mg/g FW on day 9, the rate of increase subsequently slowing between day 9 and day 14 (Fig. 1B). Glucose and fructose increased gradually after the lag period and reached approximately 1.7 mg/g FW on day 14 (Fig. 1B). These results are in accordance with our previous observation on Toyoshiro stored at 4 °C, in which sucrose began to increase markedly about one week earlier than glucose and fructose, reaching a peak within approximately 2 weeks of storage, while the reducing sugars continued to increase up to 60 days of storage. These changes can indicate that starch breaks down and sucrose is formed, before sucrose is converted into reducing sugars by a vacuolar acid invertase. The level of acrylamide in chips also gradually increased after 3 days of storage, reaching over 5 μg/g of chips on day 6 and 18 μg/g of chips on day 14 (Fig. 1C).

Noti et al. have also investigated the effect of cold storage of potato tubers on the potential for acrylamide formation and reported a lag phase of around 5 days before the increase in the potential of acrylamide formation for several cultivars stored at 4 °C. The lag period observed in this present study was shorter than that observed by Noti et al., probably due to the lower storage temperature. These results show that even 3 days of cold storage accelerated sugar accumulation in the tubers and increased the potential for acrylamide formation.

Individual data for 2 weeks of storage at 2 °C in this test (n = 28) were used to analyze the correlation between the components in tubers and acrylamide in chips. The correlation between the concentration of reducing sugars in the tubers and acrylamide level in chips was very high: R² = 0.890 (P < 0.001) for fructose and R² = 0.889 (P < 0.001) for glucose. The correlation between the sucrose content and acrylamide level was also high, R² = 0.779 (P < 0.001), although the concentration of the four amino acids showed no significant correlation with acrylamide concentration.

Comparison between early and regular harvesting

The early-harvested tubers, whose haulms had not completely senesced, are sometimes used for a year-round supply of chips in Japan. The effect of early harvesting of the tubers on the acrylamide level after cooking is thus also a matter of interest. Our previous study has shown that the contents of glucose and sucrose...
were high in young tubers and decreased according to maturation. Burton has also described that the maximum contents of sugars were obtained 1–2 weeks after tuber initiation, then the sugars decreased along with growth of the tubers and reached a minimum before the end of the growing season. We compared the sugar and amino acid components between the early-harvested and regular-harvested tubers, as well as acrylamide level in chips made from them. No significant difference in sugar contents was apparent between the early-harvested and regular harvested tubers because of large differences among samples, although the average values were somewhat higher in the early-harvested tubers (Table 1). The early-harvested tubers, which had been harvested approximately one month earlier than the regular harvest date, seemed to be at a growth stage in which the sugar contents had not completely decreased. On the other hand, the contents of asparagine and glutamic acid in the early-harvested tubers were significantly lower than those in the regular-harvested tubers (Table 1). The acrylamide level in the chips showed no significant difference between the harvesting times due to the large deviations as observed for the sugar contents (Table 1).

**Effect of curing on the tuber components and acrylamide in chips**

Curing is the process for healing tissues that have been wounded during lifting and transport. The tuber tissue forms a protective layer (wound periderm) over the damaged area during the curing period. The regular-harvested tubers were used to compare the components in the tubers before and after curing, and the acrylamide level in chips made from them. After curing, only slight changes in each average value were found: sucrose decreased, and amino acids and acrylamide increased slightly (Table 1). These results show that the curing treatment had little effect on the components or acrylamide level in this study.

**Tuber components during 4 weeks of storage at 2°C or 18°C and the acrylamide level in chips**

The contents of amino acids showed a slight increase in asparatic acid and decrease in glutamic acid after 4 weeks of storage at 2°C over the levels before storage, while there was no change in the amount of asparagine or glutamine (Table 1). The reducing sugar contents showed a marked increase during the storage at 2°C. The contents of fructose and glucose were 0.03 and 0.25 mg/g FW on day 0, before respectively increasing to 1.59 and 1.34 mg/g FW after 2 weeks of storage. After 4 weeks of storage, the contents were more than 4 times higher than those in the tubers stored for 2 weeks (Table 1). The sucrose content, which was 0.84 mg/g FW on day 0, had increased to 9.53 mg/g FW after 2 weeks of storage, but was less than one half of that figure in the tubers after 4 weeks of storage (Table 1). The acrylamide content in the chips was 1.32 μg/g of chips on day 0 and had increased to 11.95 μg/g of chips.

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**Table 1. Effect of Harvest Time, Curing and Short-Term Storage on Sugars and Amino Acids in Tuber and on Acrylamide in Chips**

<table>
<thead>
<tr>
<th>Harvest Time</th>
<th>Curing</th>
<th>Short-term storage</th>
<th>Sugar (mg/g FW)</th>
<th>Amino acid (μmol/g FW)</th>
<th>Acrylamide (μg/g chips)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early</td>
<td>Early</td>
<td>2 C weeks</td>
<td>Fructose</td>
<td>0.15 ± 0.01</td>
<td>0.33 ± 0.33</td>
</tr>
<tr>
<td>Early</td>
<td>Early</td>
<td>2 C weeks</td>
<td>Glucose</td>
<td>1.36 ± 0.11</td>
<td>0.29 ± 0.09</td>
</tr>
<tr>
<td>Regular</td>
<td>Early</td>
<td>2 C weeks</td>
<td>Fructose</td>
<td>0.03 ± 0.04</td>
<td>0.25 ± 0.08</td>
</tr>
<tr>
<td>Regular</td>
<td>Early</td>
<td>2 C weeks</td>
<td>Glucose</td>
<td>1.34 ± 0.31</td>
<td>0.34 ± 0.21</td>
</tr>
<tr>
<td>Regular</td>
<td>Regular</td>
<td>2 C weeks</td>
<td>Fructose</td>
<td>1.39 ± 0.21</td>
<td>0.34 ± 0.21</td>
</tr>
<tr>
<td>Regular</td>
<td>Regular</td>
<td>2 C weeks</td>
<td>Glucose</td>
<td>1.34 ± 0.31</td>
<td>0.34 ± 0.21</td>
</tr>
<tr>
<td>Regular</td>
<td>Regular</td>
<td>2 C weeks</td>
<td>Fructose</td>
<td>1.30 ± 0.21</td>
<td>0.34 ± 0.21</td>
</tr>
<tr>
<td>Regular</td>
<td>Regular</td>
<td>2 C weeks</td>
<td>Glucose</td>
<td>1.30 ± 0.21</td>
<td>0.34 ± 0.21</td>
</tr>
</tbody>
</table>

Early harvesting (99 days after planting) was around one month earlier than regular harvesting (136 days after planting). Curing was for two weeks at 18°C in the dark. The data were used to analyze the data for the analysis of amino acids and sugars. Values followed by the same letter within each component are not significantly different at the 5% level by Tukey multiple-comparison test.
after 2 weeks of storage. After 4 weeks of storage, the content had increased to three times of the values of that in chips stored for 2 weeks (Table 1). These results confirm those obtained from the 2-week storage experiment at 2 °C already described. At 18 °C, however, little change was observed in the contents of amino acids and sugars in the tubers during storage, and no increase in the acrylamide content in chips was apparent during 4 weeks of storage (Table 1).

The individual data for 4 weeks of storage at 2 °C or 18 °C in this test (n = 20) were used to analyze the correlation between the components in tubers and acrylamide in chips. High correlation was found between the contents of reducing sugars in the tubers and the acrylamide level in chips: \( R^2 = 0.910 \) (P < 0.001) for fructose and \( R^2 = 0.878 \) (P < 0.001) for glucose. The correlation between the sucrose content and acrylamide level was much smaller at \( R^2 = 0.214 \) (P < 0.05) than that between the reducing sugars and acrylamide, because the sucrose content had dropped after 4 weeks of storage at 2 °C, in spite of the increase in acrylamide content. These results suggest that sucrose in the tubers is not strongly linked to the formation of acrylamide in chips, although sucrose could form acrylamide with asparagine in a model system.12 Amrein et al.22 who have investigated the relationship between the contents of sugars and asparagine in potato and acrylamide formation, have described that the far more efficient fructose and glucose seemed to completely surpass the sucrose activity for forming acrylamide in potato chips.

Factors determining acrylamide in chips

To understand which component in tubers had the most effect on the acrylamide level, we analyzed the correlation between the components in tubers and acrylamide in chips according to all the data generated in this study (n = 56). The contents of amino acids showed no significant correlation with the acrylamide level: \( R^2 = 0.018 \) for asparatic acid, \( R^2 = 0.033 \) for asparagine (Fig. 2), \( R^2 = 0.097 \) for glutamic acid, and \( R^2 = 0.011 \) for glutamine, P < 0.01 for all. On the other hand, there was strong correlation between the contents of reducing sugars and the acrylamide level: \( R^2 = 0.873 \) for fructose (Fig. 3) and \( R^2 = 0.836 \) for glucose, P < 0.001 for both. These results confirmed those obtained in our previous study.15 Amrein et al.22 and Becalski et al.23 have reported that incorporating the asparagine concentration with those of the reducing sugars in the calculation improved the correlation a little. Using Amrein's formula \((0.5 \times \text{glucose} + \text{fructose}) \times \text{asparagine}\), in which glucose was weighted as half of fructose based on their efficiency for acrylamide formation,24 we analyzed our data and found a small increase in the coefficient, \( R^2 = 0.923 \). The results suggest asparagine in potato tubers as the main precursor for acrylamide formation during frying, as model studies have shown that acrylamide was formed from asparagine in the presence of reducing sugars.11–14) However, the asparagine content is generally more steady and substantially higher than those of the reducing sugars in potato tubers.22,25 It was also found in this study that the asparagine contents on a molecular basis were higher than those of the reducing sugars, with the exception of those shown after four weeks of storage at 2 °C. Therefore, the asparagine content alone had no correlation with the acrylamide level. Although there was a small increase in the coefficient between \((0.5 \times \text{glucose} + \text{fructose}) \times \text{asparagine} \) and acrylamide as just mentioned, it is likely in practice that the contents of reducing sugars may determine the degree of acrylamide formation in potato chips.

The sucrose content showed weak correlation with the acrylamide level: \( R^2 = 0.317 \) (P < 0.001). However, during 4 weeks of storage at 2 °C, the sucrose content decreased to less than one half of that in tubers stored for
2 weeks, although the acrylamide content continued to increase. This result suggests that sucrose in the tubers did not primarily take part in the formation of acrylamide during frying.

**Relationship between the chip color and acrylamide content**

Our previous study has shown that chips colored extremely dark brown contained a high level of acrylamide.\(^{1,5}\) Chip color, as estimated with \(L^*\) (lightness), had a high correlation with the acrylamide content in this study (Fig. 4). This result is consistent with the fact that acrylamide is formed in the Maillard reactions,\(^{11–14,19}\) which determine browning.\(^{3,5}\) The analysis of chip color is much easier than the measurement of acrylamide. Companies producing chips usually measure the chip color to remove discolored or burned chips before packing.\(^{26}\) This process is practically effective for decreasing the acrylamide level in the products.

**Conclusion**

We monitored in this study the changes in sugars and amino acids in tubers during short-term storage and their effects on the acrylamide level in chips. The acrylamide content in chips began to increase after 3 days of storage at 2°C in response to the increase of reducing sugars in the tubers. There was strong correlation between the contents of reducing sugars and the acrylamide level. The four amino acids tested in this study showed no significant correlation with the acrylamide level. These results indicate that the contents of reducing sugars in potato tubers determined the degree of acrylamide formation in chips. Early harvesting and the curing treatment had little effect on the components and acrylamide level in this study. The acrylamide content correlated well with the chip color as estimated by the lightness (\(L^*\)) reflecting the progress of the Maillard reactions.

A temperature of 2°C was selected for cold storage to emphasize the relationship between the compositional factors and acrylamide formation. This 2°C storage induced a marked accumulation of reducing sugars in the tubers and an increase in acrylamide after frying. Potatoes are generally stored at approximately 8 to 12°C for crispness.\(^{6,7}\) Tubers stored at 2°C are not processed into chips as commercial products. In fact, the chip color data (\(L^*\)) obtained from tubers stored for 2 and 4 weeks at 2°C were 47 and 38, respectively, these chips being extremely dark brown and inedible.

**Acknowledgments**

We thank Dr. Kiyofumi Mori and his co-workers at the Ohsumi branch of Kagoshima Agricultural Experiment Station for kindly providing the potato tubers from Kagoshima Prefecture. This study was financially supported by the “Integrated Research Program for Functionality and Safety of Food toward an Establishment of Healthy Diet” from the Ministry of Agriculture, Forestry and Fisheries of Japan.

**References**


